Physiology in Health and Disease
Published on behalf of the American Physiological Society by Springer

Sarah M. Greising
Jarrod A. Call *Editors*

Regenerative Rehabilitation

From Basic Science to the Clinic

american physiological society

Springer

Physiology in Health and Disease

Published on behalf of the American Physiological Society by Springer

This book series is published on behalf of the American Physiological Society (APS) by Springer. Access to APS books published with Springer is free to APS members.

APS publishes three book series in partnership with Springer: *Physiology in Health and Disease* (formerly *Clinical Physiology*), *Methods in Physiology*, and *Perspectives in Physiology* (formerly *People and Ideas*), as well as general titles.

Sarah M. Greising • Jarrod A. Call
Editors

Regenerative Rehabilitation

From Basic Science to the Clinic

Springer

american physiological society

Editors
Sarah M. Greising
School of Kinesiology
University of Minnesota
Minneapolis, MN, USA

Jarrod A. Call
Department of Kinesiology
University of Georgia
Athens, GA, USA

ISSN 2625-252X ISSN 2625-2538 (electronic)
Physiology in Health and Disease
ISBN 978-3-030-95883-1 ISBN 978-3-030-95884-8 (eBook)
https://doi.org/10.1007/978-3-030-95884-8

Published on behalf of the American Physiological Society

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Our ultimate goal with this book is to collectively draw attention to the current field of regenerative rehabilitation across a broad range of neuromusculoskeletal tissues. The currently accepted field definition of regenerative rehabilitation is a therapy that "*... integrates regenerative technologies with rehabilitation clinical practices to restitute function and quality of life in individuals with disabilities due to otherwise irreparable tissues or organs damaged by disease or trauma*." The stated primary goal of all regenerative rehabilitation is functional restoration to a pre-morbid state. This field broadly covers bone, skeletal muscle, cartilage, ligaments/tendons, vasculature, and the central and peripheral nervous tissue. Fundamental to the physiology of these neuromusculoskeletal tissue types is their plasticity, or the ability of these tissues to adapt to mechanical and/or chemical cues to improve functional capacity. In practice, rehabilitation is contingent on tissue plasticity as various rehabilitative stimuli are expected to impart positive changes on tissue function. We hope this work will advance evidence-based approaches by emphasizing physiologic opportunities and limitations to date.

Minneapolis, MN, USA — Sarah M. Greising
Athens, GA, USA — Jarrod A. Call

Acknowledgments

First, we would like to thank the American Physiologic Society (APS) and the eBook committee for their guidance and support through this process. One aspect we sought to specifically highlight in this work is the importance of physiology in the regenerative rehabilitation field. Physiological outcomes must be the cornerstone of any field hoping to advance evidence-based approaches to reach clinical integration. With this, we would like to thank our past mentors Drs. Robert Grange, Dawn Lowe, Carlos Mantilla, Gary Sieck, and Gordon Warren who have helped us to appreciate the importance of physiology.

Finally, a heartfelt thanks to every author who contributed to this work. Especially all the trainees, who supported chapters in this work, and represent the future of the regenerative rehabilitation field.

Contents

Chapter 1
Historical Perspectives of Regenerative Rehabilitation: Recovering and Restoring Functional Capacity

Christiana J. Raymond-Pope, Daniel B. Hoffman, David L. Saunders, and Sarah M. Greising

Abstract Regenerative rehabilitation is an emerging field combining regenerative medicine strategies with evidence-based rehabilitation practices. The overall aim of regenerative rehabilitation is to repair and regenerate organ systems and recover function following injury or illness. This review presents recent advances in regenerative rehabilitation with attention to historical context. The state of the field of development and implementation of therapeutic strategies across anatomic and physiologic systems is discussed. Providing an overview of relevant strategies and examples of early successes, the importance of physiologic functional outcomes is emphasized.

Keywords Physical therapy · Regenerative medicine · Skeletal muscle · Bone · Tendon · Vasculature · Nervous system

1.1 Introduction

In his statements on the history of rehabilitation from the Vietnam War, Paul Brown noted, "Leaders and innovators in the field were asking the question—'Where does treatment cease and rehabilitation start?' The true significance of this basically rhetorical question lay in the implication that treatment and rehabilitation were indistinguishable. . ." (Burkhalter 1994). Using a more thoughtful approach to the importance of the indistinguishable aspects of treatment and rehabilitation, regenerative rehabilitation has recently expanded as a field to provide patients with multi-pronged approaches to restore function after injury, disease, or illness. While the origins of rehabilitation and regenerative medicine treatments can be traced back

C. J. Raymond-Pope · D. B. Hoffman · S. M. Greising (✉)
School of Kinesiology, University of Minnesota, Minneapolis, MN, USA
e-mail: grei0064@umn.edu

D. L. Saunders
Department of Medicine, Uniformed Services University, Bethesda, MD, USA

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_1

thousands of years, the establishment of regenerative rehabilitation as a discipline has occurred relatively recently.

Strategies for rehabilitation can be traced back to the ancient Chinese, who used movement to relieve pain thousands of years ago through the practice of Qigong. Greek physician Herodicus used exercise to prevent disease in the fifth century B.C.E.; Roman physician Galen used rehabilitation for military injuries in the second century C.E., and during the Middle Ages philosopher-physician Maimonides used exercise for health (Kleisiaris et al. 2014). Similarly, the use of regenerative medicine techniques for various treatments can be found in the early use of biomaterials by the Egyptians during the Neolithic age, the Europeans during the Middle Ages, and as early as 600 A.D. by the Mayan civilization (Marin et al. 2020). The historic review of the field of regenerative rehabilitation herein seeks to draw attention to notable recent successes and describe progress in preclinical research and clinical translation. Through this review, we focus on clinically meaningful and system-specific functional outcomes as the field collectively works to develop regenerative rehabilitation practices that repair, regenerate, and rejuvenate the body following injury or disease.

1.2 Rehabilitation

The objective of rehabilitation, or physical therapy, is to act upon the systems of the body to facilitate physiologically beneficial adaptations. The medical specialty of Physical Medicine and Rehabilitation aims to restore functional limitations resulting from various pathological conditions or injuries by incorporating the expertise of practitioners involved in all stages of the rehabilitative process (Atanelov et al. 2015). Examples include skeletal muscle hypertrophy and/or bone mineral deposition following injury or surgery, or to attenuate declines in patients suffering chronic diseases, such as sarcopenia or heart failure. Toward the beginning of the twentieth century, patients were often restricted to bedrest and prolonged immobilization following acute or chronic injury, subsequently followed by a slow progression back into activity. Unfortunately, bedrest reduces the physiological load on the musculoskeletal system needed to induce physiological adaptations. This results in atrophy, net bone resorption, and poor functional outcomes (Koukourikos et al. 2014). In contrast, early weight-bearing and ambulation following surgical procedures to repair injuries, such as ankle fracture and anterior cruciate ligament (ACL) injury, have been associated with reduced in-patient hospitalization time, earlier return to full ambulation, and earlier return to work or sport (Simanski et al. 2006; Dehghan et al. 2016).

1.2.1 Early History of Rehabilitation

The practice of rehabilitation dates back to written accounts of physical healing techniques around 400 B.C. However, it was not until World War I that rehabilitation methodologies and education became more prominent in the United States as physicians began practicing formal physiotherapy, or physical medicine and rehabilitation, to rehabilitate injured and disabled military personnel. Although physicians prescribed various physical treatments, no standard physical therapy practices had yet been established. Consequently, the American Medical Association (AMA) Council on Physical Therapy was established in 1926 to broadly identify effective physical rehabilitative treatments. At the forefront of this movement was Dr. John Coulter, a military physician who served during World War I and as faculty at Northwestern University Medical School. Coulter was a leader in the educational development of physical therapy practices. Notably, his collaboration with basic scientists and other medical and surgical practitioners helped to establish the legitimacy of rehabilitative practices. In particular, his collaboration with Dr. Frank Krusen, founder of one of the first academic departments of physical medicine in the United States at Temple University Medical School, later resulted in establishing the American Academy of Physical Medicine and Rehabilitation in 1947. The practice of rehabilitation became more prevalent and accelerated during and immediately following World War II. During this time period, more injuries were survivable. The focus of physical medicine shifted from the recovery of ambulation to improving patients' physical, mental, emotional, vocational, and social capacities.

1.2.2 Progressing to Evidence-Based Practices in Rehabilitation

In recent years, the rehabilitation field has moved to incorporate sound methodological research and evidence-based practice (EBP) into the clinic. One of the first descriptions of EBP in rehabilitation was in 1992 at McMaster University in Ontario, Canada. Since then, EBP has been defined as "the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients/clients" (Sackett et al. 1996). Evidence-based practice encompasses three main principles. First, it integrates therapists' individual expertise, proficiency, and judgment based on clinical experience, with the best available clinical evidence from systematic and sound methodological research. Second, individual patient preferences and values must be considered in selecting the best procedures and practices for the patients' condition and the severity of that condition. Third, healthcare economics must be considered, with specific attention paid to the availability, quality and cost of treatments, facilities, and health insurance. Taken together, multiple factors must be considered when implementing an EBP

rehabilitation program for patients across a wide spectrum of pathological conditions and injuries.

It is well known that physical activity and resistance exercise confer substantial health benefits, with consistent, progressive training shown to improve a variety of health and physical outcomes. These outcomes include muscular strength, endurance, power, and cardiorespiratory fitness. The latter includes increased maximal oxygen uptake and decreased heart rate and blood pressure for a given absolute submaximal intensity, among other physiological variables. Increased levels of physical activity and exercise are inversely associated with premature mortality, cardiovascular disease, stroke, osteoporosis, type II diabetes, and metabolic syndrome, among other progressive deleterious conditions. Specific types of exercises are recommended to improve long-term health and functional outcomes across a wide spectrum of pathological conditions and injuries.

The purpose of prescribing rehabilitative exercise is to impart various mechanical stimuli, loads, and/or forces on body systems to effect beneficial responses. These stimuli are needed to transmit biochemical signals and subsequently activate intracellular signaling cascades; a process referred to as mechanotransduction. These include tensile (pulling), compressive (pushing), shear (parallel, opposite), torsional (twisting), and vibrational forces. Mechanotransduction is critical for eliciting a molecular response involving the activation of transcription factors necessary for net protein production, extracellular matrix (ECM) synthesis, skeletal or cardiac muscle hypertrophy, and bone mineral deposition, among others. For example, protein synthesis rates within skeletal muscle fibers are primarily controlled by the mechanistic target of rapamycin (mTOR) enzyme, which is activated by the mechanical stretch of muscle and several anabolic hormones, including insulin-like growth factor and growth hormone (Schoenfeld 2010; Mavalli et al. 2010). In fact, mTOR has been reported (Dreyer et al. 2006) to increase during and immediately following an acute bout of resistance exercise, subsequently leading to phosphorylation and activation of downstream target proteins to increase net protein production. Ultimately, the application of whole-body or regional mechanical stimuli must be specifically tailored to obtain the desired beneficial adaptations.

To impart specific adaptations on physiologic systems, different rehabilitation regimens are used in patients depending on age, specific pathological conditions, and injuries. The foundation of most common types of rehabilitation includes body weight, resistance, flexibility exercises, and aerobic activity. Body weight and resistance exercises impart tensile and compressive forces that activate intracellular signaling cascades. These induce skeletal muscle hypertrophy, increase net bone mineral deposition, and improve cardiovascular function. For example, loading specific skeletal muscle groups and bones has been shown to prevent, attenuate, or reverse bone mass loss in patients with osteoporosis. It has also been shown to reduce pain and disability in patients with osteoarthritis (American College of Sports Medicine et al. 2009; Garber et al. 2011; Messier 2008). Common indications for body weight and resistance exercises include vascular (e.g., myocardial infarction, heart failure), neurological (e.g., multiple sclerosis, stroke, amyotrophic sclerosis), and various musculoskeletal conditions (e.g., fractures, joint arthroplasty). Dynamic

and static flexibility exercises are prescribed to improve range of motion (ROM) and enhance patients' ability to perform weight-bearing activities, activities of daily living, and, in the case of athletes, improve sports performance through increased speed and rate of force production. In addition to commonly implemented rehabilitation methods, other therapies have been developed to improve functional outcomes, including electrical, magnetic, or mechanical stimulation, whole-body or targeted vibration, and blood flow restriction. Although these approaches have demonstrated some promise in improving functional outcomes across clinical populations, there is a lack of evidence to suggest specific timing and dosing guidelines to optimize therapeutic benefit. A multimodal rehabilitation program may act synergistically to confer optimal physiological adaptations on the body, specific to the stimuli enacted, to enhance functional outcomes.

1.2.3 Overarching Goals of Rehabilitation

Rehabilitation programs are designed to include distinct phases to progressively improve outcomes related to pain, joint ROM, muscular strength, and overall function by the end of each stage. Prior to the advancement from one phase to the next, a series of functional tests are typically administered to ensure patients are ready to progress. Tests may include loading percentage, number of repetitions for resistance exercises, or performance intensity of a particular task, such as walking or jogging.

Generally, the goal of the first phase of rehabilitation following injury or a surgical procedure is to protect the injured area. In this phase, immobilization may be prescribed, such as unloading of lower extremity injuries with a cast or a sling for upper extremity injuries. The goal of the second phase is to increase tissue tolerance to loading by slowly progressing to weight-bearing exercises such as walking. During this phase, activities may include performing active and passive ROM exercises, submaximal isometric exercises for affected muscle groups, and progressive loading. Examples of the latter include unloaded cycling or abdominal stability exercises for lower extremity injuries. Goals of the third phase include achieving full ROM of the affected joint, increasing tissue tolerance to loading, and improving strength and endurance with exercises such as jogging or biking. The final rehabilitation phase is aimed to return patients to performing activities at pre-injury or pre-surgery levels.

Each progressive phase of rehabilitation varies in length of time and types of exercises performed based upon the heterogeneity, complexity and severity of the patients' injury, and pain tolerance level (Myer et al. 2006). For example, mild hamstring strains generally require up to 1 month to rehabilitate. More complex injuries such as ACL injury with subsequent surgical reconstruction may require 9 months or longer to rehabilitate before patients return to full activity. As hamstring strain injuries primarily impact the muscle, do not involve bone or require surgery, an unloading phase is generally not prescribed. Instead, the first rehabilitative phase

(acute management) includes early loading of the injured muscle (Heiderscheit et al. 2010). The functional recovery phase follows with the goal of recovering muscle strength and ROM, in addition to graded running to maintain cardiorespiratory fitness. Then activities specific to an individual's sport or vocation are implemented. Finally, testing to return to full activity is performed. Individuals are encouraged to continue specific functional exercises to prevent re-injury following clearance for full activity. Rehabilitation phases are general guidelines. Even across studies examining optimal strategies to rehabilitate homogeneous mild hamstring injuries, varying numbers of phases and types of loading have been reported (Heiderscheit et al. 2010; Lightsey et al. 2018; Wangensteen et al. 2017). Further investigations are required to determine the optimal timing and loading strategies for each stage of rehabilitation across various injuries and pathological conditions to promote efficient repair.

1.3 Regenerative Medicine

Regenerative medicine is based on the principles of cell and tissue biology to facilitate the restoration of tissue and function. Regenerative medicine has been described as the "process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects" by the United States National Institutes of Health (NIH). Regenerative medicine includes an array of emerging technologies, such as biomaterials, stem cell therapies, engineered organs (e.g., trachea, urinary bladder), and/or tissues (e.g., skin, muscle, cartilage) to promote regeneration in affected body regions. While rehabilitation can improve tissue restoration and functional outcomes, there are countless conditions for which rehabilitation alone is not sufficient.

1.3.1 History of Biomaterials

The use of natural materials in medical procedures had a long history of success before modern biomaterial-based regenerative medicine emerged as a field in the mid-twentieth century. The use of sutures may be the oldest form of a regenerative biomaterial, with some reports indicating ancient Egyptians used linen sutures during the Neolithic age. Europeans used catgut sutures during the Middle Ages, and nacre dental implants were successfully used as early as 600 A.D. by the Mayan civilization (Biomaterials Science 2020). Although the first contact lenses were not developed until the nineteenth century, Leonardo Da Vinci is credited for developing the concept in 1508. Many synthetic polymers were developed for military use during World War II and subsequently used by surgeons as experimental treatments when necessary. For example, polymethylmethacrylate (PMMA) was found to be biologically inert, or producing minimal foreign body reaction, after fighter pilots

were injured with PMMA windshield shrapnel (Williams and Isaacson 2014). This prompted the use of PMMA as intraocular lens replacements while various other materials began to be tested for biocompatibility.

Historically, biomaterials were designed to be inert and simply replace damaged tissue rather than assisting in regeneration. To be successful, biomaterials need to have the necessary mechanical properties for a specific application in addition to being biocompatible. If a material is cytotoxic, the surrounding tissue will die and thus it is not biocompatible. Even when not cytotoxic, materials may prove incompatible by activating the immune system's foreign body response. A foreign body response may create a fibrous capsule around the implant, altering its physical properties and damaging the surrounding tissue. In many applications today, surface modifications are made to the biomaterial to reduce the foreign body response and/or provoke beneficial effects in the surrounding tissue.

While attempting to solve the problem of inert metallic bone implants from being rejected, Hench et al. developed a bioactive glass material that integrates with the existing bone (Hench et al. 1971). The interface between the glass and bone was bound with an active hydroxyapatite layer instead of a fibrous capsule. The bound interface was not only stronger but also reduced the risk of rejection compared to inert metallic implants. This study provided a new framework for thinking about biomaterials by demonstrating inertness is not always advantageous. Instead of trying to avoid biological interaction, materials can be designed to positively influence the surrounding tissue. Currently, the most widely accepted definition of a biomaterial is "any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual" (Marin et al. 2020).

1.3.2 Modern Biomaterials

Modern biomaterials can be broken down broadly into three classes: ceramics/glasses, metals, and polymers. Ceramics have the highest tensile strength but lowest ductility of the three biomaterial categories, making them suitable for bone and dental implants. They are often used in articulations of metal joint replacements because of their friction resistance. Porous ceramics are not suitable for load-bearing but may provide an ideal bone implant by allowing existing bone to grow within the pores and create a strong interface. Glasses and glass-ceramics have become popular choices for bone implants due to the mechanical properties and ability to integrate bioactive components. As of 2016, it was reported Hench's 45S5 Bioglass® has been used in over 1.5 million patients (Hench et al. 1971).

Metallic biomaterials are used commonly in joint replacements and bone fixation, dental implants, and vascular stents. Common types include stainless steel, cobalt-based alloys, and titanium-based alloys. These are generally preferred over other materials because of their mechanical strength and toughness, corrosion resistance,

and light weight. In addition, they can be processed below their recrystallization temperature, known as cold working. This creates imperfections in the crystalline structure that inhibit the movement of atoms, further increasing strength and hardness (Biomaterials Science 2020). Stainless steels are commonly used as expandable vascular stents because of their ductility and subsequent strain hardening. Furthermore, metals allow for endothelial growth over their surface which reduces the risk of blood clotting. By the early 2000s, drug-eluting metallic stents proved superior to bare metal stents for reducing clots and are regularly used today (Silvain et al. 2014). Metallic implants are thought to promote the regeneration of healthy vascular tissue. However, due to their strength and general inability to degrade, metallic biomaterials used for applications such as fracture fixation do not promote regeneration. Instead, metallic fixation has a stress shielding effect, limiting the load placed on the bone, and consequently reduces bone remodeling due to Wolff's law. Therefore, degradable materials, such as polymers, have begun to be tested for viability in bone fixation to encourage bone remodeling and ultimately function (Kulkarni et al. 1971; Cai et al. 2019).

Polymers comprise a wide range of possible bulk material and surface properties and may be more useful for tissue regeneration than metals or ceramics due to potential biodegradability. Polymers consist of multiple repeating molecular subunits, or "*mers*," which form chains that cross-link together to form the bulk material. They can be synthetically made (e.g., polyethylene, polypropylene, etc.) or occur naturally (e.g., collagen, silk, wool, etc.). Polymers can consist of a single type of repeat unit, called a homopolymer, or can be made up of two or more types of repeat units called a copolymer. The amount of cross-linking within a polymer material can dictate the elastic modulus and toughness of that material. Polymers with lower cross-linking density have a low modulus and are known as elastomers, as they can stretch and return to the prior shape. Plastic polymers can withstand higher stress than elastomers with greater ductility and toughness. Finally, brittle polymers are heavily cross-linked, resulting in lower ductility but increased strength, similar to ceramics. Because of this wide range, polymers have been used in tissue engineering, drug delivery, vascular and skin grafts, and joint replacements, among others. Moreover, highly cross-linked and hydrophilic polymers can form hydrogels, which are advantageous in a variety of applications. The highly cross-linked structure of hydrogels allows for withstanding tensile stress, while hydrophilicity allows for stretch and elasticity.

1.3.3 Tissue Engineering

The field of tissue engineering began to emerge in the 1970s. Bell et al. first reported that collagen hydrogels cultured with fibroblasts undergo contraction and form skin-like structures (Bell et al. 1979). Around the same time, Yannas and Burke examined the ability of a porous, cross-linked collagen-glycosaminoglycan scaffold to regenerate skin in vivo. The scaffolds were implanted into the wound beds of excised skin

of guinea pigs and found to regenerate skin remarkably well (Yannas et al. 1982; Yannas and Burke 1980). These findings led to the first clinical uses of artificial skin and provided a basis for engineering other types of tissue.

Providing a conceptual framework, the tissue engineering triad guides the successful development of new constructs (Almouemen et al. 2019). The first pillar of the triad is a biomaterial scaffold, or matrix, which provides structure and an organized template upon which stem cells and regenerated tissue can align. The scaffold's mechanical properties should closely resemble the native ECM of the desired tissue. Thus, natural polymers such as collagen, silk, cellulose, and various proteins are often used as tissue engineering scaffolds due to the resemblance of native ECM. Importantly, because natural polymers are comprised of proteins, they have the advantage of innate bioactivity. This encompasses the second pillar of the tissue engineering triad: appropriate signaling and biophysical cues. Natural materials contain binding sites for various growth factors and cell attachment, which can dictate cellular differentiation and proliferation. They can also be enzymatically degraded, allowing for replacement by an endogenously produced matrix. Additionally, modifying polymer alignment and pore size within the scaffold can enhance organization of regenerated tissue and allow nutrients and metabolites to enter and exit the region. The third pillar of the triad is the need for cells; specifically, progenitor cells that will regenerate the desired tissue. This can be accomplished by the scaffold attracting endogenous cells when implanted, or by seeding the scaffold before implantation.

Today, tissue engineering using decellularized scaffolds has been attempted for just about every organ in the body with varying degrees of success (Yu et al. 2016; Corona and Greising 2016). Although these naturally derived scaffolds offer many advantages as previously discussed, they typically lack the necessary mechanical properties for some applications and are difficult to produce with consistent properties. Synthetic polymer scaffolds can be produced with more precision, consistency, and stronger mechanical properties, yet do not degrade as readily and are not innately bioactive materials. Consequently, composite scaffolds and hydrogels which combine natural and synthetic polymeric aspects are becoming more common in tissue engineering.

1.3.4 Cellular Therapy

Perhaps a more simplified approach to regeneration than transplantation of engineered tissues is the use of stem cell therapy. Instead of introducing a foreign biomaterial and managing the potential host responses, stem cells can be injected or delivered to the desired tissue and directly contribute to regeneration. Hematopoietic (HSCs) and mesenchymal stem cells (MSCs) are the most common types used for tissue regeneration today, though embryonic (ESCs) and induced pluripotent stem cells (iPSCs) theoretically have greater potential. The first use of the term "stem cell" in the literature dates back to the late nineteenth century by Ernst Haeckel

(Ramalho-Santos and Willenbring 2007). However, stem cell research did not truly gain momentum until the late twentieth century. Today MSCs as they are referred to, are "adult" stem cells primarily harvested from bone marrow. It is now known that other types of tissue can be sources of MSCs, including adipose, umbilical cord, amniotic fluid, and dental pulp. While the term MSC was first coined by Arnold Caplan in 1991 (Caplan 1991), previous studies identified the osteogenic capacity of bone marrow, likely due to the presence of such cells (Tavassoli and Crosby 1968). MSCs are multipotent, meaning they can differentiate to form multiple types of tissue, including muscle, bone, cartilage, fat, and connective tissue. While MSCs have been useful for the aforementioned applications, pluripotent stem cells' ability to differentiate into any type of tissue presents a likely regenerative advantage. Thomson et al. were the first to isolate human ESCs (Thomson et al. 1998), which are pluripotent and can self-renew indefinitely. However, because the embryo (blastocyst) from which these cells are taken needs to be destroyed, there is controversy regarding the ethics of harvesting ESCs. In 2006, Takahashi and Yamanaka developed a method for converting mature fibroblast cells to induced pluripotent stem cells (iPSCs) for which Yamanaka received the Nobel Prize in 2012 (Takahashi and Yamanaka 2006). This was a breakthrough in the field of stem cell research, as iPSCs have the virtually unlimited proliferative ability, and avoid ethical issues surrounding the use of ESCs, significantly advancing the field of stem cell biology and regeneration.

In theory, multipotent and pluripotent stem cells have the ability to cure a host of diseases, yet the only current US FDA-approved stem cell products are HSCs derived from cord blood to treat blood disorders, mainly for end-stage cancer patients. Common adverse reactions to HSC treatment include acute and chronic bacterial infections and graft-versus-host disease, with a range of severity (Omrani and Almaghrabi 2017; Zhao et al. 2019). Thus, the primary limitations of obtaining FDA approval are safety and efficacy, as numerous severe complications exist with treatment and little to no improvement has been demonstrated with treatment for some conditions. Nonetheless, hundreds of clinics providing autologous stem cell therapies have opened throughout the U.S. and globally. While often justified by the use of autologous tissue, the legality under which many operate is questionable. Though long-term complications are not well understood at this point, numerous patients have reported tumor formation years after an unapproved treatment (Bauer et al. 2018). Likewise, MSCs have been shown to promote metastatic growth in vitro and in vivo (Wang et al. 2015).

While there is a promising future for stem cell therapies, refinements need to be made to reduce adverse complications and improve efficacy. Appropriate methods to effectively deliver cells remain a significant barrier to use. MSCs have been tested in animal models for the treatment of various conditions including skeletal muscle injury, stroke, peripheral nerve injury, cartilage damage, and osteoarthritis among others (Goldman et al. 2017; Wilke et al. 2007; Guercio et al. 2012; Horita et al. 2006). The most common methods of cell delivery are injection either directly into the tissue of interest or the systemic circulation. Yet only a small proportion of cells appear to engraft at the treated site in most cases with, approximately 90% of cells

lost within the first few hours following transplantation (Mooney and Vandenburgh 2008). Moreover, cells display a weak ability to migrate from the injection site. Inconsistent results between studies with similar treatments are perhaps linked to inconsistencies in cell engraftment due to these issues.

1.3.5 Combined Approaches in Regenerative Medicine

Combinatorial approaches that use stem cells in conjunction with biomaterials may help address the problem of stem cell loss by promoting cell adhesion and encouraging cell engraftment within the body region of interest. Current means of the combination include seeding a 3D biomaterial scaffold with stem cells *ex vivo* and subsequent implantation of the engineered tissue construct. Challenges of creating a useful tissue construct include the efficiency of cell seeding and obtaining a uniform distribution of cells within the 3D scaffold (Martin et al. 2004). Early attempts at cell seeding often used static loading methods, resulting in an uneven distribution of cells throughout the scaffold and poor seeding efficiency. This process is better accomplished by using a bioreactor, which typically provides mechanical stimulation to the cells in addition to maintaining an appropriate physiological environment. Mechanical stress or stimulation has been shown to increase in vitro bioactivity of cells in 3D matrices (Butler et al. 2000), and thus allows improved tissue regeneration outcomes.

Bioreactors designed to grow tissues "in situ" have also been widely employed in regenerative medicine research. Considerations for bioreactor designs include scaffold type, environmental control, mass transport of nutrients and regulatory molecules, physical signals, and scale (Biomaterials Science 2020). It is important to note that ideal bioreactor conditions will vary based on the specific type of tissue desired. As previously discussed, scaffold types can include naturally- or synthetically-derived 3D polymer matrices or a composite of the two. Environmental control of parameters including temperature, pH, and gas diffusion is an advantageous feature of the bioreactor approach compared to standard laboratory tissue culture systems. Gas exchange units can precisely regulate oxygen and carbon dioxide at physiological levels, in turn controlling pH of the culture medium and metabolic activity of seeded cells. Moreover, mass transport of molecules, gas, and nutrients into the porous scaffold and subsequent waste removal has been a major obstacle. Under static conditions, little substance successfully migrates into scaffold pores, including cells, resulting in a shell on the exterior of the scaffold. By manipulating fluid flow rates, proper transport and uniform distribution of cells and molecules can occur. One recent approach has been to surgically implant bioreactors into patients themselves during the incubation period in an effort to overcome some of these limitations (Watson et al. 2020), with bioreactor harvest prior to tissue implantation.

1.3.6 Composite Tissue Regeneration

A long-term unrealized goal of regenerative medicine will be the complete regeneration of composite tissues in the manner of amphibians and other self-regenerating animals. Strategies to unlock the body's natural healing responses have been pursued using various approaches. While pioneering work has been done, clinically significant results have remained elusive. Early work in electrical stimulation replicated the processes of limb regeneration from salamanders through the formation of a blastemal of germ cells, akin to ESCs which then recapitulated severed limbs. Reversal of the naturally occurring biological currents could likewise abort amphibian limb regeneration (Becker and Spadaro 1972). This work progressed to mammals with some indication of a partial healing response akin to blastemal formation (Becker and Spadaro 1972), and this work has been replicated recently (Leppik et al. 2015). Recent investigations have attempted to refine this approach through the use of tailored small molecule modulation of the underlying bioelectrical circuits that have been shown to direct spatial patterning and direct anatomic regrowth (Mathews and Levin 2018).

Building on this work, a more in-depth study of the underlying mechanisms of these early experiments has been pursued. A host of growth factor, cellular, and small molecule approaches have been pursued. Cultured human placental cells are believed to exert a favorable paracrine effect from a host of endogenous growth factors. Given their intrinsic lack of immunogenicity, they have been found safe and effective for use in lower extremity critical limb ischemia and hip fracture when applied locally (Modarai and Patel 2019; Winkler et al. 2018). Signaling molecules such as small interfering RNA (siRNA) have been tested preclinically for the healing of a variety of insults by stimulating endogenous repair processes (Hu et al. 2019). Small molecule interventions have also found application in this regard (Billin et al. 2016). Further advances in composite tissue regeneration are expected to result in simultaneous regrowth of multiple tissues, with the potential to reduce or eliminate the need for wound micromanagement following injury and pathology.

1.4 Regenerative Rehabilitation

Regenerative medicine and rehabilitation science were established and have evolved primarily as separate disciplines. Yet, when implemented alone, each may fail to fully recover tissue function. Recent calls have been made to combine these two approaches into a single therapy, termed *regenerative rehabilitation*. The concept of regenerative rehabilitation broadly covers all tissues and organ systems of the body. It is defined as therapy that "integrates regenerative technologies with rehabilitation clinical practices to restitute function and quality of life in patients with disabilities due to otherwise irreparable tissues or organs damaged by disease or trauma" (Perez-Terzic and Childers 2014). Regenerative rehabilitation is an emerging and rapidly

expanding field, as evidenced by the rise in publications per year since 2000. The overarching goal of this combined approach is to synergistically improve clinical outcomes by restoring damaged or lost tissue and recovering tissue functionality to a pre-pathological or pre-injured state.

The concept of regenerative rehabilitation was initially formalized and integrated within the past decade (Perez-Terzic and Childers 2014). The first department of Rehabilitation and Regenerative Medicine was established at Columbia University in New York and has been led by Dr. Joel Stein since 2008 (Perez-Terzic and Childers 2014). Subsequently, Columbia's Stem Cell Initiative research program became integrated with the University's rehabilitation medicine and education entities. Since this establishment and the integration of these entities, several institutions throughout the United States have integrated regenerative medicine techniques into academic rehabilitation departments, including for example: the Rehabilitation Medicine Research Center within the Physical Medicine and Rehabilitation Department at Mayo Clinic; the McGowan Institute for Regenerative Medicine at the University of Pittsburgh; the Stanford Institute for Stem Cell Biology and Regenerative Medicine; and the Department of Physical Therapy and Rehabilitation Science at the University of California, San Francisco. These institutions, along with others, are part of a larger International Consortium for Regenerative Rehabilitation. The Consortium's mission is to bring together leading scientists and clinicians to form new interdisciplinary collaborations and exchange ideas for the development and translation of technologies that restore function and enhance patients' quality of life (Willett et al. 2020). Beginning in 2015, the Alliance of Regenerative Rehabilitation Research and Training (AR^3T) program at the University of Pittsburgh received NIH funding to support the expansion of research and scientific knowledge, expertise, and methodologies across regenerative medicine and rehabilitation science disciplines. Broadly advancing the field of regenerative rehabilitation, a growing number of researchers have recently begun investigating the efficacy of integrating regenerative and rehabilitation strategies for various traumatic injuries and chronic pathologies (Ambrosio and Rando 2018). Prominent examples from various fields are subsequently discussed.

1.4.1 Early Successes of Regenerative Rehabilitation

Despite its relative infancy, progress has been made in the study and implementation of regenerative rehabilitation approaches. The purpose of these combined approaches is to enhance the local microenvironment, tissue plasticity, and functional capacity following injury or in a pathological condition (Fig. 1.1). To date, most of this research has been conducted in the preclinical setting. There are currently only limited clinical research reports, largely case studies and series. Combined approaches implemented across fields of study have included rehabilitation in the form of physical activity, electrical, magnetic, or mechanical stimulation, and ultrasonography. These rehabilitation practices have been performed prior to

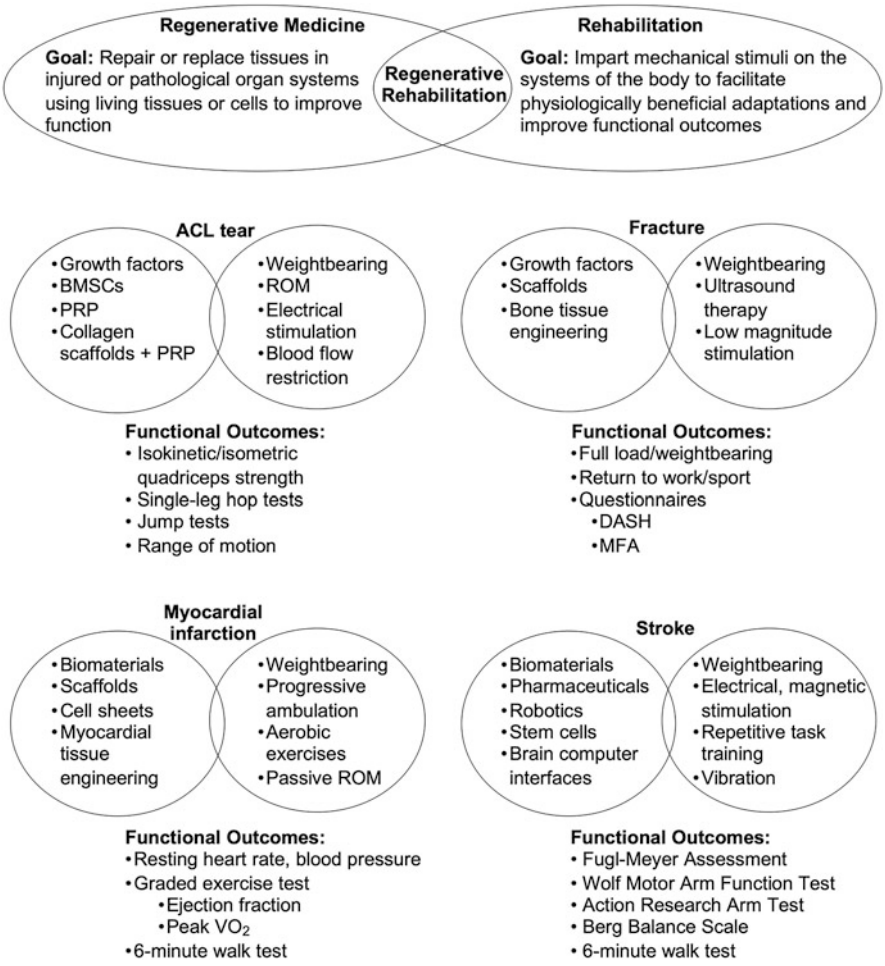

Fig. 1.1 The goal of regenerative rehabilitation is to elicit a synergistic effect when combining regenerative medicine approaches with rehabilitation practices to enhance tissue repair and regeneration and recover muscle function. Regenerative rehabilitation practices have been investigated across fields, with various techniques employed across injuries and pathologies. *BMSC* bone marrow-derived stem cells, *PRP* platelet-rich plasma, *ROM* range of motion, *DASH* disabilities of the arm, shoulder, and hand, *MFA* musculoskeletal function assessment, VO_2 oxygen consumption

and/or following a regenerative strategy, such as stem cell or growth factor delivery and/or scaffold implantation, with varying success reported. Examples of injuries and pathologies for which initial regenerative rehabilitation therapies have made substantial progress include ischemic conditions, such as stroke and peripheral arterial disease (PAD).

In the central nervous system (CNS), the primary effectors of neural plasticity and remodeling are electrical and neurotrophic signaling. It is therefore the goal to elicit

plasticity through these mechanisms following CNS trauma, such as ischemic stroke. Stroke is a leading cause of disability worldwide (Krause et al. 2019). In the United States, approximately 800,000 people suffer from a stroke yearly, and at least two-thirds of stroke survivors require rehabilitation (Virani et al. 2020). During an ischemic stroke, vessel occlusion and cessation of cerebral blood flow leads to a lack of oxygen and glucose to the area fed by the occluded vessel. This results in neuronal cell death by necrosis in the initial phase following injury and long-term apoptosis due to oxidative stress, chronic inflammation, and glial scar formation at and surrounding the infarct region (Portis and Sanberg 2017; Tam et al. 2014). Following stroke, patients may experience various disabilities, including paralysis, hemiplegia, or hemiparesis, all leading to decreased ambulation; lack of coordination or balance; sensory disturbances such as pain; difficulty using or understanding language; and problems with thinking and memory, among others. Currently, the only FDA-approved treatment for ischemic stroke is tissue plasminogen activator (tPA), a naturally occurring protein that must be administered within approximately three hours of a stroke event to dissolve the clot and restore blood flow (Powers et al. 2018). Patients are generally then prescribed rehabilitation that includes aerobic exercise, such as treadmill walking or ergometer cycling. Patients may also be prescribed non-invasive repetitive transcranial magnetic stimulation (rTMS) and/or transcranial direct current stimulation (tDCS) to enhance neuromuscular activation and cortical reorganization. Stimulation-based rehabilitation has been reported to improve, but not fully recover ambulation, muscle strength, balance, and postural control following stroke (Moritz and Ambrosio 2017; Teskey et al. 2003). The clinical study of regenerative strategies has been limited to small trials or case studies, primarily using stem cell therapies over the past two decades. Significantly more research has been conducted in the preclinical setting using rodent models of stroke.

Early animal models of ischemic stroke, or cerebral ischemia (e.g., middle cerebral arterial occlusion; MCAO), were developed in the late 1970s. These stroke models have brought insight into tissue damage mechanisms following cerebral vessel occlusion. The models have also been used to examine the efficacy of regenerative therapies, both alone and in combination with rehabilitation strategies, to improve structural and functional outcomes following stroke (Fluri et al. 2015). Preclinical regenerative strategies implemented following MCAO have included: stem cells, such as MSCs, neural stem cells (NSC), and neural progenitor cells (NPC); decellularized or hydrogel-based scaffolds seeded with or without stem cells; and pharmacological treatments (Gopalakrishnan et al. 2019). Preclinical rehabilitation regimens have included: treadmill running; enriched environments that provide various sensory, social, motor, and visual stimuli; and electrical stimulation and rTMS. In a rat model of MCAO and mouse model of hypoxic-ischemic brain injury, the efficacy of various combinations of regenerative rehabilitation strategies has been examined. The majority of these studies reported combining MSCs derived from various sources, such as adipose tissue and bone marrow with treadmill exercise or with an enriched environment (Cho et al. 2016; Zhang et al. 2015; Sasaki et al. 2016).

Across studies, results have demonstrated improved angiogenesis, neurogenesis, and synaptogenesis while modulating inflammation and decreasing apoptosis, glial scar formation, and infarct volume. Additionally, in most studies, a beneficial synergistic effect was reported on behavioral, sensory, and motor function outcomes. Tests to examine these outcomes included: the limb placement test; cylinder and ladder walking tests to evaluate forelimb symmetry; Roger's test to assess simple motor function, such as reflexes; grip strength; and the rotarod test to examine coordination and balance. More recently, the use of MSCs combined with ipsilesional cathodal current stimulation (Morimoto et al. 2018) as well as NSCs combined with rTMS (Peng et al. 2019) supports similar improved structural and functional outcomes to studies combining stem cell therapies and rehabilitation, such as treadmill running. It is evident that regenerative rehabilitation approaches can synergistically facilitate neuroprotection and enhance structural and functional outcomes following stroke. This is an important observation, as stem cells, particularly MSCs, are being considered for stroke treatment in the clinical setting. Phase I/II trials have shown initial safety and efficacy of bone-marrow-derived MSCs in combination with rehabilitation (e.g., physical, occupational, and speech therapies) to improve clinical outcomes following stroke, including scores on the NIH Stroke Scale (NIHSS) and Fugle-Meyer assessment (Steinberg et al. 2016). More investigations are needed to identify the optimal combination of regenerative rehabilitation strategies for patients following stroke and the appropriate timing to initiate these therapies in the clinic.

The field of cardiology has also seen success in implementing regenerative rehabilitation approaches. One such area is limb PAD, a major vascular complication that affects over 200 million people worldwide and up to 20% of individuals over the age of 65 (Shu and Santulli 2018). PAD is characterized by progressive blockage of at least one peripheral artery in the lower extremity by plaque, resulting in stenosis or occlusion and leading to decreased blood flow to leg muscles. This disease can lead to chronic limb ischemia and tissue loss, requiring amputation in severe cases. Approximately 40% of PAD patients have intermittent claudication, or muscle pain, cramping, or aching in the calf or thigh. Currently, rehabilitation prescribed for PAD patients includes walking, with intensity guided by pain. Regenerative strategies have included delivery of endothelial progenitor cells (EPCs) in animal models (Yu et al. 2009; Hu et al. 2008) and in clinical trials (Lara-Hernandez et al. 2010). Endothelial progenitor cells, which are isolated from mononuclear cells, are well known for their ability to self-renew and their potential to differentiate into functional endothelial cells (Napoli et al. 2011), supporting the effectiveness of EPCs to improve ischemia-related organ dysfunction through enhanced angiogenesis. In clinical trials, EPCs have been shown to be safe and effective in improving tissue perfusion and ankle-brachial index measurements, the gold standard method by which PAD is diagnosed, while decreasing leg pain at rest and/or improving pain-free walking time (Van Tongeren et al. 2008; Higashi et al. 2004; Bartsch et al. 2007). Others, however, have reported null results for these measures (Franz et al. 2011). It is noteworthy that study endpoints have ranged between 1- and 13-months post-EPC treatment, and different processing techniques and EPC concentrations

have been used, which may explain conflicting results across clinical studies. Preclinically, in a rat model of critical limb ischemia, investigators (Yeh et al. 2012) implemented a combined approach delivering bone marrow-derived EPCs and extracorporeal shock wave therapy, providing mechanical stimulation through high-energy acoustic waves. This combined approach has been observed to improve hindlimb angiogenesis and restore blood flow to levels observed in uninjured animals. Importantly, these improvements were greater than with either approach alone. However, no measures of function were included in these analyses. These findings are important as EPCs and shock wave therapy are clinically available (Sun et al. 2011; Napoli et al. 2011); further clinical investigation is warranted to determine the efficacy of this combined therapy along with other approaches.

1.5 Historic Systems Biology Approach to Regenerative Rehabilitation

Across injuries and pathological conditions, it is evident that substantial progress has been made in the fields of regenerative medicine and rehabilitation. Initial findings suggest that regenerative rehabilitation approaches can act synergistically to improve tissue architecture and function. Currently, approaches are at various stages of success and translation across the body. Herein, using a systems biology approach, we provide highlights of recent work and emphasize functional evaluation of regenerative rehabilitation.

1.5.1 Central and Peripheral Nervous System

A host of debilitating conditions can affect the CNS and PNS. Regenerative rehabilitation for previously intractable nervous system injuries has come a long way in the past decade, yet still faces substantial challenges. Despite the development of stem cell therapies, for the most part, significant injury to the brain and spinal cord remains largely irreversible from a functional standpoint. Historically, focus has been on the management of long-term disability, reduction of the rate of loss of residual function, and preservation of quality of life. Physical and occupational therapy plays a critical role, along with appropriate medical management and social support. However, substantial progress in regenerative rehabilitation for injuries to the brain, spinal cord, and nerves promises to substantially improve the outlook in the coming years.

1.5.1.1 Central Nervous System

Vascular insults and cellular degenerative processes can lead to a vast number of CNS lesions affecting nearly every possible function. Until recently, the mainstays of therapy have been medical management to prevent ongoing declines, and physical and occupational rehabilitation to preserve activities of daily living. Due to the complexity of injuries, there is a need to study the relative contributions of rehabilitation and regenerative therapies independently. Improvements in functional recovery have been demonstrated clinically following stem cell transplantation. Combination with growth factors may further reduce inflammation, stimulate neurogenesis and improve stem cell survival (Asgharzade et al. 2020). Repetitive transcranial stimulation to alter brain excitability is also now being used clinically and found to have use in subcortical stroke, particularly when applied to the unaffected hemisphere (Ito et al. 2020). Pharmacologic interventions may serve as adjuvants to recovery and some including citicoline, fluoxetine, niacin, and levodopa are in clinical trials or current use (Szelenberger et al. 2020). Exercise, including increasing use of robotic assistance has been demonstrated to induce neural plasticity and improve motor function by regenerating neurons and intra-hemispheric connections, along with functional reorganization in unaffected areas (Xing and Bai 2020). The window for exercise rehabilitation intervention is now thought to be greater than 6 months (Szelenberger et al. 2020). Stem cell therapies have also been used to treat neurodegenerative diseases including various forms of dementia, with some initial promise demonstrated (Sivandzade and Cucullo 2021).

1.5.1.1.1 Spinal Cord

More than 300,000 people are estimated to live with spinal cord injury in the United States, with new cases approaching 20,000 per year due primarily to falls, gunshot, and motor vehicle accidents (Kiyotake et al. 2020). The current standard of care includes: early decompression surgery to repair vertebrae, medical management attempting to increase spinal perfusion, hypothermia, and rehabilitation. The outlook for recovery generally remains poor for moderate to severe injuries (Khorasanizadeh et al. 2019). While there are a great deal of potential solutions currently being studied, neither regenerative medicine or rehabilitation approaches have yet led to full recovery from moderate to severe injuries (Chhabra and Sarda 2017). Regenerative approaches have included a wide range of cellular products alone and in combination with biomaterials, molecular therapies and/or drugs (Ashammakhi et al. 2019), including some in clinical stage development. The wide array of rehabilitation approaches currently in development includes direct electrical (Courtine and Sofroniew 2019), transcranial (de Araujo et al. 2020), and pharmacologic stimulation (Hayashi et al. 2010), as well as conventional locomotor rehabilitation techniques. Numerous electrically conductive biomaterials are also in

preclinical development in an effort to merge regenerative and rehabilitative approaches (Kiyotake et al. 2020).

1.5.1.1.2 Traumatic Brain Injury

Traumatic brain injury (TBI) has been a signature insult of recent wars and is common in civilians as well, with more than 280,000 hospitalizations and 56,000 deaths in 2014 (Capizzi et al. 2020). Roughly 20% of injuries are moderate to severe. Traumatic injuries to the CNS, including the brain (i.e., TBI) and spinal cord, are less common and less well studied than medical causes of CNS injury. As a result, progress in regenerative rehabilitation has not been as great as with medical causes. As a more diffuse process, TBI is more poorly understood, associated more commonly with cognitive deficits, and is more difficult to treat. Despite this, there is limited evidence emerging for modest improvements in even severe TBI symptoms, cognitive function, and favorable neuroplasticity with both cognitive rehabilitation and non-invasive brain stimulation techniques (Galetto and Sacco 2017). Immersive virtual reality rehabilitation has also played an increasing role (Maggio et al. 2019). Vagal nerve stimulation has demonstrated limited benefit in recovery from both stroke and TBI (Wu et al. 2020; Pruitt et al. 2016) and may have a favorable impact on a number of inflammatory conditions as well (Johnson and Wilson 2018).

1.5.1.2 Peripheral Nerve Injury

While injured peripheral nerves tend to regenerate reliably, the slow speed of Wallerian degeneration limits the speed of distal axonal regrowth past the point of injury to 1 mm/day. Even with modern diagnostics, including electromyograms and nerve conduction testing, it remains impossible to determine with confidence whether or not injured nerves will recover function (Saunders and Rose 2021). As a result, watchful waiting generally remains the norm for injury repair due to ongoing diagnostic limitations. Treatment with autologous nerve grafts may commit patients to longer periods of denervation with greater risk for atrophy of target muscles, ultimately rendering such repairs useless. Decellularized cadaveric human nerve grafts have proven to be effective tissue sparing solutions and an early win for repair of traumatic injuries. Emerging regenerative technologies aim to overturn this paradigm. Interventions to improve diagnostic accuracy, preserve partial nerve conduction, and support denervated target end organs during regrowth are currently in development (Gurjar et al. 2021). Methods to preserve nerve continuity and short-circuit the process of Wallerian degeneration, including fusion of the nerve ends with chemical sealants, could potentially shift the current watchful waiting paradigm to one of surgical emergency akin to that of traumatic vascular repair (Riley et al. 2017).

1.5.2 Cardiovascular System

Meaningful regenerative and rehabilitative outcomes are dependent on well-perfused tissues. In serious injuries and vascular occlusive events early, definitive, and sustained restoration of blood flow is at the heart of limb and vital organ salvage. Failure to do so invariably leads to loss of target organ function and in some cases amputation with a permanent disability. In serious traumatic injuries, arterial damage, laceration, and thrombosis often require urgent vascular reconstruction to save tissues from ischemia, necrosis, and further amputation. Once the large vessels have been restored, attention must be turned to microvascular damage to prevent tissue ischemia. On the macro level, while regenerative vascular replacements have advanced to clinical-stage investigational therapies including trauma indications (Amiel et al. 2006), little has changed in surgical practice in the last 30 years. There are two current methods for vascular reconstruction: harvesting of autologous vein or using synthetic graft materials. While each has advantages, there are important limitations for both. Autologous vessels can be time-consuming to harvest, in some cases inadequate for the intended use, and difficult or impossible to use, particularly in complex multi-limb trauma (Fox et al. 2005). Donor-site morbidity is also an issue including infection, scarring, leg edema, and loss of potential graft material in young patients who later develop atherosclerotic disease (Terada et al. 1999). Contamination of open traumatic injuries likewise creates challenges for use of synthetic grafts, commonly of teflon (ePTFE) or dacron due to high risk of bacterial infection (Owens et al. 2007). In addition to conventional open repair procedures, endovascular techniques have gained increasing use in trauma in recent years (Johnson 2010; DuBose et al. 2015).

Advances in biomaterials are currently being investigated in large animal models for the potential to improve clinical outcomes when coated onto synthetic vascular grafts and reduce infection rates (Liu et al. 2018). These have had greater application in recent years for developing interventions for cardiovascular disease (Stapleton et al. 2020). Recent advances in decellularized scaffolds, vascular cell seeding, and the design of bioactive polymers for in situ arterial regeneration have yielded promising results, but are not yet approved for clinical use (Ong et al. 2017). The ideal engineered vascular graft material would behave like a native vessel, but spare the harvest of autologous tissue. Originally developed as hemodialysis grafts for patients with end-stage renal disease (Kirkton et al. 2019), investigational regenerative medicine technologies advancing through the clinic (i.e., the human acellular vessel, or HAV) have shown significant preliminary promise for battlefield trauma applications (Morrison et al. 2019).

While truly novel regenerative options for vascular trauma remain largely experimental, options for those requiring rehabilitation from cardiovascular disease are more numerous. The primary financial interest for developing treatment modalities for injured vessels is the proliferation of cardiovascular disease, including coronary heart, cerebrovascular, and PAD. The emphasis on cardiovascular disease has resulted in a technology gap concerning vascular trauma. However, conventional

grafts, and even those that are tissue-engineered suffer from patency issues as smaller calibers below 6 mm vessel diameter (Pashneh-Tala et al. 2016). Many current vascular repair treatments focus on treating occluded or stenosed vessels via stent-graft insertion, angioplasty, or vascular grafts (Pashneh-Tala et al. 2016). Importantly, many vascular repair solutions have been studied in the context of diseased rather than injured vessels. As a result, many products are indicated to repair, bypass, or improve vessel patency. Although many of these solutions can be used to replace vessels in cases of traumatic vascular wounds, they may not have been expressly designed to do so. The primary disadvantage in this regard is the need to grow vessels *ex vivo*, often based on tissue culture from patient biopsies and/or bioreactors. An immediately implantable off-the-shelf solution is needed in severe trauma, though only one example of a tissue-engineered product has made it to clinical stage development to date (Morrison et al. 2019).

Traumatic injury rehabilitation tends to be focused more on specific rehabilitation of the effector organs, primarily muscle, nerve, and soft tissue. While there are no rehabilitation interventions specifically aimed at blood vessels currently, recent findings suggest exercise under partial ischemic conditions may improve limb rehabilitation outcomes (Day 2018). Rehabilitation programs for PAD affecting the limbs are better defined, with supervised treadmill rehabilitation found to be of benefit across a meta-analysis of randomized trials (McDermott 2018). Cardiovascular injury rehabilitation paradigms vary between traumatic injury and occlusive disease. Cardiac rehabilitation programs are the norm with graded exercise programs, increasingly performed at home following revascularization procedures, including surgical coronary artery bypass grafting, endovascular interventions, and medically managed vascular events (Ambrosetti et al. 2020). These programs in combination with lipid management, dietary interventions, and tobacco cessation have been shown to improve outcomes as well as physiologic indicators, such as increased peak VO_2 (Izawa 2020). Vascular rehabilitation related to stroke specifically is described above.

1.5.3 Skeletal Muscle System

Skeletal muscle makes up approximately 40% of body mass and is essential for movement and locomotion, postural control, respiratory activity, and heat production. Although skeletal muscle has a robust capacity for regeneration and repair following acute injury, endogenous healing mechanisms are impaired in more complex or traumatic skeletal muscle injuries or chronic pathologies, such as volumetric muscle loss (VML), muscular dystrophies (e.g., Duchenne's and limb girdle), and sarcopenia. These conditions contribute to muscle atrophy and associated deficits in muscle function, leading to poor quality of life. Patients may also suffer from a host of related chronic comorbidities, such as fibrosis and inflammation. The heterogenous nature of skeletal muscle pathologies and related comorbidities make the establishment of standardized care difficult. Therefore, it is

essential to investigate various regenerative rehabilitation approaches to treat complex skeletal muscle pathologies.

Duchenne and limb girdle muscular dystrophies are debilitating conditions characterized by skeletal muscle atrophy and weakness. Duchenne muscular dystrophy (DMD) is the most common genetic disease among pediatric onset dystrophies. Patients have a mutation in the dystrophin gene, resulting in a lack of the structural protein, dystrophin, contributing to fragile muscle tissue, atrophy, and weakness (Emery 2002; Hoffman et al. 1987). Although rehabilitation is currently not prescribed for patients with DMD due to lack of standard guidelines and perhaps fear of exacerbating outcomes (Markert et al. 2012), clinical trials have recently been conducted to examine the safety and efficacy of aerobic (NCT03319030) and isometric resistance (NCT02421523) exercise to improve muscle strength in ambulatory and non-ambulatory boys with DMD. In the preclinical setting, rehabilitation, including treadmill and wheel running and isometric resistance exercise, has been shown to improve ambulation, maximal isometric force and contractility rates, as well as decrease the number of regenerating muscle fibers as determined by histology (Lindsay et al. 2019; Call et al. 2010).

In addition to rehabilitation, regenerative strategies, including cellular therapies, have also been examined. The first attempt to transplant cells in a dystrophin-deficient *mdx* mouse model occurred in 1989 with the transplantation of healthy mouse satellite cells which improved numbers of dystrophin positive muscle cells (Partridge et al. 1989). More recently, iPS cells have been investigated. Within the past decade, human artificial chromosome (HAC) and CRISPR technologies have been used to successfully repair the dystrophin gene of DMD patient-derived iPS cells (Li et al. 2015; Kazuki et al. 2010). The corrected iPS cells were then differentiated into skeletal muscle cells, with subsequent analysis showing the full-length dystrophin mRNA to be present (Li et al. 2015). These results provide an important framework for iPS cell-based gene therapy for genetic disorders such as DMD. In the *mdx* mouse model, transplantation of autologous iPS cells has restored dystrophin to the diseased muscle, while improving, but not fully recovering, muscle function compared to wild-type mice (Darabi et al. 2012). Collectively, this work indicates that strategies are needed to improve dystrophin levels and that rehabilitation is needed to enhance muscle function to improve long-term outcomes for patients with DMD.

Severe skeletal muscle injury, such as VML, is another example of a muscle pathology that would benefit from a combined regenerative rehabilitation strategy (Saunders and Rose 2021). Volumetric muscle loss was initially defined in 2011 as "the traumatic or surgical loss of skeletal muscle with resultant functional impairment" (Grogan et al. 2011). Current treatment strategies include wound closure to mitigate infection and scar tissue debridement. Following initial wound repair the most common approach to treat the muscle remaining after VML is rehabilitation, but limited functional recovery has been reported preclinically and clinically with rehabilitation alone (Garg et al. 2015). Regenerative strategies have also been investigated in treating skeletal muscle injury. A recent systematic review and meta-analysis (Greising et al. 2019) reviewed the effectiveness of various

regenerative approaches in animal models of VML injury. Decellularized scaffolds combined with stem cells and/or progenitor cells were found to have the greatest probability to improve muscle function compared to untreated animals. Although a treatment approach that combines the preceding regenerative strategy with rehabilitation may work to synergistically improve functional outcomes following VML, few studies have examined this combinatorial approach. Combined approaches have reported functional improvements resulting from, for example, implantation of biomaterials within the defect area, seeded with or without stem cells, followed by rehabilitation, although functional deficits persist (Greising et al. 2019). In the clinic, a paucity of case studies and series have been conducted to investigate the efficacy of regenerative rehabilitation strategies to improve functional outcomes post-VML. A case study of a combat injured soldier who underwent rehabilitation prior to and following surgical implantation of a decellularized scaffold into the VML defect area indicated an ~20% improvement in isometric force 27 weeks post-operatively, but functional deficits were still evident (Gentile et al. 2014). More recently, a clinical trial (Dziki et al. 2016) conducted in 13 VML-afflicted patients, 7–120 months removed from the injury date, received a decellularized scaffold followed by early rehabilitation. Despite ~37% and 27% improvements in force production and ROM, respectively, significant functional deficits remained. While it is evident that this type of heterogenous injury necessitates a more involved regenerative rehabilitation approach, it is necessary to investigate the combination of therapies that optimally enhance tissue regeneration and functionality following injury.

1.5.4 Skeletal System

Fractures of the bone are one of the most common traumatic injuries in humans and result from high-energy trauma such as motor vehicle accidents, military combat, or hard hits in contact sports. Like skeletal muscle, bone displays incredible plasticity following injury. According to the mechanostat theory, bone adapts its strength and geometry when subjected to mechanical loading to meet the functional demands (Frost 2003). Clinically, rehabilitation, including early weight-bearing exercise, low magnitude electrical stimulation, and low-intensity pulsed ultrasonography (LIPUS), is generally prescribed across the healing process to elicit various stimuli for adaptation (Kristiansen et al. 1997; Heckman et al. 1994; Kubiak et al. 2013). However, complex or compromised fractures, including open fractures that involve not only bone but also the surrounding muscle, nerves, and vasculature, fail to heal, resulting in a non-union fracture. In cases of open fracture, infection mitigation and internal or external fixation is the first treatment priority. Patients also commonly receive a bone graft, currently considered a gold standard treatment, using either an autograft or allograft. Yet, bone grafts have limitations, including donor site morbidity, failed bone tissue integration from the host, and vascularization issues, often leading to a delay in healing and further delayed rehabilitation (Ho-Shui-Ling et al. 2018).

Complex fractures are an ideal instance where regenerative rehabilitation can improve return to function sooner. Potential regenerative medicine strategies to treat complex bone fracture have been largely investigated in the preclinical setting, including use of bone tissue engineering, gene, and growth factor (e.g., bone morphogenetic protein-2 and 7) therapies, MSC delivery (e.g., adipose-, bone marrow-derived), and bioengineered decellularized scaffold implantation (Trohatou and Roubelakis 2017). Some of these strategies have been evaluated in ongoing or recently completed clinical case studies or trials. Clinically, the first report of an autologous bone marrow-derived MSC-seeded hydroxyapatite biomaterial implanted in large (4–8 cm) bone segmental defects of three patients was published in 2001 (Quarto et al. 2001). By 12 months following implantation, all patients had experienced complete bone union and full recovery of limb function. Although similar strategies have been used in the clinic since this report, various issues have been raised, including the high complexity and high-cost burdens associated with implementing cell-based engineering therapies.

Strategies implementing regenerative and rehabilitation approaches in combination have been investigated in animal models of fracture. These strategies have included electrical stimulation initiated following implantation of scaffolds incorporated with MSCs (Leppik et al. 2018) and a LIPUS regimen initiated following MSC injection (Cheung et al. 2013). A combined strategy of electrical stimulation applied after implantation of a scaffold seeded with adipose tissue-derived MSCs was first investigated in 2018 in a rat model of femoral fracture (Leppik et al. 2018). Initial in vitro analysis showed electrical stimulation following cell seeding on the scaffold increased osteogenic differentiation, similar to findings from previous studies (Hardy et al. 2015). A subsequent in vivo analysis showed the combined treatment strategy to improve bone healing up to 8 weeks following fracture compared to uninjured animals. Evidence of healing included increased bone formation and vessel density assessed histologically; bone strength evaluated by 3-point bending tests; and osteogenic gene expression. Finally, others (Cheung et al. 2013) have shown MSC injection followed by 4 weeks of LIPUS in a rat model of femoral fracture improves fracture healing. Healing was evidenced by increased callus width and area, evaluated by radiology, and greater bone volume, measured via microcomputed tomography, compared to uninjured animals and injured animals administered MSCs without LIPUS. Although it is evident that these combined strategies may facilitate improved structural and functional bone outcomes following fracture, further study is needed to elucidate the mechanisms underlying these combined approaches and to determine an optimal therapy combination for translation into the clinical setting.

1.5.5 Connective Tissue

Connective tissue comes in a variety of types and can be found in all systems of the body; however, this section will focus on the dense connective tissues of the

musculoskeletal system, specifically tendons and ligaments. While both tissues are comprised of collagen, elastin, proteoglycans, fibroblasts, and water, the percentage of each component differs, leading to different mechanical properties. Tendons typically have a higher collagen composition coupled with lower elastin, proteoglycan, and water content (Rumian et al. 2007). This leads to high stiffness and tensile strength, advantageous for force transmission from muscle to bone that generally occurs in a uniaxial direction. Ligaments serve as support structures for joints, connecting bone to bone, and thus not only need high strength but also enough elasticity to withstand forces from various directions. Therefore, ligaments typically have lower collagen and higher elastin content than tendons. When injured, both tendon and ligaments display a range of outcomes based on anatomical location and severity of injury, but in most cases, the injured tissue never regains full strength. Rehabilitation alone in some combination of targeted exercise strengthening, stretching, ultrasound, cryotherapy, or massage is typically recommended for partial tears and mild tendinopathy (Papadopoulos and Mani 2020; Edwards et al. 2016; Maffulli et al. 2004). Full-thickness tears and more progressive tendinopathy often require surgical treatment followed by rehabilitation modalities. While the most common surgical approach for ligament rupture is reconstruction using autologous or allogenic tendon grafts, donor site morbidity and infection risk remain significant limitations (Hardy et al. 2017; Weitzel et al. 2002). Furthermore, of athletes that undergo ACL reconstruction, only about a third achieve pre-injury level of play after 2 years and the long-term risk of osteoarthritis increases dramatically (Sepulveda et al. 2017), particularly for athletes that experience more than one ACL rupture. Tendon ruptures are commonly repaired with similar methods and limitations, or by anchoring the tendon to the bone. In all cases, the prevalence of re-rupture is high.

Novel strategies for tendon and ligament regeneration have included synthetic biomaterial grafts, cell therapy (Agung et al. 2006), growth factors and/or gene therapy, or a combination of these in tissue engineering approaches. Though synthetic ACL grafts have been developed and experimentally used for over a century (Corner 1914), they have been remarkably unsuccessful to date (Legnani et al. 2010; Ventura et al. 2010). Likewise, optimism regarding a standalone cell therapy for tendon and ligament regeneration has generally faded (Hirzinger et al. 2014; Pas et al. 2017), yet there remains a promise for its use in tissue engineering. Furthermore, while platelet-rich plasma (PRP) emerged in the 1980s to combat blood loss during cardiac surgery (Ferrari et al. 1987), its use as a therapy for musculoskeletal injuries has grown in popularity over the past decade (Kia et al. 2018). Platelet-rich plasma is proposed to stimulate healing by the release and subsequent regulation of various growth factors from platelets (Boswell et al. 2012). In a rat model of Achilles tendon injury, PRP treatment improved outcomes of stiffness and force at failure after 3 and 5 days of muscle unloading using Botox injections; yet, mechanical loading was necessary for these effects to continue after 14 days (Virchenko and Aspenberg 2006). Similarly, animal models of tendon tissue engineering have shown improved outcomes when stem cells are used in conjunction with mechanical stimulation prior to implantation (Juncosa-Melvin et al. 2006; Juncosa-Melvin et al. 2007) however, when mechanical tension is removed, these beneficial changes are

lost and inflammation increases (Bayer et al. 2014). Together these findings highlight the potential importance of a structured rehabilitation program in combination with regenerative strategies to maintain mechanical loading and thus the tissue architecture.

1.6 Importance of Functional Outcomes

As an emerging field leveraging the disciplines of regenerative medicine and rehabilitation, goals to optimize functional repair, recovery, and/or regeneration of various tissues across the body must be centered on the functional physiologic outcomes to advance EBP approaches. As more and more regenerative rehabilitation interventions reach the clinic, there is an ongoing need for investigations to determine how best to evaluate recovery and restoration of functional capacity. Ultimately, standardized physiologic outcomes are required and should dove-tail into clinical outcome assessment and patient-centered outcomes as outlined by the United States FDA. Requiring evaluations both prior to and following the implementation of regenerative rehabilitation strategies is essential. Often patient-focused outcomes can be impactful in these instances, and qualitative self or caregiver assessments of: daily physical activity, extremity function, physical function, and walking speed can be used to understand ongoing efficacy.

Again, using a systems biology approach (Fig. 1.1), functional measures commonly assessed in the clinical setting to determine improvements during the rehabilitative process are discussed. Across the cardiovascular system, well-established outcomes evaluate changes in exercise capacity and endurance by the use of the submaximal and maximal VO_2 tests, resting and submaximal heart rate, blood pressure, and ejection fraction. Additionally, these are often combined with respiratory function tests, such as vital capacity, maximal inspiratory pressure, and maximal expiratory pressures. Within the central and peripheral nervous systems, a variety of functional tests are used, including the Fugl-Meyers Assessment, Wolf Motor Arm Function Test, Action Research Arm Test, 6-min walk test, and Berg Balance Scale. All in efforts to assess the ongoing efficacy of interventions. Moving into the musculoskeletal system, broadly function of the extremities is often predicated on skeletal muscle function. Muscle function, or strength, is often evaluated using isokinetic or isometric testing or task-specific movements, such as single-leg jumps. The evaluation of muscle function in these ways is useful for skeletal muscle, bone, and connective tissue.

1.7 Future Directions in Regenerative Rehabilitation

The field of regenerative rehabilitation has come a long way in the last two decades with the advent of cellular and regenerative therapies. Automation, adjunctive therapies, and a greater understanding of effective rehabilitation paradigms through

study have advanced recovery of functional deficits. The long-term goal is to translate these combined strategies to the clinical setting to optimize patients' function and quality of life. Given the wide array of potential debilitating insults and illnesses, a highly ambitious and broad research agenda lies ahead before the true promise of converging regenerative and rehabilitative modalities can be realized.

The rehabilitation field continues to suffer from insufficient outcome evidence from randomized clinical trials. The ideal 'prescription' of therapy regimens likely to be beneficial in many cases remains an educated guess, often tied to traditional approaches based on an intuitive understanding of the pathology. Studying and standardizing function outcomes in controlled experiments will help to better quantify the timing, intensity, and specific physiologic approaches to physical medicine regimens. Randomized clinical trials of regenerative rehabilitation regimens will be essential in this regard, comparing novel physical medicine prescriptions to commonly accepted standards of care. The variability encountered will also help to better define the need for patient-centric approaches based on specific responses encountered during locomotor interventions. Advances in automated, individualized self-guided rehabilitation programs are likely to be of great benefit in this regard, by providing data-driven feedback to both the patient and the treatment team to guide therapy. This will in turn improve the capacity for self-care and adherence to prescribed regimens. Unfortunately, funding for large, controlled trials remains anemic, in part due to the relative lack of potential commercial indications. In contrast, emerging rehabilitative adjuncts including device-based electrical and mechanical stimulation, immersive virtual reality, and robotic assistance are likely to attract significant commercial backing and help to lead the field forward.

In contrast, the closely aligned field of regenerative medicine has rapidly expanded into a multibillion-dollar industry and promises to grow exponentially over the coming decade. As more potential approaches to unlocking the body's natural healing powers are discovered, the pace of development is only expected to increase. Simple autologous therapies such as PRP injection for soft tissue injury, or MSCs for cosmetic and medical applications have gained substantial clinical acceptance in recent years. Autologous *ex vivo* constructs including personalized meniscal cartilage replacements grown from biopsy specimens are likely to be approved soon. Decellularized scaffolds are also on the verge of approval for several applications. Products combining scaffolds, growth factors, and small molecules are also on the horizon, along with adjunctive therapies to support immunomodulation and growth. These early interventions are ultimately likely to be supplanted by composite tissue-agnostic approaches. Proof of principle has already been found for a plethora of technologies to include siRNAs, modulation of bioelectrical circuits, electrical stimulation, antiaging factors, and pluripotent stem cell therapies. These are likely to dominate the field in coming decades, promising to unlock the potential for full regeneration of lost and damaged tissues.

Acknowledgments The authors gratefully acknowledge helpful comments and review from Dr. Jarrod Call. Additionally, we acknowledge the work of many colleagues who contributed to

the improvement of the understanding of the current state of the field for regenerative rehabilitation, across our collective goals for the advancement of new solutions.

Funding

This work was supported by funding from the Department of Defense, Clinical & Rehabilitative Medicine Research Program: W81XWH-18-1-0710 and W81XWH-20-1-0885 (SMG) and National Institutes of Health T32-AR050938 (CJRP). Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense or National Institutes of Health.

Declaration of Interests The authors declare that they have no potential or actual conflict of interest.

References

Agung M, Ochi M, Yanada S, Adachi N, Izuta Y, Yamasaki T, Toda K (2006) Mobilization of bone marrow-derived mesenchymal stem cells into the injured tissues after intraarticular injection and their contribution to tissue regeneration. Knee Surg Sports Traumatol Arthrosc 14(12): 1307–1314. https://doi.org/10.1007/s00167-006-0124-8

Almouemen N, Kelly HM, O'Leary C (2019) Tissue engineering: understanding the role of biomaterials and biophysical forces on cell functionality through computational and structural biotechnology analytical methods. Comput Struct Biotechnol J 17:591–598. https://doi.org/10.1016/j.csbj.2019.04.008

Ambrosetti M, Abreu A, Corra U, Davos CH, Hansen D, Frederix I, Iliou MC, Pedretti RF, Schmid JP, Vigorito C, Voller H, Wilhelm M, Piepoli MF, Bjarnason-Wehrens B, Berger T, Cohen-Solal A, Cornelissen V, Dendale P, Doehner W, Gaita D, Gevaert AB, Kemps H, Kraenkel N, Laukkanen J, Mendes M, Niebauer J, Simonenko M, Zwisler AO (2020) Secondary prevention through comprehensive cardiovascular rehabilitation: from knowledge to implementation. 2020 update. A position paper from the Secondary Prevention and Rehabilitation Section of the European Association of Preventive Cardiology. Eur J Prev Cardiol. https://doi.org/10.1177/2047487320913379

Ambrosio F, Rando TA (2018) The regenerative rehabilitation collection: a forum for an emerging field. NPJ Regen Med 3:20. https://doi.org/10.1038/s41536-018-0058-z

American College of Sports Medicine, Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, Salem GJ, Skinner JS (2009) American College of Sports Medicine position stand. Exercise and physical activity for older adults. Med Sci Sports Exerc 41(7): 1510–1530. https://doi.org/10.1249/MSS.0b013e3181a0c95c

Amiel GE, Komura M, Shapira O, Yoo JJ, Yazdani S, Berry J, Kaushal S, Bischoff J, Atala A, Soker S (2006) Engineering of blood vessels from acellular collagen matrices coated with human endothelial cells. Tissue Eng 12(8):2355–2365. https://doi.org/10.1089/ten.2006.12.2355

Asgharzade S, Talaei A, Farkhondeh T, Forouzanfar F (2020) Combining growth factor and stem cell therapy for stroke rehabilitation, a review. Curr Drug Targets 21(8):781–791. https://doi.org/10.2174/1389450121666200107100747

Ashammakhi N, Kim HJ, Ehsanipour A, Bierman RD, Kaarela O, Xue C, Khademhosseini A, Seidlits SK (2019) Regenerative therapies for spinal cord injury. Tissue Eng Part B Rev 25(6): 471–491. https://doi.org/10.1089/ten.TEB.2019.0182

Atanelov L, Stiens SA, Young MA (2015) History of physical medicine and rehabilitation and its ethical dimensions. AMA J Ethics 17(6):568–574. https://doi.org/10.1001/journalofethics.2015.17.6.mhst1-1506

Bartsch T, Brehm M, Zeus T, Kogler G, Wernet P, Strauer BE (2007) Transplantation of autologous mononuclear bone marrow stem cells in patients with peripheral arterial disease (the TAM-PAD study). Clin Res Cardiol 96(12):891–899. https://doi.org/10.1007/s00392-007-0569-x

Bauer G, Elsallab M, Abou-El-Enein M (2018) Concise review: a comprehensive analysis of reported adverse events in patients receiving unproven stem cell-based interventions. Stem Cells Transl Med 7(9):676–685. https://doi.org/10.1002/sctm.17-0282

Bayer ML, Schjerling P, Herchenhan A, Zeltz C, Heinemeier KM, Christensen L, Krogsgaard M, Gullberg D, Kjaer M (2014) Release of tensile strain on engineered human tendon tissue disturbs cell adhesions, changes matrix architecture, and induces an inflammatory phenotype. PLoS One 9(1):e86078. https://doi.org/10.1371/journal.pone.0086078

Becker RO, Spadaro JA (1972) Electrical stimulation of partial limb regeneration in mammals. Bull N Y Acad Med 48(4):627–641

Bell E, Ivarsson B, Merrill C (1979) Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci U S A 76(3):1274–1278. https://doi.org/10.1073/pnas.76.3.1274

Billin AN, Bantscheff M, Drewes G, Ghidelli-Disse S, Holt JA, Kramer HF, McDougal AJ, Smalley TL, Wells CI, Zuercher WJ, Henke BR (2016) Discovery of novel small molecules that activate satellite cell proliferation and enhance repair of damaged muscle. ACS Chem Biol 11(2):518–529. https://doi.org/10.1021/acschembio.5b00772

Biomaterials Science (2020) A history of biomaterials, 4th edn. Academic. https://doi.org/10.1016/B978-0-12-816137-1.00002-7

Boswell SG, Cole BJ, Sundman EA, Karas V, Fortier LA (2012) Platelet-rich plasma: a milieu of bioactive factors. Arthroscopy 28(3):429–439. https://doi.org/10.1016/j.arthro.2011.10.018

Burkhalter W (1994) Surgery in Vietnam: orthopaedic surgery. OTSG Department of the Army, Washington, DC

Butler DL, Goldstein SA, Guilak F (2000) Functional tissue engineering: the role of biomechanics. J Biomech Eng 122(6):570–575. https://doi.org/10.1115/1.1318906

Cai M, Liu H, Jiang Y, Wang J, Zhang S (2019) A high-strength biodegradable thermoset polymer for internal fixation bone screws: preparation, in vitro and in vivo evaluation. Colloids Surf B Biointerfaces 183:110445. https://doi.org/10.1016/j.colsurfb.2019.110445

Call JA, McKeehen JN, Novotny SA, Lowe DA (2010) Progressive resistance voluntary wheel running in the mdx mouse. Muscle Nerve 42(6):871–880

Capizzi A, Woo J, Verduzco-Gutierrez M (2020) Traumatic brain injury: an overview of epidemiology, pathophysiology, and medical management. Med Clin North Am 104(2):213–238. https://doi.org/10.1016/j.mcna.2019.11.001

Caplan AI (1991) Mesenchymal stem cells. J Orthop Res 9(5):641–650. https://doi.org/10.1002/jor.1100090504

Cheung WH, Chin WC, Wei FY, Li G, Leung KS (2013) Applications of exogenous mesenchymal stem cells and low intensity pulsed ultrasound enhance fracture healing in rat model. Ultrasound Med Biol 39(1):117–125. https://doi.org/10.1016/j.ultrasmedbio.2012.08.015

Chhabra HS, Sarda K (2017) Clinical translation of stem cell based interventions for spinal cord injury—are we there yet? Adv Drug Deliv Rev 120:41–49. https://doi.org/10.1016/j.addr.2017.09.021

Cho SR, Suh H, Yu JH, Kim HH, Seo JH, Seo CH (2016) Astroglial activation by an enriched environment after transplantation of mesenchymal stem cells enhances angiogenesis after hypoxic-ischemic brain injury. Int J Mol Sci 17(9). https://doi.org/10.3390/ijms17091550

Corner EM (1914) Notes of a case illustrative of an artificial anterior crucial ligament, demonstrating the action of that ligament. Proc R Soc Med 7 (Clin Sect):120–121

Corona BT, Greising SM (2016) Challenges to acellular biological scaffold mediated skeletal muscle tissue regeneration. Biomaterials 104:238–246. https://doi.org/10.1016/j.biomaterials.2016.07.020

Courtine G, Sofroniew MV (2019) Spinal cord repair: advances in biology and technology. Nat Med 25(6):898–908. https://doi.org/10.1038/s41591-019-0475-6

Darabi R, Arpke RW, Irion S, Dimos JT, Grskovic M, Kyba M, Perlingeiro RC (2012) Human ES- and iPS-derived myogenic progenitors restore DYSTROPHIN and improve contractility upon transplantation in dystrophic mice. Cell Stem Cell 10(5):610–619. https://doi.org/10.1016/j.stem.2012.02.015

Day B (2018) Personalized blood flow restriction therapy: how, when and where can it accelerate rehabilitation after surgery? Arthroscopy 34(8):2511–2513. https://doi.org/10.1016/j.arthro.2018.06.022

de Araujo AVL, Ribeiro FPG, Massetti T, Potter-Baker KA, Cortes M, Plow EB, da Silva TD, Tonks J, Anghinah R, Magalhaes FH, Fregni F, de Mello Monteiro CB (2020) Effectiveness of anodal transcranial direct current stimulation to improve muscle strength and motor functionality after incomplete spinal cord injury: a systematic review and meta-analysis. Spinal Cord 58(6):635–646. https://doi.org/10.1038/s41393-020-0438-2

Dehghan N, McKee MD, Jenkinson RJ, Schemitsch EH, Stas V, Nauth A, Hall JA, Stephen DJ, Kreder HJ (2016) Early weightbearing and range of motion versus non-weightbearing and immobilization after open reduction and internal fixation of unstable ankle fractures: a randomized controlled trial. J Orthop Trauma 30(7):345–352. https://doi.org/10.1097/BOT.0000000000000572

Dreyer HC, Fujita S, Cadenas JG, Chinkes DL, Volpi E, Rasmussen BB (2006) Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. J Physiol 576(Pt 2):613–624. https://doi.org/10.1113/jphysiol.2006.113175

DuBose JJ, Savage SA, Fabian TC, Menaker J, Scalea T, Holcomb JB, Skarupa D, Poulin N, Chourliaras K, Inaba K, Rasmussen TE, Group APS (2015) The American Association for the Surgery of Trauma PROspective Observational Vascular Injury Treatment (PROOVIT) registry: multicenter data on modern vascular injury diagnosis, management, and outcomes. J Trauma Acute Care Surg 78(2):215–222.; Discussion 222–213. https://doi.org/10.1097/TA.0000000000000520

Dziki J, Badylak S, Yabroudi M, Sicari B, Ambrosio F, Stearns K, Turner N, Wyse A, Boninger ML, Brown EHP, Rubin JP (2016) An acellular biologic scaffold treatment for volumetric muscle loss: results of a 13-patient cohort study. NPJ Regen Med 1:16008. https://doi.org/10.1038/npjregenmed.2016.8

Edwards P, Ebert J, Joss B, Bhabra G, Ackland T, Wang A (2016) Exercise rehabilitation in the non-operative management of rotator cuff tears: a review of the literature. Int J Sports Phys Ther 11(2):279–301

Emery AE (2002) The muscular dystrophies. Lancet 359(9307):687–695

Ferrari M, Zia S, Valbonesi M, Henriquet F, Venere G, Spagnolo S, Grasso MA, Panzani I (1987) A new technique for hemodilution, preparation of autologous platelet-rich plasma and intraoperative blood salvage in cardiac surgery. Int J Artif Organs 10(1):47–50

Fluri F, Schuhmann MK, Kleinschnitz C (2015) Animal models of ischemic stroke and their application in clinical research. Drug Des Devel Ther 9:3445–3454. https://doi.org/10.2147/DDDT.S56071

Fox CJ, Gillespie DL, O'Donnell SD, Rasmussen TE, Goff JM, Johnson CA, Galgon RE, Sarac TP, Rich NM (2005) Contemporary management of wartime vascular trauma. J Vasc Surg 41(4):638–644. https://doi.org/10.1016/j.jvs.2005.01.010

Franz RW, Shah KJ, Johnson JD, Pin RH, Parks AM, Hankins T, Hartman JF, Wright ML (2011) Short- to mid-term results using autologous bone-marrow mononuclear cell implantation therapy as a limb salvage procedure in patients with severe peripheral arterial disease. Vasc Endovascular Surg 45(5):398–406. https://doi.org/10.1177/1538574411405545

Frost HM (2003) Bone's mechanostat: a 2003 update. Anat Rec A Discov Mol Cell Evol Biol 275(2):1081–1101. https://doi.org/10.1002/ar.a.10119

Galetto V, Sacco K (2017) Neuroplastic changes induced by cognitive rehabilitation in traumatic brain injury: a review. Neurorehabil Neural Repair 31(9):800–813. https://doi.org/10.1177/1545968317723748

Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, Nieman DC, Swain DP, American College of Sports Medicine (2011) American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc 43(7):1334–1359. https://doi.org/10.1249/MSS.0b013e318213fefb

Garg K, Ward CL, Hurtgen BJ, Wilken JM, Stinner DJ, Wenke JC, Owens JG, Corona BT (2015) Volumetric muscle loss: persistent functional deficits beyond frank loss of tissue. J Orthop Res 33(1):40–46. https://doi.org/10.1002/jor.22730

Gentile NE, Stearns KM, Brown EH, Rubin JP, Boninger ML, Dearth CL, Ambrosio F, Badylak SF (2014) Targeted rehabilitation after extracellular matrix scaffold transplantation for the treatment of volumetric muscle loss. Am J Phys Med Rehabil 93(11 Suppl 3):S79–S87. https://doi.org/10.1097/PHM.0000000000000145

Goldman SM, Henderson BEP, Corona BT (2017) Evaluation of bone marrow mononuclear cells as an adjunct therapy to minced muscle graft for the treatment of volumetric muscle loss injuries. Stem Cell Res Ther 8(1):142. https://doi.org/10.1186/s13287-017-0589-z

Gopalakrishnan A, Shankarappa SA, Rajanikant GK (2019) Hydrogel scaffolds: towards restitution of ischemic stroke-injured brain. Transl Stroke Res 10(1):1–18. https://doi.org/10.1007/s12975-018-0655-6

Greising SM, Corona BT, McGann C, Frankum JK, Warren GL (2019) Therapeutic approaches for volumetric muscle loss injury: a systematic review and meta-analysis. Tissue Eng Part B Rev. https://doi.org/10.1089/ten.TEB.2019.0207

Grogan BF, Hsu JR, Skeletal Trauma Research Consortium (2011) Volumetric muscle loss. J Am Acad Orthop Surg 19(Suppl 1):S35–S37

Guercio A, Di Marco P, Casella S, Cannella V, Russotto L, Purpari G, Di Bella S, Piccione G (2012) Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints. Cell Biol Int 36(2):189–194. https://doi.org/10.1042/CBI20110304

Gurjar AA, Manto KM, Estrada JA, Kaufman M, Sun D, Talukder MAH, Elfar JC (2021) 4-aminopyridine: a single-dose diagnostic agent to differentiate axonal continuity in nerve injuries. Mil Med 186(Suppl 1):479–485. https://doi.org/10.1093/milmed/usaa310

Hardy JG, Villancio-Wolter MK, Sukhavasi RC, Mouser DJ, Aguilar D Jr, Geissler SA, Kaplan DL, Schmidt CE (2015) Electrical stimulation of human mesenchymal stem cells on conductive nanofibers enhances their differentiation toward osteogenic outcomes. Macromol Rapid Commun 36(21):1884–1890. https://doi.org/10.1002/marc.201500233

Hardy A, Casabianca L, Andrieu K, Baverel L, Noailles T, Junior French Arthroscopy S (2017) Complications following harvesting of patellar tendon or hamstring tendon grafts for anterior cruciate ligament reconstruction: systematic review of literature. Orthop Traumatol Surg Res 103(8S):S245–S248. https://doi.org/10.1016/j.otsr.2017.09.002

Hayashi Y, Jacob-Vadakot S, Dugan EA, McBride S, Olexa R, Simansky K, Murray M, Shumsky JS (2010) 5-HT precursor loading, but not 5-HT receptor agonists, increases motor function after spinal cord contusion in adult rats. Exp Neurol 221(1):68–78. https://doi.org/10.1016/j.expneurol.2009.10.003

Heckman JD, Ryaby JP, McCabe J, Frey JJ, Kilcoyne RF (1994) Acceleration of tibial fracture-healing by non-invasive, low-intensity pulsed ultrasound. J Bone Joint Surg Am 76(1):26–34. https://doi.org/10.2106/00004623-199401000-00004

Heiderscheit BC, Sherry MA, Silder A, Chumanov ES, Thelen DG (2010) Hamstring strain injuries: recommendations for diagnosis, rehabilitation, and injury prevention. J Orthop Sports Phys Ther 40(2):67–81. https://doi.org/10.2519/jospt.2010.3047

Hench LL, Splinter RJ, Allen WC, Greenlee TK (1971) Bonding mechanisms at the interface of ceramic prosthetic materials. J Biomed Mater Res 5(6):117–141

Higashi Y, Kimura M, Hara K, Noma K, Jitsuiki D, Nakagawa K, Oshima T, Chayama K, Sueda T, Goto C, Matsubara H, Murohara T, Yoshizumi M (2004) Autologous bone-marrow

mononuclear cell implantation improves endothelium-dependent vasodilation in patients with limb ischemia. Circulation 109(10):1215–1218. https://doi.org/10.1161/01.CIR.0000121427.53291.78

Hirzinger C, Tauber M, Korntner S, Quirchmayr M, Bauer HC, Traweger A, Tempfer H (2014) ACL injuries and stem cell therapy. Arch Orthop Trauma Surg 134(11):1573–1578. https://doi.org/10.1007/s00402-014-2060-2

Hoffman EP, Brown RH Jr, Kunkel LM (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 51(6):919–928

Horita Y, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD (2006) Intravenous administration of glial cell line-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in the adult rat. J Neurosci Res 84(7):1495–1504. https://doi.org/10.1002/jnr.21056

Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C (2018) Bone regeneration strategies: engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. Biomaterials 180:143–162. https://doi.org/10.1016/j.biomaterials.2018.07.017

Hu Z, Zhang F, Yang Z, Yang N, Zhang D, Zhang J, Cao K (2008) Combination of simvastatin administration and EPC transplantation enhances angiogenesis and protects against apoptosis for hindlimb ischemia. J Biomed Sci 15(4):509–517. https://doi.org/10.1007/s11373-008-9243-1

Hu B, Weng Y, Xia XH, Liang XJ, Huang Y (2019) Clinical advances of siRNA therapeutics. J Gene Med 21(7):e3097. https://doi.org/10.1002/jgm.3097

Ito A, Kubo N, Liang N, Aoyama T, Kuroki H (2020) Regenerative rehabilitation for stroke recovery by inducing synergistic effects of cell therapy and neurorehabilitation on motor function: a narrative review of pre-clinical studies. Int J Mol Sci 21(9). https://doi.org/10.3390/ijms21093135

Izawa H (2020) Cardiac rehabilitation as therapeutic strategy after coronary artery bypass grafting. Circ J 84(3):378–379. https://doi.org/10.1253/circj.CJ-20-0046

Johnson CA (2010) Endovascular management of peripheral vascular trauma. Semin Intervent Radiol 27(1):38–43. https://doi.org/10.1055/s-0030-1247887

Johnson RL, Wilson CG (2018) A review of vagus nerve stimulation as a therapeutic intervention. J Inflamm Res 11:203–213. https://doi.org/10.2147/JIR.S163248

Juncosa-Melvin N, Shearn JT, Boivin GP, Gooch C, Galloway MT, West JR, Nirmalanandhan VS, Bradica G, Butler DL (2006) Effects of mechanical stimulation on the biomechanics and histology of stem cell-collagen sponge constructs for rabbit patellar tendon repair. Tissue Eng 12(8):2291–2300. https://doi.org/10.1089/ten.2006.12.2291

Juncosa-Melvin N, Matlin KS, Holdcraft RW, Nirmalanandhan VS, Butler DL (2007) Mechanical stimulation increases collagen type I and collagen type III gene expression of stem cell-collagen sponge constructs for patellar tendon repair. Tissue Eng 13(6):1219–1226. https://doi.org/10.1089/ten.2006.0339

Kazuki Y, Hiratsuka M, Takiguchi M, Osaki M, Kajitani N, Hoshiya H, Hiramatsu K, Yoshino T, Kazuki K, Ishihara C, Takehara S, Higaki K, Nakagawa M, Takahashi K, Yamanaka S, Oshimura M (2010) Complete genetic correction of ips cells from Duchenne muscular dystrophy. Mol Ther 18(2):386–393. https://doi.org/10.1038/mt.2009.274

Khorasanizadeh M, Yousefifard M, Eskian M, Lu Y, Chalangari M, Harrop JS, Jazayeri SB, Seyedpour S, Khodaei B, Hosseini M, Rahimi-Movaghar V (2019) Neurological recovery following traumatic spinal cord injury: a systematic review and meta-analysis. J Neurosurg Spine 1–17. https://doi.org/10.3171/2018.10.SPINE18802

Kia C, Baldino J, Bell R, Ramji A, Uyeki C, Mazzocca A (2018) Platelet-rich plasma: review of current literature on its use for tendon and ligament pathology. Curr Rev Musculoskelet Med 11(4):566–572. https://doi.org/10.1007/s12178-018-9515-y

Kirkton RD, Santiago-Maysonet M, Lawson JH, Tente WE, Dahl SLM, Niklason LE, Prichard HL (2019) Bioengineered human acellular vessels recellularize and evolve into living blood vessels

after human implantation. Sci Transl Med 11(485). https://doi.org/10.1126/scitranslmed.aau6934
Kiyotake EA, Martin MD, Detamore MS (2020) Regenerative rehabilitation with conductive biomaterials for spinal cord injury. Acta Biomater. https://doi.org/10.1016/j.actbio.2020.12.021
Kleisiaris CF, Sfakianakis C, Papathanasiou IV (2014) Health care practices in ancient Greece: the hippocratic ideal. J Med Ethics Hist Med 7:6
Koukourikos K, Tsaloglidou A, Kourkouta L (2014) Muscle atrophy in intensive care unit patients. Acta Inform Med 22(6):406–410. https://doi.org/10.5455/aim.2014.22.406-410
Krause M, Phan TG, Ma H, Sobey CG, Lim R (2019) Cell-based therapies for stroke: are we there yet? Front Neurol 10:656. https://doi.org/10.3389/fneur.2019.00656
Kristiansen TK, Ryaby JP, McCabe J, Frey JJ, Roe LR (1997) Accelerated healing of distal radial fractures with the use of specific, low-intensity ultrasound. A multicenter, prospective, randomized, double-blind, placebo-controlled study. J Bone Joint Surg Am 79(7):961–973. https://doi.org/10.2106/00004623-199707000-00002
Kubiak EN, Beebe MJ, North K, Hitchcock R, Potter MQ (2013) Early weight bearing after lower extremity fractures in adults. J Am Acad Orthop Surg 21(12):727–738. https://doi.org/10.5435/JAAOS-21-12-727
Kulkarni RK, Moore EG, Hegyeli AF, Leonard F (1971) Biodegradable poly(lactic acid) polymers. J Biomed Mater Res 5(3):169–181. https://doi.org/10.1002/jbm.820050305
Lara-Hernandez R, Lozano-Vilardell P, Blanes P, Torreguitart-Mirada N, Galmes A, Besalduch J (2010) Safety and efficacy of therapeutic angiogenesis as a novel treatment in patients with critical limb ischemia. Ann Vasc Surg 24(2):287–294. https://doi.org/10.1016/j.avsg.2009.10.012
Legnani C, Ventura A, Terzaghi C, Borgo E, Albisetti W (2010) Anterior cruciate ligament reconstruction with synthetic grafts. A review of literature. Int Orthop 34(4):465–471. https://doi.org/10.1007/s00264-010-0963-2
Leppik LP, Froemel D, Slavici A, Ovadia ZN, Hudak L, Henrich D, Marzi I, Barker JH (2015) Effects of electrical stimulation on rat limb regeneration, a new look at an old model. Sci Rep 5:18353. https://doi.org/10.1038/srep18353
Leppik L, Zhihua H, Mobini S, Thottakkattumana Parameswaran V, Eischen-Loges M, Slavici A, Helbing J, Pindur L, Oliveira KMC, Bhavsar MB, Hudak L, Henrich D, Barker JH (2018) Combining electrical stimulation and tissue engineering to treat large bone defects in a rat model. Sci Rep 8(1):6307. https://doi.org/10.1038/s41598-018-24892-0
Li HL, Fujimoto N, Sasakawa N, Shirai S, Ohkame T, Sakuma T, Tanaka M, Amano N, Watanabe A, Sakurai H, Yamamoto T, Yamanaka S, Hotta A (2015) Precise correction of the dystrophin gene in Duchenne muscular dystrophy patient induced pluripotent stem cells by TALEN and CRISPR-Cas9. Stem Cell Rep 4(1):143–154. https://doi.org/10.1016/j.stemcr.2014.10.013
Lightsey HM, Kantrowitz DE, Swindell HW, Trofa DP, Ahmad CS, Lynch TS (2018) Variability of United States online rehabilitation protocols for proximal hamstring tendon repair. Orthop J Sports Med 6(2):2325967118755116. https://doi.org/10.1177/2325967118755116
Lindsay A, Larson AA, Verma M, Ervasti JM, Lowe DA (2019) Isometric resistance training increases strength and alters histopathology of dystrophin-deficient mouse skeletal muscle. J Appl Physiol (1985) 126(2):363–375. https://doi.org/10.1152/japplphysiol.00948.2018
Liu RH, Ong CS, Fukunishi T, Ong K, Hibino N (2018) Review of vascular graft studies in large animal models. Tissue Eng Part B Rev 24(2):133–143. https://doi.org/10.1089/ten.TEB.2017.0350
Maffulli N, Sharma P, Luscombe KL (2004) Achilles tendinopathy: aetiology and management. J R Soc Med 97(10):472–476. https://doi.org/10.1258/jrsm.97.10.472
Maggio MG, De Luca R, Molonia F, Porcari B, Destro M, Casella C, Salvati R, Bramanti P, Calabro RS (2019) Cognitive rehabilitation in patients with traumatic brain injury: a narrative review on the emerging use of virtual reality. J Clin Neurosci 61:1–4. https://doi.org/10.1016/j.jocn.2018.12.020

Marin E, Boschetto F, Pezzotti G (2020) Biomaterials and biocompatibility: an historical overview. J Biomed Mater Res A 108(8):1617–1633. https://doi.org/10.1002/jbm.a.36930

Markert CD, Case LE, Carter GT, Furlong PA, Grange RW (2012) Exercise and Duchenne muscular dystrophy: where we have been and where we need to go. Muscle Nerve 45(5): 746–751. https://doi.org/10.1002/mus.23244

Martin I, Wendt D, Heberer M (2004) The role of bioreactors in tissue engineering. Trends Biotechnol 22(2):80–86. https://doi.org/10.1016/j.tibtech.2003.12.001

Mathews J, Levin M (2018) The body electric 2.0: recent advances in developmental bioelectricity for regenerative and synthetic bioengineering. Curr Opin Biotechnol 52:134–144. https://doi.org/10.1016/j.copbio.2018.03.008

Mavalli MD, DiGirolamo DJ, Fan Y, Riddle RC, Campbell KS, van Groen T, Frank SJ, Sperling MA, Esser KA, Bamman MM, Clemens TL (2010) Distinct growth hormone receptor signaling modes regulate skeletal muscle development and insulin sensitivity in mice. J Clin Invest 120(11):4007–4020. https://doi.org/10.1172/JCI42447

McDermott MM (2018) Exercise rehabilitation for peripheral artery disease: a review. J Cardiopulm Rehabil Prev 38(2):63–69. https://doi.org/10.1097/HCR.0000000000000343

Messier SP (2008) Obesity and osteoarthritis: disease genesis and nonpharmacologic weight management. Rheum Dis Clin North Am 34(3):713–729. https://doi.org/10.1016/j.rdc.2008.04.007

Modarai B, Patel AS (2019) PLX-PAD treatment of critical limb ischaemia: a clinically effective cell therapy at long last? Eur J Vasc Endovasc Surg 57(4):546. https://doi.org/10.1016/j.ejvs.2019.02.004

Mooney DJ, Vandenburgh H (2008) Cell delivery mechanisms for tissue repair. Cell Stem Cell 2(3):205–213. https://doi.org/10.1016/j.stem.2008.02.005

Morimoto J, Yasuhara T, Kameda M, Umakoshi M, Kin I, Kuwahara K, Kin K, Okazaki M, Takeuchi H, Sasaki T, Toyoshima A, Tajiri N, Agari T, Borlongan CV, Date I (2018) Electrical stimulation enhances migratory ability of transplanted bone marrow stromal cells in a rodent ischemic stroke model. Cell Physiol Biochem 46(1):57–68. https://doi.org/10.1159/000488409

Moritz CT, Ambrosio F (2017) Regenerative rehabilitation: combining stem cell therapies and activity-dependent stimulation. Pediatr Phys Ther 29(Suppl 3):S10–S15. https://doi.org/10.1097/PEP.0000000000000378

Morrison JJ, McMahon J, DuBose JJ, Scalea TM, Lawson JH, Rasmussen TE (2019) Clinical implementation of the Humacyte human acellular vessel: implications for military and civilian trauma care. J Trauma Acute Care Surg 87(1S Suppl 1):S44–S47. https://doi.org/10.1097/TA.0000000000002350

Myer GD, Paterno MV, Ford KR, Quatman CE, Hewett TE (2006) Rehabilitation after anterior cruciate ligament reconstruction: criteria-based progression through the return-to-sport phase. J Orthop Sports Phys Ther 36(6):385–402. https://doi.org/10.2519/jospt.2006.2222

Napoli C, Hayashi T, Cacciatore F, Casamassimi A, Casini C, Al-Omran M, Ignarro LJ (2011) Endothelial progenitor cells as therapeutic agents in the microcirculation: an update. Atherosclerosis 215(1):9–22. https://doi.org/10.1016/j.atherosclerosis.2010.10.039

Omrani AS, Almaghrabi RS (2017) Complications of hematopoietic stem cell transplantation: bacterial infections. Hematol Oncol Stem Cell Ther 10(4):228–232. https://doi.org/10.1016/j.hemonc.2017.05.018

Ong CS, Zhou X, Huang CY, Fukunishi T, Zhang H, Hibino N (2017) Tissue engineered vascular grafts: current state of the field. Expert Rev Med Devices 14(5):383–392. https://doi.org/10.1080/17434440.2017.1324293

Owens BD, Kragh JF Jr, Macaitis J, Svoboda SJ, Wenke JC (2007) Characterization of extremity wounds in operation Iraqi freedom and operation enduring freedom. J Orthop Trauma 21(4): 254–257. https://doi.org/10.1097/BOT.0b013e31802f78fb

Papadopoulos ES, Mani R (2020) The role of ultrasound therapy in the management of musculoskeletal soft tissue pain. Int J Low Extrem Wounds 19(4):350–358. https://doi.org/10.1177/1534734620948343

Partridge TA, Morgan JE, Coulton GR, Hoffman EP, Kunkel LM (1989) Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts. Nature 337(6203):176–179. https://doi.org/10.1038/337176a0

Pas H, Moen MH, Haisma HJ, Winters M (2017) No evidence for the use of stem cell therapy for tendon disorders: a systematic review. Br J Sports Med 51(13):996–1002. https://doi.org/10.1136/bjsports-2016-096794

Pashneh-Tala S, MacNeil S, Claeyssens F (2016) The tissue-engineered vascular graft-past, present, and future. Tissue Eng Part B Rev 22(1):68–100. https://doi.org/10.1089/ten.teb.2015.0100

Peng JJ, Sha R, Li MX, Chen LT, Han XH, Guo F, Chen H, Huang XL (2019) Repetitive transcranial magnetic stimulation promotes functional recovery and differentiation of human neural stem cells in rats after ischemic stroke. Exp Neurol 313:1–9. https://doi.org/10.1016/j.expneurol.2018.12.002

Perez-Terzic C, Childers MK (2014) Regenerative rehabilitation: a new future? Am J Phys Med Rehabil 93(11 Suppl 3):S73–S78. https://doi.org/10.1097/PHM.0000000000000211

Portis SM, Sanberg PR (2017) Regenerative rehabilitation: an innovative and multifactorial approach to recovery from stroke and brain injury. Cell Med 9(3):67–71. https://doi.org/10.3727/215517917X693393

Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, Biller J, Brown M, Demaerschalk BM, Hoh B, Jauch EC, Kidwell CS, Leslie-Mazwi TM, Ovbiagele B, Scott PA, Sheth KN, Southerland AM, Summers DV, Tirschwell DL, American Heart Association Stroke Council (2018) 2018 guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 49(3):e46–e110. https://doi.org/10.1161/STR.0000000000000158

Pruitt DT, Schmid AN, Kim LJ, Abe CM, Trieu JL, Choua C, Hays SA, Kilgard MP, Rennaker RL (2016) Vagus nerve stimulation delivered with motor training enhances recovery of function after traumatic brain injury. J Neurotrauma 33(9):871–879. https://doi.org/10.1089/neu.2015.3972

Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E, Marcacci M (2001) Repair of large bone defects with the use of autologous bone marrow stromal cells. N Engl J Med 344(5):385–386. https://doi.org/10.1056/NEJM200102013440516

Ramalho-Santos M, Willenbring H (2007) On the origin of the term "stem cell". Cell Stem Cell 1(1):35–38. https://doi.org/10.1016/j.stem.2007.05.013

Riley DC, Boyer RB, Deister CA, Pollins AC, Cardwell NL, Kelm ND, Does MD, Dortch RD, Bamba R, Shack RB, Thayer WP (2017) Immediate enhancement of nerve function using a novel axonal fusion device after neurotmesis. Ann Plast Surg 79(6):590–599. https://doi.org/10.1097/SAP.0000000000001242

Rumian AP, Wallace AL, Birch HL (2007) Tendons and ligaments are anatomically distinct but overlap in molecular and morphological features—a comparative study in an ovine model. J Orthop Res 25(4):458–464. https://doi.org/10.1002/jor.20218

Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS (1996) Evidence based medicine: what it is and what it isn't. BMJ 312(7023):71–72. https://doi.org/10.1136/bmj.312.7023.71

Sasaki Y, Sasaki M, Kataoka-Sasaki Y, Nakazaki M, Nagahama H, Suzuki J, Tateyama D, Oka S, Namioka T, Namioka A, Onodera R, Mikami T, Wanibuchi M, Kakizawa M, Ishiai S, Kocsis JD, Honmou O (2016) Synergic effects of rehabilitation and intravenous infusion of mesenchymal stem cells after stroke in rats. Phys Ther 96(11):1791–1798. https://doi.org/10.2522/ptj.20150504

Saunders D, Rose L (2021) Regenerative rehabilitation of catastrophic extremity injury in military conflicts and a review of recent developmental efforts. Connect Tissue Res 62(1):83–98. https://doi.org/10.1080/03008207.2020.1776707

Schoenfeld BJ (2010) The mechanisms of muscle hypertrophy and their application to resistance training. J Strength Cond Res 24(10):2857–2872. https://doi.org/10.1519/JSC.0b013e3181e840f3

Sepulveda F, Sanchez L, Amy E, Micheo W (2017) Anterior cruciate ligament injury: return to play, function and long-term considerations. Curr Sports Med Rep 16(3):172–178. https://doi.org/10.1249/JSR.0000000000000356

Shu J, Santulli G (2018) Update on peripheral artery disease: epidemiology and evidence-based facts. Atherosclerosis 275:379–381. https://doi.org/10.1016/j.atherosclerosis.2018.05.033

Silvain J, Cayla G, Collet JP, Fargeot C, Montalescot G (2014) [Coronary stents: 30 years of medical progress]. Med Sci (Paris) 30(3):303–310. https://doi.org/10.1051/medsci/20143003019

Simanski CJ, Maegele MG, Lefering R, Lehnen DM, Kawel N, Riess P, Yucel N, Tiling T, Bouillon B (2006) Functional treatment and early weightbearing after an ankle fracture: a prospective study. J Orthop Trauma 20(2):108–114. https://doi.org/10.1097/01.bot.0000197701.96954.8c

Sivandzade F, Cucullo L (2021) Regenerative stem cell therapy for neurodegenerative diseases: an overview. Int J Mol Sci 22(4). https://doi.org/10.3390/ijms22042153

Stapleton L, Zhu Y, Woo YJ, Appel E (2020) Engineered biomaterials for heart disease. Curr Opin Biotechnol 66:246–254. https://doi.org/10.1016/j.copbio.2020.08.008

Steinberg GK, Kondziolka D, Wechsler LR, Lunsford LD, Coburn ML, Billigen JB, Kim AS, Johnson JN, Bates D, King B, Case C, McGrogan M, Yankee EW, Schwartz NE (2016) Clinical outcomes of transplanted modified bone marrow-derived mesenchymal stem cells in stroke: a phase 1/2a study. Stroke 47(7):1817–1824. https://doi.org/10.1161/STROKEAHA.116.012995

Sun CK, Shao PL, Wang CJ, Yip HK (2011) Study of vascular injuries using endothelial denudation model and the therapeutic application of shock wave: a review. Am J Transl Res 3(3):259–268

Szelenberger R, Kostka J, Saluk-Bijak J, Miller E (2020) Pharmacological Interventions and Rehabilitation Approach for Enhancing Brain Self-repair and Stroke Recovery. Curr Neuropharmacol 18(1):51–64. https://doi.org/10.2174/1570159X17666190726104139

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676. https://doi.org/10.1016/j.cell.2006.07.024

Tam RY, Fuehrmann T, Mitrousis N, Shoichet MS (2014) Regenerative therapies for central nervous system diseases: a biomaterials approach. Neuropsychopharmacology 39(1):169–188. https://doi.org/10.1038/npp.2013.237

Tavassoli M, Crosby WH (1968) Transplantation of marrow to extramedullary sites. Science 161(3836):54–56. https://doi.org/10.1126/science.161.3836.54

Terada Y, Fukuda S, Tohda E, Kigawa I, Wanibuchi Y, Mitsui T (1999) Venous function and delayed leg swelling following saphenectomy in coronary artery bypass grafting. Jpn J Thorac Cardiovasc Surg 47(11):559–562. https://doi.org/10.1007/BF03218062

Teskey GC, Flynn C, Goertzen CD, Monfils MH, Young NA (2003) Cortical stimulation improves skilled forelimb use following a focal ischemic infarct in the rat. Neurol Res 25(8):794–800. https://doi.org/10.1179/016164103771953871

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391):1145–1147. https://doi.org/10.1126/science.282.5391.1145

Trohatou O, Roubelakis MG (2017) Mesenchymal stem/stromal cells in regenerative medicine: past, present, and future. Cell Reprogram 19(4):217–224. https://doi.org/10.1089/cell.2016.0062

Van Tongeren RB, Hamming JF, Fibbe WE, Van Weel V, Frerichs SJ, Stiggelbout AM, Van Bockel JH, Lindeman JH (2008) Intramuscular or combined intramuscular/intra-arterial administration of bone marrow mononuclear cells: a clinical trial in patients with advanced limb ischemia. J Cardiovasc Surg (Torino) 49(1):51–58

Ventura A, Terzaghi C, Legnani C, Borgo E, Albisetti W (2010) Synthetic grafts for anterior cruciate ligament rupture: 19-year outcome study. Knee 17(2):108–113. https://doi.org/10.1016/j.knee.2009.07.013

Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Delling FN, Djousse L, MSV E, Ferguson JF, Fornage M, Khan SS, Kissela BM, Knutson KL, Kwan TW, Lackland DT, Lewis TT, Lichtman JH, Longenecker CT, Loop MS, Lutsey PL, Martin SS, Matsushita K, Moran AE, Mussolino ME, Perak AM, Rosamond WD, Roth GA, Sampson UKA, Satou GM, Schroeder EB, Shah SH, Shay CM, Spartano NL, Stokes A, Tirschwell DL, VanWagner LB, Tsao CW, American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee (2020) Heart disease and stroke statistics-2020 update: a report from the American Heart Association. Circulation 141(9):e139–e596. https://doi.org/10.1161/CIR.0000000000000757

Virchenko O, Aspenberg P (2006) How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation. Acta Orthop 77(5):806–812. https://doi.org/10.1080/17453670610013033

Wang W, Zhong W, Yuan J, Yan C, Hu S, Tong Y, Mao Y, Hu T, Zhang B, Song G (2015) Involvement of Wnt/beta-catenin signaling in the mesenchymal stem cells promote metastatic growth and chemoresistance of cholangiocarcinoma. Oncotarget 6(39):42276–42289. https://doi.org/10.18632/oncotarget.5514

Wangensteen A, Bahr R, Van Linschoten R, Almusa E, Whiteley R, Witvrouw E, Tol JL (2017) MRI appearance does not change in the first 7 days after acute hamstring injury-a prospective study. Br J Sports Med 51(14):1087–1092. https://doi.org/10.1136/bjsports-2016-096881

Watson E, Tatara AM, van den Beucken J, Jansen JA, Wong ME, Mikos AG (2020) An ovine model of in vivo bioreactor-based bone generation. Tissue Eng Part C Methods 26(7):384–396. https://doi.org/10.1089/ten.TEC.2020.0125

Weitzel PP, Richmond JC, Altman GH, Calabro T, Kaplan DL (2002) Future direction of the treatment of ACL ruptures. Orthop Clin North Am 33(4):653–661. https://doi.org/10.1016/s0030-5898(02)00017-2

Wilke MM, Nydam DV, Nixon AJ (2007) Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. J Orthop Res 25(7):913–925. https://doi.org/10.1002/jor.20382

Willett NJ, Boninger ML, Miller LJ, Alvarez L, Aoyama T, Bedoni M, Brix KA, Chisari C, Christ G, Dearth CL, Dyson-Hudson TA, Evans CH, Goldman SM, Gregory K, Gualerzi A, Hart J, Ito A, Kuroki H, Loghmani MT, Mack DL, Malanga GA, Noble-Haeusslein L, Pasquina P, Roche JA, Rose L, Stoddart MJ, Tajino J, Terzic C, Topp KS, Wagner WR, Warden SJ, Wolf SL, Xie H, Rando TA, Ambrosio F (2020) Taking the next steps in regenerative rehabilitation: establishment of a new interdisciplinary field. Arch Phys Med Rehabil 101(5):917–923. https://doi.org/10.1016/j.apmr.2020.01.007

Williams D, Isaacson B (2014) The 5 hallmarks of biomaterials success: an emphasis on orthopaedics. Adv Biosci Biotechnol 5:283–293

Winkler T, Perka C, von Roth P, Agres AN, Plage H, Preininger B, Pumberger M, Geissler S, Hagai EL, Ofir R, Pinzur L, Eyal E, Stoltenburg-Didinger G, Meisel C, Consentius C, Streitz M, Reinke P, Duda GN, Volk HD (2018) Immunomodulatory placental-expanded, mesenchymal stromal cells improve muscle function following hip arthroplasty. J Cachexia Sarcopenia Muscle 9(5):880–897. https://doi.org/10.1002/jcsm.12316

Wu D, Ma J, Zhang L, Wang S, Tan B, Jia G (2020) Effect and safety of transcutaneous auricular vagus nerve stimulation on recovery of upper limb motor function in subacute ischemic stroke patients: a randomized pilot study. Neural Plast 2020:8841752. https://doi.org/10.1155/2020/8841752

Xing Y, Bai Y (2020) A review of exercise-induced neuroplasticity in ischemic stroke: pathology and mechanisms. Mol Neurobiol 57(10):4218–4231. https://doi.org/10.1007/s12035-020-02021-1

Yannas IV, Burke JF (1980) Design of an artificial skin. I. Basic design principles. J Biomed Mater Res 14(1):65–81. https://doi.org/10.1002/jbm.820140108

Yannas IV, Burke JF, Orgill DP, Skrabut EM (1982) Wound tissue can utilize a polymeric template to synthesize a functional extension of skin. Science 215(4529):174–176. https://doi.org/10.1126/science.7031899

Yeh KH, Sheu JJ, Lin YC, Sun CK, Chang LT, Kao YH, Yen CH, Shao PL, Tsai TH, Chen YL, Chua S, Leu S, Yip HK (2012) Benefit of combined extracorporeal shock wave and bone marrow-derived endothelial progenitor cells in protection against critical limb ischemia in rats. Crit Care Med 40(1):169–177. https://doi.org/10.1097/CCM.0b013e31822d74d0

Yu JX, Huang XF, Lv WM, Ye CS, Peng XZ, Zhang H, Xiao LB, Wang SM (2009) Combination of stromal-derived factor-1alpha and vascular endothelial growth factor gene-modified endothelial progenitor cells is more effective for ischemic neovascularization. J Vasc Surg 50(3):608–616. https://doi.org/10.1016/j.jvs.2009.05.049

Yu Y, Alkhawaji A, Ding Y, Mei J (2016) Decellularized scaffolds in regenerative medicine. Oncotarget 7(36):58671–58683. https://doi.org/10.18632/oncotarget.10945

Zhang YX, Yuan MZ, Cheng L, Lin LZ, Du HW, Chen RH, Liu N (2015) Treadmill exercise enhances therapeutic potency of transplanted bone mesenchymal stem cells in cerebral ischemic rats via anti-apoptotic effects. BMC Neurosci 16:56. https://doi.org/10.1186/s12868-015-0196-9

Zhao L, Chen S, Yang P, Cao H, Li L (2019) The role of mesenchymal stem cells in hematopoietic stem cell transplantation: prevention and treatment of graft-versus-host disease. Stem Cell Res Ther 10(1):182. https://doi.org/10.1186/s13287-019-1287-9

Chapter 2
Considerations for Small Animal Physical Rehabilitation

Sarah M. Greising, Alec M. Basten, Albino G. Schifino, and Jarrod A. Call

Abstract Translational and preclinical investigations of regenerative rehabilitation approaches are dependent on an ability to appropriately design and implement physical rehabilitation intervention able to facilitate physiologically beneficial adaptations. To continue to drive success and translation in the field of regenerative rehabilitation a comprehensive understanding of rehabilitation approaches in rodents is necessary. The goal of this chapter is to provide an overview on commonly used physical rehabilitation techniques in mice and rats, with specific emphasis on ideal physiologic overload and tissue-specific targets.

Keywords Physical therapy · Running · Electrical stimulation · Methodology · Task-specific rehabilitation

2.1 Introduction

Regenerative rehabilitation can affect all tissues and organ systems of the body. As noted in Chap. 1, it is operationally defined by the field as a therapy that "*integrates regenerative technologies with rehabilitation clinical practices to restitute function and quality of life in patients with disabilities due to otherwise irreparable tissues or organs damaged by disease or trauma*" (Perez-Terzic and Childers 2014). The ideal physical rehabilitation approach for a given investigation depends on the objective of the strategy. To begin, a fundamental understanding of targeted effects (Thompson 2002) for whole-body function, tissue morphology or size, or functional capacity is necessary to address the question at hand. Targeting whole-body function would include physical rehabilitation modalities aimed at improving aerobic capacity (i.e.,

S. M. Greising (✉) · A. M. Basten
School of Kinesiology, University of Minnesota, Minneapolis, MN, USA
e-mail: grei0064@umn.edu

A. G. Schifino · J. A. Call
Regenerative Bioscience Center, University of Georgia, Athens, GA, USA

Department of Kinesiology, University of Georgia, Athens, GA, USA

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_2

VO_{2max}), endurance (e.g., time maintaining a consistent speed), and/or anaerobic capacity (e.g., O_2 deficit). While improving functional capacity is more related to skeletal muscle endurance, power, strength, and motor performance, for example. The goal of this chapter is to provide a broad overview of commonly used physical rehabilitation modalities in rodents. We provide considerations for their use, physiologic targets, and general pros and cons to consider in methodological design; spanning methodology for conscious and unconscious rodents. Indeed, many of these approaches are addressed in subsequent chapters of this work.

2.2 Rehabilitation in Rodents

Rodents, both mice and rats, are a common research animal model with many benefits for their use (e.g., lifespan, abundant genetic resources, physiology) (Bryda 2013). The homogeneous nature of rodents allows for easy randomization into experimental groups. Additionally, significant experimental control over diet, genetics, environment, and training protocols is a primary advantage of using rodents. All of which supports research designs that are highly sensitive and reproducible within a lab or across many labs. In studies utilizing various physical rehabilitation approaches rodents provide an attractive model system for reproducible training paradigms. These rehabilitation paradigms being evaluated in rodents have the potential for scale-up to large animals, as well as translation into the clinical population to further support evidence-based rehabilitation for injured patients.

2.3 What Is the Ideal Rehabilitation Method?

In both rodent models and the clinical population, the objective of rehabilitation, or physical therapy, is to act upon the systems of the body to facilitate physiologically beneficial adaptations. Adaptations can be targeted to a specific physiologic system or to the whole body. It is imperative for investigators to weigh the pros and cons of each rehabilitation approach to best fit with their research question. For example, if the goal of rehabilitation is to improve endurance performance and aerobic capacity as in disrupted metabolic signaling cases, then treadmill or wheel running may be the ideal rehabilitation. Alternatively, if the goal of rehabilitation is to improve strength, as in the case of sarcopenia (i.e., the age-related loss of muscle size and function), then resistance mimicking activities or neuromuscular electrical stimulation may be more appropriate. We will overview commonly used rehabilitation modalities and their benefits and limitations for consideration in this chapter, with the foundation that the ideal rehabilitation method is the one that physiologically addresses the research question posed.

2.4 Common Rehabilitation Methods Implemented in Rodents

Physical rehabilitation in rodents will be discussed in two categories, those modalities conducted while the rodent is conscious and those conducted when the rodent is unconscious.

2.4.1 Conscious Methods

Short- and long-term interventions commonly utilize conscious rodents. Various methodologies range from rodents conducting tasks in a fully voluntary manner, to tasks requiring external motivation. Methodologies such as treadmill running and voluntary wheel running represent widespread approaches to impart whole-body or tissue-specific (i.e., bone and skeletal muscle) adaptations to rodents.

2.4.1.1 Treadmill Running

A common physical rehabilitation methodology that has direct clinical translation is treadmill walking and running. During a typical rehabilitation session, rodents will be placed on a motorized belt and the investigator will have precise control of belt speed and grade (e.g., uphill/downhill). Similar to human treadmill exercise, the session will provide a stimulus to the cardiovascular (Feng et al. 2019; Lund et al. 2015; Kemi et al. 2002; Wisloff et al. 2002), respiratory, and musculoskeletal systems (Kemi et al. 2002; Davies et al. 1981). There are two primary utilization of treadmill running often observed in the literature: acute endurance tests and chronic aerobic training. Acute endurance test protocols are one-time sessions in which treadmill belt speed, and/or grade, are progressively increased until rodents, despite external motivation provided from the investigator, can no longer participate/sustain treadmill running. At the time of exhaustion, the duration and total distance covered are recorded, often in conjunction with a measure of blood lactate taken from venous tail blood as an indirect assessment of anaerobic metabolism/fatigue (Ferreira et al. 2007). Acute endurance tests are sensitive to detecting the adaptations to aerobic exercise training and detraining, and thus serve as a measure of the efficacy of exercise training and/or exercise mimetic programs (Seldeen et al. 2018, 2019).

Treadmill aerobic training protocols vary in intensity, duration, frequency, and progression (Poole et al. 2020). For example, there are protocols available for high-intensity interval training (Seldeen et al. 2018, 2019; Picoli et al. 2018) as well as moderate intensity (Wang et al. 2017; Navarro et al. 2004; Boveris and Navarro 2008) and ramped intensities (Poole et al. 2020). Regardless, treadmill aerobic training has considerable utility and widespread efficacy as a model for inducing

beneficial adaptations across pathologies and injuries (Lund et al. 2015; Wisloff et al. 2002; Goh et al. 2019; Davies et al. 1981).

A primary benefit of treadmill rehabilitation is that the investigator can control the rehabilitation dose by modulating the frequency, duration, and intensity of the sessions. For example, if the goal is to achieve one kilometer of distance per day, that can be precisely administered. This approach can also be helpful when working with rodent models that do not demonstrate consistent voluntary running behavior if provided a running wheel (e.g., rats) (Rodnick et al. 1989). However, the "involuntary" nature of this approach is also a limitation to consider, as rodents are neither free to modulate their time engaging with the treadmill, nor are they able to self-select a running speed. Some studies also utilize shock grids, stiff bristle brushes, and puffs of air to motivate rodents to accomplish a standardized distance or to maintain a certain speed for a given amount of time. These motivation tactics to ensure training compliance may induce stress responses (Svensson et al. 2016) that should be considered as a factor in study design. Indeed, researchers have demonstrated that 100% training compliance can be accomplished without motivational assistance; however, considerable time must be spent familiarizing rodents with the desired task (>10 sessions) (Arnold and Salvatore 2014). When utilizing the treadmill as a rehabilitation tool, it might be necessary to briefly acclimate rodents in 2–3 short sessions prior to initiating treadmill training. Furthermore, identifying "good runners" and then allocating these specific rodents into the experimental groups can aid in reducing the influence of intraspecies variability in exercise compliance/capacity.

2.4.1.2 Voluntary Wheel Running

Voluntary wheel running involves placing a wheel in the cage of a singly housed rodent and recording the number of revolutions completed per day. Similar to treadmill running, voluntary wheel running is a whole-body activity that provides a stimulus to the cardiovascular (Allen et al. 2001; Judge et al. 2005), respiratory, and musculoskeletal systems (Ikeda et al. 2006; Boveris and Navarro 2008; Gurley et al. 2016). Rodents are nocturnal animals, and the majority of the total running distance will occur during the vivarium lights-off phase. Initially designed as a purely aerobic training paradigm, voluntary wheels can be modified to add a load or resistance training component (Ishihara et al. 1998). Resistance or load components, i.e., high-resistance wheel running, can be modulated by adhering weights to the circumference of the wheel (White et al. 2016; Soffe et al. 2016), fastening a manual tensioning device (Call et al. 2010; Konhilas et al. 2005), or through servomotor generated resistance brakes (Fig. 2.1a–c) (Ishihara et al. 1998; Mobley et al. 2018). Both low- and high-resistance wheel running paradigms induce beneficial adaptations across several diseases and injuries (White et al. 2016; Call et al. 2010; Brooks et al. 2018).

A primary benefit of wheel running is that the rodent can self-select running speed and engagement with the running wheel (in contrast to treadmill running), creating a

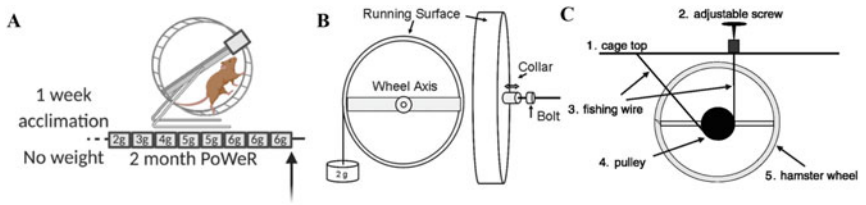

Fig. 2.1 Examples of unweighted and weighted (resistance) voluntary wheels. (**a**) A schematic showing a series of masses that are adhered to the circumference of the wheel to add resistance (Murach et al. 2020). (**b**) A schematic showing a threaded cuff on the wheel axle that is turned to add resistance (Call et al. 2010). (**c**) A schematic of a pulley system that is used to add resistance to a wheel (Konhilas et al. 2005). Schematics are reprinted with permission from John Wiley & Sons

less stressful rehabilitation environment. Twenty-four-hour access to the running wheel means that the rodent can assume its natural pattern aligning to circadian rhythms, while the training volume (i.e., distance covered) is often much greater than that which can be reasonably accomplished with treadmill running (e.g., 10 km/day) (Lightfoot et al. 2004; Lerman et al. 2002). Although, it is important to note beneficial adaptations are possible with as little as 1.5 km/day (Warren et al. 2007; Landisch et al. 2008; Goh and Ladiges 2013). The time demand on the investigator is also much less compared to the treadmill as the rehabilitation sessions happen spontaneously and independent of investigator input. Some limitations of voluntary wheel running include study design and investigator tolerance for the potential of considerable between- and within-animal running distance variability. In contrast to treadmill running where a set distance can be prescribed each day, it is common for there to be 1–2 km/day differences among rodents and sometimes for the same rodents across days of the week (Manzanares et al. 2018). In fact, taking advantage of the between-animal distance variability by the selective breeding of high-distance voluntary wheel running mice has demonstrated several unique characteristics across organ systems compared to normal distance runners (Swallow et al. 2005; Garland et al. 2002; Rhodes et al. 2005; Lightfoot et al. 2004).

2.4.1.3 Whole-Body Vibration

Vibration therapy is an emerging therapeutic modality that requires a mechanical device to augment oscillatory movement (accelerations). Vibration therapy can be applied locally to specific regions with handheld devices, mimicking massage therapy. More often, vibration therapy is administered at the whole-body level via a standing platform (Fig. 2.2). Whole-body vibration therapy requires a cyclic mechanical device (actuator or motor) that can be adjusted to specific magnitudes (g) and frequencies (Hz), which is applied through the standing platform or surface (Novotny et al. 2013). Vibration therapy is administered passively to rodents independently housed and fully conscious inside compartments atop the vibrating platform (Novotny et al. 2013). Vibration supplied through a platform provides

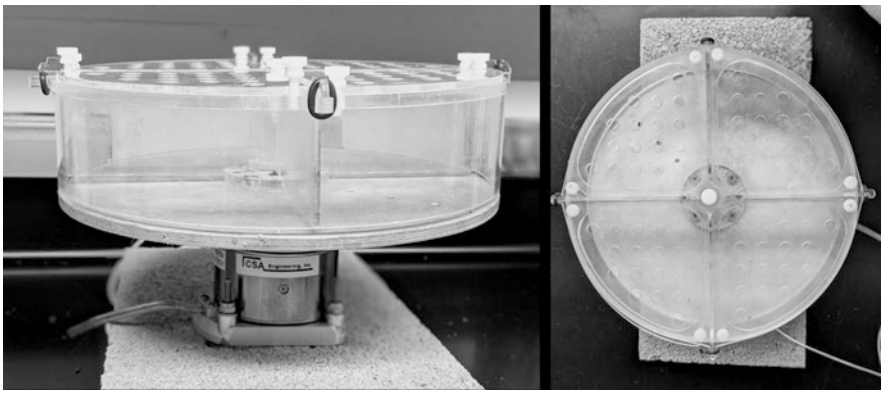

Fig. 2.2 Example of a custom-built small-animal vibration plate (Novotny et al. 2013). A concrete base is used to anchor a linear actuator. A Plexiglas cage for mice is centered on an aluminum platform and has a low ceiling height (6.3 cm) to limit rearing and jumping by the mice during the rehabilitation sessions. This custom-built vibration plate has four compartments to deliver rehabilitation to four mice simultaneously

mechanical stimuli to semi-rigid body structures and can elicit low levels of muscle activation, and metabolic stimulation (Park and Martin 1993; McKeehen et al. 2013; Ren et al. 2020). The biological response of skeletal muscles, muscle spindles, and nerve-endings to vibration stimulus is characterized as the "tonic vibration reflex" (Park and Martin 1993; Bongiovanni and Hagbarth 1990; Zaidell et al. 2013). This passive reflex is proposed to be the result of minor alterations to muscle length under tension, activating muscle stretch-receptors, known as muscle spindles, and coordinating the proprioceptive response of the musculoskeletal system (Burke et al. 1976; Weill et al. 1976). When administered as a chronic therapy, vibration has been shown to improve bone integrity, wound healing (Xie et al. 2006; Vanleene and Shefelbine 2013; Chung et al. 2014; Rubin et al. 2001), skeletal muscle function (McKeehen et al. 2013; Xie et al. 2008; Novotny et al. 2014), and even mimics exercise in rodent models of disease and disuse (McGee-Lawrence et al. 2017; Novotny et al. 2014; Ren et al. 2020).

Vibration therapy serves as a passive modality that can induce muscle activation and increase physical activity independent of voluntary participation or central governing processes. Vibration therapy is a time-effective (<60 min/session), specified mechanotherapy that can be administered free of anesthetics to fully conscious, ambulatory rodents. Vibration therapy can be precisely modulated in frequency, magnitude, and duration, which aids in further understanding vibration stimulus parameters that are crucial for favorable adaptation. Notably, vibration therapy can be both beneficial and destructive, as vibration administered beyond what is considered "low magnitude" (<1.0 g) can instigate muscle injury and impair circulation/vasculature (e.g., Raynaud's phenomenon) (Murfee et al. 2005; Necking et al. 1996). The risk of overexposure to vibration is well-documented, and thus any utilization of vibration for rehabilitation purposes should be aware of overexposure risk and

consider utilizing an external accelerometer to provide live feedback of vibrational accelerations. Commercially available vibration platforms for human use lack the precision to administer vibration therapy suitable for rodents and other small animals. Therefore, custom-designed systems developed by research teams are required to consistently and reliably administer whole-body vibration therapy in rodents (Novotny et al. 2013). Vibration therapy can cause mild stress and exacerbate aggressive behavior; thus, animals should receive therapy either individually or simultaneously in separated compartments. Vibration therapy serves as a clinically relevant rehabilitation approach, with minimal risk, and clear evidence for application across various tissues, diseases, and species of small animals (Xie et al. 2006; Weinheimer-Haus et al. 2014; McGee-Lawrence et al. 2017; Xie et al. 2008).

2.4.1.4 Alternative Resistance Training Approaches

Rehabilitation modalities that target skeletal muscle size and strength absent from cardiovascular and respiratory stimuli (such as that from treadmill and voluntary wheel running) are at times needed to better model a clinical setting or because the research design is not powered to deal with variance from other organ system adaptations. Several resistance training approaches have been validated with the specific intent to overload the skeletal muscle system and produce muscle fiber hypertrophy (i.e., increase in cross-sectional area) and greater muscle force/strength. A weighted vertical ladder climbing technique (Fig. 2.3a) uses a cylinder or plank with horizontal wooden ladder pegs and a top platform with a food reward to encourage rodents to climb (Hellyer et al. 2012). Upon acclimation, weight (e.g., 80% of body mass) can be attached to the tail. Cage tops have also been modified to lift a weight in order for rodents to reach their food supply, effectively creating a squat rack (Fig. 2.3b) (Cui et al. 2020; Barauna et al. 2005; Tamaki et al. 1992). There is also a weight-pulling system where rodents pull weighted carts down a narrow corridor (Fig. 2.3c) (Zhu et al. 2021). Flywheel pulley systems have also been created to allow for resistance training in muscle atrophy study designs (e.g., hindlimb suspension) (Fluckey et al. 2002; Dupont-Versteegden et al. 2006). Across the preceding techniques, investigators should adhere to the training principle of progressive overload (i.e., adjusting the weight-load overtime to maximize muscle overload) to optimize muscle size and strength adaptations in healthy and diseased mice (Leite et al. 2013; Souza et al. 2014; Duncan et al. 1998).

A primary benefit of these alternative resistance training modalities is their ability to specifically target the neuromusculoskeletal system for adaptation. Modalities such as the squat rack (Fig. 2.1b) have the benefit of being a self-selected rehabilitation in which sessions can occur spontaneously without extensive investigator input (similar to voluntary wheel running). When the distance the weight is being moved vertically (squat rack and vertical ladder) or horizontally (weighted carts) is known, along with the timing/velocity of the movement, then both work and power can be calculated serving as complementary outcomes to clinical settings. Similar to treadmill running, a potential consideration for vertical ladder and weighted-cart

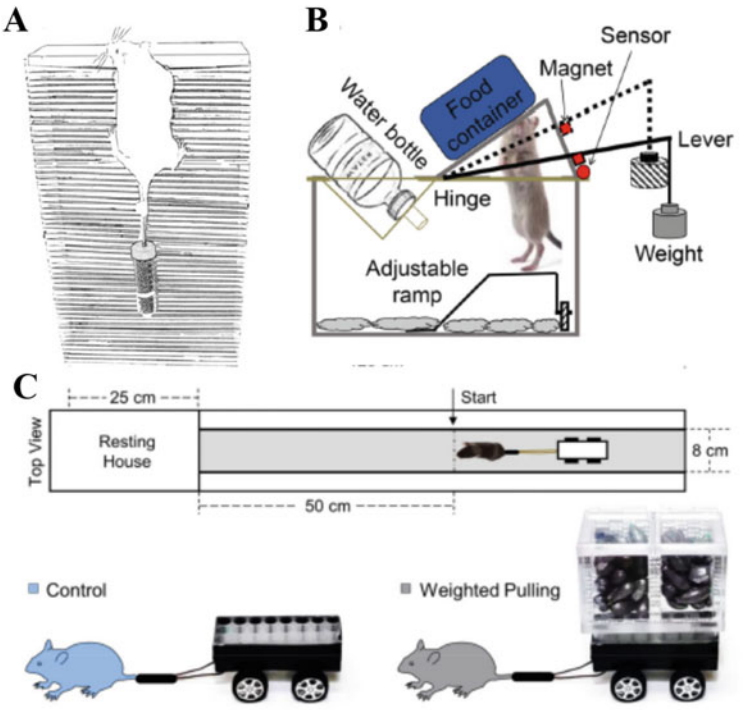

Fig. 2.3 Examples of resistance training methods. (**a**) A drawing depicting an animal climbing a vertical ladder with a weighted conical tube attached to its tail (Hellyer et al. 2012). (**b**) A cartoon depicting a mouse performing a squat-like movement in order to access food against the load of a weighted lever (Cui et al. 2020). (**c**) A schematic depicting the length of a narrow track for a weight-pulling exercise. Cartoons depict animals with unweighted and weighted carts attached to their tails (Zhu et al. 2021). Drawings, cartoons, and schematics are reprinted with permission from MDPI and LWW publishers (references noted).

pulling is animal stress, especially if significant encouragement is needed for animals to complete the task (i.e., strong bristled brushes, electrical shocks). These two techniques also require active participation by the investigator.

2.4.1.5 Swimming

The use of swimming to promote whole-body cardiovascular and oxidative improvements is common in rodents (Kaplan et al. 1994; Dawson and Horvath 1970; Wang et al. 2020; Strickland and Smith 2016), especially in rats. Rats will naturally swim without further intervention, allowing for a physical rehabilitation modality that is self-motivating but lower impact than wheel running. Although less common, mice have also been used in swimming intervention (Spaulding and Selsby 2018; Hsu et al. 2021). More akin to treadmill exercise bouts, swimming bouts are prescribed to

a single whole-body activity and can mimic an aerobic intervention. In contrast to wheel running that consists of multiple small sessions over a period of time, swimming does not have a stop-and-go aspect. This allows the investigator precise control of the rehabilitation dose, which can be progressively overloaded over a multi-week intervention. Additionally, akin to the progressive overload rehabilitation described above, an external load can be added to the body or tail of the rodents during their swimming bouts (Hsu et al. 2021). In rodent models of neuromuscular trauma, swimming is beneficial when an injury to limbs is present that could preclude treadmill running, and there is no potential additional injury to the rodent's feet (Seo et al. 2014). Swimming interventions can be utilized after incomplete spinal cord injury and have shown improvements in motor recovery (Loy and Bareyre 2019).

Necessary considerations for utilizing swimming protocols are water temperature, and prompt drying (and possibly warming) of the rodent following the swimming bout to maintain body temperature. Often swimming interventions can be accomplished in a laboratory with less sophisticated and costly equipment than other intervention types described here. Dedicated investigator observation during all swimming bouts is necessary, requiring active participation during all interventions. Investigators must monitor rodents for noncontinuous swimming behaviors such as diving, floating, or bobbing throughout the rehabilitation bout. Prior work has suggested implementing varying levels of swimming overload in rodents spanning low to high intensity based on time (20 min, 60 min, >90 min per bout, respectively) (Seo et al. 2014; Wang et al. 2020). As a non-weight bearing activity, swimming is not imparting stress on the skeletal system and thus bone overload is not expected, with the exception of the load produced from muscle contractions. Indeed, no impacts on bone mineral density have been indicated in rodent models of swimming (Portier et al. 2020). Swimming may induce stressful stimuli to some rodents (Kaplan et al. 1994), that can confound potential results (Strickland and Smith 2016). Swimming interventions also introduce the risk of drowning, which may be increased in highly diseased rodent models. Finally, for nontreatment groups, investigators should consider placing rodents in shallow water to control for the impact of water exposure on experimental outcomes.

2.4.1.6 Task-Specific Rehabilitation

Increasingly, physical rehabilitation is becoming highly specialized more akin to occupational therapy in the clinical setting. In rodent models of stroke, traumatic brain injury, and spinal cord injury, these specialized rehabilitation tasks are designed primarily to target and strengthen neural pathways, improving motor recovery (Fenrich et al. 2021). Notably, in some models, these tasks can be utilized as overuse designs for musculoskeletal models (Xin et al. 2017; Barbe et al. 2021). In both cases, the use of task-based rehabilitation often requires rodent motivation much like the resistance-style rehabilitation approaches noted in previous sections. The tasks the rodents are trained to complete can be vast but commonly they revolve

around the forelimb to include grasping to retrieve food/treats (Okabe et al. 2017; Dutcher et al. 2021; Joa et al. 2017) or lever pulling (Xin et al. 2017; Barbe et al. 2021). In some instances of neurovascular injuries, the research design may be most appropriate for the initial task training to occur to induction of injury, representing a "re-learning" of the tasks as rehabilitation occurs (DeBoer et al. 2021), more akin to occupational therapy in the clinic.

During familiarization and training for tasks, ongoing modification to the ability to obtain the reward may be necessary, starting from no threshold to a further reach that requires a greater force of activation. While the investigators have an initial involvement in the familiarization as the tasks are learned, the rehabilitation can become more rodent-driven as time progresses. Targeted tasks, such as grasping, represent a more appropriate rehabilitation modality than wheel running, for example, which stresses larger muscle groups responsible primarily for weight-bearing and whole-body physiology. In part, this represents a limitation of wheel running, as it imparts limited neurologic stimulus. Conversely, forelimb rehabilitation is more appropriate to target small muscle groups, responsible for fine motor skills.

2.4.2 Unconscious Methods

Methodologies for physical rehabilitation can also be undertaken in unconscious, or anesthetized, rodents. In many instances, the ability to motivate rodents is limited or not possible and other modalities are necessary to impart physiologic changes. Collectively, there is a major limitation to the use and repeated use of anesthetics, due to the various effects that dose and type of anesthetic have on neuromuscular function (Ingalls et al. 1996). With this, a major investigator consideration is consistency in dose and type of anesthetic across studies and experimental groups. Additionally, limitations with repeated anesthesia are known and provide various stressors to the rodents (Hohlbaum et al. 2017, 2018; Peng et al. 2021).

2.4.2.1 Repeated In Vivo Electrical Stimulation

In vivo functional assessment of skeletal muscle (e.g., isometric, isokinetic, fatigability) is often used to evaluate torque (Corona et al. 2021; Lovering et al. 2011; Mintz et al. 2016; Call et al. 2011, 2013; Ingalls et al. 2004; Warren et al. 1999). The methodology also has the utility to conduct repeated rehabilitation sessions in rodents in a minimally invasive manner to stimulate muscle hypertrophy, muscle-specific metabolism, and neuromuscular function. In vivo muscle stimulation using needle electrodes (Greising et al. 2018), implantable nerve cuffs (Walters et al. 1991; Warren et al. 1998), and fully implantable wireless stimulating electrodes (Deshmukh et al. 2020; Koo et al. 2018) all provide options to directly and precisely stimulate terminal nerves innervating of skeletal muscles across the body. For this, investigators use an external stimulator to precisely control the frequency of the

nerve stimulation and thus the action potential and magnitude of force generation in a species-dependent manner. Specifically for 50% and 100% activation of peak isometric force in healthy skeletal muscle in the mouse stimulation at 30 Hz and 125 Hz are required, while the rat would need 50 Hz and 150 Hz, respectively. The ability for the investigator to also ensure maximal activation of all motor units is possible by stimulating at high frequencies (e.g., >200 Hz). Investigators also have the ability to control strictly all aspects of the rehabilitation bout, such as contraction number, rest periods, and muscle activation.

The primary advantage to in vivo electrical stimulation as a repeated rehabilitation is the independence of rodent motivation which is in direct contrast to training protocols that stimulate resistance style training and require motivation (Sect. 2.4.1.4) in some form in conscious models as noted in previous sections. Additionally, electrical stimulation is capable of specifically reproducing effects of resistance training overload unilaterally allowing for an intra-animal control (Lowe and Alway 2002). For electrical stimulation via percutaneous electrodes, needle electrodes are inserted through the skin to directly stimulate a nerve. One benefit to the use of needle electrodes is that it does not require invasive surgical implantation of a device. Needle electrodes are also limited to superficial nerves, such as the peroneal nerve, which branches off the sciatic to innervate the tibialis anterior muscle. Additionally, stimulation of muscle groups or units could be possible with stimulation of the sciatic nerve superiorly. Repeated insertions of needle electrodes can result in scar tissue formation and accumulation, and the exact electrode placement between sessions may vary slightly compared to an electrode that is surgically implanted. A nerve cuff is a surgically implanted electrode cuff that surrounds a nerve of interest. While nerve cuffs have been utilized on superficial nerves, surgical implantation does allow for targeting nerves that may not be accessible to needle electrodes, for example, the tibial nerve or the phrenic nerve (Fenik et al. 2001). Electrodes are often housed externally on the back of the neck and the nerve cuff method for electrical stimulation permits chronic and direct stimulation of a nerve for months (Warren et al. 1998). However, this method may be more invasive than using percutaneous needle electrodes that do not require surgery for implantation. Additionally, time between implantation and rehabilitation initiation is needed. Recently, newer methods for wireless implantation of electrodes have been developed. These methods are similar to the implantable nerve cuff except that the electrodes are controlled by radio waves and powered by batteries using wireless power transmission technology to eliminate the need for external wires (Deshmukh et al. 2020). An important consideration for translating any electrode implantation into a human population is that a secondary surgery is necessary for the removal of the device (Ju et al. 2020). However, developing technologies such as bioresorbable wireless electrodes may become more available, eliminating the need for a secondary surgery for removal (Koo et al. 2018).

2.4.2.2 Range of Motion

Continuous passive range of motion uses passive joint movement to mitigate muscle, tendon, and joint stiffness. This type of physical rehabilitation is common in knee pathologies clinically (D'Amore et al. 2021). Although less common than other rehabilitation methods in rodents, the use of passive range of motion in rehabilitation is known to improve joint range of motion following eccentric and rotator cuff injuries (Chang et al. 2015; Matsuo et al. 2015). Using various techniques, most of which involve computer-controlled servomotors, investigators control the angle and duration of movement at specific joints. An advantage of using passive range of motion is that it is non-weight bearing in nature and does not require muscle fiber innervation to be effective (Greising et al. 2018). Notably, in rat models of spinal cord injury, range of motion exercise has been conducted while the rat is conscious (Keller et al. 2017), with investigators manually performing all the exercises; for this, timing in each position was accomplished using a standard metronome. However, investigator-controlled range of motion exercise could induce more variability than the servomotor-controlled rehabilitations. It is also important to note that timing (i.e., start of rehabilitation post-injury, duration, frequency) and range of motion parameters will play a role in the effectiveness and physiological response. For example, earlier implementation of passive range of motion caused a higher rate of recurrent tendon tears in rotator cuff injury (Chang et al. 2015), suggesting a delayed start of range of motion rehabilitation should be considered. Additionally, fast repetitive stretching was more effective in suppressing muscle fibrosis in rats with denervated sciatic nerve than slower stretching (Tanaka et al. 2017). Passive range of motion could also be performed in conjunction with other modes of rehabilitation, such as intermittent in vivo electrical stimulation, which represents a rehabilitation regimen that is readily translatable to the clinic, even while patients are non-weight bearing (Greising et al. 2018).

2.5 Importance of Functional Outcomes and Future Considerations

To fully leverage preclinical animal studies in building a foundation to develop evidence-based rehabilitation practices for humans, there needs to be a focus on functional outcomes and clinically relevant techniques. Functional outcomes will vary depending on the organ system of interest. For example, function outcomes for skeletal muscle are typically measurements of muscle force and/or torque (Call and Lowe 2016), whereas functional outcomes for tendons may include in vivo passive stiffness about a joint or assessing ex vivo stiffness properties of isolated tendons (Wang et al. 2006). Some organ systems require indirect functional outcome measurements such as the central and peripheral nervous systems where outcomes such as grasping task successes vs. failures, novel object recognition tasks, and/or maze

orientation are valuable (Noble et al. 2019; Zemmar et al. 2015; Wolf et al. 2016). A functional outcome is perhaps the strongest way to advance the efficacy of any rehabilitation approach.

Many of the approaches emphasized in this chapter can serve a dual role as both rehabilitative approaches and endpoint measurements of adaptation (i.e., functional outcomes). For example, a treadmill can be used to administer weekly rehabilitation of a set distance (e.g., 1 km/day), and an acute treadmill fatigue test can be an important endpoint outcome to evaluate the extent to which rehabilitation resulted in whole-body adaptations (i.e., greater treadmill running endurance compared to a control) (Dougherty et al. 2016). Similarly, a stimulating electrical nerve cuff can be used to precisely activate a particular muscle group and provide repeated rehabilitation. The nerve cuff approach can be combined with a torque transducer to measure muscle strength prior to and at the conclusion of the rehabilitation window to determine beneficial remodeling of the targeted muscle group (Call et al. 2011).

The relationship between the preclinical and clinical rehabilitation environments is dynamic and ever-evolving. Sometimes techniques evolve or manifest first in the clinical or commercial rehabilitation environments prior to preclinical testing. For example, whole-body cryotherapy, in which the body is treated with extremely cold air in hopes of lessening muscle soreness, is a commercial practice with little to no evidence-based proof of effectiveness (Costello et al. 2015). For their part, preclinical researchers should consider the scalability and clinical relevance of their rehabilitative approach. Several techniques highlighted in this chapter both scale up well and are clinically feasible. For example, treadmill exercise is effective in the mouse, pig, and humans across different disease conditions (Hyzewicz et al. 2015; McDermott 2018; Robles and Heaps 2015). Vibration, discussed as an option for mice in this chapter, has served as a rehabilitation approach in both equines (Halsberghe 2017) and humans (Ritzmann et al. 2018). The future of developing and validating rehabilitative techniques can be strengthened by partnerships between the researcher and clinician to best deliver a bench to clinic model.

Funding

This work was supported by funding from the Department of Defense, Clinical & Rehabilitative Medicine Research Program: W81XWH-18-1-0710 and W81XWH-20-1-0885 (SMG and JAC) Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense.

Declaration of Interests The authors declare that they have no potential or actual conflict of interest.

References

Allen DL, Harrison BC, Maass A, Bell ML, Byrnes WC, Leinwand LA (2001) Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. J Appl Physiol (1985) 90(5): 1900–1908. https://doi.org/10.1152/jappl.2001.90.5.1900

Arnold JC, Salvatore MF (2014) Getting to compliance in forced exercise in rodents: a critical standard to evaluate exercise impact in aging-related disorders and disease. J Vis Exp 90. https://doi.org/10.3791/51827

Barauna VG, Batista ML Jr, Costa Rosa LF, Casarini DE, Krieger JE, Oliveira EM (2005) Cardiovascular adaptations in rats submitted to a resistance-training model. Clin Exp Pharmacol Physiol 32(4):249–254. https://doi.org/10.1111/j.1440-1681.2005.04180.x

Barbe MF, Harris MY, Cruz GE, Amin M, Billett NM, Dorotan JT, Day EP, Kim SY, Bove GM (2021) Key indicators of repetitive overuse-induced neuromuscular inflammation and fibrosis are prevented by manual therapy in a rat model. BMC Musculoskelet Disord 22(1):417. https://doi.org/10.1186/s12891-021-04270-0

Bongiovanni LG, Hagbarth KE (1990) Tonic vibration reflexes elicited during fatigue from maximal voluntary contractions in man. J Physiol 423:1–14. https://doi.org/10.1113/jphysiol.1990.sp018007

Boveris A, Navarro A (2008) Systemic and mitochondrial adaptive responses to moderate exercise in rodents. Free Radic Biol Med 44(2):224–229. https://doi.org/10.1016/j.freeradbiomed.2007.08.015

Brooks MJ, Hajira A, Mohamed JS, Alway SE (2018) Voluntary wheel running increases satellite cell abundance and improves recovery from disuse in gastrocnemius muscles from mice. J Appl Physiol (1985) 124(6):1616–1628. https://doi.org/10.1152/japplphysiol.00451.2017

Bryda EC (2013) The Mighty Mouse: the impact of rodents on advances in biomedical research. Mo Med 110(3):207–211

Burke D, Hagbarth KE, Lofstedt L, Wallin BG (1976) The responses of human muscle spindle endings to vibration of non-contracting muscles. J Physiol 261(3):673–693. https://doi.org/10.1113/jphysiol.1976.sp011580

Call JA, Lowe DA (2016) Eccentric contraction-induced muscle injury: reproducible, quantitative, physiological models to impair skeletal muscle's capacity to generate force. Methods Mol Biol 1460:3–18. https://doi.org/10.1007/978-1-4939-3810-0_1

Call JA, McKeehen JN, Novotny SA, Lowe DA (2010) Progressive resistance voluntary wheel running in the mdx mouse. Muscle Nerve 42(6):871–880

Call JA, Eckhoff MD, Baltgalvis KA, Warren GL, Lowe DA (2011) Adaptive strength gains in dystrophic muscle exposed to repeated bouts of eccentric contraction. J Appl Physiol (1985) 111(6):1768–1777. https://doi.org/10.1152/japplphysiol.00942.2011

Call JA, Warren GL, Verma M, Lowe DA (2013) Acute failure of action potential conduction in mdx muscle reveals new mechanism of contraction-induced force loss. J Physiol 591(Pt 15):3765–3776. https://doi.org/10.1113/jphysiol.2013.254656

Chang KV, Hung CY, Han DS, Chen WS, Wang TG, Chien KL (2015) Early versus delayed passive range of motion exercise for arthroscopic rotator cuff repair: a meta-analysis of randomized controlled trials. Am J Sports Med 43(5):1265–1273. https://doi.org/10.1177/0363546514544698

Chung SL, Leung KS, Cheung WH (2014) Low-magnitude high-frequency vibration enhances gene expression related to callus formation, mineralization and remodeling during osteoporotic fracture healing in rats. J Orthop Res 32(12):1572–1579. https://doi.org/10.1002/jor.22715

Corona BT, Call JA, Borkowski M, Greising SM (2021) In vivo measurement of hindlimb dorsiflexor isometric torque from pig. J Vis Exp. https://doi.org/10.3791/62905

Costello JT, Baker PR, Minett GM, Bieuzen F, Stewart IB, Bleakley C (2015) Whole-body cryotherapy (extreme cold air exposure) for preventing and treating muscle soreness after exercise in adults. Cochrane Database Syst Rev (9):CD010789. https://doi.org/10.1002/14651858.CD010789.pub2

Cui D, Drake JC, Wilson RJ, Shute RJ, Lewellen B, Zhang M, Zhao H, Sabik OL, Onengut S, Berr SS, Rich SS, Farber CR, Yan Z (2020) A novel voluntary weightlifting model in mice promotes muscle adaptation and insulin sensitivity with simultaneous enhancement of autophagy and mTOR pathway. FASEB J 34(6):7330–7344. https://doi.org/10.1096/fj.201903055R

D'Amore T, Rao S, Corvi J, Jack RA 2nd, Tjoumakaris FP, Ciccotti MG, Freedman KB (2021) The utility of continuous passive motion after anterior cruciate ligament reconstruction: a systematic review of comparative studies. Orthop J Sports Med 9(6):23259671211013841. https://doi.org/10.1177/23259671211013841

Davies KJ, Packer L, Brooks GA (1981) Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. Arch Biochem Biophys 209(2):539–554. https://doi.org/10.1016/0003-9861(81)90312-x

Dawson CA, Horvath SM (1970) Swimming in small laboratory animals. Med Sci Sports 2(2): 51–78

DeBoer SR, Hubbard R, Mersha M, Pinilla Monsalve G, Winter S, Zeiler SR (2021) Enhanced spontaneous motor recovery after stroke in mice treated with cerebrolysin. Neurorehabil Neural Repair 35(6):525–533. https://doi.org/10.1177/15459683211000734

Deshmukh A, Brown L, Barbe MF, Braverman AS, Tiwari E, Hobson L, Shunmugam S, Armitage O, Hewage E, Ruggieri MR Sr, Morizio J (2020) Fully implantable neural recording and stimulation interfaces: peripheral nerve interface applications. J Neurosci Methods 333: 108562. https://doi.org/10.1016/j.jneumeth.2019.108562

Dougherty JP, Springer DA, Gershengorn MC (2016) The treadmill fatigue test: a simple, high-throughput assay of fatigue-like behavior for the mouse. J Vis Exp (111). https://doi.org/10.3791/54052

Duncan ND, Williams DA, Lynch GS (1998) Adaptations in rat skeletal muscle following long-term resistance exercise training. Eur J Appl Physiol Occup Physiol 77(4):372–378. https://doi.org/10.1007/s004210050347

Dupont-Versteegden EE, Fluckey JD, Knox M, Gaddy D, Peterson CA (2006) Effect of flywheel-based resistance exercise on processes contributing to muscle atrophy during unloading in adult rats. J Appl Physiol (1985) 101(1):202–212. https://doi.org/10.1152/japplphysiol.01540.2005

Dutcher AM, Truong KV, Miller DD, Allred RP, Nudi E, Jones TA (2021) Training in a cooperative bimanual skilled reaching task, the popcorn retrieval task, improves unimanual function after motor cortical infarcts in rats. Behav Brain Res 396:112900. https://doi.org/10.1016/j.bbr.2020.112900

Feng R, Wang L, Li Z, Yang R, Liang Y, Sun Y, Yu Q, Ghartey-Kwansah G, Sun Y, Wu Y, Zhang W, Zhou X, Xu M, Bryant J, Yan G, Isaacs W, Ma J, Xu X (2019) A systematic comparison of exercise training protocols on animal models of cardiovascular capacity. Life Sci 217:128–140. https://doi.org/10.1016/j.lfs.2018.12.001

Fenik V, Fenik P, Kubin L (2001) A simple cuff electrode for nerve recording and stimulation in acute experiments on small animals. J Neurosci Methods 106(2):147–151. https://doi.org/10.1016/s0165-0270(01)00340-5

Fenrich KK, Hallworth BW, Vavrek R, Raposo PJF, Misiaszek JE, Bennett DJ, Fouad K, Torres-Espin A (2021) Self-directed rehabilitation training intensity thresholds for efficient recovery of skilled forelimb function in rats with cervical spinal cord injury. Exp Neurol 339:113543. https://doi.org/10.1016/j.expneurol.2020.113543

Ferreira JC, Rolim NP, Bartholomeu JB, Gobatto CA, Kokubun E, Brum PC (2007) Maximal lactate steady state in running mice: effect of exercise training. Clin Exp Pharmacol Physiol 34(8):760–765. https://doi.org/10.1111/j.1440-1681.2007.04635.x

Fluckey JD, Dupont-Versteegden EE, Montague DC, Knox M, Tesch P, Peterson CA, Gaddy-Kurten D (2002) A rat resistance exercise regimen attenuates losses of musculoskeletal mass during hindlimb suspension. Acta Physiol Scand 176(4):293–300. https://doi.org/10.1046/j.1365-201X.2002.01040.x

Garland T Jr, Morgan MT, Swallow JG, Rhodes JS, Girard I, Belter JG, Carter PA (2002) Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. Evolution 56(6):1267–1275. https://doi.org/10.1111/j.0014-3820.2002.tb01437.x

Goh J, Ladiges WC (2013) A novel long term short interval physical activity regime improves body composition in mice. BMC Res Notes 6:66. https://doi.org/10.1186/1756-0500-6-66

Goh Q, Song T, Petrany MJ, Cramer AA, Sun C, Sadayappan S, Lee SJ, Millay DP (2019) Myonuclear accretion is a determinant of exercise-induced remodeling in skeletal muscle. Elife 8. https://doi.org/10.7554/eLife.44876

Greising SM, Warren GL, Southern WM, Nichenko AS, Qualls AE, Corona BT, Call JA (2018) Early rehabilitation for volumetric muscle loss injury augments endogenous regenerative aspects of muscle strength and oxidative capacity. BMC Musculoskelet Disord 19(1):173. https://doi.org/10.1186/s12891-018-2095-6

Gurley JM, Griesel BA, Olson AL (2016) Increased skeletal muscle GLUT4 expression in obese mice after voluntary wheel running exercise is posttranscriptional. Diabetes 65(10):2911–2919. https://doi.org/10.2337/db16-0305

Halsberghe BT (2017) Long-term and immediate effects of whole body vibration on chronic lameness in the horse: a pilot study. J Equine Vet Sci 48:121–128.e122. https://doi.org/10.1016/j.jevs.2015.12.007

Hellyer NJ, Nokleby JJ, Thicke BM, Zhan WZ, Sieck GC, Mantilla CB (2012) Reduced ribosomal protein s6 phosphorylation after progressive resistance exercise in growing adolescent rats. J Strength Cond Res 26(6):1657–1666. https://doi.org/10.1519/JSC.0b013e318231abc9

Hohlbaum K, Bert B, Dietze S, Palme R, Fink H, Thone-Reineke C (2017) Severity classification of repeated isoflurane anesthesia in C57BL/6JRj mice-assessing the degree of distress. PLoS One 12(6):e0179588. https://doi.org/10.1371/journal.pone.0179588

Hohlbaum K, Bert B, Dietze S, Palme R, Fink H, Thone-Reineke C (2018) Impact of repeated anesthesia with ketamine and xylazine on the well-being of C57BL/6JRj mice. PLoS One 13(9):e0203559. https://doi.org/10.1371/journal.pone.0203559

Hsu YJ, Lee MC, Huang CC, Ho CS (2021) The effects of different types of aquatic exercise training interventions on a high-fructose diet-fed mice. Int J Med Sci 18(3):695–705. https://doi.org/10.7150/ijms.52347

Hyzewicz J, Ruegg UT, Takeda S (2015) Comparison of experimental protocols of physical exercise for mdx mice and duchenne muscular dystrophy patients. J Neuromuscul Dis 2(4):325–342. https://doi.org/10.3233/JND-150106

Ikeda S, Kawamoto H, Kasaoka K, Hitomi Y, Kizaki T, Sankai Y, Ohno H, Haga S, Takemasa T (2006) Muscle type-specific response of PGC-1 alpha and oxidative enzymes during voluntary wheel running in mouse skeletal muscle. Acta Physiol (Oxf) 188(3–4):217–223. https://doi.org/10.1111/j.1748-1716.2006.01623.x

Ingalls CP, Warren GL, Lowe DA, Boorstein DB, Armstrong RB (1996) Differential effects of anesthetics on in vivo skeletal muscle contractile function in the mouse. J Appl Physiol 80(1):332–340

Ingalls CP, Wenke JC, Nofal T, Armstrong RB (2004) Adaptation to lengthening contraction-induced injury in mouse muscle. J Appl Physiol (1985) 97(3):1067–1076. https://doi.org/10.1152/japplphysiol.01058.2003

Ishihara A, Roy RR, Ohira Y, Ibata Y, Edgerton VR (1998) Hypertrophy of rat plantaris muscle fibers after voluntary running with increasing loads. J Appl Physiol (1985) 84(6):2183–2189. https://doi.org/10.1152/jappl.1998.84.6.2183

Joa KL, Moon S, Kim JH, Shin DW, Lee KH, Jung S, Kim MO, Kim CH, Jung HY, Kang JH (2017) Effects of task-specific rehabilitation training on tau modification in rat with photothrombotic cortical ischemic damage. Neurochem Int 108:309–317. https://doi.org/10.1016/j.neuint.2017.05.004

Ju C, Park E, Kim T, Kim T, Kang M, Lee KS, Park SM (2020) Effectiveness of electrical stimulation on nerve regeneration after crush injury: comparison between invasive and non-invasive stimulation. PLoS One 15(5):e0233531. https://doi.org/10.1371/journal.pone.0233531

Judge S, Jang YM, Smith A, Selman C, Phillips T, Speakman JR, Hagen T, Leeuwenburgh C (2005) Exercise by lifelong voluntary wheel running reduces subsarcolemmal and interfibrillar mitochondrial hydrogen peroxide production in the heart. Am J Physiol Regul Integr Comp Physiol 289(6):R1564–R1572. https://doi.org/10.1152/ajpregu.00396.2005

Kaplan ML, Cheslow Y, Vikstrom K, Malhotra A, Geenen DL, Nakouzi A, Leinwand LA, Buttrick PM (1994) Cardiac adaptations to chronic exercise in mice. Am J Physiol 267(3 Pt 2):H1167–H1173. https://doi.org/10.1152/ajpheart.1994.267.3.H1167

Keller A, Rees K, Prince D, Morehouse J, Shum-Siu A, Magnuson D (2017) Dynamic "range of motion" hindlimb stretching disrupts locomotor function in rats with moderate subacute spinal cord injuries. J Neurotrauma 34(12):2086–2091. https://doi.org/10.1089/neu.2016.4951

Kemi OJ, Loennechen JP, Wisloff U, Ellingsen O (2002) Intensity-controlled treadmill running in mice: cardiac and skeletal muscle hypertrophy. J Appl Physiol (1985) 93(4):1301–1309. https://doi.org/10.1152/japplphysiol.00231.2002

Konhilas JP, Widegren U, Allen DL, Paul AC, Cleary A, Leinwand LA (2005) Loaded wheel running and muscle adaptation in the mouse. Am J Physiol Heart Circ Physiol 289(1):H455–H465. https://doi.org/10.1152/ajpheart.00085.2005

Koo J, MacEwan MR, Kang SK, Won SM, Stephen M, Gamble P, Xie Z, Yan Y, Chen YY, Shin J, Birenbaum N, Chung S, Kim SB, Khalifeh J, Harburg DV, Bean K, Paskett M, Kim J, Zohny ZS, Lee SM, Zhang R, Luo K, Ji B, Banks A, Lee HM, Huang Y, Ray WZ, Rogers JA (2018) Wireless bioresorbable electronic system enables sustained nonpharmacological neuroregenerative therapy. Nat Med 24(12):1830–1836. https://doi.org/10.1038/s41591-018-0196-2

Landisch RM, Kosir AM, Nelson SA, Baltgalvis KA, Lowe DA (2008) Adaptive and nonadaptive responses to voluntary wheel running by mdx mice. Muscle Nerve 38(4):1290–1303

Leite RD, Durigan Rde C, de Souza Lino AD, de Souza Campos MV, Souza M, Selistre-de-Araujo HS, Bouskela E, Kraemer-Aguiar LG (2013) Resistance training may concomitantly benefit body composition, blood pressure and muscle MMP-2 activity on the left ventricle of high-fat fed diet rats. Metabolism 62(10):1477–1484. https://doi.org/10.1016/j.metabol.2013.05.009

Lerman I, Harrison BC, Freeman K, Hewett TE, Allen DL, Robbins J, Leinwand LA (2002) Genetic variability in forced and voluntary endurance exercise performance in seven inbred mouse strains. J Appl Physiol (1985) 92(6):2245–2255. https://doi.org/10.1152/japplphysiol.01045.2001

Lightfoot JT, Turner MJ, Daves M, Vordermark A, Kleeberger SR (2004) Genetic influence on daily wheel running activity level. Physiol Genomics 19(3):270–276. https://doi.org/10.1152/physiolgenomics.00125.2004

Lovering RM, Roche JA, Goodall MH, Clark BB, McMillan A (2011) An in vivo rodent model of contraction-induced injury and non-invasive monitoring of recovery. J Vis Exp (51). https://doi.org/10.3791/2782

Lowe DA, Alway SE (2002) Animal models for inducing muscle hypertrophy: are they relevant for clinical applications in humans? J Orthop Sports Phys Ther 32(2):36–43. https://doi.org/10.2519/jospt.2002.32.2.36

Loy K, Bareyre FM (2019) Rehabilitation following spinal cord injury: how animal models can help our understanding of exercise-induced neuroplasticity. Neural Regen Res 14(3):405–412. https://doi.org/10.4103/1673-5374.245951

Lund J, Hafstad AD, Boardman NT, Rossvoll L, Rolim NP, Ahmed MS, Florholmen G, Attramadal H, Wisloff U, Larsen TS, Aasum E (2015) Exercise training promotes cardioprotection through oxygen-sparing action in high fat-fed mice. Am J Physiol Heart Circ Physiol 308(8):H823–H829. https://doi.org/10.1152/ajpheart.00734.2014

Manzanares G, Brito-da-Silva G, Gandra PG (2018) Voluntary wheel running: patterns and physiological effects in mice. Braz J Med Biol Res 52(1):e7830. https://doi.org/10.1590/1414-431X20187830

Matsuo S, Suzuki S, Iwata M, Hatano G, Nosaka K (2015) Changes in force and stiffness after static stretching of eccentrically-damaged hamstrings. Eur J Appl Physiol 115(5):981–991. https://doi.org/10.1007/s00421-014-3079-3

McDermott MM (2018) Exercise rehabilitation for peripheral artery disease: a review. J Cardiopulm Rehabil Prev 38(2):63–69. https://doi.org/10.1097/HCR.0000000000000343

McGee-Lawrence ME, Wenger KH, Misra S, Davis CL, Pollock NK, Elsalanty M, Ding K, Isales CM, Hamrick MW, Wosiski-Kuhn M, Arounleut P, Mattson MP, Cutler RG, Yu JC, Stranahan AM (2017) Whole-body vibration mimics the metabolic effects of exercise in male leptin receptor-deficient mice. Endocrinology 158(5):1160–1171. https://doi.org/10.1210/en.2016-1250

McKeehen JN, Novotny SA, Baltgalvis KA, Call JA, Nuckley DJ, Lowe DA (2013) Adaptations of mouse skeletal muscle to low-intensity vibration training. Med Sci Sports Exerc 45(6): 1051–1059. https://doi.org/10.1249/MSS.0b013e3182811947

Mintz EL, Passipieri JA, Lovell DY, Christ GJ (2016) Applications of in vivo functional testing of the rat tibialis anterior for evaluating tissue engineered skeletal muscle repair. J Vis Exp (116). https://doi.org/10.3791/54487

Mobley CB, Holland AM, Kephart WC, Mumford PW, Lowery RP, Kavazis AN, Wilson JM, Roberts MD (2018) Progressive resistance-loaded voluntary wheel running increases hypertrophy and differentially affects muscle protein synthesis, ribosome biogenesis, and proteolytic markers in rat muscle. J Anim Physiol Anim Nutr (Berl) 102(1):317–329. https://doi.org/10.1111/jpn.12691

Murach KA, McCarthy JJ, Peterson CA, Dungan CM (2020) Making mice mighty: recent advances in translational models of load-induced muscle hypertrophy. J Appl Physiol 129(3):516–521. https://doi.org/10.1152/japplphysiol.00319.2020

Murfee WL, Hammett LA, Evans C, Xie L, Squire M, Rubin C, Judex S, Skalak TC (2005) High-frequency, low-magnitude vibrations suppress the number of blood vessels per muscle fiber in mouse soleus muscle. J Appl Physiol (1985) 98(6):2376–2380. https://doi.org/10.1152/japplphysiol.01135.2004

Navarro A, Gomez C, Lopez-Cepero JM, Boveris A (2004) Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. Am J Physiol Regul Integr Comp Physiol 286(3):R505–R511. https://doi.org/10.1152/ajpregu.00208.2003

Necking LE, Lundstrom R, Lundborg G, Thornell LE, Friden J (1996) Skeletal muscle changes after short term vibration. Scand J Plast Reconstr Surg Hand Surg 30(2):99–103. https://doi.org/10.3109/02844319609056390

Noble EE, Hsu TM, Liang J, Kanoski SE (2019) Early-life sugar consumption has long-term negative effects on memory function in male rats. Nutr Neurosci 22(4):273–283. https://doi.org/10.1080/1028415X.2017.1378851

Novotny SA, Mehta H, Lowe DA, Nuckley DJ (2013) Vibration platform for mice to deliver precise, low intensity mechanical signals to the musculoskeleton. J Musculoskelet Neuronal Interact 13(4):412–417

Novotny SA, Mader TL, Greising AG, Lin AS, Guldberg RE, Warren GL, Lowe DA (2014) Low intensity, high frequency vibration training to improve musculoskeletal function in a mouse model of Duchenne muscular dystrophy. PLoS One 9(8):e104339. https://doi.org/10.1371/journal.pone.0104339

Okabe N, Himi N, Maruyama-Nakamura E, Hayashi N, Narita K, Miyamoto O (2017) Rehabilitative skilled forelimb training enhances axonal remodeling in the corticospinal pathway but not the brainstem-spinal pathways after photothrombotic stroke in the primary motor cortex. PLoS One 12(11):e0187413. https://doi.org/10.1371/journal.pone.0187413

Park HS, Martin BJ (1993) Contribution of the tonic vibration reflex to muscle stress and muscle fatigue. Scand J Work Environ Health 19(1):35–42. https://doi.org/10.5271/sjweh.1506

Peng L, Zhu M, Yang Y, Lu F, Liu X, Guo Q, Zhong T (2021) Repeated neonatal isoflurane exposure is associated with higher susceptibility to chronic variable stress-induced behavioural and neuro-inflammatory alterations. Neuroscience 465:166–176. https://doi.org/10.1016/j.neuroscience.2021.04.024

Perez-Terzic C, Childers MK (2014) Regenerative rehabilitation: a new future? Am J Phys Med Rehabil 93(11 Suppl 3):S73–S78. https://doi.org/10.1097/PHM.0000000000000211

Picoli CC, Romero P, Gilio GR, Guariglia DA, Tofolo LP, de Moraes SMF, Machado FA, Peres SB (2018) Peak velocity as an alternative method for training prescription in mice. Front Physiol 9: 42. https://doi.org/10.3389/fphys.2018.00042

Poole DC, Copp SW, Colburn TD, Craig JC, Allen DL, Sturek M, O'Leary DS, Zucker IH, Musch TI (2020) Guidelines for animal exercise and training protocols for cardiovascular studies. Am J Physiol Heart Circ Physiol 318(5):H1100–H1138. https://doi.org/10.1152/ajpheart.00697.2019

Portier H, Benaitreau D, Pallu S (2020) Does physical exercise always improve bone quality in rats? Life (Basel) 10(10). https://doi.org/10.3390/life10100217

Ren Z, Lan Q, Chen Y, Chan YWJ, Mahady GB, Lee SM (2020) Low-magnitude high-frequency vibration decreases body weight gain and increases muscle strength by enhancing the p38 and AMPK pathways in db/db mice. Diabetes Metab Syndr Obes 13:979–989. https://doi.org/10.2147/DMSO.S228674

Rhodes JS, Gammie SC, Garland T Jr (2005) Neurobiology of mice selected for high voluntary wheel-running activity. Integr Comp Biol 45(3):438–455. https://doi.org/10.1093/icb/45.3.438

Ritzmann R, Stark C, Krause A (2018) Vibration therapy in patients with cerebral palsy: a systematic review. Neuropsychiatr Dis Treat 14:1607–1625. https://doi.org/10.2147/NDT.S152543

Robles JC, Heaps CL (2015) Adaptations of the endothelin system after exercise training in a porcine model of ischemic heart disease. Microcirculation 22(1):68–78. https://doi.org/10.1111/micc.12174

Rodnick KJ, Reaven GM, Haskell WL, Sims CR, Mondon CE (1989) Variations in running activity and enzymatic adaptations in voluntary running rats. J Appl Physiol (1985) 66(3):1250–1257. https://doi.org/10.1152/jappl.1989.66.3.1250

Rubin C, Turner AS, Bain S, Mallinckrodt C, McLeod K (2001) Anabolism. Low mechanical signals strengthen long bones. Nature 412(6847):603–604. https://doi.org/10.1038/35088122

Seldeen KL, Lasky G, Leiker MM, Pang M, Personius KE, Troen BR (2018) High intensity interval training improves physical performance and frailty in aged mice. J Gerontol A Biol Sci Med Sci 73(4):429–437. https://doi.org/10.1093/gerona/glx120

Seldeen KL, Redae YZ, Thiyagarajan R, Berman RN, Leiker MM, Troen BR (2019) Short session high intensity interval training and treadmill assessment in aged mice. J Vis Exp (144). https://doi.org/10.3791/59138

Seo DY, Lee SR, Kim N, Ko KS, Rhee BD, Han J (2014) Humanized animal exercise model for clinical implication. Pflugers Arch 466(9):1673–1687. https://doi.org/10.1007/s00424-014-1496-0

Soffe Z, Radley-Crabb HG, McMahon C, Grounds MD, Shavlakadze T (2016) Effects of loaded voluntary wheel exercise on performance and muscle hypertrophy in young and old male C57Bl/6J mice. Scand J Med Sci Sports 26(2):172–188. https://doi.org/10.1111/sms.12416

Souza MV, Leite RD, Souza Lino AD, Marqueti Rde C, Bernardes CF, Araujo HS, Bouskela E, Shiguemoto GE, Andrade Perez SE, Kraemer-Aguiar LG (2014) Resistance training improves body composition and increases matrix metalloproteinase 2 activity in biceps and gastrocnemius muscles of diet-induced obese rats. Clinics (Sao Paulo) 69(4):265–270. https://doi.org/10.6061/clinics/2014(04)08

Spaulding HR, Selsby JT (2018) Is exercise the right medicine for dystrophic muscle? Med Sci Sports Exerc 50(9):1723–1732. https://doi.org/10.1249/MSS.0000000000001639

Strickland JC, Smith MA (2016) Animal models of resistance exercise and their application to neuroscience research. J Neurosci Methods 273:191–200. https://doi.org/10.1016/j.jneumeth.2016.08.003

Svensson M, Rosvall P, Boza-Serrano A, Andersson E, Lexell J, Deierborg T (2016) Forced treadmill exercise can induce stress and increase neuronal damage in a mouse model of global cerebral ischemia. Neurobiol Stress 5:8–18. https://doi.org/10.1016/j.ynstr.2016.09.002

Swallow JG, Rhodes JS, Garland T Jr (2005) Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. Integr Comp Biol 45(3):426–437. https://doi.org/10.1093/icb/45.3.426

Tamaki T, Uchiyama S, Nakano S (1992) A weight-lifting exercise model for inducing hypertrophy in the hindlimb muscles of rats. Med Sci Sports Exerc 24(8):881–886

Tanaka A, Yoshimura Y, Aoki K, Okamoto M, Kito M, Suzuki S, Takazawa A, Ishida T, Kato H (2017) Prediction of muscle strength and postoperative function after knee flexor muscle resection for soft tissue sarcoma of the lower limbs. Orthop Traumatol Surg Res 103(7): 1081–1085. https://doi.org/10.1016/j.otsr.2017.07.005

Thompson LV (2002) Skeletal muscle adaptations with age, inactivity, and therapeutic exercise. J Orthop Sports Phys Ther 32(2):44–57. https://doi.org/10.2519/jospt.2002.32.2.44

Vanleene M, Shefelbine SJ (2013) Therapeutic impact of low amplitude high frequency whole body vibrations on the osteogenesis imperfecta mouse bone. Bone 53(2):507–514. https://doi.org/10.1016/j.bone.2013.01.023

Walters TJ, Sweeney HL, Farrar RP (1991) Influence of electrical stimulation on a fast-twitch muscle in aging rats. J Appl Physiol (1985) 71(5):1921–1928. https://doi.org/10.1152/jappl.1991.71.5.1921

Wang VM, Banack TM, Tsai CW, Flatow EL, Jepsen KJ (2006) Variability in tendon and knee joint biomechanics among inbred mouse strains. J Orthop Res 24(6):1200–1207. https://doi.org/10.1002/jor.20167

Wang N, Liu Y, Ma Y, Wen D (2017) High-intensity interval versus moderate-intensity continuous training: superior metabolic benefits in diet-induced obesity mice. Life Sci 191:122–131. https://doi.org/10.1016/j.lfs.2017.08.023

Wang R, Tian H, Guo D, Tian Q, Yao T, Kong X (2020) Impacts of exercise intervention on various diseases in rats. J Sport Health Sci 9(3):211–227. https://doi.org/10.1016/j.jshs.2019.09.008

Warren GL, Ingalls CP, Armstrong RB (1998) A stimulating nerve cuff for chronic in vivo measurements of torque produced about the ankle in the mouse. J Appl Physiol 84(6): 2171–2176

Warren GL, Lowe DA, Armstrong RB (1999) Measurement tools used in the study of eccentric contraction-induced injury. Sports Med 27(1):43–59. https://doi.org/10.2165/00007256-199927010-00004

Warren GL, Moran AL, Hogan HA, Lin AS, Guldberg RE, Lowe DA (2007) Voluntary run training but not estradiol deficiency alters the tibial bone-soleus muscle functional relationship in mice. Am J Physiol Regul Integr Comp Physiol 293(5):R2015–R2026

Weill F, Bourgoin A, Eisenscher A, Aucant D, Tuetey JB, Duval JM (1976) [Ultrasonic aspect of abdominal fluid collections. Ascites, abscess, hematoma]. J Radiol Electrol Med Nucl 57 (8–9): 618

Weinheimer-Haus EM, Judex S, Ennis WJ, Koh TJ (2014) Low-intensity vibration improves angiogenesis and wound healing in diabetic mice. PLoS One 9(3):e91355. https://doi.org/10.1371/journal.pone.0091355

White Z, Terrill J, White RB, McMahon C, Sheard P, Grounds MD, Shavlakadze T (2016) Voluntary resistance wheel exercise from mid-life prevents sarcopenia and increases markers of mitochondrial function and autophagy in muscles of old male and female C57BL/6J mice. Skelet Muscle 6(1):45. https://doi.org/10.1186/s13395-016-0117-3

Wisloff U, Loennechen JP, Currie S, Smith GL, Ellingsen O (2002) Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca2+ sensitivity and SERCA-2 in rat after myocardial infarction. Cardiovasc Res 54(1):162–174. https://doi.org/10.1016/s0008-6363(01)00565-x

Wolf A, Bauer B, Abner EL, Ashkenazy-Frolinger T, Hartz AM (2016) A comprehensive behavioral test battery to assess learning and memory in 129S6/Tg2576 mice. PLoS One 11(1): e0147733. https://doi.org/10.1371/journal.pone.0147733

Xie L, Jacobson JM, Choi ES, Busa B, Donahue LR, Miller LM, Rubin CT, Judex S (2006) Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. Bone 39(5):1059–1066. https://doi.org/10.1016/j.bone.2006.05.012

Xie L, Rubin C, Judex S (2008) Enhancement of the adolescent murine musculoskeletal system using low-level mechanical vibrations. J Appl Physiol (1985) 104(4):1056–1062. https://doi.org/10.1152/japplphysiol.00764.2007

Xin DL, Hadrevi J, Elliott ME, Amin M, Harris MY, Barr-Gillespie AE, Barbe MF (2017) Effectiveness of conservative interventions for sickness and pain behaviors induced by a high repetition high force upper extremity task. BMC Neurosci 18(1):36. https://doi.org/10.1186/s12868-017-0354-3

Zaidell LN, Mileva KN, Sumners DP, Bowtell JL (2013) Experimental evidence of the tonic vibration reflex during whole-body vibration of the loaded and unloaded leg. PLoS One 8(12):e85247. https://doi.org/10.1371/journal.pone.0085247

Zemmar A, Kast B, Lussi K, Luft AR, Schwab ME (2015) Acquisition of a high-precision skilled forelimb reaching task in rats. J Vis Exp (100):e53010. https://doi.org/10.3791/53010

Zhu WG, Hibbert JE, Lin KH, Steinert ND, Lemens JL, Jorgenson KW, Newman SM, Lamming DW, Hornberger TA (2021) Weight pulling: a novel mouse model of human progressive resistance exercise. Cells 10(9). https://doi.org/10.3390/cells10092459

Chapter 3
Regenerative Rehabilitation for Nonlethal Muscular Dystrophies

Joseph A. Roche

Abstract The muscular dystrophies are a group of inherited conditions that are associated with progressive weakness and wasting of muscle tissue. Muscular dystrophies are placed under a larger umbrella of disorders known as inherited neuromuscular diseases, but differ from other neuromuscular diseases in one key aspect—they are caused by genetic, structural, and functional changes primarily within muscle cells themselves and not in the motor and sensory nerves that innervate muscle. This difference is significant because it has a bearing on the interventions aimed at slowing down, stopping, or reversing muscle weakness and wasting. For example, muscle cell fragility seems to be a conserved feature across many muscular dystrophies irrespective of the genetic basis—this makes it important to adjust the type, intensity, and duration of muscle contractions, during exercise and functional activities in order to protect muscle from damage caused by its own contractions. On the plus side, skeletal muscle cells can regenerate from damage and death through muscle-generating stem cells (known as muscle satellite cells) present in muscle tissue, whereas nerve cells have limited regenerative potential. Therefore, regenerative and rehabilitative interventions aimed at regrowing muscle fibers in persons with muscular dystrophies holds promise. Such induced regeneration is likely to translate to improved function because the motor and sensory nerve connections to muscle and the connections of those nerves to motor control areas in the brain are not directly affected in muscular dystrophies.

Keywords Muscular dystrophies · Functional muscle mass · Regenerative rehabilitation · Regenerative muscle biology · Muscle protection

J. A. Roche (✉)
Physical Therapy Program, Department of Health Care Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, MI, USA
e-mail: joseph.roche@wayne.edu

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_3

3.1 Epidemiological Data on the Muscular Dystrophies

For many clinicians, scientists, and lay people alike, the term "muscular dystrophy" conjures the image of a biologically male child who walks up his legs with his hands due to weak antigravity muscles (Gower's sign); has difficulty walking (pelvic instability and risk of falls); has muscles that appear large but are actually weak (pseudohypertrophy); and most unfortunately, does not live beyond his twenties (Emery 2002). In reality, this phenotype is characteristic of only one type of muscular dystrophy known as Duchenne muscular dystrophy (DMD) (Emery 2002). DMD is caused by the absence of functional dystrophin, a protein that is encoded by the human dystrophin gene, which is also abbreviated DMD like the disease (Emery 2002; National Center for Biotechnology Information 2021). Since the dystrophin gene is located on the X chromosome and since inheritance of DMD is recessive, biological males born to mothers who carry DMD-related mutations (carriers) are at risk for getting DMD (Emery 2002). This is because biological males inherit the only X chromosome they have from their mothers. The prevalence of DMD is estimated at ~1:3500 live male births (National Organization for Rare Disorders (NORD) 2021a, b). As of 2019, the estimated population of the United States (US) was 328,239,523 with 161,493,845 (49.2%) being male (United States Census Bureau 2021), thus putting the prevalence of DMD at ~46,000 in the US (National Organization for Rare Disorders (NORD) 2021a, b). The National Organization for Rare Disorders (NORD) considers a disease rare if <200,000 people in the US have that disease, which makes DMD a rare disease (National Organization for Rare Disorders (NORD) 2021a, b). However, many other forms of muscular dystrophy exist (Mercuri et al. 2019). Several forms of muscular dystrophy are linked to autosomes rather than the X and Y sex chromosomes, thereby making it likely for biological males and females to get those forms of muscular dystrophy (Mercuri et al. 2019; Kanagawa and Toda 2006). When all different forms of muscular dystrophy are taken into account, ~250,000 individuals in the US population have some form of muscular dystrophy (National Organization for Rare Disorders (NORD) 2021a, b). Therefore, the muscular dystrophies, which will be abbreviated henceforth as MDs, are not rare. Furthermore, not all MDs are lifespan limiting as DMD. Therefore, addressing the changing needs of individuals with MDs through lifespan should be a priority in biomedical research and healthcare delivery.

3.2 Defining the Main Problem in MDs in Order to Find Solutions

One of the key tenets of problem solving is to first precisely define what the problem is (Hewitt-Taylor 2012). In terms of defining the key problem in MDs, it would require an understanding of the pathophysiology of MDs. However, as mentioned earlier, MDs are a collection of different disease conditions, which means, each form

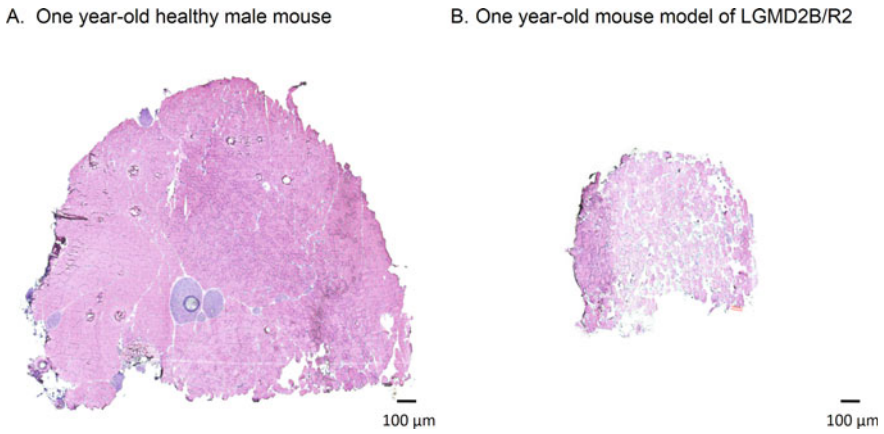

Fig. 3.1 Muscle wasting in a mouse model of limb girdle muscular dystrophy type 2B or R2 (LGMD2B/R2). (**a**) Cross section of a psoas major muscle harvested from a 1-year-old wild-type male mouse. (**b**) Cross section of the psoas major muscle harvested from a 1-year-old B6.A-*Dysf*^{prmd}/GeneJ (a.k.a. BLAJ) male mouse. The BLAJ mouse is a model of human LGMD2B/R2, which is a nonlethal form of MD (Harris et al. 2016; Nagy et al. 2017; Begam et al. 2020). Muscle weakness and wasting usually begin after 15 years of age. The disease progresses rapidly after onset. Individuals with LGMD2B/R2 typically need a wheelchair for mobility by the age of 40 years. LGMD2B/R2 is autosomal recessive in inheritance, and therefore affects both males and females. In this figure, the qualitative data indicate that, the psoas major muscle of a 1-year-old BLAJ mouse is several-fold smaller than the same muscle in a healthy mouse due to dystrophic muscle wasting. Source of images: unpublished data from author's laboratory

of MD has its own unique set of pathophysiological changes. There is indeed widespread heterogeneity in terms of the etiological factors and pathophysiological changes associated with various MDs (Emery 2002; Lovering et al. 2005; Sahenk and Mendell 2011; Wicklund 2013). However, there are certain commonalities among MDs that give MDs their unique identity in comparison to other disorders that are placed under the common umbrella term called inherited neuromuscular diseases (Dowling et al. 2018; Wallace and McNally 2009; Burr and Molkentin 2015).

By definition, MDs are inherited diseases, which cause progressive muscle weakness and wasting (Eunice Kennedy Shriver National Institute of Child Health and Human Development 2021). The term inherited means that, a key etiological factor in MDs is altered genomic information (a genetic defect) that is passed from parent to offspring. The term progressive means that, the signs and symptoms of MDs will become worse over time. And finally, the term muscle weakness and wasting describes the two main symptoms seen in most MDs, which is reduced contractile force generated by muscles and a loss in both numbers and cross sectional areas of muscle fibers. It must be noted that dystrophic muscle degeneration and wasting is not the same as disuse muscle atrophy (Fig. 3.1). The definition of MDs is not trivial because it precisely presents the problems that need to be solved if we wish to cure MDs. In theory, fixing the specific genetic defect associated with the

particular type of MD should stop the pathophysiological changes downstream to the genetic defect, and should therefore stop progressive muscle weakness and wasting. Unfortunately, by the time most individuals with MD receive a diagnosis (typically when walking is affected), the disease has already caused muscle weakness and muscle wasting (Genetic and Rare Diseases Information Center (GARD) (2021)). It is worth noting that in congenital forms of MD (congenital MDs, CMDs), symptoms manifest at birth, significant neurological involvement and delayed motor milestones might be observed, and sometimes symptoms improvement in symptoms may be seen over time rather than progressive worsening—these features make CMDs atypical MDs (Reed 2009). What further complicates things in MDs is the fact that, the primary genetic bases for certain MDs have not yet been identified, making it impossible to zero in on a genetic target that can be fixed. Nonetheless, through aggressive scientific efforts, promising genetic therapies for certain MDs have successfully passed through the drug discovery and testing process and are close to approval for clinical use in humans (Duan 2018). With the assumption that some of these therapies can successfully fix the primary genetic defect associated with a particular MD, the logical question to ask is—will fixing the genetic defect automatically restore muscle strength and mass that is lost? This question is difficulty to answer since we cannot be certain regarding whether or not muscle mass and strength will be restored once the genetic defect associated with a particular MD has been fixed. What we can assume is that, if an MD is diagnosed early, prior to the onset of muscle weakness and wasting, there will be more muscle fibers available to genetically modify, thus increasing the probability of better functional outcomes from genetic therapies (Fig. 3.2). Conversely, it would be logical to argue that severe muscle loss creates a conundrum in the sense that, muscle fibers that do not exist cannot be genetically transformed (Begam et al. 2020). For these reasons, it is imperative that promising regenerative interventions aimed at regrowing muscle tissue in individuals with MDs must be aggressively explored and studied along with promising genetic therapies. Furthermore, it is amply clear that regenerative interventions are more successful when combined with rehabilitative interventions like physical exercise (Dziki et al. 2016; Ambrosio and Russell 2010). Therefore, a synergistic approach involving genetic, regenerative, and rehabilitative therapies is likely to produce the best outcomes for individuals with MDs, and help arrest and reverse muscle weakness and wasting (Ambrosio and Russell 2010).

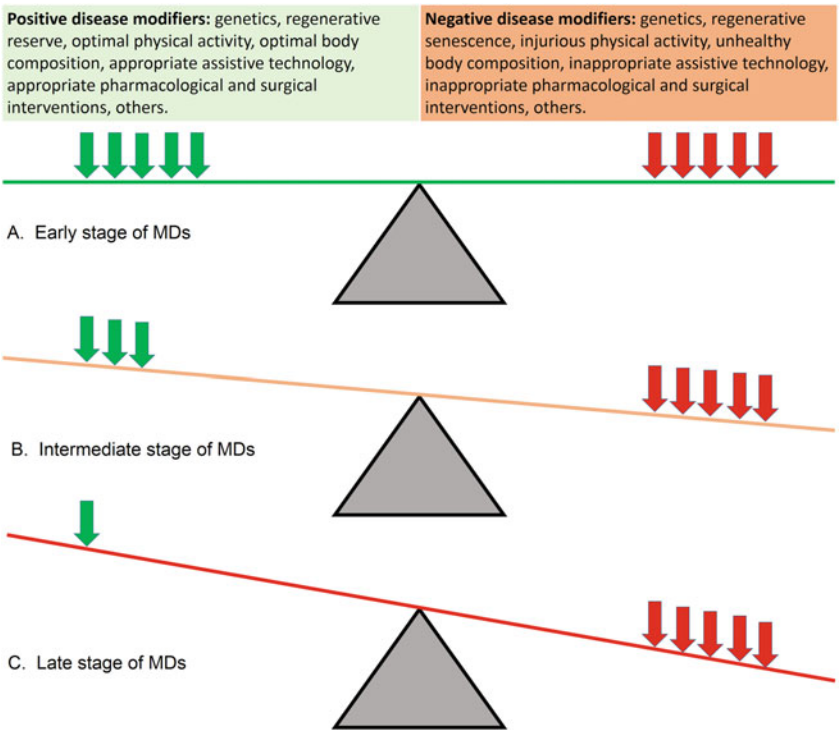

Fig. 3.2 An illustration of how and why progressive muscle wasting occurs in MDs. (**a**–**c**) In the early stages of most typical MDs, muscle fiber regeneration likely matches muscle fiber degeneration due to which symptoms seem mild, static, or even absent (Wicklund and Kissel 2014). Over time, various factors contribute to regeneration losing out to degeneration, which manifests as progressive muscle weakness and wasting (Wallace and McNally 2009; Krag et al. 2011; Tichy et al. 2017)

3.3 A Strengths, Weaknesses, Opportunities, and Threats (SWOT) Analysis for the Regenerative Rehabilitation Research Community to Solve the Muscle Mass Conundrum in MDs

When an organization embarks on a new project, the organization should assess what it can and cannot do, in order to identify the resources it needs from within and outside, to ultimately solve the problem at hand. In the previous section, the main problem in MDs was identified based on the definition of MDs—i.e. inherited genetic defects leading to progressive muscle weakness and wasting. Additionally, the conundrum posed by muscle mass that might be permanently lost was also presented. Then, the possible solution to solving the muscle mass conundrum was presented, in the form of a synergistic approach that leverages the advances that have been made so far in the areas of genetic, regenerative, and rehabilitative therapies. If

Table 3.1 SWOT matrix for the regenerative rehabilitation research community (R3C) to address the challenge of muscle loss in MDs

Strengths	Weaknesses
1. Knowledge of myogenesis 2. Methods to culture myogenic cells in 2D and 3D	1. Cardiorespiratory complications in certain MDs 2. Knowledge gaps regarding genetics of certain MDs
Opportunities	**Threats**
1. Synergize and leverage combined strengths of MD and VML research 2. Capitalize on gene therapies to correct MD-associated genetic defects in myogenic cells	1. Difficulty in getting funding due to challenges associated with studying molecular mechanisms in regenerative rehabilitation strategies 2. Unknowns
Aim Statement based on SWOT analysis: To develop safe, effective, and practical regenerative rehabilitation strategies, to regrow muscle fibers in muscles that are most essential for independence in basic activities of daily living, in individuals with nonlethal muscular dystrophies	

In this chapter, the author characterized the R3C as a unified organization whose goal is to reverse muscle loss in MDs. A SWOT analysis was performed to identify strengths, weaknesses, opportunities, and threats with regard to the R3C's mission of reversing muscle loss in MDs. The main factors identified by the author, based on clinical practice, research literature, and the author's personal experiences as a physical therapist, regenerative muscle biologist, and delegate in the International Consortium for Regenerative Rehabilitation (ICRR 2021)

the regenerative rehabilitation community was a single organization that sought to find the most effective way to regrow muscle mass in individuals with MDs, then a SWOT analysis would help identify the resources we already have within our community (strengths), the resources that we lack (weaknesses), the unique opportunities that make success a strong possibility (opportunities), and the barriers that are likely to thwart success (threats) (Giusti et al. 2020). In the following sections, each component of the SWOT matrix will be discussed (MindTools 2021) (Table 3.1).

3.3.1 Strengths: Resources Available Within the Regenerative Rehabilitation Community That Will Likely Help Solve the Muscle Mass Conundrum in MDs

MDs affect skeletal muscle and sometimes cardiac muscle (Emery 2002; Lovering et al. 2005; Sahenk and Mendell 2011; Wicklund 2013). Cardiac muscle poses challenges that are far more complex than those seen in skeletal muscle, and will be discussed under the weaknesses and threats portions of the SWOT analysis. Overall, the focus of this chapter will be on regrowing skeletal muscle that is lost in MDs.

Myogenesis is the process through which new muscle fibers are generated or regenerated, and myogenic capacity of cells refers to the ability of cells to promote

myogenesis (Charge and Rudnicki 2004). In MDs, there might be repeated rounds of muscle fiber degeneration and regeneration, which depletes regenerative potential sooner than would occur through normal aging (Krag et al. 2011; Morgan and Partridge 2003). Therefore, restoring lost regenerative potential in MDs and stimulating muscle growth through exercise/rehabilitation, would likely promote myogenesis and increase muscle mass (Distefano et al. 2013; Sakellariou et al. 2015). One of the greatest strengths of the regenerative rehabilitation research community, which can be leveraged, is the knowledge that has been generated on myogenesis in skeletal muscle tissue (Brack and Rando 2012; Dumont et al. 2015). Through pioneering efforts from the regenerative rehabilitation research community (henceforth abbreviated "the R3C"), which includes all scholars who are interested in combining regenerative and rehabilitative strategies to optimize structure and function in humans, it is now established that skeletal muscle fibers possess excellent regenerative capacity despite being post-mitotic (post-mitotic cells cannot or do not divide and form new daughter cells) (Collins et al. 2005; Grounds and Partridge 1983; Partridge et al. 1978). The ability of muscle fibers to regenerate is attributed to myogenic progenitors, known as muscle stem cells or muscle satellite cells (MuSCs) (Mauro 1961; Lipton and Schultz 1979). In the regenerative muscle biology literature, MuSCs are sometimes simply called satellite cells (SCs) (Relaix and Zammit 2012; Rathbone et al. 2003). However, since the term satellite cells may also refer to certain glial cells in the nervous system (Hanani 2010), and since the stem cells in muscle tissue can refer to progenitor cells other than muscle satellite cells (e.g., non-myogenic progenitor cells present in the vascular, neural, and connective tissue components of muscle tissue) (Qu-Petersen et al. 2002), in this chapter, myogenic progenitor cells in muscle tissue will be referred to as muscle satellite cells and will be abbreviated as MuSCs (Gilbert et al. 2010; Quarta et al. 2016).

In addition to the knowledge that has been generated on the regenerative capacity of skeletal muscle and the various molecular signals involved in myogenesis, highly reproducible research methods have been developed to isolate MuSCs from animal and human muscle tissue (Skuk and Tremblay 2019; Sherwood et al. 2004; Jackson et al. 1999). Not only have methods been developed to isolate MuSCs from muscle tissue, but clever methods based on the titration of serum levels in culture media have been developed to maintain MuSCs in a state of repeated division (high serum) or to induce them to fuse (low serum) to form multinucleated muscle fiber-like structures called myotubes (Sherwood et al. 2004). The daughter cells generated from in vitro division of MuSCs, which may be referred to as myoblasts, can be placed in cryogenic storage and revived later as needed. The myotubes can be electrically and/or chemically stimulated to generate action potentials and contract similar to how mature muscle fibers function in vivo (Evers-van Gogh et al. 2015).

Based on the methods discussed above to isolate MuSCs and generate myotubes, cutting-edge techniques have been developed to generate three-dimensional (3D) muscle tissue mimetics (a muscle tissue mimetic mimics muscle tissue) (Juhas et al. 2014; Machingal et al. 2011; VanDusen et al. 2014). These engineered 3D muscle tissue mimetics might resemble membranous bands, which have several thousands of small caliber muscle fibers arranged in multiple layers within them

(Juhas et al. 2014). These engineered muscle tissue bands are generated in bioreactors instead of Petri dishes, and have structure and function that more closely resembles actual muscle tissue when compared to myotubes in a dish (Juhas et al. 2014; Machingal et al. 2011). In addition to possessing structure and function similar to native muscle tissue, some types of engineered muscle tissue can regenerate following injury in the bioreactor, and can promote donor-cell-derived myogenesis when grafted into a live animal (Juhas et al. 2014). The ability to generate engineered muscle tissue that can promote donor-cell-derived muscle regeneration in a living host, is the greatest strength possessed by the R3C, which can help reverse muscle loss in MDs.

3.3.2 Weaknesses: Resources Required from Outside of the Regenerative Rehabilitation Community to Solve the Muscle Mass Conundrum in MDs

Research requires funding. While funding for regenerative medicine and stem cell biology research is robust, specific funding for regenerative rehabilitation is scarce. The Alliance for Regenerative Rehabilitation Research and Training (AR^3T), which is supported by the National Institutes of Health (NIH) through a Resource-Related Research Multi-Component Projects and Centers (P2C) grant, and the Department of Defense through The Joint Program Committee-8/Clinical and Rehabilitative Medicine Research Program (JPC-8/CRMRP), have been spearheading the efforts to build the R3C and help investigators obtain more funding specifically for regenerative rehabilitation research (National Institutes of Health 2021a; Willett et al. 2020; Department of Defense 2021). NIH Institutional National Research Service Award grants aimed at training the next generation of R3C scientists (e.g., T32 grants) and special requests for proposals (RFPs) for regenerative rehabilitation research will likely expedite the discovery, testing, and clinical translation of regenerative rehabilitation therapies that can reverse muscle loss in MDs. Fortunately, the 2016 NIH Research Plan on Rehabilitation specifically mentions increasing the use of regenerative rehabilitation techniques and therapies to optimize function in persons with disabilities (Eunice Kennedy Shriver National Institute of Child Health and Human Development and the NIH Medical Rehabilitation Coordinating Committee 2016).

In addition to the lack of research funding earmarked specifically for regenerative rehabilitation, one of the major weaknesses with reference to solving the muscle mass conundrum in MDs is the limited knowledge that is currently available on MDs. Given that the link between DMD and the dystrophin gene was identified only in the late 1980s (Hoffman et al. 1987), it is remarkable that in just over 30 years, the genetic bases for over 30 different MDs have been identified (Mercuri et al. 2019; Kanagawa and Toda 2006). Nonetheless, there are still knowledge gaps in terms of the genetic bases for certain MDs, which is further complicated by the fact that a particular form of MD might be caused by several different types of alterations to the

gene that is implicated. These knowledge gaps make it difficult to design precise genetic therapies to address the primary genetic defects and protect existing muscle tissue from progressive weakness and wasting. Finally, despite the numerous experimental strategies that have been developed to deliver gene therapies systemically in animal models of MDs, there still remain concerns about whether or not the therapies would give sustained benefit throughout the lifespan in humans. For example, if a gene therapy is only able to affect mature muscle fibers and not MuSCs, there is the possibility that the effect of the therapy might be lost with damage and regeneration of those muscle fibers over many years (Crudele and Chamberlain 2019). There is also the concern that the immune system might negate the effects of viral vectors if antibodies to those vectors exist already in a person's circulation (Crudele and Chamberlain 2019). These knowledge and technical limitations make it necessary for the R3C to rely on other research communities to address the genetic factors that cause MDs. More importantly, the limitations make it necessary to further refine the problem that we are trying to address in MDs.

As discussed above, due to the limitations that exist in terms of fixing the genetic problems associated with MDs, it is necessary that the problem we aim to solve in MDs is further refined. Refining the problem would have to start with acknowledging that without addressing the genetic problem that causes a particular MD, it would not be possible to protect all skeletal muscles in the body. By extension, it would then not be possible to develop regenerative rehabilitation strategies to regrow every muscle that is affected by a particular MD. Therefore, when attempting to regrow muscles that are lost, the R3C will have to target muscles and/or muscle groups that are likely to give the best possible results in terms of increasing independence in mobility and activities of daily living. For example: targeting the biceps and triceps brachii is likely to improve upper extremity function and independence in feeding, dressing, personal hygiene, and transfers (Radomski and Latham 2014; Peters et al. 2018); and, targeting large antigravity muscles in the lower extremity is likely to improve independence and safety during standing transfers (Ploutz-Snyder et al. 2002; Cuesta-Vargas et al. 2013). Refining the problem this way preemptively addresses the weaknesses identified in this SWOT analysis, and increases the probability of success.

As part of refining the muscle mass problem in MDs to set an achievable goal, it is important to acknowledge the involvement of the heart and lungs in certain MDs (Verhaert et al. 2011; Birnkrant et al. 2018; Bushby et al. 2007; Wicklund and Kissel 2014). Early death in DMD is mainly attributed to respiratory and cardiac complications, due to which, preservation of cardiorespiratory function is a top priority (Buyse et al. 2015). The heart functions as a pump to move blood around the body and through the lungs. The heart's pumping action is achieved through synchronized action potentials and contractions of cardiac muscle cells known as cardiomyocytes, which populate the muscular layer of the heart known as the myocardium. The myocardium thus generates the contractile force needed to push blood out of the heart's chambers. Since cardiomyocytes share certain structural, functional, and biochemical properties with skeletal muscle fibers, the myocardium is susceptible to progressive degeneration and weakness in certain MDs. This is further

complicated by the fact that, the specialized cardiomyocytes present in the areas of the heart that generate and conduct signals for cardiac rhythm might also be affected in certain MDs. Therefore, severe cardiac involvement in certain MDs can reduce lifespan. The lungs do not have a muscular layer equivalent to the myocardium, which can pull in and push out air during respiration. The filling and emptying of the lungs is achieved through contraction and relaxation of skeletal muscles, such as the diaphragm, the abdominal muscles, and the intercostal muscles, which increase and decrease the volume of the chest. Progressive degeneration of the skeletal muscles needed for respiration can thus cause respiratory complications and reduce lifespan in certain MDs. Completely solving the cardiac and respiratory complications in lifespan-limiting lethal MDs will require highly effective genetic therapies, which the R3C or other research communities do not have yet in their toolkit. Therefore, as part of planning, an achievable goal for the R3C could be written as the following Aim Statement:

To develop safe, effective, and practical regenerative rehabilitation strategies to regrow muscle fibers in muscles that are most essential for independence in basic activities of daily living, in individuals with nonlethal muscular dystrophies (Table 3.1).

The process that has been followed in arriving at the specific goal defined above, is consistent with the planning aspect of the Plan-Do-Study-Act (PDSA) model of continuous program quality improvement (Taylor et al. 2014). The Aim Statement addresses the three main questions that an organization must ask as part of the planning phase in PDSA, namely:

- Question 1: What are we trying to accomplish?
- Answer: To regrow muscle fibers in muscles that are most essential for independence in basic activities of daily living (e.g., the biceps and triceps brachii in the upper extremity and the vastus medialis in the lower extremity).
- Question 2: How will we know if a change is an improvement?
- Answer: Through preclinical and clinical trials, to demonstrate that the regenerative rehabilitative strategies developed are safe, effective, and practical, in relation to increasing functional muscle mass in animal models of MDs and in human patients.
- Question 3: What change can we make that will result in an improvement?
- Answer: Increase the probability of success by focusing on reversing muscle loss in a few high priority muscles in nonlethal MDs. Educate patients, caregivers, and healthcare professionals on the importance of protecting existing muscle mass.

Excluding lethal muscular dystrophies from the Aim Statement does not imply that lifespan-limiting muscular dystrophies should not be of concern to the R3C. Rather, it emphasizes the urgency required in first addressing the lethal cardiorespiratory implications before addressing the issue of muscle weakness and wasting in limb muscles in certain MDs (Gordish-Dressman et al. 2018). Furthermore, addressing cardiorespiratory complications and limb muscle weakness in MDs would be mutually beneficial rather than exclusive, since advances made in one domain are most likely to benefit the other. In subsequent sections of this chapter,

when the term nonlethal MDs is used, it refers to MDs that are naturally nonlethal (e.g., dysferlinopathy or dysferlin-linked muscular dystrophy, previously abbreviated as LGMD2B for limb girdle muscular dystrophy type 2B, now abbreviated as LGMDR2 for limb girdle muscular dystrophy recessive type 2) (Straub et al. 2018; Moore et al. 2021), as well as MDs that will be or have been made nonlethal by addressing cardiorespiratory problems (e.g., DMD) (Amoasii et al. 2018).

3.3.3 Opportunities: Leveraging the Resources of the R3C and Other Research Communities to Solve the Mass Conundrum in MDs

In the preceding sections, an Aim Statement was drafted based on the R3C's strengths and weaknesses. In this section, the resources that are already available and can be optimized and synergized to achieve the R3C's aim will be discussed in greater detail.

In another chapter that is part of this series, the condition known as volumetric muscle loss (VML) has been discussed in detail (see Chap. 6). VML is a term that is used to describe the loss of a large amount of muscle tissue arising from trauma (e.g., blast injuries, fractures, removal of neoplasms, etc.) (Greising et al. 2017; Grogan et al. 2011). Even though skeletal muscle possesses excellent regenerative capacity and is capable of undergoing hypertrophy with intense resistance training, muscle mass and strength that is lost in VML does not show clinically meaningful improvement with standard treatments (Corona et al. 2015). The reason for this lack of recovery in VML may be explained by the loss of MuSCs along with lost muscle fibers, discontinuity of muscle fibers and the extracellular matrix templates for each muscle fiber, replacement of native tissue with scar tissue, altered neural and vascular connections to the remaining muscle fibers, and biomechanical and metabolic alterations in the remnant tissue (Garg et al. 2015; Southern et al. 2019). When compared to VML, the muscle loss seen in nonlethal MDs is less complex because there is no primary disruption of the neurovascular connections in MDs. Furthermore, in VML, due to the traumatic etiology, scar tissue formation is a barrier to recovery (Dziki et al. 2016); whereas, in MDs, there is no scar tissue formation per se, but rather fibrous and fatty tissue replacement of muscle fibers that occurs over a prolonged time course. The lack of scarring is a plus in MDs since a more organized extracellular matrix is conducive for muscle fiber regeneration (Skuk and Tremblay 2019). Despite the differences in the etiology and pathophysiology of muscle loss in VML and MDs, the problem of muscle loss that does not reverse spontaneously is common between the two conditions. Therefore, the vast amount of knowledge and research methods that have been developed to address VML can be modified and repurposed for addressing muscle loss in nonlethal MDs.

If we synergized the strengths of the R3C, and other research communities shared their resources with the R3C, an action plan to regrow muscle fibers in the context of nonlethal MDs could be (Fig. 3.3):

- Collect a muscle biopsy from patients, isolate MuSCs from harvested muscle
- Correct or compensate the MD-causing genetic defect in MuSCs
- Enrich for MuSCs that have been successfully corrected or compensated
- Generate large numbers of gene-corrected autologous (from patient itself) daughter cells in culture
- Seed the corrected cells onto tissue engineering templates
- Stimulate the templates to form engineered muscle tissue with myogenic potential
- Implant engineered myogenic tissue into high priority muscles of patients with procedures that can be repeated to scale up donor-cell-derived myogenesis
- Provide precision rehabilitation to patients to maximize induced muscle regeneration and muscle function
- Develop and implement standardized outcome measures to evaluate treatment effectiveness

Since the implanted cells will be derived from the patient's own MuSCs, immune rejection is not a major concern. There is reason for optimism that this plan will succeed because numerous basic science, translational, preclinical, and clinical breakthroughs have been made in each of the different components of the action plan—some of these breakthroughs are highlighted in the paragraphs that follow.

Highlight 1. In a single case study, investigators injected ~1.35 billion healthy MuSC-derived myoblasts into the gastrocnemius of a 26-year-old biologically male patient with DMD (Skuk et al. 2007). Since the donor myoblasts were not autologous, the patient received pharmacological immunosuppression. The results at 18 months post-procedure were promising, with ~35% of the fibers biopsied from the treated region being positive for dystrophin, suggesting that there was indeed donor-cell-derived myogenesis. Even though this was a single case study, it was an important first step in demonstrating that dystrophin-expressing myoblasts implanted into a dystrophin-deficient human host muscle can generate donor-cell-derived muscle fibers. Incidentally, the donor tissue from which MuSCs were harvested and expanded to form numerous myoblasts was biopsied from the patient's father.

Highlight 2. In a clinical trial, five biologically male patients ranging from 27 to 32 years old with established VML (13–85 months since injury) in the thigh or leg, were given an experimental surgical treatment that involved scar removal and grafting of porcine bladder acellular matrix in the VML defect (Dziki et al. 2016). All patients performed at least 3 months of customized physical therapy before and after surgical VML repair with the acellular matrix. At 6–8 months post-surgery plus physical therapy, non-invasive imaging with magnetic resonance or computed tomography of the VML defect gave signals similar to that of muscle tissue. Furthermore, biopsies of the graft at 6–8 months after surgery had structures that resembled muscle fibers and expressed muscle specific proteins. This study was an important step in demonstrating that a tissue matrix, even if not seeded with

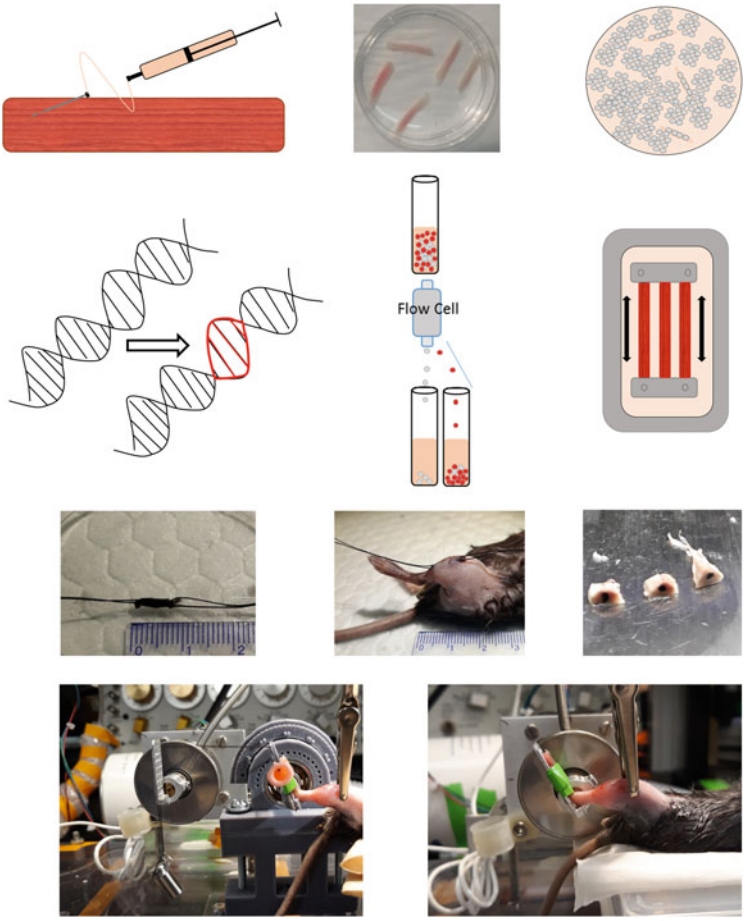

Fig. 3.3 Suggested action plan for the R3C to achieve its goal of reversing muscle loss in certain muscles in individuals with nonlethal MDs. (**a**) Collect muscle biopsies from individuals with MD. (**b**) Use mechanical and enzymatic methods to release MuSCs from tissue. (**c**) Isolate MuSCs based on methods perfected by the R3C. (**d**) Correct or compensate the MD-causing genetic defect (s) in MuSCs based on methods perfected by the molecular biology research community. (**e**) Enrich and expand gene-corrected MuSCs based on methods developed by the R3C and the immunology research community. (**f**) Seed gene-corrected cells onto tissue engineering templates, and "exercise" the templates with stretch-shortening cycles or other means. (**g**–**i**) Implant engineered muscle tissue with myogenic potential into targeted muscles in animal models (basic and preclinical research stage) or patients (clinical research stage) through minimally invasive methods. (**g**) A roll of bladder acellular matrix doped with black India ink, prepared for implantation into the TA muscle of a mouse. (**h**) Use of the MIME™ technique to implant bladder acellular matrix within the TA muscle of a mouse along the host muscle's long axis. (**i**) Host mouse TA muscle showing the ink-doped tissue implant in the proximal, middle, and distal third of the muscle, confirming placement of engineered donor tissue along the long axis of the host TA muscle. (**j**, **k**) Provide precision rehabilitation (e.g., dosage-adjusted resisted training, DART™) to host animal models or patients before and after tissue implantation to stimulate autologous donor-cell-derived muscle regeneration and monitor functional improvement. Muscular contractions for resistance training can be elicited by electrical stimulation in animal models and through voluntary activation in humans. Note: In the proposed system, engineered muscle is a delivery vehicle for myogenic cells and is not intended to

myoblasts before grafting, provides a conducive environment for de novo myogenesis. This study also emphasized the value of rehabilitative therapy coupled with regenerative therapy—it must be noted however that, there were no "scar removal only" or "no rehabilitation" control groups in this study.

Highlight 3. One possible reason for myoblast injections not translating into meaningful clinical therapies could be the low number of donor-derived myofibers that are generated and the formation of fibrous tissue instead of muscle fibers (Skuk and Tremblay 2019). Intriguingly, even when small amounts of actual human or mouse muscle tissue along with native MuSCs are implanted into mouse host muscles, robust donor-cell-derived myogenesis is achieved (Collins et al. 2005; Zhang et al. 2014; Roche et al. 2020; Roche et al. 2019). Not surprisingly, a common theme in regenerative biology seems to be that, regenerative cells perform better when they are constrained by a matrix and then implanted into hosts versus being delivered as separate cells (Prestwich and Healy 2015; Lin et al. 2014; Williams 2019; Scarritt et al. 2019). It could then be argued that implanting engineered muscle tissue, which has MuSC like cells, would likely be more effective at promoting donor-derived myogenesis than injecting the same number of isolated myoblasts. At this time, to the best of the author's knowledge, this prediction has not been tested experimentally with stringent controls. However, a promising breakthrough has been made in developing a tissue mimetic that can regenerate from a toxin-induced injury performed in a bioreactor, implying that it mimics the regenerative ability of native muscle tissue (Juhas et al. 2014). This engineered muscle tissue becomes vascularized and retains its myogenic potential when implanted under the skin of immunodeficient host mice (Juhas et al. 2014). More recent studies with this muscle tissue mimetic suggest that it even responds to exercise in a bioreactor similar to actual biological muscle tissue (Khodabukus et al. 2019). A synergy of this technology with the gains that have been made at the preclinical level through tissue-engineered muscle repair in the VML field, would likely move the R3C closer to our goal of reversing muscle loss in nonlethal MDs (Quarta et al. 2017; Nakayama et al. 2018; Alcazar et al. 2020; Corona et al. 2012; Corona et al. 2014; Machingal et al. 2011).

Highlight 4. In a preclinical study, viral vectors were used to deliver a CRISPR/Cas-9-based strategy to edit the dystrophin gene in a canine model of DMD (Amoasii et al. 2018). Both systemic and intramuscular delivery of the gene editing strategy induced the expression of dystrophin in numerous muscle fibers in muscles that were studied, which included cardiac muscle and the diaphragm. The experimental therapy also reduced spontaneous muscle fiber degeneration and regeneration when compared to untreated control animals. Studies by other research groups have demonstrated that CRISPR/Cas-9-based gene editing strategies can be used to express dystrophin in dystrophin-deficient mouse and human myoblasts

Fig. 3.3 (continued) graft cultured fibers onto host muscles. Source of images: unpublished art and images from the author's laboratory

(Tabebordbar et al. 2016), and that edited human DMD myoblasts can generate muscle fibers in a host mouse (Ousterout et al. 2015). These studies suggest that in the future, it might be possible to convert lethal MDs into nonlethal MDs through techniques like gene editing. They also demonstrate that myogenic cells can be manipulated to express MD-related proteins and then be used in regenerative therapies along with rehabilitation to promote the growth of healthy muscle fibers in patients with MD.

Highlight 5. Numerous surgical procedures that were once performed as open surgical procedures have now been refined so that they can be performed in a minimally invasive manner (Tsui et al. 2013). The increasing availability of modern surgical instruments and technologies, shorter recovery periods, and better cosmetic results for outcomes comparable to traditional open surgical procedures, have all led to the increased adoption of minimally invasive surgical protocols (Tonutti et al. 2017; Tan et al. 2014). The author's laboratory has developed a novel minimally invasive procedure known as minimally invasive muscle embedding (MIME™) for preclinical testing of tissue-based regenerative rehabilitation therapies aimed at promoting donor-cell-derived myogenesis. MIME™ involves passing a sterile hypodermic needle through the long axis of the tibialis anterior (TA) muscle in a host mouse, and placing a segment of donor tissue with myogenic potential in the needle track (Roche et al. 2017). MIME™ promotes robust donor-cell-derived myogenesis when donor mouse extensor digitorum longus (EDL) muscle or segments of human cadaveric TA muscle are implanted into host mouse TA muscles (Roche et al. 2020; Roche et al. 2019). Furthermore, human muscle fibers generated in a host mouse through MIME™, promote functional recovery and persist even 3 months after the procedure (Roche et al. 2019). The goal of MIME™ is not to graft mature muscle fibers into host muscle, but rather, to use actual or engineered muscle tissue as a medium to deliver myogenic cells into host muscle with minimal disruption to the host muscle's neurovascular connections and extracellular matrix templates. The eventual goal of the author's laboratory is to adapt the MIME™ procedure for experimental and clinical use in humans.

3.3.4 Threats: Factors That Might Prevent the R3C from Achieving Its Goal of Solving the Muscle Mass Conundrum in MDs

The 2013 Walter Cannon Lecture at the Annual Meeting of the American Physiological Society (The APS, the sponsor of this book) was titled "Is Physiology Redundant"? (Joyner 2013). One might wonder why someone would even ask such a question. However, this talk was presented at a time when physiologists were struggling to convince the scientific community of their relevance and the need to retain physiology departments at universities. For the author of this chapter who was in the audience during the lecture, it was astounding to learn that many

physiology departments across the US were shutting down or being amalgamated with other departments due to the difficult funding situation and the low chances of physiological research receiving funding if proposals did not have a strong molecular mechanism component. Thus, the danger of regenerative rehabilitation research ideas with high translational impact not being funded because they lack a strong molecular mechanism component, might threaten the R3C's ability to achieve its goal of reversing muscle loss in MDs. The National Center for Medical Rehabilitation Research (NCMRR) (National Institutes of Health 2021d), the NIH-supported Medical Rehabilitation Research Resource (MR3) Network (National Institutes of Health 2021c), and the NIH-supported Training in Grantsmanship in Rehabilitation Researcher (TIGRR) program (National Institutes of Health 2021b), are all doing yeoman's service in building the R3C, promoting regenerative rehabilitation research, and helping investigators identify sources of funding for their research programs. The R3C must work closely with supporting agencies to ensure that translational potential is weighted heavily in relation to regenerative rehabilitation research projects aimed at reversing muscle loss in MDs.

The APS lecture, mentioned in the previous paragraph, is held each year in honor of the famed physiologist Walter B. Cannon, who is credited for coining the term "homeostasis" and describing its key aspects (Cannon 1932). Cannon described homeostasis as the "wisdom of the body," which helps maintain stability in physiological measurements like body temperature, blood pressure, blood pH, blood glucose, etc. (Billman 2020). The pathophysiological features of MDs such as muscle degeneration and regeneration suggests that muscle tissue tries to restore a homeostatic state, against the odds created by upstream changes in MD-associated genes (Grounds 2014). For example, in DMD, mutations in the dystrophin gene cause downstream consequences to synthesis of the dystrophin protein, which affects homeostasis of the muscle cell plasma membrane, which affects calcium ion homeostasis in muscle fibers, which causes muscle fiber death, which triggers repeated cycles of muscle degeneration and regeneration, which causes replacement of muscle fibers with fibrous and fatty tissue that can transmit but not produce force, and after many more intermediate steps, ultimately affects whole body homeostasis (Allen et al. 2010; Burr and Molkentin 2015; Maciel et al. 2021; Boldrin et al. 2015). Each skeletal muscle is a multicellular organ, which possesses complex homeostatic circuits within itself and connections with the rest of the body (Hoffmann and Weigert 2017; Grounds 2014). This complexity creates many unknowns in relation to regrowing functional muscle mass, even if we work only on the biceps and triceps brachii and the vastus medialis (Grounds 2014). Human skeletal muscle is very capable of hypertrophy (an increase in functional muscle mass mediated by an increase in muscle fiber diameter), but is not capable of hyperplasia (an increase in muscle fiber number) (Grounds 2014). This homeostatic limitation in the ability of human skeletal muscle to undergo hyperplasia is something the R3C will have to proactively address as we work toward our goal of reversing muscle loss in nonlethal MDs. Furthermore, due to this concern, it is imperative that patients with MDs, their caregivers, and healthcare are made aware of the importance of preserving functional muscle mass as best as possible through healthy habits (modifiers of MDs

highlighted in Fig. 3.2) (Lott et al. 2021; Begam et al. 2020; Grounds 2014). It could be argued that, preserving functional muscle mass through healthy habits might not only improve outcomes from promising gene therapies, but might also improve outcomes from regenerative rehabilitation interventions aimed at regrowing lost muscle (Grounds 2014). The reader is directed to the Discussion section of an article by Begam et al., which describes the muscle mass conundrum posed by MDs, in greater detail with illustrations (Begam et al. 2020).

3.4 Conclusions

In this chapter, the author defined MDs, briefly described the pathophysiological processes that are common to MDs, and identified that the irreversible loss of functional muscle mass in MDs is a major concern. The author then used the strengths and weaknesses parts of a SWOT analysis to present an achievable goal for the R3C in relation to MDs—i.e. to develop safe, effective, and practical regenerative rehabilitation strategies to regrow muscle fibers in muscles that are most essential for independence in basic activities of daily living, in individuals with nonlethal muscular dystrophies. The author next discussed the unique opportunities available to the R3C to synergize and leverage their strengths to achieve their goal. Finally, the author expressed concern that if regenerative rehabilitation ideas that have strong translational potential are not funded because the studies lack a strong molecular mechanism component, it might threaten the ability of the R3C to achieve its goal of reversing muscle loss in nonlethal MDs. Furthermore, because each muscle is a complex multicellular organ with internal and external homeostatic connections, there are unknown factors that might threaten the ability of the R3C to reverse muscle loss in nonlethal MDs.

Conflict of Interest Statement

The author has no conflicts to declare.

Funding Sources and Acknowledgments The author acknowledges research grants from the Jain Foundation Inc., pilot funding from AR3T (NIH P2C HD086843), R03HD091648 from the NIH, and FRAP and startup funds from Wayne State University, Detroit, MI, USA. The author acknowledges Visiting Scholar Awards from the Jain Foundation, Inc. and AR3T, through which he received training in methods to isolate and culture myogenic and other mononucleated cells from skeletal muscle tissue, at the laboratories of Dr. Amy J. Wagers (Joslin Diabetes Center, Harvard Medical School, Boston, MA) and Dr. Thomas A. Rando (Veterans Affairs Medical Center, Stanford Medicine, Palo Alto, CA), respectively. The author also expresses his gratitude to individuals with MDs and their caregivers, who have through their activism contributed to the vast body of knowledge on MDs. The author acknowledges Renuka Roche, PhD, OTR/L (Associate Professor, Occupational Therapy Program, Eastern Michigan University, Ypsilanti, MI, USA) for reading this chapter and providing scholarly input. The author acknowledges Mr. Senthil Kuppuswamy (Clinical Lead Occupational Therapist, Manchester University NHS Foundation Trust, Manchester, England, UK), and Sivaramkumar Shanmugam, PhD (Senior Lecturer in Physiotherapy, Glasgow Caledonian University, Glasgow, Scotland, UK), for insights shared on continuous quality improvement. The author expresses his sincere thanks to Antoine de Morrée, PhD, for sharing his expertise on culturing myogenic cells.

References

Alcazar CA, Hu C, Rando TA, Huang NF, Nakayama KH (2020) Transplantation of insulin-like growth factor-1 laden scaffolds combined with exercise promotes neuroregeneration and angiogenesis in a preclinical muscle injury model. Biomater Sci 8(19):5376–5389. https://doi.org/10.1039/d0bm00990c

Allen DG, Gervasio OL, Yeung EW, Whitehead NP (2010) Calcium and the damage pathways in muscular dystrophy. Can J Physiol Pharmacol 88(2):83–91. https://doi.org/10.1139/Y09-058

Ambrosio F, Russell A (2010) Regenerative rehabilitation: a call to action. J Rehabil Res Dev 47(3): xi–xv. https://doi.org/10.1682/jrrd.2010.03.0021

Amoasii L, Hildyard JCW, Li H, Sanchez-Ortiz E, Mireault A, Caballero D, Harron R, Stathopoulou TR, Massey C, Shelton JM, Bassel-Duby R, Piercy RJ, Olson EN (2018) Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. Science 362(6410):86–91. https://doi.org/10.1126/science.aau1549

Begam M, Roche R, Hass JJ, Basel CA, Blackmer JM, Konja JT, Samojedny AL, Collier AF, Galen SS, Roche JA (2020) The effects of concentric and eccentric training in murine models of dysferlin-associated muscular dystrophy. Muscle Nerve. https://doi.org/10.1002/mus.26906

Billman GE (2020) Homeostasis: the underappreciated and far too often ignored central organizing principle of physiology. Front Physiol 11:200

Birnkrant DJ, Bushby K, Bann CM, Alman BA, Apkon SD, Blackwell A, Case LE, Cripe L, Hadjiyannakis S, Olson AK, Sheehan DW, Bolen J, Weber DR, Ward LM, Group DMDCCW (2018) Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. Lancet Neurol 17(4):347–361. https://doi.org/10.1016/S1474-4422(18)30025-5

Boldrin L, Zammit PS, Morgan JE (2015) Satellite cells from dystrophic muscle retain regenerative capacity. Stem Cell Res 14(1):20–29. https://doi.org/10.1016/j.scr.2014.10.007

Brack AS, Rando TA (2012) Tissue-specific stem cells: lessons from the skeletal muscle satellite cell. Cell Stem Cell 10(5):504–514. https://doi.org/10.1016/j.stem.2012.04.001

Burr AR, Molkentin JD (2015) Genetic evidence in the mouse solidifies the calcium hypothesis of myofiber death in muscular dystrophy. Cell Death Differ 22(9):1402–1412. https://doi.org/10.1038/cdd.2015.65

Bushby K, Norwood F, Straub V (2007) The limb-girdle muscular dystrophies—diagnostic strategies. Biochim Biophys Acta 1772(2):238–242. https://doi.org/10.1016/j.bbadis.2006.09.009

Buyse GM, Voit T, Schara U, Straathof CSM, D'Angelo MG, Bernert G, Cuisset JM, Finkel RS, Goemans N, McDonald CM, Rummey C, Meier T, Group DS (2015) Efficacy of idebenone on respiratory function in patients with Duchenne muscular dystrophy not using glucocorticoids (DELOS): a double-blind randomised placebo-controlled phase 3 trial. Lancet (London, England) 385(9979):1748–1757. https://doi.org/10.1016/S0140-6736(15)60025-3

Cannon WB (1932) The wisdom of the body. The wisdom of the body. W W Norton, New York

Charge SB, Rudnicki MA (2004) Cellular and molecular regulation of muscle regeneration. Physiol Rev 84(1):209–238. https://doi.org/10.1152/physrev.00019.2003

Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, Morgan JE (2005) Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell 122(2):289–301. https://doi.org/10.1016/j.cell.2005.05.010

Corona BT, Machingal MA, Criswell T, Vadhavkar M, Dannahower AC, Bergman C, Zhao W, Christ GJ (2012) Further development of a tissue engineered muscle repair construct in vitro for enhanced functional recovery following implantation in vivo in a murine model of volumetric muscle loss injury. Tissue Eng Part A 18(11-12):1213–1228. https://doi.org/10.1089/ten.TEA.2011.0614

Corona BT, Ward CL, Baker HB, Walters TJ, Christ GJ (2014) Implantation of in vitro tissue engineered muscle repair constructs and bladder acellular matrices partially restore in vivo skeletal muscle function in a rat model of volumetric muscle loss injury. Tissue Eng Part A 20(3–4):705–715. https://doi.org/10.1089/ten.TEA.2012.0761

Corona BT, Rivera JC, Owens JG, Wenke JC, Rathbone CR (2015) Volumetric muscle loss leads to permanent disability following extremity trauma. J Rehabil Res Dev 52(7):785–792. https://doi.org/10.1682/jrrd.2014.07.0165

Crudele JM, Chamberlain JS (2019) AAV-based gene therapies for the muscular dystrophies. Hum Mol Genet 28(R1):R102–R107. https://doi.org/10.1093/hmg/ddz128

Cuesta-Vargas AI, Cano-Herrera CL, Heywood S (2013) Analysis of the neuromuscular activity during rising from a chair in water and on dry land. J Electromyogr Kinesiol 23(6):1446–1450. https://doi.org/10.1016/j.jelekin.2013.06.001

Department of Defense (2021) CDMRP—JPC-8 clinical and rehabilitative medicine. Department of Defense—congressionally directed medical research programs. https://cdmrp.army.mil/dmrdp/jpc8crmrp. Accessed 16 Aug 2021

Distefano G, Ferrari RJ, Weiss C, Deasy BM, Boninger ML, Fitzgerald GK, Huard J, Ambrosio F (2013) Neuromuscular electrical stimulation as a method to maximize the beneficial effects of muscle stem cells transplanted into dystrophic skeletal muscle. PLoS One 8(3):e54922. https://doi.org/10.1371/journal.pone.0054922

Dowling JJ, Gonorazky HD, Cohn RD, Campbell C (2018) Treating pediatric neuromuscular disorders: the future is now. Am J Med Genet A 176(4):804–841. https://doi.org/10.1002/ajmg.a.38418

Duan D (2018) Systemic AAV micro-dystrophin gene therapy for duchenne muscular dystrophy. Mol Ther 26(10):2337–2356. https://doi.org/10.1016/j.ymthe.2018.07.011

Dumont NA, Bentzinger CF, Sincennes MC, Rudnicki MA (2015) Satellite cells and skeletal muscle regeneration. Compr Physiol 5(3):1027–1059. https://doi.org/10.1002/cphy.c140068

Dziki J, Badylak S, Yabroudi M, Sicari B, Ambrosio F, Stearns K, Turner N, Wyse A, Boninger ML, Brown EHP, Rubin JP (2016) An acellular biologic scaffold treatment for volumetric muscle loss: results of a 13-patient cohort study. NPJ Regen Med 1(1):16008. https://doi.org/10.1038/npjregenmed.2016.8

Emery AE (2002) The muscular dystrophies. Lancet (London, England) 359(9307):687–695. https://doi.org/10.1016/S0140-6736(02)07815-7

Eunice Kennedy Shriver National Institute of Child Health and Human Development (2021) Muscular dystrophy. https://www.nichd.nih.gov/health/topics/musculardys. Accessed 14 Mar 2021

Eunice Kennedy Shriver National Institute of Child Health and Human Development and the NIH Medical Rehabilitation Coordinating Committee (2016) National Institutes of Health research plan on rehabilitation 2016. https://www.nichd.nih.gov/sites/default/files/publications/pubs/Documents/NIH_ResearchPlan_Rehabilitation.pdf. Accessed 14 Mar 2021

Evers-van Gogh IJA, Alex S, Stienstra R, Brenkman AB, Kersten S, Kalkhoven E (2015) Electric pulse stimulation of myotubes as an in vitro exercise model: cell-mediated and non-cell-mediated effects. Sci Rep 5(1):10944. https://doi.org/10.1038/srep10944

Garg K, Ward CL, Hurtgen BJ, Wilken JM, Stinner DJ, Wenke JC, Owens JG, Corona BT (2015) Volumetric muscle loss: persistent functional deficits beyond frank loss of tissue. J Orthop Res 33(1):40–46. https://doi.org/10.1002/jor.22730

Genetic and Rare Diseases Information Center (GARD) (2021) Duchenne muscular dystrophy. https://rarediseases.info.nih.gov/diseases/6291/duchenne-muscular-dystrophy. Accessed 14 Mar 2021

Gilbert PM, Havenstrite KL, Magnusson KEG, Sacco A, Leonardi NA, Kraft P, Nguyen NK, Thrun S, Lutolf MP, Blau HM (2010) Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science 329(5995):1078. https://doi.org/10.1126/science.1191035

Giusti A, Maggini M, Colaceci S (2020) The burden of chronic diseases across Europe: what policies and programs to address diabetes? A SWOT analysis. Health Res Policy Syst 18(1):12. https://doi.org/10.1186/s12961-019-0523-1

Gordish-Dressman H, Willmann R, Dalle Pazze L, Kreibich A, van Putten M, Heydemann A, Bogdanik L, Lutz C, Davies K, Demonbreun AR, Duan D, Elsey D, Fukada S-i, Girgenrath M, Patrick Gonzalez J, Grounds MD, Nichols A, Partridge T, Passini M, Sanarica F, Schnell FJ, Wells DJ, Yokota T, Young CS, Zhong Z, Spurney C, Spencer M, De Luca A, Nagaraju K, Aartsma-Rus A (2018) "Of mice and measures": a project to improve how we advance Duchenne muscular dystrophy therapies to the clinic. J Neuromuscul Dis 5:407–417. https://doi.org/10.3233/JND-180324

Greising SM, Rivera JC, Goldman SM, Watts A, Aguilar CA, Corona BT (2017) Unwavering pathobiology of volumetric muscle loss injury. Sci Rep 7(1):13179–13179. https://doi.org/10.1038/s41598-017-13306-2

Grogan BF, Hsu JR, Skeletal Trauma Research Consortium (2011) Volumetric muscle loss. JAAOS J Am Acad Orthop Surg 19:S35–S37

Grounds MD (2014) The need to more precisely define aspects of skeletal muscle regeneration. Int J Biochem Cell Biol 56:56–65. https://doi.org/10.1016/j.biocel.2014.09.010

Grounds MD, Partridge TA (1983) Isoenzyme studies of whole muscle grafts and movement of muscle precursor cells. Cell Tissue Res 230(3):677–688

Hanani M (2010) Satellite glial cells: more than just 'rings around the neuron'. Neuron Glia Biol 6(1):1–2. https://doi.org/10.1017/S1740925X10000104

Harris E, Bladen CL, Mayhew A, James M, Bettinson K, Moore U, Smith FE, Rufibach L, Cnaan A, Bharucha-Goebel DX, Blamire AM, Bravver E, Carlier PG, Day JW, Diaz-Manera J, Eagle M, Grieben U, Harms M, Jones KJ, Lochmuller H, Mendell JR, Mori-Yoshimura M, Paradas C, Pegoraro E, Pestronk A, Salort-Campana E, Schreiber-Katz O, Semplicini C, Spuler S, Stojkovic T, Straub V, Takeda S, Rocha CT, Walter MC, Bushby K, Jain COSC (2016) The clinical outcome study for dysferlinopathy: an international multicenter study. Neurol Genet 2(4):e89. https://doi.org/10.1212/NXG.0000000000000089

Hewitt-Taylor J (2012) Identifying, analysing and solving problems in practice. Nurs Stand 26(40):35–41. https://doi.org/10.7748/ns2012.06.26.40.35.c9139

Hoffman EP, Brown RH Jr, Kunkel LM (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 51(6):919–928. https://doi.org/10.1016/0092-8674(87)90579-4

Hoffmann C, Weigert C (2017) Skeletal muscle as an endocrine organ: the role of myokines in exercise adaptations. Cold Spring Harb Perspect Med 7(11). https://doi.org/10.1101/cshperspect.a029793

International Consortium for Regenerative Rehabilitation (ICRR) (2021) https://ar3t.pitt.edu/icrr/. Accessed 14 Mar 2021

Jackson KA, Mi T, Goodell MA (1999) Hematopoietic potential of stem cells isolated from murine skeletal muscle. Proc Natl Acad Sci USA 96(25):14482. https://doi.org/10.1073/pnas.96.25.14482

Joyner MJ (2013) Physiology and redundancy. Physiology 28(3):136–137. https://doi.org/10.1152/physiol.00015.2013

Juhas M, Engelmayr GC Jr, Fontanella AN, Palmer GM, Bursac N (2014) Biomimetic engineered muscle with capacity for vascular integration and functional maturation in vivo. Proc Natl Acad Sci U S A 111(15):5508–5513. https://doi.org/10.1073/pnas.1402723111

Kanagawa M, Toda T (2006) The genetic and molecular basis of muscular dystrophy: roles of cell-matrix linkage in the pathogenesis. J Hum Genet 51(11):915–926. https://doi.org/10.1007/s10038-006-0056-7

Khodabukus A, Madden L, Prabhu NK, Koves TR, Jackman CP, Muoio DM, Bursac N (2019) Electrical stimulation increases hypertrophy and metabolic flux in tissue-engineered human skeletal muscle. Biomaterials 198:259–269. https://doi.org/10.1016/j.biomaterials.2018.08.058

Krag TO, Hauerslev S, Sveen ML, Schwartz M, Vissing J (2011) Level of muscle regeneration in limb-girdle muscular dystrophy type 2I relates to genotype and clinical severity. Skelet Muscle 1(1):31. https://doi.org/10.1186/2044-5040-1-31

Lin H, Cheng AW, Alexander PG, Beck AM, Tuan RS (2014) Cartilage tissue engineering application of injectable gelatin hydrogel with in situ visible-light-activated gelation capability in both air and aqueous solution. Tissue Eng Part A 20(17-18):2402–2411. https://doi.org/10.1089/ten.TEA.2013.0642

Lipton BH, Schultz E (1979) Developmental fate of skeletal muscle satellite cells. Science 205(4412):1292–1294

Lott DJ, Taivassalo T, Cooke KD, Park H, Moslemi Z, Batra A, Forbes SC, Byrne BJ, Walter GA, Vandenborne K (2021) Safety, feasibility, and efficacy of strengthening exercise in Duchenne muscular dystrophy. Muscle Nerve 63(3):320–326. https://doi.org/10.1002/mus.27137

Lovering RM, Porter NC, Bloch RJ (2005) The muscular dystrophies: from genes to therapies. Phys Ther 85(12):1372–1388

Machingal MA, Corona BT, Walters TJ, Kesireddy V, Koval CN, Dannahower A, Zhao W, Yoo JJ, Christ GJ (2011) A tissue-engineered muscle repair construct for functional restoration of an irrecoverable muscle injury in a murine model. Tissue Eng Part A 17(17–18):2291–2303. https://doi.org/10.1089/ten.TEA.2010.0682

Maciel FKL, Santos A, Artilheiro MC, Sá C (2021) Motor compensation strategies for reduced upper limb function among individuals with Duchenne muscular dystrophy. Percept Mot Skills. https://doi.org/10.1177/0031512521998278

Mauro A (1961) Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 9:493–495

Mercuri E, Bonnemann CG, Muntoni F (2019) Muscular dystrophies. Lancet (London, England) 394(10213):2025–2038. https://doi.org/10.1016/S0140-6736(19)32910-1

MindTools (2021) SWOT analysis. https://www.mindtools.com/pages/article/newTMC_05.htm. Accessed 14 Mar 2021

Moore U, Gordish H, Diaz-Manera J, James MK, Mayhew AG, Guglieri M, Fernandez-Torron R, Rufibach LE, Feng J, Blamire AM, Carlier PG, Spuler S, Day JW, Jones KJ, Bharucha-Goebel DX, Salort-Campana E, Pestronk A, Walter MC, Paradas C, Stojkovic T, Mori-Yoshimura M, Bravver E, Pegoraro E, Lowes LP, Mendell JR, Bushby K, Straub V (2021) Miyoshi myopathy and limb girdle muscular dystrophy R2 are the same disease. Neuromuscul Disord 31(4):265–280. https://doi.org/10.1016/j.nmd.2021.01.009

Morgan JE, Partridge TA (2003) Muscle satellite cells. Int J Biochem Cell Biol 35(8):1151–1156. https://doi.org/10.1016/s1357-2725(03)00042-6

Nagy N, Nonneman RJ, Llanga T, Dial CF, Riddick NV, Hampton T, Moy SS, Lehtimaki KK, Ahtoniemi T, Puolivali J, Windish H, Albrecht D, Richard I, Hirsch ML (2017) Hip region muscular dystrophy and emergence of motor deficits in dysferlin-deficient Bla/J mice. Physiol Rep 5(6). https://doi.org/10.14814/phy2.13173

Nakayama KH, Alcazar C, Yang G, Quarta M, Paine P, Doan L, Davies A, Rando TA, Huang NF (2018) Rehabilitative exercise and spatially patterned nanofibrillar scaffolds enhance vascularization and innervation following volumetric muscle loss. NPJ Regen Med 3:16. https://doi.org/10.1038/s41536-018-0054-3

National Center for Biotechnology Information (2021) DMD dystrophin. https://www.ncbi.nlm.nih.gov/gene/1756. Accessed 14 Mar 2021

National Institutes of Health (2021a) Alliance for regenerative rehabilitation research & training 2.0. https://grantome.com/grant/NIH/P2C-HD086843-06. Accessed 14 Mar 2021

National Institutes of Health (2021b) Intensive Rehabilitation Research Grant Writing Workshops in the United States (TIGRR). https://www.grantome.com/grant/NIH/R25-HD074546-07. Accessed 14 Mar 2021

National Institutes of Health (2021c) Medical Rehabilitation Research Resource Network. https://ncmrr.org/. Accessed 14 Mar 2021

National Institutes of Health (2021d) National Center for Medical Rehabilitation Research (NCMRR). https://www.nichd.nih.gov/about/org/ncmrr. Accessed 14 Mar 2021

National Organization for Rare Disorders (NORD) (2021a) https://www.nidcd.nih.gov/directory/national-organization-rare-disorders-nord. Accessed 14 Mar 2021

National Organization for Rare Disorders (NORD) (2021b) Duchenne muscular dystrophy. https://rarediseases.org/rare-diseases/duchenne-muscular-dystrophy/. Accessed 14 Mar 2021

Ousterout DG, Kabadi AM, Thakore PI, Majoros WH, Reddy TE, Gersbach CA (2015) Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. Nat Commun 6(1):6244. https://doi.org/10.1038/ncomms7244

Partridge TA, Grounds M, Sloper JC (1978) Evidence of fusion between host and donor myoblasts in skeletal muscle grafts. Nature 273(5660):306–308

Peters KM, Kelly VE, Chang T, Weismann MC, Westcott McCoy S, Steele KM (2018) Muscle recruitment and coordination during upper-extremity functional tests. J Electromyogr Kinesiol 38:143–150. https://doi.org/10.1016/j.jelekin.2017.12.002

Ploutz-Snyder LL, Manini T, Ploutz-Snyder RJ, Wolf DA (2002) Functionally relevant thresholds of quadriceps femoris strength. J Gerontol A Biol Sci Med Sci 57(4):B144–B152. https://doi.org/10.1093/gerona/57.4.b144

Prestwich GD, Healy KE (2015) Why regenerative medicine needs an extracellular matrix. Expert Opin Biol Ther 15(1):3–7. https://doi.org/10.1517/14712598.2015.975200

Quarta M, Brett JO, DiMarco R, De Morree A, Boutet SC, Chacon R, Gibbons MC, Garcia VA, Su J, Shrager JB, Heilshorn S, Rando TA (2016) An artificial niche preserves the quiescence of muscle stem cells and enhances their therapeutic efficacy. Nat Biotechnol 34(7):752–759. https://doi.org/10.1038/nbt.3576

Quarta M, Cromie M, Chacon R, Blonigan J, Garcia V, Akimenko I, Hamer M, Paine P, Stok M, Shrager JB, Rando TA (2017) Bioengineered constructs combined with exercise enhance stem cell-mediated treatment of volumetric muscle loss. Nat Commun 8:15613. https://doi.org/10.1038/ncomms15613

Qu-Petersen Z, Deasy B, Jankowski R, Ikezawa M, Cummins J, Pruchnic R, Mytinger J, Cao B, Gates C, Wernig A, Huard J (2002) Identification of a novel population of muscle stem cells in mice : potential for muscle regeneration. J Cell Biol 157(5):851–864. https://doi.org/10.1083/jcb.200108150

Radomski MV, Latham CAT (2014) Occupational therapy for physical dysfunction, 7th edn. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia

Rathbone CR, Wenke JC, Warren GL, Armstrong RB (2003) Importance of satellite cells in the strength recovery after eccentric contraction-induced muscle injury. Am J Physiol Regul Integr Comp Physiol 285(6):R1490–R1495. https://doi.org/10.1152/ajpregu.00032.2003

Reed UC (2009) Congenital muscular dystrophy. Part I: a review of phenotypical and diagnostic aspects. Arq Neuropsiquiatr 67:144–168

Relaix F, Zammit PS (2012) Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage. Development 139(16):2845–2856. https://doi.org/10.1242/dev.069088

Roche JA, Begam M, Galen SS (2017) Minimally invasive muscle embedding (MIME)—a novel experimental technique to facilitate donor-cell-mediated myogenesis. J Vis Exp 126. https://doi.org/10.3791/55731

Roche JA, Begam M, Eaton AK, Elkins CJ, Johnson JP, Rosinski MM, Sartwell JA, Rosso-Norgan BJ, Galen SS (2019) Minimally invasive muscle embedding (MIME), facilitates the development of functional muscle fibers of human cadaveric origin, in host mice. FASEB J 33-(1_supplement):602.604. https://doi.org/10.1096/fasebj.2019.33.1_supplement.602.4

Roche JA, Begam M, Eaton AK, Elkins CJ, Johnson JP, Rosinski MM, Galen SS (2020) Minimally invasive muscle embedding generates donor-cell-derived muscle fibers that express desmin and dystrophin. Mil Med 185(Suppl 1):423–429. https://doi.org/10.1093/milmed/usz203

Sahenk Z, Mendell JR (2011) The muscular dystrophies: distinct pathogenic mechanisms invite novel therapeutic approaches. Curr Rheumatol Rep 13(3):199–207. https://doi.org/10.1007/s11926-011-0178-6

Sakellariou P, O'Neill A, Mueller AL, Stadler G, Wright WE, Roche JA, Bloch RJ (2015) Neuromuscular electrical stimulation promotes development in mice of mature human muscle from immortalized human myoblasts. Skelet Muscle 6(1):4. https://doi.org/10.1186/s13395-016-0078-6

Scarritt M, Murdock M, Badylak SF (2019) Chapter 35—Biologic scaffolds composed of extracellular matrix for regenerative medicine. In: Atala A, Lanza R, Mikos AG, Nerem R (eds) Principles of regenerative medicine, 3rd edn. Academic, Boston, pp 613–626. https://doi.org/10.1016/B978-0-12-809880-6.00035-7

Sherwood RI, Christensen JL, Conboy IM, Conboy MJ, Rando TA, Weissman IL, Wagers AJ (2004) Isolation of adult mouse myogenic progenitors: functional heterogeneity of cells within and engrafting skeletal muscle. Cell 119(4):543–554. https://doi.org/10.1016/j.cell.2004.10.021

Skuk D, Tremblay JP (2019) Chapter 55—Myoblast transplantation in skeletal muscles. In: Atala A, Lanza R, Mikos AG, Nerem R (eds) Principles of regenerative medicine, 3rd edn. Academic, Boston, pp 971–986. https://doi.org/10.1016/B978-0-12-809880-6.00055-2

Skuk D, Goulet M, Roy B, Piette V, Cote CH, Chapdelaine P, Hogrel JY, Paradis M, Bouchard JP, Sylvain M, Lachance JG, Tremblay JP (2007) First test of a "high-density injection" protocol for myogenic cell transplantation throughout large volumes of muscles in a Duchenne muscular dystrophy patient: eighteen months follow-up. Neuromuscul Disord 17(1):38–46. https://doi.org/10.1016/j.nmd.2006.10.003

Southern WM, Nichenko AS, Tehrani KF, McGranahan MJ, Krishnan L, Qualls AE, Jenkins NT, Mortensen LJ, Yin H, Yin A, Guldberg RE, Greising SM, Call JA (2019) PGC-1α overexpression partially rescues impaired oxidative and contractile pathophysiology following volumetric muscle loss injury. Sci Rep 9(1):4079. https://doi.org/10.1038/s41598-019-40606-6

Straub V, Murphy A, Udd B (2018) 229th ENMC international workshop: limb girdle muscular dystrophies—Nomenclature and reformed classification Naarden, the Netherlands, 17-19 March 2017. Neuromuscul Disord 28(8):702–710. https://doi.org/10.1016/j.nmd.2018.05.007

Tabebordbar M, Zhu K, Cheng JKW, Chew WL, Widrick JJ, Yan WX, Maesner C, Wu EY, Xiao R, Ran FA, Cong L, Zhang F, Vandenberghe LH, Church GM, Wagers AJ (2016) In vivo gene editing in dystrophic mouse muscle and muscle stem cells. Science 351(6271):407–411. https://doi.org/10.1126/science.aad5177

Tan H-J, Wolf JS, Ye Z, Hafez Khaled S, Miller David C (2014) Population level assessment of hospital based outcomes following laparoscopic versus open partial nephrectomy during the adoption of minimally invasive surgery. J Urol 191(5):1231–1237. https://doi.org/10.1016/j.juro.2013.11.002

Taylor MJ, McNicholas C, Nicolay C, Darzi A, Bell D, Reed JE (2014) Systematic review of the application of the plan–do–study–act method to improve quality in healthcare. BMJ Qual Saf 23(4):290. https://doi.org/10.1136/bmjqs-2013-001862

Tichy ED, Sidibe DK, Tierney MT, Stec MJ, Sharifi-Sanjani M, Hosalkar H, Mubarak S, Johnson FB, Sacco A, Mourkioti F (2017) Single stem cell imaging and analysis reveals telomere length differences in diseased human and mouse skeletal muscles. Stem Cell Rep 9(4):1328–1341. https://doi.org/10.1016/j.stemcr.2017.08.003

Tonutti M, Elson DS, Yang G-Z, Darzi AW, Sodergren MH (2017) The role of technology in minimally invasive surgery: state of the art, recent developments and future directions. Postgrad Med J 93(1097):159. https://doi.org/10.1136/postgradmedj-2016-134311

Tsui C, Klein R, Garabrant M (2013) Minimally invasive surgery: national trends in adoption and future directions for hospital strategy. Surg Endosc 27(7):2253–2257. https://doi.org/10.1007/s00464-013-2973-9

United States Census Bureau (2021) Quick facts. https://www.census.gov/quickfacts/fact/table/US/PST045219. Accessed 14 Mar 2021

VanDusen KW, Syverud BC, Williams ML, Lee JD, Larkin LM (2014) Engineered skeletal muscle units for repair of volumetric muscle loss in the tibialis anterior muscle of a rat. Tissue Eng Part A 20(21-22):2920–2930. https://doi.org/10.1089/ten.TEA.2014.0060

Verhaert D, Richards K, Rafael-Fortney JA, Raman SV (2011) Cardiac involvement in patients with muscular dystrophies: magnetic resonance imaging phenotype and genotypic considerations. Circ Cardiovasc Imaging 4(1):67–76. https://doi.org/10.1161/CIRCIMAGING.110.960740

Wallace GQ, McNally EM (2009) Mechanisms of muscle degeneration, regeneration, and repair in the muscular dystrophies. Annu Rev Physiol 71(1):37–57. https://doi.org/10.1146/annurev.physiol.010908.163216

Wicklund MP (2013) The muscular dystrophies. Continuum (Minneap Minn) 19(6 Muscle Disease):1535–1570. https://doi.org/10.1212/01.CON.0000440659.41675.8b

Wicklund MP, Kissel JT (2014) The limb-girdle muscular dystrophies. Neurol Clin 32(3):729–749, ix. https://doi.org/10.1016/j.ncl.2014.04.005

Willett NJ, Boninger ML, Miller LJ, Alvarez L, Aoyama T, Bedoni M, Brix KA, Chisari C, Christ G, Dearth CL, Dyson-Hudson TA, Evans CH, Goldman SM, Gregory K, Gualerzi A, Hart J, Ito A, Kuroki H, Loghmani MT, Mack DL, Malanga GA, Noble-Haeusslein L, Pasquina P, Roche JA, Rose L, Stoddart MJ, Tajino J, Terzic C, Topp KS, Wagner WR, Warden SJ, Wolf SL, Xie H, Rando TA, Ambrosio F (2020) Taking the next steps in regenerative rehabilitation: establishment of a new interdisciplinary field. Arch Phys Med Rehabil 101(5):917–923. https://doi.org/10.1016/j.apmr.2020.01.007

Williams DF (2019) Chapter 36—Hydrogels in regenerative medicine. In: Atala A, Lanza R, Mikos AG, Nerem R (eds) Principles of regenerative medicine, 3rd edn. Academic, Boston, pp 627–650. https://doi.org/10.1016/B978-0-12-809880-6.00036-9

Zhang Y, King OD, Rahimov F, Jones TI, Ward CW, Kerr JP, Liu N, Emerson CP Jr, Kunkel LM, Partridge TA, Wagner KR (2014) Human skeletal muscle xenograft as a new preclinical model for muscle disorders. Hum Mol Genet 23(12):3180–3188. https://doi.org/10.1093/hmg/ddu028

Chapter 4
Regenerative Rehabilitation for Duchenne Muscular Dystrophy

Kristy Swiderski, Justin P. Hardee, and Gordon S. Lynch

Abstract Duchenne muscular dystrophy (DMD) is a severe, progressive, genetic muscle wasting disorder arising from the absence of the membrane stabilizing protein, dystrophin, that renders muscle fibers susceptible to damage and degeneration. Since the discovery of the *dystrophin* gene, research efforts have focused on the development of regenerative gene- and cell-based therapies for DMD, although many obstacles need to be overcome before they can be considered for clinical application. The development of adjunct therapies that can slow the pathologic progression, preserve muscle mass, enhance muscle regeneration, and promote muscle growth, is therefore essential. Rehabilitation through physical exercise or muscle contraction protocols may help attenuate muscle weakness and dysfunction in DMD, with evidence supporting rehabilitation as an adjunct treatment to gene- and cell-mediated therapies. This chapter summarizes the current state of research for DMD therapy and explores the potential for combined regenerative and rehabilitation therapies to improve outcomes for DMD patients.

Keywords Duchenne muscular dystrophy · Gene therapy · Cell therapy · Rehabilitation · Exercise

Kristy Swiderski and Justin P. Hardee contributed equally with all other contributors.

K. Swiderski · J. P. Hardee
Centre for Muscle Research, Department of Anatomy and Physiology, The University of Melbourne, Melbourne, VIC, Australia

G. S. Lynch (✉)
Centre for Muscle Research, Department of Anatomy and Physiology, The University of Melbourne, Melbourne, VIC, Australia

Department of Anatomy and Physiology, The University of Melbourne, Melbourne, VIC, Australia
e-mail: gsl@unimelb.edu.au

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_4

4.1 Introduction

Duchenne muscular dystrophy (DMD) is a severe and progressive muscle wasting disorder caused by mutations in the *dystrophin* gene resulting in the absence of the membrane stabilizing protein, dystrophin. Loss of dystrophin renders muscle fibers fragile and susceptible to membrane tears that facilitate an influx of Ca^{2+} that can activate inflammatory and muscle degenerative pathways. Although considerable efforts are being directed to the development of gene- and cell-based therapies for DMD, these techniques are far from perfect, and many obstacles need to be overcome in order for them to advance to clinical application. In the interim, it is essential that alternative therapies also be developed, including research directed to slowing the pathologic progression, preserving muscle mass, enhancing muscle regeneration, and promoting muscle growth. This chapter will highlight the current state of research for regenerative medicine (e.g., gene-, cell-, and pharmacologic therapies), the current state of research for rehabilitation (e.g., exercise modalities), and explore the potential for combined regenerative and rehabilitation therapies to improve outcomes for DMD patients.

4.2 Duchenne Muscular Dystrophy: A Difficult Disease to Treat

DMD is a debilitating X-linked genetic disorder affecting 1:3500–6000 males worldwide, caused by mutations in the dystrophin (*DMD*) gene that result in an absence of the dystrophin protein. In striated muscle, the dystrophin protein is a component of the dystrophin-glycoprotein complex (DGC), a multimeric protein complex located at the sarcolemma of striated muscle fibers which links the intracellular actin cytoskeleton to the extracellular matrix (ECM) to transmit the forces generated by muscle contraction (Ervasti and Campbell 1993). The loss of dystrophin destabilizes this link, causing failure of the DGC to assemble at the sarcolemma (Ohlendieck and Campbell 1991). The loss of integrity leads to a progressive loss of muscle mass and function and early lethality in DMD patients. The current gold-standard treatment for most DMD patients is administration of corticosteroids that can slow the pathologic progression and prolong ambulation (Manzur et al. 2004), but does not cure the disease.

DMD is not an easy disease to treat or cure. Within affected patients, all muscle fibers and stem cells within the body contain a defective *DMD* gene. Therefore, a vast volume of tissue requires restorative, lifelong treatment. This is further complicated by the fact that the dystrophin protein is expressed locally (i.e., it does not circulate) and must therefore be delivered into every muscle cell, or at least a significant proportion of muscle fibers. Furthermore, although DMD is a monogenic disorder, over 1800 different mutations have been reported within the *DMD* gene that result in DMD, including nonsense and missense mutations, duplications, and

deletions. It is therefore unlikely that a single therapy will be effective for all patients. Clinical trials have also demonstrated that the therapeutic dystrophin protein may be identified as "foreign" by the immune system of some patients, resulting in immune-mediated destruction of the therapeutic protein. Dystrophin is a large, complex protein, originating from the largest known gene in the human genome, and it is therefore a difficult gene to reintroduce into the body. Therapeutic approaches must therefore consider factors such as: the route of delivery; the carrying capacity of vector systems; the ideal timing for administration; the requirement for patient-specific versus generic therapies; and the potential for an immune response to both the delivery vehicle and the therapeutic protein. Efforts to identify a cure, via gene, cell, pharmacologic, and/or physical therapies remain ongoing and are discussed in detail.

4.3 Gene Therapies for DMD

The development of dystrophin restoration therapies for DMD has received much attention since the discovery of the causative mutation in dystrophin over 30 years ago (Hoffman et al. 1987a). This discovery was a key milestone in the development of a potential cure for DMD, with patient advocates and research communities working on the assumption that once the underlying genetic defect was identified, a cure would soon follow. This proved not to be the case, with the development of gene replacement therapies for DMD facing many hurdles. However, over in the most recent 10–15 years, significant progress has been made toward developing both viral and non-viral gene correction/replacement strategies, with a small number being approved for conditional use in DMD patients.

4.3.1 Gene Correction Strategies

4.3.1.1 Stop Codon Readthrough

Stop codon readthrough refers to the process by which various drugs facilitate the continuation of mRNA translation to restore protein expression. The process, originally referred to as "phenotypic suppression" was first observed for the aminoglycoside antibiotics in bacteria and yeast (Palmer et al. 1979; Singh et al. 1979). Within the population, it is estimated that approximately 10–15% of all DMD cases arise from nonsense mutations, in which mutations result in the introduction of a premature stop codon (Aartsma-Rus et al. 2006). Therefore, a significant proportion of DMD patients would benefit from stop codon readthrough-based therapy. Gentamicin, an aminoglycoside antibiotic, was the first such drug tested for treating DMD, and was shown to restore dystrophin expression in DMD myoblasts in vitro and restore sarcolemmal dystrophin expression with some functional benefit in *mdx*

dystrophic mice (Barton-Davis et al. 1999). Several clinical trials have assessed the potential for gentamicin to increase dystrophin protein expression and improve pathology in DMD patients (Dunant et al. 2003; Malik et al. 2010; Wagner et al. 2001; Politano et al. 2003). Of these, the most comprehensive showed increases in dystrophin protein expression up to 15% of normal levels, with reduced serum creatine kinase (CK) levels and stabilization of muscle strength after 6 months of intravenous administration (Malik et al. 2010). Based on the success of these studies, more potent drugs were developed with the potential to drive stop codon readthrough, the most successful being ataluren (PTC142, Translarna®). Ataluren received approval from the European Medicines Agency (EMA) in 2014 for the treatment of ambulant patients aged 5 years and older with DMD resulting from a nonsense mutation in the *DMD* gene. Compared with gentamicin which requires intravenous administration, ataluren is orally bioavailable and therefore repeat administration is more feasible. The therapeutic potential of ataluren remains under investigation in clinical trials, particularly to determine efficacy in non-ambulatory patients. Early studies suggest that earlier intervention with ataluren leads to the best outcomes (Ruggiero et al. 2018).

4.3.1.2 Exon Skipping

The concept of exon skipping was first proposed in the mid-to-late 1990s, as a method to reduce the severity of some genetic mutations. A large majority of DMD-causative mutations arise from missense mutations, in which a mutation induces a shift in the mRNA reading frame of the *DMD* gene, resulting in a lack of dystrophin protein production. Exon skipping aims to correct the reading frame by inducing the "skipping" of mutation-containing exons during pre-mRNA splicing using antisense oligonucleotides (AONs) that interfere with the splicing of targeted exons. Therefore, skipping over exons containing these frame-shift mutations facilitates the restoration of a smaller, but functional, dystrophin protein that can reduce disease severity. While mutations have been identified throughout the *DMD* gene, 70% of DMD patients have a mutation in a "hot-spot" in the central genomic region, between exons 45 and 53 (Den Dunnen et al. 1989; Koenig et al. 1989; Nobile et al. 1997). Therefore, the majority of exon skipping strategies have to date focused on this region of the gene, with the aim of treating as large a percentage of the DMD patient population as possible with a small number of drugs.

The most studied tools for exon skipping include the AONs, the phosphorodiamidate morpholino oligomers (PMOs), and the peptide conjugated PMOs (PPMOs). Exon skipping with AONs was first shown in the early 2000s to restore dystrophin protein expression in human myotubes in vitro and reduce disease severity in *mdx* mice in vivo (van Deutekom et al. 2001; Lu et al. 2003), but initial clinical trials demonstrated variable benefit and FDA approval was subsequently denied in 2016 (Flanigan et al. 2014; Goemans et al. 2011, 2016, 2018; Voit et al. 2014). Subsequent testing of a morpholino-based drug showed levels of dystrophin protein restoration between 10 and 50% and improved skeletal muscle function in

the *mdx* mouse (Alter et al. 2006). This was followed by significant improvements in DMD patient ambulation and respiration in clinical trials (Mendell et al. 2013), leading to FDA approval in 2016 of the first exon skipping therapy for DMD patients amenable to exon 51 skipping, eteplirsen. Since then, a further three drugs have been approved by the FDA for treatment of DMD patients amenable to exon 53 (golodirsen, Vyondys 53®, approved in 2019; viltolarsen, Viltepso®, approved in 2020) and exon 45 skipping (casimersen, Amondys 45®, approved in 2021). Exon skipping is at the forefront of DMD therapeutics, with significant promise to improve the lives of many DMD patients.

4.3.1.3 Gene Editing

The most recent strategy for gene correction in DMD utilizes clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing technology. CRISPR/Cas9-mediated genomic editing was first demonstrated to correct the dystrophin mutation in the *mdx* mouse in 2014 (Long et al. 2014). Injection of guide RNAs (sgRNA) and the Cas9 enzyme into *mdx* mouse zygotes, to correct the mutation in the germ line, restored dystrophin protein expression 17–83% in skeletal muscles and the hearts of treated mice (Long et al. 2014). Studies are currently focussed on optimizing the therapeutic potential for CRISPR/Cas9 gene editing to correct dystrophin mutations in postnatal mice (Xu et al. 2016; Long et al. 2016). Investigations are currently focussed on combining gene editing technology with adeno-associated viral (AAV) vector delivery.

4.3.2 Gene Replacement Strategies

4.3.2.1 Delivery of Plasmid DNA

Early methods attempted to reintroduce the *DMD* gene by direct injection of a plasmid containing the *DMD* gene into the skeletal muscles of *mdx* mice. While this restored dystrophin expression in injected muscles, efficiency was only around one percent due to the limited ability of skeletal muscle fibers to take up the large *DMD* gene (Acsadi et al. 1991; Danko et al. 1993). Subsequent attempts utilized adjunct methods to improve uptake of the plasmid DNA, including electroporation, chemical induction of injury, liposome encapsulation, copolymer administration, and co-administration of hyaluronidase (Wells 1993; Yanagihara et al. 1996; Baranov et al. 1999; Vilquin et al. 2001; Danialou et al. 2002; Gollins et al. 2003; Murakami et al. 2003; Ferrer et al. 2004; Molnar et al. 2004; Richard et al. 2005; Wong et al. 2005). While these methods enabled delivery of the full-length dystrophin cDNA to the muscle, the process was highly inefficient and not amenable to systemic gene restoration. More recently, groups have demonstrated the potential for systemic delivery of plasmid DNA to reach multiple muscles, including the

diaphragm (Liu et al. 2001; Zhang et al. 2004, 2010). While exciting, these methods are invasive and therefore currently limited in their potential for clinical translation.

4.3.2.2 Viral-Mediated Gene Therapy

Viral-mediated gene delivery strategies utilizing vectors and viruses carrying genetic material into cells, are at the forefront of DMD gene therapies. While some viral vectors contain large genetic constructs, the carrying capacity of most vectors is a limiting factor. The *DMD* gene, at 14 kb, is the largest gene in the human genome and therefore presents significant challenges for gene delivery systems, with most viral vectors being too small to carry such a large gene. However, in 1990, a patient with very mild Becker muscular dystrophy (BMD, a dystrophinopathy like DMD, but with a milder phenotype) was identified with a mutation that resulted in deletion of 46% of the *DMD* gene, demonstrating that large portions of the gene could be removed yet continue to make a reasonably functional protein (England et al. 1990). Subsequent mutagenesis studies revealed a series of dystrophin mini- and micro-genes that were small enough to be packaged into even the smallest of viral vectors (Harper et al. 2002).

4.3.2.2.1 Adenoviral Vectors

The first viral vectors to deliver dystrophin to *mdx* mice were based on the adenoviruses. The adenoviruses have a double stranded (ds)DNA genome with a 35 kb capacity, and therefore the capacity to carry the entire *DMD* gene. First-generation adenoviral vectors were generated by removal of the E1 and/or E3 viral genes, resulting in a carrying capacity of approximately 8 kb. These vectors successfully delivered a miniaturized *DMD* gene to *mdx* mice via intramuscular injection (Ragot et al. 1993; Vincent et al. 1993). However, the presence of the remaining viral genes limited the potential for these vectors to be delivered systemically due to risk of an immune response. The newer generation vectors, termed "gutted" adenoviral vectors, lack all viral genes and therefore have the capacity to deliver the full-length *DMD* gene (Hartigan-O'Connor et al. 2002). Gutted adenoviral vectors have effectively delivered the full-length dystrophin protein to skeletal muscle after direct intramuscular injection into both adult and newborn mice, resulting in stable protein expression and functional improvements up to a year after injection (Dudley et al. 2004; Gilbert et al. 2001, 2003). Unfortunately, the use of these vectors is limited by the ongoing risk of an immune response, and therefore not suitable for systemic administration and potential clinical application.

4.3.2.2.2 Lentiviral Vectors

Vectors based on lentiviruses, which have an RNA genome with an 8 kb capacity, have also been utilized for delivery of dystrophin mini-genes. Early studies demonstrated that injection of lentiviral vectors containing a dystrophin mini-gene could confer almost lifelong restoration of dystrophin protein expression and functional improvement when subcutaneously injected into a newborn mouse; proposed to be a result of in vivo targeting of the muscle stem cells (Kobinger et al. 2003; Kimura et al. 2010). This benefit was reduced when injected intramuscularly into an adult mouse (Kobinger et al. 2003). As lentiviral vectors have the capacity to integrate into the genome, they possess the ability to confer lifelong protection by integrating into the DNA of muscle stem cells, enabling restoration of dystrophin protein into muscle fibers as a consequence of injury-repair. However, this comes with a significant risk of insertional mutagenesis, and so in vivo systemic delivery of lentiviral vectors to treat skeletal muscle is not therapeutically viable. Instead, the use of lentiviral vectors is widespread in the treatment of muscle stem cells, facilitating autologous muscle stem cell transplantation.

4.3.2.2.3 Adeno-Associated Viral Vectors

The adeno-associated viruses (AAVs) are small, DNA parvoviruses with a single stranded (ss)DNA genome with a 5 kb capacity. Although vectors based on AAV are limited to the delivery of only small dystrophin micro-genes, these vectors have shown the most promise for viral-mediated DMD gene therapy. There are many different AAV capsid serotypes, with AAV1, 6, 7, 8, 9, and 10 having a high tropism for striated muscle, effectively transducing both skeletal and cardiac muscle after systemic administration (Muraine et al. 2020; Zincarelli et al. 2008). Delivery of different mini- and micro-dystrophin genes with various AAV serotypes, restored dystrophin protein expression and ameliorated disease in skeletal and cardiac muscle (of mice) after systemic administration (Fabb et al. 2002; Lai et al. 2005; Liu et al. 2005; Wang et al. 2000; Watchko et al. 2002; Yoshimura et al. 2004; Yue et al. 2003). Systemic delivery of a micro-dystrophin gene with an AAV6 vector rescued the pathology and improve lifespan in the severely dystrophic dystrophin/utrophin double knockout mouse (Gregorevic et al. 2006). This was the first evidence that AAV-mediated micro-dystrophin gene therapy might be a viable treatment for DMD. Early clinical trials with an optimized AAV vector and micro-dystrophin gene failed to restore dystrophin protein expression and improve pathology, which was attributed to patients developing an immune response to the therapeutic dystrophin protein (Mendell et al. 2010). Both the AAV vectors and dystrophin micro-genes underwent significant optimization and three different clinical trials were underway as of 2021, with Sarepta, Pfizer, and Solid Biosciences, demonstrating therapeutic potential of AAV-micro-dystrophin gene therapy for DMD.

While AAV-mediated micro-dystrophin gene therapy is progressing through clinical trials, researchers are investigating the therapeutic potential of combining the AAV-mediated delivery system with exon skipping and CRISPR technologies, to enable systemic in vivo gene editing. In vivo gene editing was first reported by two groups in 2016 and 2017, using AAV9 and AAV6 to systemically deliver the CRISPR/Cas9 machinery to muscles of dystrophic mice (Bengtsson et al. 2017; Tabebordbar et al. 2016). These studies showed improved functional outcomes (Bengtsson et al. 2017) and correction in both muscle stem cells and differentiated muscle fibers of dystrophic mice (Tabebordbar et al. 2016). Subsequent studies have aimed to improve the efficacy of this methodology using self-complementary (sc)AAV vectors (Zhang et al. 2020), testing the smaller *Streptococcus aureus* Cas9 to enable packaging into a single AAV vector (Zhang et al. 2021), and utilizing a Pax7nGFP;Ai9 dual reporter to specifically correct mutations within muscle stem cells via CRISPR/Cas9 delivery using AAV9 in vivo, thus conferring lifelong disease correction (Kwon et al. 2020). Together, this combination of viral delivery of gene editing tools provides a powerful and exciting toolkit for DMD gene therapy.

4.4 Cell-Mediated Therapies for DMD

While gene therapies should eventually provide a cure for DMD, not all patients may benefit, depending on the progression of the disease. For example, older DMD patients will likely present with significant replacement of their skeletal muscle fibers with fat and fibrotic tissue, which not only limit the amount of muscle able to be corrected but serve as physical barriers for some treatments. Cell-mediated therapies with potential to replace skeletal muscle fibers will be similarly important in the treatment of DMD, especially for older patients. Although cell therapies for DMD have not advanced as far as gene therapies, several advances have been made over the past 10–15 years.

4.4.1 Myoblast/Muscle Stem Cell Transplantation

Myoblasts were the initial choice for muscle cell transplantation to restore muscle due to their ease of isolation, and demonstrated potential when injected into the muscles of *mdx* mice (Partridge et al. 1989). Unfortunately, subsequent clinical trials identified only 10% dystrophin-positive fibers in muscles of immunosuppressed DMD patients despite multiple cell injections. These disappointing results were attributed to the poor survival of the injected cells in vivo, limited migration from the injection sites, and an inability to participate in long-term regeneration as the muscle stem cell population was not restored (Mendell et al. 1995). Myoblasts therefore have limited therapeutic potential as a cell population for treating DMD.

The ability to successfully isolate muscle stem cells from skeletal muscle, first characterized in a *Pax3*-GFP knock-in mouse (Montarras et al. 2005), was a significant advance in the mid-2000s. Injection of freshly isolated cells into muscles of *mdx* mice resulted in significant engraftment and improvements in muscle function (Cerletti et al. 2008), as well as successful engraftment into the muscle stem cell niche (Montarras et al. 2005; Sacco et al. 2008), demonstrating potential for long-term therapeutic benefit. While these early studies were encouraging, it became apparent that the myogenic potential and survival/migration of these cells were severely reduced if the cells were cultured prior to injection (Montarras et al. 2005). Studies have since focused on developing strategies to improve the survival and engraftment of these cells, most of which have attempted to mimic the conditions within the muscle stem cell niche to promote a more quiescent, self-renewal state. Both culture substrate rigidity and oxygen levels have been demonstrated to be important in the maintenance of "stemness" in cultured muscle stem cells to enhance the efficacy of myoblast transplantation in vivo (Duguez et al. 2012; Gilbert et al. 2010; Liu et al. 2012). Investigations are ongoing to identify the "ideal" culture conditions to expand muscle stem cells for transplantation.

While allogenic transplants have been successful in immunocompetent mice (Vilquin et al. 1995), autologous transplantations are preferred because they limit the possibility of immune responses to the transplanted cells. Advances in the development of induced pluripotent stem cells (iPSCs) and their differentiation into myogenic cells have led to further developments. Isolation and differentiation of patient-derived iPS cells into myogenic progenitors have been successful in vitro and genetic correction of both these and patient-derived myoblasts has been shown feasible using lentiviral, transposon, and CRISPR-based technologies (Filareto et al. 2013; Ifuku et al. 2018; Kazuki et al. 2010; Li et al. 2015; Quenneville et al. 2007; Young et al. 2016). Myoblast transplants remain a viable option for targeted replacement of lost muscle tissue in DMD patients.

4.4.2 Mesoangioblasts and Pericytes

While myoblast transplantation continues to be optimized in preclinical and clinical studies, one major drawback is that myoblasts are not suitable for systemic delivery, meaning their use is limited to local, intramuscular delivery. As DMD patients will require dystrophin restoration in the entire musculature, including the diaphragm, this reduces their potential as a standalone therapy. Mesoangioblasts, and a related cell population, termed "pericytes," are alternative cell populations which became attractive candidates for muscle cell transplantation due to their myogenic potential and suitability for systemic delivery (Dellavalle et al. 2007; Minasi et al. 2002). Mesoangioblasts engraft efficiently into skeletal muscle, restore dystrophin expression, and improve functional and histological parameters in dystrophic mice and dogs when delivered either intramuscularly or systemically (Berry et al. 2007; Sampaolesi et al. 2006). Based on these encouraging studies, an initial clinical trial

tested the efficacy of intraarterial delivery of human leukocyte antigen (HLA)-matched donor mesoangioblasts in five DMD patients, demonstrating delivery of the cells to be reasonably safe and well-tolerated, but with little or no restoration of dystrophin expression. This was attributed to the advanced disease progression in the patients, the impact of steroid therapy on the cells (reduced extravasation), and an insufficient number of injected cells (Cossu et al. 2015). The therapeutic potential of these cells to treat DMD remains under investigation. The potential for autologous transplantation has been demonstrated using PiggyBac transposons to correct the genetic defect in mesoangioblasts from dystrophic SCID-*mdx* mice. This resulted in 11–44% restoration of dystrophin expression after transplantation back into the mice which was stable for up to 5 months (Iyer et al. 2018). Other studies turned to differentiating iPS cells into mesoangioblasts for transplantation, with and without lentiviral transduction to restore genetic insufficiencies, in mouse models of other muscular dystrophies (Tedesco et al. 2012; Gerli et al. 2014). These blood-vessel associated progenitors hold promise for DMD, and their therapeutic potential continues to be investigated.

4.5 Pharmacological Therapies for DMD

Although gene- and/or cell-mediated therapies have significant potential to eventually cure DMD, the success of these approaches will likely be reduced in patients with a more advanced pathology. Therefore, pharmacologic interventions that can delay the disease progression by tackling different aspects of the dystrophic pathology, remain crucial treatments for DMD patients.

4.5.1 Targeting Myostatin

Myostatin signaling is a potent negative regulator of skeletal muscle mass. Inhibition of the myostatin signaling pathway has proved promising in preclinical studies in dystrophic mice, with antibody treatments increasing muscle mass and force production, and decreasing fibrosis (Morine et al. 2010; Murphy et al. 2010b; Pistilli et al. 2011; Nakatani et al. 2008; Parsons et al. 2006; Bogdanovich et al. 2002). Various strategies to inhibit myostatin signaling have since moved into clinical trials. AAV-mediated overexpression of the myostatin inhibitor, follistatin, using AAV1-FS344 increased muscle mass and strength in dystrophic mice and non-human primates (Haidet et al. 2008; Kota et al. 2009), and was subsequently proven safe and efficacious in BMD patients, with improved outcomes in the six-minute walk test (Mendell et al. 2015). AAV1-FS344 has subsequently progressed to Phase I/II trials with intramuscular injections in DMD patients, although the results have yet to be reported.

Antibody-directed myostatin inhibition also attenuated muscle atrophy in mouse models of muscle atrophy, including cachexia, disuse, sarcopenia, and muscular dystrophy (Murphy et al. 2010a, b, 2011a, b), leading to the development of anti-myostatin antibodies (MYO-029, PF-06252616) which had variable success in clinical trials (Krivickas et al. 2009; Singh et al. 2016; Wagner et al. 2008). Fusion of a soluble activin receptor inhibitor to IgG (ActRIIB-IgG), which reduces signaling through the TGF-β pathway, increased muscle mass in injected muscles of dystrophic mice with reduced off-target effects (Pearsall et al. 2019), and was shown safe in healthy volunteers in clinical trials (Glasser et al. 2018). Disappointingly, despite myostatin inhibition showing great promise in preclinical models, clinical trials have not progressed due to a lack of demonstrated efficacy (reviewed in Wagner 2020). The reasons for the lack of efficacy in patients are unclear but are thought to be attributed to differences in myostatin expression in mice relative to humans, a lack of improvement in muscle function despite increases in mass, and potential interference between corticosteroid therapy and myostatin inhibition (Rybalka et al. 2020).

4.5.2 Reducing Inflammation and Fibrosis

4.5.2.1 HDAC Inhibition

Compounds that inhibit class I and/or II histone deacetylases (HDACs) have been shown to ameliorate dystrophic pathology in the *mdx* mouse (Colussi et al. 2008; Johnson et al. 2013; Minetti et al. 2006; Vianello et al. 2014). In preclinical studies using two murine models of DMD, the HDAC inhibitor, givinostat, increased muscle mass and muscle fiber size, reduced fat and collagen deposition, and improved fatigue resistance after oral administration (Consalvi et al. 2013; Licandro et al. 2021). High dose administration of givinostat improved muscle function and pathology to a greater extent than conventional steroid (glucocorticoid) therapy in severely dystrophic mice (Licandro et al. 2021). Givinostat is in clinical trials to test efficacy in DMD patients. Oral administration of givinostat to ambulant DMD boys aged 7–11, who were already receiving corticosteroid treatment, for greater than 1 year, reduced fibrosis, necrosis, and fatty tissue deposition in muscle biopsies, showing the drug to be safe for long-term administration and effective in delaying aspects of the pathology (Bettica et al. 2016). Givinostat is a promising intervention for DMD patients and Phase III trials are ongoing with results expected in 2022.

4.5.2.2 NF-κB Inhibition

Signaling via the nuclear factor kappa B (NF-κB) pathway is tightly linked to changes in skeletal muscle mass. Transgenic mice with chronically elevated NF-κB signaling have severe wasting of limb and trunk muscles and conversely, NF-κB inhibition protects against wasting (Cai et al. 2004; Mourkioti et al. 2006).

Increased NF-κB signaling is linked to disease progression in DMD patients and in mouse models of DMD, and targeted inhibition of NF-κB is an attractive therapeutic option for skeletal muscle atrophy. Preclinical studies in dystrophic mice showed inhibition of NF-κB improved skeletal muscle structure and function (Hammers et al. 2016). Edasalonexent (CAT-1004) is an oral NF-κB inhibitor under investigation for treatment of DMD patients, shown to effectively inhibit NF-κB signaling and to be well-tolerated in Phase I safety studies in healthy adults and pediatric DMD patients (Donovan et al. 2017; Finanger et al. 2019).

Another promising NF-κB inhibitor, VBP15, is in development by ReveraGen BioPharma as an alternative to current glucocorticoids for DMD. In *mdx* mice, VBP15 produced consistent improvements in muscle inflammation and function, with increased fore- and hind-limb grip strength and improved ex vivo contractile properties in the extensor digitorum longus (EDL) muscle (Heier et al. 2013). Importantly, VBP15 has been proposed to inhibit NF-κB signaling to a greater extent than traditional steroid therapy, with reduced off-target effects (Conklin et al. 2018; Hoffman et al. 2018). Phase I and Phase IIa studies demonstrate VBP15 to be well-tolerated with benefits to motor function in young DMD patients (Smith et al. 2020; Mavroudis et al. 2019). Phase III trials are currently underway.

4.5.2.3 Inhibition of Collagen

Halofuginone (HT-100) had potent anti-fibrotic properties in multiple mouse models of disease with fibrosis and was tested in dystrophic mice to determine its efficacy for attenuating fibrosis in skeletal muscle. In *mdx* mice, halofuginone administration inhibited muscle fibrosis and improved muscle histopathology and strength (Turgeman et al. 2008). In addition, halofuginone has been shown to have direct effects on muscle cells, enhancing cell survival and myoblast fusion in both primary and C2C12 myoblasts (Bodanovsky et al. 2014; Roffe et al. 2010). HT-100 was in clinical trials to test safety and efficacy in DMD patients, but extended Phase II studies to study long-term impacts of HT-100 administration were terminated in 2016 after the unexpected death of a patient. No further updates have been provided.

4.5.2.4 Sodium/Proton Exchanger Type 1 (NHE-1) Inhibition

In the skeletal muscles and hearts of patients and mouse models of DMD, membrane tears caused by mechanical stress during contraction can lead to Ca^{2+} influx and consequent increase in $[Na^{+}]$. Rimeporide, an NHE-1 inhibitor, has potent anti-inflammatory and anti-fibrotic effects in both skeletal and cardiac muscles in *mdx* mice (Porte-Thome et al. 2015), and demonstrated improved cardiac function in dystrophic dogs (Ghaleh et al. 2020). Phase 1b studies found Rimeporide to be well-tolerated and positive indications as a cardioprotective treatment in DMD patients (Previtali et al. 2020). Further clinical development is underway.

4.5.3 Upregulation of Utrophin

Since dystrophin gene replacement therapies may induce immune responses in some DMD patients, a possible safer alternative could be the therapeutic upregulation of the related protein, utrophin. In the *mdx* mouse, upregulation of utrophin can compensate for the loss of dystrophin (Tinsley et al. 1996, 1998). This was the basis of the development of the first orally bioavailable small molecule upregulator of utrophin, SMT-C1100, which was shown to reduce inflammation and fibrosis and improve force production in muscles of sedentary and exercised *mdx* mice (Tinsley et al. 2011). Phase Ia and Phase Ib trials confirmed SMT-C1100 to be safe and well-tolerated in healthy adults and in DMD patients (Ricotti et al. 2016; Tinsley et al. 2015). However, drug blood plasma concentrations were found to be lower in DMD patients than in healthy adults when administered at the same dose; an effect attributed to differences in diet and other disease-related factors. Absorption was subsequently improved in patients on a controlled diet (Muntoni et al. 2019). Unfortunately, development of SMT-C1100 was discontinued in 2018 due to it not meeting primary and secondary endpoints at the conclusion of the Phase II trial (Babbs et al. 2020). Preclinical development is continuing on a second-generation compound, SMT022357, which improved the dystrophic phenotype in *mdx* mice along with improved absorption, distribution, metabolism, and excretion profiles compared to SMT-C1100 (Babbs et al. 2020; Guiraud et al. 2015).

4.5.4 Improving Membrane Stability

As calcium ion (Ca^{2+}) influx is thought to be a primary initiator of skeletal and cardiac muscle cell degeneration in DMD, compounds able to seal damaged membranes have therapeutic potential. Poloxamer-188 (P-188) is a non-ionic tri-block copolymer that can act as a membrane sealant after different types of injury. P-188 was cardioprotective after systemic administration in both mouse and dog models of muscular dystrophy. One- or 2-week treatments improved left ventricle function and promoted survival in mice after challenge with cardiac stimulants and reduced myocardial fibrosis and left ventricle remodeling in dystrophic dogs after chronic infusion for 8 weeks (Spurney et al. 2011; Townsend et al. 2010; Yasuda et al. 2005). In addition, in vitro studies showed improvements in dystrophic skeletal muscle after P-188 treatment (Spurney et al. 2011; Ng et al. 2008). P-188 (Carmeseal-MD™) improved respiratory and cardiac function in dystrophic mice (Markham et al. 2015), although some studies have reported less success with respect to ameliorating contraction-induced injury in muscles of *mdx* mice (Terry et al. 2014). Although most studies in animal models report positive benefits, studies examining the safety and efficacy of P-188 for DMD, are warranted.

4.6 Physical Therapies

Skeletal muscle is comprised of functionally diverse fibers that can differ in their size, metabolism, and contractility; with extremes being classically referred to as "*slow oxidative*" or "*fast glycolytic*" (Egan and Zierath 2013). Based on myosin heavy chain (MyHC) protein isoforms, which largely dictate the rate of force development, shortening velocity and the rate of cross-bridge cycling, slow oxidative (type I) fibers are typically small with a high oxidative capacity and fatigue resistance compared with fast glycolytic (type II) fibers that are typically larger, reliant on glycolysis and highly fatigable (Egan and Zierath 2013). Subtypes of the fast fibers (type IIa, type IIx) vary in their reliance on oxidative and glycolytic metabolism. Most mammalian muscles are usually comprised of different proportions of these four main fiber types (type I, type IIa, type IIb, and type IIx), although muscle fibers can exist along a continuum, with subtypes exhibiting different variations of the main attributes, especially metabolic features, as revealed by single fiber proteomics (Schiaffino et al. 2020).

Muscle fibers can exhibit remarkable plasticity, capable of altering their intrinsic structural, functional, metabolic, and molecular properties in response to changes in loading, contractile activity, and circulating hormones (Pette and Vrbova 1999; Blaauw et al. 2013; Lynch 2017; Schiaffino and Reggiani 2011). This plasticity was first demonstrated through pioneering nerve cross-reinnervation studies in cats, which revealed when fast muscles were innervated by a slow nerve, the muscle transformed from a fast (glycolytic) to a slower, more oxidative phenotype and contracted more slowly. When slow muscles were innervated by a fast nerve, the muscle transformed from an oxidative to a more glycolytic phenotype and contracted more quickly. Such phenotypic changes were attributed to the specific impulse patterns delivered to the muscle via the motor neuron (Buller et al. 1960). Muscular contractions through physical activity (exercise) can be an effective stimulus to induce adaptations in muscle if exercise duration and intensity are sufficient. Endurance exercise (e.g., running, cycling) can increase muscle oxidative capacity and fatigue resistance, while resistance exercise (e.g., lifting weights) increases fiber size (hypertrophy) and strength (Egan and Zierath 2013).

In DMD and well-characterized murine models of the disease linked to the genetic loss of the protein dystrophin, fast muscle fibers are more susceptible to contraction-mediated damage and pathological progression than slow muscle fibers, which are resistant to injury and relatively spared (Webster et al. 1988). Although a cure for DMD will eventually come from the corrective gene therapies described earlier, limitations of delivery systems, gene carrying capacity, dissemination efficiency, expression persistence, and immunological tolerance, all pose significant obstacles for clinical application. There remains an urgent and unmet clinical need for therapies that can ameliorate the pathology, preserve and protect dystrophic muscles from damage. Physical modalities such as exercise have many localized and systemic health benefits, and therefore may ultimately serve as adjuvant therapies for any gene- or cell-based approaches.

Much of our understanding as to whether physical activity and exercise training interventions can improve quality of life in DMD patients has come from exercise studies conducted in appropriate mouse models, particularly *mdx* dystrophic mice. These studies have examined: (1) whether exercise exacerbates the dystrophic pathology, typically from high-intensity, involuntary exercise protocols; or (2) the therapeutic potential of low-intensity, exercise protocols (voluntary and involuntary) to attenuate the dystrophic pathology (Grange and Call 2007). Several exercise protocols in *mdx* mice have demonstrated beneficial adaptations, with some of these low-intensity exercises having translational relevance for DMD.

4.6.1 Involuntary Exercise

It is generally accepted that low-intensity, low-weight bearing exercise promote beneficial adaptations for the dystrophic pathology, whereas exercises involving potentially injurious lengthening (i.e., eccentric) contractions may aggravate the pathology (Markert et al. 2012). Several models of exercise training have been developed and utilized in preclinical studies that have improved our understanding of the therapeutic potential of exercise for muscular dystrophy (reviewed in Hyzewicz et al. 2015; Markert et al. 2011).

4.6.1.1 Treadmill Running

Treadmill exercise training in healthy mice promotes adaptations similar to endurance exercise in humans. One main advantage of treadmill exercise is that it allows researchers to precisely control the training workload (e.g., frequency, intensity, duration) to interrogate specific muscle adaptations to submaximal or maximal workloads. Most studies have demonstrated detrimental effects of treadmill exercise training in *mdx* mice (reviewed in Hyzewicz et al. 2015), especially since, in most cases, exercise intensity was matched to levels achieved by otherwise healthy wild-type mice. When performed at lower intensities, treadmill running has been shown to promote beneficial adaptations to the dystrophic pathology, including decreasing intramuscular collagen deposition (Fernandes et al. 2019; Gaiad et al. 2017), reducing serum creatine kinase levels (Hall et al. 2007), and increasing activity of antioxidant enzymes (Fernandes et al. 2019).

4.6.1.2 Swimming

Swimming exercise training has been widely used in *mdx* mice to examine acute responses and training adaptations to endurance exercise. Swimming activity recruits muscle groups throughout body and can be used to monitor adaptations in the heart, diaphragm, and limb skeletal muscles. Endurance swimming (2 h/day,

5 days/week, for 10–20 weeks) improved the functional capacity of hindlimb muscles (in *mdx* mice) through adaptations arising from an increased proportion of oxidative fibers and reducing muscle fatigue (Lynch et al. 1993; Hayes et al. 1993). Favorable adaptations to low-intensity, swim exercise could also be achieved in older *mdx* mice (Hayes and Williams 1998), at a time when the progressive pathology more closely mimics that of DMD. Swim training was shown to improve the dystrophic pathology when combined with pharmacologic (Hayes and Williams 1997) and cell-based interventions (Bouchentouf et al. 2006). These and many other confirmative studies support the contention that low-intensity exercise, when performed alone or in combination with other interventions, may have therapeutic potential for muscle wasting conditions, including the muscular dystrophies.

4.6.1.3 Electrical Stimulation

Despite physical activity and muscular contraction, especially endurance exercise training, having many beneficial effects on muscle health, the sad reality is that many patients with neuromuscular diseases are simply unable to exercise, especially boys with DMD who are often confined to a wheelchair before their teens. Therefore, protocols of muscle contractions that can mimic the benefits of exercise are under investigation to determine whether such interventions can attenuate the loss of muscle health and function and potentially improve quality of life for patients.

Electrical stimulation to induce muscular contractions can provide an exercise-like stimulus that has been shown to induce beneficial muscle adaptations and clinical outcomes in adults with advanced progressive disease (Jones et al. 2016). Electrical stimulation at varying frequencies can be used to induce concentric, isometric, and/or eccentric muscle contractions, and several variations have been examined in preclinical mouse models and in DMD patients. Isometric contractions generate force or torque without a change in muscle length or joint angle. Repeated bouts of isometric contractions induced by percutaneous stimulation of the peroneal nerve in *mdx* mice improved aspects of the dystrophic pathology, including increased force production, satellite cell number, and myofiber hypertrophy, while reducing fibrosis and injury susceptibility (Lindsay et al. 2019).

Chronic low-frequency stimulation (LFS) mimics the electrical discharge pattern of slow motor neurons innervating slow muscles and induces downstream molecular signaling pathways that promote transcription of slow, more oxidative fiber-specific genes (Pette and Vrbova 1999). The resultant fast-to-slow adaptations include increased oxidative metabolism and mitochondrial biogenesis concurrent with fiber transitions in the type IIb > type IId/x > type IIa > type I direction (Leeuw and Pette 1993; Termin et al. 1989). While LFS can challenge a muscle to its full adaptive potential, it can do so efficiently and typically in the absence of injury and regeneration (Pette and Vrbova 1999). Therefore, LFS is an ideal model for investigating the therapeutic potential of promoting a slower, more oxidative muscle phenotype to ameliorate the dystrophic pathology. Initial studies of LFS in dystrophic mice were not conducted on mouse models of DMD (Dangain and Vrbova

1989; Luthert et al. 1980; Reichmann et al. 1981, 1983). Nonetheless, these studies showed that LFS exerted beneficial effects on laminin-deficient muscles in C57BL/6J-dy2j (*dy/dy*) mice, including improved strength (Dangain and Vrbova 1989; Luthert et al. 1980), and normalized enzyme activities (Reichmann et al. 1981, 1983).

More recently, we and others have evaluated the therapeutic merit of LFS in well-characterized mouse models of DMD. In dystrophin-deficient *mdx* mice, LFS (10 Hz, 12 h/day, 7 days/week, 28 days) was sufficient to induce remodeling of mitochondrial respiratory chain complexes, enhanced fiber respiration, and conferred protection from eccentric contraction-mediated damage (Hardee et al. 2021). However, this adaptive remodeling was attenuated or abrogated in dystrophic muscles lacking both dystrophin and utrophin (i.e., *dko* mice), highlighting a role for utrophin in the adaptations of dystrophic skeletal muscles. Using an alternate approach via transcutaneous surface stimulation, others have found that neuromuscular electrical stimulation (NMES) to *mdx* mice improved muscle-derived stem cell (MDSC) engraftment sufficient to enhance muscle strength, and, in combination with MDSC transplantation, improve recovery from fatigue (Distefano et al. 2013). Collectively, these findings highlight the therapeutic potential of LFS to ameliorate the dystrophic pathology and protect from contraction-induced injury with important implications for DMD and related muscle disorders.

4.6.1.4 LFS in DMD Patients

From a clinical perspective, there was considerable interest in LFS as a therapy for DMD during the 1970s through to the early 1990s, but after the discovery of dystrophin in 1987, unsurprisingly the field focused on addressing the dystrophic pathophysiology through molecular biochemical approaches (Hoffman et al. 1987a–c). These early studies found that electrical stimulation reduced the rate of deterioration of ankle dorsiflexors and quadriceps muscles in boys with DMD (Scott et al. 1990; Zupan 1992; Zupan et al. 1993, 1995), provided that the stimulation was performed before the patients were not severely disabled (Scott et al. 1986). Similar findings related to safety, practicality, and improved muscular strength and endurance have been reported in facioscapulohumeral muscular dystrophy (FSHD) and myotonic dystrophy type 1 patients (Colson et al. 2010; Cudia et al. 2016). While the initial studies on DMD patients were encouraging (e.g., some showing preserved strength), they were largely preliminary in nature (with few patients and studies of limited duration) with a lack of rigorous scientific and statistical clarity (Lynch 2017). Nonetheless, there remains a dearth of information on the application of such a well-described and utilized intervention like LFS (with existing applications in rehabilitation medicine and physical therapy) to ameliorate aspects of the dystrophic pathology.

4.6.2 Voluntary Exercise

4.6.2.1 Wheel Running

Providing access to running wheels in cages is one approach to elicit low-intensity endurance training adaptations in rodents. While this model permits normal physiological patterns of motor unit recruitment, it is dependent on intrinsic, voluntary physical activity and therefore total work performed can vary dependent on the animal's volition based on age and disease severity (Wineinger et al. 1998). Voluntary wheel running in *mdx* mice has been shown to promote favorable adaptations in plasma CK levels (Carter et al. 1995), improve force production (Baltgalvis et al. 2012; Call et al. 2010; Carter et al. 1995; Dupont-Versteegden et al. 1994; Hayes and Williams 1996; Hourde et al. 2013), decrease injury susceptibility (Hourde et al. 2013; Delacroix et al. 2018), improve fatigue resistance (Baltgalvis et al. 2012; Hayes and Williams 1996; Wineinger et al. 1998), and alter myofiber size and type (Delacroix et al. 2018; Hayes and Williams 1996; Landisch et al. 2008). Mechanisms mediating these functional outcomes were identified, such as increased utrophin protein expression (Gordon et al. 2014) and muscle oxidative capacity (Baltgalvis et al. 2012). A recent study combining voluntary wheel running and micro-dystrophin gene therapy reported improved running capacity, increased muscle contractile properties, protection from eccentric contraction-induced injury, and enhanced mitochondrial respiration (Hamm et al. 2021). Thus, exercise may be a complementary intervention for enhancing the efficacy of micro-dystrophin gene therapies. Further studies are warranted to examine the efficacy of combined interventions, including investigations of different aged mice and disease severities before this might be considered for potential clinical application.

While it is generally accepted that low-intensity exercise may be more beneficial for attenuating the dystrophic process, resisted wheel running exercise may also induce favorable adaptations such as enhanced muscle growth through myofiber hypertrophy, improved regeneration, and force production. Indeed, voluntary wheel running with progressive resistance in *mdx* mice was shown to improve grip strength and increase specific force of the soleus muscle (Call et al. 2010). Overall, these studies support the contentions that: (1) voluntary exercise is not detrimental to dystrophic pathology in mice; and (2) dystrophic skeletal muscles retain the adaptive potential to respond to muscular contractions, with favorable, clinically relevant outcomes.

4.6.2.2 Exercise in DMD Patients

4.6.2.2.1 Resistance Exercise

Initial studies examining physical training interventions in DMD patients utilized resistance-type exercise. At the medical conference for Muscular Dystrophy

Associations of America in 1952, Abramson and Rogoff (Abramson and Rogoff 1952) first reported in 27 patients with muscular dystrophy that active, assisted active, and resistive exercises (3 days/week, 7 months) led to improvements ($n = 13$) and/or no changes ($n = 13$) in the manual muscle chart. Based on these encouraging findings, Hoberman (Hoberman 1955) studied ten children with progressive muscular dystrophy. The patients performed 4 months of physical medicine and rehabilitation tests, instruction and training. While no changes in muscle strength were observed, there were improvements in performance of activities of daily living, vital capacity, and endurance (Hoberman 1955). Vignos and Watkins (1966) studied 24 patients with muscular dystrophy (14 DMD, 6 limb-girdle, and 4 facioscapulohumeral), who performed home-based resistance exercise program for 12 months. In the year leading up to the study, DMD patients exhibited declines in muscle strength. Unexercised controls continued to exhibit a decline in muscle strength, while the exercised group maintained or improved slightly from their initial muscle strength (Vignos and Watkins 1966). Most of the improvements in muscle strength occurred within the first 4 months of the exercise program and the degree of improvement was related to the initial strength of the exercised muscle; i.e., a stronger muscle improved more, while weaker muscles improved less. Unfortunately, functional improvements were not permanent and only occurred in 7/52 patients at 4 months and 1/52 patients at 12 months (Vignos and Watkins 1966). To determine if submaximal exercise could improve strength, four DMD patients performed unilateral isokinetic exercise of the quadriceps (4–5 days/week, 6 months). The authors reported a modest, though not statistically significant, increase in strength during the 6-month period, which was maintained for 3 months after the cessation of training (de Lateur and Giaconi 1979). More recently, Lott et al. (2021) reported that a 12-week in-home, remotely-supervised, mild-moderate intensity resistance isometric leg exercise program was safe, feasible, and increased strength (knee extension, knee flexion) and function (descending step) in ambulatory boys with DMD. Overall, these studies indicate that resistance-type exercise programs can improve aspects of activities of daily living and functional performance. It is noted and that the exercise program should be started earlier in the disease trajectory when muscles are most functional. Importantly, it also highlighted that type of regimen was feasible (e.g., no ill events reported) and did not cause deleterious effects on muscle strength in the patients. Regardless, exercise should be prescribed cautiously and the therapeutic merit of any resistance training for patients with neuromuscular diseases must be assessed against the risk for overwork and potential for exacerbating the pathology.

4.6.2.2.2 Endurance Exercise

More recently, the "No Use is Disuse" study was the first randomized control trial in ambulant and wheelchair-dependent DMD boys that examined whether low-intensity physical training through assisted cycling training using the arms and legs (5 days/week, 24 weeks) could improve muscle endurance and functional

capacity (Jansen et al. 2010). The authors found all participants could complete the training protocol (except one) with no serious adverse events reported. Importantly, the primary outcome of total Motor Function Measure remained stable with physical training, whereas it decreased in the control group (Jansen et al. 2013). However, no improvements in the Assisted 6-Min Cycling Test were observed. These findings suggest that assisted bicycle training of the legs and arms was feasible and safe for both ambulant and wheelchair-dependent DMD patients and that physical training helped maintain functional capacity.

Others have demonstrated improvements in clinically relevant outcomes with different types of endurance exercise modalities. Compared to range of motion exercises alone, upper extremity training with an arm ergometer (40 min/session, 3 days/week, 8 weeks) was more effective in preserving and improving the functional level of early-stage DMD patients (Alemdaroglu et al. 2015). Overall, the studies performed to date demonstrate that dystrophic skeletal muscles retain the capacity to adapt favorably to exercise training and this can attenuate the functional decline with disease progression. However, as highlighted in a recent Cochrane Review of exercise in muscle diseases (Voet et al. 2019), the evidence regarding endurance and resistance exercise training interventions in muscle diseases remains uncertain, and more research with robust methodology and greater numbers of participants are still required.

4.7 Conclusions

Improving quality of life for DMD patients through exercise requires activities that can improve function in all muscles of the body, ideally including the heart and respiratory muscles. Exercise has the capacity to improve or maintain physical function, body composition, and overall quality of life for patients. While endurance and resistance exercise training can individually promote health benefits, the muscle adaptive responses are unique to the stimulus/intervention provided. Exercise interventions could attenuate muscle weakness and dysfunction in DMD, and current international guidelines recommend regular submaximal exercise activities for boys with DMD (Bushby et al. 2010). Although studies support rehabilitation as an adjunct treatment to gene- and cell-mediated therapies for DMD patients (see Fig. 4.1), current recommendations are based on theories, practical experience of the practitioners, and knowledge gained from animal studies. Randomized, controlled trials are warranted to investigate the therapeutic merit of adjunct rehabilitation in conjunction with other interventions based on the benefits observed in preclinical models and DMD patients.

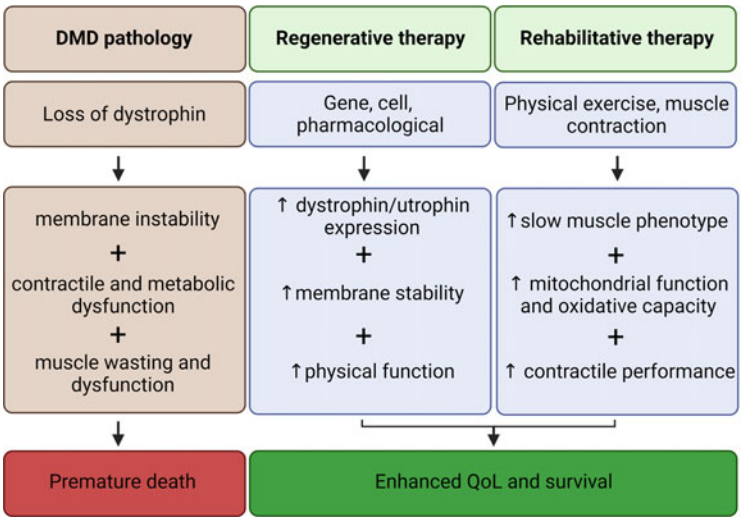

Fig. 4.1 Therapeutic combinations for DMD. Striated muscle pathology in DMD arises from a loss of the dystrophin protein causing membrane instability, contractile and metabolic dysfunction, and muscle wasting and weakness, ultimately resulting in premature death. Regenerative gene, cell, and/or pharmacologic therapies have varying efficacy for increasing dystrophin/utrophin protein expression to improve membrane stability and physical function. In addition, rehabilitative therapies including physical exercise and muscle contraction protocols elicited by electrical stimulation (e.g., neuromuscular electrical stimulation, functional electrical stimulation, or low-frequency stimulation) have also shown promise for improving skeletal muscle pathology by potentially driving a slow muscle phenotype that improves mitochondrial function, oxidative capacity, and contractile performance. Combinations of regenerative and rehabilitative therapies have significant potential to improve quality of life and survival in DMD patients. Created with BioRender.com

References

Aartsma-Rus A, Van Deutekom JC, Fokkema IF, Van Ommen GJ, Den Dunnen JT (2006) Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. Muscle Nerve 34(2):135–144. https://doi.org/10.1002/mus.20586

Abramson AS, Rogoff J (1952) An approach to the rehabilitation of children with muscular dystrophy. Physical treatment in muscular dystrophy. Paper presented at the 1st and 2nd medical conferences, Muscular Dystrophy Association of America, New York

Acsadi G, Dickson G, Love DR, Jani A, Walsh FS, Gurusinghe A, Wolff JA, Davies KE (1991) Human dystrophin expression in mdx mice after intramuscular injection of DNA constructs. Nature 352(6338):815–818. https://doi.org/10.1038/352815a0

Alemdaroglu I, Karaduman A, Yilmaz OT, Topaloglu H (2015) Different types of upper extremity exercise training in Duchenne muscular dystrophy: effects on functional performance, strength, endurance, and ambulation. Muscle Nerve 51(5):697–705. https://doi.org/10.1002/mus.24451

Alter J, Lou F, Rabinowitz A, Yin H, Rosenfeld J, Wilton SD, Partridge TA, Lu QL (2006) Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. Nat Med 12(2):175–177. https://doi.org/10.1038/nm1345

Babbs A, Berg A, Chatzopoulou M, Davies KE, Davies SG, Edwards B, Elsey DJ, Emer E, Figuccia ALA, Fletcher AM, Guiraud S, Harriman S, Moir L, Robinson N, Rowley JA, Russell

AJ, Squire SE, Thomson JE, Tinsley JM, Wilson FX, Wynne GM (2020) Synthesis of SMT022357 enantiomers and in vivo evaluation in a Duchenne muscular dystrophy mouse model. Tetrahedron 76(2):130819. https://doi.org/10.1016/j.tet.2019.130819

Baltgalvis KA, Call JA, Cochrane GD, Laker RC, Yan Z, Lowe DA (2012) Exercise training improves plantar flexor muscle function in mdx mice. Med Sci Sports Exerc 44(9):1671–1679. https://doi.org/10.1249/MSS.0b013e31825703f0

Baranov A, Glazkov P, Kiselev A, Ostapenko O, Mikhailov V, Ivaschenko T, Sabetsky V, Baranov V (1999) Local and distant transfection of mdx muscle fibers with dystrophin and LacZ genes delivered in vivo by synthetic microspheres. Gene Ther 6(8):1406–1414. https://doi.org/10.1038/sj.gt.3300954

Barton-Davis ER, Cordier L, Shoturma DI, Leland SE, Sweeney HL (1999) Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice. J Clin Invest 104(4):375–381. https://doi.org/10.1172/JCI7866

Bengtsson NE, Hall JK, Odom GL, Phelps MP, Andrus CR, Hawkins RD, Hauschka SD, Chamberlain JR, Chamberlain JS (2017) Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. Nat Commun 8:14454. https://doi.org/10.1038/ncomms14454

Berry SE, Liu J, Chaney EJ, Kaufman SJ (2007) Multipotential mesoangioblast stem cell therapy in the mdx/utrn-/- mouse model for Duchenne muscular dystrophy. Regen Med 2(3):275–288. https://doi.org/10.2217/17460751.2.3.275

Bettica P, Petrini S, D'Oria V, D'Amico A, Catteruccia M, Pane M, Sivo S, Magri F, Brajkovic S, Messina S, Vita GL, Gatti B, Moggio M, Puri PL, Rocchetti M, De Nicolao G, Vita G, Comi GP, Bertini E, Mercuri E (2016) Histological effects of givinostat in boys with Duchenne muscular dystrophy. Neuromuscul Disord 26(10):643–649. https://doi.org/10.1016/j.nmd.2016.07.002

Blaauw B, Schiaffino S, Reggiani C (2013) Mechanisms modulating skeletal muscle phenotype. Compr Physiol 3(4):1645–1687. https://doi.org/10.1002/cphy.c130009

Bodanovsky A, Guttman N, Barzilai-Tutsch H, Genin O, Levy O, Pines M, Halevy O (2014) Halofuginone improves muscle-cell survival in muscular dystrophies. Biochim Biophys Acta 1843(7):1339–1347. https://doi.org/10.1016/j.bbamcr.2014.03.025

Bogdanovich S, Krag TO, Barton ER, Morris LD, Whittemore LA, Ahima RS, Khurana TS (2002) Functional improvement of dystrophic muscle by myostatin blockade. Nature 420(6914):418–421. https://doi.org/10.1038/nature01154

Bouchentouf M, Benabdallah BF, Mills P, Tremblay JP (2006) Exercise improves the success of myoblast transplantation in mdx mice. Neuromuscul Disord 16(8):518–529. https://doi.org/10.1016/j.nmd.2006.06.003

Buller AJ, Eccles JC, Eccles RM (1960) Interactions between motoneurones and muscles in respect of the characteristic speeds of their responses. J Physiol 150:417–439. https://doi.org/10.1113/jphysiol.1960.sp006395

Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, Kaul A, Kinnett K, McDonald C, Pandya S, Poysky J, Shapiro F, Tomezsko J, Constantin C, Group DMDCCW (2010) Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lancet Neurol 9(2):177–189. https://doi.org/10.1016/S1474-4422(09)70272-8

Cai D, Frantz JD, Tawa NE Jr, Melendez PA, Oh BC, Lidov HG, Hasselgren PO, Frontera WR, Lee J, Glass DJ, Shoelson SE (2004) IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 119(2):285–298. https://doi.org/10.1016/j.cell.2004.09.027

Call JA, McKeehen JN, Novotny SA, Lowe DA (2010) Progressive resistance voluntary wheel running in the mdx mouse. Muscle Nerve 42(6):871–880. https://doi.org/10.1002/mus.21764

Carter GT, Wineinger MA, Walsh SA, Horasek SJ, Abresch RT, Fowler WM Jr (1995) Effect of voluntary wheel-running exercise on muscles of the mdx mouse. Neuromuscul Disord 5(4):323–332. https://doi.org/10.1016/0960-8966(94)00063-f

Cerletti M, Jurga S, Witczak CA, Hirshman MF, Shadrach JL, Goodyear LJ, Wagers AJ (2008) Highly efficient, functional engraftment of skeletal muscle stem cells in dystrophic muscles. Cell 134(1):37–47. https://doi.org/10.1016/j.cell.2008.05.049

Colson SS, Benchortane M, Tanant V, Faghan JP, Fournier-Mehouas M, Benaim C, Desnuelle C, Sacconi S (2010) Neuromuscular electrical stimulation training: a safe and effective treatment for facioscapulohumeral muscular dystrophy patients. Arch Phys Med Rehabil 91(5):697–702. https://doi.org/10.1016/j.apmr.2010.01.019

Colussi C, Mozzetta C, Gurtner A, Illi B, Rosati J, Straino S, Ragone G, Pescatori M, Zaccagnini G, Antonini A, Minetti G, Martelli F, Piaggio G, Gallinari P, Steinkuhler C, Clementi E, Dell'Aversana C, Altucci L, Mai A, Capogrossi MC, Puri PL, Gaetano C (2008) HDAC2 blockade by nitric oxide and histone deacetylase inhibitors reveals a common target in Duchenne muscular dystrophy treatment. Proc Natl Acad Sci U S A 105(49):19183–19187. https://doi.org/10.1073/pnas.0805514105

Conklin LS, Damsker JM, Hoffman EP, Jusko WJ, Mavroudis PD, Schwartz BD, Mengle-Gaw LJ, Smith EC, Mah JK, Guglieri M, Nevo Y, Kuntz N, McDonald CM, Tulinius M, Ryan MM, Webster R, Castro D, Finkel RS, Smith AL, Morgenroth LP, Arrieta A, Shimony M, Jaros M, Shale P, McCall JM, Hathout Y, Nagaraju K, van den Anker J, Ward LM, Ahmet A, Cornish MR, Clemens PR (2018) Phase IIa trial in Duchenne muscular dystrophy shows vamorolone is a first-in-class dissociative steroidal anti-inflammatory drug. Pharmacol Res 136:140–150. https://doi.org/10.1016/j.phrs.2018.09.007

Consalvi S, Mozzetta C, Bettica P, Germani M, Fiorentini F, Del Bene F, Rocchetti M, Leoni F, Monzani V, Mascagni P, Puri PL, Saccone V (2013) Preclinical studies in the mdx mouse model of Duchenne muscular dystrophy with the histone deacetylase inhibitor givinostat. Mol Med 19:79–87. https://doi.org/10.2119/molmed.2013.00011

Cossu G, Previtali SC, Napolitano S, Cicalese MP, Tedesco FS, Nicastro F, Noviello M, Roostalu U, Natali Sora MG, Scarlato M, De Pellegrin M, Godi C, Giuliani S, Ciotti F, Tonlorenzi R, Lorenzetti I, Rivellini C, Benedetti S, Gatti R, Marktel S, Mazzi B, Tettamanti A, Ragazzi M, Imro MA, Marano G, Ambrosi A, Fiori R, Sormani MP, Bonini C, Venturini M, Politi LS, Torrente Y, Ciceri F (2015) Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy. EMBO Mol Med 7(12):1513–1528. https://doi.org/10.15252/emmm.201505636

Cudia P, Weis L, Baba A, Kiper P, Marcante A, Rossi S, Angelini C, Piccione F (2016) Effects of functional electrical stimulation lower extremity training in myotonic dystrophy type I: a pilot controlled study. Am J Phys Med Rehabil 95(11):809–817. https://doi.org/10.1097/PHM.0000000000000497

Dangain J, Vrbova G (1989) Long term effect of low frequency chronic electrical stimulation on the fast hind limb muscles of dystrophic mice. J Neurol Neurosurg Psychiatry 52(12):1382–1389. https://doi.org/10.1136/jnnp.52.12.1382

Danialou G, Comtois AS, Dudley RW, Nalbantoglu J, Gilbert R, Karpati G, Jones DH, Petrof BJ (2002) Ultrasound increases plasmid-mediated gene transfer to dystrophic muscles without collateral damage. Mol Ther 6(5):687–693

Danko I, Fritz JD, Latendresse JS, Herweijer H, Schultz E, Wolff JA (1993) Dystrophin expression improves myofiber survival in mdx muscle following intramuscular plasmid DNA injection. Hum Mol Genet 2(12):2055–2061. https://doi.org/10.1093/hmg/2.12.2055

Delacroix C, Hyzewicz J, Lemaitre M, Friguet B, Li Z, Klein A, Furling D, Agbulut O, Ferry A (2018) Improvement of dystrophic muscle fragility by short-term voluntary exercise through activation of calcineurin pathway in mdx mice. Am J Pathol 188(11):2662–2673. https://doi.org/10.1016/j.ajpath.2018.07.015

de Lateur BJ, Giaconi RM (1979) Effect on maximal strength of submaximal exercise in Duchenne muscular dystrophy. Am J Phys Med 58(1):26–36

Dellavalle A, Sampaolesi M, Tonlorenzi R, Tagliafico E, Sacchetti B, Perani L, Innocenzi A, Galvez BG, Messina G, Morosetti R, Li S, Belicchi M, Peretti G, Chamberlain JS, Wright WE, Torrente Y, Ferrari S, Bianco P, Cossu G (2007) Pericytes of human skeletal muscle are

myogenic precursors distinct from satellite cells. Nat Cell Biol 9(3):255–267. https://doi.org/10.1038/ncb1542

Den Dunnen JT, Grootscholten PM, Bakker E, Blonden LA, Ginjaar HB, Wapenaar MC, van Paassen HM, van Broeckhoven C, Pearson PL, van Ommen GJ (1989) Topography of the Duchenne muscular dystrophy (DMD) gene: FIGE and cDNA analysis of 194 cases reveals 115 deletions and 13 duplications. Am J Hum Genet 45(6):835–847

Distefano G, Ferrari RJ, Weiss C, Deasy BM, Boninger ML, Fitzgerald GK, Huard J, Ambrosio F (2013) Neuromuscular electrical stimulation as a method to maximize the beneficial effects of muscle stem cells transplanted into dystrophic skeletal muscle. PLoS One 8(3):e54922. https://doi.org/10.1371/journal.pone.0054922

Donovan JM, Zimmer M, Offman E, Grant T, Jirousek M (2017) A novel NF-kappaB inhibitor, edasalonexent (CAT-1004), in development as a disease-modifying treatment for patients with Duchenne muscular dystrophy: phase 1 safety, pharmacokinetics, and pharmacodynamics in adult subjects. J Clin Pharmacol 57(5):627–639. https://doi.org/10.1002/jcph.842

Dudley RW, Lu Y, Gilbert R, Matecki S, Nalbantoglu J, Petrof BJ, Karpati G (2004) Sustained improvement of muscle function one year after full-length dystrophin gene transfer into mdx mice by a gutted helper-dependent adenoviral vector. Hum Gene Ther 15(2):145–156. https://doi.org/10.1089/104303404772679959

Duguez S, Duddy WJ, Gnocchi V, Bowe J, Dadgar S, Partridge TA (2012) Atmospheric oxygen tension slows myoblast proliferation via mitochondrial activation. PLoS One 7(8):e43853. https://doi.org/10.1371/journal.pone.0043853

Dunant P, Walter MC, Karpati G, Lochmuller H (2003) Gentamicin fails to increase dystrophin expression in dystrophin-deficient muscle. Muscle Nerve 27(5):624–627. https://doi.org/10.1002/mus.10341

Dupont-Versteegden EE, McCarter RJ, Katz MS (1994) Voluntary exercise decreases progression of muscular dystrophy in diaphragm of mdx mice. J Appl Physiol (1985) 77(4):1736–1741. https://doi.org/10.1152/jappl.1994.77.4.1736

Egan B, Zierath JR (2013) Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab 17(2):162–184. https://doi.org/10.1016/j.cmet.2012.12.012

England SB, Nicholson LV, Johnson MA, Forrest SM, Love DR, Zubrzycka-Gaarn EE, Bulman DE, Harris JB, Davies KE (1990) Very mild muscular dystrophy associated with the deletion of 46% of dystrophin. Nature 343(6254):180–182. https://doi.org/10.1038/343180a0

Ervasti JM, Campbell KP (1993) A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. J Cell Biol 122(4):809–823. https://doi.org/10.1083/jcb.122.4.809

Fabb SA, Wells DJ, Serpente P, Dickson G (2002) Adeno-associated virus vector gene transfer and sarcolemmal expression of a 144 kDa micro-dystrophin effectively restores the dystrophin-associated protein complex and inhibits myofibre degeneration in nude/mdx mice. Hum Mol Genet 11(7):733–741. https://doi.org/10.1093/hmg/11.7.733

Fernandes DC, Cardoso-Nascimento JJA, Garcia BCC, Costa KB, Rocha-Vieira E, Oliveira MX, Machado ASD, Santos AP, Gaiad TP (2019) Low intensity training improves redox status and reduces collagen fibers on dystrophic muscle. J Exerc Rehabil 15(2):213–223. https://doi.org/10.12965/jer.1938060.030

Ferrer A, Foster H, Wells KE, Dickson G, Wells DJ (2004) Long-term expression of full-length human dystrophin in transgenic mdx mice expressing internally deleted human dystrophins. Gene Ther 11(11):884–893. https://doi.org/10.1038/sj.gt.3302242

Filareto A, Parker S, Darabi R, Borges L, Iacovino M, Schaaf T, Mayerhofer T, Chamberlain JS, Ervasti JM, McIvor RS, Kyba M, Perlingeiro RC (2013) An ex vivo gene therapy approach to treat muscular dystrophy using inducible pluripotent stem cells. Nat Commun 4:1549. https://doi.org/10.1038/ncomms2550

Finanger E, Vandenborne K, Finkel RS, Lee Sweeney H, Tennekoon G, Yum S, Mancini M, Bista P, Nichols A, Liu H, Fretzen A, Donovan JM (2019) Phase 1 study of edasalonexent

(CAT-1004), an oral NF-kappaB inhibitor, in pediatric patients with Duchenne muscular dystrophy. J Neuromuscul Dis 6(1):43–54. https://doi.org/10.3233/JND-180341

Flanigan KM, Voit T, Rosales XQ, Servais L, Kraus JE, Wardell C, Morgan A, Dorricott S, Nakielny J, Quarcoo N, Liefaard L, Drury T, Campion G, Wright P (2014) Pharmacokinetics and safety of single doses of drisapersen in non-ambulant subjects with Duchenne muscular dystrophy: results of a double-blind randomized clinical trial. Neuromuscul Disord 24(1): 16–24. https://doi.org/10.1016/j.nmd.2013.09.004

Gaiad TP, Oliveira MX, Lobo AR Jr, Liborio LR, Pinto PAF, Fernandes DC, Santos AP, Ambrosio CE, Machado ASD (2017) Low-intensity training provokes adaptive extracellular matrix turnover of a muscular dystrophy model. J Exerc Rehabil 13(6):693–703. https://doi.org/10.12965/jer.1735094.547

Gerli MF, Maffioletti SM, Millet Q, Tedesco FS (2014) Transplantation of induced pluripotent stem cell-derived mesoangioblast-like myogenic progenitors in mouse models of muscle regeneration. J Vis Exp 83:e50532. https://doi.org/10.3791/50532

Ghaleh B, Barthelemy I, Wojcik J, Sambin L, Bize A, Hittinger L, Tran TD, Thome FP, Blot S, Su JB (2020) Protective effects of rimeporide on left ventricular function in golden retriever muscular dystrophy dogs. Int J Cardiol 312:89–95. https://doi.org/10.1016/j.ijcard.2020.03.031

Gilbert R, Nalbantoglu J, Howell JM, Davies L, Fletcher S, Amalfitano A, Petrof BJ, Kamen A, Massie B, Karpati G (2001) Dystrophin expression in muscle following gene transfer with a fully deleted ("gutted") adenovirus is markedly improved by trans-acting adenoviral gene products. Hum Gene Ther 12(14):1741–1755. https://doi.org/10.1089/104303401750476249

Gilbert R, Dudley RW, Liu AB, Petrof BJ, Nalbantoglu J, Karpati G (2003) Prolonged dystrophin expression and functional correction of mdx mouse muscle following gene transfer with a helper-dependent (gutted) adenovirus-encoding murine dystrophin. Hum Mol Genet 12(11): 1287–1299. https://doi.org/10.1093/hmg/ddg141

Gilbert PM, Havenstrite KL, Magnusson KE, Sacco A, Leonardi NA, Kraft P, Nguyen NK, Thrun S, Lutolf MP, Blau HM (2010) Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science 329(5995):1078–1081. https://doi.org/10.1126/science.1191035

Glasser CE, Gartner MR, Wilson D, Miller B, Sherman ML, Attie KM (2018) Locally acting ACE-083 increases muscle volume in healthy volunteers. Muscle Nerve 57(6):921–926. https://doi.org/10.1002/mus.26113

Goemans NM, Tulinius M, van den Akker JT, Burm BE, Ekhart PF, Heuvelmans N, Holling T, Janson AA, Platenburg GJ, Sipkens JA, Sitsen JM, Aartsma-Rus A, van Ommen GJ, Buyse G, Darin N, Verschuuren JJ, Campion GV, de Kimpe SJ, van Deutekom JC (2011) Systemic administration of PRO051 in Duchenne's muscular dystrophy. N Engl J Med 364(16): 1513–1522. https://doi.org/10.1056/NEJMoa1011367

Goemans NM, Tulinius M, van den Hauwe M, Kroksmark AK, Buyse G, Wilson RJ, van Deutekom JC, de Kimpe SJ, Lourbakos A, Campion G (2016) Long-term efficacy, safety, and pharmacokinetics of drisapersen in Duchenne muscular dystrophy: results from an open-label extension study. PLoS One 11(9):e0161955. https://doi.org/10.1371/journal.pone.0161955

Goemans N, Mercuri E, Belousova E, Komaki H, Dubrovsky A, McDonald CM, Kraus JE, Lourbakos A, Lin Z, Campion G, Wang SX, Campbell C, Group DIs (2018) A randomized placebo-controlled phase 3 trial of an antisense oligonucleotide, drisapersen, in Duchenne muscular dystrophy. Neuromuscul Disord 28(1):4–15. https://doi.org/10.1016/j.nmd.2017.10.004

Gollins H, McMahon J, Wells KE, Wells DJ (2003) High-efficiency plasmid gene transfer into dystrophic muscle. Gene Ther 10(6):504–512. https://doi.org/10.1038/sj.gt.3301927

Gordon BS, Lowe DA, Kostek MC (2014) Exercise increases utrophin protein expression in the mdx mouse model of Duchenne muscular dystrophy. Muscle Nerve 49(6):915–918. https://doi.org/10.1002/mus.24151

Grange RW, Call JA (2007) Recommendations to define exercise prescription for Duchenne muscular dystrophy. Exerc Sport Sci Rev 35(1):12–17. https://doi.org/10.1249/01.jes.0000240020.84630.9d

Gregorevic P, Allen JM, Minami E, Blankinship MJ, Haraguchi M, Meuse L, Finn E, Adams ME, Froehner SC, Murry CE, Chamberlain JS (2006) rAAV6-microdystrophin preserves muscle function and extends lifespan in severely dystrophic mice. Nat Med 12(7):787–789. https://doi.org/10.1038/nm1439

Guiraud S, Squire SE, Edwards B, Chen H, Burns DT, Shah N, Babbs A, Davies SG, Wynne GM, Russell AJ, Elsey D, Wilson FX, Tinsley JM, Davies KE (2015) Second-generation compound for the modulation of utrophin in the therapy of DMD. Hum Mol Genet 24(15):4212–4224. https://doi.org/10.1093/hmg/ddv154

Haidet AM, Rizo L, Handy C, Umapathi P, Eagle A, Shilling C, Boue D, Martin PT, Sahenk Z, Mendell JR, Kaspar BK (2008) Long-term enhancement of skeletal muscle mass and strength by single gene administration of myostatin inhibitors. Proc Natl Acad Sci U S A 105(11):4318–4322. https://doi.org/10.1073/pnas.0709144105

Hall JE, Kaczor JJ, Hettinga BP, Isfort RJ, Tarnopolsky MA (2007) Effects of a CRF2R agonist and exercise on mdx and wildtype skeletal muscle. Muscle Nerve 36(3):336–341. https://doi.org/10.1002/mus.20820

Hamm SE, Fathalikhani DD, Bukovec KE, Addington AK, Zhang H, Perry JB, McMillan RP, Lawlor MW, Prom MJ, Vanden Avond MA, Kumar SN, Coleman KE, Dupont JB, Mack DL, Brown DA, Morris CA, Gonzalez JP, Grange RW (2021) Voluntary wheel running complements microdystrophin gene therapy to improve muscle function in mdx mice. Mol Ther Methods Clin Dev 21:144–160. https://doi.org/10.1016/j.omtm.2021.02.024

Hammers DW, Sleeper MM, Forbes SC, Coker CC, Jirousek MR, Zimmer M, Walter GA, Sweeney HL (2016) Disease-modifying effects of orally bioavailable NF-kappaB inhibitors in dystrophin-deficient muscle. JCI Insight 1(21):e90341. https://doi.org/10.1172/jci.insight.90341

Hardee JP, Martins KJB, Miotto PM, Ryall JG, Gehrig SM, Reljic B, Naim T, Chung JD, Trieu J, Swiderski K, Philp AM, Philp A, Watt MJ, Stroud DA, Koopman R, Steinberg GR, Lynch GS (2021) Metabolic remodeling of dystrophic skeletal muscle reveals biological roles for dystrophin and utrophin in adaptation and plasticity. Mol Metab 45:101157. https://doi.org/10.1016/j.molmet.2020.101157

Harper SQ, Hauser MA, DelloRusso C, Duan D, Crawford RW, Phelps SF, Harper HA, Robinson AS, Engelhardt JF, Brooks SV, Chamberlain JS (2002) Modular flexibility of dystrophin: implications for gene therapy of Duchenne muscular dystrophy. Nat Med 8(3):253–261. https://doi.org/10.1038/nm0302-253

Hartigan-O'Connor D, Barjot C, Crawford R, Chamberlain JS (2002) Efficient rescue of gutted adenovirus genomes allows rapid production of concentrated stocks without negative selection. Hum Gene Ther 13(4):519–531. https://doi.org/10.1089/10430340252809810

Hayes A, Williams DA (1996) Beneficial effects of voluntary wheel running on the properties of dystrophic mouse muscle. J Appl Physiol (1985) 80(2):670–679. https://doi.org/10.1152/jappl.1996.80.2.670

Hayes A, Williams DA (1997) Contractile properties of clenbuterol-treated mdx muscle are enhanced by low-intensity swimming. J Appl Physiol (1985) 82(2):435–439. https://doi.org/10.1152/jappl.1997.82.2.435

Hayes A, Williams DA (1998) Contractile function and low-intensity exercise effects of old dystrophic (mdx) mice. Am J Physiol 274(4):C1138–C1144. https://doi.org/10.1152/ajpcell.1998.274.4.C1138

Hayes A, Lynch GS, Williams DA (1993) The effects of endurance exercise on dystrophic mdx mice. I Contractile and histochemical properties of intact muscles. Proc Biol Sci 253(1336):19–25. https://doi.org/10.1098/rspb.1993.0077

Heier CR, Damsker JM, Yu Q, Dillingham BC, Huynh T, Van der Meulen JH, Sali A, Miller BK, Phadke A, Scheffer L, Quinn J, Tatem K, Jordan S, Dadgar S, Rodriguez OC, Albanese C,

Calhoun M, Gordish-Dressman H, Jaiswal JK, Connor EM, McCall JM, Hoffman EP, Reeves EK, Nagaraju K (2013) VBP15, a novel anti-inflammatory and membrane-stabilizer, improves muscular dystrophy without side effects. EMBO Mol Med 5(10):1569–1585. https://doi.org/10.1002/emmm.201302621

Hoberman M (1955) Physical medicine and rehabilitation: its value and limitations in progressive muscular dystrophy. Am J Phys Med 34(1):109–115

Hoffman EP, Brown RH Jr, Kunkel LM (1987a) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 51(6):919–928. https://doi.org/10.1016/0092-8674(87)90579-4

Hoffman EP, Knudson CM, Campbell KP, Kunkel LM (1987b) Subcellular fractionation of dystrophin to the triads of skeletal muscle. Nature 330(6150):754–758. https://doi.org/10.1038/330754a0

Hoffman EP, Monaco AP, Feener CC, Kunkel LM (1987c) Conservation of the Duchenne muscular dystrophy gene in mice and humans. Science 238(4825):347–350. https://doi.org/10.1126/science.3659917

Hoffman EP, Riddle V, Siegler MA, Dickerson D, Backonja M, Kramer WG, Nagaraju K, Gordish-Dressman H, Damsker JM, McCall JM (2018) Phase 1 trial of vamorolone, a first-in-class steroid, shows improvements in side effects via biomarkers bridged to clinical outcomes. Steroids 134:43–52. https://doi.org/10.1016/j.steroids.2018.02.010

Hourde C, Joanne P, Medja F, Mougenot N, Jacquet A, Mouisel E, Pannerec A, Hatem S, Butler-Browne G, Agbulut O, Ferry A (2013) Voluntary physical activity protects from susceptibility to skeletal muscle contraction-induced injury but worsens heart function in mdx mice. Am J Pathol 182(5):1509–1518. https://doi.org/10.1016/j.ajpath.2013.01.020

Hyzewicz J, Ruegg UT, Takeda S (2015) Comparison of experimental protocols of physical exercise for mdx mice and Duchenne muscular dystrophy patients. J Neuromuscul Dis 2(4):325–342. https://doi.org/10.3233/JND-150106

Ifuku M, Iwabuchi KA, Tanaka M, Lung MSY, Hotta A (2018) Restoration of dystrophin protein expression by exon skipping utilizing CRISPR-Cas9 in myoblasts derived from DMD patient iPS cells. Methods Mol Biol 1828:191–217. https://doi.org/10.1007/978-1-4939-8651-4_12

Iyer PS, Mavoungou LO, Ronzoni F, Zemla J, Schmid-Siegert E, Antonini S, Neff LA, Dorchies OM, Jaconi M, Lekka M, Messina G, Mermod N (2018) Autologous cell therapy approach for Duchenne muscular dystrophy using PiggyBac transposons and mesoangioblasts. Mol Ther 26(4):1093–1108. https://doi.org/10.1016/j.ymthe.2018.01.021

Jansen M, de Groot IJ, van Alfen N, Geurts A (2010) Physical training in boys with Duchenne muscular dystrophy: the protocol of the no use is disuse study. BMC Pediatr 10:55. https://doi.org/10.1186/1471-2431-10-55

Jansen M, van Alfen N, Geurts AC, de Groot IJ (2013) Assisted bicycle training delays functional deterioration in boys with Duchenne muscular dystrophy: the randomized controlled trial "no use is disuse". Neurorehabil Neural Repair 27(9):816–827. https://doi.org/10.1177/1545968313496326

Johnson NM, Farr GH 3rd, Maves L (2013) The HDAC inhibitor TSA ameliorates a zebrafish model of Duchenne muscular dystrophy. PLoS Curr 5. https://doi.org/10.1371/currents.md.8273cf41db10e2d15dd3ab827cb4b027

Jones S, Man WD, Gao W, Higginson IJ, Wilcock A, Maddocks M (2016) Neuromuscular electrical stimulation for muscle weakness in adults with advanced disease. Cochrane Database Syst Rev 10:CD009419. https://doi.org/10.1002/14651858.CD009419.pub3

Kazuki Y, Hiratsuka M, Takiguchi M, Osaki M, Kajitani N, Hoshiya H, Hiramatsu K, Yoshino T, Kazuki K, Ishihara C, Takehara S, Higaki K, Nakagawa M, Takahashi K, Yamanaka S, Oshimura M (2010) Complete genetic correction of ips cells from Duchenne muscular dystrophy. Mol Ther 18(2):386–393. https://doi.org/10.1038/mt.2009.274

Kimura E, Li S, Gregorevic P, Fall BM, Chamberlain JS (2010) Dystrophin delivery to muscles of mdx mice using lentiviral vectors leads to myogenic progenitor targeting and stable gene expression. Mol Ther 18(1):206–213. https://doi.org/10.1038/mt.2009.253

Kobinger GP, Louboutin JP, Barton ER, Sweeney HL, Wilson JM (2003) Correction of the dystrophic phenotype by in vivo targeting of muscle progenitor cells. Hum Gene Ther 14(15): 1441–1449. https://doi.org/10.1089/104303403769211655

Koenig M, Beggs AH, Moyer M, Scherpf S, Heindrich K, Bettecken T, Meng G, Muller CR, Lindlof M, Kaariainen H, de la Chapellet A, Kiuru A, Savontaus ML, Gilgenkrantz H, Recan D, Chelly J, Kaplan JC, Covone AE, Archidiacono N, Romeo G, Liechti-Gailati S, Schneider V, Braga S, Moser H, Darras BT, Murphy P, Francke U, Chen JD, Morgan G, Denton M, Greenberg CR, Wrogemann K, Blonden LA, van Paassen MB, van Ommen GJ, Kunkel LM (1989) The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. Am J Hum Genet 45(4):498–506

Kota J, Handy CR, Haidet AM, Montgomery CL, Eagle A, Rodino-Klapac LR, Tucker D, Shilling CJ, Therlfall WR, Walker CM, Weisbrode SE, Janssen PM, Clark KR, Sahenk Z, Mendell JR, Kaspar BK (2009) Follistatin gene delivery enhances muscle growth and strength in nonhuman primates. Sci Transl Med 1(6):6ra15. https://doi.org/10.1126/scitranslmed.3000112

Krivickas LS, Walsh R, Amato AA (2009) Single muscle fiber contractile properties in adults with muscular dystrophy treated with MYO-029. Muscle Nerve 39(1):3–9. https://doi.org/10.1002/mus.21200

Kwon JB, Ettyreddy AR, Vankara A, Bohning JD, Devlin G, Hauschka SD, Asokan A, Gersbach CA (2020) In vivo gene editing of muscle stem cells with adeno-associated viral vectors in a mouse model of Duchenne muscular dystrophy. Mol Ther Methods Clin Dev 19:320–329. https://doi.org/10.1016/j.omtm.2020.09.016

Lai Y, Yue Y, Liu M, Ghosh A, Engelhardt JF, Chamberlain JS, Duan D (2005) Efficient in vivo gene expression by trans-splicing adeno-associated viral vectors. Nat Biotechnol 23(11): 1435–1439. https://doi.org/10.1038/nbt1153

Landisch RM, Kosir AM, Nelson SA, Baltgalvis KA, Lowe DA (2008) Adaptive and nonadaptive responses to voluntary wheel running by mdx mice. Muscle Nerve 38(4):1290–1303. https://doi.org/10.1002/mus.21141

Leeuw T, Pette D (1993) Coordinate changes in the expression of troponin subunit and myosin heavy-chain isoforms during fast-to-slow transition of low-frequency-stimulated rabbit muscle. Eur J Biochem 213(3):1039–1046. https://doi.org/10.1111/j.1432-1033.1993.tb17851.x

Li HL, Fujimoto N, Sasakawa N, Shirai S, Ohkame T, Sakuma T, Tanaka M, Amano N, Watanabe A, Sakurai H, Yamamoto T, Yamanaka S, Hotta A (2015) Precise correction of the dystrophin gene in Duchenne muscular dystrophy patient induced pluripotent stem cells by TALEN and CRISPR-Cas9. Stem Cell Rep 4(1):143–154. https://doi.org/10.1016/j.stemcr.2014.10.013

Licandro SA, Crippa L, Pomarico R, Perego R, Fossati G, Leoni F, Steinkuhler C (2021) The pan HDAC inhibitor Givinostat improves muscle function and histological parameters in two Duchenne muscular dystrophy murine models expressing different haplotypes of the LTBP4 gene. Skelet Muscle 11(1):19. https://doi.org/10.1186/s13395-021-00273-6

Lindsay A, Larson AA, Verma M, Ervasti JM, Lowe DA (2019) Isometric resistance training increases strength and alters histopathology of dystrophin-deficient mouse skeletal muscle. J Appl Physiol (1985) 126(2):363–375. https://doi.org/10.1152/japplphysiol.00948.2018

Liu F, Nishikawa M, Clemens PR, Huang L (2001) Transfer of full-length Dmd to the diaphragm muscle of Dmd(mdx/mdx) mice through systemic administration of plasmid DNA. Mol Ther 4(1):45–51. https://doi.org/10.1006/mthe.2001.0419

Liu M, Yue Y, Harper SQ, Grange RW, Chamberlain JS, Duan D (2005) Adeno-associated virus-mediated microdystrophin expression protects young mdx muscle from contraction-induced injury. Mol Ther 11(2):245–256. https://doi.org/10.1016/j.ymthe.2004.09.013

Liu W, Wen Y, Bi P, Lai X, Liu XS, Liu X, Kuang S (2012) Hypoxia promotes satellite cell self-renewal and enhances the efficiency of myoblast transplantation. Development 139(16): 2857–2865. https://doi.org/10.1242/dev.079665

Long C, McAnally JR, Shelton JM, Mireault AA, Bassel-Duby R, Olson EN (2014) Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. Science 345(6201):1184–1188. https://doi.org/10.1126/science.1254445

Long C, Amoasii L, Mireault AA, McAnally JR, Li H, Sanchez-Ortiz E, Bhattacharyya S, Shelton JM, Bassel-Duby R, Olson EN (2016) Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science 351(6271):400–403. https://doi.org/10.1126/science.aad5725

Lott DJ, Taivassalo T, Cooke KD, Park H, Moslemi Z, Batra A, Forbes SC, Byrne BJ, Walter GA, Vandenborne K (2021) Safety, feasibility, and efficacy of strengthening exercise in Duchenne muscular dystrophy. Muscle Nerve 63(3):320–326. https://doi.org/10.1002/mus.27137

Lu QL, Mann CJ, Lou F, Bou-Gharios G, Morris GE, Xue SA, Fletcher S, Partridge TA, Wilton SD (2003) Functional amounts of dystrophin produced by skipping the mutated exon in the mdx dystrophic mouse. Nat Med 9(8):1009–1014. https://doi.org/10.1038/nm897

Luthert P, Vrbova G, Ward KM (1980) Effects of slow frequency electrical stimulation on muscles of dystrophic mice. J Neurol Neurosurg Psychiatry 43(9):803–809. https://doi.org/10.1136/jnnp.43.9.803

Lynch GS (2017) Therapeutic potential of skeletal muscle plasticity and slow muscle programming for muscular dystrophy and related muscle conditions. In: Sakuma K (ed) The plasticity of skeletal muscle. Springer, Singapore, pp 277–292

Lynch GS, Hayes A, Lam MH, Williams DA (1993) The effects of endurance exercise on dystrophic mdx mice. II Contractile properties of skinned muscle fibres. Proc Biol Sci 253(1336):27–33. https://doi.org/10.1098/rspb.1993.0078

Malik V, Rodino-Klapac LR, Viollet L, Wall C, King W, Al-Dahhak R, Lewis S, Shilling CJ, Kota J, Serrano-Munuera C, Hayes J, Mahan JD, Campbell KJ, Banwell B, Dasouki M, Watts V, Sivakumar K, Bien-Willner R, Flanigan KM, Sahenk Z, Barohn RJ, Walker CM, Mendell JR (2010) Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy. Ann Neurol 67(6):771–780. https://doi.org/10.1002/ana.22024

Manzur AY, Kuntzer T, Pike M, Swan A (2004) Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane Database Syst Rev (2):CD003725. https://doi.org/10.1002/14651858.CD003725.pub2

Markert CD, Ambrosio F, Call JA, Grange RW (2011) Exercise and Duchenne muscular dystrophy: toward evidence-based exercise prescription. Muscle Nerve 43(4):464–478. https://doi.org/10.1002/mus.21987

Markert CD, Case LE, Carter GT, Furlong PA, Grange RW (2012) Exercise and Duchenne muscular dystrophy: where we have been and where we need to go. Muscle Nerve 45(5):746–751. https://doi.org/10.1002/mus.23244

Markham BE, Kernodle S, Nemzek J, Wilkinson JE, Sigler R (2015) Chronic Dosing with Membrane Sealant Poloxamer 188 NF Improves Respiratory Dysfunction in Dystrophic Mdx and Mdx/Utrophin-/- Mice. PLoS One 10(8):e0134832. https://doi.org/10.1371/journal.pone.0134832

Mavroudis PD, van den Anker J, Conklin LS, Damsker JM, Hoffman EP, Nagaraju K, Clemens PR, Jusko WJ (2019) Population pharmacokinetics of vamorolone (VBP15) in healthy men and boys with Duchenne muscular dystrophy. J Clin Pharmacol 59(7):979–988. https://doi.org/10.1002/jcph.1388

Mendell JR, Kissel JT, Amato AA, King W, Signore L, Prior TW, Sahenk Z, Benson S, McAndrew PE, Rice R et al (1995) Myoblast transfer in the treatment of Duchenne's muscular dystrophy. N Engl J Med 333(13):832–838. https://doi.org/10.1056/NEJM199509283331303

Mendell JR, Campbell K, Rodino-Klapac L, Sahenk Z, Shilling C, Lewis S, Bowles D, Gray S, Li C, Galloway G, Malik V, Coley B, Clark KR, Li J, Xiao X, Samulski J, McPhee SW, Samulski RJ, Walker CM (2010) Dystrophin immunity in Duchenne's muscular dystrophy. N Engl J Med 363(15):1429–1437. https://doi.org/10.1056/NEJMoa1000228

Mendell JR, Rodino-Klapac LR, Sahenk Z, Roush K, Bird L, Lowes LP, Alfano L, Gomez AM, Lewis S, Kota J, Malik V, Shontz K, Walker CM, Flanigan KM, Corridore M, Kean JR, Allen

HD, Shilling C, Melia KR, Sazani P, Saoud JB, Kaye EM, Eteplirsen Study Group (2013) Eteplirsen for the treatment of Duchenne muscular dystrophy. Ann Neurol 74(5):637–647. https://doi.org/10.1002/ana.23982

Mendell JR, Sahenk Z, Malik V, Gomez AM, Flanigan KM, Lowes LP, Alfano LN, Berry K, Meadows E, Lewis S, Braun L, Shontz K, Rouhana M, Clark KR, Rosales XQ, Al-Zaidy S, Govoni A, Rodino-Klapac LR, Hogan MJ, Kaspar BK (2015) A phase 1/2a follistatin gene therapy trial for Becker muscular dystrophy. Mol Ther 23(1):192–201. https://doi.org/10.1038/mt.2014.200

Minasi MG, Riminucci M, De Angelis L, Borello U, Berarducci B, Innocenzi A, Caprioli A, Sirabella D, Baiocchi M, De Maria R, Boratto R, Jaffredo T, Broccoli V, Bianco P, Cossu G (2002) The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. Development 129(11):2773–2783

Minetti GC, Colussi C, Adami R, Serra C, Mozzetta C, Parente V, Fortuni S, Straino S, Sampaolesi M, Di Padova M, Illi B, Gallinari P, Steinkuhler C, Capogrossi MC, Sartorelli V, Bottinelli R, Gaetano C, Puri PL (2006) Functional and morphological recovery of dystrophic muscles in mice treated with deacetylase inhibitors. Nat Med 12(10):1147–1150. https://doi.org/10.1038/nm1479

Molnar MJ, Gilbert R, Lu Y, Liu AB, Guo A, Larochelle N, Orlopp K, Lochmuller H, Petrof BJ, Nalbantoglu J, Karpati G (2004) Factors influencing the efficacy, longevity, and safety of electroporation-assisted plasmid-based gene transfer into mouse muscles. Mol Ther 10(3):447–455. https://doi.org/10.1016/j.ymthe.2004.06.642

Montarras D, Morgan J, Collins C, Relaix F, Zaffran S, Cumano A, Partridge T, Buckingham M (2005) Direct isolation of satellite cells for skeletal muscle regeneration. Science 309(5743):2064–2067. https://doi.org/10.1126/science.1114758

Morine KJ, Bish LT, Selsby JT, Gazzara JA, Pendrak K, Sleeper MM, Barton ER, Lee SJ, Sweeney HL (2010) Activin IIB receptor blockade attenuates dystrophic pathology in a mouse model of Duchenne muscular dystrophy. Muscle Nerve 42(5):722–730. https://doi.org/10.1002/mus.21743

Mourkioti F, Kratsios P, Luedde T, Song YH, Delafontaine P, Adami R, Parente V, Bottinelli R, Pasparakis M, Rosenthal N (2006) Targeted ablation of IKK2 improves skeletal muscle strength, maintains mass, and promotes regeneration. J Clin Invest 116(11):2945–2954. https://doi.org/10.1172/JCI28721

Muntoni F, Tejura B, Spinty S, Roper H, Hughes I, Layton G, Davies KE, Harriman S, Tinsley J (2019) A phase 1b trial to assess the pharmacokinetics of ezutromid in pediatric Duchenne muscular dystrophy patients on a balanced diet. Clin Pharmacol Drug Dev 8(7):922–933. https://doi.org/10.1002/cpdd.642

Muraine L, Bensalah M, Dhiab J, Cordova G, Arandel L, Marhic A, Chapart M, Vasseur S, Benkhelifa-Ziyyat S, Bigot A, Butler-Browne G, Mouly V, Negroni E, Trollet C (2020) Transduction efficiency of adeno-associated virus serotypes after local injection in mouse and human skeletal muscle. Hum Gene Ther 31(3–4):233–240. https://doi.org/10.1089/hum.2019.173

Murakami T, Nishi T, Kimura E, Goto T, Maeda Y, Ushio Y, Uchino M, Sunada Y (2003) Full-length dystrophin cDNA transfer into skeletal muscle of adult mdx mice by electroporation. Muscle Nerve 27(2):237–241. https://doi.org/10.1002/mus.10283

Murphy KT, Koopman R, Naim T, Leger B, Trieu J, Ibebunjo C, Lynch GS (2010a) Antibody-directed myostatin inhibition in 21-mo-old mice reveals novel roles for myostatin signaling in skeletal muscle structure and function. FASEB J 24(11):4433–4442. https://doi.org/10.1096/fj.10-159608

Murphy KT, Ryall JG, Snell SM, Nair L, Koopman R, Krasney PA, Ibebunjo C, Holden KS, Loria PM, Salatto CT, Lynch GS (2010b) Antibody-directed myostatin inhibition improves diaphragm pathology in young but not adult dystrophic mdx mice. Am J Pathol 176(5):2425–2434. https://doi.org/10.2353/ajpath.2010.090932

Murphy KT, Chee A, Gleeson BG, Naim T, Swiderski K, Koopman R, Lynch GS (2011a) Antibody-directed myostatin inhibition enhances muscle mass and function in tumor-bearing mice. Am J Physiol Regul Integr Comp Physiol 301(3):R716–R726. https://doi.org/10.1152/ajpregu.00121.2011

Murphy KT, Cobani V, Ryall JG, Ibebunjo C, Lynch GS (2011b) Acute antibody-directed myostatin inhibition attenuates disuse muscle atrophy and weakness in mice. J Appl Physiol (1985) 110(4):1065–1072. https://doi.org/10.1152/japplphysiol.01183.2010

Nakatani M, Takehara Y, Sugino H, Matsumoto M, Hashimoto O, Hasegawa Y, Murakami T, Uezumi A, Takeda S, Noji S, Sunada Y, Tsuchida K (2008) Transgenic expression of a myostatin inhibitor derived from follistatin increases skeletal muscle mass and ameliorates dystrophic pathology in mdx mice. FASEB J 22(2):477–487. https://doi.org/10.1096/fj.07-8673com

Ng R, Metzger JM, Claflin DR, Faulkner JA (2008) Poloxamer 188 reduces the contraction-induced force decline in lumbrical muscles from mdx mice. Am J Physiol Cell Physiol 295(1):C146–C150. https://doi.org/10.1152/ajpcell.00017.2008

Nobile C, Marchi J, Nigro V, Roberts RG, Danieli GA (1997) Exon-intron organization of the human dystrophin gene. Genomics 45(2):421–424. https://doi.org/10.1006/geno.1997.4911

Ohlendieck K, Campbell KP (1991) Dystrophin-associated proteins are greatly reduced in skeletal muscle from mdx mice. J Cell Biol 115(6):1685–1694. https://doi.org/10.1083/jcb.115.6.1685

Palmer E, Wilhelm JM, Sherman F (1979) Phenotypic suppression of nonsense mutants in yeast by aminoglycoside antibiotics. Nature 277(5692):148–150. https://doi.org/10.1038/277148a0

Parsons SA, Millay DP, Sargent MA, McNally EM, Molkentin JD (2006) Age-dependent effect of myostatin blockade on disease severity in a murine model of limb-girdle muscular dystrophy. Am J Pathol 168(6):1975–1985. https://doi.org/10.2353/ajpath.2006.051316

Partridge TA, Morgan JE, Coulton GR, Hoffman EP, Kunkel LM (1989) Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts. Nature 337(6203):176–179. https://doi.org/10.1038/337176a0

Pearsall RS, Davies MV, Cannell M, Li J, Widrick J, Mulivor AW, Wallner S, Troy ME, Spaits M, Liharska K, Sako D, Castonguay R, Keates S, Grinberg AV, Suragani R, Kumar R (2019) Follistatin-based ligand trap ACE-083 induces localized hypertrophy of skeletal muscle with functional improvement in models of neuromuscular disease. Sci Rep 9(1):11392. https://doi.org/10.1038/s41598-019-47818-w

Pette D, Vrbova G (1999) What does chronic electrical stimulation teach us about muscle plasticity? Muscle Nerve 22(6):666–677. https://doi.org/10.1002/(sici)1097-4598(199906)22:6<666::aid-mus3>3.0.co;2-z

Pistilli EE, Bogdanovich S, Goncalves MD, Ahima RS, Lachey J, Seehra J, Khurana T (2011) Targeting the activin type IIB receptor to improve muscle mass and function in the mdx mouse model of Duchenne muscular dystrophy. Am J Pathol 178(3):1287–1297. https://doi.org/10.1016/j.ajpath.2010.11.071

Politano L, Nigro G, Nigro V, Piluso G, Papparella S, Paciello O, Comi LI (2003) Gentamicin administration in Duchenne patients with premature stop codon. Preliminary results. Acta Myol 22(1):15–21

Porte-Thome F, Nagaraju K, Yu Q, Tatem K, Bkaily G, Scholz W, Slade A, Bot N, Kant C (2015) Development of Rimeporide, a sodium-hydrogen exchanger (NHE-1) inhibitor, for patients with Duchenne muscular dystrophy. Neuromuscul Disord 25:S259–S260. Abstract.

Previtali SC, Gidaro T, Diaz-Manera J, Zambon A, Carnesecchi S, Roux-Lombard P, Spitali P, Signorelli M, Szigyarto CA, Johansson C, Gray J, Labolle D, Porte Thome F, Pitchforth J, Domingos J, Muntoni F (2020) Rimeporide as a first-in-class NHE-1 inhibitor: results of a phase Ib trial in young patients with Duchenne Muscular Dystrophy. Pharmacol Res 159:104999. https://doi.org/10.1016/j.phrs.2020.104999

Quenneville SP, Chapdelaine P, Skuk D, Paradis M, Goulet M, Rousseau J, Xiao X, Garcia L, Tremblay JP (2007) Autologous transplantation of muscle precursor cells modified with a

lentivirus for muscular dystrophy: human cells and primate models. Mol Ther 15(2):431–438. https://doi.org/10.1038/sj.mt.6300047

Ragot T, Vincent N, Chafey P, Vigne E, Gilgenkrantz H, Couton D, Cartaud J, Briand P, Kaplan JC, Perricaudet M et al (1993) Efficient adenovirus-mediated transfer of a human minidystrophin gene to skeletal muscle of mdx mice. Nature 361(6413):647–650. https://doi.org/10.1038/361647a0

Reichmann H, Pette D, Vrbova G (1981) Effects of low frequency electrical stimulation on enzyme and isozyme patterns of dystrophic mouse muscle. FEBS Lett 128(1):55–58. https://doi.org/10.1016/0014-5793(81)81078-2

Reichmann H, Pette D, Vrbova G (1983) Electrostimulation-induced changes in the enzyme activity pattern of dystrophic mouse muscles. Acta Histochem Suppl 28:125–127

Richard P, Bossard F, Desigaux L, Lanctin C, Bello-Roufai M, Pitard B (2005) Amphiphilic block copolymers promote gene delivery in vivo to pathological skeletal muscles. Hum Gene Ther 16(11):1318–1324. https://doi.org/10.1089/hum.2005.16.1318

Ricotti V, Spinty S, Roper H, Hughes I, Tejura B, Robinson N, Layton G, Davies K, Muntoni F, Tinsley J (2016) Safety, tolerability, and pharmacokinetics of SMT C1100, a 2-arylbenzoxazole utrophin modulator, following single- and multiple-dose administration to pediatric patients with Duchenne muscular dystrophy. PLoS One 11(4):e0152840. https://doi.org/10.1371/journal.pone.0152840

Roffe S, Hagai Y, Pines M, Halevy O (2010) Halofuginone inhibits Smad3 phosphorylation via the PI3K/Akt and MAPK/ERK pathways in muscle cells: effect on myotube fusion. Exp Cell Res 316(6):1061–1069. https://doi.org/10.1016/j.yexcr.2010.01.003

Ruggiero L, Iodice R, Esposito M, Dubbioso R, Tozza S, Vitale F, Santoro L, Manganelli F (2018) One-year follow up of three Italian patients with Duchenne muscular dystrophy treated with ataluren: is earlier better? Ther Adv Neurol Disord 11:1756286418809588. https://doi.org/10.1177/1756286418809588

Rybalka E, Timpani CA, Debruin DA, Bagaric RM, Campelj DG, Hayes A (2020) The failed clinical story of myostatin inhibitors against Duchenne muscular dystrophy: exploring the biology behind the battle. Cell 9(12). https://doi.org/10.3390/cells9122657

Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM (2008) Self-renewal and expansion of single transplanted muscle stem cells. Nature 456(7221):502–506. https://doi.org/10.1038/nature07384

Sampaolesi M, Blot S, D'Antona G, Granger N, Tonlorenzi R, Innocenzi A, Mognol P, Thibaud JL, Galvez BG, Barthelemy I, Perani L, Mantero S, Guttinger M, Pansarasa O, Rinaldi C, Cusella De Angelis MG, Torrente Y, Bordignon C, Bottinelli R, Cossu G (2006) Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. Nature 444(7119):574–579. https://doi.org/10.1038/nature05282

Schiaffino S, Reggiani C (2011) Fiber types in mammalian skeletal muscles. Physiol Rev 91(4):1447–1531. https://doi.org/10.1152/physrev.00031.2010

Schiaffino S, Reggiani C, Murgia M (2020) Fiber type diversity in skeletal muscle explored by mass spectrometry-based single fiber proteomics. Histol Histopathol 35(3):239–246. https://doi.org/10.14670/HH-18-170

Scott OM, Vrbova G, Hyde SA, Dubowitz V (1986) Responses of muscles of patients with Duchenne muscular dystrophy to chronic electrical stimulation. J Neurol Neurosurg Psychiatry 49(12):1427–1434. https://doi.org/10.1136/jnnp.49.12.1427

Scott OM, Hyde SA, Vrbova G, Dubowitz V (1990) Therapeutic possibilities of chronic low frequency electrical stimulation in children with Duchenne muscular dystrophy. J Neurol Sci 95(2):171–182. https://doi.org/10.1016/0022-510x(90)90240-n

Singh A, Ursic D, Davies J (1979) Phenotypic suppression and misreading Saccharomyces cerevisiae. Nature 277(5692):146–148. https://doi.org/10.1038/277146a0

Singh P, Rong H, Gordi T, Bosley J, Bhattacharya I (2016) Translational pharmacokinetic/pharmacodynamic analysis of MYO-029 antibody for muscular dystrophy. Clin Transl Sci 9(6):302–310. https://doi.org/10.1111/cts.12420

Smith EC, Conklin LS, Hoffman EP, Clemens PR, Mah JK, Finkel RS, Guglieri M, Tulinius M, Nevo Y, Ryan MM, Webster R, Castro D, Kuntz NL, Kerchner L, Morgenroth LP, Arrieta A, Shimony M, Jaros M, Shale P, Gordish-Dressman H, Hagerty L, Dang UJ, Damsker JM, Schwartz BD, Mengle-Gaw LJ, McDonald CM, Cinrg VBP, Investigators D (2020) Efficacy and safety of vamorolone in Duchenne muscular dystrophy: an 18-month interim analysis of a non-randomized open-label extension study. PLoS Med 17(9):e1003222. https://doi.org/10.1371/journal.pmed.1003222

Spurney CF, Guerron AD, Yu Q, Sali A, van der Meulen JH, Hoffman EP, Nagaraju K (2011) Membrane sealant Poloxamer P188 protects against isoproterenol induced cardiomyopathy in dystrophin deficient mice. BMC Cardiovasc Disord 11:20. https://doi.org/10.1186/1471-2261-11-20

Tabebordbar M, Zhu K, Cheng JKW, Chew WL, Widrick JJ, Yan WX, Maesner C, Wu EY, Xiao R, Ran FA, Cong L, Zhang F, Vandenberghe LH, Church GM, Wagers AJ (2016) In vivo gene editing in dystrophic mouse muscle and muscle stem cells. Science 351(6271):407–411. https://doi.org/10.1126/science.aad5177

Tedesco FS, Gerli MF, Perani L, Benedetti S, Ungaro F, Cassano M, Antonini S, Tagliafico E, Artusi V, Longa E, Tonlorenzi R, Ragazzi M, Calderazzi G, Hoshiya H, Cappellari O, Mora M, Schoser B, Schneiderat P, Oshimura M, Bottinelli R, Sampaolesi M, Torrente Y, Broccoli V, Cossu G (2012) Transplantation of genetically corrected human iPSC-derived progenitors in mice with limb-girdle muscular dystrophy. Sci Transl Med 4(140):140ra189. https://doi.org/10.1126/scitranslmed.3003541

Termin A, Staron RS, Pette D (1989) Changes in myosin heavy chain isoforms during chronic low-frequency stimulation of rat fast hindlimb muscles. A single-fiber study. Eur J Biochem 186(3):749–754. https://doi.org/10.1111/j.1432-1033.1989.tb15269.x

Terry RL, Kaneb HM, Wells DJ (2014) Poloxamer [corrected] 188 has a deleterious effect on dystrophic skeletal muscle function. PLoS One 9(3):e91221. https://doi.org/10.1371/journal.pone.0091221

Tinsley JM, Potter AC, Phelps SR, Fisher R, Trickett JI, Davies KE (1996) Amelioration of the dystrophic phenotype of mdx mice using a truncated utrophin transgene. Nature 384(6607):349–353. https://doi.org/10.1038/384349a0

Tinsley J, Deconinck N, Fisher R, Kahn D, Phelps S, Gillis JM, Davies K (1998) Expression of full-length utrophin prevents muscular dystrophy in mdx mice. Nat Med 4(12):1441–1444. https://doi.org/10.1038/4033

Tinsley JM, Fairclough RJ, Storer R, Wilkes FJ, Potter AC, Squire SE, Powell DS, Cozzoli A, Capogrosso RF, Lambert A, Wilson FX, Wren SP, De Luca A, Davies KE (2011) Daily treatment with SMTC1100, a novel small molecule utrophin upregulator, dramatically reduces the dystrophic symptoms in the mdx mouse. PLoS One 6(5):e19189. https://doi.org/10.1371/journal.pone.0019189

Tinsley J, Robinson N, Davies KE (2015) Safety, tolerability, and pharmacokinetics of SMT C1100, a 2-arylbenzoxazole utrophin modulator, following single- and multiple-dose administration to healthy male adult volunteers. J Clin Pharmacol 55(6):698–707. https://doi.org/10.1002/jcph.468

Townsend D, Turner I, Yasuda S, Martindale J, Davis J, Shillingford M, Kornegay JN, Metzger JM (2010) Chronic administration of membrane sealant prevents severe cardiac injury and ventricular dilatation in dystrophic dogs. J Clin Invest 120(4):1140–1150. https://doi.org/10.1172/JCI41329

Turgeman T, Hagai Y, Huebner K, Jassal DS, Anderson JE, Genin O, Nagler A, Halevy O, Pines M (2008) Prevention of muscle fibrosis and improvement in muscle performance in the mdx mouse by halofuginone. Neuromuscul Disord 18(11):857–868. https://doi.org/10.1016/j.nmd.2008.06.386

van Deutekom JC, Bremmer-Bout M, Janson AA, Ginjaar IB, Baas F, den Dunnen JT, van Ommen GJ (2001) Antisense-induced exon skipping restores dystrophin expression in DMD patient

derived muscle cells. Hum Mol Genet 10(15):1547–1554. https://doi.org/10.1093/hmg/10.15.1547

Vianello S, Consolaro F, Bich C, Cancela JM, Roulot M, Lanchec E, Touboul D, Brunelle A, Israel M, Benoit E, de la Porte S (2014) Low doses of arginine butyrate derivatives improve dystrophic phenotype and restore membrane integrity in DMD models. FASEB J 28(6):2603–2619. https://doi.org/10.1096/fj.13-244798

Vignos PJ Jr, Watkins MP (1966) The effect of exercise in muscular dystrophy. JAMA 197(11):843–848

Vilquin JT, Wagner E, Kinoshita I, Roy R, Tremblay JP (1995) Successful histocompatible myoblast transplantation in dystrophin-deficient mdx mouse despite the production of antibodies against dystrophin. J Cell Biol 131(4):975–988. https://doi.org/10.1083/jcb.131.4.975

Vilquin JT, Kennel PF, Paturneau-Jouas M, Chapdelaine P, Boissel N, Delaere P, Tremblay JP, Scherman D, Fiszman MY, Schwartz K (2001) Electrotransfer of naked DNA in the skeletal muscles of animal models of muscular dystrophies. Gene Ther 8(14):1097–1107. https://doi.org/10.1038/sj.gt.3301484

Vincent N, Ragot T, Gilgenkrantz H, Couton D, Chafey P, Gregoire A, Briand P, Kaplan JC, Kahn A, Perricaudet M (1993) Long-term correction of mouse dystrophic degeneration by adenovirus-mediated transfer of a minidystrophin gene. Nat Genet 5(2):130–134. https://doi.org/10.1038/ng1093-130

Voet NB, van der Kooi EL, van Engelen BG, Geurts AC (2019) Strength training and aerobic exercise training for muscle disease. Cochrane Database Syst Rev 12:CD003907. https://doi.org/10.1002/14651858.CD003907.pub5

Voit T, Topaloglu H, Straub V, Muntoni F, Deconinck N, Campion G, De Kimpe SJ, Eagle M, Guglieri M, Hood S, Liefaard L, Lourbakos A, Morgan A, Nakielny J, Quarcoo N, Ricotti V, Rolfe K, Servais L, Wardell C, Wilson R, Wright P, Kraus JE (2014) Safety and efficacy of drisapersen for the treatment of Duchenne muscular dystrophy (DEMAND II): an exploratory, randomised, placebo-controlled phase 2 study. Lancet Neurol 13(10):987–996. https://doi.org/10.1016/S1474-4422(14)70195-4

Wagner KR (2020) The elusive promise of myostatin inhibition for muscular dystrophy. Curr Opin Neurol 33(5):621–628. https://doi.org/10.1097/WCO.0000000000000853

Wagner KR, Hamed S, Hadley DW, Gropman AL, Burstein AH, Escolar DM, Hoffman EP, Fischbeck KH (2001) Gentamicin treatment of Duchenne and Becker muscular dystrophy due to nonsense mutations. Ann Neurol 49(6):706–711

Wagner KR, Fleckenstein JL, Amato AA, Barohn RJ, Bushby K, Escolar DM, Flanigan KM, Pestronk A, Tawil R, Wolfe GI, Eagle M, Florence JM, King WM, Pandya S, Straub V, Juneau P, Meyers K, Csimma C, Araujo T, Allen R, Parsons SA, Wozney JM, Lavallie ER, Mendell JR (2008) A phase I/IItrial of MYO-029 in adult subjects with muscular dystrophy. Ann Neurol 63(5):561–571. https://doi.org/10.1002/ana.21338

Wang B, Li J, Xiao X (2000) Adeno-associated virus vector carrying human minidystrophin genes effectively ameliorates muscular dystrophy in mdx mouse model. Proc Natl Acad Sci U S A 97(25):13714–13719. https://doi.org/10.1073/pnas.240335297

Watchko J, O'Day T, Wang B, Zhou L, Tang Y, Li J, Xiao X (2002) Adeno-associated virus vector-mediated minidystrophin gene therapy improves dystrophic muscle contractile function in mdx mice. Hum Gene Ther 13(12):1451–1460. https://doi.org/10.1089/10430340260185085

Webster C, Silberstein L, Hays AP, Blau HM (1988) Fast muscle fibers are preferentially affected in Duchenne muscular dystrophy. Cell 52(4):503–513. https://doi.org/10.1016/0092-8674(88)90463-1

Wells DJ (1993) Improved gene transfer by direct plasmid injection associated with regeneration in mouse skeletal muscle. FEBS Lett 332(1–2):179–182. https://doi.org/10.1016/0014-5793(93)80508-r

Wineinger MA, Abresch RT, Walsh SA, Carter GT (1998) Effects of aging and voluntary exercise on the function of dystrophic muscle from mdx mice. Am J Phys Med Rehabil 77(1):20–27. https://doi.org/10.1097/00002060-199801000-00004

Wong SH, Lowes KN, Quigley AF, Marotta R, Kita M, Byrne E, Kornberg AJ, Cook MJ, Kapsa RM (2005) DNA electroporation in vivo targets mature fibres in dystrophic mdx muscle. Neuromuscul Disord 15(9-10):630–641. https://doi.org/10.1016/j.nmd.2005.04.008

Xu L, Park KH, Zhao L, Xu J, El Refaey M, Gao Y, Zhu H, Ma J, Han R (2016) CRISPR-mediated genome editing restores dystrophin expression and function in mdx mice. Mol Ther 24(3): 564–569. https://doi.org/10.1038/mt.2015.192

Yanagihara I, Inui K, Dickson G, Turner G, Piper T, Kaneda Y, Okada S (1996) Expression of full-length human dystrophin cDNA in mdx mouse muscle by HVJ-liposome injection. Gene Ther 3(6):549–553

Yasuda S, Townsend D, Michele DE, Favre EG, Day SM, Metzger JM (2005) Dystrophic heart failure blocked by membrane sealant poloxamer. Nature 436(7053):1025–1029. https://doi.org/10.1038/nature03844

Yoshimura M, Sakamoto M, Ikemoto M, Mochizuki Y, Yuasa K, Miyagoe-Suzuki Y, Takeda S (2004) AAV vector-mediated microdystrophin expression in a relatively small percentage of mdx myofibers improved the mdx phenotype. Mol Ther 10(5):821–828. https://doi.org/10.1016/j.ymthe.2004.07.025

Young CS, Hicks MR, Ermolova NV, Nakano H, Jan M, Younesi S, Karumbayaram S, Kumagai-Cresse C, Wang D, Zack JA, Kohn DB, Nakano A, Nelson SF, Miceli MC, Spencer MJ, Pyle AD (2016) A single CRISPR-Cas9 deletion strategy that targets the majority of DMD patients restores dystrophin function in hiPSC-derived muscle cells. Cell Stem Cell 18(4):533–540. https://doi.org/10.1016/j.stem.2016.01.021

Yue Y, Li Z, Harper SQ, Davisson RL, Chamberlain JS, Duan D (2003) Microdystrophin gene therapy of cardiomyopathy restores dystrophin-glycoprotein complex and improves sarcolemma integrity in the mdx mouse heart. Circulation 108(13):1626–1632. https://doi.org/10.1161/01.CIR.0000089371.11664.27

Zhang G, Ludtke JJ, Thioudellet C, Kleinpeter P, Antoniou M, Herweijer H, Braun S, Wolff JA (2004) Intraarterial delivery of naked plasmid DNA expressing full-length mouse dystrophin in the mdx mouse model of Duchenne muscular dystrophy. Hum Gene Ther 15(8):770–782. https://doi.org/10.1089/1043034041648408

Zhang G, Wooddell CI, Hegge JO, Griffin JB, Huss T, Braun S, Wolff JA (2010) Functional efficacy of dystrophin expression from plasmids delivered to mdx mice by hydrodynamic limb vein injection. Hum Gene Ther 21(2):221–237. https://doi.org/10.1089/hum.2009.133

Zhang Y, Li H, Min YL, Sanchez-Ortiz E, Huang J, Mireault AA, Shelton JM, Kim J, Mammen PPA, Bassel-Duby R, Olson EN (2020) Enhanced CRISPR-Cas9 correction of Duchenne muscular dystrophy in mice by a self-complementary AAV delivery system. Sci Adv 6(8): eaay6812. https://doi.org/10.1126/sciadv.aay6812

Zhang Y, Nishiyama T, Li H, Huang J, Atmanli A, Sanchez-Ortiz E, Wang Z, Mireault AA, Mammen PPA, Bassel-Duby R, Olson EN (2021) A consolidated AAV system for single-cut CRISPR correction of a common Duchenne muscular dystrophy mutation. Mol Ther Methods Clin Dev 22:122–132. https://doi.org/10.1016/j.omtm.2021.05.014

Zincarelli C, Soltys S, Rengo G, Rabinowitz JE (2008) Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection. Mol Ther 16(6):1073–1080. https://doi.org/10.1038/mt.2008.76

Zupan A (1992) Long-term electrical stimulation of muscles in children with Duchenne and Becker muscular dystrophy. Muscle Nerve 15(3):362–367. https://doi.org/10.1002/mus.880150316

Zupan A, Gregoric M, Valencic V, Vandot S (1993) Effects of electrical stimulation on muscles of children with Duchenne and Becker muscular dystrophy. Neuropediatrics 24(4):189–192. https://doi.org/10.1055/s-2008-1071537

Zupan A, Gregoric M, Valencic V (1995) Long-lasting effects of electrical stimulation upon muscles of patients suffering from progressive muscular dystrophy. Clin Rehabil 9(2):102–109

Chapter 5
Regenerative Rehabilitation in Sarcopenia, Dynapenia, and Frailty

Cory W. Baumann, Brian C. Clark, Bethan E. Phillips, Nathaniel J. Szewczyk, and Leslie A. Consitt

Abstract Overall lifespan and health span are dependent on maintaining neuromuscular function. Degeneration in neural and muscular etiological factors with advancing age can result in sarcopenia, dynapenia, and physical frailty. The relative contribution of these neuromuscular mechanisms to clinically meaningful skeletal muscle function with aging is poorly understood. Here, we posit that optimal neuromuscular function, which is critical for healthy aging, includes domains of motor function, contractile quality, muscle mass, and muscle metabolism. Thus, it is essential that research efforts identify mechanisms to attenuate neuromuscular dysfunction and skeletal muscle weakness so effective therapeutics can be provided in the clinical setting. This chapter highlights five major components of neuromuscular function that are potential targets for regenerative rehabilitation in relation to sarcopenia, dynapenia, and physical frailty. These are (1) neuromuscular excitation, (2) excitation–contraction (EC) coupling, (3) mitochondrial function, (4) protein homeostasis and (5) glucose metabolism.

Keywords Aging · Atrophy · Metabolism · Neuromuscular · Physical Function · Skeletal Muscle · Strength

C. W. Baumann (✉) · B. C. Clark · N. J. Szewczyk · L. A. Consitt
Ohio Musculoskeletal and Neurological Institute (OMNI), Ohio University, Athens, OH, USA

Department of Biomedical Sciences, Ohio University, Athens, OH, USA
e-mail: baumann@ohio.edu

B. E. Phillips
Centre for Musculoskeletal Ageing and National Institute of Health Research, Nottingham Biomedical Research Centre, University of Nottingham, Derby, UK

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_5

5.1 Introduction

5.1.1 The Aging Neuromuscular System

The neuromuscular system, consisting of the nervous and skeletal muscle tissues, experiences many deficits with advancing age. These deficits contribute to skeletal muscle weakness that manifests in pervasive chronic conditions and disability observed in many older adults (defined as 65 years and older by the National Institutes of Health) (Guidelines for the Review of Inclusion on the Basis of Sex/Gender, Race, Ethnicity, and Age in Clinical Research 2019). For instance, around 30% of women and 15% of men in the USA over 60 years self-report that they are unable to lift or carry 10 pounds (Louie and Ward 2010). Furthermore, over 40% of seniors have limitations in performing one or more daily tasks (e.g., walking two to three blocks, transferring from sitting to standing) that are essential for maintaining physical independence (Seeman et al. 2010). Scientific and medical communities agree that weakness (frequently referred to as dynapenia) is a major determinant of physical limitations and general poor health in older adults (Rantanen 2003; Rantanen et al. 1998, 1999, 2002; Visser et al. 2005; Newman et al. 2006; Manini et al. 2007; McGrath et al. 2018, 2019a, b). The neuromuscular mechanisms of dynapenia are known to be multi-factorial. One well-known contributor to dynapenia is the age-related loss of muscle mass (collectively referred to as sarcopenia[1]) (Clark and Manini 2008). Muscle wasting is not, however, the sole determinant of age-related weakness. In fact, when changes in quadriceps muscle size and leg extensor strength were assessed over a 5-year period ($n = 1678$, 70–79 years at baseline), it was observed that decreases in strength were two–five times greater than loss of muscle size in those who lost or maintained weight (Delmonico et al. 2009). Further, individuals that gained weight ($n = 333$) actually exhibited a small increase in muscle size, yet were not spared from dynapenia. These findings indicate that age-related loss of strength is not only attributed to muscle atrophy, but also complex neurologic and other skeletal muscle factors (Enoka et al. 2003; Tieland et al. 2018; Russ et al. 2012; Clark and Manini 2008; Narici and Maffulli 2010).

With continued and progressive loss of neuromuscular function, the risk of developing physical frailty increases, with physical frailty defined as "a medical syndrome with multiple causes and contributors that is characterized by diminished strength, endurance, and reduced physiologic function that increases an individual's vulnerability for developing increased dependency and/or death" (Morley et al. 2013). Physical frailty is often considered to be on a continuum, in that frailty status

[1] In this article we use terms that have been more commonly used over the past decade and longer to specifically refer to age-related muscle weakness (i.e., dynapenia) and wasting (i.e., sarcopenia). We should note, however, that the operational definition of "sarcopenia" is still fluid and that recent consensus statements now emphasizing low muscle strength as the primary characteristic of sarcopenia (Cruz-Jentoft et al. 2019).

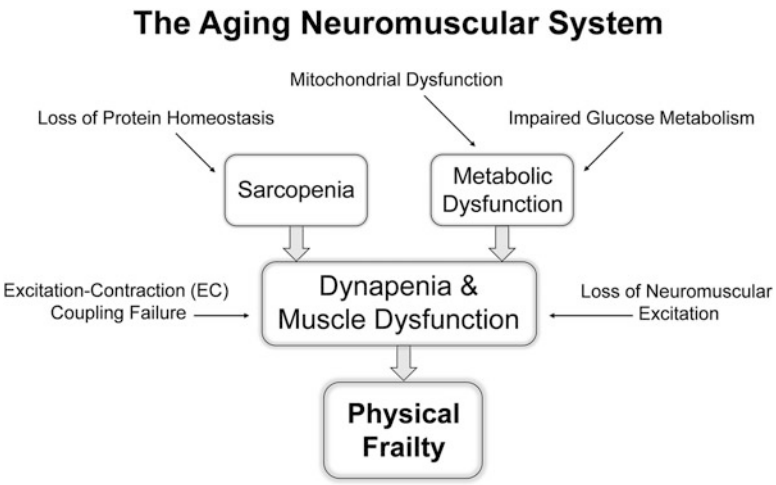

Fig. 5.1 Domains that contribute to neuromuscular deficits with age. Loss of neuromuscular excitation, EC coupling failure, sarcopenia (via loss of protein homeostasis) and metabolic dysfunction (via mitochondrial dysfunction and impaired glucose metabolism) contribute to physical frailty, as measured by dynapenia and muscle dysfunction

can transition between non-frail, pre-frail (or intermediate) and frail, with status corresponding to mortality risk (Fried et al. 2001; Baumann et al. 2020). In a recent systematic review and meta-analysis, these transition states predicted mortality, with hazard ratios of 1.335 (95% CI: 1.260–1.414) and 2.000 (95% CI: 1.727–2.316) for pre-frail and frail individuals when compared to those that were non-frail, respectively (Chang and Lin 2015). Similar findings have also been observed in the C57BL/6 mouse (Baumann et al. 2018; Kwak et al. 2020; Baumann et al. 2020), a common strain used by laboratories to study aging (Mitchell et al. 2015). In both humans (Fried et al. 2001) and rodents (Liu et al. 2014; Baumann et al. 2020), skeletal muscle weakness and loss of function are salient components in the development of physical frailty. Therefore, if sarcopenia and dynapenia are left untreated, a vicious cycle of weakness and neuromuscular dysfunction will continue, leading to physical frailty, loss of dependence and ultimately, an early death.

5.1.2 Regenerative Rehabilitation in the Aging Neuromuscular System

Maintaining neuromuscular function is an important component to overall lifespan and health span. Sarcopenia, dynapenia and resultant physical frailty may result from primary neural or muscular etiological factors or a combination thereof (Fig. 5.1). The relative contribution of these neuromuscular mechanisms to clinically meaningful skeletal muscle deficits in advancing age is poorly understood. Moreover,

muscle mass has broad health benefits that extend beyond just force generation capacity and locomotion, such as the critical role it plays in metabolism, specifically glucose regulation. Optimal neuromuscular function in the domains of motor function, contractile quality, muscle mass, and muscle metabolism are therefore critical to healthy aging. Thus, identifying and validating therapeutic approaches that focus on attenuating or delaying neuromuscular dysfunction and subsequently dynapenia are essential goals in laboratory and clinical settings. This chapter highlights five major components of neuromuscular function that are potential targets for regenerative rehabilitation in sarcopenia, dynapenia, and physical frailty. These are (1) neuromuscular excitation, (2) excitation-contraction (EC) coupling, (3) mitochondrial function, (4) protein homeostasis, and (5) glucose metabolism. Each component or section within this chapter is further divided into the following subsections: Introduction, Age-induced Pathophysiology, and Regenerative Rehabilitation.

5.2 Neuromuscular Excitation

5.2.1 Introduction to Neuromuscular Excitation

There are two important constructs to muscular strength, the ability to generate sufficient force and the ability to generate force rapidly. The capacity of the central nervous system to excite motor units (i.e., muscle fibers innervated by a motor neuron) is critical to both. It is also important to recognize that the ability to selectively engage muscles in a coordinated, context sensitive manner is critical to motor control of skeletal muscle. For instance, if a perturbation occurs during gait, the adaptive response that is observed extends across the whole body, involving coordinated lower limb muscle activity, but also goal-directed engagement of torso and upper limb muscles (Marigold and Misiaszek 2009). The pattern of muscle activation under such circumstances is often described with the term "synergy." Synergy is defined as mechanisms used by the central nervous system to coordinate groups of motor units into functional assemblies (Windhort et al. 1991). While it is perhaps obvious that measures of muscle strength depend upon the ability to recruit motor units, it is less widely appreciated that all voluntary contractions and movements reflect the organization of muscle synergies. Thus, strength is the accumulation of both skeletal muscle and the motor system (Enoka 1988). Voluntary engagement occurs through activity in brain networks (e.g., in the primary motor cortex), which results in elevated firing of (descending) corticospinal neurons and the consequential recruitment of spinal motor neurons and muscle fibers. As descending neural drive increases, a greater number of spinal motor neurons are recruited, discharge more rapidly, and increase contractile force (Ashe 1997). When a motor neuron fires sufficiently fast, the muscle fibers it innervates produce a fused contraction. In this context, the state of the spinal motor neurons (in addition to descending drive from the motor cortex and other supraspinal centers) are influenced by many factors, such as those mediated by excitatory and inhibitory afferent

projections and alterations in motor neuron properties that may make them more or less responsive to synaptic input (Berardelli et al. 2001).

5.2.2 *Age-Induced Pathophysiology of Neuromuscular Excitation*

5.2.2.1 Age-Induced Loss of Voluntary (Central) Muscle Activation

Numerous studies have compared electrically-stimulated force production vs. voluntary force production to examine whether aging results in an impaired ability of the nervous system to fully activate skeletal muscle volitionally (Clark and Taylor 2011). These reports have yielded discrepant findings, likely due to variations in the muscle groups investigated, as well as the inherent heterogeneity of aging (Lowsky et al. 2014). In a recent report, older adults with clinically meaningful leg extensor weakness, exhibited deficits in the ability of their nervous system to fully activate their leg extensor muscles, while stronger older adults did not (Clark et al. 2019). Data such as this support the notion that the nervous system is a key culprit in older adults with clinically meaningful age-related weakness.

In addition to the leg extensor musculature, there is also evidence that grip strength measures are largely reflective of the integrity of the nervous system (Carson 2018). For instance, there is a well-described decline in the flexion force that can be generated by an individual finger, as the number of other fingers that contribute to the grip is increased (Ohtsuki 1981). The observation that the magnitude of this deficit is greater in older adults than in the young (Shinohara et al. 2003b) is consistent with evidence that deficiencies in muscle synergy formation contribute to the difficulties experienced by older adults in many movement tasks (Shinohara et al. 2003a; Barry et al. 2005). This finding is particularly intriguing when one considers that low hand grip strength is associated with a wide range of negative health outcomes in older adults, including cardiovascular disease (Celis-Morales et al. 2018), diabetes (McGrath et al. 2017), dementia (Buchman et al. 2007; Carson 2018), functional disability (McGrath et al. 2018; Al Snih et al. 2004), depression (Fukumori et al. 2015), mobility limitations (Hicks et al. 2012; Bhasin et al. 2020), and early all-cause mortality (Duchowny 2019; McGrath et al. 2019b).

5.2.2.2 Age-Induced Loss of Neural Excitability

Neural excitability can broadly be defined as the readiness of a nerve cell or a neural circuit to respond to a stimulus (Konstantinovic and Fliipovic 2019; Kandel et al. 2013; Schulz et al. 2006). The response is typically in the form of an action potential, a transient change of electrical charge (polarization) of the neuronal membrane. The action potential can be measured either individually, at the level of an individual nerve cell, or as the sum of action potentials in the form of a compound action

potential or an evoked potential, at the level of groups of neurons or neural circuits (Kandel et al. 2013). There is a strong theoretical basis for the contention that neural hypoexcitability serves as a key contributor to weakness. That is, a neuron with low excitability will, conceptually, have a lower maximal steady-state firing frequency (Schulz et al. 2006).

Dynapenia, as well as a myriad of other disorders and conditions (e.g., disuse, injury, and sepsis), may be due, in part, to neural hypoexcitability (Clark et al. 2020; Clark et al. 2014; Nardelli et al. 2013; Stefanelli et al. 2019). In the context of aging, older adults with clinically meaningful leg extensor weakness exhibit indexes indicative of corticospinal hypoexcitability (e.g., magnetic brain stimulation derived motor evoked potentials about half that of the strong older adults) (Clark et al. 2020). Moreover, these indexes could explain ~33% of the between-subject variability in older adult's leg extensor strength, which was slightly more than that explained by thigh lean mass (Clark et al. 2020). These findings suggest that weakness is mechanistically, partially mediated by neural hypoexcitability. Whether dysfunction is at the level of the cortical or spinal motor neurons has yet to be determined; though it has been hypothesized that age-induced hypoexcitability occurs in both upper and lower motor neurons. For instance, paired-pulse brain stimulation paradigms that permit inferences in relation to intracortical excitability demonstrate that older adults have greater indices of cortical hypoexcitability in comparison to young adults (McGinley et al. 2010; Clark et al. 2015). Human and animal studies also suggest that aging results in a reduction in α-motor neuron excitability (e.g., greater and longer hyperpolarization potentials and lower minimal and maximal steady-state firing frequencies) (Kalmar et al. 2009; Christie and Kamen 2006, 2010; Kamen et al. 1995).

5.2.2.3 Age-Induced Loss of Dopaminergic Function

Within the central nervous system, the basal ganglia may theoretically be linked to age-related reductions in mobility capacity via its associated dopaminergic function. Progressive degeneration of mid-brain dopaminergic neurons has been associated with deficits in the initiation, speed, and fluidity of voluntary movement (Berardelli et al. 2001; Buhusi and Meck 2005; Turner and Desmurget 2010). With regards to aging, slower rates of voluntary force development have been linked to risk of falls (Kamo et al. 2019), and ability of the nervous system to rapidly drive muscle force production is associated with overall mobility (Moskowitz et al. 2020). Moreover, peak horizontal saccade velocity, which theoretically should not be impacted by musculoskeletal mechanisms and processes, has been reported to decrease with age (Irving et al. 2006). In fact, older adults have a saccade velocity about half that of young adults (Irving et al. 2006). Studies of the aging human brain have also shown that regulation of dopamine action is significantly reduced in older age via structural degradation, including neuronal loss, fewer neuroreceptor sites, and loss of transporter molecules (Kaasinen and Rinne 2002). For instance, age-dependent declines of brain dopamine agonist (DA) levels have been reported in the basal ganglia,

specifically the dorsal striatum post-mortem (Carlsson and Winblad 1976). In vivo imaging studies have since confirmed these findings (Kaasinen and Rinne 2002).

After the age of 20, the availability of dopamine D1-like receptors also declines in the human striatum at a rate of ~7% per decade (Suhara et al. 1991; Wang et al. 1998). The D2-like family demonstrates a similar decrease in receptor density (~5–10% per decade) (Rinne et al. 1993; Wong et al. 1997) and receptor binding potential (~6–8% per decade) (Rinne et al. 1993; Antonini and Leenders 1993; Volkow et al. 1996). There are several studies that have directly examined the relationship between striatal DA and age-related changes in gait and other parameters of motor function. Volkow et al. (1998) reported that age-related decreases in brain dopamine activity in non-Parkinsonian older adults, coincided with reductions in finger tapping speed. Similar studies have reported that lower striatal dopamine transporter activity explained ~23% and 35% of the between-subject variance in "comfortable pace" gait speed and cadence, respectively (Cham et al. 2008). Moreover, several investigations have reported a relationship between dopaminergic genotype (catechol-O-methyltransferase (COMT) genotype) and mobility (Moskowitz et al. 2020; Metti et al. 2017; Holtzer et al. 2010). These studies suggest that the genotype resulting in intermediate levels of tonic DA (i.e., the Val158Met polymorphism) is associated with faster gait and movement speeds, which is likely due to it balancing the roles of tonic and phasic DA (i.e., tonic–phasic regulation of DA transmission) (Bilder et al. 2004; Grace 1991; Schacht 2016).

5.2.3 Regenerative Rehabilitation and Neuromuscular Excitation

Physical exercise is known to enhance motor control and function via neural adaptations in older adults, and therefore cannot be overemphasized (Watson 2017). However, general physical exercise is beyond the scope of this chapter. Here, we discuss goal-directed motor training as well as other novel neurotherapeutic approaches that may be leveraged to improve neural excitation of muscle. We should note at the outset that, to our knowledge, there are no FDA-approved neuro-based therapies that have been approved for use in situations of muscle weakness or mobility limitations. All approaches that we will discuss should be considered experimental and only used in the context of research studies (as opposed to clinical practice per se). There are several potential approaches for enhancing neural excitability; herein, we discuss; goal-based motor training, non-invasive brain stimulation and pharmacological compounds.

5.2.3.1 Goal-Based Motor Training

The attachments between neurons are incredibly dynamic; they change and grow (or shrink) constantly. Working together in a network, neurons organize themselves into specialized groups to form different kinds of information processing. When one neuron sends a signal to another, the synapse between the two strengthens (hence the adage "neurons that fire together, wire together"). The more often a particular signal is sent between them, the stronger the connection grows. Novel experiences and learning cause new dendrites to form, whereas repeated behavior and learning cause existing dendrites to become more entrenched. These basic principles are fundamental to the development and rationale of exercise training strategies for enhancing motor function.

Motor representations (i.e., movement memories) are formed and stored in the brain, just like our memories of people and events. Motor representations are created by a series of remarkably complex coordinated processes dispersed throughout the brain that involve multiple neural networks that interact to help individuals perform learned movements. Thus, memory is the cornerstone of all learning. Motor adaptation refers to learning a new movement skill. When someone practices a movement over and over again, they perform better, partly because they develop new motor memories.

Progressive resistance exercise is largely considered to be the first-line therapy to manage sarcopenia (Dent et al. 2018). We believe this is rational in that it has been shown to have innumerable benefits (Churchward-Venne et al. 2015; Law et al. 2016). However, advancing age has been suggested to result in increased cortical processing for mobility tasks (i.e., less automaticity) (Sorond et al. 2015). Thus, we encourage increased attention to be given to interventional strategies that incorporate goal-based motor skill training (note: "goal-based" refers to the practice of certain activities that lead to improved performance). There are many exercise modalities that can incorporate aspects of goal-based motor skill training, but the more classic approaches are exercise modalities such as Tai Chi, dancing, boxing, and mixed martial arts. The central premise of goal-based motor skill training is that it facilitates learning through feedback (reinforcement learning), involves engagement of the prefrontal cognitive circuits that are involved in early phases of motor learning, and incorporates parameters important for experience-dependent neuroplasticity (e.g., intensity, repetition, difficulty, specificity, complexity of practice, etc.). Feedback (verbal cueing, proprioceptive, etc.) has numerous purposes, such as challenging individuals beyond their self-selected levels of perceived capability, maintaining motivation, and increasing cognitive awareness of movements that were previously automatic and unconscious. Figure 5.2 provides an illustration of the neural structures and connections involved in the cognitive and automatic aspects of motor control in relation to goal-directed learning. Goal-based exercise strategies have become a mainstay in neurorehabilitation for the improvement or recovery of impaired or lost motor function in overt neurological disease (e.g., Petzinger et al.

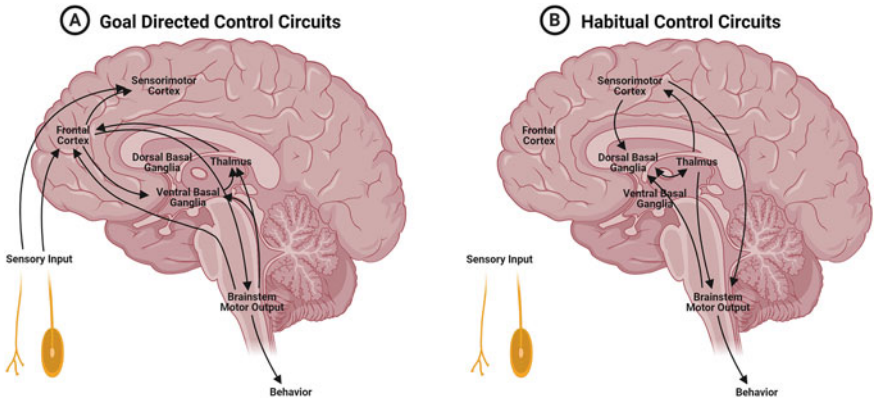

Fig. 5.2 Goal directed and habitual control circuits of behavior. Motor control incorporates numerous cortical and subcortical structures with the most critical connections being those between the basal ganglia and cortex as these deeply involved in the cognitive (**a**) and automatic (**b**) aspects of motor control. In **a**, arrows represent the cognitive (or volitional) circuits. In **b**, the arrows represent the automatic (or the unconscious/habitual) circuits. Aging has been suggested to result in impaired automaticity. As such, we advocate for increased investigations on whether goal-based motor training programs can be used to mitigate and treat dynapenia. Figure recreated based on that of Petzinger et al. (2013). This image was created using BioRender (https://BioRender.com)

2013), and for the reasons stated in this chapter, we believe these strategies hold promise for dynapenia.

5.2.3.2 Non-invasive Brain Stimulation

Non-invasive brain stimulation consists of techniques including transcranial magnetic stimulation and transcranial direct current stimulation (tDCS). High-frequency (e.g., 10–20 Hz) magnetic brain stimulation and anodal transcranial direct current stimulation both have been demonstrated to transiently increase excitability of the motor cortex (Reis et al. 2008; Kobayashi and Pascual-Leone 2003; Lefebvre and Liew 2017). Whether they have the potential to modify strength and physical function, for use during rehabilitation or as a stand-alone therapy is not clear. In relatively small studies (based on sample size) consecutive sessions of anodal transcranial direct current stimulation enhanced hand dexterity (i.e., ~20–25% improvement on the Purdue Pegboard Test performance) (Rostami et al. 2020) and improved elbow flexor fatigue-resistance (i.e., ~15% increase the time to task failure of a sustained, submaximal contraction) (Oki et al. 2016) (Fig 5.3a). Thus, while this is far from conclusive data, it does suggest that non-invasive brain stimulation has the ability to modify indexes of motoric and muscle function in older adults.

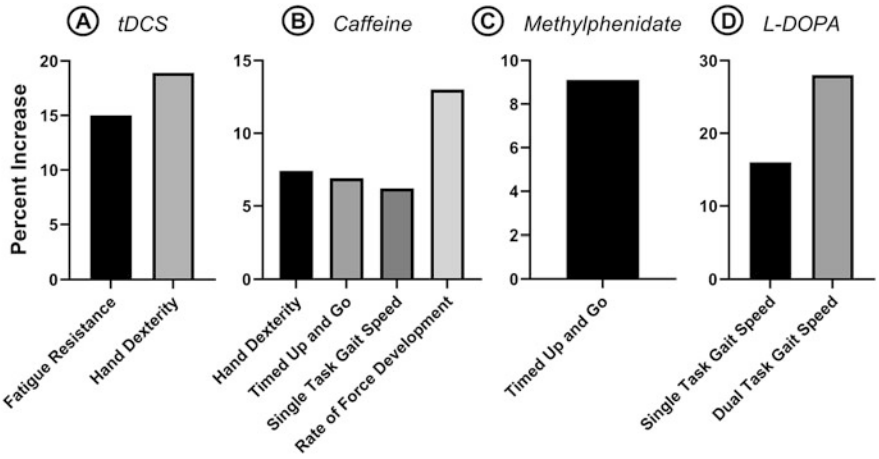

Fig. 5.3 Mean effects of various approaches that increase neural excitability on enhancing measures of physical function in older adults. (**a**) Single session of anodal transcranial direct current stimulation (tDCS), which increases cortical excitability, increased the time to task failure of a sustained, submaximal elbow flexion contraction (Oki et al. 2016), and five consecutive days of anodal tDCS improved the Purdue Pegboard Task performance (a measure of hand dexterity) (Rostami et al. 2020). (**b**) Acute ingestion of caffeine (3 mg/kg, which is the equivalence of 2–3 cups of coffee) increased manual dexterity, timed up and go time, six-minute walk gait speed (Duncan et al. 2014), as well as the rate of force development during a sit to stand task (Tallis et al. 2020). (**c**) A single dose of methylphenidate improved timed up and go performance (Ben-Itzhak et al. 2008). (**d**) Three weeks of L-DOPA improves single (blue) and dual task (violet) usual gait speed in older adults with depression (Rutherford et al. 2020). Graphs recreated based on data published in the respective articles

5.2.3.3 Pharmacological Compounds

Compounds that increase neural excitability can also enhance motor function. One such potential compound is caffeine. Caffeine is a well-established performance enhancing nutritional compound in young, healthy humans. There are limited data on the effects of caffeine on measures of human performance in older adults. The few studies that do exist are small-scale trials (e.g., 10–30 subjects) and have reported mixed effects. Specifically, some studies suggest that caffeine supplementation (typically around 3 mg/kg, or the equivalence of two–three cups of coffee) does not alter leg extensor muscle strength, static balance, as well as various measures of physical performance (Tallis et al. 2020). Conversely, some studies have reported that caffeine, when compared to a placebo, modestly enhances (~6–8%) manual dexterity, timed up and go time, and six-minute walk gait speed (Duncan et al. 2014), and more robustly increases the rate of force development during a sit to stand task around 13% (Tallis et al. 2020) (Fig. 5.3b). It should be noted that all of the prior work has been conducted in relatively young older adults (e.g., mean age of late 60s to early 70s) who were extremely high functioning. Thus,

it is possible that a ceiling effect may exist as it relates to any enhancement in function amongst older adults with such high levels of physical function and mobility. Thus, more work is needed, and the existing results must be interpreted cautiously and critically.

Another potential compound of interest is the serotonin 5-HT2c receptor agonist, although the effects of serotonin 5-HT2c receptor agonist on motor function in aged rodents or humans have yet to be tested. However, motor neuron hypoexcitability has been reported to contribute to sepsis-induced weakness in rat muscle (Nardelli et al. 2013, 2016), and subthreshold voltage-activated currents are a key mechanism underlying defective repetitive firing observed in this model (Nardelli et al. 2017). More recently, it was demonstrated that treatment of septic rats with injection of a selective serotonin 5-HT2c agonist (lorcaserin; 3 mg/kg) significantly improved repetitive motor neuronal firing and dramatically increased motor unit force production. To our knowledge, only one prior study has examined the effects of lorcaserin on any behavioral measure related to motor function. In that study, young rats ($n = 8$) received single, varying 5-HT2c agonist doses, and a 21% increase in Rotarod performance (i.e., walking speed, motor performance) was observed at a dose of 0.6 mg/kg (Higgins et al. 2012). However, worsened performance was observed at higher doses, consistent with reports that high doses result in malaise (Higgins et al. 2020). Thus, additional research is needed.

With regards to the dopaminergic system, there are several studies that support the potential modulation of this system to enhance physical function and mobility outcomes in older adults. The earliest of these evaluated the potential of a single dose of methylphenidate (20 mg) on measures of physical function (Ben-Itzhak et al. 2008). Methylphenidate has multiple mechanisms of action, yielding its stimulant effect, with one of the mechanisms of action being inhibition of dopamine uptake (PubChem 2021a). This study reported that methylphenidate improved timed up and go performance and gait (stride time variability) in 26 community dwelling older adults when compared to placebo (Ben-Itzhak et al. 2008) (Fig. 5.3c). The second of these studies also examined the effects of a single dose of methylphenidate (short-acting 10 mg) on gait in thirty healthy older adults (Shorer et al. 2013). Here, it was also observed that methylphenidate improved mobility by reducing step errors during a standard gait task as well as when the gait task was overlayed with a cognitive load component. The findings that the effect was most robust in a dual task requiring higher executive control suggests the effects could be due to improvements in sustained attention as well as potential direct effects on the motor system. While beyond the scope of this chapter, it should be noted that there is growing scientific interest in the role of cognitive and motor system interactions and their interrelationship with age-related declines in both systems (Cohen and Verghese 2019). Lastly, there is one study that has examined the potential utility of levodopa (L-DOPA), an amino acid precursor of dopamine (PubChem 2021b), for enhancing physical function in older adult. This recent pilot study treated sixteen older adults who suffered from depression with L-DOPA for 3-weeks, and reported a 16% and

28% significant increase in single task and dual task usual gait speed, respectively (Rutherford et al. 2020) (Fig. 5.3d). These above-mentioned findings clearly indicate the need for further work examining the role of dopaminergic function in age-related mobility capacity.

With the above-mentioned said, a pharmaceutical approach to enhancing physical function in older adults could, in and of itself, be problematic as polypharmacy, defined as being prescribed five or more medications, has been shown to be associated with a decline in mental and physical functioning in elderly patients (Williams et al. 2019). In particular, anticholinergics, benzodiazepines, antipsychotics, and opioids were all found to have significant adverse effects in the elderly population (Williams et al. 2019). It should be noted that, in general, these classes of medications have sedative properties that reducing neural excitability. Thus, it is very possible that a "less is more" pharmaceutical approach may be the most beneficial approach to enhancing physical function in older adults.

5.3 Excitation–Contraction (EC) Coupling

5.3.1 Introduction to EC Coupling

In addition to the loss of neuromuscular excitation, sites and structures within the EC coupling pathway have also been linked to age-induced weakness (Delbono et al. 1995; Renganathan et al. 1997a; Ryan and Ohlendieck 2004; Delbono 2011; Baumann et al. 2016). EC coupling can broadly be defined as the sequence of events linking plasmalemma depolarization to the release of Ca^{2+} from the sarcoplasmic reticulum (SR) (Sandow 1952; Hernández-Ochoa and Schneider 2018; Calderón et al. 2014). Briefly, after a threshold potential is initiated at the motor endplate, a membrane action potential propagates along the plasmalemma down the transverse (T)-tubules. Action potential conduction is dependent on a coordinated response of various plasmalemmal ion channels and pumps. Depolarization of the plasma membrane stimulates the voltage-sensitive dihydropyridine receptors (DHPRs) located in the T-tubules, which in turn, activate the ryanodine receptors (RyRs). In skeletal muscle, the DHPRs and RyRs are in close proximity as to permit physical contact, thereby allowing direct interaction and communication (Fill and Copello 2002; Grabner and Dayal 2010; Calderón et al. 2014). The RyR is a Ca^{2+} release channel composed of four monomers embedded in the SR membrane. Each RyR monomer interacts with numerous ancillary proteins (e.g., calstabin, calmodulin) and enzymes (e.g., phosphatase; PP1, phosphodiesterase; PDE4D3) known to alter its gating (Bellinger et al. 2008a; Santulli et al. 2018). Upon RyR activation, Ca^{2+} is released into the cytosol and cross-bridge formation subsequently occurs. Theoretically, if any step in the EC coupling pathway is disrupted (termed EC coupling

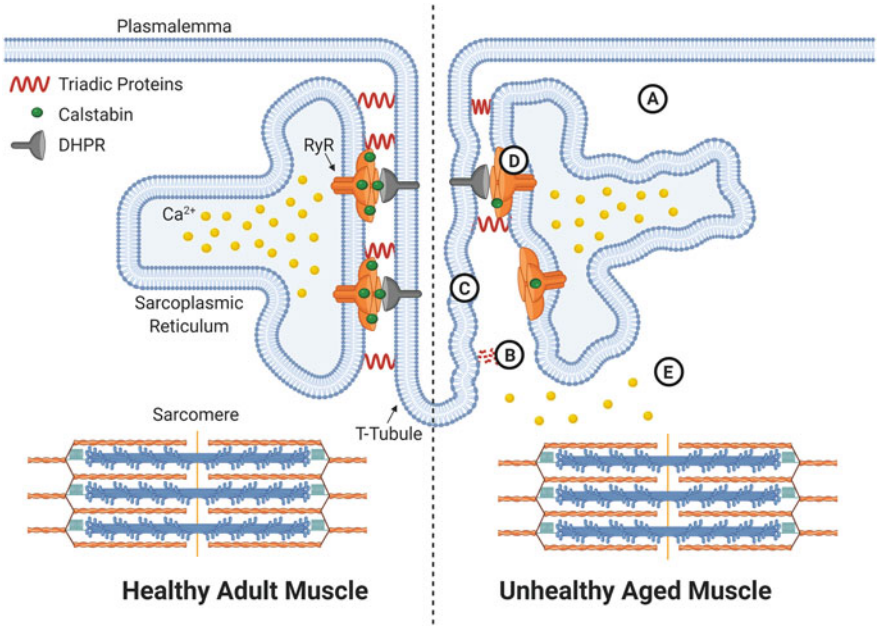

Fig. 5.4 Possible sites and structures within the excitation-contraction (EC) coupling pathway implicated in age-induced skeletal muscle weakness (i.e., dynapenia). EC coupling failure with advanced age this thought to stem from DHPR-RyR uncoupling via the loss of DHPR, RyR and/or the triad structure. (A) Loss of triad structure with (B) corresponding reductions in triadic proteins, such as JP45 and MG29. (C) Loss of the DHPR and/or RyR causing a decrease in the DHPR/RyR ratio (in figure, DHPR content is reduced). (D) Loss or depletion of calstabin from RyR causing SR Ca^{2+} leak and (E) increased intracellular Ca^{2+} concentrations. This image was created using BioRender (https://BioRender.com)

failure), voltage-induced SR Ca^{2+} release will be impaired, and less cytosolic Ca^{2+} will be available for cross-bridge formation and force generation (Baumann et al. 2016). With increasing age, peak intracellular Ca^{2+} transients evoked by plasmalemmal depolarization have been shown to decrease (Wang et al. 2000; Gonzalez et al. 2003; Delbono et al. 1995; Russ et al. 2011; Umanskaya et al. 2014), indicative of impaired SR Ca^{2+} release. Age-induced EC coupling failure can be observed as dynapenia without a concomitant change in muscle mass, as measured by reductions in skeletal muscle-specific force (i.e., force normalized to muscle mass or cross-sectional area; CSA) (Lynch et al. 1999; Moran et al. 2005; Hill et al. 2020; Goodpaster et al. 2006; Russ et al. 2011). Potential mechanisms for EC coupling failure in aged muscle include reduced content of EC coupling proteins, loss of EC coupling protein-protein interactions and/or modifications to EC coupling proteins (Fig. 5.4).

5.3.2 *Age-Induced Pathophysiology of EC Coupling Failure*

5.3.2.1 Age-Induced Loss of EC Coupling Proteins and Protein-Protein Interactions

Any reduction in EC coupling proteins or the ability of these proteins to interact could result in EC coupling failure. Although many sites of age-induced EC coupling failure have yet to be established, several research groups have reported reduced expression, lowered content, and altered protein-protein interactions among the DHPR and RyR proteins (Fig. 5.4). Reductions in either DHPR (O'Connell et al. 2008; Renganathan et al. 1997a; Ryan et al. 2000), specifically the $Ca_V1.1$ subunit of DHPR ($DHPR\alpha_{1s}$) (Catterall et al. 2005), or RyR (Fodor et al. 2020) expression or protein content have been reported by many, but not by all (Lamboley et al. 2016; Ryan et al. 2003, 2011). With loss of DHPR and/or RyR, the DHPR-RyR interaction is uncoupled, as measured by decreases in the DHPR/RyR ratio (Renganathan et al. 1997a; Ryan et al. 2000). Delbono and colleagues (Renganathan et al. 1997a) reported that reduced DHPR expression in rat skeletal muscle is the molecular basis for age-induced EC coupling failure. Decreased expression of DHPR was suggested to cause significant impairment in action potential transduction into a DHPR signal, consequently reducing RyR activation, SR Ca^{2+} release and cross-bridge formation.

Besides loss of DHPR and/or RyR, age-induced uncoupling between DHPR and RyR may stem from diminished communication between these two proteins (Fig. 5.4). This is supported by fragmented SR (Weisleder et al. 2006) and disarranged triads (Boncompagni et al. 2006; Zampieri et al. 2015) observed using electron microscopy. Essentially, with triad disarrangement, the proximity of the DHPRs to the RyRs will be lost, reducing the number of proteins that can physically interact. Disorganized alignment of SR and T-tubule membranes coincide with reductions in the triadic proteins JP-45 (Anderson et al. 2003) and MG29 (Weisleder et al. 2006), proteins thought to interact with DHPR and RyR or maintain the structure of triad junction. Loss of these triadic proteins would inevitably increase the distance between the T-tubule and SR, and subsequently the DHPR-RyR interaction. Aligning with these aforementioned changes, Ca^{2+} handling proteins (e.g., sarcalumenin) (O'Connell et al. 2008), plasmalemmal ion channels (e.g., chloride channels) (Pierno et al. 1999) and plasmalemmal pumps (Ca^{2+} ATPase and the Na^+-Ca^{2+} exchanger) (O'Connell et al. 2008) have also been reported to exhibit age-related reductions in content. Further research will be needed to elucidate whether all these age-related changes occur in synchronization or if there is an initiating event. Taken together, many EC coupling structures and proteins appear to be influenced by age, but most data suggest EC coupling failure stems from DHPR-RyR uncoupling via the loss of DHPR, RyR and/or the triad structure.

5.3.2.2 Age-Induced Modifications to EC Coupling Proteins

Modifications that occur to EC coupling proteins will also result in EC coupling failure. The most documented proteins are at the triad junction, specifically the RyRs. The RyR is a macromolecule complex that interacts with numerous proteins and is prone to various modifications (Bellinger et al. 2008a; Santulli et al. 2018). With advancing age, the RyR becomes increasingly oxidized and nitrosylated, promoting RyR dysfunction (Andersson et al. 2011; Umanskaya et al. 2014; Lamboley et al. 2016). Interestingly, these redox modifications do not appear to directly alter RyR function per se, but rather through their effects on the ancillary proteins associated with the channel's activity. One in particular is calstabin (FK506 binding protein, FKBP12) (Andersson et al. 2011), a 12-kDa protein that normally binds to each RyR monomer (Fig. 5.4). Calstabin is thought to stabilize the channel, preventing it from opening to subconductance states (Ahern et al. 1997; Brillantes et al. 1994). Age-associated oxidation and nitrosylation of RyR depletes the channel of calstabin, diminishing the calstabin-RyR interaction (Andersson et al. 2011; Umanskaya et al. 2014). These changes are thought to result in "leaky" RyRs that manifest in increased single-channel open probability, increased Ca^{2+} spark frequency (Andersson et al. 2011; Umanskaya et al. 2014) and reduced SR Ca^{2+} content (Lamboley et al. 2015, 2016). Under these conditions, voltage-mediated SR Ca^{2+} release appears to be impaired through loss of RyR function rather than RyR content (Andersson et al. 2011; Lamboley et al. 2016; Russ et al. 2011). In support of the loss in the calstabin-RyR interaction causing SR Ca^{2+} leak, others have reported resting Ca^{2+} concentrations are elevated in aged muscle (Mijares et al. 2020). Importantly, RyR dysfunction mediated by depletion of calstabin is not only observed with age (Russ et al. 2011; Andersson et al. 2011; Umanskaya et al. 2014), but also heart failure (Shan et al. 2010), muscular dystrophy (Bellinger et al. 2009), chronic muscle fatigue (Bellinger et al. 2008b), and contraction-induced injury (Baumann et al. 2014). These results suggest that age-related EC coupling failure predominantly stems from redox-induced RyR dysfunction.

5.3.3 Regenerative Rehabilitation and EC Coupling

Data suggests age-induced weakness due to EC coupling failure stems from (1) a reduction in the DHPR-RyR interaction via the loss of the DHPR, RyR and/or triad structure and (2) redox-induced RyR dysfunction. Although the initiating events that induce EC coupling failure are likely many, loss of mitochondria homeostasis and oxidative stress appear to be central catalysts (Andersson et al. 2011; Qaisar et al. 2018; Umanskaya et al. 2014). With advancing age, oxidative stress is thought to occur due to an overproduction of reactive oxygen and nitrogen species (ROS/RNS) and an impaired ability to neutralize them (Mijares et al. 2020; Vasilaki et al. 2006). ROS/RNS can increase with age due to mitochondrial dysfunction caused by

age-related mitochondrial DNA mutations, deletions and/or damage (Boengler et al. 2017) (see Sect. 5.4.2.1). With the accumulation of ROS/RNS, the RyRs are subject to oxidative stress, which leads to SR Ca^{2+} leak and elevated cytosolic Ca^{2+} concentrations (see Sect. 5.3.2.2) (Andersson et al. 2011; Umanskaya et al. 2014). These events can lead to a vicious cycle that exacerbates mitochondrial dysfunction by causing mitochondrial Ca^{2+} overload, stimulation of additional ROS/RNS and greater SR Ca^{2+} leak (i.e., further depleting RyR of its stabilizing subunit calstabin). These data indicate that overproduction of ROS/RNS occurring in aged skeletal muscle may alter the expression and function of key EC coupling proteins, thereby causing dynapenia.

When considering regenerative rehabilitation to combat age-associated EC coupling failure, it is essential to reduce oxidative stress via lowering ROS/RNS production, increasing ROS/RNS clearance or by attenuating the negative effects of ROS/RNS. Likely one of the most robust and potent strategies is modifying lifestyle with physical exercise (Distefano and Goodpaster 2018; Lanza et al. 2008). For instance, muscle from older adults who exercised regularly had lower expression of genes related to oxygen species detoxification, better preserved fiber morphology, better preserved ultrastructure of intracellular organelles involved in Ca^{2+} handling and produced greater maximal isometric knee extensor force when compared to age-matched sedentary participants (Zampieri et al. 2015). Moreover, long-term training (i.e., access to a voluntary running wheel) promoted maintenance of the triad junction (Boncompagni et al. 2020), prevented age-induced elevations in resting intracellular Ca^{2+}, loss of RyR content and reductions in ex vivo specific force in C57BL/6 mouse muscle (Fodor et al. 2020). Although physical exercise is a robust lifestyle modifier that improves function, it is difficult to pinpoint its precise mechanisms of action, especially if it is lifelong. Moreover, physical exercise may not be practical or feasible for all older adults. Methods to mimic physical activity have therefore become attractive therapies to attenuate age-induced oxidative stress and its downstream effects. Here, we highlight the use of antioxidants and the pharmaceutical S107 as potential therapeutic approaches, and briefly discuss the role of insulin-like growth factor-1 (IGF-1) in EC coupling maintenance with age.

5.3.3.1 Antioxidants

Preventing or restoring the redox environment of skeletal muscle has been reported to improve EC coupling and muscular strength in aged muscle. Recently, in vivo supplementation of selenium for 2 months, a trace element with antioxidant properties, was found to increase the maximal rate of Ca^{2+} efflux and specific force in aged C57BL/6 mouse muscle (Fodor et al. 2020). Furthermore, antioxidant treatment of dithiothreitol (DTT; a strong reducing agent) reduced RyR channel oxidation, SR Ca^{2+} leak and RyR Ca^{2+} sparks in aged C57BL/6 mouse fibers (Umanskaya et al. 2014) and increased maximal SR Ca^{2+} accumulation in human fibers of old subjects (Lamboley et al. 2016). To determine if increasing lifelong mitochondrial antioxidant activity would prevent age-related EC coupling failure and dynapenia,

Umanskaya et al. (Umanskaya et al. 2014) overexpressed the human catalase gene in mitochondria. Catalase is an antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen. When compared to aged-matched controls, mice overexpressing catalase had less mitochondrial ROS (Lee et al. 2010), increased SR Ca^{2+} load, increased Ca^{2+} transients and reduced SR Ca^{2+} leak, all of which translated into greater skeletal muscle-specific force (Umanskaya et al. 2014). Taken together, these studies indicate mitochondrial ROS production is a molecular mechanism for age-related EC coupling failure and that reducing ROS through antioxidants (particularly those that target the mitochondrial or RyRs) may improve Ca^{2+} homeostasis and attenuate or delay the onset of dynapenia.

5.3.3.2 S107

Pharmacological agents can also be used to improve age-induced EC coupling failure caused by excessive oxidative stress. Marks and colleagues (Andersson et al. 2011) eloquently accomplished this by treating aged mice with S107, a drug that preserves the calstabin-RyR interaction. Impressively, 4 weeks of S107 treatment in old C57BL/6 mice was able to stabilize calstabin to RyR, prevent SR Ca^{2+} leak, restore tetanic Ca^{2+} release and increase muscle-specific force when compared to muscle of age-matched, untreated mice. These beneficial effects were observed despite persistent RyR oxidation and nitrosylation, indicating treatment was not able to reduce RyR modifications, but was able to ameliorate the detrimental effects of oxidative stress. These reports align with data demonstrating that when catalase is overexpressed in aged C57BL/6 mice, less RyR is oxidized and depleted of calstabin (Umanskaya et al. 2014). Reports such as these indicate EC coupling failure due to RyR dysfunction mediated by depletion of calstabin can be improved through preventing the loss of calstabin (via the increased capacity to neutralize oxidative stress) or by restoring calstabin to RyR (via S107). From a practical perspective, pharmacological agents like S107 yield significant promise for individuals that may have already experienced dynapenia or passed the timepoint when age-related RyR oxidation or nitrosylation is reversible.

5.3.3.3 Insulin-Like Growth Factor-1 (IGF-1)

An alternative strategy, not directly associated with the mitochondria or oxidative stress, is the use of IGF-1. Insulin-like growth factor (IGF-1) is a peptide primarily known for its canonical role in promoting skeletal muscle differentiation and growth (Florini et al. 1996). Another, less recognized function of IGF-1 is expression of DHPR. Data from S1S2 mice overexpressing human IGF-1 (20–30-fold increase in concentration) in skeletal muscle resulted in a significant increase (over 50%) in the number of DHPRs, specifically the $Ca_V1.1$ subunit (Renganathan et al. 1997b). Moreover, with advancing age, muscle of these IGF-1 overexpressing mice maintained a high DHPR number, DHPR/RyR ratio and muscle specific force

when compared to the reductions observed in muscle of control mice (i.e., wildtype) (Renganathan et al. 1998). However, more recent reports in human skeletal muscle did not observe age-related reductions in DHPR expression (Ryan et al. 2003) or content (Lamboley et al. 2016), contrary to what has been measured in rodent (O'Connell et al. 2008; Renganathan et al. 1997a) and rabbit muscle (Ryan et al. 2000). These equivocal findings question the utility of IGF-1 as a beneficial therapy for improving EC coupling in aging skeletal muscle, however, additional research is needed before definitive conclusions can be drawn.

The use of antioxidants, the pharmaceutical S107 and IGF-1 all appear, to some extent, to improve EC coupling and strength in aged skeletal muscle. However, some caution should be considered when looking at the breadth of literature as several caveats will be noted. Some include the model used (human vs. animal models) and muscle/fibers analyzed (fast twitch vs., slow twitch)—as differences are likely to be observed. Despite these, there remains great potential for regenerative rehabilitation to improve EC coupling failure, loss of Ca^{2+} homeostasis and strength (i.e., attenuate dynapenia) in aging skeletal muscle.

5.4 Mitochondrial Function

5.4.1 Introduction to Mitochondrial Function

Skeletal muscles require energy to carry out their functions. Amongst other things, energy is required for the myofilament contractions that enable movement, for the interconversion and storage of nutrients that contribute to maintaining whole-body homeostasis, and for the continual maintenance of muscle itself (for example, repair and replacement of old and damaged contractile proteins). As with most cells, the main source of energy employed by muscle is adenosine triphosphate (ATP) which is produced by oxidative phosphorylation in the mitochondria and glycolysis in the cytosol (Baker et al. 2010). The number of mitochondria varies from muscle to muscle in accordance with its function. For example, cells with constant high energy demand such as oxidative, slow twitch, type I fibers are rich in mitochondria, whereas cells with low, sporadic energy demand such as glycolytic, fast twitch, type II fibers contain fewer mitochondria (Carter et al. 2015).

Unlike other cells, skeletal muscle contains two pools of mitochondria, one in the cytoplasm and one in the sarcomeric contractile fibers (Carter et al. 2015). The cytoplasmic mitochondria, or subplasmalemmal, are believed to be more responsive to the energy needs of the cell for growth and metabolism (Crescenzo et al. 2006; Koves et al. 2005; Ritov et al. 2005), whereas the intrasarcomeric or intermyofibrillar mitochondria are believed to provide energy specifically to the contractile fibers (Ferreira et al. 2010). Interestingly, in addition to producing ATP, mitochondria also provide cells with an ability to buffer Ca^{2+} gradients and are often found localized in areas of Ca^{2+} influx (Parekh 2003a). Thus, another possible function of having both cytoplasmic and intrasarcomeric mitochondria is to

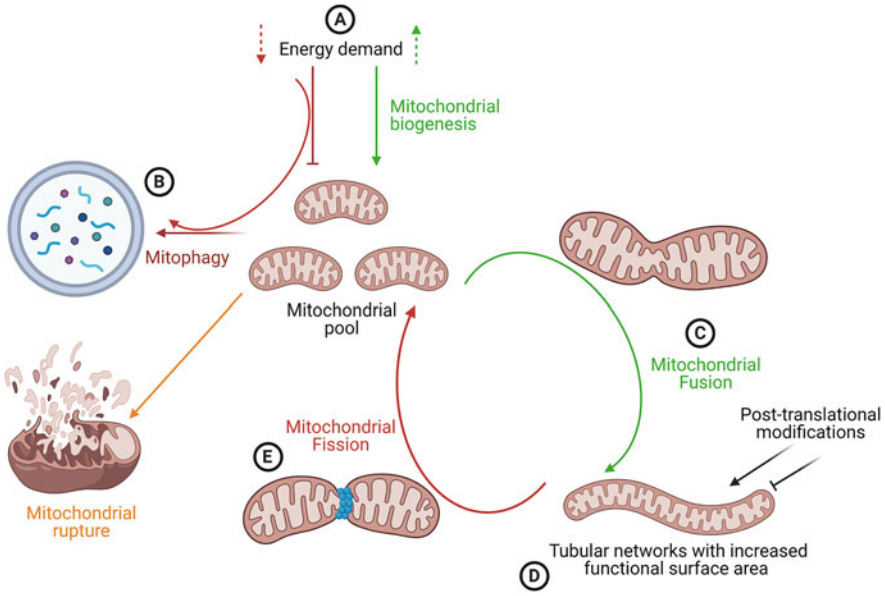

Fig. 5.5 Conceptual diagram of control of mitochondrial structure and function. Cellular energy demand (A) drives rates of mitochondrial biogenesis with increased demand increasing the mitochondrial pool and decreased demand leading to the mitochondrial pool to tend towards mitophagy (B). Increases in energy demand can lead to mitochondrial fusion (C) which increases the mitochondrial surface area to enable increased proton motive force and derived functions (D). Mitochondrial function can also be affected by post-translational modifications. Impaired mitochondria function or needs to remodel mitochondrial location can lead to mitochondrial fission (E) enabling mitochondria to renter the pool where they can undergo further fusion, mitophagy, or rupture with smaller consequences that rupture of fussed mitochondria

provide an additional level of regulation of Ca^{2+} levels during EC coupling (see example in Sect. 5.3.3) via the interplay of the plasmalemma, SR and mitochondria (Parekh 2003b).

Mitochondrial content is established and increased via mitochondrial biogenesis (Gureev et al. 2019) and is decreased by degradation via a specialized form of autophagy termed mitophagy (Chen et al. 2020b) (Fig. 5.5). Mitochondrial biogenesis is controlled by the activity of several transcription factors, including PGC-1α, PGC-1β, NRF1, and NRF2 (Gureev et al. 2019). These, and other, transcription factors regulate the transcription of roughly 1500 nuclear encoded genes (Hendrickson et al. 2010; Hill 2014) that are translated and translocated into the mitochondria, largely through the mitochondrial TOM20/TOM40 import complex (Boengler et al. 2011). Slightly more than a dozen mitochondrial encoded proteins also contribute to maintaining mitochondrial structure and function, which are regulated by TFAM (Ngo et al. 2014). Removal of mitochondrial via autophagy requires regulation/induction of autophagy and the ability of autophagic vesicles to

recognize mitochondrial via surface proteins such as the PINK1-PARKIN complex, NIX, and BNIP3 and thus target them for degradation (Chen et al. 2020b).

Mitochondrial structure is dynamic with mitochondria undergoing fission to separate into smaller structures and fusion to establish larger ones (Hood et al. 2019). Fission is controlled by DRP1 and associated binding proteins. Fusion is controlled by OPA1, MFN1 and MFN2 and associated regulator proteins. Fission produces smaller structures that can be more easily targeted for degradation by mitophagy and/or act independently (Fig. 5.5). Fusion produces larger structures with increased surface area which allows for more coordinated action and more efficient establishment, maintenance, and utilization of membrane gradients for ATP production, heat production, and Ca^{2+} buffering. Mitochondria function then is a function of cellular environment/demands (e.g., fiber type, recruitment pattern, etc.), content (regulated by biogenesis and mitophagy), structure (regulated by fission and fusion), and also post-translational modifications (Stram and Payne 2016); for example, oxidation, persulfidation, and Ca^{2+} binding.

5.4.2 Age-Induced Alterations in Mitochondria

Loss of physiologic functions and increased risk of death are observed with age. Molecularly, these losses of molecular physiologic function have been characterized into nine hallmarks of aging (Lopez-Otin et al. 2013); reductions in mitochondria being one of the hallmarks. These reductions in mitochondria appear to take place across species and tissues. Below we discuss alterations in human skeletal muscle mitochondria with advancing age that have been shown to contribute to reduced physical function, increased fatigability, sarcopenia, dynapenia, and cardiorespiratory fitness (Coen et al. 2018).

5.4.2.1 Age-Associated Loss in Mitochondrial Number and Volume

With age there is a reduction of mitochondria in human muscle as assessed by mitochondrial DNA content, mitochondrial enzyme activity assays, and the gold standard method of counting mitochondria in electron micrographs (Seo et al. 2016). However, more research is needed to understand when, chronologically, mitochondria may be lost and at what rate. Human muscle appears to retain the ability to invoke mitochondrial biogenesis with age (Konopka et al. 2014), however, this response may be blunted when compared to younger human muscle (Deane et al. 2019). Thus, it may be the case that muscle loses the ability to rejuvenate/maintain mitochondria with age and that this also impacts the ability to rejuvenate/maintain muscle as a whole (Chen et al. 2020c); some speculate that this may be related to reductions in stem cell function with age (Lopez-Otin et al. 2013). While more research remains to be done to understand why mitochondrial response to exercise may be blunted with age, it is clear that mitochondria reman responsive to exercise

interventions. Therefore, there is reason to suspect that reductions in mitochondrial number and volume can be overcome by further understanding how exercise influences mitochondrial structure in old human muscle.

5.4.2.2 Age-Associated Loss in Mitochondrial Function

Loss of mitochondrial function with age can be observed as reductions in ATP production, ability to buffer intramuscular Ca^{2+}, ability to process energetic substrates, and in efficiency of oxygen use (Short et al. 2005; Seo et al. 2016; Hood et al. 2019). These declines in function are seen in both humans and animal models and occur prior to age-associated functional decline and the onset of frailty (Andreux et al. 2018). Mitochondrial dysfunction has been tied to cellular senescence (Chapman et al. 2019), another hallmark of aging (Lopez-Otin et al. 2013). While alterations in mitochondrial structure and function are both observed in human muscle with age, it is not clear if one causes the other and/or if individual specific (genetic and/or epigenetic) differences occur. For example, single nucleotide polymorphisms in nuclear encoded mitochondrial genes have been linked with disease progression (Hendrickson et al. 2010). As structural defects can cause functional defects and vice versa, it is entirely possible that one causing the other may differ between individuals. Similarly, as different individuals may have different causes of mitochondrial dysfunctions, they may show similar changes in mitochondrial structure but as the result of different dysfunctions. As discussed above, the ability of exercise to invoke similar improvements in mitochondrial function, in addition to structure, suggests that mitochondrial function can be restored in aged human muscle.

5.4.3 Regenerative Rehabilitation and Mitochondrial Function

5.4.3.1 Exercise Mimetics

It is clear that exercise promotes mitochondrial biogenesis and mitochondrial function, and that some of these improvements via the action of the transcription factor PGC-1α (Konopka et al. 2014; Seo et al. 2016; Gureev et al. 2019; Hood et al. 2019). Thus, pharmaceutical or nutraceutical activation of PGC-1α could be a potential mimetic for exercise. PGC-1α stimulation has also been suggested as a potential therapeutic for mitochondrial disease and diabetes (Wenz 2009; Yuan et al. 2019). To date, drugs in preclinical trials have targeted PPAR (bezafibrate, rosiglitazone), AMPK (AICAR, metformin), and Sirt1 (resveratrol) (Hofer et al. 2014). See Sect. 5.6.3 for more details on some of these treatments. Additionally, there are novel small molecular activators for PGC-1α under development (for example, Zhang et al. 2013). Another interesting mimetic is carbon monoxide (CO). As a

gasotransmitter, CO has recently been shown to have mitochondrial biogenesis effects and enhance the effects of exercise alone (Rhodes et al. 2009).

5.4.3.2 Hormone and Diet Supplementation

A number of hormones regulate mitochondrial content and/or function (Ritz et al. 2005). Some of these, like the androgens change with age and are present at different levels in across biological sex and gender. Correction of clinical deficiencies in hormones has clear benefits in promoting mitochondrial health. For instance, a recent study supplementing testosterone found improved efficacy of the response to the exercise training when the supplement was provided as an adjunct (Gharahdaghi et al. 2019). Similarly, clinical deficiencies in most nutrients (protein, fatty acids, vitamins) lead to impaired metabolism and correction of these had benefits in promoting mitochondrial health (Du et al. 2016). For example, in relation to Vitamin D, intramuscular Vitamin D receptor concentration correlates with the extent of muscle improvement in response to exercise training (Bass et al. 2020). As another example, supplementation of NAD, a pyridine-nucleoside similar to vitamin B3, has been found to have beneficial effects across species (Romani et al. 2021). The reason for this beneficial effect has been suggested to be due to an age-associated decline in NAD levels (Schultz and Sinclair 2016), which may or may not be associated with mitochondrial deficits.

5.5 Protein Homeostasis

5.5.1 Introduction to Protein Homeostasis

Muscle is the largest store of nitrogen, in the form of protein, in the human body. In a young, healthy individual muscle comprises roughly 40% of all the protein in the body (Brook et al. 2016b). Accordingly, protein turnover accounts for roughly 35% of daily whole-body resting energy expenditure (Carbone et al. 2019). Within muscle, more than 40% of protein can be found in the contractile apparatus (i.e., sarcomeres) (Ojima 2019). Thus, there is a strong link between muscle protein content and sarcomere content (Sweeney and Hammers 2018). This means that the human body needs to balance regulation of protein content for maintenance of whole-body energy homeostasis. Muscle lengths tend to be dictated by the skeletal attachment points, whereas muscle volume can change based upon hydration/inflammatory status and growth, termed hypertrophy (Haun et al. 2019). When muscle does grow in length, sarcomeres are added in series, and this results in alterations in the strength and contraction velocity of the muscles (Wisdom et al. 2015). Thus, muscle growth for increased strength requires energy at the whole-body level to maintain whole-body energy balance. Conversely, when muscle atrophies (Atherton et al. 2016), strength will generally be lost as the result of breakdown of sarcomeres

in parallel. As muscle size is largely dictated by balancing energy and physical performance needs, muscle hypertrophy and atrophy are largely dictated by alterations in muscle protein synthesis, with or without increased protein degradation (Brook et al. 2016b). Rates of muscle protein synthesis and/or degradation are controlled by a wide variety of anabolic (growth promoting) and catabolic (atrophy promoting) stimuli (McCarthy and Esser 2010).

A number of signaling pathways acting downstream of growth factor receptors have been demonstrated to regulate protein synthesis in animal models and cell culture. In human adult muscle, it is clear that the mechanistic target of rapamycin (mTOR) is a central regulator of muscle protein synthesis, serving to integrate anabolic signals such as amino acid content and energy status, as signaled by 5′ AMP-activated protein kinase (AMPK) and likely other growth and mechanical signals (Brook et al. 2016b). mTOR regulates protein synthesis via 4EBP1, S6K and downstream signals.

The control of protein degradation in human muscle is less well defined, in part due to greater technical challenges in studying degradation in human muscle. While the signals regulating the enzymes that carry out protein degradation, termed proteases, may not be well defined, the proteases themselves are (Szewczyk and Jacobson 2005). Lysosomes and proteasomes are the major proteases within muscle. Both lysosomes and proteasomes are normally "on" but only degrade proteins that are sent to them. Thus, regulation of lysosomal and proteasomal degradation is largely at the level of trafficking proteins to the proteases rather that at the level of turning these proteases "on" or "off" (Szewczyk and Jacobson 2005). In the case of lysosomes, this occurs largely via autophagy and increased autophagy appears to be a basal state in the absence of positive growth factors in yeast, *C. elegans*, and rodents, so it may similarly be the case for humans (Klionsky et al. 2021). For proteasomes, proteins destined for degradation are typically targeted to the proteasome by tagging the protein with ubiquitin via ubiquitin conjugating enzymes (Lecker et al. 2006). Importantly, at least 30% of all newly synthesized proteins do not properly mature and are degraded by the proteasome, meaning that the proteasome plays a significant role in the degradation of newly synthesized improperly folded proteins (Schubert et al. 2000). Calpains and caspases are the other two main proteolytic systems in muscle and both are constitutively inactive, or "off" (Szewczyk and Jacobson 2005), meaning that their activation results in the degradation of proteins in the immediate vicinity of the proteases once activated. The majority of proteins degraded by calpains (Goll et al. 2003) and caspases are cytoskeletal proteins, implying that a major role of each protease is in the structural remodeling of muscle (Crawford and Wells 2011). In the case of calpains, these tend to be membrane associated and activated by Ca^{2+}, suggesting they may have a role in maintaining the structures necessary for proper EC coupling (Verburg et al. 2009) (see Sect. 5.3.1 for EC coupling) and other Ca^{2+} signaling. For caspases, these are associated with mitochondria and appear to be activated in response to mitochondrial Ca^{2+} overload and/or other toxic stressors to mitochondria (Vringer and Tait 2019), suggesting that caspases may have a role in appropriately localizing mitochondria within muscle.

5.5.2 Age-Induced Loss of Protein Homeostasis

Declines in maintaining protein homeostasis, termed proteostasis, is one of nine hallmarks of aging (Lopez-Otin et al. 2013). Like other hallmarks of aging, declines in proteostasis appear to take place across species and tissues. Loss of proteostasis results in accumulation of protein aggregates, disruption of cellular structures, and leads to general declines in the ability to maintain cellular homeostasis (Santra et al. 2019). Below we discuss two aspects of altered protein homeostasis in muscle with advancing age: (1) loss of muscle size and (2) increased anabolic resistance. As previously mentioned, sarcopenia (Evans et al. 2019) and dynapenia are associated with increased physical frailty, increased risk of morbidity, and increased risk of mortality (Wilkinson et al. 2018).

5.5.2.1 Age-Loss of Muscle Size (Sarcopenia)

Human muscle grows in size from birth and reaches a peak in the mid-20s to early-30s depending on the individual. After this point muscle size declines for reasons not still fully understood (Nair 2005). It has been suggested that this may be due to lack of evolutionary pressure for prolonged muscle function with age based upon shorter human life expectancy as little as 2000 years ago (Goldspink 2012). Some of the loss of muscle mass with age may be due to biological effects of aging such as alterations in hormonal signaling and/or loss of mitochondrial or protein homeostasis, and some of this may be determined by lifestyle changes such as nutritional excesses or deficits and/or level of activity (Andreux et al. 2018; Brook et al. 2016b; Carter et al. 2015; Coen et al. 2018; Gharahdaghi et al. 2019; Wilkinson et al. 2018). Once individuals reach the age of 60, there is an exponential decline in muscle size which when associated with declines in muscle function is termed sarcopenia (Nair 2005; Wilkinson et al. 2018). For reasons currently unknown (Papadopoulou 2020), this decline differs between males and females. Males usually display changes earlier than females, yet females usually displaying greater rates of decline than males. Recent studies examining the effect of exercise on human muscle growth have found that older human muscle does not respond the same as younger muscle in terms of signaling growth of the extracellular matrix that surrounds muscle, possibly signifying that alterations in the extramuscular scaffold underlie decreased growth with age (Deane et al. 2019; Wessner et al. 2019). This suggestion is yet to be tested with an intervention to restore growth.

5.5.2.2 Age-Associated Anabolic Resistance

Despite proteostasis being altered with age and across species (Lopez-Otin et al. 2013), it is not immediately clear that the causes and consequences are identical across species. For example, wide spread protein aggregation is not reported for

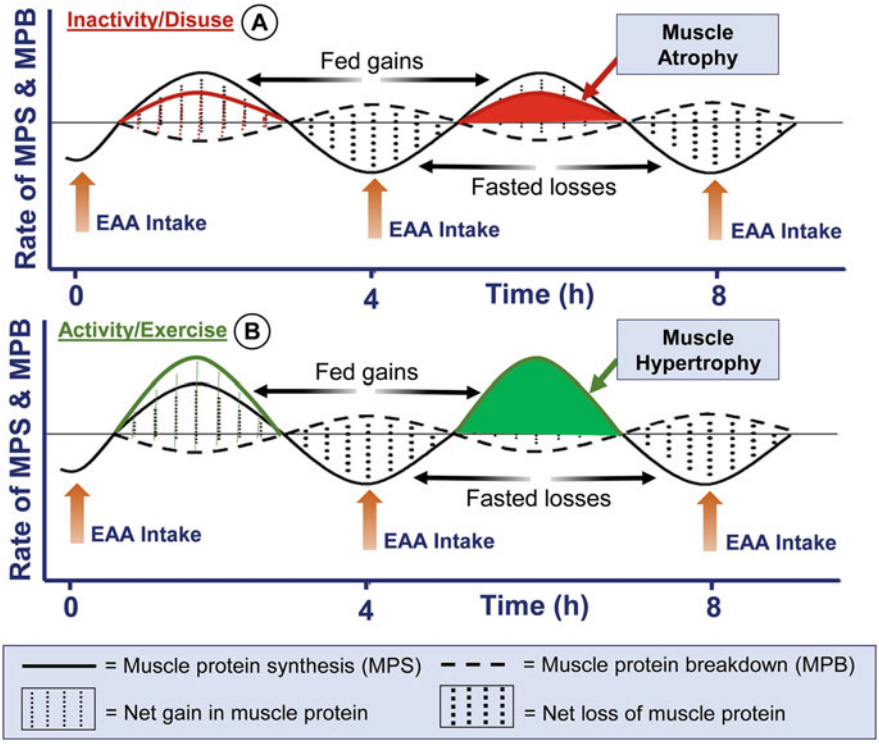

Fig. 5.6 Conceptual diagram of how changes in human muscle protein homeostasis drive changes in muscle size. Rates of Muscle Protein Synthesis (MPS) and Muscle Protein Breakdown (MPB) fluctuate during the day with rates of MPS increasing and rates of MPB decreasing immediately after a meal or ingestion of Essential Amino Acids (EAA). Decreases (**a**) or increases (**b**) in physical activity can decrease or increase the rate at which MPS is increased in response to eating a meal or ingesting EAA. Net losses (**a**, *red curve*) in the responsiveness of MPS (termed anabolic blunting) lead to muscle atrophy, whereas net increases (**b**, *green curve*) in the responsiveness of MPS lead to muscle hypertrophy. Note that in all cases, there is a net loss of muscle protein between meals (bottom, red) and a net increase in muscle protein post-meal (bottom, green). The anabolic blunting displayed in response to inactivity is also observed with age and disease, raising the issue of how much anabolic blunting with age or disease is due to decreased physical activity

human muscle with age (Wilkinson et al. 2018), unlike in some human neurodegenerative diseases (Currais et al. 2017). Further, baseline rates of muscle protein synthesis and degradation are not widely reported to be altered in older versus younger human muscle (Wilkinson et al. 2018). Rather, the responsiveness of muscle to anabolic stimuli such as feeding (Mitchell et al. 2016) and exercise (Durham et al. 2010) seems to be blunted (Wilkinson et al. 2018). That is, the increase in protein synthesis in response to anabolic stimuli is less in old muscle than in young. Thus, with similar rates of basal protein synthesis and degradation in young vs. old muscle but smaller post-meal increases in synthesis, muscle protein content gradually declines over time (Fig. 5.6). Accordingly, this gradual decline is

sufficient to account for loss of muscle size which contributes to decreased function. It remains to be determined what causes the blunted anabolic responses in older muscle, presumably this is at the level of translation or later as anabolic signaling pathways via mTOR seem normally activated in older human muscle (Brook et al. 2016b). A recent study has suggested that impaired ribosomal biogenesis with advancing age may underly anabolic resistance (Brook et al. 2016a), which could lead to novel therapies for combating age-associated anabolic resistance.

5.5.3 Regenerative Rehabilitation and Protein Homeostasis

5.5.3.1 Hormone and Dietary Strategies

As discussed in Sect. 5.4.3.2, correction of clinical deficiencies in hormone levels or dietary intake have clear implications for muscle health, including size and protein content. It is possible that in the absence of clinical deficiencies supplementation combined with exercise may have positive effects, with both testosterone (Gharahdaghi et al. 2019) and vitamin D (McKendry et al. 2020) supplementation enhancing skeletal muscle responses to exercise. In addition, protein intake or absorption is often deficient in older individuals (Mitchell et al. 2016), especially in frail individuals. The amino acid leucine and its metabolite HMB have both been shown to be sufficient to produce the same anabolic muscle protein synthesis response as eating a protein-rich meal (Mitchell et al. 2016). Thus, targeted strategies using these nutrients and others, particularly as adjuncts to activity level, have been proposed and are under study, with a focus on particular factors such as concentration and timing (McKendry et al. 2020).

5.5.3.2 Ribosomal Biogenesis

The full cause of anabolic blunting currently remains unknown, but is associated with decreased levels of ribosomes that cause further declines in ribosome biogenesis (Brook et al. 2016a). As rRNA comprises the bulk of cellular RNA, which is a major component of ribosomes (Figueiredo and McCarthy 2019), it is possible that age-related declines in nucleic acids and/or RNA (Tahoe et al. 2004) manifest most notably as declines in rRNA. This would be entirely in keeping with the recent findings of cross-species NAD declines with advancing age (Schultz and Sinclair 2016). The idea that declines in ribosomal biogenesis underly age-related muscular deficits has been proposed as a general feature of aging (Steffen and Dillin 2016). If this proves true, then supplementation of nucleic acids rather than proteins, lipids, carbohydrates, or vitamins may be a viable strategy for increasing anabolic responses to exercise with age.

5.6 Glucose Metabolism

5.6.1 Introduction to Glucose Metabolism

Glucose homeostasis is critical to human health and survival due to the important role that glucose has in providing cellular energy. Any disturbances in glucose homeostasis can result in disease, most notably type 2 diabetes, but can also contribute to other life-altering conditions, including sarcopenia (Sugimoto et al. 2019) and dynapenia (Nebuloni et al. 2020; Kalyani et al. 2015). In healthy individuals, systemic glucose homeostasis is intricately regulated between tissue (i.e., muscle, liver, adipose, pancreas). Liver plays a primary role in regulating glucose homeostasis (glycogenolysis and gluconeogenesis) in the fasted state, while peripheral tissue plays a critical role in promoting glucose uptake and restoring normoglycemia after eating. As the largest organ in the body, skeletal muscle is considered a major regulator of whole-body glucose homeostasis, with 70–80% of insulin-stimulated glucose uptake occurring in this tissue (DeFronzo et al. 1981). Therefore, it is not surprising that the muscle atrophy that occurs with aging, plays a contributing role in the disruption of glucose homeostasis and pathogenesis of insulin resistance in these individuals (Sugimoto et al. 2019; Nebuloni et al. 2020; Kalyani et al. 2015).

In healthy adults, insulin-mediated glucose uptake occurs when insulin binds to the insulin receptor, initiating a signaling cascade including phosphorylation of the insulin receptor, insulin receptor substrate-1 (IRS-1) association with p85 subunit of phosphoinositide 3-kinase (PI3K), Akt2 phosphorylation on threonine 308 and serine 473 sites, and phosphorylation of the TBC1 domain family member 4 (TBC1D4) on multiple sites, subsequently allowing the translocation of glucose transporter type 4 (GLUT4) to the plasma membrane (Thorell et al. 1999; Kramer et al. 2006) (Fig. 5.7). In resting muscle, the majority of insulin-stimulated glucose undergoes nonoxidative metabolism (primarily converted to glycogen), while the remaining is oxidized (DeFronzo and Tripathy 2009). Impairments in any of these signaling pathways can cause skeletal muscle insulin resistance, which is believed to be the primary defect leading to the development of type 2 diabetes (DeFronzo and Tripathy 2009).

It is well known that the aging process is associated with whole-body insulin resistance (Consitt et al. 2013; Petersen et al. 2003; Fink et al. 1983, 1986; Rowe et al. 1983; Petersen et al. 2015). The factors contributing to age-related insulin resistance are likely multifaceted and include chronological age (Consitt et al. 2013), reduced physical activity (Amati et al. 2009), inflammation (Greiwe et al. 2001), and/or increased body fat (Kohrt et al. 1993; Amati et al. 2009). The obesity epidemic combined with the growing prevalence of sarcopenia in older adults has resulted in the concept of "sarcopenic obesity" (i.e., coexistence of obesity and sarcopenia conditions) (Stenholm et al. 2008; Cauley 2015). These individuals experience the negative metabolic effects of obesity, plus they have reduced skeletal muscle mass available for insulin-stimulated glucose uptake (Reaven 1988), putting

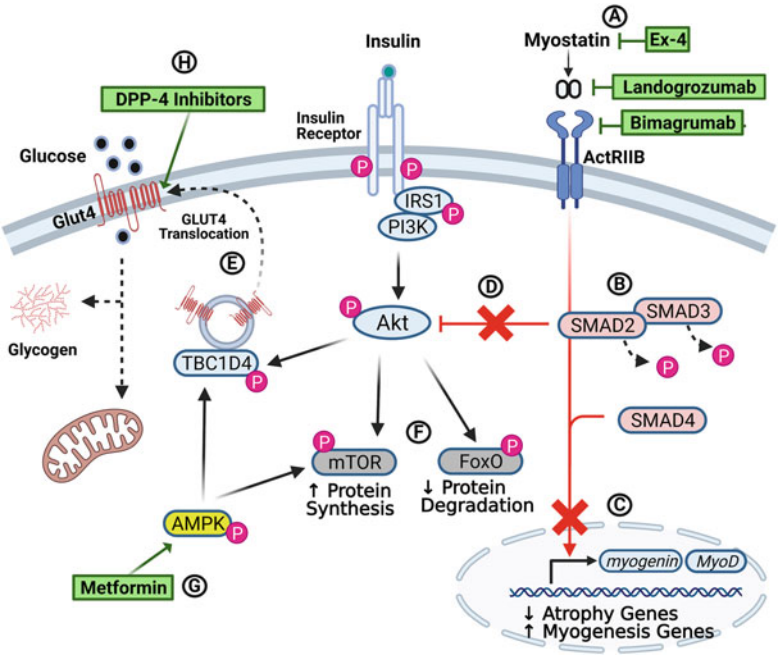

Fig. 5.7 Potential cellular mechanisms for therapeutic enhancement of glucose metabolism and prevention of age-induced skeletal muscle dysfunction. (A) Decreased myostatin protein expression (i.e., via Ex-4), inhibition of myostatin (i.e., via anti-myostatin antibody landogrozumab), or inhibition of the myostatin receptor (i.e., via anti-ActRII antibody bimagrumab) results in (B) reductions in SMAD2/SMAD3 phosphorylation leading to (C) inhibition of the SMAD2/SMAD3/SMAD4 complex entering the nucleus, preventing the upregulation of atrophy genes involved in sarcopenia. SMAD2/SMAD3 dephosphorylation also (D) suppresses myostatin's inhibition of insulin-stimulated Akt phosphorylation leading to (E) TBC1D4 phosphorylation, GLUT4 translocation to the plasma membrane, and increased glucose uptake, as well as, (F) increased protein synthesis (via mTOR signaling) and suppression of protein degradation (phosphorylation of FoxO inhibits its translocation to the nucleus where it would upregulate genes involved in autophagy and protein degradation). (G) Metformin increases the phosphorylation of AMPK, which increases glucose uptake (via TBC1D4) and protein synthesis (via mTOR signaling). (H) DPP-4 inhibitors increase GLUT4 content and insulin-stimulated glucose uptake. This image was created using BioRender (https://BioRender.com)

them at even greater risk for developing insulin resistance and type 2 diabetes. While the prevalence of sarcopenic obesity varies depending on the operational definition, the National Health and Nutrition Examination Survey (NHANES) III study reported that 19% of women and 28% of men over the age of 60 suffered from this condition (Batsis et al. 2015), further highlighting the need to understand the interaction between metabolic dysfunction and sarcopenia.

A discussion on the effects of glucose metabolism and age-related muscle dysfunction would be remiss without mentioning that over 70% of Americans over the age of 65 are classified as either type 2 diabetic or prediabetic (Cowie

et al. 2009). While the detrimental effects of insulin resistance and type 2 diabetes have been well documented for the general population, the consequences are even more dire for the elderly. Poor glycemic control and insulin resistance are well-known risk factors for sarcopenia (Sugimoto et al. 2019) and dynapenia (Nebuloni et al. 2020; Kalyani et al. 2015) and have been shown to accelerate age-related losses in both skeletal muscle mass (Park et al. 2009) and strength (Park et al. 2007). Therefore, the aging adult is often caught in a vicious cycle of glucose dysregulation and muscle dysfunction, which may be further exacerbated by conditions of obesity. The mechanism(s) responsible for age-related related impairments in glucose metabolism and muscle function remain unclear and are likely complex. Here, age-related impairments in insulin-stimulated glucose metabolism, accumulation of advanced glycation end-products (AGEs) and increases in myostatin are discussed.

5.6.2 Age-Induced Pathophysiology of Impaired Glucose Metabolism

5.6.2.1 Age-Related Impairments in Insulin-Stimulated Glucose Metabolism

Age-related impairments in the skeletal muscle insulin signaling cascade have been reported in both human (Consitt et al. 2013; Petersen et al. 2015) and animal models (Gupte et al. 2008; Sharma et al. 2010; Consitt et al. 2018). In aging humans, several impairments in distal insulin signaling have been reported. Peterson et al. (Petersen et al. 2015) reported diminished Akt phosphorylation after 20 min of hyperinsulinemia in aged individuals compared to their younger counterparts when body mass index (BMI), fat mass, and habitual physical activity were matched (Petersen et al. 2015). Additionally, in a cross-sectional study including individuals across a wide range of the adult life span (18–84 years of age), insulin-stimulated phosphorylation of AS160 on multiple sites (serine-588, threonine-642, and serine-666) were impaired in conjunction with age-related whole-body insulin resistance (Consitt et al. 2013). Skeletal muscle GLUT4 has also been reported to decrease with age in some (Consitt et al. 2013; Gaster et al. 2000; Houmard et al. 1995; Xu et al. 2017) but not all (Houmard et al. 1995; Cox et al. 1999; Dela et al. 1994) studies, and at least one study has suggested that age-related changes may be fiber-type specific with older adults having reduced GLUT4 in type II, but not type I fibers (Gaster et al. 2000).

Age-related impairments have been reported in both the insulin-stimulated oxidative and nonoxidative pathways (Bryhni et al. 2005; Poulsen et al. 2005; Franssila-Kallunki et al. 1992; Petersen et al. 2015; Consitt et al. 2016). Early findings that older adults had ~60% less skeletal muscle glycogen stores compared to their younger counterparts (Meredith et al. 1989), led to subsequent findings of diminished insulin-stimulated glycogen synthase activity in aged (Poulsen et al. 2005; Bienso et al. 2015) compared to younger adults (Poulsen et al. 2005; Pehleman et al.

2005). Reduced skeletal muscle pyruvate dehydrogenase (PDH) flux during insulin stimulation has also been reported in the elderly compared to young individuals (Petersen et al. 2015; Consitt et al. 2016), suggesting that under hyperinsulinemic conditions, PDH regulation may be compromised in older adults putting them at increased risk for metabolic inflexibility and the development of insulin resistance (Constantin-Teodosiu et al. 2015; Hansen et al. 2019).

Deletion of the insulin receptor in the skeletal muscle of mice has been reported to reduce skeletal muscle mass and grip strength by approximately 20% (O'Neill et al. 2016), supporting a direct role for insulin signaling on muscle mass and function. Besides stimulating glucose uptake, insulin-mediated Akt phosphorylation can promote protein synthesis through the Akt/mTOR/p70S6K signaling pathway (Wang and Proud 2006) and inhibit protein atrophy through the Akt/FoxO pathway (O'Neill et al. 2016). Given the crosstalk between these pathways, it is conceivable that age-related impairments in insulin signaling are responsible for both reduced glucose uptake and sarcopenia. Animal research suggests insulin acts to maintain muscle mass by suppressing Akt/FoxO1/3/4-mediated autophagy and protein degradation pathways (O'Neill et al. 2016). In humans, there remains considerable debate regarding which insulin-stimulated pathway is responsible for age-related sarcopenia and may be related additionally to insulin's effect on blood flow, as well as amino acid availability and uptake (Rasmussen et al. 2006; Fujita et al. 2009; Wilkes et al. 2009).

5.6.2.2 Age-Related Accumulation of Advanced Glycation End-Products (AGEs)

With age-related insulin resistance, postprandial blood glucose levels become elevated for pronged periods of time. Poor glycemic control and chronic hyperglycemia (often measured as hemoglobin A1c, HbA1c) lead to the accumulation of advanced glycation end-products (AGEs). Elevated serum AGEs have been associated with decreased muscular performance, including reduced grip strength (Dalal et al. 2009), and slower walking speed (Semba et al. 2010) in adults aged 65 or older. Incubation of C2C12 myotubes (muscle cells) with AGE has been reported to reduce cell diameter (muscle atrophy) and increase atrogin-1 (protein involved in muscle atrophy) (Chiu et al. 2016), suggesting AGEs have a direct role in skeletal muscle wasting. While the mechanism remains to be fully elucidated, Chiu et al. (2016) proposed AGEs act through their receptor to activate AMPK and downregulate Akt signaling, resulting in atrophy and impairments in myogenesis. Recently, it was suggested that AGEs may promote mitochondrial dysfunction in skeletal muscle (Daussin et al. 2021), which theoretically would contribute further to loss of muscle mass and function. It should also be noted that chronic hyperglycemia and AGEs are believed to play an essential role in the development of diabetic peripheral neuropathy, a condition reported to be an independent risk factor for sarcopenia (Yang et al. 2020; Erbas et al. 2011; Addison et al. 2018; Sugimoto et al. 2008).

5.6.2.3 Age-Related Increases in Myostatin

The myokine myostatin, also referred to as growth and differentiation factor-8 (GDF-8), is a member of the transforming growth factor β (TGF-β) superfamily and is well-known negative regulator of skeletal muscle growth (McPherron et al. 1997). A number of studies have reported a link between increased myostatin levels, age (Yarasheski et al. 2002; Leger et al. 2008), declining muscle mass (Yarasheski et al. 2002; Leger et al. 2008), decreased muscular strength (Han et al. 2011; Patel et al. 2014), as well as, obesity (Hittel et al. 2009) and insulin resistance (Hjorth et al. 2016; Hittel et al. 2009; Ryan et al. 2013; Guo et al. 2009), suggesting this protein contributes to both muscle atrophy and insulin resistance in the elderly, especially those suffering from sarcopenic obesity.

At the cellular level, muscle growth is dependent on myogenesis which includes the differentiation and fusion of single-nucleated muscle cells (myoblasts) into multi-nucleated, mature myotubes. Myostatin is believed to promote muscle atrophy by suppressing the expression of genes involved in myogenesis through its canonical signaling pathway. Myostatin binds to the activin type IIB receptor (ActRIIB), leading to phosphorylation of Small Mothers Against Decapentaplegic (Smad) 2 and Smad3, which form a complex with Smad4 and translocate to the nucleus to inhibit the transcription of myogenic genes, including myogenic differentiation factor (*MyoD)* and *myogenin* (Langley et al. 2002; Trendelenburg et al. 2009) (Fig. 5.7). Myostatin-induced SMAD3 phosphorylation can also interact and inhibit the IGF-1/Akt/mTOR pathway, via inhibition of Akt phosphorylation (Trendelenburg et al. 2009; Goodman et al. 2013; Morissette et al. 2009). Since signaling pathways for protein synthesis and glucose uptake converge on Akt, it is not surprising that several studies have also provided convincing evidence that myostatin can inhibit insulin signaling by reducing Akt phosphorylation (Hittel et al. 2010; Zhang et al. 2011; Guo et al. 2009). Taken together, these findings support myostatin-induced inhibition of Akt as a mechanism for age-related muscle wasting and insulin resistance, especially in conditions of sarcopenic obesity.

5.6.3 Regenerative Medicine and Age-Impaired Glucose Metabolism

Therapeutic interventions to combat muscle wasting and insulin resistance have been historically studied independent of each other. For example, the discovery of myostatin as a negative regulator of skeletal muscle growth (McPherron et al. 1997) led to therapeutic strategies to target the myostatin signaling pathway, which focused on muscle mass/strength outcomes but neglected potential metabolic effects. The lack of translation of promising preclinical data to muscular dystrophy models (Wagner et al. 2008; St Andre et al. 2017; Wagner et al. 2020) combined with more recent findings that myostatin acts as a negative regulator of insulin

signaling (Guo et al. 2009; Zhang et al. 2011) led to speculation that these therapeutics could be more beneficial in older adults, especially those suffering from sarcopenic obesity. In contrast to the lack of FDA-approved drugs to treat sarcopenia, several pharmacological treatments are available for older adults with impaired glucose metabolism. Whereas the metabolic outcomes from these treatments have been well documented (Musi et al. 2002; Kim et al. 2002; Kulkarni et al. 2018; Hirst et al. 2012; Sasaki et al. 2019; Huthmacher et al. 2020; Gedulin et al. 2005; Henry et al. 2018), research continues to examine if these therapeutics could also prevent sarcopenia in older adults. The following provides examples of each of these types of therapeutics with special attention towards how each can regulate glucose metabolism, as well as skeletal muscle mass and function.

5.6.3.1 Myostatin/ActIIB Inhibitors

There has been considerable interest in developing therapeutics to block myostatin's inhibitory action on glucose uptake and muscle growth. Promising preclinical data demonstrated that mice treated with an anti-myostatin polyclonal antibody had a reversal of diet-induced whole-body insulin resistance, in conjunction with skeletal muscle changes including increased muscle mass, enhanced PI3K activity, Akt phosphorylation, GLUT4 protein expression, and increased phosphorylation of mTOR (Tang et al. 2014). Unfortunately, no known clinical studies have examined the effectiveness of these therapeutics in older sarcopenic patients with accompanying insulin resistance; however, early studies examining each condition separately have provided beneficial insight.

Phase II clinical studies blocking myostatin or its receptor (ActRIIB) suggest that despite increases in muscle mass, improvements in muscular function in non-obese, older adults, may be dependent on mobility/activity levels preceding or during treatment. For example, old (75 years or older), frail adults that had experienced at least one fall in the previous year, treated with the human monoclonal anti-myostatin antibody landogrozumab (LY2495655, Eli Lilly) demonstrated increased appendicular lean mass (~2.5%) and improvements in muscular performance on 12-step stair climb time (~ −1.3 s) (Becker et al. 2015). In contrast, when older non-obese, sarcopenic patients added bimagrumab (BYM338; Novartis), a human monoclonal anti-ActRII antibody, to nutritional counseling and a home-based exercise program, no additional improvements in muscular strength or mobility were observed, despite increases in lean mass (Rooks et al. 2020). This latter finding is of interest given an earlier proof-of-concept study that reported bimagrumab treatment improved muscle function in older sarcopenic patients when combined with nutritional counselling alone (Rooks et al. 2017). The effectiveness of myostatin inhibition on specific muscle contractile properties has been less clear with preclinical data producing concern that myostatin deletion could reduce specific force (Mendias et al. 2006; Mendias et al. 2011). Reduced SR Ca^{2+} release, possibly due to a decreased ability to refill the SR, has been suggested as a mechanism to explain the reduced specific force observed in hypermuscular ("compact") mice with a mutation in the *myostatin*

gene (Sztretye et al. 2017; Bodnar et al. 2014). It is possible that postnatal therapeutic blockade may be less detrimental on specific force, at least in mice (Bogdanovich et al. 2002, 2005; Mendias et al. 2015).

Early clinical studies suggest that therapeutics that block myostatin's receptor can improve insulin sensitivity and glycemic control in individuals with impaired glucose metabolism. For example, a single intravenous dose of bimagrubmab increased insulin sensitivity by ~20% (measured by hyperinsulinemic-euglycemic clamp) and reduced HbA1C in middle-aged (mean age: ~45 years), insulin-resistant individuals (Garito et al. 2018). These metabolic improvements were recently extended to overweight and obese type 2 diabetics when bimagrubmab treatment was added to a nutritional and exercise counseling (Heymsfield et al. 2021). Given recent findings that increased intramuscular lipids play an inhibitory role (Choi et al. 2016) in the age-related reductions in specific force (Ochala et al. 2007; Larsson et al. 1997; Choi et al. 2016) and that myostatin inhibition consistently demonstrates reductions in fat mass (Garito et al. 2018; Heymsfield et al. 2021), future research in obese, sarcopenic adults is warranted.

While purely speculative, these clinical findings suggest that older adults without co-existing conditions of obesity or uncontrolled glycemia can achieve increases in muscle mass with therapeutics that block myostatin or its receptor; however, improvements in muscle performance appear to be specific to frail or inactive older adults where these therapeutics could compensate for the lack of metabolic adaptations that regular muscle contractions induce. In contrast, it is possible that these therapeutics can improve insulin sensitivity and glycemic control above the benefits achieved by lifestyle modifications in insulin-resistant individuals; however, the potential effects on muscle mass, contractile properties, and muscle function in these individuals remain to be elucidated.

5.6.3.2 Pharmacological Agents

Metformin, an AMPK agonist, is often the first-line of treatment for insulin resistance, including those of advancing age due to its ability to enhance insulin sensitivity (Musi et al. 2002; Kim et al. 2002), improve glucose tolerance (Kulkarni et al. 2018), lower HbA1c levels (Hirst et al. 2012) and reduce type 2 diabetes incidence rates (Diabetes Prevention Program Research Group 2015). The mechanism (s) through which metformin improves glucose homeostasis are still under debate but likely involves the drug's ability to target numerous tissues, including the liver, skeletal muscle, and adipose tissue (Konopka and Miller 2019). Metformin is commonly cross-referenced as a calorie restrictive or exercise mimetic since it increases AMPK activity and has been shown to extend lifespan (Novelle et al. 2016; Martin-Montalvo et al. 2013; Sharoff et al. 2010). Additionally, metformin treatment has improved glucose intolerance in older adults (Kulkarni et al. 2018) and whole-body insulin sensitivity in type 2 diabetics (Musi et al. 2002; Kim et al. 2002). Of interest, these improvements in glucose metabolism do not appear to be related to changes in proximal insulin signaling (Kim et al. 2002). Instead, metformin

incubation of muscle cells has demonstrated increases in AS160 phosphorylation (Lee et al. 2011b), suggesting improvements may occur distally in the insulin signaling cascade where older adults appear to be most susceptible (Consitt et al. 2013). The effects of metformin on sarcopenia and dynapenia are less clear with reports that it may prevent sarcopenia in elderly adults with diabetes (Chen et al. 2020a; Lee et al. 2011a), but may also prevent gains in muscle mass (Walton et al. 2019; Long et al. 2021) and strength (Long et al. 2021) when prescribed in combination with resistance training, potentially due to the lack of increase in type II fiber frequency in older adults (Long et al. 2021) and/or hyperphosphorylation of AMPK and dampening of mTOR signaling (Walton et al. 2019).

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are effective in lowering blood glucose and HbA1c levels (Sasaki et al. 2019) by preventing glucose absorption in the kidneys, but their impact on sarcopenia and dynapenia remains unclear. Despite studies in middle to older aged type 2 diabetics reporting increased grip strength (Sano et al. 2016) and no loss in muscle mass (Sugiyama et al. 2018) with SGLT2 treatment, others have reported these drugs may accelerate diabetes-associated sarcopenia, especially in the elderly (Yabe et al. 2015; Sasaki et al. 2019). While the mechanisms responsible for reductions in muscle mass remain unknown, Yabe et al. (2015) speculated it may be related hypoglycemia events causing low circulating insulin levels to reduce glucose and amino acid entry into the skeletal muscle.

Treatment with GLP-1 receptor (GLP-1R) agonists has proven to be useful in managing hyperglycemia and decreasing HbA1c levels (Huthmacher et al. 2020; Gedulin et al. 2005; Henry et al. 2018). Recent in vitro research suggests these agonists may have a direct role on muscle. Treatment of C2C12 myotubes with the GLP1R agonist, exendin-4 (Ex-4), resulted in the suppression of myostatin and muscle atrophic genes (*atrogin-1* and *Murf-1*) along with increasing protein synthesis (Hong et al. 2019). In addition, it has also been suggested Ex-4 may have neuroprotective properties based on findings that treatment of a mouse model of Parkinson's disease protected dopaminergic neurons against degeneration and improved motor function (Li et al. 2009). DPP-4 inhibitors block the DPP-4 degradation of circulating incretins, including GLP-1 (Herman et al. 2005; Berg et al. 2011) and have proven effective in reducing HbA1c levels (Dicker 2011), increasing skeletal muscle insulin-stimulated glucose uptake (Sato et al. 2016) and increasing GLUT4 expression (Giannocco et al. 2013). In addition, DPP-4 inhibitors have been reported to reduce the progression of sarcopenia in elderly type 2 diabetics (Rizzo et al. 2016). Although it remains unclear if DPP-4 inhibitors act directly on the muscle to provide this protective effect on muscle mass, these findings are encouraging and warrant future research.

Taken together, there is promising data suggesting inhibitors that target the myostatin pathway and some pharmacologic agents traditionally used to regulate glycemic control could provide improvements in both glucose metabolism, as well as muscle mass and strength in older adults. However, it is evident that despite a clear link between glucose dysregulation, sarcopenia and dynapenia, the cellular mechanisms contributing to this relationship and the type of therapeutics required

may differ among individuals. Factors including, but not limited to, obesity, degree of glucose dysregulation, previous and current physical activity can all impact responses depending on the therapeutic used and should be considered.

5.7 Conclusions of the Chapter

Deficits in neuromuscular physiological factors contribute to skeletal muscle dysfunction that manifests in pervasive chronic conditions and disability are observed in many older adults. Moreover, scientific and medical communities agree that dynapenia is a major determinant of physical limitations and general poor health in older adults. The neuromuscular mechanisms of dynapenia and more broadly, muscle dysfunction, are multi-factorial and likely include several domains that are affected by the aging process. We propose some of these domains are loss of neuromuscular excitation, EC coupling failure, sarcopenia (via loss of protein homeostasis) and metabolic dysfunction (via mitochondrial dysfunction and impaired glucose metabolism) (Fig. 5.1). However, we acknowledge these domains are likely a few of many, and are all interconnected. It appears that if dynapenia and sarcopenia continue to worsen with age that a vicious cycle of continued neuromuscular deficits ensues, which can ultimately lead to physical frailty. Thus, maintenance of neuromuscular function is a critical component to healthy aging. Within this chapter, we have listed several approaches that yield promise in delaying or preventing many domains of age-related neuromuscular deficits. However, we urge caution in that many of our discussed approaches are speculative and in the early stages of investigations. With that stated, regenerative rehabilitation in sarcopenia, dynapenia, and frailty in both basic laboratory and clinical settings that utilizes a team science approach is essential to advancing the aging field forward.

References

Addison O, Prior SJ, Kundi R, Serra MC, Katzel LI, Gardner AW, Ryan AS (2018) Sarcopenia in peripheral arterial disease: prevalence and effect on functional status. Arch Phys Med Rehabil 99(4):623–628. https://doi.org/10.1016/j.apmr.2017.10.017

Ahern GP, Junankar PR, Dulhunty AF (1997) Subconductance states in single-channel activity of skeletal muscle ryanodine receptors after removal of FKBP12. Biophys J 72(1):146–162. https://doi.org/10.1016/s0006-3495(97)78654-5

Al Snih S, Markides KS, Ottenbacher KJ, Raji MA (2004) Hand grip strength and incident ADL disability in elderly Mexican Americans over a seven-year period. Aging Clin Exp Res 16(6): 481–486. https://doi.org/10.1007/BF03327406

Amati F, Dube JJ, Coen PM, Stefanovic-Racic M, Toledo FG, Goodpaster BH (2009) Physical inactivity and obesity underlie the insulin resistance of aging. Diabetes Care 32(8):1547–1549. https://doi.org/10.2337/dc09-0267

Anderson AA, Treves S, Biral D, Betto R, Sandonà D, Ronjat M, Zorzato F (2003) The novel skeletal muscle sarcoplasmic reticulum JP-45 protein. Molecular cloning, tissue distribution,

developmental expression, and interaction with alpha 1.1 subunit of the voltage-gated calcium channel. J Biol Chem 278(41):39987–39992. https://doi.org/10.1074/jbc.M305016200

Andersson DC, Betzenhauser MJ, Reiken S, Meli AC, Umanskaya A, Xie W, Shiomi T, Zalk R, Lacampagne A, Marks AR (2011) Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. Cell Metab 14(2):196–207. https://doi.org/10.1016/j.cmet.2011.05.014

Andreux PA, van Diemen MPJ, Heezen MR, Auwerx J, Rinsch C, Groeneveld GJ, Singh A (2018) Mitochondrial function is impaired in the skeletal muscle of pre-frail elderly. Sci Rep 8(1):8548. https://doi.org/10.1038/s41598-018-26944-x

Antonini A, Leenders KL (1993) Dopamine D2 receptors in normal human brain: effect of age measured by positron emission tomography (PET) and [11C]-raclopride. Ann N Y Acad Sci 695:81–85. https://doi.org/10.1111/j.1749-6632.1993.tb23033.x

Ashe J (1997) Force and the motor cortex. Behav Brain Res 87(2):255–269. https://doi.org/10.1016/s0166-4328(97)00752-3

Atherton PJ, Greenhaff PL, Phillips SM, Bodine SC, Adams CM, Lang CH (2016) Control of skeletal muscle atrophy in response to disuse: clinical/preclinical contentions and fallacies of evidence. Am J Physiol Endocrinol Metab 311(3):E594–E604. https://doi.org/10.1152/ajpendo.00257.2016

Baker JS, McCormick MC, Robergs RA (2010) Interaction among skeletal muscle metabolic energy systems during intense exercise. J Nutr Metab 2010:905612. https://doi.org/10.1155/2010/905612

Barry BK, Riek S, Carson RG (2005) Muscle coordination during rapid force production by young and older adults. J Gerontol A Biol Sci Med Sci 60(2):232–240. https://doi.org/10.1093/gerona/60.2.232

Bass JJ, Nakhuda A, Deane CS, Brook MS, Wilkinson DJ, Phillips BE, Philp A, Tarum J, Kadi F, Andersen D, Garcia AM, Smith K, Gallagher IJ, Szewczyk NJ, Cleasby ME, Atherton PJ (2020) Overexpression of the vitamin D receptor (VDR) induces skeletal muscle hypertrophy. Mol Metab 42:101059. https://doi.org/10.1016/j.molmet.2020.101059

Batsis JA, Mackenzie TA, Lopez-Jimenez F, Bartels SJ (2015) Sarcopenia, sarcopenic obesity, and functional impairments in older adults: National Health and Nutrition Examination Surveys 1999-2004. Nutr Res 35(12):1031–1039. https://doi.org/10.1016/j.nutres.2015.09.003

Baumann CW, Rogers RG, Gahlot N, Ingalls CP (2014) Eccentric contractions disrupt FKBP12 content in mouse skeletal muscle. Physiol Rep 2(7). https://doi.org/10.14814/phy2.12081

Baumann CW, Kwak D, Liu HM, Thompson LV (2016) Age-induced oxidative stress: how does it influence skeletal muscle quantity and quality? J Appl Physiol (1985) 121(5):1047–1052. https://doi.org/10.1152/japplphysiol.00321.2016

Baumann CW, Kwak D, Thompson LV (2018) Assessing onset, prevalence and survival in mice using a frailty phenotype. Aging (Albany NY) 10(12):4042–4053. https://doi.org/10.18632/aging.101692

Baumann CW, Kwak D, Thompson LV (2020) Phenotypic frailty assessment in mice: development, discoveries, and experimental considerations. Physiology (Bethesda) 35(6):405–414. https://doi.org/10.1152/physiol.00016.2020

Becker C, Lord SR, Studenski SA, Warden SJ, Fielding RA, Recknor CP, Hochberg MC, Ferrari SL, Blain H, Binder EF, Rolland Y, Poiraudeau S, Benson CT, Myers SL, Hu L, Ahmad QI, Pacuch KR, Gomez EV, Benichou O, STEADY Group (2015) Myostatin antibody (LY2495655) in older weak fallers: a proof-of-concept, randomised, phase 2 trial. Lancet Diabetes Endocrinol 3(12):948–957. https://doi.org/10.1016/S2213-8587(15)00298-3

Bellinger AM, Mongillo M, Marks AR (2008a) Stressed out: the skeletal muscle ryanodine receptor as a target of stress. J Clin Invest 118(2):445–453. https://doi.org/10.1172/jci34006

Bellinger AM, Reiken S, Dura M, Murphy PW, Deng SX, Landry DW, Nieman D, Lehnart SE, Samaru M, LaCampagne A, Marks AR (2008b) Remodeling of ryanodine receptor complex causes “leaky” channels: a molecular mechanism for decreased exercise capacity. Proc Natl Acad Sci U S A 105(6):2198–2202. https://doi.org/10.1073/pnas.0711074105

Bellinger AM, Reiken S, Carlson C, Mongillo M, Liu X, Rothman L, Matecki S, Lacampagne A, Marks AR (2009) Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. Nat Med 15(3):325–330. https://doi.org/10.1038/nm.1916

Ben-Itzhak R, Giladi N, Gruendlinger L, Hausdorff JM (2008) Can methylphenidate reduce fall risk in community-living older adults? A double-blind, single-dose cross-over study. J Am Geriatr Soc 56(4):695–700. https://doi.org/10.1111/j.1532-5415.2007.01623.x

Berardelli A, Rothwell JC, Thompson PD, Hallett M (2001) Pathophysiology of bradykinesia in Parkinson's disease. Brain 124(Pt 11):2131–2146. https://doi.org/10.1093/brain/124.11.2131

Berg JK, Shenouda SK, Heilmann CR, Gray AL, Holcombe JH (2011) Effects of exenatide twice daily versus sitagliptin on 24-h glucose, glucoregulatory and hormonal measures: a randomized, double-blind, crossover study. Diabetes Obes Metab 13(11):982–989. https://doi.org/10.1111/j.1463-1326.2011.01428.x

Bhasin S, Travison TG, Manini TM, Patel S, Pencina KM, Fielding RA, Magaziner JM, Newman AB, Kiel DP, Cooper C, Guralnik JM, Cauley JA, Arai H, Clark BC, Landi F, Schaap LA, Pereira SL, Rooks D, Woo J, Woodhouse LJ, Binder E, Brown T, Shardell M, Xue QL, D'Agostino RB Sr, Orwig D, Gorsicki G, Correa-De-Araujo R, Cawthon PM (2020) Sarcopenia definition: the position statements of the sarcopenia definition and outcomes consortium. J Am Geriatr Soc. https://doi.org/10.1111/jgs.16372

Bienso RS, Olesen J, Gliemann L, Schmidt JF, Matzen MS, Wojtaszewski JF, Hellsten Y, Pilegaard H (2015) Effects of exercise training on regulation of skeletal muscle glucose metabolism in elderly men. J Gerontol A Biol Sci Med Sci 70(7):866–872. https://doi.org/10.1093/gerona/glv012

Bilder RM, Volavka J, Lachman HM, Grace AA (2004) The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. Neuropsychopharmacology 29(11):1943–1961. https://doi.org/10.1038/sj.npp.1300542

Bodnar D, Geyer N, Ruzsnavszky O, Olah T, Hegyi B, Sztretye M, Fodor J, Dienes B, Balogh A, Papp Z, Szabo L, Muller G, Csernoch L, Szentesi P (2014) Hypermuscular mice with mutation in the myostatin gene display altered calcium signalling. J Physiol 592(6):1353–1365. https://doi.org/10.1113/jphysiol.2013.261958

Boengler K, Heusch G, Schulz R (2011) Nuclear-encoded mitochondrial proteins and their role in cardioprotection. Biochim Biophys Acta 1813(7):1286–1294. https://doi.org/10.1016/j.bbamcr.2011.01.009

Boengler K, Kosiol M, Mayr M, Schulz R, Rohrbach S (2017) Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. J Cachexia Sarcopenia Muscle 8(3):349–369. https://doi.org/10.1002/jcsm.12178

Bogdanovich S, Krag TO, Barton ER, Morris LD, Whittemore LA, Ahima RS, Khurana TS (2002) Functional improvement of dystrophic muscle by myostatin blockade. Nature 420(6914):418–421. https://doi.org/10.1038/nature01154

Bogdanovich S, Perkins KJ, Krag TO, Whittemore LA, Khurana TS (2005) Myostatin propeptide-mediated amelioration of dystrophic pathophysiology. FASEB J 19(6):543–549. https://doi.org/10.1096/fj.04-2796com

Boncompagni S, d'Amelio L, Fulle S, Fanò G, Protasi F (2006) Progressive disorganization of the excitation-contraction coupling apparatus in aging human skeletal muscle as revealed by electron microscopy: a possible role in the decline of muscle performance. J Gerontol A Biol Sci Med Sci 61(10):995–1008. https://doi.org/10.1093/gerona/61.10.995

Boncompagni S, Pecorai C, Michelucci A, Pietrangelo L, Protasi F (2020) Long-term exercise reduces formation of tubular aggregates and promotes maintenance of Ca(2+) entry units in aged muscle. Front Physiol 11:601057. https://doi.org/10.3389/fphys.2020.601057

Brillantes AB, Ondrias K, Scott A, Kobrinsky E, Ondriasová E, Moschella MC, Jayaraman T, Landers M, Ehrlich BE, Marks AR (1994) Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. Cell 77(4):513–523. https://doi.org/10.1016/0092-8674(94)90214-3

Brook MS, Wilkinson DJ, Mitchell WK, Lund JN, Phillips BE, Szewczyk NJ, Greenhaff PL, Smith K, Atherton PJ (2016a) Synchronous deficits in cumulative muscle protein synthesis and ribosomal biogenesis underlie age-related anabolic resistance to exercise in humans. J Physiol 594(24):7399–7417. https://doi.org/10.1113/JP272857

Brook MS, Wilkinson DJ, Phillips BE, Perez-Schindler J, Philp A, Smith K, Atherton PJ (2016b) Skeletal muscle homeostasis and plasticity in youth and ageing: impact of nutrition and exercise. Acta Physiol (Oxf) 216(1):15–41. https://doi.org/10.1111/apha.12532

Bryhni B, Jenssen TG, Olafsen K, Bendikssen A (2005) Oxidative and nonoxidative glucose disposal in elderly vs younger men with similar and smaller body mass indices and waist circumferences. Metabolism 54(6):748–755. https://doi.org/10.1016/j.metabol.2005.01.016

Buchman AS, Wilson RS, Boyle PA, Bienias JL, Bennett DA (2007) Grip strength and the risk of incident Alzheimer's disease. Neuroepidemiology 29(1–2):66–73. https://doi.org/10.1159/000109498

Buhusi CV, Meck WH (2005) What makes us tick? Functional and neural mechanisms of interval timing. Nat Rev Neurosci 6(10):755–765. https://doi.org/10.1038/nrn1764

Calderón JC, Bolaños P, Caputo C (2014) The excitation-contraction coupling mechanism in skeletal muscle. Biophys Rev 6(1):133–160. https://doi.org/10.1007/s12551-013-0135-x

Carbone JW, McClung JP, Pasiakos SM (2019) Recent advances in the characterization of skeletal muscle and whole-body protein responses to dietary protein and exercise during negative energy balance. Adv Nutr 10(1):70–79. https://doi.org/10.1093/advances/nmy087

Carlsson A, Winblad B (1976) Influence of age and time interval between death and autopsy on dopamine and 3-methoxytyramine levels in human basal ganglia. J Neural Transm 38(3–4):271–276. https://doi.org/10.1007/BF01249444

Carson RG (2018) Get a grip: individual variations in grip strength are a marker of brain health. Neurobiol Aging 71:189–222. https://doi.org/10.1016/j.neurobiolaging.2018.07.023

Carter HN, Chen CC, Hood DA (2015) Mitochondria, muscle health, and exercise with advancing age. Physiology (Bethesda) 30(3):208–223. https://doi.org/10.1152/physiol.00039.2014

Catterall WA, Goldin AL, Waxman SG (2005) International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. Pharmacol Rev 57(4):397–409. https://doi.org/10.1124/pr.57.4.4

Cauley JA (2015) An overview of sarcopenic obesity. J Clin Densitom 18(4):499–505. https://doi.org/10.1016/j.jocd.2015.04.013

Celis-Morales CA, Welsh P, Lyall DM, Steell L, Petermann F, Anderson J, Iliodromiti S, Sillars A, Graham N, Mackay DF, Pell JP, Gill JMR, Sattar N, Gray SR (2018) Associations of grip strength with cardiovascular, respiratory, and cancer outcomes and all cause mortality: prospective cohort study of half a million UK Biobank participants. BMJ 361:k1651. https://doi.org/10.1136/bmj.k1651

Cham R, Studenski SA, Perera S, Bohnen NI (2008) Striatal dopaminergic denervation and gait in healthy adults. Exp Brain Res 185(3):391–398. https://doi.org/10.1007/s00221-007-1161-3

Chang SF, Lin PL (2015) Frail phenotype and mortality prediction: a systematic review and meta-analysis of prospective cohort studies. Int J Nurs Stud 52(8):1362–1374. https://doi.org/10.1016/j.ijnurstu.2015.04.005

Chapman J, Fielder E, Passos JF (2019) Mitochondrial dysfunction and cell senescence: deciphering a complex relationship. FEBS Lett 593(13):1566–1579. https://doi.org/10.1002/1873-3468.13498

Chen F, Xu S, Wang Y, Chen F, Cao L, Liu T, Huang T, Wei Q, Ma G, Zhao Y, Wang D (2020a) Risk factors for sarcopenia in the elderly with type 2 diabetes mellitus and the effect of metformin. J Diabetes Res 2020:3950404. https://doi.org/10.1155/2020/3950404

Chen G, Kroemer G, Kepp O (2020b) Mitophagy: an emerging role in aging and age-associated diseases. Front Cell Dev Biol 8:200. https://doi.org/10.3389/fcell.2020.00200

Chen W, Datzkiw D, Rudnicki MA (2020c) Satellite cells in ageing: use it or lose it. Open Biol 10(5):200048. https://doi.org/10.1098/rsob.200048

Chiu CY, Yang RS, Sheu ML, Chan DC, Yang TH, Tsai KS, Chiang CK, Liu SH (2016) Advanced glycation end-products induce skeletal muscle atrophy and dysfunction in diabetic mice via a RAGE-mediated, AMPK-down-regulated, Akt pathway. J Pathol 238(3):470–482. https://doi.org/10.1002/path.4674

Choi SJ, Files DC, Zhang T, Wang ZM, Messi ML, Gregory H, Stone J, Lyles MF, Dhar S, Marsh AP, Nicklas BJ, Delbono O (2016) Intramyocellular lipid and impaired myofiber contraction in normal weight and obese older adults. J Gerontol A Biol Sci Med Sci 71(4):557–564. https://doi.org/10.1093/gerona/glv169

Christie A, Kamen G (2006) Doublet discharges in motoneurons of young and older adults. J Neurophysiol 95(5):2787–2795. https://doi.org/10.1152/jn.00685.2005. 00685.2005 [pii]

Christie A, Kamen G (2010) Short-term training adaptations in maximal motor unit firing rates and afterhyperpolarization duration. Muscle Nerve 41(5):651–660. https://doi.org/10.1002/mus.21539

Churchward-Venne TA, Tieland M, Verdijk LB, Leenders M, Dirks ML, de Groot LC, van Loon LJ (2015) There are no nonresponders to resistance-type exercise training in older men and women. J Am Med Dir Assoc 16(5):400–411. https://doi.org/10.1016/j.jamda.2015.01.071

Clark BC, Manini TM (2008) Sarcopenia =/= dynapenia. J Gerontol A Biol Sci Med Sci 63(8):829–834. 63/8/829 [pii]

Clark BC, Taylor JL (2011) Age-related changes in motor cortical properties and voluntary activation of skeletal muscle. Curr Aging Sci. https://doi.org/10.2174/1874609811104030192

Clark BC, Mahato NK, Nakazawa M, Law TD, Thomas JS (2014) The power of the mind: the cortex as a critical determinant of muscle strength/weakness. J Neurophysiol 112(12):3219–3226. https://doi.org/10.1152/jn.00386.2014

Clark BC, Taylor JL, Hong SL, Law TD, Russ DW (2015) Weaker seniors exhibit motor cortex hypoexcitability and impairments in voluntary activation. J Gerontol A Biol Sci Med Sci 70(9):1112–1119. https://doi.org/10.1093/gerona/glv030

Clark BC, Manini TM, Wages NP, Simon JE, Clark LA (2019) Voluntary vs electrically stimulated activation in age-related muscle weakness. JAMA Netw Open 2(9):e1912052. https://doi.org/10.1001/jamanetworkopen.2019.12052

Clark LA, Manini TM, Wages NP, Simon JE, Russ DW, Clark BC (2020) Reduced neural excitability and activation contribute to clinically-meaningful weakness in older adults. J Gerontol A Biol Sci Med Sci. https://doi.org/10.1093/gerona/glaa157

Coen PM, Musci RV, Hinkley JM, Miller BF (2018) Mitochondria as a target for mitigating sarcopenia. Front Physiol 9:1883. https://doi.org/10.3389/fphys.2018.01883

Cohen JA, Verghese J (2019) Gait and dementia. Handb Clin Neurol 167:419–427. https://doi.org/10.1016/B978-0-12-804766-8.00022-4

Consitt LA, Van Meter J, Newton CA, Collier DN, Dar MS, Wojtaszewski JF, Treebak JT, Tanner CJ, Houmard JA (2013) Impairments in site-specific AS160 phosphorylation and effects of exercise training. Diabetes 62(10):3437–3447. https://doi.org/10.2337/db13-0229

Consitt LA, Saxena G, Saneda A, Houmard JA (2016) Age-related impairments in skeletal muscle PDH phosphorylation and plasma lactate are indicative of metabolic inflexibility and the effects of exercise training. Am J Physiol Endocrinol Metab 311(1):E145–E156. https://doi.org/10.1152/ajpendo.00452.2015

Consitt LA, Saxena G, Slyvka Y, Clark BC, Friedlander M, Zhang Y, Nowak FV (2018) Paternal high-fat diet enhances offspring whole-body insulin sensitivity and skeletal muscle insulin signaling early in life. Physiol Rep 6(5):doi:10.14814/phy2.13583

Constantin-Teodosiu D, Stephens FB, Greenhaff PL (2015) Perpetual muscle PDH activation in PDH kinase knockout mice protects against high-fat feeding-induced muscle insulin resistance. Proc Natl Acad Sci U S A 112(8):E824. https://doi.org/10.1073/pnas.1422929112

Cowie CC, Rust KF, Ford ES, Eberhardt MS, Byrd-Holt DD, Li C, Williams DE, Gregg EW, Bainbridge KE, Saydah SH, Geiss LS (2009) Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. Diabetes Care 32(2):287–294. https://doi.org/10.2337/dc08-1296

Cox JH, Cortright RN, Dohm GL, Houmard JA (1999) Effect of aging on response to exercise training in humans: skeletal muscle GLUT-4 and insulin sensitivity. J Appl Physiol (1985) 86(6):2019–2025. https://doi.org/10.1152/jappl.1999.86.6.2019

Crawford ED, Wells JA (2011) Caspase substrates and cellular remodeling. Annu Rev Biochem 80: 1055–1087. https://doi.org/10.1146/annurev-biochem-061809-121639

Crescenzo R, Lionetti L, Mollica MP, Ferraro M, D'Andrea E, Mainieri D, Dulloo AG, Liverini G, Iossa S (2006) Altered skeletal muscle subsarcolemmal mitochondrial compartment during catch-up fat after caloric restriction. Diabetes 55(8):2286–2293. https://doi.org/10.2337/db06-0312

Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, Cooper C, Landi F, Rolland Y, Sayer AA (2019) Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 48(1):16–31

Currais A, Fischer W, Maher P, Schubert D (2017) Intraneuronal protein aggregation as a trigger for inflammation and neurodegeneration in the aging brain. FASEB J 31(1):5–10. https://doi.org/10.1096/fj.201601184

Dalal M, Ferrucci L, Sun K, Beck J, Fried LP, Semba RD (2009) Elevated serum advanced glycation end products and poor grip strength in older community-dwelling women. J Gerontol A Biol Sci Med Sci 64(1):132–137. https://doi.org/10.1093/gerona/gln018

Daussin FN, Boulanger E, Lancel S (2021) From mitochondria to sarcopenia: role of inflammaging and RAGE-ligand axis implication. Exp Gerontol 146:111247. https://doi.org/10.1016/j.exger.2021.111247

Deane CS, Ames RM, Phillips BE, Weedon MN, Willis CRG, Boereboom C, Abdulla H, Bukhari SSI, Lund JN, Williams JP, Wilkinson DJ, Smith K, Gallagher IJ, Kadi F, Szewczyk NJ, Atherton PJ, Etheridge T (2019) The acute transcriptional response to resistance exercise: impact of age and contraction mode. Aging (Albany NY) 11(7):2111–2126. https://doi.org/10.18632/aging.101904

DeFronzo RA, Tripathy D (2009) Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 32(Suppl 2):S157–S163. https://doi.org/10.2337/dc09-S302

DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP (1981) The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 30(12):1000–1007

Dela F, Ploug T, Handberg A, Petersen LN, Larsen JJ, Mikines KJ, Galbo H (1994) Physical training increases muscle GLUT4 protein and mRNA in patients with NIDDM. Diabetes 43(7): 862–865. https://doi.org/10.2337/diab.43.7.862

Delbono O (2011) Expression and regulation of excitation-contraction coupling proteins in aging skeletal muscle. Curr Aging Sci 4(3):248–259. https://doi.org/10.2174/1874609811104030248

Delbono O, O'Rourke KS, Ettinger WH (1995) Excitation-calcium release uncoupling in aged single human skeletal muscle fibers. J Membr Biol 148(3):211–222. https://doi.org/10.1007/bf00235039

Delmonico MJ, Harris TB, Visser M, Park SW, Conroy MB, Velasquez-Mieyer P, Boudreau R, Manini TM, Nevitt M, Newman AB, Goodpaster BH (2009) Longitudinal study of muscle strength, quality, and adipose tissue infiltration. Am J Clin Nutr 90(6):1579–1585. https://doi.org/10.3945/ajcn.2009.28047. ajcn.2009.28047 [pii]

Dent E, Morley JE, Cruz-Jentoft AJ, Arai H, Kritchevsky SB, Guralnik J, Bauer JM, Pahor M, Clark BC, Cesari M, Ruiz J, Sieber CC, Aubertin-Leheudre M, Waters DL, Visvanathan R, Landi F, Villareal DT, Fielding R, Won CW, Theou O, Martin FC, Dong B, Woo J, Flicker L, Ferrucci L, Merchant RA, Cao L, Cederholm T, Ribeiro SML, Rodriguez-Manas L, Anker SD, Lundy J, Gutierrez Robledo LM, Bautmans I, Aprahamian I, Schols J, Izquierdo M, Vellas B (2018) International Clinical Practice Guidelines for Sarcopenia (ICFSR): screening, diagnosis and management. J Nutr Health Aging 22(10):1148–1161. https://doi.org/10.1007/s12603-018-1139-9

Diabetes Prevention Program Research Group (2015) Long-term effects of lifestyle intervention or metformin on diabetes development and microvascular complications over 15-year follow-up:

the Diabetes Prevention Program Outcomes Study. Lancet Diabetes Endocrinol 3(11):866–875. https://doi.org/10.1016/S2213-8587(15)00291-0

Dicker D (2011) DPP-4 inhibitors: impact on glycemic control and cardiovascular risk factors. Diabetes Care 34(Suppl 2):S276–S278. https://doi.org/10.2337/dc11-s229

Distefano G, Goodpaster BH (2018) Effects of exercise and aging on skeletal muscle. Cold Spring Harb Perspect Med 8(3). https://doi.org/10.1101/cshperspect.a029785

Du J, Zhu M, Bao H, Li B, Dong Y, Xiao C, Zhang GY, Henter I, Rudorfer M, Vitiello B (2016) The role of nutrients in protecting mitochondrial function and neurotransmitter signaling: implications for the treatment of depression, PTSD, and suicidal behaviors. Crit Rev Food Sci Nutr 56(15):2560–2578. https://doi.org/10.1080/10408398.2013.876960

Duchowny K (2019) Do nationally representative cutpoints for clinical muscle weakness predict mortality? Results from 9 years of follow-up in the health and retirement study. J Gerontol A Biol Sci Med Sci 74(7):1070–1075. https://doi.org/10.1093/gerona/gly169

Duncan MJ, Clarke ND, Tallis J, Guimaraes-Ferreira L, Leddington Wright S (2014) The effect of caffeine ingestion on functional performance in older adults. J Nutr Health Aging 18(10): 883–887. https://doi.org/10.1007/s12603-014-0474-8

Durham WJ, Casperson SL, Dillon EL, Keske MA, Paddon-Jones D, Sanford AP, Hickner RC, Grady JJ, Sheffield-Moore M (2010) Age-related anabolic resistance after endurance-type exercise in healthy humans. FASEB J 24(10):4117–4127. https://doi.org/10.1096/fj.09-150177

Enoka RM (1988) Muscle strength and its development. New perspectives. Sports Med 6(3): 146–168

Enoka RM, Christou EA, Hunter SK, Kornatz KW, Semmler JG, Taylor AM, Tracy BL (2003) Mechanisms that contribute to differences in motor performance between young and old adults. J Electromyogr Kinesiol 13(1):1–12. S1050641102000846 [pii]

Erbas T, Ertas M, Yucel A, Keskinaslan A, Senocak M, TURNEP Study Group (2011) Prevalence of peripheral neuropathy and painful peripheral neuropathy in Turkish diabetic patients. J Clin Neurophysiol 28(1):51–55. https://doi.org/10.1097/WNP.0b013e3182051334

Evans WJ, Hellerstein M, Orwoll E, Cummings S, Cawthon PM (2019) D3 -Creatine dilution and the importance of accuracy in the assessment of skeletal muscle mass. J Cachexia Sarcopenia Muscle 10(1):14–21. https://doi.org/10.1002/jcsm.12390

Ferreira R, Vitorino R, Alves RM, Appell HJ, Powers SK, Duarte JA, Amado F (2010) Subsarcolemmal and intermyofibrillar mitochondria proteome differences disclose functional specializations in skeletal muscle. Proteomics 10(17):3142–3154. https://doi.org/10.1002/pmic.201000173

Figueiredo VC, McCarthy JJ (2019) Regulation of ribosome biogenesis in skeletal muscle hypertrophy. Physiology (Bethesda) 34(1):30–42. https://doi.org/10.1152/physiol.00034.2018

Fill M, Copello JA (2002) Ryanodine receptor calcium release channels. Physiol Rev 82(4): 893–922. https://doi.org/10.1152/physrev.00013.2002

Fink RI, Kolterman OG, Griffin J, Olefsky JM (1983) Mechanisms of insulin resistance in aging. J Clin Invest 71(6):1523–1535. https://doi.org/10.1172/jci110908

Fink RI, Wallace P, Olefsky JM (1986) Effects of aging on glucose-mediated glucose disposal and glucose transport. J Clin Invest 77(6):2034–2041. https://doi.org/10.1172/JCI112533

Florini JR, Ewton DZ, Coolican SA (1996) Growth hormone and the insulin-like growth factor system in myogenesis. Endocr Rev 17(5):481–517. https://doi.org/10.1210/edrv-17-5-481

Fodor J, Al-Gaadi D, Czirják T, Oláh T, Dienes B, Csernoch L, Szentesi P (2020) Improved calcium homeostasis and force by selenium treatment and training in aged mouse skeletal muscle. Sci Rep 10(1):1707. https://doi.org/10.1038/s41598-020-58500-x

Franssila-Kallunki A, Schalin-Jantti C, Groop L (1992) Effect of gender on insulin resistance associated with aging. Am J Physiol 263(4 Pt 1):E780–E785. https://doi.org/10.1152/ajpendo.1992.263.4.E780

Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA (2001) Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 56(3):M146–M156. https://doi.org/10.1093/gerona/56.3.m146

Fujita S, Glynn EL, Timmerman KL, Rasmussen BB, Volpi E (2009) Supraphysiological hyperinsulinaemia is necessary to stimulate skeletal muscle protein anabolism in older adults: evidence of a true age-related insulin resistance of muscle protein metabolism. Diabetologia 52(9):1889–1898. https://doi.org/10.1007/s00125-009-1430-8

Fukumori N, Yamamoto Y, Takegami M, Yamazaki S, Onishi Y, Sekiguchi M, Otani K, Konno S, Kikuchi S, Fukuhara S (2015) Association between hand-grip strength and depressive symptoms: locomotive syndrome and health outcomes in Aizu Cohort Study (LOHAS). Age Ageing 44(4):592–598. https://doi.org/10.1093/ageing/afv013

Garito T, Roubenoff R, Hompesch M, Morrow L, Gomez K, Rooks D, Meyers C, Buchsbaum MS, Neelakantham S, Swan T, Filosa LA, Laurent D, Petricoul O, Zakaria M (2018) Bimagrumab improves body composition and insulin sensitivity in insulin-resistant individuals. Diabetes Obes Metab 20(1):94–102. https://doi.org/10.1111/dom.13042

Gaster M, Poulsen P, Handberg A, Schroder HD, Beck-Nielsen H (2000) Direct evidence of fiber type-dependent GLUT-4 expression in human skeletal muscle. Am J Physiol Endocrinol Metab 278(5):E910–E916. https://doi.org/10.1152/ajpendo.2000.278.5.E910

Gedulin BR, Nikoulina SE, Smith PA, Gedulin G, Nielsen LL, Baron AD, Parkes DG, Young AA (2005) Exenatide (exendin-4) improves insulin sensitivity and {beta}-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. Endocrinology 146(4):2069–2076. https://doi.org/10.1210/en.2004-1349

Gharahdaghi N, Rudrappa S, Brook MS, Idris I, Crossland H, Hamrock C, Abdul Aziz MH, Kadi F, Tarum J, Greenhaff PL, Constantin-Teodosiu D, Cegielski J, Phillips BE, Wilkinson DJ, Szewczyk NJ, Smith K, Atherton PJ (2019) Testosterone therapy induces molecular programming augmenting physiological adaptations to resistance exercise in older men. J Cachexia Sarcopenia Muscle 10(6):1276–1294. https://doi.org/10.1002/jcsm.12472

Giannocco G, Oliveira KC, Crajoinas RO, Venturini G, Salles TA, Fonseca-Alaniz MH, Maciel RM, Girardi AC (2013) Dipeptidyl peptidase IV inhibition upregulates GLUT4 translocation and expression in heart and skeletal muscle of spontaneously hypertensive rats. Eur J Pharmacol 698(1–3):74–86. https://doi.org/10.1016/j.ejphar.2012.09.043

Goldspink G (2012) Age-related loss of muscle mass and strength. J Aging Res 2012:158279. https://doi.org/10.1155/2012/158279

Goll DE, Thompson VF, Li H, Wei W, Cong J (2003) The calpain system. Physiol Rev 83(3): 731–801. https://doi.org/10.1152/physrev.00029.2002

Gonzalez E, Messi ML, Zheng Z, Delbono O (2003) Insulin-like growth factor-1 prevents age-related decrease in specific force and intracellular Ca2+ in single intact muscle fibres from transgenic mice. J Physiol 552(Pt 3):833–844. https://doi.org/10.1113/jphysiol.2003.048165

Goodman CA, McNally RM, Hoffmann FM, Hornberger TA (2013) Smad3 induces atrogin-1, inhibits mTOR and protein synthesis, and promotes muscle atrophy in vivo. Mol Endocrinol 27(11):1946–1957. https://doi.org/10.1210/me.2013-1194

Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, Simonsick EM, Tylavsky FA, Visser M, Newman AB (2006) The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J Gerontol A Biol Sci Med Sci 61(10):1059–1064. https://doi.org/10.1093/gerona/61.10.1059

Grabner M, Dayal A (2010) Crosstalk via the sarcoplasmic gap: the DHPR-RyR interaction. Curr Top Membr 66:115–138. https://doi.org/10.1016/s1063-5823(10)66006-1

Grace AA (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41(1):1–24. https://doi.org/10.1016/0306-4522(91)90196-u

Greiwe JS, Cheng B, Rubin DC, Yarasheski KE, Semenkovich CF (2001) Resistance exercise decreases skeletal muscle tumor necrosis factor alpha in frail elderly humans. FASEB J 15(2): 475–482. https://doi.org/10.1096/fj.00-0274com

Guidelines for the Review of Inclusion on the Basis of Sex/Gender, Race, Ethnicity, and Age in Clinical Research (2019) Older adults. National Institutes of Health (NIH)

Guo T, Jou W, Chanturiya T, Portas J, Gavrilova O, McPherron AC (2009) Myostatin inhibition in muscle, but not adipose tissue, decreases fat mass and improves insulin sensitivity. PLoS One 4(3):e4937. https://doi.org/10.1371/journal.pone.0004937

Gupte AA, Bomhoff GL, Geiger PC (2008) Age-related differences in skeletal muscle insulin signaling: the role of stress kinases and heat shock proteins. J Appl Physiol (1985) 105(3): 839–848. https://doi.org/10.1152/japplphysiol.00148.2008

Gureev AP, Shaforostova EA, Popov VN (2019) Regulation of mitochondrial biogenesis as a way for active longevity: interaction between the Nrf2 and PGC-1alpha signaling pathways. Front Genet 10:435. https://doi.org/10.3389/fgene.2019.00435

Han DS, Chen YM, Lin SY, Chang HH, Huang TM, Chi YC, Yang WS (2011) Serum myostatin levels and grip strength in normal subjects and patients on maintenance haemodialysis. Clin Endocrinol (Oxf) 75(6):857–863. https://doi.org/10.1111/j.1365-2265.2011.04120.x

Hansen SL, Svendsen PF, Jeppesen JF, Hoeg LD, Andersen NR, Kristensen JM, Nilas L, Lundsgaard AM, Wojtaszewski JFP, Madsbad S, Kiens B (2019) Molecular mechanisms in skeletal muscle underlying insulin resistance in women who are lean with polycystic ovary syndrome. J Clin Endocrinol Metab 104(5):1841–1854. https://doi.org/10.1210/jc.2018-01771

Haun CT, Vann CG, Roberts BM, Vigotsky AD, Schoenfeld BJ, Roberts MD (2019) A critical evaluation of the biological construct skeletal muscle hypertrophy: size matters but so does the measurement. Front Physiol 10:247. https://doi.org/10.3389/fphys.2019.00247

Hendrickson SL, Lautenberger JA, Chinn LW, Malasky M, Sezgin E, Kingsley LA, Goedert JJ, Kirk GD, Gomperts ED, Buchbinder SP, Troyer JL, O'Brien SJ (2010) Genetic variants in nuclear-encoded mitochondrial genes influence AIDS progression. PLoS One 5(9):e12862. https://doi.org/10.1371/journal.pone.0012862

Henry RR, Rosenstock J, Denham DS, Prabhakar P, Kjems L, Baron MA (2018) Clinical impact of ITCA 650, a novel drug-device GLP-1 Receptor agonist, in uncontrolled type 2 diabetes and very high baseline HbA1c: the FREEDOM-1 HBL (high baseline) study. Diabetes Care 41(3): 613–619. https://doi.org/10.2337/dc17-1519

Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, Snyder K, Hilliard D, Tanen M, Tanaka W, Wang AQ, Zeng W, Musson D, Winchell G, Davies MJ, Ramael S, Gottesdiener KM, Wagner JA (2005) Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clin Pharmacol Ther 78(6): 675–688. https://doi.org/10.1016/j.clpt.2005.09.002

Hernández-Ochoa EO, Schneider MF (2018) Voltage sensing mechanism in skeletal muscle excitation-contraction coupling: coming of age or midlife crisis? Skelet Muscle 8(1):22. https://doi.org/10.1186/s13395-018-0167-9

Heymsfield SB, Coleman LA, Miller R, Rooks DS, Laurent D, Petricoul O, Praestgaard J, Swan T, Wade T, Perry RG, Goodpaster BH, Roubenoff R (2021) Effect of bimagrumab vs placebo on body fat mass among adults with type 2 diabetes and obesity: a phase 2 randomized clinical trial. JAMA Netw Open 4(1):e2033457. https://doi.org/10.1001/jamanetworkopen.2020.33457

Hicks GE, Shardell M, Alley DE, Miller RR, Bandinelli S, Guralnik J, Lauretani F, Simonsick EM, Ferrucci L (2012) Absolute strength and loss of strength as predictors of mobility decline in older adults: the InCHIANTI study. J Gerontol A Biol Sci Med Sci 67(1):66–73. https://doi.org/10.1093/gerona/glr055

Higgins GA, Silenieks LB, Rossmann A, Rizos Z, Noble K, Soko AD, Fletcher PJ (2012) The 5-HT2C receptor agonist lorcaserin reduces nicotine self-administration, discrimination, and reinstatement: relationship to feeding behavior and impulse control. Neuropsychopharmacology 37(5):1177–1191. https://doi.org/10.1038/npp.2011.303

Higgins GA, Fletcher PJ, Shanahan WR (2020) Lorcaserin: a review of its preclinical and clinical pharmacology and therapeutic potential. Pharmacol Ther 205:107417. https://doi.org/10.1016/j.pharmthera.2019.107417

Hill GE (2014) Sex linkage of nuclear-encoded mitochondrial genes. Heredity (Edinb) 112(5): 469–470. https://doi.org/10.1038/hdy.2013.125

Hill C, James RS, Cox VM, Seebacher F, Tallis J (2020) Age-related changes in isolated mouse skeletal muscle function are dependent on sex, muscle, and contractility mode. Am J Physiol Regul Integr Comp Physiol 319(3):R296–r314. https://doi.org/10.1152/ajpregu.00073.2020

Hirst JA, Farmer AJ, Ali R, Roberts NW, Stevens RJ (2012) Quantifying the effect of metformin treatment and dose on glycemic control. Diabetes Care 35(2):446–454. https://doi.org/10.2337/dc11-1465

Hittel DS, Berggren JR, Shearer J, Boyle K, Houmard JA (2009) Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. Diabetes 58(1):30–38. https://doi.org/10.2337/db08-0943

Hittel DS, Axelson M, Sarna N, Shearer J, Huffman KM, Kraus WE (2010) Myostatin decreases with aerobic exercise and associates with insulin resistance. Med Sci Sports Exerc 42(11):2023–2029. https://doi.org/10.1249/MSS.0b013e3181e0b9a8

Hjorth M, Pourteymour S, Gorgens SW, Langleite TM, Lee S, Holen T, Gulseth HL, Birkeland KI, Jensen J, Drevon CA, Norheim F (2016) Myostatin in relation to physical activity and dysglycaemia and its effect on energy metabolism in human skeletal muscle cells. Acta Physiol (Oxf) 217(1):45–60. https://doi.org/10.1111/apha.12631

Hofer A, Noe N, Tischner C, Kladt N, Lellek V, Schauss A, Wenz T (2014) Defining the action spectrum of potential PGC-1alpha activators on a mitochondrial and cellular level in vivo. Hum Mol Genet 23(9):2400–2415. https://doi.org/10.1093/hmg/ddt631

Holtzer R, Ozelius L, Xue X, Wang T, Lipton RB, Verghese J (2010) Differential effects of COMT on gait and executive control in aging. Neurobiol Aging 31(3):523–531. https://doi.org/10.1016/j.neurobiolaging.2008.05.011

Hong Y, Lee JH, Jeong KW, Choi CS, Jun HS (2019) Amelioration of muscle wasting by glucagon-like peptide-1 receptor agonist in muscle atrophy. J Cachexia Sarcopenia Muscle 10(4):903–918. https://doi.org/10.1002/jcsm.12434

Hood DA, Memme JM, Oliveira AN, Triolo M (2019) Maintenance of skeletal muscle mitochondria in health, exercise, and aging. Annu Rev Physiol 81:19–41. https://doi.org/10.1146/annurev-physiol-020518-114310

Houmard JA, Weidner MD, Dolan PL, Leggett-Frazier N, Gavigan KE, Hickey MS, Tyndall GL, Zheng D, Alshami A, Dohm GL (1995) Skeletal muscle GLUT4 protein concentration and aging in humans. Diabetes 44(5):555–560. https://doi.org/10.2337/diab.44.5.555

Huthmacher JA, Meier JJ, Nauck MA (2020) Efficacy and safety of short- and long-acting glucagon-like peptide 1 receptor agonists on a background of basal insulin in type 2 diabetes: a meta-analysis. Diabetes Care 43(9):2303–2312. https://doi.org/10.2337/dc20-0498

Irving EL, Steinbach MJ, Lillakas L, Babu RJ, Hutchings N (2006) Horizontal saccade dynamics across the human life span. Invest Ophthalmol Vis Sci 47(6):2478–2484. https://doi.org/10.1167/iovs.05-1311

Kaasinen V, Rinne JO (2002) Functional imaging studies of dopamine system and cognition in normal aging and Parkinson's disease. Neurosci Biobehav Rev 26(7):785–793. https://doi.org/10.1016/s0149-7634(02)00065-9

Kalmar JM, Button DC, Gardiner K, Cahill F, Gardiner PF (2009) Caloric restriction does not offset age-associated changes in the biophysical properties of motoneurons. J Neurophysiol 101(2):548–557. https://doi.org/10.1152/jn.90617.2008

Kalyani RR, Metter EJ, Egan J, Golden SH, Ferrucci L (2015) Hyperglycemia predicts persistently lower muscle strength with aging. Diabetes Care 38(1):82–90. https://doi.org/10.2337/dc14-1166

Kamen G, Sison SV, Du CC, Patten C (1995) Motor unit discharge behavior in older adults during maximal-effort contractions. J Appl Physiol 79(6):1908–1913. https://doi.org/10.1152/jappl.1995.79.6.1908

Kamo T, Asahi R, Azami M, Ogihara H, Ikeda T, Suzuki K, Nishida Y (2019) Rate of torque development and the risk of falls among community dwelling older adults in Japan. Gait Posture 72:28–33. https://doi.org/10.1016/j.gaitpost.2019.05.019

Kandel ER, Schwartz JH, Jessel TM, Siegelbaum SA, Hudspeth AJ (2013) Principles of neural science, 5th edn. Mc-Graw Hill, New York

Kim YB, Ciaraldi TP, Kong A, Kim D, Chu N, Mohideen P, Mudaliar S, Henry RR, Kahn BB (2002) Troglitazone but not metformin restores insulin-stimulated phosphoinositide 3-kinase activity and increases p110beta protein levels in skeletal muscle of type 2 diabetic subjects. Diabetes 51(2):443–448. https://doi.org/10.2337/diabetes.51.2.443

Klionsky DJ et al (2021) Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). Autophagy 17(1):1–382. https://doi.org/10.1080/15548627.2020.1797280

Kobayashi M, Pascual-Leone A (2003) Transcranial magnetic stimulation in neurology. Lancet Neurol 2(3):145–156. S1474442203003211 [pii]

Kohrt WM, Kirwan JP, Staten MA, Bourey RE, King DS, Holloszy JO (1993) Insulin resistance in aging is related to abdominal obesity. Diabetes 42(2):273–281

Konopka AR, Miller BF (2019) Taming expectations of metformin as a treatment to extend healthspan. Geroscience 41(2):101–108. https://doi.org/10.1007/s11357-019-00057-3

Konopka AR, Suer MK, Wolff CA, Harber MP (2014) Markers of human skeletal muscle mitochondrial biogenesis and quality control: effects of age and aerobic exercise training. J Gerontol A Biol Sci Med Sci 69(4):371–378. https://doi.org/10.1093/gerona/glt107

Konstantinovic LM, Fliipovic SR (2019) Effects of near-infrared low-level laser stimulation on neuronal excitability. In: Photobiomodulation in the brain. Elsevier, San Diego

Koves TR, Noland RC, Bates AL, Henes ST, Muoio DM, Cortright RN (2005) Subsarcolemmal and intermyofibrillar mitochondria play distinct roles in regulating skeletal muscle fatty acid metabolism. Am J Physiol Cell Physiol 288(5):C1074–C1082. https://doi.org/10.1152/ajpcell.00391.2004

Kramer HF, Witczak CA, Fujii N, Jessen N, Taylor EB, Arnolds DE, Sakamoto K, Hirshman MF, Goodyear LJ (2006) Distinct signals regulate AS160 phosphorylation in response to insulin, AICAR, and contraction in mouse skeletal muscle. Diabetes 55(7):2067–2076. https://doi.org/10.2337/db06-0150

Kulkarni AS, Brutsaert EF, Anghel V, Zhang K, Bloomgarden N, Pollak M, Mar JC, Hawkins M, Crandall JP, Barzilai N (2018) Metformin regulates metabolic and nonmetabolic pathways in skeletal muscle and subcutaneous adipose tissues of older adults. Aging Cell 17(2). https://doi.org/10.1111/acel.12723

Kwak D, Baumann CW, Thompson LV (2020) Identifying characteristics of frailty in female mice using a phenotype assessment tool. J Gerontol A Biol Sci Med Sci 75(4):640–646. https://doi.org/10.1093/gerona/glz092

Lamboley CR, Wyckelsma VL, Dutka TL, McKenna MJ, Murphy RM, Lamb GD (2015) Contractile properties and sarcoplasmic reticulum calcium content in type I and type II skeletal muscle fibres in active aged humans. J Physiol 593(11):2499–2514. https://doi.org/10.1113/jp270179

Lamboley CR, Wyckelsma VL, McKenna MJ, Murphy RM, Lamb GD (2016) Ca(2+) leakage out of the sarcoplasmic reticulum is increased in type I skeletal muscle fibres in aged humans. J Physiol 594(2):469–481. https://doi.org/10.1113/jp271382

Langley B, Thomas M, Bishop A, Sharma M, Gilmour S, Kambadur R (2002) Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. J Biol Chem 277(51):49831–49840. https://doi.org/10.1074/jbc.M204291200

Lanza IR, Short DK, Short KR, Raghavakaimal S, Basu R, Joyner MJ, McConnell JP, Nair KS (2008) Endurance exercise as a countermeasure for aging. Diabetes 57(11):2933–2942. https://doi.org/10.2337/db08-0349

Larsson L, Li X, Frontera WR (1997) Effects of aging on shortening velocity and myosin isoform composition in single human skeletal muscle cells. Am J Physiol 272(2 Pt 1):C638–C649. https://doi.org/10.1152/ajpcell.1997.272.2.C638

Law TD, Clark LA, Clark BC (2016) Resistance exercise to prevent and manage sarcopenia and dynapenia. Annu Rev Gerontol Geriatr 36(1):205–228. https://doi.org/10.1891/0198-8794.36.205

Lecker SH, Goldberg AL, Mitch WE (2006) Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. J Am Soc Nephrol 17(7):1807–1819. https://doi.org/10.1681/ASN.2006010083

Lee HY, Choi CS, Birkenfeld AL, Alves TC, Jornayvaz FR, Jurczak MJ, Zhang D, Woo DK, Shadel GS, Ladiges W, Rabinovitch PS, Santos JH, Petersen KF, Samuel VT, Shulman GI (2010) Targeted expression of catalase to mitochondria prevents age-associated reductions in mitochondrial function and insulin resistance. Cell Metab 12(6):668–674. https://doi.org/10.1016/j.cmet.2010.11.004

Lee CG, Boyko EJ, Barrett-Connor E, Miljkovic I, Hoffman AR, Everson-Rose SA, Lewis CE, Cawthon PM, Strotmeyer ES, Orwoll ES, Osteoporotic Fractures in Men Study Research Group (2011a) Insulin sensitizers may attenuate lean mass loss in older men with diabetes. Diabetes Care 34(11):2381–2386. https://doi.org/10.2337/dc11-1032

Lee JO, Lee SK, Jung JH, Kim JH, You GY, Kim SJ, Park SH, Uhm KO, Kim HS (2011b) Metformin induces Rab4 through AMPK and modulates GLUT4 translocation in skeletal muscle cells. J Cell Physiol 226(4):974–981. https://doi.org/10.1002/jcp.22410

Lefebvre S, Liew SL (2017) Anatomical parameters of tDCS to modulate the motor system after stroke: a review. Front Neurol 8:29. https://doi.org/10.3389/fneur.2017.00029

Leger B, Derave W, De Bock K, Hespel P, Russell AP (2008) Human sarcopenia reveals an increase in SOCS-3 and myostatin and a reduced efficiency of Akt phosphorylation. Rejuvenation Res 11(1):163–175B. https://doi.org/10.1089/rej.2007.0588

Li Y, Perry T, Kindy MS, Harvey BK, Tweedie D, Holloway HW, Powers K, Shen H, Egan JM, Sambamurti K, Brossi A, Lahiri DK, Mattson MP, Hoffer BJ, Wang Y, Greig NH (2009) GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. Proc Natl Acad Sci U S A 106(4):1285–1290. https://doi.org/10.1073/pnas.0806720106

Liu H, Graber TG, Ferguson-Stegall L, Thompson LV (2014) Clinically relevant frailty index for mice. J Gerontol A Biol Sci Med Sci 69(12):1485–1491. https://doi.org/10.1093/gerona/glt188

Long DE, Peck BD, Tuggle SC, Villasante Tezanos AG, Windham ST, Bamman MM, Kern PA, Peterson CA, Walton RG (2021) Associations of muscle lipid content with physical function and resistance training outcomes in older adults: altered responses with metformin. Geroscience. https://doi.org/10.1007/s11357-020-00315-9

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153(6):1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Louie GH, Ward MM (2010) Sex disparities in self-reported physical functioning: true differences, reporting bias, or incomplete adjustment for confounding? J Am Geriatr Soc 58(6):1117–1122. https://doi.org/10.1111/j.1532-5415.2010.02858.x

Lowsky DJ, Olshansky SJ, Bhattacharya J, Goldman DP (2014) Heterogeneity in healthy aging. J Gerontol A Biol Sci Med Sci 69(6):640–649. https://doi.org/10.1093/gerona/glt162

Lynch NA, Metter EJ, Lindle RS, Fozard JL, Tobin JD, Roy TA, Fleg JL, Hurley BF (1999) Muscle quality. I. Age-associated differences between arm and leg muscle groups. J Appl Physiol (1985) 86(1):188–194. https://doi.org/10.1152/jappl.1999.86.1.188

Manini TM, Visser M, Won-Park S, Patel KV, Strotmeyer ES, Chen H, Goodpaster B, De Rekeneire N, Newman AB, Simonsick EM, Kritchevsky SB, Ryder K, Schwartz AV, Harris TB (2007) Knee extension strength cutpoints for maintaining mobility. J Am Geriatr Soc 55(3):451–457. https://doi.org/10.1111/j.1532-5415.2007.01087.x. JGS1087 [pii]

Marigold DS, Misiaszek JE (2009) Whole-body responses: neural control and implications for rehabilitation and fall prevention. Neuroscientist 15(1):36–46. https://doi.org/10.1177/1073858408322674

Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, Gomes AP, Ward TM, Minor RK, Blouin MJ, Schwab M, Pollak M, Zhang Y, Yu Y, Becker

KG, Bohr VA, Ingram DK, Sinclair DA, Wolf NS, Spindler SR, Bernier M, de Cabo R (2013) Metformin improves healthspan and lifespan in mice. Nat Commun 4:2192. https://doi.org/10.1038/ncomms3192

McCarthy JJ, Esser KA (2010) Anabolic and catabolic pathways regulating skeletal muscle mass. Curr Opin Clin Nutr Metab Care 13(3):230–235. https://doi.org/10.1097/MCO.0b013e32833781b5

McGinley M, Hoffman RL, Russ DW, Thomas JS, Clark BC (2010) Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. Exp Gerontol 45(9):671–678. https://doi.org/10.1016/j.exger.2010.04.005

McGrath R, Vincent BM, Al Snih S, Markides KS, Peterson MD (2017) The association between muscle weakness and incident diabetes in older Mexican Americans. J Am Med Dir Assoc 18(5):452 e457–452 e412. https://doi.org/10.1016/j.jamda.2017.01.017

McGrath RP, Vincent BM, Lee IM, Kraemer WJ, Peterson MD (2018) Handgrip strength, function, and mortality in older adults: a time-varying approach. Med Sci Sports Exerc 50(11):2259–2266. https://doi.org/10.1249/MSS.0000000000001683

McGrath R, Erlandson KM, Vincent BM, Hackney KJ, Herrmann SD, Clark BC (2019a) Decreased handgrip strength is associated with impairments in each autonomous living task for aging adults in the United States. J Frailty Aging 8(3):141–145. https://doi.org/10.14283/jfa.2018.47

McGrath R, Vincent BM, Peterson MD, Jurivich DA, Dahl LJ, Hackney KJ, Clark BC (2019b) Weakness may have a causal association with early mortality in older Americans: a matched cohort analysis. J Am Med Dir Assoc. https://doi.org/10.1016/j.jamda.2019.10.016

McKendry J, Currier BS, Lim C, McLeod JC, Thomas ACQ, Phillips SM (2020) Nutritional supplements to support resistance exercise in countering the sarcopenia of aging. Nutrients 12(7). https://doi.org/10.3390/nu12072057

McPherron AC, Lawler AM, Lee SJ (1997) Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387(6628):83–90. https://doi.org/10.1038/387083a0

Mendias CL, Marcin JE, Calerdon DR, Faulkner JA (2006) Contractile properties of EDL and soleus muscles of myostatin-deficient mice. J Appl Physiol (1985) 101(3):898–905. https://doi.org/10.1152/japplphysiol.00126.2006

Mendias CL, Kayupov E, Bradley JR, Brooks SV, Claflin DR (2011) Decreased specific force and power production of muscle fibers from myostatin-deficient mice are associated with a suppression of protein degradation. J Appl Physiol (1985) 111(1):185–191. https://doi.org/10.1152/japplphysiol.00126.2011

Mendias CL, Bakhurin KI, Gumucio JP, Shallal-Ayzin MV, Davis CS, Faulkner JA (2015) Haploinsufficiency of myostatin protects against aging-related declines in muscle function and enhances the longevity of mice. Aging Cell 14(4):704–706. https://doi.org/10.1111/acel.12339

Meredith CN, Frontera WR, Fisher EC, Hughes VA, Herland JC, Edwards J, Evans WJ (1989) Peripheral effects of endurance training in young and old subjects. J Appl Physiol (1985) 66(6):2844–2849. https://doi.org/10.1152/jappl.1989.66.6.2844

Metti AL, Rosano C, Boudreau R, Massa R, Yaffe K, Satterfield S, Harris T, Rosso AL (2017) Catechol-O-methyltransferase genotype and gait speed changes over 10 years in older adults. J Am Geriatr Soc 65(9):2016–2022. https://doi.org/10.1111/jgs.14980

Mijares A, Allen PD, Lopez JR (2020) Senescence is associated with elevated intracellular resting [Ca(2 +)] in mice skeletal muscle fibers. An in vivo study. Front Physiol 11:601189. https://doi.org/10.3389/fphys.2020.601189

Mitchell SJ, Scheibye-Knudsen M, Longo DL, de Cabo R (2015) Animal models of aging research: implications for human aging and age-related diseases. Annu Rev Anim Biosci 3:283–303. https://doi.org/10.1146/annurev-animal-022114-110829

Mitchell WK, Wilkinson DJ, Phillips BE, Lund JN, Smith K, Atherton PJ (2016) Human skeletal muscle protein metabolism responses to amino acid nutrition. Adv Nutr 7(4):828S–838S. https://doi.org/10.3945/an.115.011650

Moran AL, Warren GL, Lowe DA (2005) Soleus and EDL muscle contractility across the lifespan of female C57BL/6 mice. Exp Gerontol 40(12):966–975. https://doi.org/10.1016/j.exger.2005.09.005

Morissette MR, Cook SA, Buranasombati C, Rosenberg MA, Rosenzweig A (2009) Myostatin inhibits IGF-I-induced myotube hypertrophy through Akt. Am J Physiol Cell Physiol 297(5):C1124–C1132. https://doi.org/10.1152/ajpcell.00043.2009

Morley JE, Vellas B, van Kan GA, Anker SD, Bauer JM, Bernabei R, Cesari M, Chumlea WC, Doehner W, Evans J, Fried LP, Guralnik JM, Katz PR, Malmstrom TK, McCarter RJ, Gutierrez Robledo LM, Rockwood K, von Haehling S, Vandewoude MF, Walston J (2013) Frailty consensus: a call to action. J Am Med Dir Assoc 14(6):392–397. https://doi.org/10.1016/j.jamda.2013.03.022

Moskowitz S, Russ DW, Clark LA, Wages NP, Grooms DR, Woods AJ, Suhr J, Simon JE, O'Shea A, Criss CR, Fadda P, Clark BC (2020) Is impaired dopaminergic function associated with mobility capacity in older adults? GeroScience. https://doi.org/10.1007/s11357-020-00303-z

Musi N, Hirshman MF, Nygren J, Svanfeldt M, Bavenholm P, Rooyackers O, Zhou G, Williamson JM, Ljunqvist O, Efendic S, Moller DE, Thorell A, Goodyear LJ (2002) Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes 51(7):2074–2081. https://doi.org/10.2337/diabetes.51.7.2074

Nair KS (2005) Aging muscle. Am J Clin Nutr 81(5):953–963. https://doi.org/10.1093/ajcn/81.5.953

Nardelli P, Khan J, Powers R, Cope TC, Rich MM (2013) Reduced motoneuron excitability in a rat model of sepsis. J Neurophysiol 109(7):1775–1781. https://doi.org/10.1152/jn.00936.2012

Nardelli P, Vincent JA, Powers R, Cope TC, Rich MM (2016) Reduced motor neuron excitability is an important contributor to weakness in a rat model of sepsis. Exp Neurol 282:1–8. https://doi.org/10.1016/j.expneurol.2016.04.020

Nardelli P, Powers R, Cope TC, Rich MM (2017) Increasing motor neuron excitability to treat weakness in sepsis. Ann Neurol 82(6):961–971. https://doi.org/10.1002/ana.25105

Narici MV, Maffulli N (2010) Sarcopenia: characteristics, mechanisms and functional significance. Br Med Bull 95:139–159. https://doi.org/10.1093/bmb/ldq008

Nebuloni CC, Maximo RO, de Oliveira C, Alexandre TDS (2020) Uncontrolled diabetes as an associated factor with dynapenia in adults aged 50 years or older: sex differences. J Gerontol A Biol Sci Med Sci 75(6):1191–1197. https://doi.org/10.1093/gerona/glz257

Newman AB, Simonsick EM, Naydeck BL, Boudreau RM, Kritchevsky SB, Nevitt MC, Pahor M, Satterfield S, Brach JS, Studenski SA, Harris TB (2006) Association of long-distance corridor walk performance with mortality, cardiovascular disease, mobility limitation, and disability. JAMA 295(17):2018–2026. https://doi.org/10.1001/jama.295.17.2018

Ngo HB, Lovely GA, Phillips R, Chan DC (2014) Distinct structural features of TFAM drive mitochondrial DNA packaging versus transcriptional activation. Nat Commun 5:3077. https://doi.org/10.1038/ncomms4077

Novelle MG, Ali A, Dieguez C, Bernier M, de Cabo R (2016) Metformin: a hopeful promise in aging research. Cold Spring Harb Perspect Med 6(3):a025932. https://doi.org/10.1101/cshperspect.a025932

Ochala J, Frontera WR, Dorer DJ, Van Hoecke J, Krivickas LS (2007) Single skeletal muscle fiber elastic and contractile characteristics in young and older men. J Gerontol A Biol Sci Med Sci 62(4):375–381. https://doi.org/10.1093/gerona/62.4.375

O'Connell K, Gannon J, Doran P, Ohlendieck K (2008) Reduced expression of sarcalumenin and related Ca2+ -regulatory proteins in aged rat skeletal muscle. Exp Gerontol 43(10):958–961. https://doi.org/10.1016/j.exger.2008.07.006

Ohtsuki T (1981) Inhibition of individual fingers during grip strength exertion. Ergonomics 24(1):21–36. https://doi.org/10.1080/00140138108924827

Ojima K (2019) Myosin: formation and maintenance of thick filaments. Anim Sci J 90(7):801–807. https://doi.org/10.1111/asj.13226

Oki K, Mahato NK, Nakazawa M, Amano S, France CR, Russ DW, Clark BC (2016) Preliminary evidence that excitatory transcranial direct current stimulation extends time to task failure of a sustained, submaximal muscular contraction in older adults. J Gerontol A Biol Sci Med Sci 71(8):1109–1112. https://doi.org/10.1093/gerona/glw011

O'Neill BT, Lee KY, Klaus K, Softic S, Krumpoch MT, Fentz J, Stanford KI, Robinson MM, Cai W, Kleinridders A, Pereira RO, Hirshman MF, Abel ED, Accili D, Goodyear LJ, Nair KS, Kahn CR (2016) Insulin and IGF-1 receptors regulate FoxO-mediated signaling in muscle proteostasis. J Clin Invest 126(9):3433–3446. https://doi.org/10.1172/JCI86522

Papadopoulou SK (2020) Sarcopenia: a contemporary health problem among older adult populations. Nutrients 12(5). https://doi.org/10.3390/nu12051293

Parekh AB (2003a) Mitochondrial regulation of intracellular Ca2+ signaling: more than just simple Ca2+ buffers. News Physiol Sci 18:252–256. https://doi.org/10.1152/nips.01458.2003

Parekh AB (2003b) Store-operated Ca2+ entry: dynamic interplay between endoplasmic reticulum, mitochondria and plasma membrane. J Physiol 547(Pt 2):333–348. https://doi.org/10.1113/jphysiol.2002.034140

Park SW, Goodpaster BH, Strotmeyer ES, Kuller LH, Broudeau R, Kammerer C, de Rekeneire N, Harris TB, Schwartz AV, Tylavsky FA, Cho YW, Newman AB, Health A, Body Composition S (2007) Accelerated loss of skeletal muscle strength in older adults with type 2 diabetes: the health, aging, and body composition study. Diabetes Care 30(6):1507–1512. https://doi.org/10.2337/dc06-2537

Park SW, Goodpaster BH, Lee JS, Kuller LH, Boudreau R, de Rekeneire N, Harris TB, Kritchevsky S, Tylavsky FA, Nevitt M, Cho YW, Newman AB, Health A, Body Composition S (2009) Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. Diabetes Care 32(11):1993–1997. https://doi.org/10.2337/dc09-0264

Patel HP, Al-Shanti N, Davies LC, Barton SJ, Grounds MD, Tellam RL, Stewart CE, Cooper C, Sayer AA (2014) Lean mass, muscle strength and gene expression in community dwelling older men: findings from the Hertfordshire Sarcopenia Study (HSS). Calcif Tissue Int 95(4):308–316. https://doi.org/10.1007/s00223-014-9894-z

Pehleman TL, Peters SJ, Heigenhauser GJ, Spriet LL (2005) Enzymatic regulation of glucose disposal in human skeletal muscle after a high-fat, low-carbohydrate diet. J Appl Physiol (1985) 98(1):100–107. https://doi.org/10.1152/japplphysiol.00686.2004

Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI (2003) Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 300(5622):1140–1142. https://doi.org/10.1126/science.1082889

Petersen KF, Morino K, Alves TC, Kibbey RG, Dufour S, Sono S, Yoo PS, Cline GW, Shulman GI (2015) Effect of aging on muscle mitochondrial substrate utilization in humans. Proc Natl Acad Sci U S A 112(36):11330–11334. https://doi.org/10.1073/pnas.1514844112

Petzinger GM, Fisher BE, McEwen S, Beeler JA, Walsh JP, Jakowec MW (2013) Exercise-enhanced neuroplasticity targeting motor and cognitive circuitry in Parkinson's disease. Lancet Neurol 12(7):716–726. https://doi.org/10.1016/S1474-4422(13)70123-6

Pierno S, De Luca A, Beck CL, George AL Jr, Conte Camerino D (1999) Aging-associated down-regulation of ClC-1 expression in skeletal muscle: phenotypic-independent relation to the decrease of chloride conductance. FEBS Lett 449(1):12–16. https://doi.org/10.1016/s0014-5793(99)00202-1

Poulsen P, Wojtaszewski JF, Petersen I, Christensen K, Richter EA, Beck-Nielsen H, Vaag A (2005) Impact of genetic versus environmental factors on the control of muscle glycogen synthase activation in twins. Diabetes 54(5):1289–1296. https://doi.org/10.2337/diabetes.54.5.1289

PubChem (2021a) PubChem Compound Summary for CID 4158, Methylphenidate. National Library of Medicine (US), National Center for Biotechnology Information. https://pubchem.ncbi.nlm.nih.gov/compound/Methylphenidate. Accessed 29 Jan 2021

PubChem (2021b) PubChem Compound Summary for CID 6047, Levodopa. National Library of Medicine (US), National Center for Biotechnology Information. https://pubchem.ncbi.nlm.nih.gov/compound/Levodopa. Accessed 29 Jan 2021

Qaisar R, Bhaskaran S, Premkumar P, Ranjit R, Natarajan KS, Ahn B, Riddle K, Claflin DR, Richardson A, Brooks SV, Van Remmen H (2018) Oxidative stress-induced dysregulation of excitation-contraction coupling contributes to muscle weakness. J Cachexia Sarcopenia Muscle 9(5):1003–1017. https://doi.org/10.1002/jcsm.12339

Rantanen T (2003) Muscle strength, disability and mortality. Scand J Med Sci Sports 13(1):3–8

Rantanen T, Guralnik JM, Izmirlian G, Williamson JD, Simonsick EM, Ferrucci L, Fried LP (1998) Association of muscle strength with maximum walking speed in disabled older women. Am J Phys Med Rehabil 77(4):299–305. https://doi.org/10.1097/00002060-199807000-00008

Rantanen T, Guralnik JM, Foley D, Masaki K, Leveille S, Curb JD, White L (1999) Midlife hand grip strength as a predictor of old age disability. JAMA 281(6):558–560. jbr80447 [pii]

Rantanen T, Avlund K, Suominen H, Schroll M, Frandin K, Pertti E (2002) Muscle strength as a predictor of onset of ADL dependence in people aged 75 years. Aging Clin Exp Res 14(3 Suppl):10–15

Rasmussen BB, Fujita S, Wolfe RR, Mittendorfer B, Roy M, Rowe VL, Volpi E (2006) Insulin resistance of muscle protein metabolism in aging. FASEB J 20(6):768–769. https://doi.org/10.1096/fj.05-4607fje

Reaven GM (1988) Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37(12):1595–1607

Reis J, Swayne OB, Vandermeeren Y, Camus M, Dimyan MA, Harris-Love M, Perez MA, Ragert P, Rothwell JC, Cohen LG (2008) Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. J Physiol 586(2):325–351. https://doi.org/10.1113/jphysiol.2007.144824. jphysiol.2007.144824 [pii]

Renganathan M, Messi ML, Delbono O (1997a) Dihydropyridine receptor-ryanodine receptor uncoupling in aged skeletal muscle. J Membr Biol 157(3):247–253. https://doi.org/10.1007/s002329900233

Renganathan M, Messi ML, Schwartz R, Delbono O (1997b) Overexpression of hIGF-1 exclusively in skeletal muscle increases the number of dihydropyridine receptors in adult transgenic mice. FEBS Lett 417(1):13–16. https://doi.org/10.1016/s0014-5793(97)01225-8

Renganathan M, Messi ML, Delbono O (1998) Overexpression of IGF-1 exclusively in skeletal muscle prevents age-related decline in the number of dihydropyridine receptors. J Biol Chem 273(44):28845–28851. https://doi.org/10.1074/jbc.273.44.28845

Rhodes MA, Carraway MS, Piantadosi CA, Reynolds CM, Cherry AD, Wester TE, Natoli MJ, Massey EW, Moon RE, Suliman HB (2009) Carbon monoxide, skeletal muscle oxidative stress, and mitochondrial biogenesis in humans. Am J Physiol Heart Circ Physiol 297(1):H392–H399. https://doi.org/10.1152/ajpheart.00164.2009

Rinne JO, Hietala J, Ruotsalainen U, Sako E, Laihinen A, Nagren K, Lehikoinen P, Oikonen V, Syvalahti E (1993) Decrease in human striatal dopamine D2 receptor density with age: a PET study with [11C]raclopride. J Cereb Blood Flow Metab 13(2):310–314. https://doi.org/10.1038/jcbfm.1993.39

Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE (2005) Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes 54(1):8–14. https://doi.org/10.2337/diabetes.54.1.8

Ritz P, Dumas JF, Ducluzeau PH, Simard G (2005) Hormonal regulation of mitochondrial energy production. Curr Opin Clin Nutr Metab Care 8(4):415–418. https://doi.org/10.1097/01.mco.0000172582.86890.19

Rizzo MR, Barbieri M, Fava I, Desiderio M, Coppola C, Marfella R, Paolisso G (2016) Sarcopenia in elderly diabetic patients: role of dipeptidyl peptidase 4 inhibitors. J Am Med Dir Assoc 17(10):896–901. https://doi.org/10.1016/j.jamda.2016.04.016

Romani M, Sorrentino V, Oh CM, Li H, de Lima TI, Zhang H, Shong M, Auwerx J (2021) NAD(+) boosting reduces age-associated amyloidosis and restores mitochondrial homeostasis in muscle. Cell Rep 34(3):108660. https://doi.org/10.1016/j.celrep.2020.108660

Rooks D, Praestgaard J, Hariry S, Laurent D, Petricoul O, Perry RG, Lach-Trifilieff E, Roubenoff R (2017) Treatment of sarcopenia with bimagrumab: results from a phase II, randomized, controlled, proof-of-concept study. J Am Geriatr Soc 65(9):1988–1995. https://doi.org/10.1111/jgs.14927

Rooks D, Swan T, Goswami B, Filosa LA, Bunte O, Panchaud N, Coleman LA, Miller RR, Garcia Garayoa E, Praestgaard J, Perry RG, Recknor C, Fogarty CM, Arai H, Chen LK, Hashimoto J, Chung YS, Vissing J, Laurent D, Petricoul O, Hemsley S, Lach-Trifilieff E, Papanicolaou DA, Roubenoff R (2020) Bimagrumab vs optimized standard of care for treatment of sarcopenia in community-dwelling older adults: a randomized clinical trial. JAMA Netw Open 3(10): e2020836. https://doi.org/10.1001/jamanetworkopen.2020.20836

Rostami M, Mosallanezhad Z, Ansari S, Kidgell D, Rezaeian T, Bakhshi E, Ghodrati M, Jaberzadeh S (2020) The effects of consecutive sessions of anodal transcranial direct current stimulation over the primary motor cortex on hand function in healthy older adults. Arch Gerontol Geriatr 89:104063. https://doi.org/10.1016/j.archger.2020.104063

Rowe JW, Minaker KL, Pallotta JA, Flier JS (1983) Characterization of the insulin resistance of aging. J Clin Invest 71(6):1581–1587. https://doi.org/10.1172/jci110914

Russ DW, Grandy JS, Toma K, Ward CW (2011) Ageing, but not yet senescent, rats exhibit reduced muscle quality and sarcoplasmic reticulum function. Acta Physiol (Oxf) 201(3): 391–403. https://doi.org/10.1111/j.1748-1716.2010.02191.x

Russ DW, Gregg-Cornell K, Conaway MJ, Clark BC (2012) Evolving concepts on the age-related changes in "muscle quality". J Cachexia Sarcopenia Muscle. https://doi.org/10.1007/s13539-011-0054-2

Rutherford BR, Choi J, Slifstein M, O'Boyle K, Abi-Dargham A, Brown PJ, Wall MW, Vanegas-Arroyave N, Sakhardande J, Stern Y, Roose SP (2020) Neuroanatomical predictors of L-DOPA response in older adults with psychomotor slowing and depression: a pilot study. J Affect Disord 265:439–444. https://doi.org/10.1016/j.jad.2020.01.066

Ryan M, Ohlendieck K (2004) Excitation-contraction uncoupling and sarcopenia. Basic Appl Myol 14(3):141–154

Ryan M, Carlson BM, Ohlendieck K (2000) Oligomeric status of the dihydropyridine receptor in aged skeletal muscle. Mol Cell Biol Res Commun 4(4):224–229. https://doi.org/10.1006/mcbr.2001.0282

Ryan M, Butler-Browne G, Erzen I, Mouly V, Thornell LE, Wernig A, Ohlendieck K (2003) Persistent expression of the alpha1S-dihydropyridine receptor in aged human skeletal muscle: implications for the excitation-contraction uncoupling hypothesis of sarcopenia. Int J Mol Med 11(4):425–434

Ryan AS, Li G, Blumenthal JB, Ortmeyer HK (2013) Aerobic exercise + weight loss decreases skeletal muscle myostatin expression and improves insulin sensitivity in older adults. Obesity (Silver Spring) 21(7):1350–1356. https://doi.org/10.1002/oby.20216

Sandow A (1952) Excitation-contraction coupling in muscular response. Yale J Biol Med 25(3): 176–201

Sano M, Meguro S, Kawai T, Suzuki Y (2016) Increased grip strength with sodium-glucose cotransporter 2. J Diabetes 8(5):736–737. https://doi.org/10.1111/1753-0407.12402

Santra M, Dill KA, de Graff AMR (2019) Proteostasis collapse is a driver of cell aging and death. Proc Natl Acad Sci U S A 116(44):22173–22178. https://doi.org/10.1073/pnas.1906592116

Santulli G, Lewis D, des Georges A, Marks AR, Frank J (2018) Ryanodine receptor structure and function in health and disease. Subcell Biochem 87:329–352. https://doi.org/10.1007/978-981-10-7757-9_11

Sasaki T, Sugawara M, Fukuda M (2019) Sodium-glucose cotransporter 2 inhibitor-induced changes in body composition and simultaneous changes in metabolic profile: 52-week

prospective LIGHT (Luseogliflozin: the Components of Weight Loss in Japanese Patients with Type 2 Diabetes Mellitus) Study. J Diabetes Invest 10(1):108–117. https://doi.org/10.1111/jdi.12851

Sato H, Kubota N, Kubota T, Takamoto I, Iwayama K, Tokuyama K, Moroi M, Sugi K, Nakaya K, Goto M, Jomori T, Kadowaki T (2016) Anagliptin increases insulin-induced skeletal muscle glucose uptake via an NO-dependent mechanism in mice. Diabetologia 59(11):2426–2434. https://doi.org/10.1007/s00125-016-4071-8

Schacht JP (2016) COMT val158met moderation of dopaminergic drug effects on cognitive function: a critical review. Pharmacogenomics J 16(5):430–438. https://doi.org/10.1038/tpj.2016.43

Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR (2000) Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature 404(6779):770–774. https://doi.org/10.1038/35008096

Schultz MB, Sinclair DA (2016) Why NAD(+) declines during aging: it's destroyed. Cell Metab 23(6):965–966. https://doi.org/10.1016/j.cmet.2016.05.022

Schulz DJ, Baines RA, Hempel CM, Li L, Liss B, Misonou H (2006) Cellular excitability and the regulation of functional neuronal identity: from gene expression to neuromodulation. J Neurosci 26(41):10362–10367. https://doi.org/10.1523/JNEUROSCI.3194-06.2006

Seeman TE, Merkin SS, Crimmins EM, Karlamangla AS (2010) Disability trends among older Americans: National Health And Nutrition Examination Surveys, 1988-1994 and 1999-2004. Am J Public Health 100(1):100–107. https://doi.org/10.2105/AJPH.2008.157388

Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L (2010) Relationship of an advanced glycation end product, plasma carboxymethyl-lysine, with slow walking speed in older adults: the InCHIANTI study. Eur J Appl Physiol 108(1):191–195. https://doi.org/10.1007/s00421-009-1192-5

Seo DY, Lee SR, Kim N, Ko KS, Rhee BD, Han J (2016) Age-related changes in skeletal muscle mitochondria: the role of exercise. Integr Med Res 5(3):182–186. https://doi.org/10.1016/j.imr.2016.07.003

Shan J, Betzenhauser MJ, Kushnir A, Reiken S, Meli AC, Wronska A, Dura M, Chen BX, Marks AR (2010) Role of chronic ryanodine receptor phosphorylation in heart failure and β-adrenergic receptor blockade in mice. J Clin Invest 120(12):4375–4387. https://doi.org/10.1172/jci37649

Sharma N, Arias EB, Sajan MP, MacKrell JG, Bhat AD, Farese RV, Cartee GD (2010) Insulin resistance for glucose uptake and Akt2 phosphorylation in the soleus, but not epitrochlearis, muscles of old vs. adult rats. J Appl Physiol (1985) 108(6):1631–1640. https://doi.org/10.1152/japplphysiol.01412.2009

Sharoff CG, Hagobian TA, Malin SK, Chipkin SR, Yu H, Hirshman MF, Goodyear LJ, Braun B (2010) Combining short-term metformin treatment and one bout of exercise does not increase insulin action in insulin-resistant individuals. Am J Physiol Endocrinol Metab 298(4):E815–E823. https://doi.org/10.1152/ajpendo.00517.2009

Shinohara M, Latash ML, Zatsiorsky VM (2003a) Age effects on force produced by intrinsic and extrinsic hand muscles and finger interaction during MVC tasks. J Appl Physiol (1985) 95(4):1361–1369. https://doi.org/10.1152/japplphysiol.00070.2003

Shinohara M, Li S, Kang N, Zatsiorsky VM, Latash ML (2003b) Effects of age and gender on finger coordination in MVC and submaximal force-matching tasks. J Appl Physiol (1985) 94(1):259–270. https://doi.org/10.1152/japplphysiol.00643.2002

Shorer Z, Bachner Y, Guy T, Melzer I (2013) Effect of single dose methylphenidate on walking and postural stability under single- and dual-task conditions in older adults—a double-blind randomized control trial. J Gerontol A Biol Sci Med Sci 68(10):1271–1280. https://doi.org/10.1093/gerona/glt035

Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102(15):5618–5623. https://doi.org/10.1073/pnas.0501559102

Sorond FA, Cruz-Almeida Y, Clark DJ, Viswanathan A, Scherzer CR, De Jager P, Csiszar A, Laurienti PJ, Hausdorff JM, Chen WG, Ferrucci L, Rosano C, Studenski SA, Black SE, Lipsitz LA (2015) Aging, the central nervous system, and mobility in older adults: neural mechanisms of mobility impairment. J Gerontol A Biol Sci Med Sci 70(12):1526–1532. https://doi.org/10.1093/gerona/glv130

St Andre M, Johnson M, Bansal PN, Wellen J, Robertson A, Opsahl A, Burch PM, Bialek P, Morris C, Owens J (2017) A mouse anti-myostatin antibody increases muscle mass and improves muscle strength and contractility in the mdx mouse model of Duchenne muscular dystrophy and its humanized equivalent, domagrozumab (PF-06252616), increases muscle volume in cynomolgus monkeys. Skelet Muscle 7(1):25. https://doi.org/10.1186/s13395-017-0141-y

Stefanelli L, Lockyer EJ, Collins BW, Snow NJ, Crocker J, Kent C, Power KE, Button DC (2019) Delayed-onset muscle soreness and topical analgesic alter corticospinal excitability of the biceps brachii. Med Sci Sports Exerc 51(11):2344–2356. https://doi.org/10.1249/MSS.0000000000002055

Steffen KK, Dillin A (2016) A ribosomal perspective on proteostasis and aging. Cell Metab 23(6):1004–1012. https://doi.org/10.1016/j.cmet.2016.05.013

Stenholm S, Harris TB, Rantanen T, Visser M, Kritchevsky SB, Ferrucci L (2008) Sarcopenic obesity: definition, cause and consequences. Curr Opin Clin Nutr Metab Care 11(6):693–700. https://doi.org/10.1097/MCO.0b013e328312c37d

Stram AR, Payne RM (2016) Post-translational modifications in mitochondria: protein signaling in the powerhouse. Cell Mol Life Sci 73(21):4063–4073. https://doi.org/10.1007/s00018-016-2280-4

Sugimoto K, Yasujima M, Yagihashi S (2008) Role of advanced glycation end products in diabetic neuropathy. Curr Pharm Des 14(10):953–961. https://doi.org/10.2174/138161208784139774

Sugimoto K, Tabara Y, Ikegami H, Takata Y, Kamide K, Ikezoe T, Kiyoshige E, Makutani Y, Onuma H, Gondo Y, Ikebe K, Ichihashi N, Tsuboyama T, Matsuda F, Kohara K, Kabayama M, Fukuda M, Katsuya T, Osawa H, Hiromine Y, Rakugi H (2019) Hyperglycemia in non-obese patients with type 2 diabetes is associated with low muscle mass: the multicenter study for clarifying evidence for sarcopenia in patients with diabetes mellitus. J Diabetes Invest 10(6):1471–1479. https://doi.org/10.1111/jdi.13070

Sugiyama S, Jinnouchi H, Kurinami N, Hieshima K, Yoshida A, Jinnouchi K, Nishimura H, Suzuki T, Miyamoto F, Kajiwara K, Jinnouchi T (2018) Dapagliflozin reduces fat mass without affecting muscle mass in type 2 diabetes. J Atheroscler Thromb 25(6):467–476. https://doi.org/10.5551/jat.40873

Suhara T, Fukuda H, Inoue O, Itoh T, Suzuki K, Yamasaki T, Tateno Y (1991) Age-related changes in human D1 dopamine receptors measured by positron emission tomography. Psychopharmacology (Berl) 103(1):41–45. https://doi.org/10.1007/BF02244071

Sweeney HL, Hammers DW (2018) Muscle contraction. Cold Spring Harb Perspect Biol 10(2). https://doi.org/10.1101/cshperspect.a023200

Szewczyk NJ, Jacobson LA (2005) Signal-transduction networks and the regulation of muscle protein degradation. Int J Biochem Cell Biol 37(10):1997–2011. https://doi.org/10.1016/j.biocel.2005.02.020

Sztretye M, Geyer N, Vincze J, Al-Gaadi D, Olah T, Szentesi P, Kis G, Antal M, Balatoni I, Csernoch L, Dienes B (2017) SOCE is important for maintaining sarcoplasmic calcium content and release in skeletal muscle fibers. Biophys J 113(11):2496–2507. https://doi.org/10.1016/j.bpj.2017.09.023

Tahoe NM, Mokhtarzadeh A, Curtsinger JW (2004) Age-related RNA decline in adult Drosophila melanogaster. J Gerontol A Biol Sci Med Sci 59(9):B896–B901. https://doi.org/10.1093/gerona/59.9.b896

Tallis J, Bradford C, Duncan MJ, Leddington-Wright S, Higgins MF, Hill M (2020) The effect of acute caffeine ingestion on cognitive dual task performance during assessment of static and dynamic balance in older adults. Nutrients 12(12). https://doi.org/10.3390/nu12123653

Tang L, Liu CT, Wang XD, Luo K, Zhang DD, Chi AP, Zhang J, Sun LJ (2014) A prepared anti-MSTN polyclonal antibody reverses insulin resistance of diet-induced obese rats via regulation of PI3K/Akt/mTOR&FoxO1 signal pathways. Biotechnol Lett 36(12):2417–2423. https://doi.org/10.1007/s10529-014-1617-z

Thorell A, Hirshman MF, Nygren J, Jorfeldt L, Wojtaszewski JF, Dufresne SD, Horton ES, Ljungqvist O, Goodyear LJ (1999) Exercise and insulin cause GLUT-4 translocation in human skeletal muscle. Am J Physiol 277(4):E733–E741. https://doi.org/10.1152/ajpendo.1999.277.4.E733

Tieland M, Trouwborst I, Clark BC (2018) Skeletal muscle performance and ageing. J Cachexia Sarcopenia Muscle 9(1):3–19. https://doi.org/10.1002/jcsm.12238

Trendelenburg AU, Meyer A, Rohner D, Boyle J, Hatakeyama S, Glass DJ (2009) Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. Am J Physiol Cell Physiol 296(6):C1258–C1270. https://doi.org/10.1152/ajpcell.00105.2009

Turner RS, Desmurget M (2010) Basal ganglia contributions to motor control: a vigorous tutor. Curr Opin Neurobiol 20(6):704–716. https://doi.org/10.1016/j.conb.2010.08.022

Umanskaya A, Santulli G, Xie W, Andersson DC, Reiken SR, Marks AR (2014) Genetically enhancing mitochondrial antioxidant activity improves muscle function in aging. Proc Natl Acad Sci U S A 111(42):15250–15255. https://doi.org/10.1073/pnas.1412754111

Vasilaki A, McArdle F, Iwanejko LM, McArdle A (2006) Adaptive responses of mouse skeletal muscle to contractile activity: the effect of age. Mech Ageing Dev 127(11):830–839. https://doi.org/10.1016/j.mad.2006.08.004

Verburg E, Murphy RM, Richard I, Lamb GD (2009) Involvement of calpains in Ca2+-induced disruption of excitation-contraction coupling in mammalian skeletal muscle fibers. Am J Physiol Cell Physiol 296(5):C1115–C1122. https://doi.org/10.1152/ajpcell.00008.2009

Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, Simonsick EM, Harris TB (2005) Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. J Gerontol A Biol Sci Med Sci 60(3):324–333. 60/3/324 [pii]

Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, MacGregor RR, Schlyer DJ, Hitzemann R, Wolf AP (1996) Measuring age-related changes in dopamine D2 receptors with 11C-raclopride and 18F-N-methylspiroperidol. Psychiatry Res 67(1):11–16. https://doi.org/10.1016/0925-4927(96)02809-0

Volkow ND, Gur RC, Wang GJ, Fowler JS, Moberg PJ, Ding YS, Hitzemann R, Smith G, Logan J (1998) Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. Am J Psychiatry 155(3):344–349. https://doi.org/10.1176/ajp.155.3.344

Vringer E, Tait SWG (2019) Mitochondria and inflammation: cell death heats up. Front Cell Dev Biol 7:100. https://doi.org/10.3389/fcell.2019.00100

Wagner KR, Fleckenstein JL, Amato AA, Barohn RJ, Bushby K, Escolar DM, Flanigan KM, Pestronk A, Tawil R, Wolfe GI, Eagle M, Florence JM, King WM, Pandya S, Straub V, Juneau P, Meyers K, Csimma C, Araujo T, Allen R, Parsons SA, Wozney JM, Lavallie ER, Mendell JR (2008) A phase I/IItrial of MYO-029 in adult subjects with muscular dystrophy. Ann Neurol 63(5):561–571. https://doi.org/10.1002/ana.21338

Wagner KR, Abdel-Hamid HZ, Mah JK, Campbell C, Guglieri M, Muntoni F, Takeshima Y, McDonald CM, Kostera-Pruszczyk A, Karachunski P, Butterfield RJ, Mercuri E, Fiorillo C, Bertini ES, Tian C, Statland J, Sadosky AB, Purohit VS, Sherlock SP, Palmer JP, Binks M, Charnas L, Marraffino S, Wong BL (2020) Randomized phase 2 trial and open-label extension of domagrozumab in Duchenne muscular dystrophy. Neuromuscul Disord 30(6):492–502. https://doi.org/10.1016/j.nmd.2020.05.002

Walton RG, Dungan CM, Long DE, Tuggle SC, Kosmac K, Peck BD, Bush HM, Villasante Tezanos AG, McGwin G, Windham ST, Ovalle F, Bamman MM, Kern PA, Peterson CA (2019) Metformin blunts muscle hypertrophy in response to progressive resistance exercise training in

older adults: a randomized, double-blind, placebo-controlled, multicenter trial: the MASTERS trial. Aging Cell 18(6):e13039. https://doi.org/10.1111/acel.13039

Wang X, Proud CG (2006) The mTOR pathway in the control of protein synthesis. Physiology (Bethesda) 21:362–369. https://doi.org/10.1152/physiol.00024.2006

Wang Y, Chan GL, Holden JE, Dobko T, Mak E, Schulzer M, Huser JM, Snow BJ, Ruth TJ, Calne DB, Stoessl AJ (1998) Age-dependent decline of dopamine D1 receptors in human brain: a PET study. Synapse 30(1):56–61. https://doi.org/10.1002/(SICI)1098-2396(199809)30:1<56::AID-SYN7>3.0.CO;2-J

Wang ZM, Messi ML, Delbono O (2000) L-type Ca(2+) channel charge movement and intracellular Ca(2+) in skeletal muscle fibers from aging mice. Biophys J 78(4):1947–1954. https://doi.org/10.1016/s0006-3495(00)76742-7

Watson R (2017) Physical activity and the aging brain: effects of exercise on neurological function. Elsevier, London

Weisleder N, Brotto M, Komazaki S, Pan Z, Zhao X, Nosek T, Parness J, Takeshima H, Ma J (2006) Muscle aging is associated with compromised Ca2+ spark signaling and segregated intracellular Ca2+ release. J Cell Biol 174(5):639–645. https://doi.org/10.1083/jcb.200604166

Wenz T (2009) PGC-1alpha activation as a therapeutic approach in mitochondrial disease. IUBMB Life 61(11):1051–1062. https://doi.org/10.1002/iub.261

Wessner B, Liebensteiner M, Nachbauer W, Csapo R (2019) Age-specific response of skeletal muscle extracellular matrix to acute resistance exercise: a pilot study. Eur J Sport Sci 19(3):354–364. https://doi.org/10.1080/17461391.2018.1526974

Wilkes EA, Selby AL, Atherton PJ, Patel R, Rankin D, Smith K, Rennie MJ (2009) Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. Am J Clin Nutr 90(5):1343–1350. https://doi.org/10.3945/ajcn.2009.27543

Wilkinson DJ, Piasecki M, Atherton PJ (2018) The age-related loss of skeletal muscle mass and function: measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans. Ageing Res Rev 47:123–132. https://doi.org/10.1016/j.arr.2018.07.005

Williams S, Miller G, Khoury R, Grossberg GT (2019) Rational deprescribing in the elderly. Ann Clin Psychiatry 31(2):144–152

Windhort U, Burke R, Dieringer N, Evinger C, Feldman A, Hasan Z, Humphrey DR, Freund H-J (1991) What are the outputs of motor behavior and how are they controlled? In: Humphrey DR, Freund H-J (eds) Motor control: concepts and issues. Wiley, New York, pp 101–119

Wisdom KM, Delp SL, Kuhl E (2015) Use it or lose it: multiscale skeletal muscle adaptation to mechanical stimuli. Biomech Model Mechanobiol 14(2):195–215. https://doi.org/10.1007/s10237-014-0607-3

Wong DF, Young D, Wilson PD, Meltzer CC, Gjedde A (1997) Quantification of neuroreceptors in the living human brain: III. D2-like dopamine receptors: theory, validation, and changes during normal aging. J Cereb Blood Flow Metab 17(3):316–330. https://doi.org/10.1097/00004647-199703000-00009

Xu H, Lamb GD, Murphy RM (2017) Changes in contractile and metabolic parameters of skeletal muscle as rats age from 3 to 12 months. J Muscle Res Cell Motil 38(5–6):405–420. https://doi.org/10.1007/s10974-017-9484-6

Yabe D, Nishikino R, Kaneko M, Iwasaki M, Seino Y (2015) Short-term impacts of sodium/glucose co-transporter 2 inhibitors in Japanese clinical practice: considerations for their appropriate use to avoid serious adverse events. Expert Opin Drug Saf 14(6):795–800. https://doi.org/10.1517/14740338.2015.1034105

Yang Q, Zhang Y, Zeng Q, Yang C, Shi J, Zhang C, Ni X, Du Z, Tang Z, Hu J, Li X, Cai J, Li Q, Cheng Q (2020) Correlation between diabetic peripheral neuropathy and sarcopenia in patients with type 2 diabetes mellitus and diabetic foot disease: a cross-sectional study. Diabetes Metab Syndr Obes 13:377–386. https://doi.org/10.2147/DMSO.S237362

Yarasheski KE, Bhasin S, Sinha-Hikim I, Pak-Loduca J, Gonzalez-Cadavid NF (2002) Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting. J Nutr Health Aging 6(5):343–348

Yuan D, Xiao D, Gao Q, Zeng L (2019) PGC-1alpha activation: a therapeutic target for type 2 diabetes? Eat Weight Disord 24(3):385–395. https://doi.org/10.1007/s40519-018-0622-y

Zampieri S, Pietrangelo L, Loefler S, Fruhmann H, Vogelauer M, Burggraf S, Pond A, Grim-Stieger M, Cvecka J, Sedliak M, Tirpáková V, Mayr W, Sarabon N, Rossini K, Barberi L, De Rossi M, Romanello V, Boncompagni S, Musarò A, Sandri M, Protasi F, Carraro U, Kern H (2015) Lifelong physical exercise delays age-associated skeletal muscle decline. J Gerontol A Biol Sci Med Sci 70(2):163–173. https://doi.org/10.1093/gerona/glu006

Zhang C, McFarlane C, Lokireddy S, Bonala S, Ge X, Masuda S, Gluckman PD, Sharma M, Kambadur R (2011) Myostatin-deficient mice exhibit reduced insulin resistance through activating the AMP-activated protein kinase signalling pathway. Diabetologia 54(6):1491–1501. https://doi.org/10.1007/s00125-011-2079-7

Zhang LN, Zhou HY, Fu YY, Li YY, Wu F, Gu M, Wu LY, Xia CM, Dong TC, Li JY, Shen JK, Li J (2013) Novel small-molecule PGC-1alpha transcriptional regulator with beneficial effects on diabetic db/db mice. Diabetes 62(4):1297–1307. https://doi.org/10.2337/db12-0703

Chapter 6
Pathophysiology of Volumetric Muscle Loss and Targets for Regenerative Rehabilitation

Jacob R. Sorensen, Jennifer Mcfaline-Figueroa, and Jarrod A. Call

Abstract Orthopedic extremity trauma is a major problem resulting in both long-term functional disability and substantial medical cost in various populations. One such injury is volumetric muscle loss (VML), which is clinically identified as a chronic and irrecoverable loss of skeletal muscle tissue resulting in functional impairments. VML is coupled with clinical outcomes related to long-term dysfunction, reduced mobility and physical activity, comorbidities, and often delayed amputation. The objectives for this chapter are to (1) summarize known pathophysiologies associated with VML injury and tools for their clinical and experimental assessment, (2) review current rehabilitation and regenerative medicine strategies for VML injury, and (3) explore the evidence supporting regenerative rehabilitation as a strategy to maximize functional recovery of the VML-injured limb and discuss knowledge gaps and areas for scientific advancements.

Keywords Muscle strength · Mitochondria · Physical therapy · Exercise training · Muscle adaptation

6.1 Introduction

The clinically accepted definition for volumetric muscle loss (VML) injury is "the traumatic or surgical loss of skeletal muscle with resultant functional impairment" (Grogan and Hsu 2011). Traumatic muscle injuries like VML often result from blunt

J. R. Sorensen
School of Kinesiology, University of Minnesota, Minneapolis, MN, USA

J. Mcfaline-Figueroa
Department of Physiology and Pharmacology, University of Georgia, Athens, GA, USA

Regenerative Bioscience Center, University of Georgia, Athens, GA, USA

J. A. Call (✉)
Regenerative Bioscience Center, University of Georgia, Athens, GA, USA

Department of Kinesiology, University of Georgia, Athens, GA, USA
e-mail: call@uga.edu

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_6

force trauma in which an object strikes the body, from penetrating traumas in which an object pierces the body creating an open wound, or controlled, surgical trauma in which an object is removed from the body. Although traumatic injuries often cross tissue boundaries such as bone, nerve, vascular, tendinous, ligamentous, and/or cartilaginous, this chapter focuses on skeletal muscle pathology and regenerative rehabilitation strategies to improve skeletal muscle function. Skeletal muscle makes up ~40% of the body's mass and contributes to locomotion, thermoregulation, and whole-body metabolism. Any loss of skeletal muscle function can negatively affect mobility and increase an individual's risk of all-cause morbidity and mortality.

VML injury can result secondary to any of the 150,000 open fractures or 30,000 gunshot wounds (trauma), 36,000 chainsaw accidents (industrial/farm), and 13,000 soft-tissue sarcomas (cancer) that occur annually in the USA. Approximately 77% of all military casualties are musculoskeletal injuries, many that have a component of debilitating VML injuries (Cross et al. 2011; Owens et al. 2007; Owens et al. 2008). Data from the US trauma centers indicate that two-thirds of traumatic injuries occur to extremities with 32% and 40% to the upper and lower extremities, respectively. Collectively, the US economic burden related to trauma and injury is $400 billion yearly (Seifert 2007).

This chapter (1) describes the pathophysiology of VML injury, (2) discusses common VML study outcome measurements, (3) reviews rehabilitation and regenerative medicine approaches to improve VML, and (4) highlights the need for regenerative rehabilitation studies with functionally relevant outcome measurements to advance evidence-based approaches for VML.

6.2 Current Standards of Care

The current practices for definitive care following VML injury and orthopedic injuries involving concomitant VML injury can be generalized into three phases: (1) immediate field care, (2) acute surgical care, and (3) short-term rehabilitative care (Saunders and Rose 2021; Dolan et al. 2021). Standardization within these phases is complicated by the site of the injury and poly-traumatic nature of the injury that can include damage to the bone, muscle, skin, nerve, and/or vascular network. Prioritization of care among the phases starts with patient survival, minimizing infection risk, limb salvage, and finally tissue rehabilitation. Immediate field care often takes place at the site of injury (e.g., field of play, battlefield) and involves fixation of the limb for emergency transport, field dressing to minimize infection risk, and possible tourniquets to reduce blood loss from the injury. These practices prioritize patient survival. Acute surgical care involves surgical procedures and postoperative procedures conducted at a trauma center. Standard practices include blood transfusion, bone union, muscle flaps, nerve allografts, and limb amputation if required for survival (e.g., infection). Evidence of rehabilitative approaches applied in the intensive care unit immediately following acute surgical care is nonexistent, although could involve neuromuscular stimulation and/or mobilization. Short-term

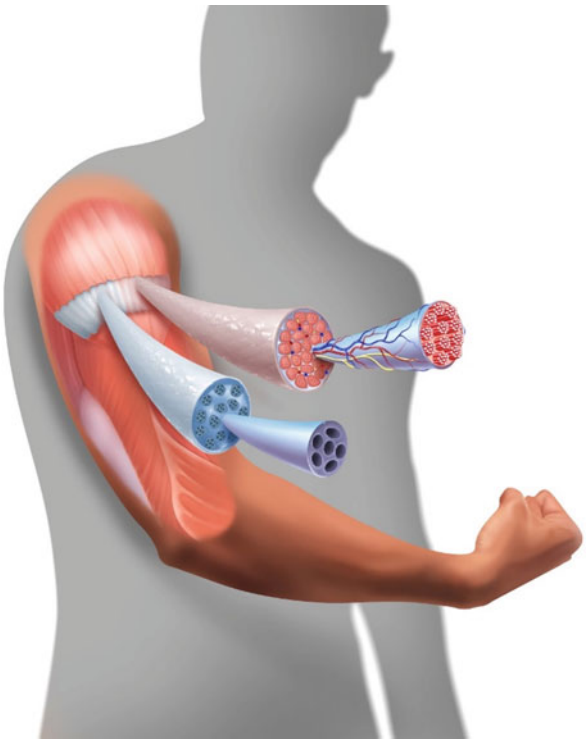

Fig. 6.1 The goal of regenerative rehabilitation. Regenerative rehabilitation seeks to leverage a regenerative medicine technology (exemplified here as a biomaterial-based muscle fascicle) with validated rehabilitation approaches to maximize muscle restoration and functional capacity. Ideally, a regenerative rehabilitation approach has a greater effectiveness than either regenerative medicine or rehabilitation alone. This is exemplified here by a biomaterial-based muscle fascicle with vascularized (yellow) and perfused (red/vessels) muscle fibers

rehabilitative care often prioritizes task rehabilitation, e.g., regaining mobility or ability to navigate stairs, as opposed to functional rehabilitation (i.e., improving strength) due to a lack of evidence showing robust improvements in muscle function with current strategies. To address this challenge, there is an urgent need to identify new or existing strategies in combination that can bolster functional recovery. One idea is to explore regenerative rehabilitation approaches.

Regenerative rehabilitation was broadly defined in Chap. 1, so here a more specific definition will be advanced for VML injury. *Regenerative rehabilitation for VML injury is a focused effort to improve skeletal muscle quality and overall muscle function and advance mobility and quality of life for the patient by integrating regenerative technologies with rehabilitation clinical practices* (Fig. 6.1). For the VML-injured patient, any functional gain beyond those achieved with rehabilitation are expected to have a considerable impact on quality of life and as such regenerative rehabilitation may be an ideal tool to accomplish this goal.

6.3 Pathophysiology

In this section, we highlight primary pathologies associated with VML injuries, and then summarize outcome measurement commonly used to evaluate the effectiveness of an intervention. VML injuries are idiosyncratic and any one injury may not involve all of the pathologies discussed below. Additionally, investigators should consider the pros and cons of each technique in relation to their study design questions and anticipated outcomes. Throughout this section we will reference Fig. 6.2 that provides examples of common pathologies after VML injuries at four different regions of the VML-injured muscle. These four regions include (1) the defect, representing the area in which a volume of muscle was removed; (2) the border, representing the tissue layer between the defect and remaining muscle; (3) the near defect, representing the remaining muscle in close proximity to the defect; and (4) the distant to defect, representing the remaining muscle far enough from the defect area that it was not injured by the initial VML injury. The pathologies taking place at these four muscle regions can all contribute to the long-lasting functional deficits observed after VML injury and the lack of full muscle restoration, and are in contrast to the full muscle recovery capable after more common contraction-induced muscle injuries.

6.3.1 Loss of Muscle Function

Skeletal muscle has many roles, perhaps the most important is to facilitate movement. Thus, the drastic loss of muscle function following a VML injury is considered the most consequential (Corona et al. 2016). Clinically, individuals that have some form of VML injury display a wide spectrum of functional deficits including strength loss, limited range of motion, and muscle stiffness. The gold standard for quantifying the extent of an injury or the efficacy of a regenerative or rehabilitative intervention is by testing the functional capacity of the muscle. Follow-up assessments also show conflicting results related to the ability of the muscle to restore function several months post-injury. In some VML injuries, the limb may be completely void of any initial functional strength or intervention-induced strength gains, while other VML injuries, though limited, can show promise for a restoration of function over time. Overall, the loss of function is typically disproportionally greater than the size of muscle removed (Corona et al. 2016). The most unfortunate finding from clinical work is that muscle function may actually decline chronically, despite the use of rehabilitative and/or regenerative interventions (Mase et al. 2010; Tanaka et al. 2017; Sicari et al. 2014; Gentile et al. 2014; Garg et al. 2015).

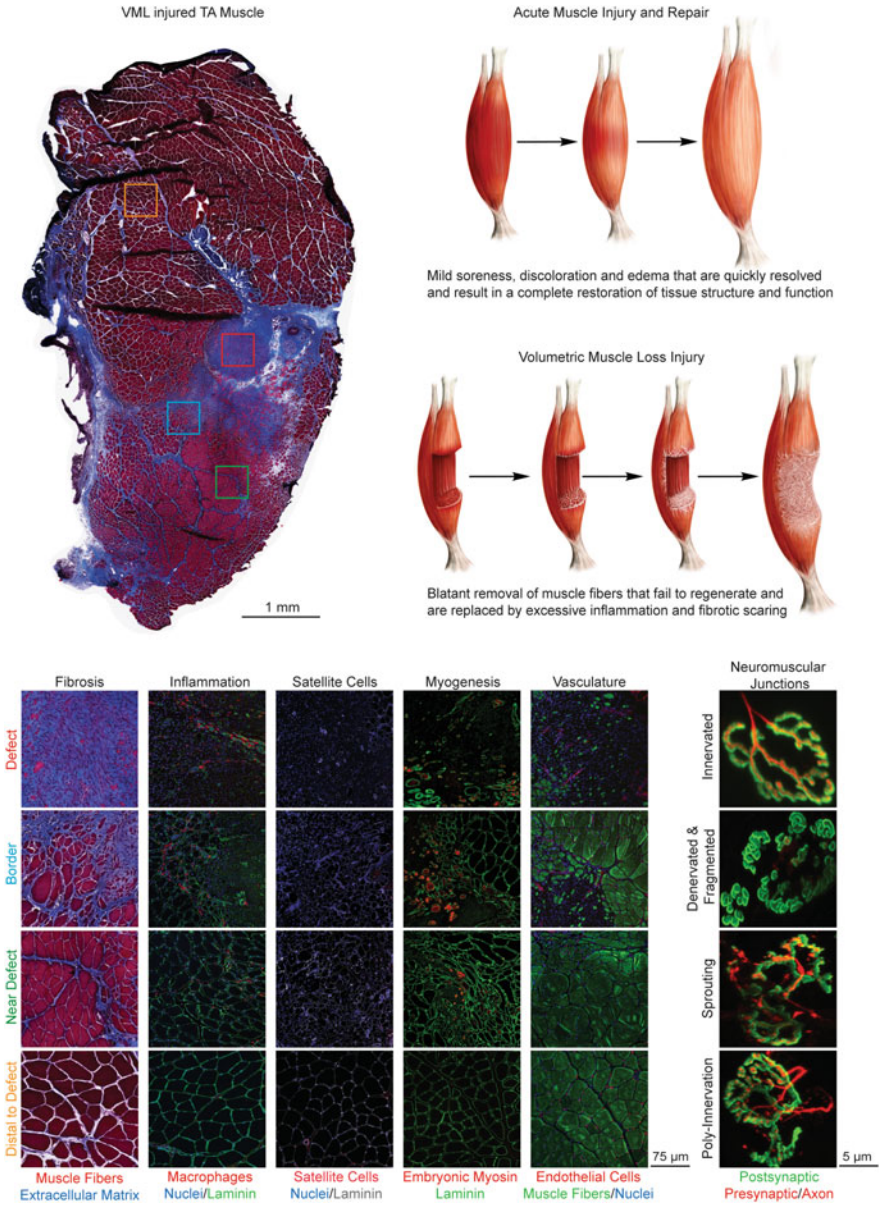

Fig. 6.2 Pathophysiology of volumetric muscle loss injury. Acute skeletal muscle injuries (e.g., strains, contusions, and exercise induced) follow a canonical sequence of cellular events that typically result in the successful restoration of the damaged tissue to its previously uninjured and functional state. Conversely, VML injuries are plagued with an irregular pathologic response that overwhelms the remaining tissue. The VML pathology is greatest in and around the defect area and gradually declines at the more distal regions of the injured tissue. Characteristics include excessive fibrosis, persistent and irregular inflammation, poor satellite cell migration, and activation, limited formation of new muscle fibers, changes in vascularity, and dysregulation of the remaining neuromuscular junctions. Ultimately, VML-injured muscle fails to recover the structural and function components of healthy skeletal muscle tissue, often resulting in long-term disability. The

6.3.1.1 Experimental and/or Clinical Outcome Measurements

Clinically, muscle function is measured using video photography, a goniometer, and a biodex dynamometer for assessing gait, range of motion, and functional strength, respectively. The standardized use of these modalities allows for continual evaluation of VML strength for understanding long-term complications and/or progress of a physical therapy. It also serves as a mechanism to facilitate coordinated and individualized care by the medical staff.

The development and standardization of VML injury models and equipment have advanced preclinical VML research (Garg et al. 2015; Greising et al. 2019). For example, VML injuries in the tibialis anterior (TA), gastrocnemius, quadriceps, and latissimus dorsi muscles of animals have been developed to allow neuromuscular strength testing (Greising et al. 2019). While it is possible to measure grip strength and forced treadmill running, the use of a functional muscle testing system provides the most accurate results for testing force generation of specific muscles of interest and can be performed in vivo, in situ, or in vitro (e.g., Quarta et al. 2017; Corona et al. 2013a; Corona et al. 2013b). As muscles vary in size and strength, absolute strength is often normalized to body or muscle mass. Importantly, the use of a functional muscle testing system with preclinical VML models enables specific examination of various rehabilitation and regenerative medicine paradigms that are not yet suitable clinical trials (e.g., a new biological scaffold).

An emerging method for measuring muscle function is the biomechanical assessment of ambulation and gait kinematics. Two different approaches have been used to accomplish this, both include the use of videography, and it is recommended that treadmill acclimation be performed prior to testing (Dienes et al. 2019; Quarta et al. 2017). In the first approach, reflective markers are placed on bony landmarks that correspond with the pelvis, femur, tibia, and foot. Animals are then placed on a treadmill and a multi-camera setup is used to identify and record the reflective markers with their corresponding joint angles and segment lengths for gait kinematic analysis. This method has been used to detect differences in gait between healthy and VML-injured limbs and can be used in combination with force data or separately to evaluate muscle function (Dienes et al. 2019). In the second approach, a video imaging system captures gait kinematics from the lateral and ventral perspective in rodents using a transparent treadmill belt (e.g., Digigate; Mouse Specifics Inc.). This method provides a unique view of the animal's gait while also generating digital paw prints that allow for the identification of gait abnormalities (Quarta et al. 2017). The data collected from both of these approaches can be used to assess strength, balance, gait, and coordination, making it a useful tool for evaluating the effectiveness of a regenerative and/or rehabilitative intervention.

Fig. 6.2 (continued) images in this figure are representative of a VML injury at different locations in a rat tibialis anterior muscle 21 days following the surgical removal of a full thickness, 6 mm biopsy punch. Cell identification markers used in this figure include the colocalization of nuclei (DAPI) with $CD68^+$ macrophages, $Pax7^+$ satellite cells, or $CD146^+$ endothelial cells

The drastic loss of muscle function brought on by VML is thought to be mostly attributed to the abrupt removal of muscle fibers. Thus, the majority of regenerative interventions focus on stimulating de novo myofiber formation using biomimetic scaffolds and various stem cell interventions. However, after accounting for the mass of tissue or the number of fibers removed and/or damaged due to the VML injury, there remains a glaring discrepancy in strength loss that is unaccounted for, possibly a result of metabolic deficiencies, satellite cell dysfunction, denervation, impaired excitation-contraction coupling, extensive inflammation, connective tissue accumulation, and/or alterations in the length tension relationship of the myosin/actin cross-bridge. As the study of VML injury is a relatively young and growing field, gaps currently exist in understanding the long-term consequences of VML injury. As such, pathologies moving forward are supported by the literature, but it is entirely possible that this list will grow along with advanced knowledge of the VML injury.

6.3.2 Loss of Metabolic Function

The effects of VML injuries are not constrained to mobility deficits. Loss of functional muscle fibers is analogous to a stark reduction of metabolically active cells that, for example, contribute to basal metabolic rate and circulating glucose levels. At a cellular level, skeletal muscle's metabolic efficiency is regulated by the mitochondria. Within this organelle, several factors contribute to this metabolism, including enzyme kinetics of the components of the electron transport chain, oxygen consumption, and mitochondrial membrane polarization. Oxidative capacity has been shown to decrease by roughly 25% in VML-injured muscle when compared to uninjured muscle (Greising et al. 2018; Southern et al. 2019). This could have potential whole-body implications as VML-injured mice are reported to have a lower overall metabolic rate despite similar levels of physical activity (Dalske et al. 2021). Such whole-body changes can lead to metabolic deficits associated with an increased likelihood of developing diabetes, cardiovascular disease, and metabolic syndrome. The muscle-specific and whole-body characterization of metabolic changes after VML is relatively new and there is a large knowledge gap relating to clinical manifestations and cellular mechanisms.

6.3.2.1 Experimental and/or Clinical Outcome Measurements

Muscle metabolism is orchestrated, in large part, by a collection of enzymes found within the mitochondria that aid in the process of breaking down pyruvate to create ATP. Briefly, pyruvate dehydrogenase oxidizes pyruvate to create acetyl-CoA by way of the reduction of NAD+ to NADH. The action of the enzyme citrate synthase condenses acetyl-CoA and oxaloacetate into a six-carbon molecule, citrate. Citrate will undergo stepwise oxidation at the hands of the enzymes isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and succinate dehydrogenase, among others,

until oxaloacetate is recovered. Rates of activity from these enzymes have been successfully measured in skeletal muscle tissue previously and are prime targets to monitor the disease progression and subsequent recovery of skeletal muscle in models of VML injury (Corona et al. 2018a; Southern et al. 2019; Chao et al. 2019).

Products from the tri-carboxylic cycle are used in the mitochondria to further produce ATP through oxidative phosphorylation. Here, the reduction of NADH and FADH2 will produce electrons that will be shuttled between the respiratory complexes and H+ ions that are pumped into the intermembrane space, with oxygen acting as the final electron acceptor. The shuttling of electrons through the respiratory complexes allows for more H+ to be pumped into the intermembrane space and for additional electrons to be shuttled down the pathway. Therefore, a decrease in oxygen consumption, the final step in the process, can point toward deficiencies in skeletal muscle metabolism. To this point, studies have reported a decrease in oxygen consumption at State III in VML-injured muscle compared to uninjured controls (Greising et al. 2018; Southern et al. 2019; Chao et al. 2019).

To date, studies have relied on the substrate-uncoupler-inhibitor titration (SUIT) method in high-resolution respirometry to determine oxygen consumption. Substrates that feed each complex within the electron transport chain are added into a closed system with a known amount of oxygen. Rate of oxygen consumption at each titration is compared to a control to identify changes in mitochondrial function. While this method offers the ability to observe mitochondrial deficiencies on a complex-by-complex basis, it also paints an idealistic picture of oxygen consumption. This is due to the fact that substrates provided into the experimental system are at supra-physiological concentrations where you assume the limiting factor is the materials needed to balance the ADP/ATP ratio. A more recent respirometry method, known as the creatine kinase clamp (CK clamp), utilizes known facts about ADP/ATP and Cr/PCr ratios during rest and exercise to calculate energetic demand and the tissue's ability to adapt when that energetic demand increases or decreases (Fisher-Wellman et al. 2018). In this assay, the ADP/ATP ratio is maintained constant, while titrations of phosphocreatine lower energetic demand, and, likewise, oxygen consumption will also decrease. Other indirect measures for the activity if CK in skeletal muscle following VML have also been achieved through in vitro systems (Li et al. 2017).

Hand in hand with oxygen consumption, mitochondrial membrane potential flux is another measure of mitochondrial function. The mitochondrial membrane potential is created by the traffic of electrons from Complexes I, III, and IV into the intermembrane space. This inequity of protons in the outside of the mitochondrial matrix creates a proton gradient that provides the transmembrane potential needed to create ATP. Consequently, the changes in mitochondrial membrane potential are tied with the accumulation and later alleviation of the pressure produced by the H+ in the intermembrane space and the shuttling of electrons down the respiratory complexes. Several colorimetric methods have been developed to track mitochondrial membrane potential (Krumschnabel et al. 2014). Most commonly used is tetramethylrhodamine methyl ester (TMRM e.g., (Tehrani et al. 2019)), a molecule

small enough to be taken into the mitochondrial matrix during times of hyperpolarization and released upon membrane depolarization.

Previous clinical examination of large-scale orthopedic trauma has been postulated to increase sedentarism (Maggio et al. 2017), and subsequently the risk of metabolic comorbidities (Rynders et al. 2018), but measuring whole-body metabolic parameters in animal models has been historically challenging. Advancements in equipment have made it possible to overcome some of these challenges and were recently used to study VML-injured mice (Dalske et al. 2021). The equipment used is called a comprehensive lab animal monitoring system (CLAMS), which is a closed environmental and metabolic chamber that can control and measure temperature and ventilatory gases (O_2 and CO_2) used to calculate VO_2, respiratory exchange ratios (RER), and metabolic rate. The chamber also includes infrared beams in the X, Y, and Z planes that can track rest and movement patterns, which are used to determine physical activity. Other modular features include periodic body mass measurements, wheel/treadmill running, and food/fluid intake monitoring. The versatility of the metabolic chambers combined with robust monitoring of animal activity makes it an excellent tool for assessing animals after injury such as VML. In fact, the aforementioned study revealed that VML-injured mice maintain similar physical activity levels to uninjured controls (Dalske et al. 2021), which is supported by wheel running experiments (Southern et al. 2019), but is an unexpected finding based on clinical observations following traumatic injury (Ceroni et al. 2011). Despite similarities in physical activity, VML-injured mice demonstrated whole-body metabolic impairments, referred to as metabolic inflexibility (Kelley et al. 1999). Specifically, VML-injured mice were unable to effectively shift between carbohydrate and lipid utilization during active hours, suggesting that metabolic impairments previously observed at the cellular level extend beyond the relatively small injury site to negatively impact whole-body metabolism. Of course, additional studies using advanced whole-body metabolism and physical activity monitoring are needed to connect VML with chronic metabolic outcomes. On the other hand, equipment such as the CLAMS units could also be used in combination with regenerative and rehabilitative interventions following VML injury to see if physical activity and/or whole-body metabolism can be improved.

6.3.3 Loss of Regenerative Capacity

Successful restoration of injured muscle tissue requires the repair and/or regeneration of muscle fibers, which are highly organized in bundles with specific structural and contractile properties. Typically, skeletal muscle has a robust endogenous regenerative response following acute injuries (exercise-induced, strains, contusions), which follow a canonical regenerative order that ultimately restores tissue structure and function to, or even above, pre-injury levels (Fig. 6.2) (Wosczyna and Rando 2018). This regenerative process occurs over the course of several days to weeks in mammals and follows a time-dependent, energy-demanding sequence

characterized by robust infiltration and adaptation of immune cells (McLoughlin et al. 2003), expansion and migration of muscle progenitor cells (i.e., satellite cells, Fig. 6.2), and remodeling of the vasculature, motor units, and extracellular matrix (ECM) (Hyldahl et al. 2015). Unfortunately, VML injuries do not follow the same regenerative time course and ultimately fail to restore functional muscle tissue to its original form (Corona et al. 2013a).

A number of preclinical VML studies do show an initial increase in muscle function following VML injury, suggesting that the endogenous regenerative repair response may be present in the early phase of the injury (Aguilar et al. 2018; Greising et al. 2017; Corona et al. 2018a). Upon further inspection however, this response is characterized by irregular waves of inflammation, an absence of progenitor cell expansion and migration into the defect area, and excessive formation of ECM collagens that fill the VML defect area with scar tissue (Greising et al. 2017). Thus, the early improvements in function may not indicate regeneration, but instead an unregulated cellular response with a passive increase in force transmitting collagens that ultimately overwhelm the tissues endogenous regenerative capacity.

6.3.3.1 Experimental and/or Clinical Outcome Measurements

A significant portion of VML-related work is focused on developing treatments to restore order and function to the cellular response within the VML defect area. One of the primary techniques used to assess regenerative capacity is the histological identification of cells and their structures using various cellular identification techniques (e.g., flow cytometry) and imaging (e.g., microscopy, ultrasound, MRI).

For the quantification of mononuclear cell populations, the most promising method of interest would be to use flow or mass cytometry. These techniques use fluorescently labeled antibodies or unique metals, respectively, to identify cell populations with high efficiency. What makes this approach valuable is that it can accurately identify and differentiate between multiple cell populations in a given sample or whole muscle, drastically reducing the amount of time spent analyzing images. Pathological studies have shown extensive infiltration and persistence of immune cells into the VML injury using flow cytometry (Hurtgen et al. 2017; Hymel et al. 2021). If possible, more studies should consider using this technique to better understand the dynamic inflammatory response of VML injury following the use of regenerative and/or rehabilitative interventions. However, a specific limitation to this method with VML injury is that it lacks the temporo-spatial benefits found using microscopy. For example, histological samples reveal that Pax7-expressing satellite cells are found in the remaining muscle and border regions of the VML injury, yet they are rarely seen infiltrating the defect area (Greising et al. 2017). This type of spatial observation would go unnoticed using flow cytometry alone. Thus, the benefit of microscopy is that it provides temporo-spatial context to the dynamic response in cellular populations. Furthermore, microscopy can be used to assess other muscle fiber characteristics.

Quantifiable measures of muscle fiber regeneration are typically done using microscopy, and include the identification of newly forming muscle fibers, total muscle fiber number, the measurement of muscle fiber cross-sectional area, and in some cases the appearance of centralized nuclei. To differentiate between existing and newly forming muscle, various myosin heavy chain (MyHC) isoforms can be used to identify mature fibers, while embryonic MyHC is acutely expressed in regenerating fibers. If a regenerative cell therapy approach is used, such as stem cells or minced muscle graft transplants, investigators should consider using fluorescently tagged cells or tissue. Thus, the contribution of the cell therapy or tissue can be easily distinguished from the endogenous fibers. In otherwise healthy muscle, muscle function is highly correlated with muscle size and number, and the cross-sectional area of the muscle fibers is a good indication of the muscle's capacity to generate force and adapt. The extent to which these relationships are maintained in VML-injured muscle is unclear.

6.3.4 Loss of Functional ECM Replaced with Pathologic Fibrosis

The complex web-like structure that surrounds the muscle tissue is known as the extracellular matrix (ECM), which is an extension of the tendon, transmitting the contractile forces generated from muscle fiber contraction to the bone for movement. Beyond the ECM's contribution to movement, there is mounting evidence to support its critical role in the healing of injury muscle tissue (Gillies and Lieber 2011; Hyldahl et al. 2015). Skeletal muscle ECM is composed of various collagen isoforms, proteoglycans, and glycosaminoglycans that serve as a scaffolding to provide structural support for the muscle fibers, vasculature, nerves, and the diverse cellular populations that reside within the tissue micro-environment. Healthy muscle has a relatively consistent ratio of ECM in comparison to myofiber cross-sectional area. As muscle fibers hypertrophy, there is a corresponding increase and complex remodeling of ECM content (Mendias et al. 2017). However, muscle wasting conditions such as muscular dystrophies appear to disrupt this relationship, resulting in greater ECM accumulation with smaller muscle fibers. The unbalanced accumulation of ECM (pathologic fibrosis) results in deterioration of muscle quality and poor muscle function by reducing joint range of motion and maximal force production. Excessive ECM accumulation is one of the primary characteristics of VML injury (Corona et al. 2018b). Following the blunt removal of muscle fibers, mononuclear cells infiltrate the defect area and deposit thick layers of ECM that create a ball of scar tissue to replace the muscle fibers.

Following acute injuries (i.e., strains, or exercise induced) the ECM undergoes a remodeling phase that is vastly important for healthy regeneration and repair of the damaged myofibers. This transient remodeling phase is referred to as the transitional ECM, and includes the enzymatic breakdown and cellular upregulation of collagens

and adhesion-related proteoglycans (i.e., tenascin-c, fibronectin, hyaluronic acid) (Calve et al. 2010). The transitional ECM acts as a highway for cells to migrate toward the injury sight and facilitate the repair/regeneration process. The disappearance of a transitional matrix can lead to widespread fibrogenesis, as is observed in other skeletal muscle disorders (i.e., muscular dystrophy) and aging (Sorensen et al. 2018). There appears to be a tipping point in more severe pathologies, where muscle regeneration is repressed, and ECM deposition progresses uninhibited (Fig. 6.2). For example, the blunt removal of muscle fibers, resident mononuclear cells, and ECM components following VML injuries results in the aggressive formation of highly cross-linked or dense collagens (Hoffman et al. 2021). Indeed, the loss of functional ECM appears to limit the regenerative potential of the muscle, likely contributing to failed migration of satellite cells from the remaining tissue into the defect area. Notably, the frank removal of the existing ECM eliminates the structural support system that is needed for new muscle fibers to grow and survive. Therefore, regenerative medicine interventions have attempted to address these issues with an approach of inserting various biomimetic scaffolds into the VML defect area to establish a physical support system for angiogenesis, myogenesis, and reinnervation (Aurora et al. 2015; Haas et al. 2019). This approach has had varying levels of success, specifically in the ability to restore function. Going forward, it will be critical to find a balance between functional ECM remodeling and inhibition of fibrogenesis.

6.3.4.1 Experimental and/or Clinical Outcome Measurements

As work progresses to better understand and mitigate the accumulation of fibrosis following VML injury, there are several methods that can be used to evaluate ECM content in skeletal muscle. For clinical or repeated measures less invasive techniques are available; these include ultrasound or passive muscle function examinations (Corona et al. 2020). Modern advancements in technology, real-time imaging, and wide spread availability make ultrasound an excellent option for human studies. Briefly, images are collected utilizing B-mode and shearwave elastography as a mechanism to directly examine mechanical properties of skeletal muscle. This is done at various joint angles when an external force is applied to the tissue being imaged and the amount of displacement or "stiffness" is measured. Stiff tissue tends to deform less and show less strain compared to compliant tissue (Corona et al. 2020). To carry out passive muscle stiffness examinations, the limb of interest is secured to a dynamometer and passively rotated at the joint. Passive force measurements are collected at various joint angles where an increase in resistance for a given joint angle indicates greater muscle stiffness (Greising et al. 2018; Corona et al. 2020). Importantly, these measures can be examined longitudinally in humans to determine the efficacy of a given treatment or the progression of the VML pathology.

The assessment of ECM content at the cellular level can be performed using more invasive techniques that require the collection of muscle samples. A commonly used method is histological analysis of skeletal muscle cross sections. Notably, different

histological techniques are used to quantify ECM content; these include electron microscopy, Masson's trichrome stain, picrosirius red stain, and antibody labeling of specific ECM markers. Each approach uses a corresponding microscope to take full advantage of the muscle sample. Generally speaking, electron microscopy allows for high definition qualitative assessment of the ECM structure (Gillies et al. 2017; Gao et al. 2008; Järvinen et al. 2002; Purslow and Trotter 1994), while Masson's trichrome and picrosirius red quantitatively assess overall ECM content (Ramaswamy et al. 2011; Greising et al. 2017). With the addition of a polarizing filter, the picrosirius red stain can be further used to assess loosely vs. densely packed collagen (Hoffman et al. 2021; Dearth et al. 2016; Smith and Barton 2014; Arruda et al. 2007). Lastly, immunohistochemistry can be broadly used to label specific collagen isoforms and other ECM proteins (e.g., laminin, fibronectin, dystrophin) (Piñol-Jurado et al. 2018; Cáceres et al. 2000; Zanotti et al. 2005) or ECM-related growth factors (e.g., TGF-β or connective tissue growth factor) (Li et al. 2004). Histological quantification of ECM content is typically expressed as a measure of area.

Total collagen, collagen isoforms, and fibrotic markers can also be examined at the protein and gene level. After muscle samples are properly homogenized and processed, biochemical assessment of total collagen can be done using a hydroxyproline assay. Furthermore, antibody-based probing (e.g., ELISA) can be used to examine specific collagen isoforms and proteins of interest. Lastly, some proteins, such as TGF-β, may also be measured using multiplexing assays, which have the distinct advantage of including a host of markers in a single sample. Notably, recent advancements in proteomics and transcriptomics reveal that only a portion of the gene expression is revealed in the protein content, suggesting that protein measurements more accurately reflect the cellular response. Nonetheless, gene arrays, qRT-PCR, and next-generation sequencing analysis are widely used research techniques that provide additional insight (Aguilar et al. 2018; Corona et al. 2017b; Greising et al. 2017; Greising et al. 2018; Cherry et al. 2021; Nuutila et al. 2017; Aurora et al. 2014). The advantage of gene arrays is that a large number of genes can be tested to identify enriched pathways of differentially expressed genes that could serve as potential therapeutic targets. The advantage of sequencing is increased flexibility with hardware design and the probability of discovering unknown gene variants. Overall, the extracellular matrix is a critical and often overlooked component of skeletal muscle health. As more treatments for VML are developed and tested, the fibrotic response will continue to be an area of interest for achieving success.

6.3.5 Extensive and Irregular Inflammation

The extent of damage caused by VML injuries is typically beyond the muscle's natural ability to regenerate. Regeneration takes place under complex inflammatory conditions (Grounds 2014), entailing the activation of the innate immune system and

recruitment of effector immune cells (Fig. 6.2). Early stages of healing involve a pro-inflammatory environment, where resident macrophages are polarized toward the M1 phenotype and, along with other recruited immune cells, facilitate the repair process by breaking down cellular debris. This process is also important for the activation and migration of satellite cells to the area of injury. Under normal conditions, this pro-inflammatory milieu subsides as damaged cells and extracellular matrices are cleared away. Macrophages will then polarize toward the M2 or anti-inflammatory phenotype which will stimulate satellite cell differentiation and myofiber repair.

VML-injured muscle maintains an increased expression of chemotactic, inflammatory, and immune cell infiltration gene transcripts (Greising et al. 2016, 2017). Where other muscle injuries see a decrease in inflammatory signaling a few days after injury, VML has been shown to maintain this sustained expression even 28 days following injury (Aguilar et al. 2018). Similar to other models of severe muscle trauma, VML presents with an inflammatory profile, producing an abundance of COX1/2, CSF2, IL6, MIF, and STAT3. Moreover, immune pathways, such as complement activation, and fibrosis-inducing pathways, like TGF-β1 and Wnt, remain constantly activated after injury. This is important because TGF-β1 and Wnt control the behavior of multipotent fibro-adipogenic precursors living in the cellular niche of the muscle, which are thought to be responsible for the increased fibrosis in the VML defect area and the suppression of satellite cell activation, respectively.

Increased fibrosis in the muscle defect creates a myogenesis-inhibitive feedback loop, as fibrotic tissue secretes cytokines that block myogenic differentiation factors such as MyoD, MyoG, and Mef2. Fibrosis and prolonged immune cell infiltration and action create an unfavorable environment where remodeling enzymes are constantly activated to clear away ECM that is interfering with the growth of new myofibers, leading to further inflammation (Shayan and Huang 2020). This environment is unreceptive to therapies and causes a degenerative phenotype in satellite, immune, and fibro-adipogenic precursor cells (Larouche et al. 2018). Therefore, therapeutic candidates must be able to immunomodulate the cell niche to usher in the anti-inflammatory stages of repair, when appropriate, stave off fibrosis and adipogenesis, preserve the remaining muscle, and positively influence satellite cells.

6.3.5.1 Experimental and/or Clinical Outcome Measurements

Inflammation is a necessary component of wound and tissue healing (Greising et al. 2020). High temperature, increased fluid retention, and the recruitment of effector immune cells are all part of a highly coordinated effort to clear away debris and allow for the regeneration and remodeling period to commence. However, as it was established earlier, the inflammatory period during VML injuries tends to subsist further than what is necessary or beneficial for the tissue. This extended inflammation is thought to hinder the regeneration process by breaking down newly formed tissue and causing damage to any growth factors, cells, or adjuvants delivered to the area of injury.

Monitoring immune cell infiltration or the product of immune cell action in skeletal muscle has been previously explored in models of VML. Gene expression for protein products and proteases secreted by immune cells has been previously used to show inflammatory response in VML (Corona et al. 2013a; Hurtgen et al. 2016). Masson's trichrome and hematoxylin and eosin staining have been used to show the effects of immune cell infiltration in injured muscle. Recent reports have also used fluorescence activated cell sorting (FACS) to identify immune cell populations in a longitudinal assessment of VML injuries (Hymel et al. 2021). Additionally, the availability of ELISA and magnetic multiplex assays allows for the testing of a wide array of immune secreted factors, specifically cytokines such as IL-1β and IL-6.

6.3.6 Loss of Innervation

A motor unit is made up of a motor neuron and the skeletal muscle fibers innervated by that motor neuron's axonal terminals, i.e., individual neuromuscular junctions (NMJs) on each fiber. The NMJ is a specialized chemical synapse that plays a critical role in muscle contraction where an action potential passes from the motor neuron to the individual muscle fiber to convert chemical energy into mechanical energy via the cross-bridging of actin and myosin. Due to the critical nature of the NMJ in facilitating muscle contractions, it has been shown to be a point of failure in aging, disease, and skeletal muscle injury models such as denervation injury or tissue ischemia. Indeed, the intimate physical connection between the nerve and muscle is critical for the function, survival, and morphology of the muscle fibers. Conversely, the loss of innervation is a major contributor to the pathological loss of muscle fibers, mass, and strength.

Until recently, the pathology of innervation following VML injuries had not been thoroughly examined. It was generally assumed that a significant number of NMJs were removed or damaged in conjunction with the loss of other structural and functional properties within the muscle tissue. However, evidence to support this claim is limited and can vary depending on the injury model and sequela of the injury. In one study specifically, acetylcholine receptors were used as a marker to count NMJs throughout the muscle, revealing significantly less clusters following a VML injury when compared to the uninjured contralateral limb (Anderson et al. 2019). This data supports the idea that NMJs are removed as a result of the injury model used in this particular study. However, it is important to note that the loss of innervation due to the direct removal of tissue may vary based on the size and location of the VML injury. Furthermore, the labeling of acetylcholine receptors alone, while beneficial to determine the number of NMJs, fails to account for other critical structures important for characterizing innervation. To this end, a recent study found progressive secondary loss of innervation up to seven weeks post-VML injury in the remaining tissue (Sorensen et al. 2021). This was done by examining the colocalization of pre- and postsynaptic NMJ structures. These findings are

supported by a previous study which showed significant axotomy following VML injury (Corona et al. 2018a), suggesting that the impact of VML extends well beyond the initial injured area (Fig. 6.2). This may help explain the long-term dysfunction that persists following VML injures as well as highlight innervation as a promising therapeutic target for improved healing and restoration of function in future studies.

6.3.6.1 Experimental and/or Clinical Outcome Measurements

As muscle and nerve communicate in a bidirectional way, being able to measure motor unit defects separately from muscle defects provides insights into their pathophysiologic interplay. Therefore, considerations for future studies should understand that there are three main approaches used for identifying denervated muscle fibers. The first method is an indirect approach that combines electrical stimulation: direct muscle membrane simulation and stimulation through the nerve to test neuromuscular transmission failure (Greising et al. 2015). The second method also measures innervation indirectly using a continuous neural stimulation protocol to deplete innervated muscle fibers of glycogen, followed by histological identification of myofiber glycogen content (Corona et al. 2013a). The third method is performed by directly examining the integrity of the NMJ using immunohistological probes that identify NMJ structures. In the first method, the comparison of the muscle contractile response elicited by nerve stimulation and the response of the same muscle evoked by direct stimulation of its membrane has been proposed as an indirect measurement of NMJ functionality. Indeed, since membrane stimulation bypasses neurotransmission signaling, any differences in the two contractile responses may be ascribed to changes in the NMJ. This approach has been extensively used in porcine, rat, and mouse models of aging and disease. For the second method, the muscle undergoes an intense contractile protocol involving several bouts of electrically stimulated muscle contractions. Once the bouts are completed, the tissue is immediately harvested and probed for identification of glycogen stores. Innervated fibers will be void of glycogen, while denervated fibers will maintain glycogen stores (Corona et al. 2013a). For the third method, antibodies are used to label the presynaptic terminal of the NMJ along with the motor axons. Additionally, a neurotoxic venom from the Taiwanese many-banded krait, known as α-bungarotoxin, is used to label the postsynaptic terminal. When used in combination, investigators can determine the innervation status of the NMJ. Complete colocalization of the pre- and postsynaptic terminals indicates fully innervated, while the partial or complete absence of the presynaptic terminal would represent denervation. Furthermore, morphological characteristics such as fragmentation, poly-innervation, axon sprouting, and axon thinning, which are thought to play a role in NMJ remodeling during development or following acute injury, can be identified. When using this approach, it is highly advised to consider the anatomical structure and location of the NMJs. Indeed, the pre- and postsynaptic terminals of the NMJ and corresponding axons run longitudinally along the muscle fibers, indicating that thickly cut sections or whole tissue samples be used in the longitudinal

orientation. It is possible to use the typical cross-sectional orientation commonly seen with skeletal muscle; however, a cross-sectional orientation may fail to identify important data concerning the NMJ.

It is important to note that a number of studies have identified newly forming NMJ structures in and around the VML defect area following the use of regenerative interventions (Turner et al. 2010; VanDusen et al. 2014), suggesting that the potential to reinnervate fibers within the defect area is possible. Additionally, the discovery that NMJ size in the remaining tissue is preserved (Sorensen et al. 2021) coupled with lack of motor unit death in the spinal cord (Corona et al. 2018a) is further confirmation that innervation can likely be rescued. As such, future studies need to strongly consider targeting and performing in-depth analysis of innervation following regenerative and rehabilitative interventions, as this may be an undervalued explanation for regenerative and functional deficits.

6.3.7 Vasculature

The vascular network within skeletal muscle is highly organized into branching networks of smaller arteries, arterioles, capillaries, and venules making up the microcirculation. Microcirculation is critical for the exchange of water, gases (e.g., O_2, CO_2), nutrients (e.g., glucose, free-fatty acids), and waste products between the muscle tissue and blood. Skeletal muscle metabolic demand can increase several hundred-fold with incremental levels of physical activity, meaning perfusion and diffusion properties of microcirculation are important variables for sustained muscle function. There are three factors influencing the severity of VML injury on muscle vascularity: (1) the amount of microcirculation lost with the physical removal of the muscle, (2) disrupted microcirculation perfusion and diffusion in the remaining muscle, and (3) the extent to which re-vascularization can occur in the remaining muscle and/or tissue that replaces the muscle lost during the VML injury (Fig. 6.2). There is evidence of vascular growth near the injury site in the weeks following VML injury in rodent models, but the extent of perfusion to those and existing networks remains questionable (Anderson et al. 2019; Southern et al. 2019; Li et al. 2017). Ultimately, the loss of microcirculation after a VML injury has been implicated in contributing to mitochondrial dysfunction, impaired muscle stem cell activation, and extensive fibrosis in the remaining tissue. The microcirculation is a critical target for regenerative medicine and rehabilitation approaches alike to improve the overall quality of muscle post-VML injury.

6.3.7.1 Experimental and/or Clinical Outcome Measurements

There exist several outcome measurements for vascularization of both the remaining muscle and defect area post-VML injury that vary in accounting for the quantity of vessels and function of those vessels. Capillary density and the capillary-to-fiber

ratio can be assessed histologically with alkaline phosphate or capillary-specific antibodies (e.g., CD34) and skeletal muscle fiber membranes (e.g., dystrophin) (Dalske et al. 2021; Machingal et al. 2011). Capillary density and capillary-to-fiber ratio can indicate the theoretical perfusion and diffusion limitations within muscle, i.e., fewer capillaries increase the diffusion distance from any point within a muscle fiber to the nearest capillary. Greater diffusion distances negatively affect the ability of muscle fibers to exchange nutrients, gases, and waste products. In normal physiological settings, only 40–60% of capillaries will be perfused with blood at any time; therefore, greater physiological relevance is placed on outcome measurements of perfusion. Contrast imaging in which a radioactive dye is pushed through the left ventricle into circulation and imaged using μCT can provide a closer representation of vascular beds and their perfusion parameters (Southern et al. 2019). There are many examples of this approach being used to interrogate the pathophysiology of VML injuries as well as monitor vascularization of tissue transplants into the defect area left by a VML injury. Laser Doppler flowmetry is a noninvasive technique that relies on the Doppler shift of laser light to detect blood flow to skeletal muscle. Another noninvasive technique is near-infrared spectroscopy that measures changes in near-infrared light absorption that can be inferred as blood flow and oxy/deoxyhemoglobin levels. There are some examples of these noninvasive techniques being used to validate regenerative medicine approaches following VML injury, but in general noninvasive blood flow imaging studies are a challenge area for advancement of understanding the VML pathology.

6.3.8 Long-Term Disability and/or Delayed Amputation

The path of VML-injured patients to even modest improvements in functional capacity is currently long and hindered by comorbidities. Rehabilitation alone is unlikely to attenuate the sequela of pathology in the remaining muscle, leading to chronic limb dysfunction. A study of medically retired battlefield-injured servicemembers reported that a muscle condition contributed to 53% of the total disability rating, which reflects a servicemember's physical fitness and disability payment (Corona et al. 2015). Additional rehabilitation time does not significantly improve physical fitness and further deterioration has been reported (Rivera and Corona 2016), at which point late-stage amputation becomes a primary concern (Krueger et al. 2012; Stinner 2016; Stinner et al. 2010). Functional deficits secondary to VML injuries likely contribute to late-stage amputation. In theory, a proportion of late-stage amputations could be avoided if clinically viable treatment approaches were available to improve the regenerative potential of the remaining muscle. For the VML-injured patient, any functional gains beyond those achieved with rehabilitation are expected to have a considerable impact on quality of life and as such regenerative rehabilitation may be an ideal tool to accomplish this goal.

6.4 Rehabilitation Approaches

6.4.1 Model Limitations

Animal models are invaluable tools for disease research. They allow for the profound study of biochemical pathways and physical adaptations in a living system, and provide a platform for the screening, testing, and developing of new pharmaceuticals. Despite their usefulness, there are many instances in which disease progression or treatment response between animal models and humans generally differs (Hartung 2008; Hackam 2007; Hackam and Redelmeier 2006). For example, animal VML injury models are highly controlled and may not always translate to what is observed in clinics based on injury location, size, species, age, sex, and severity. Not to mention the anatomical and ambulatory differences between animals and humans. While these systems serve to fill a technical gap, there are disadvantages and limitations that must be kept in mind when inferring or translating study results to a human context.

6.4.1.1 Physical Constraints

A large number of orthopedic models are developed in rodents or large animals, like sheep or pigs (Hartung 2008; Moran et al. 2016; Rhrissorrakrai et al. 2015). The similarity in muscle composition offers valuable insight on the biochemical pathways activated during injury and recovery and serve as an excellent testbed to gauge biological response to therapies. However, data cannot be directly correlated to human condition. As quadrupeds, weight distribution, pennation and torque angles, and muscle position vary considerably to that of humans (Hartung 2008; Moran et al. 2016; Shayan and Huang 2020). Therefore, the strain placed on a muscle in an injury model may not accurately portray the actual injury.

The magnitude and location of VML injuries result in considerable heterogeneity and necessitate a cadre of therapies (Rodriguez et al. 2020). For instance, a small VML defect isolated to a peripheral portion of a muscle unit may be best compensated by synergist hypertrophy mediated through physical therapy, whereas VML defects have been shown to not respond to conventional physical therapy and may require regenerative medicine augmentation. This could be due to the heterogeneity of injury defect size and locations seen in clinic, which make the standardization of treatment protocols difficult. Regenerative treatments for VML have utilized various strategies but require significant further advancement to be of therapeutic benefit to patients presenting with acute or chronic VML injury.

VML injuries are known for having long periods of recovery during which the muscle undergoes several bouts of inflammation and the limb remains mostly immobile before healing due to commonly concomitant bone injury (Corona et al. 2015). By virtue of the animals these models are developed in, recovery from VML occurs in a significantly shorter time frame than their human counterparts. This is

especially true in rodents, where injured subjects are able to ambulate, run, and even jump within minutes of recovering from anesthesia. Moreover, there is no period of limb immobilization or disuse unless the animal is physically restrained or the nerve innervating the muscle group of interest is severed. While it is advantageous for researchers to have a reduced window for disease progression and healing, it can sometimes lead to treatments being suspended during clinical trials despite the fact that the preclinical data were promising.

6.4.1.2 Metabolic Constraints

A major pitfall that hinders models of nearly every disease is the determination of appropriate dosages for pharmacological adjuvants (Hartung 2008). In some cases, there are metabolic redundancies in animals not present in humans that affect the organism's response to a treatment (i.e., immune system response and robustness being distinctly different) (McGonigle and Ruggeri 2014) that leads to a discrepancy in the predicted bioavailability in humans (Musther et al. 2014). Though there are mathematical models that can help predict dosing for humans, there are still major lags in first-in-human (FIH) estimations (Zou et al. 2012) to achieve the observed effect from animal models. This is especially important when working on new dosages for off-label indications of medications already on the market.

6.4.1.3 Functional Assessments and Outcomes

In clinical settings, patients who have suffered VML injuries are evaluated on a wide range of parameters, including clinical photography and gait analysis, range of motion, muscle strength, and isometric muscle function (Sicari et al. 2014). All these evaluations are directed at assessing the muscle's performance following injury and subsequent recovery. Assessment and outcome measures are often guided by the longtime goal of the research project and are not a standard or comprehensive evaluation of muscle soundness. Therapies that aim to address filling the defect area left behind after VML injuries may not wish to include the same outcome measures as those therapies intending to prime and improve the remaining muscle tissue. As an example, a number of VML studies use histological evaluation (e.g., fiber number, fiber diameter, MyHC expression) as the metric for muscle regeneration in the defect area but fail to include measures of strength. This type of outcome not only disregards the importance of proper muscle function as the desired result, but it wrongly equates hypertrophy with functional improvement. As such, standardization of outcomes measures to match those seen in clinical settings is a necessary addition to the VML model field. In order to maximize the ability of VML studies to inform future evidence-based approaches, we propose an outcome measurements hierarchy to consult during study design (Fig. 6.3). This model prioritizes functional outcomes with clinical comparisons, e.g., gait analysis and muscle strength, and gives less priority to measurements that cannot provide direct interpretation on the

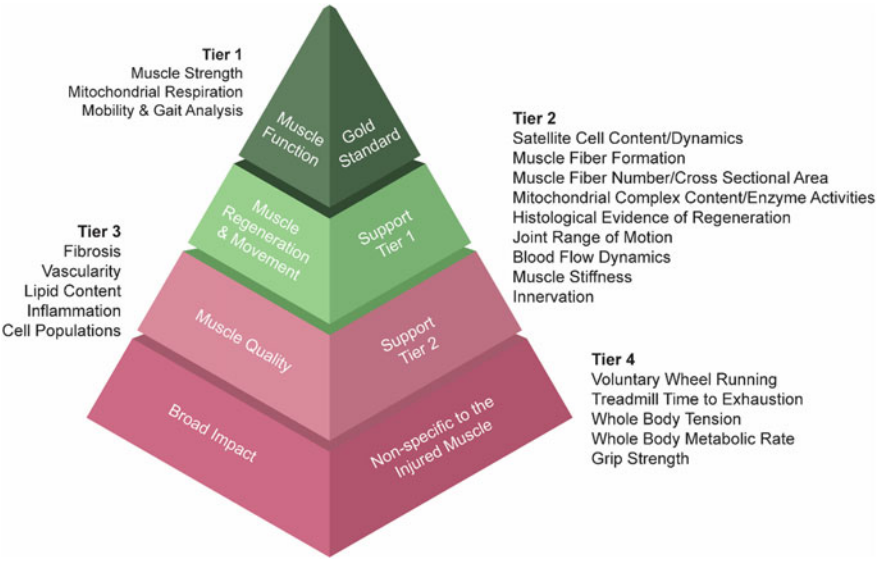

Fig. 6.3 A four-tier model for evaluating outcome measurements for VML. Tier 1 includes outcomes with clinical similarities; Tier 2 are outcomes that most directly influence Tier 1; Tier 3 provide broad evidence of muscle quality; and Tier 4 are outcomes involving other systems in the body making direct interpretation of muscle function difficult

health of skeletal muscle; e.g., treadmill running involves the cardiovascular and respiratory systems.

Models are not meant to be perfect representations. They exist to minimize the variables within a system to isolate a few tangible elements. Acknowledging the limitations within that system increases the study's robustness and prevents overselling of positive results. Moreover, it is each researcher's responsibility to create model systems that can be accurately translated to human research and that add value or improve upon the current understanding of VML pathophysiology.

6.4.2 Physical Rehabilitation

Currently, no corrective physical rehabilitation guidelines exist for the treatment of VML injuries. Perhaps, it is due to the uncertain adaptability of the remaining skeletal muscle using rehabilitation techniques. Various models of physical rehabilitation following VML have been used in preclinical models, including voluntary wheel running, forced treadmill running, chronic-intermittent electrical nerve stimulation to induce concentric or eccentric contractions, and/or passive range of motion exercises (Washington et al. 2021; Quarta et al. 2017; Southern et al. 2019; Greising et al. 2018; Aurora et al. 2015). Collectively, these diverse approaches have shown that modest contractile adaptations are possible. Meanwhile, clinical reports have

shown that VML patients see moderate improvements before their progression halts and further rehabilitation, regardless of the type or intensity, fails to improve function. Collectively, rehabilitation following VML has resulted in modest, if any, improvement in muscle function without any physiological rationale or mechanistic understanding for the lack of significant response.

Despite the discouragement of unsuccessful attempts to improve functional outcomes following VML injuries, it should not be interpreted to suggest that all forms of physical rehabilitation will fail. It is merely that the appropriate combination of rehabilitative and regenerative medicine therapies has not been identified. Furthermore, a rehabilitative standard of care does not exist and the variables that need to be considered for are extensive, including the type, timing, volume, and intensity of the therapy. Future research needs to focus on the synergistic potential of regenerative rehabilitation, where regenerative therapies are combined with rehabilitation in an attempt to restore functional muscle tissue to VML-injured patience.

6.5 Regenerative Medicine

6.5.1 Cell Therapies

Skeletal muscle stem cells, known as satellite cells, have a unique ability to fuse to existing fibers (hypertrophy) or fuse together to form new fibers (hyperplasia), making them indispensable for the endogenous repair and regeneration of skeletal muscles. Satellite cells reside underneath the basal lamina on skeletal muscle fibers, typically in a quiescent state. Following injury, satellite cells become activated and migrate to the injury site where they expand and a portion of the cells differentiate into functional muscle tissue to repair, regenerate, and restore the muscle to its previous healthy state. The remaining portion of satellite cells returns to their original quiescent state to preserve the stem cell pool. Unfortunately, the ramifications of VML on satellite cells within the remaining muscle are not completely understood and current strategies aimed at exploiting satellite cells as a source of regenerative medicine have been hampered with challenges. For instance, across various VML injury models and species, satellite cells demonstrate limited migration following injury. Specifically, all satellite cells are found within the remaining tissue, while the injury area is devoid of a satellite cell presence or meaningful regenerating muscle fibers. Studies have shown that gene expression of Pax7 (a satellite cell marker) initially increases post-VML, but its expression declines chronically, suggesting a lack of endogenous proliferative capacity (Aguilar et al. 2018). In an effort to overcome these challenges, researchers have attempted to add various donor cells (e.g., satellite cells, inducible pluripotent stem cells, mesenchymal cells) into the VML defect area (Baker et al. 2017; Merritt et al. 2010; Mori et al. 2015; Oshima et al. 2014; Passipieri et al. 2017; Qiu et al. 2018; Quarta et al. 2017; Shi et al. 2009). A variety of approaches have been used to accomplish this, the most common being the application of purified satellite cells alone or in combination with hydrogels or

scaffolds. These approaches have shown varying results with limited impact on restoring muscle function. Some of the challenges with this approach have been linked to poor satellite cell engraftment. In other words, the cells fail to adhere to the existing tissue and have been described as having lost their "stemness." A possible explanation may be that in order to generate a sufficiently large pool of satellite cells, they must first undergo in vitro expansion. While this technique can quickly expand the satellite cell pool, it also depletes the cells of their unique stem cell characteristics. Therefore, another approach which has shown some promise for improving muscle function is the use of a minced muscle graft (Corona et al. 2013a; Ward et al. 2015), which replaces all endogenous cell types and the components of the ECM. This approach does not require the in vitro expansion of the satellite cells. Additionally, it maintains the cell's natural environment by delivering the ECM and basal lamina wherein the satellite cells typically reside. Moreover, resident cells such as fibro/adipogenic progenitors, which have been shown to promote satellite cell expansion, are readily present in close proximity to the satellite cell population. However, all of these techniques still present challenging obstacles that need to be overcome, especially when considering a translational approach from animal models to humans.

6.5.2 Pharmaceutical Adjuvants

As discussed throughout the course of this chapter, a common consequence of VML injury is the overwhelming accumulation of inflammation and fibrosis that impede the endogenous regenerative response of the remaining tissue. A possible solution to restore a portion of the tissue's regenerative potential has come through the repurposing of FDA-approved pharmaceutical agents that have shown beneficial effects in other disease or injury conditions. To this end, some studies have administered anti-fibrotic agents as a primary treatment source for VML, while others have used anti-inflammatories as a mechanism to mitigate tissue donor rejection or dampen the inflammatory response when implanting a foreign material (i.e., scaffold). In fact, few studies have examined the functional impact of anti-inflammatories as a single treatment approach. What has generally been found is that functional outcomes are no different when using an anti-inflammatory versus no use, while modest improvements in muscle function are observed when combined with a muscle graft treatment (Corona et al. 2017b; Goldman et al. 2020, 2021), indicating that a small synergistic effect may occur when anti-inflammatories are used in combination with other regenerative therapies.

To date, some anti-fibrotic treatments for VML have targeted various signaling pathways such as TGF-β (Garg et al. 2014a), FGF, PDGFRα/β, and VEGF (Corona et al. 2020), which have known roles in fibrosis development. Paradoxically, many of these pathways also support other cellular functions within the muscle tissue. Perhaps, it is not surprising then that the administration of an anti-fibrotic treatment can successfully prevent fibrosis. However, the anti-fibrotic treatments do not

typically address skeletal muscle contractile function and in some cases lead to further decline in contractile force (Corona et al. 2020; Garg et al. 2014a). This supports the idea that a portion of the early improvements in muscle function are likely driven by the accumulation of force transmitting connective tissue. Despite these findings, future studies should continue to identify potential targets for preventing fibrosis or combine the currently available anti-fibrotic treatments with other regenerative and rehabilitative therapies.

6.5.3 Growth Factors

Skeletal muscles' robust capacity to adapt is dependent on the type of stress that the muscle is exposed to. For example, the ablation of the gastrocnemius muscle will result in a compensatory hypertrophic response of the remaining plantaris muscle in rodents (Degens et al. 1995), attributed to the increased demand placed on the muscle. However, the ability to adapt to stress following VML injury is lost, such that compensatory hypertrophy of the remaining muscle does not occur. It is well known that certain signaling pathways are activated as a result of injury or exercise to stimulate adaptation. The molecules that drive these adaptations are proteins commonly referred to as growth factors. It is thought that by manipulating endogenous signaling pathways following VML injury, it may be possible to rescue the adaptive potential of the remaining tissue. As a result, growth factors have been used alone or in combination with engineered muscle constructs to deliver a sustained concentration of the desired molecule during the recovery process, while also providing additional structural support to injured muscle. The results have presented both promising and conflicting outcomes, which are likely dependent on the specific molecule used. For example, a VML injury treated with a keratin construct and basic fibroblast growth factor (bFGF) showed poor force output two months after implantation (Baker et al. 2017). This may be partially explained by the discovery that molecules such as bFGF, hepatocyte growth factor (HGF), and insulin-like growth factor (IGF) are already abundantly secreted into the VML defect area. However, when the keratin and bFGF construct was further supported by IGF, there was a synergistic increase in force that was not observed by either factor alone (Baker et al. 2017). On the other hand, molecules that are downregulated following VML injury may hold greater promise for rescuing a portion of the injured muscles' adaptive capacity. One such molecule is PGC-1α, when overexpressed in VML-injured muscle there was improved oxidative capacity (Southern et al. 2019). Overall, endogenous molecules do have the potential to improve VML outcomes if the molecule is suppressed following VML injury and used in combination with other therapies.

6.5.4 *Biomaterials*

Despite the fact that the term "biomaterial" has only recently come into use, humans have been using materials to aid in healing for centuries (Ratner and Zhang 2020; Migonney 2014) as noted in Chap. 1. In early civilizations people used wood, bones, animal teeth, and sinew for dentistry applications, making prosthesis for amputated or withered limbs, and sutures. As technology evolved, so did practices. The field reached a new cusp with the scientific breakthrough of polymer synthesis. Technology has allowed for these new implants to achieve better performance, functionality, and reproducibility than the naturally derived materials of old. Nowadays, the term biomaterial is used as a broad category that includes medical implants, aids for healing and regenerating human tissue, molecular probes, biosensors, and drug delivery systems.

The study of VML injuries has brought with it a vast array of natural and synthetic polymer materials. These materials have been manipulated in a variety of shapes, including natural or native structure sponges, gelloids, and fibers. With the use of these scaffolds, researchers have sought to carry growth factors, cells, and pharmaceutical adjuvants to the injury defect in hopes of restoring muscle mass and function. More importantly, the use of biomaterials can open a new field of VML treatment, where the muscle environment can be modulated through chemical and spatial cues, priming the muscle for future physical therapy.

6.5.4.1 Biocompatibility and Biomaterials Presentation

With the increased availability of both natural and synthetic materials to aid in healing, came an interest in studying the interaction of materials with the environment in which they were to be introduced. Many early biomaterials were chosen on the basis that they remain "inert" or "non-fouling" within the body, not triggering a significant immune response or interacting with the tissue in any meaningful way (Ratner 2019). As the field has evolved, however, more importance is being put upon the materials being able to modulate the cell environment to promote healing rather than remain an inert substance (Abdulghani and Mitchell 2019). This brought about the new concept of biocompatibility–material-host synergy.

Biomaterials engineering is a highly interdisciplinary field that is under constant improvement. New synthesis and functionalization techniques allow for the modification of both naturally and synthetically derived biomaterials to fit nearly any need. Materials developed for implantable devices are tested for reactivity, as they typically go through the entire process of FDA approval, and their function may depend on their non-interaction with the organismal environment. Soft materials, non-devices such as beads, micro- and nanoparticles, gels, and scaffolds, inherently become part of the environment upon implantation. Their interaction with the host's environment is sometimes neglected, leading to failure in vivo despite its promise

in vitro. This issue may stem from the lack of knowledge of the cellular and molecular mechanisms activated by implantation leading to poor material stability.

The extracellular environment following VML injury is flooded with cell debris, signaling molecules, and immune cells simultaneously coordinating several complicated molecular mechanisms. Many of these mechanisms are tissue-specific and require different biochemical signaling pathways, macromolecules, and signal transducers. Both natural and synthetic materials can be modified or functionalized so their surfaces can positively influence the extracellular environment. Some of these modifications can include creating hydrophilic or hydrophobic interaction sites, attaching charged or protein-specific binding sites, loading the material with a payload that influences the muscle environment, and modifying degradation kinetics to fit the time frame desired for study. Additionally, the chemical properties of the base material, including the products of its degradation, can be used to the researcher's advantage to modulate cellular response.

Properties such as topographical features, stiffness, pore size, and cell binding sites will influence the effectiveness of the biomaterial (Rahmati et al. 2020). Many researchers have reported on the influence of physical characteristics of a biomaterial on the cell fate and survival of different cell types (Abdulghani and Mitchell 2019). The surface topography of a scaffold can affect the determination, adhesion, differentiation, and migration of resident stem cells, leading to unwanted cell fates, such as adipose or fibrotic tissue. The stiffness of the muscle determines the amount of torque that can be produced from voluntary contraction around an axis (Abdulghani and Mitchell 2019). For example, an overly stiff material (Young's modulus) deposited into the muscle defect of a VML injury could hinder contraction of the muscle as the tissue heals or not allow for the migration, binding, and proliferation of stem or satellite cells within the material pores. Conversely, materials pre-loaded with myoblasts or satellite cells with unfavorable physical properties may not be able to maintain cell viability in vivo or allow for the migration of those cells into the existing myofibers.

Thus, an effective biomaterial should not hinder but enhance the natural process of tissue healing. Researchers must consider the possibility that in vitro data collected on these materials may not represent the conditions present in the extracellular niche in vivo.

6.5.4.2 Natural Biomaterials

For centuries, humans have been using naturally based materials to help heal their maladies. Wood and bone were some of the first materials used to mend broken limbs and replace teeth. Currently, naturally derived materials are used as biomaterials for their availability, biocompatibility, and biomimetic properties. These materials can be divided into four broad categories: proteins, polysaccharides, glycosaminoglycans, and cellular matrices (Brovold et al. 2018). The field of VML research has seen representatives from all of these categories as vehicles for the delivery of cells and therapeutics into the injury defect. Figure 6.4 shows

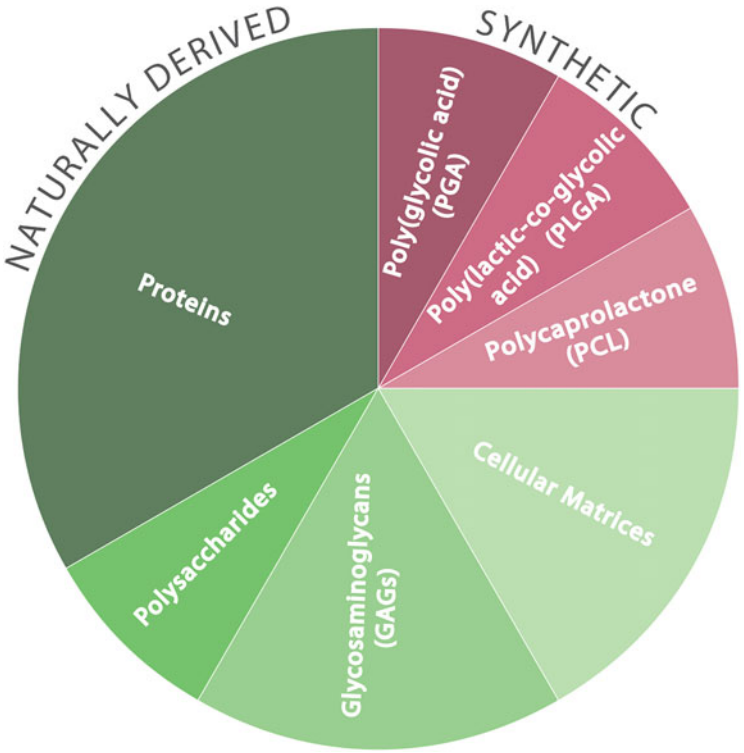

Fig. 6.4 Summary of materials tested for the treatment of volumetric muscle less injuries. The field of VML has seen a moderate number of studies where biomaterials have been engineered to either deliver a payload or encourage the growth of new tissue in the injury defect. Size and proportions of each category on the chart correlate with the prevalence for each material in research. Naturally derived materials make up the bulk of these studies, with protein scaffolds being the most popular. The use of synthetic materials has been significantly less explored, but it can be expected to increase as the field of bioengineering in VML injuries expands

different categories of scaffold materials that have been used in VML injury studies. The relative size of each category relates to the ubiquity of the material's use in research.

6.5.4.2.1 Protein Scaffolds

Collagen

Collagen is the most abundant structural protein in the body. It is secreted by fibroblasts as part of the ECM and provides support to diverse tissues, such as bones, tendons, and skin (Brovold et al. 2018). Though typically used as a scaffold for bone repair for its enhanced strength and mechanical properties, collagen can be modified to serve soft-tissue purposes. This material can be processed into beads,

sponges, meshes, and fibers that are conducive to cell growth, biocompatible, and biodegradable. It is easy to isolate and has many varieties, and its physical, structural, and chemical characteristics are well known.

Collagen is a popular material base for the construction of gels, fibers, meshes, and sponges used in VML injuries. Use of collagen sponges to treat VML muscle defects in the vastus lateralis muscle of rabbits resulted in regenerating myofibers on the periphery of the defect at 4 weeks and the partial connection of regenerating myofiber stumps at 24 weeks (Kin et al. 2007). Additionally, collagen scaffolds loaded with insulin-like growth factor-1 (IGF-1) have promoted proliferation and initiation of myogenic fusion of myoblasts, in vitro (Alcazar et al. 2020). Moreover, these biomaterials aided in the activation of satellite cells to the VML injury and scaffold site, in vivo.

Gelatin

Gelatin is a material derived from the hydrolysis of collagen (Brovold et al. 2018). Due to this, its structure and physical and chemical attributes are well known. It has an advantage over collagen in that it is readily soluble in aqueous solution, is commercially available, and can be easily cross-linked or chemically modified to fit a wide variety of applications. In a rat VML model, cross-linked gelatin hydrogels were used to deliver osteoactivin. Implantation resulted in immune cell encapsulation at 2 weeks, followed by cell infiltration into the gel at 4 weeks (Ma et al. 2017).

Gelatin has also been used in combination with other materials to create less expensive, stable, biomimetic sponges. The use of these sponges and a combination of gelatin, its polymer, collagen, and laminin have been shown to support C2C12 infiltration and survival in culture, as well as stimulate the expression of myogenic markers such as MyoD and MyoG. When implanted into VML defects, these sponges support satellite, endothelial, and inflammatory cell growth but did not offer significant myofiber regeneration after 2 weeks of treatment (Haas et al. 2019).

Keratin

Keratin encompasses a group of insoluble proteins that associate as intermediate filaments (Rouse and Van Dyke 2010). These proteins are broadly divided into two groups: hard keratins, which are embedded in cystine-rich proteins, and soft keratins which form loose bundles and offer mechanical resilience to epithelial cells. Keratin monomers have the ability to self-assemble and polymerize into porous, fibrous scaffolds with cell binding motifs that can create a three-dimensional matrix that allows for cell attachment and proliferation.

Hair-derived keratin hydrogels loaded with either/or in combination of IGF-1 and fibroblast growth factor have had a positive impact on muscle regeneration following VML injury. Implantation of these fibers resulted in significantly greater functional recovery (in both histological and contractile force analyses) over untreated muscle 8 weeks post-injury (Passipieri et al. 2017; Baker et al. 2017).

Oxidatively extracted keratin and reductively extracted keratin, keratose, and kerateine, respectively, have also been tested as therapeutic candidates for VML injuries. Though these materials possess different structural, chemical, and mechanical profiles, both keratin derivates offered protection to deliverable skeletal muscle progenitor cells and growth factors. Additionally, keratin-based biomaterials elicit minimal inflammatory and immunological response while increasing expression of muscle-specific markers (Tomblyn et al. 2016).

Fibrin

Fibrin is a protein produced from the cleavage of fibrinogen (Brovold et al. 2018). It has strong adhesive properties and promotes cell attachment and angiogenesis. Cell-laden fibrin microthreads stimulated the formation of skeletal muscle fibers, connective tissue, and Pax7-positive cells. These constructs decreased pathologic deposition of collagen in the wound and promoted the growth of new tissue. Moreover, the implantation of these cell-carrying microthreads into the site of injury restored contractile force to the muscle (Page et al. 2011).

Other reports of cell-laden fibrin microfibers implanted into VML defect sites had increased expression of total regenerating muscle marker embryonic MyHC and adult MyHC isoforms. The combination of material and adipose-derived stromal cells increased muscle reconstruction and qualitative muscle parameters in vivo in the VML defects (Gilbert-Honick et al. 2018a), while cross-linked fibrin loaded with hepatocyte growth factor significantly enhanced the force production of muscle tissue 60 days after injury, as well as enhancing myoblast differentiation and angiogenic response (Grasman et al. 2015).

6.5.4.2.2 Polysaccharides

Chitin and Chitosan

Chitin is an abundant polysaccharide that is found on the exoskeleton of arthropods and the cell wall of fungi (Brovold et al. 2018). It is biocompatible and biodegradable, and has been shown to support cell attachment and proliferation due to its porous nature and abundance of physical cues. Chitosan is the fully or partially N-deacetylated derivative of chitin and shares many of its biocompatibility characteristics with its progenitor.

Previous studies have undertaken the creation of self-healing N-carboxyethyl chitosan injectable hydrogels able to encapsulate either C2C12 myoblasts or human umbilical vein endothelial cells (HUVEC) (Guo et al. 2019). These constructs not only maintained the viability and promoted the proliferation of encapsulated cells but increased the number of centrally localized myonuclei in TA muscle at 1 and 4 weeks following VML injury.

6.5.4.2.3 Glycosaminoglycans

Hyaluronic Acid

Hyaluronic acid (HyA) is a disaccharide found in most connective tissues, synovial fluid, and vitreous of the eye (Brovold et al. 2018). It is easily chemically modifiable, viscoelastic, hydrophilic, biocompatible, and biodegradable. Studies have evaluated the additive effect of a hyaluronic acid and laminin hydrogel as a myoconductive scaffold co-delivered with minced muscle grafts (Goldman et al. 2018). While HyA gels did not offer any improvements in peak tetanic force production over minced muscle grafts, the use of HyA hydrogels has been shown to improve strength over no treatment (Dienes et al. 2021). Additionally, HyA hydrogels functionalized with the laminin peptide IKVAV can potentially enhance migration, proliferation, and muscle-specific transcription factors in myoblasts (Silva Garcia et al. 2019).

6.5.4.2.4 Cellular Matrices

Decellularized Matrices (Biomimetic Scaffolds)

Decellularized and acellular matrices or scaffolds are some of the most commonly used regenerative therapies in VML injuries. Decellularized matrices are derived from tissue explants or ECM that has been trypsinized and washed in detergent to eliminate any cells. What is left behind is the structural proteins which can then become a platform for cell culture to deliver live, proliferating cells into the injury defect or for use on its own to stimulate the growth and differentiation of progenitor and satellite cells (Wolf et al. 2015; Goldman et al. 2020). These materials have the advantage of being biocompatible and easily integrated to the host injury system.

Previous research has reported mixed results on the success of these scaffolds as delivery systems or structural templates following VML injuries. Earlier reports saw a shift in muscle healing response from deposition of scar tissue in the VML defect to the remodeling of the muscle utilizing the ECM scaffold (Sicari et al. 2012). Further investigations have demonstrated the use of acellular scaffolds to create a pro-myogenic environment that supported the de novo formation of muscle fiber (Perniconi et al. 2011) and improvements in force production of the TA muscle after transplantation of muscle-derived ECM despite the exacerbated production of fibrotic tissue (Corona et al. 2014).

On a more physiologically relevant note, one clinical study used electrodiagnosis to evaluate the effectiveness of ECM scaffold implantation in VML patients. Baseline measurements were compared before and 6 months after implantation. In 5 out of 8 patients, the implanted scaffolds had electrical activity, suggesting enervation. This increase in electrical activity was seen in combination with an improvement of muscle strength (Han et al. 2016).

Minced Muscle Grafts

Based on the mixed success of the use of decellularized matrices as biological scaffolds, various researchers have suggested the use of homologous scaffolds for skeletal muscle regeneration. Skeletal muscle-derived ECM would provide an environment similar to that missing within the tissue defect of VML injuries, secreting all the necessary growth factors and signaling molecules required for myofiber growth. Studies from various labs report improvement in muscle generated contractile force with minced muscle graft treatment following VML injury (Chen and Walters 2013; Corona et al. 2013a; Corona et al. 2017a; Ward et al. 2016). VML injuries treated with minced muscle grafts also display improvements in histological muscle quality and weight and increased myogenic and regenerative markers (Corona et al. 2013a; Corona et al. 2017a; Corona et al. 2017b; Kasukonis et al. 2016).

6.5.4.3 Synthetic Biomaterials

The early twentieth century saw the invention of synthetic polymers. These composites gave way to a brand-new field of materials for use in medical applications. Compared to naturally derived materials, these polymers are relatively easy and inexpensive to produce while maintaining low batch-to-batch variability. These synthetic polymer materials have consistent mechanical, chemical, and structural properties and are readily tailored to vary stiffness and degradation kinetics (Sarkar et al. 2017). As the synthesis and creation of synthetic biomaterials has evolved, more alternatives of host-integrative, biocompatible, and biomimetic have arisen. Some of these have been used successfully as therapeutics in VML injuries.

6.5.4.3.1 Poly(Glycolic Acid) (PGA)

PGA is a biodegradable polyester typically synthesized by the polycondensation of glycolic acid (Sarkar et al. 2017). It is commonly used for its fast degradation kinetics, which yield acidic degradations products that are thought to induce inflammation. This material can induce localized immune response from effector cells due to its degradation products, though there is no consensus on whether this response hinders tissue regeneration. Saxena et al. reported on the use of PGA meshes seeded with myoblasts as a means to engineer three-dimensional skeletal muscle tissue (Saxena et al. 2001). They demonstrated for the first time the ability of myoblasts to survive in vivo in the absence of mature skeletal muscle tissue.

6.5.4.3.2 Poly(Lactic-Co-Glycolic Acid) (PLGA)

PLGA is an ester copolymer derived from PGA which incorporates lactic acid monomers with glycolic acid monomers by means of ring-opening

co-polymerization synthesis process. It is hydrolytically biodegradable and biocompatible, elicits low toxicity, and is one of the most used synthetic biomaterials for skeletal muscle applications (Wolf et al. 2015). The use of PLGA-myoblasts constructs induced the formation of multinucleated myotubes in a study by Kamelger et al. Implanted constructs showed light fibrovascular growth without inflammation (Kamelger et al. 2004). Another study by Narayanan et al. developed an aligned electrospun fiber scaffold using PLGA that enhanced alignment, growth, and differentiation of myoblasts, in vitro, and resulted in the formation of dystrophin-positive myofibers in a TA muscle model of Duchenne muscular dystrophy (Narayanan et al. 2020). Their research on how these fiber scaffolds enhance skeletal muscle regeneration in VML injuries is ongoing.

6.5.4.3.3 Polycaprolactone (PCL)

PCL is a biodegradable polyester approved by the FDA for use in drug delivery devices and sutures. In tissue engineering, PCL is often used due to its favorable mechanical properties and slow degradation kinetics (Wolf et al. 2015). Aligned PCL nanofibers have shown promise as therapeutics for VML injuries. Muscle defects treated with these fibers have shown to have an increase in regenerating myofibers, myogenic protein expression, and HSP-70 expression, as well as an overall increase in muscle mass over non-treated controls. Moreover, VML defects treated with PCL fibers exhibited decreased CLN expression at 28 days after injury, suggesting that the scaffold decreased the severity of injury and provided mechanical support to the defect site (Patel et al. 2019).

6.6 Regenerative Rehabilitation

The status quo as it pertains to treatment strategies for VML is (1) various regenerative medicine approaches that modestly improve function preclinically and require further maturation to achieve clinically meaningful functional improvement; (2) a dearth of VML studies that include reliable, clinically relevant outcome measurements of function; and (3) no standards of care for the patient. Regenerative rehabilitation offers a departure from the status quo by combining best practices of regenerative medicine and rehabilitation, especially if future studies incorporate clinically meaningful outcome measurements of muscle function. Table 6.1 provides examples of rehabilitation and regenerative medicine studies that included at least one Tier 1 (Fig. 6.3) functional outcome comparison between VML-injured with no intervention and VML-injured with intervention. This provides context to discuss next steps toward advancing regenerative rehabilitation for VML and pursuing standards of care for patients with VML injuries.

Table 6.1 (note references within) was adapted and expanded upon from a systematic review and meta-analysis published in 2019 (Greising et al. 2019). This

Table 6.1 Summary of select rehabilitation and regenerative medicine study outcomes

Approach	Tier 1 functional outcomes	Percent Δ to VML-injured, no treatment	Tier 2–Tier 4 supporting outcomes
Rehabilitation			
Electrical stimulation plus passive range of motion (2xWk, 1-4mo duration) (Greising et al. 2018)	Peak-isometric torque; passive range of motion; mitochondrial OCR	+30% −32% NC	Tier 2: None; Tier 3: Fibrosis, inflammation; Tier 4: None
Wheel running (Southern et al. 2019)	Peak-isometric torque, mitochondrial OCR	+42% NC	Tier 2: Mitochondrial complex content/enzyme activities; Tier 3: Vascularity; Tier 4: Voluntary wheel running
Voluntary wheel running (1 or 7 weeks) (Aurora et al. 2014)	Peak-isometric torque normalized to body weight and peak-isometric torque of tibialis anterior (with and without EDL)	With EDL +9% Without EDL +17%	Tier 2: Muscle fiber number/cross-sectional area, histological evidence of regeneration; Tier 3: Fibrosis; Tier 4: None
Regenerative medicine			
Gene overexpression (PGC1) (Southern et al. 2019)	Peak-isometric torque, mitochondrial OCR	+54% +41%	Tier 2: Mitochondrial complex content/enzyme activities; Tier 3: Vascularity; Tier 4: Voluntary wheel running
Autologous mince muscle graft (Ward et al. 2016)	Peak-isometric torque	+35%	Tier 2: None; Tier 3: Inflammation; Tier 4: None
Scaffoldless multiphasic muscle units (VanDusen et al. 2014)	Peak-isometric force	+36%	Tier 2: Muscle fiber number/cross-sectional area; Tier 3: None; Tier 4: None
Acellular matrices + MSCs (Qiu et al. 2018)	Peak-isometric torque	+67%	Tier 2: Muscle fiber formation; Tier 3: Fibrosis, inflammation; Tier 4: None
Bladder acellular matrices + MDCs (Corona et al. 2012)	Peak-isometric force	+61%	Tier 2: Satellite cell content/dynamics; Tier 3: None; Tier 4: None

(continued)

Table 6.1 (continued)

Approach	Tier 1 functional outcomes	Percent Δ to VML-injured, no treatment	Tier 2–Tier 4 supporting outcomes
Muscle-derived ECM + MSCs (Corona et al. 2013b)	Peak-isometric force Peak-isometric torque	NC NC	Tier 2: Muscle fiber number/cross-sectional area; Tier 3: None; Tier 4: None
Bladder acellular matrices + MDCs (Corona et al. 2014)	Peak-isometric torque	+26%	Tier 2: None; Tier 3: None; Tier 4: None
Porcine urinary bladder matrix (Aurora et al. 2015)	Peak-isometric torque	8 weeks +29% 16 weeks NC	Tier 2: Satellite cell content/dyanmics; Tier 3: Fibrosis, vascularity; Tier 4: None
MPCand growth factor-loaded keratin hydrogels (Baker et al. 2017)	Peak-isometric torque	Keratin alone = +31% Keratin+IGF-1 = +20% Keratin+bFGF = −6% Keratin+IGF-1 + bFGF = +61%, Kertatin+MPC = +3% Keratin +MPC + bFGF = +15% Keratin+MPC + IGF-1 = +28% Keratin+MPC + IGF-1 + bFGF = 2%	Tier 2: None; tier 3: Fibrosis, histological evidence of regeneration; Tier 4: None
Acellular scaffold (Greising et al. 2017)	Normalized-isometric torque	NC	Tier 2: Histological evidence of regeneration, satellite cell content/dynamics; Tier 3: Inflammation; Tier 4: None
Anti-fibrotic (losartan) (Garg et al. 2014a)	Peak-isometric force	−17%	Tier 2: Histological evidence of regeneration, satellite cell content/dynamics, Tier 3: Fibrosis, inflammation, lipid content; Tier 4: None
HGF loaded, cross-linked fibrin microthread scaffolds (Grasman et al. 2015)	Mean twitch force, force recovery: injured ratio	+25%	Tier 2: Satellite cell content/dynamics, histological evidence of regeneration; Tier 3: Vascularity, fibrosis, cell populations; Tier 4: None

(continued)

Table 6.1 (continued)

Approach	Tier 1 functional outcomes	Percent Δ to VML-injured, no treatment	Tier 2–Tier 4 supporting outcomes
Minced muscle graft (Aguilar et al. 2018)	Peak-isometric force	+64%	Tier 2: Histological evidence of regeneration; Tier 3: Inflammation; Tier 4: None
Muscle-derived ECM (Chen and Walters 2013)	Peak-isometric force	+14%	Tier 2: Muscle fiber formation, histological evidence of regeneration; Tier 3: None; Tier 4: None
Minced muscle graft (Corona et al. 2017a)	Peak-isometric force	NC	Tier 2: Muscle fiber number/cross-sectional area, histological evidence of regeneration; Tier 3: None; Tier 4: None
Muscle-derived ECM (w/ and w/o cells) (Garg et al. 2014b)	Peak-isometric force	w/o cells +16% w/ cells +35%	Tier 2: Satellite cell content/dyanmics, histological evidence of regeneration; Tier 3: Fibrosis, inflammation, cell populations; Tier 4: None
Laminin-111 supplemented hyaluronic acid-based hydrogel w/ minced muscle graft (Goldman et al. 2018)	Peak-isometric force	HA alone +17% HA + LMN +25% 50% MMG + HA +43% 50% MMG + HA + LMN +43%	Tier 2: Histological evidence of regeneration, satellite cell content/dynamics; Tier 3: Inflammation; Tier 4: None
Autograft (Li et al. 2014)	Peak-isometric force	4 weeks morselized = +5% whole = +1%	Tier 2: Muscle fiber number/cross-sectional area, histological evidence of regeneration; Tier 3: None; Tier 4: None
Prevascularized scaffolds with and without cells (Gilbert-Honick et al. 2018b)	Peak-isometric force	Acellular = +35% C2C12 = +32%	Tier 2: Histological evidence of regeneration; Tier 3: Fibrosis, cell populations; Tier 4: None
Tissue engineered muscle repair (Machingal et al. 2011)	Peak-isometric force	+64%	Tier 2: Muscle fiber number/cross-sectional area, muscle fiber formation; Tier 3: None; Tier 4: None

(continued)

Table 6.1 (continued)

Approach	Tier 1 functional outcomes	Percent Δ to VML-injured, no treatment	Tier 2–Tier 4 supporting outcomes
Progressive muscle cell delivery (Kim et al. 2016)	Peak-isometric force	+100%	Tier 2: Muscle fiber formation, histological evidence of regeneration, innervation; Tier 3: Vascularity; Tier 4: None
Decellularized + minced muscle autograft (Kasukonis et al. 2016)	Peak-isometric force	+38%	Tier 2: Histological evidence of regeneration; Tier 3: Fibrosis; Tier 4: None

is an important study to frame the current regenerative rehabilitation field because the authors identified 2312 studies (search ended January 2019); however, once those studies were screened for the inclusion of outcome measurements of muscle function, only 44 studies remained for the meta-analysis (2%). The lack of VML studies including a clinically meaningful outcome measurement is a current challenge area for the field if providing standards of care is a goal for the future. Skeletal muscle represents ~40% of a typical individual's body mass and represents an important metabolic engine for basal metabolism, and collectively the primary job of muscle is to contract, produce force, and help move the body. Ultimately, some measurement of metabolism, contractility, and/or movement should be involved in VML studies validating a new approach to improve the pathology.

Studies highlighted within the *Rehabilitation* and *Regenerative Medicine* sections of Table 6.1 reflect strategies designed to enhance muscle function by taking into consideration the pathology of the disease. For example, Aurora et al. (2014) and Southern et al. (2019) tested the effects of voluntary wheel running on outcome measurements of muscle strength, metabolic function, fibrosis, and vascularity. These are prominent areas of VML-related pathology described above. In the *Regenerative Medicine* section of Table 6.1 there are several approaches attempting to replace the lost extracellular matrix. For example, Qui et al. created a decellularized ECM scaffold and then seeded it with mesenchymal stem cells. Peak-isometric torque was reported ~67% greater in the intervention group compared to a VML group with no intervention. A similar ECM replacement approach was conducted by Garg et al.; only their research study design tested ECM with and without endogenous cells. Peak-isometric torque was ~16% greater without cells and ~ 35% greater with cells compared to a non-intervention group. These studies exemplify a balanced approach to improving muscle function after VML injury by addressing a pathology and relying on clinically relevant outcome measurements of muscle function.

Table 6.2 Summary of regenerative rehabilitation study outcomes

Regenerative rehabilitation			
Approach	Tier 1 functional outcomes	Percent Δ to VML-injured, no treatment	Tier 2–Tier 4 supporting outcomes
Acellular biomaterial + wheel running (Quarta et al. 2017)	Gait Peak-isometric force	+27% +67%	Tier 2: Innervation, satellite cell content/dynamics; Tier 3: Fibrosis, vascularity; Tier 4: None
Stem cell amniotic membrane scaffold + HIIT on treadmill (Izadi et al. 2021b)	Peak-isometric force	+180%	Tier 2: Innervation; Tier 3: Fibrosis, vascularity; Tier 4: None
Autologous mince muscle graft + wheel running (Corona et al. 2013a)	Peak-isometric force	+30%	Tier 2: Satellite cell content/dynamics, muscle fiber formation, innervation, muscle fiber number/cross-sectional area; Tier 3: Fibrosis, inflammation; Tier 4: Voluntary wheel running
Porcine urinary bladder matrix + voluntary wheel running (Aurora et al. 2015)	Peak-isometric force	+17%	Tier 2: None; Tier 3: None; Tier 4: None
HIIT training and decellularized human amniotic membrane scaffold (Izadi et al. 2021a)	Peak-isometric torque	+103%	Tier 2: None; Tier 3: Vasculature; Tier 4: None
Scaffold and mechanical loading (Dziki et al. 2018)	Peak-isometric force	+137%	Tier 2: None; Tier 3: Inflammation; Tier 4: None

Despite the gains in muscle function with rehabilitation or regenerative medicine noted in Table 6.1, further development of these approaches is required for full recovery. Statistically significant improvements in muscle strength, for example, range from 3 to 100% with intervention compared to a VML injury with no intervention (Table 6.1). However, a deeper interrogation of the data demonstrates these advances are still modest in terms of full recovery. For example, Kim et al. provided multiple injections of C2C12 myoblasts to VML-injured muscle and reported that muscle strength was 100% greater in treated vs. VML untreated rats (1.8 N kg^{-1} vs. 0.9 N kg^{-1}). But this significant improvement in strength pales in comparison to the strength of injury-naive controls (3.7 N kg^{-1}) highlighting the need for continued optimization of interventions for VML injury.

Regenerative rehabilitation seeks to take the best practices from rehabilitation and regenerative medicine alone and combine them to optimize restoration of muscle function after VML. To date, there have only been a few regenerative rehabilitation studies that included a clinically relevant outcome measurement of muscle function and a comparison to a non-intervention VML group (Table 6.2, note references within). The range of improvement in muscle strength is noteworthy in comparison

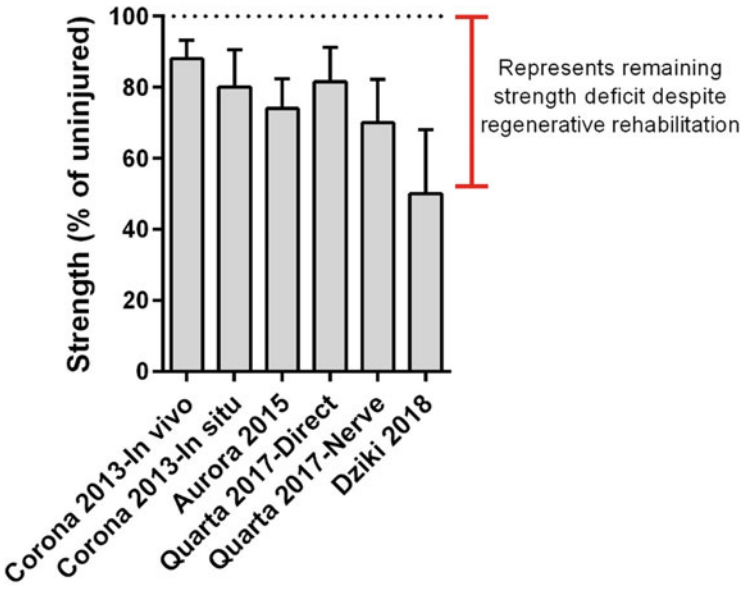

Fig. 6.5 Remaining muscle strength deficits from regenerative rehabilitation cohort of selected studies. Data are shown as a percentage of completely uninjured controls

to those in Table 6.1, 17–180%, and half of the studies report a gain above 100% compared to a non-intervention group. Though the sample size is small, these results support the notion that combined strategies can be more effective than rehabilitation or regenerative medicine strategies alone. That said, a comparison of the regenerative rehabilitation groups to uninjured cohorts, as opposed to VML untreated (Fig. 6.5), shows there is still significant room for improvement.

Beyond augmenting muscle function, these regenerative rehabilitation studies can also provide insights into the timing and/or absence of rehabilitation and the positive effects of combined approaches on other physiologically important outcomes. All of these studies, with the exception of Dziki et al., used a form of running as a rehabilitative approach (treadmill and voluntary wheel) (Dziki et al. 2018). Running stimulates adaptative responses through both mechanical and molecular cues (e.g., AMPK activation). Three studies (Aurora et al., Corona et al., Quarta et al.) initiated rehabilitation one week post-VML (Aurora et al. 2014; Corona et al. 2013a; Quarta et al. 2017), while Izadi et al. (Izadi et al. 2021b) started at two weeks post-VML. Noteworthy, Quarta et al. tested the amount of time required for VML-injured mice to return to their pre-injured daily running distances (~7 days) and noted that VML-injured mice that were forced to run daily on a treadmill immediately after injury had greater fibrosis than VML-injured mice that started forced treadmill running at 7 days post-VML (Quarta et al. 2017). They used this information to rationalize waiting at least one week to begin rehabilitation post-VML, although it should be noted that other studies have started rehab earlier with no reports of contraindicative effects (Greising et al. 2018; Southern et al. 2019). In

contrast to rehabilitation, Dziki et al. combined a bioscaffold regenerative medicine approach with hindlimb unloading that removes physical stimuli on the injured muscle and can be used to determine the effects of bedrest, inactivity, or sedentarism on muscle function post-VML (Dziki et al. 2018). Where an ECM bioscaffold improved muscle strength by 50% in VML-injured groups, hindlimb unloading completely reversed this effect. One conclusion that can be made from these studies is that there may be uncertainty on when to initiation rehabilitation, but the absence of physical stimuli, even that provided by basic ambulation, is detrimental. VML injuries in humans may indeed involve a period of recovery in which the patient is confined to a hospital bed or physically unable to voluntarily move. More clinical research is necessary to identify the extent to which assisted mobilization, rehabilitation, and other physical therapies can influence muscle function after VML.

Several of the studies noted in Table 6.2 included analysis of Tier 2–Tier 4 outcome measurements that are worth highlighting. Quarta et al. reported that rehabilitation resulted in a greater number of donor-derived myofibers being innervated following VML injury, and a greater frequency of mature NMJs in general (Quarta et al. 2017). Izadi et al. (Izadi et al. 2021b) noted that high-intensity interval rehabilitation combined with a bioscaffold and stem cell regenerative medicine approach produced greater vascular density compared to the regenerative medicine approach alone. And Corona et al. reported that voluntary wheel running in addition to an autologous minced muscle graft bolstered the immunoresponse and lessened markers of collagen deposition compared to the minced muscle graft alone. These studies exemplify how rehabilitation may improve the effectiveness of regenerative medicine approaches and be leverage to optimize functional recovery. Next, we highlight known areas for potential advancement of the regenerative rehabilitation field.

There are several known or straightforward ways to advance regenerative rehabilitation of VML. First, several regenerative medicine approaches (like those highlighted in Table 6.1) have not been combined with a rehabilitation strategy. Validating the extent to which wheel running or neuromuscular electrical stimulation influences vascularization of a biomaterial scaffold or augments a growth factor intervention is entirely feasible. Rehabilitation strategies are often considered pleiotropic, having greater than one effect on skeletal muscle, and therefore are suitable for many regenerative medicine approaches. Second, several innovative regenerative medicine approaches have not been validated to improve the functional capacity of the VML-injured muscle. These approaches can easily be advanced to the next stage of therapeutic relevancy by including any of the Tier 1 outcome measurement described above. Third, researchers should consult with physical therapists about what rehabilitation strategies are being used in a clinical setting that may require evidence-based data for further development. Approaches such as passive movement of the injured limb, vibration platform therapy, ultrasound, or weight-supported treadmill walking could be rigorously tested in animal models to expand feasible rehabilitation approaches. These approaches could then be combined with regenerative medicines to advance regenerative rehabilitation.

Finally, researchers must continue to pursue the "*unknowns*" of VML pathology. There are still knowledge gaps in the field related to the pathology of the VML injury, the plasticity of the remaining muscle, and whole-body implications that may negatively influence functional recovery. Continued holistic exploration of VML will undoubtedly produce more targets for regenerative rehabilitation. There is also a dearth of clinical data on traumatic muscle injury and the short- and long-term effects on patient health, mobility, comorbidities, and quality of life. For example, one study in animals hints that VML injury can negatively affect whole-body metabolism, yet it is unclear if VML patients have metabolic syndrome, insulin insensitivity, or changes in metabolic rate. Future researcher–clinician partnerships will be required to meet the challenges of answering these unknowns to fully understand the patient's perspective of this devastating injury.

Funding This work was supported by funding from the Department of Defense, Clinical and Rehabilitative Medicine Research Program: W81XWH-18-1-0710 and W81XWH-20-1-0885 (JAC). Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense.

References

Abdulghani S, Mitchell GR (2019) Biomaterials for in situ tissue regeneration. A Review Biomolecules 9(11):750. https://doi.org/10.3390/biom9110750

Aguilar CA, Greising SM, Watts A, Goldman SM, Peragallo C, Zook C, Larouche J, Corona BT (2018) Multiscale analysis of a regenerative therapy for treatment of volumetric muscle loss injury. Cell Death Discov 4:33. https://doi.org/10.1038/s41420-018-0027-8

Alcazar CA, Hu C, Rando TA, Huang NF, Nakayama KH (2020) Transplantation of insulin-like growth factor-1 laden scaffolds combined with exercise promotes neuroregeneration and angiogenesis in a preclinical muscle injury model. Biomater Sci 8(19):5376–5389

Anderson SE, Han WM, Srinivasa V, Mohiuddin M, Ruehle MA, Moon JY, Shin E, San Emeterio CL, Ogle ME, Botchwey EA, Willett NJ, Jang YC (2019) Determination of a critical size threshold for volumetric muscle loss in the mouse quadriceps. Tissue Eng Part C Methods 25(2):59–70. https://doi.org/10.1089/ten.TEC.2018.0324

Arruda EM, Mundy K, Calve S, Baar K (2007) Denervation does not change the ratio of collagen I and collagen III mRNA in the extracellular matrix of muscle. Am J Physiol Regul Integr Comp Physiol 292(2):R983–R987. https://doi.org/10.1152/ajpregu.00483.2006

Aurora A, Garg K, Corona BT, Walters TJ (2014) Physical rehabilitation improves muscle function following volumetric muscle loss injury. BMC Sports Sci Med Rehabil 6(1):41. https://doi.org/10.1186/2052-1847-6-41

Aurora A, Roe JL, Corona BT, Walters TJ (2015) An acellular biologic scaffold does not regenerate appreciable de novo muscle tissue in rat models of volumetric muscle loss injury. Biomaterials 67:393–407. https://doi.org/10.1016/j.biomaterials.2015.07.040

Baker HB, Passipieri JA, Siriwardane M, Ellenburg MD, Vadhavkar M, Bergman CR, Saul JM, Tomblyn S, Burnett L, Christ GJ (2017) Cell and growth factor-loaded keratin hydrogels for treatment of volumetric muscle loss in a mouse model. Tissue Eng Part A 23(11–12):572–584. https://doi.org/10.1089/ten.TEA.2016.0457

Brovold M, Almeida JI, Pla-Palacin I, Sainz-Arnal P, Sanchez-Romero N, Rivas JJ, Almeida H, Dachary PR, Serrano-Aullo T, Soker S, Baptista PM (2018) Naturally-derived biomaterials for tissue engineering applications. Adv Exp Med Biol 1077:421–449. https://doi.org/10.1007/978-981-13-0947-2_23

Cáceres S, Cuellar C, Casar JC, Garrido J, Schaefer L, Kresse H, Brandan E (2000) Synthesis of proteoglycans is augmented in dystrophic mdx mouse skeletal muscle. Eur J Cell Biol 79(3): 173–181. https://doi.org/10.1078/s0171-9335(04)70020-5

Calve S, Odelberg SJ, Simon HG (2010) A transitional extracellular matrix instructs cell behavior during muscle regeneration. Dev Biol 344(1):259–271. https://doi.org/10.1016/j.ydbio.2010.05.007

Ceroni D, Martin X, Delhumeau C, Farpour-Lambert N (2011) Decrease of physical activity level in adolescents with limb fractures: an accelerometry-based activity monitor study. BMC Musculoskelet Disord 12:87. https://doi.org/10.1186/1471-2474-12-87

Chao T, Burmeister DM, Corona BT, Greising SM (2019) oxidative pathophysiology following volumetric muscle loss injury in a porcine model. J Appl Physiol (1985) 126(6):1541–1549. https://doi.org/10.1152/japplphysiol.00026.2019

Chen XK, Walters TJ (2013) Muscle-derived decellularised extracellular matrix improves functional recovery in a rat latissimus dorsi muscle defect model. J Plast Reconstr Aesthet Surg 66(12):1750–1758. https://doi.org/10.1016/j.bjps.2013.07.037

Cherry C, Maestas DR, Han J, Andorko JI, Cahan P, Fertig EJ, Garmire LX, Elisseeff JH (2021) Computational reconstruction of the signalling networks surrounding implanted biomaterials from single-cell transcriptomics. Nat Biomed Eng. https://doi.org/10.1038/s41551-021-00770-5

Corona BT, Machingal MA, Criswell T, Vadhavkar M, Dannahower AC, Bergman C, Zhao W, Christ GJ (2012) Further development of a tissue engineered muscle repair construct in vitro for enhanced functional recovery following implantation in vivo in a murine model of volumetric muscle loss injury. Tissue Eng Part A 18(11–12):1213–1228. https://doi.org/10.1089/ten.TEA.2011.0614

Corona BT, Garg K, Ward CL, McDaniel JS, Walters TJ, Rathbone CR (2013a) Autologous minced muscle grafts: a tissue engineering therapy for the volumetric loss of skeletal muscle. Am J Physiol Cell Physiol 305(7):C761–C775. https://doi.org/10.1152/ajpcell.00189.2013

Corona BT, Wu X, Ward CL, McDaniel JS, Rathbone CR, Walters TJ (2013b) The promotion of a functional fibrosis in skeletal muscle with volumetric muscle loss injury following the transplantation of muscle-ECM. Biomaterials 34(13):3324–3335. https://doi.org/10.1016/j.biomaterials.2013.01.061

Corona BT, Ward CL, Baker HB, Walters TJ, Christ GJ (2014) Implantation of in vitro tissue engineered muscle repair constructs and bladder acellular matrices partially restore in vivo skeletal muscle function in a rat model of volumetric muscle loss injury. Tissue Eng Part A 20(3–4):705–715. https://doi.org/10.1089/ten.TEA.2012.0761

Corona BT, Rivera JC, Owens JG, Wenke JC, Rathbone CR (2015) Volumetric muscle loss leads to permanent disability following extremity trauma. J Rehabil Res Dev 52(7):785–792. https://doi.org/10.1682/jrrd.2014.07.0165

Corona BT, Wenke JC, Ward CL (2016) Pathophysiology of volumetric muscle loss injury. Cells Tissues Organs 202(3–4):180–188. https://doi.org/10.1159/000443925

Corona BT, Henderson BE, Ward CL, Greising SM (2017a) Contribution of minced muscle graft progenitor cells to muscle fiber formation after volumetric muscle loss injury in wild-type and immune deficient mice. Physiol Rep 5(7):e13249. https://doi.org/10.14814/phy2.13249

Corona BT, Rivera JC, Wenke JC, Greising SM (2017b) Tacrolimus as an adjunct to autologous minced muscle grafts for the repair of a volumetric muscle loss injury. J Exp Orthop 4(1):36. https://doi.org/10.1186/s40634-017-0112-6

Corona BT, Flanagan KE, Brininger CM, Goldman SM, Call JA, Greising SM (2018a) Impact of volumetric muscle loss injury on persistent motoneuron axotomy. Muscle Nerve 57(5): 799–807. https://doi.org/10.1002/mus.26016

Corona BT, Rivera JC, Greising SM (2018b) Inflammatory and physiological consequences of debridement of fibrous tissue after volumetric muscle loss injury. Clin Transl Sci 11(2): 208–217. https://doi.org/10.1111/cts.12519

Corona BT, Rivera JC, Dalske KA, Wenke JC, Greising SM (2020) Pharmacological mitigation of fibrosis in a porcine model of volumetric muscle loss injury. Tissue Eng Part A 26(11–12): 636–646. https://doi.org/10.1089/ten.TEA.2019.0272

Cross JD, Ficke JR, Hsu JR, Masini BD, Wenke JC (2011) Battlefield orthopaedic injuries cause the majority of long-term disabilities. J Am Acad Orthop Surg 19(Suppl 1):S1–S7. https://doi.org/10.5435/00124635-201102001-00002

Dalske KA, Raymond-Pope CJ, McFaline-Figueroa J, Basten AM, Call JA, Greising SM (2021) Independent of physical activity, volumetric muscle loss injury in a murine model impairs whole-body metabolism. PLoS One 16(6):e0253629. https://doi.org/10.1371/journal.pone.0253629

Dearth CL, Slivka PF, Stewart SA, Keane TJ, Tay JK, Londono R, Goh Q, Pizza FX, Badylak SF (2016) Inhibition of COX1/2 alters the host response and reduces ECM scaffold mediated constructive tissue remodeling in a rodent model of skeletal muscle injury. Acta Biomater 31: 50–60. https://doi.org/10.1016/j.actbio.2015.11.043

Degens H, Meessen NE, Wirtz P, Binkhorst RA (1995) The development of compensatory hypertrophy in the plantaris muscle of the rat. Ann Anat 177(3):285–289. https://doi.org/10.1016/s0940-9602(11)80203-7

Dienes JA, Hu X, Janson KD, Slater C, Dooley EA, Christ GJ, Russell SD (2019) Analysis and modeling of rat gait biomechanical deficits in response to volumetric muscle loss injury. Front Bioeng Biotechnol 7:146. https://doi.org/10.3389/fbioe.2019.00146

Dienes J, Browne S, Farjun B, Amaral Passipieri J, Mintz EL, Killian G, Healy KE, Christ GJ (2021) Semisynthetic hyaluronic acid-based hydrogel promotes recovery of the injured tibialis anterior skeletal muscle form and function. ACS Biomater Sci Eng 7(4):1587–1599. https://doi.org/10.1021/acsbiomaterials.0c01751

Dolan CP, Valerio MS, Lee Childers W, Goldman SM, Dearth CL (2021) Prolonged field care for traumatic extremity injuries: defining a role for biologically focused technologies. NPJ Regen Med 6(1):6. https://doi.org/10.1038/s41536-020-00117-9

Dziki JL, Giglio RM, Sicari BM, Wang DS, Gandhi RM, Londono R, Dearth CL, Badylak SF (2018) The effect of mechanical loading upon extracellular matrix bioscaffold-mediated skeletal muscle remodeling. Tissue Eng Part A 24(1–2):34–46. https://doi.org/10.1089/ten.TEA.2017.0011

Fisher-Wellman KH, Davidson MT, Narowski TM, Lin CT, Koves TR, Muoio DM (2018) Mitochondrial diagnostics: a multiplexed assay platform for comprehensive assessment of mitochondrial energy fluxes. Cell Rep 24(13):3593–3606.e3510. https://doi.org/10.1016/j.celrep.2018.08.091

Gao Y, Kostrominova TY, Faulkner JA, Wineman AS (2008) Age-related changes in the mechanical properties of the epimysium in skeletal muscles of rats. J Biomech 41(2):465–469. https://doi.org/10.1016/j.jbiomech.2007.09.021

Garg K, Corona BT, Walters TJ (2014a) Losartan administration reduces fibrosis but hinders functional recovery after volumetric muscle loss injury. J Appl Physiol (1985) 117(10): 1120–1131. https://doi.org/10.1152/japplphysiol.00689.2014

Garg K, Ward CL, Corona BT (2014b) Asynchronous inflammation and myogenic cell migration limit muscle tissue regeneration mediated by a cellular scaffolds. Inflamm. Cell Signal 1(4): e530. https://doi.org/10.14800/ics.530

Garg K, Ward CL, Hurtgen BJ, Wilken JM, Stinner DJ, Wenke JC, Owens JG, Corona BT (2015) Volumetric muscle loss: persistent functional deficits beyond frank loss of tissue. J Orthop Res 33(1):40–46. https://doi.org/10.1002/jor.22730

Gentile NE, Stearns KM, Brown EH, Rubin JP, Boninger ML, Dearth CL, Ambrosio F, Badylak SF (2014) Targeted rehabilitation after extracellular matrix scaffold transplantation for the treatment of volumetric muscle loss. Am J Phys Med Rehabil 93(11 Suppl 3):S79–S87. https://doi.org/10.1097/phm.0000000000000145

Gilbert-Honick J, Ginn B, Zhang Y, Salehi S, Wagner KR, Mao HQ, Grayson WL (2018a) Adipose-derived stem/stromal cells on electrospun fibrin microfiber bundles enable moderate

muscle reconstruction in a volumetric muscle loss model. Cell Transplant 27(11):1644–1656. https://doi.org/10.1177/0963689718805370

Gilbert-Honick J, Iyer SR, Somers SM, Lovering RM, Wagner K, Mao HQ, Grayson WL (2018b) Engineering functional and histological regeneration of vascularized skeletal muscle. Biomaterials 164:70–79. https://doi.org/10.1016/j.biomaterials.2018.02.006

Gillies AR, Lieber RL (2011) Structure and function of the skeletal muscle extracellular matrix. Muscle Nerve 44(3):318–331. https://doi.org/10.1002/mus.22094

Gillies AR, Chapman MA, Bushong EA, Deerinck TJ, Ellisman MH, Lieber RL (2017) High resolution three-dimensional reconstruction of fibrotic skeletal muscle extracellular matrix. J Physiol 595(4):1159–1171. https://doi.org/10.1113/jp273376

Goldman SM, Henderson BEP, Walters TJ, Corona BT (2018) Co-delivery of a laminin-111 supplemented hyaluronic acid based hydrogel with minced muscle graft in the treatment of volumetric muscle loss injury. PLoS One 13(1):e0191245. https://doi.org/10.1371/journal.pone.0191245

Goldman SM, Valerio MS, Janakiram NB, Dearth CL (2020) COX-2 inhibition does not alter wound healing outcomes of a volumetric muscle loss injury treated with a biologic scaffold. J Tissue Eng Regen Med 14(12):1929–1938. https://doi.org/10.1002/term.3144

Goldman SM, Janakiram NB, Valerio MS, Dearth CL (2021) Evaluation of licofelone as an adjunct anti-inflammatory therapy to biologic scaffolds in the treatment of volumetric muscle loss. Cell Tissue Res 385(1):149–159. https://doi.org/10.1007/s00441-021-03449-0

Grasman JM, Do DM, Page RL, Pins GD (2015) Rapid release of growth factors regenerates force output in volumetric muscle loss injuries. Biomaterials 72:49–60. https://doi.org/10.1016/j.biomaterials.2015.08.047

Greising SM, Ermilov LG, Sieck GC, Mantilla CB (2015) Ageing and neurotrophic signalling effects on diaphragm neuromuscular function. J Physiol 593(2):431–440. https://doi.org/10.1113/jphysiol.2014.282244

Greising SM, Dearth CL, Corona BT (2016) Regenerative and rehabilitative medicine: a necessary synergy for functional recovery from volumetric muscle loss injury. Cells Tissues Organs 202(3–4):237–249. https://doi.org/10.1159/000444673

Greising SM, Rivera JC, Goldman SM, Watts A, Aguilar CA, Corona BT (2017) Unwavering pathobiology of volumetric muscle loss injury. Sci Rep 7(1):13179. https://doi.org/10.1038/s41598-017-13306-2

Greising SM, Warren GL, Southern WM, Nichenko AS, Qualls AE, Corona BT, Call JA (2018) Early rehabilitation for volumetric muscle loss injury augments endogenous regenerative aspects of muscle strength and oxidative capacity. BMC Musculoskelet Disord 19(1):173. https://doi.org/10.1186/s12891-018-2095-6

Greising SM, Corona BT, McGann C, Frankum JK, Warren GL (2019) Therapeutic approaches for volumetric muscle loss injury: a systematic review and meta-analysis. Tissue Eng Part B Rev 25(6):510–525. https://doi.org/10.1089/ten.TEB.2019.0207

Greising SM, Corona BT, Call JA (2020) Musculoskeletal regeneration, rehabilitation, and plasticity following traumatic injury. Int J Sports Med 41(8):495–504. https://doi.org/10.1055/a-1128-7128

Grogan BF, Hsu JR (2011) Volumetric muscle loss. J Am Acad Orthop Surg 19(Suppl 1):S35–S37. https://doi.org/10.5435/00124635-201102001-00007

Grounds MD (2014) The need to more precisely define aspects of skeletal muscle regeneration. Int J Biochem Cell Biol 56:56–65. https://doi.org/10.1016/j.biocel.2014.09.010

Guo B, Qu J, Zhao X, Zhang M (2019) Degradable conductive self-healing hydrogels based on dextran-graft-tetraaniline and N-carboxyethyl chitosan as injectable carriers for myoblast cell therapy and muscle regeneration. Acta Biomater 84:180–193. https://doi.org/10.1016/j.actbio.2018.12.008

Haas GJ, Dunn AJ, Marcinczyk M, Talovic M, Schwartz M, Scheidt R, Patel AD, Hixon KR, Elmashhady H, McBride-Gagyi SH, Sell SA, Garg K (2019) Biomimetic sponges for

regeneration of skeletal muscle following trauma. J Biomed Mater Res A 107(1):92–103. https://doi.org/10.1002/jbm.a.36535

Hackam DG (2007) Translating animal research into clinical benefit. BMJ 334(7586):163–164. https://doi.org/10.1136/bmj.39104.362951.80

Hackam DG, Redelmeier DA (2006) Translation of research evidence from animals to humans. JAMA 296(14):1727–1732. https://doi.org/10.1001/jama.296.14.1731

Han N, Yabroudi MA, Stearns-Reider K, Helkowski W, Sicari BM, Rubin JP, Badylak SF, Boninger ML, Ambrosio F (2016) Electrodiagnostic evaluation of individuals implanted with extracellular matrix for the treatment of volumetric muscle injury: case series. Phys Ther 96(4): 540–549. https://doi.org/10.2522/ptj.20150133

Hartung T (2008) Thoughts on limitations of animal models. Parkinsonism Relat Disord 14:S81–S83

Hoffman DB, Raymond-Pope CJ, Sorensen JR, Corona BT, Greising SM (2021) Temporal changes in the muscle extracellular matrix due to volumetric muscle loss injury. Connect Tissue Res:1–14. https://doi.org/10.1080/03008207.2021.1886285

Hurtgen BJ, Ward CL, Garg K, Pollot BE, Goldman SM, McKinley TO, Wenke JC, Corona BT (2016) Severe muscle trauma triggers heightened and prolonged local musculoskeletal inflammation and impairs adjacent tibia fracture healing. J Musculoskelet Neuronal Interact 16(2): 122–134

Hurtgen BJ, Ward CL, Leopold Wager CM, Garg K, Goldman SM, Henderson BEP, McKinley TO, Greising SM, Wenke JC, Corona BT (2017) Autologous minced muscle grafts improve endogenous fracture healing and muscle strength after musculoskeletal trauma. Physiol Rep 5(14):e13362. https://doi.org/10.14814/phy2.13362

Hyldahl RD, Nelson B, Xin L, Welling T, Groscost L, Hubal MJ, Chipkin S, Clarkson PM, Parcell AC (2015) Extracellular matrix remodeling and its contribution to protective adaptation following lengthening contractions in human muscle. FASEB J 29(7):2894–2904. https://doi.org/10.1096/fj.14-266668

Hymel LA, Ogle ME, Anderson SE, San Emeterio CL, Turner TC, York WY, Liu AY, Olingy CE, Sridhar S, Lim HS, Sulchek T, Qiu P, Jang YC, Willett NJ, Botchwey EA (2021) Modulating local S1P receptor signaling as a regenerative immunotherapy after volumetric muscle loss injury. J Biomed Mater Res A 109(5):695–712. https://doi.org/10.1002/jbm.a.37053

Izadi M, Habibi A, Khodabandeh Z, Nikbakht M (2021a) Simultaneous effect of high intensity interval training and human amniotic membrane scaffold on rat tibialis anterior vascularization and innervation after volumetric muscle loss injury. Int J Organ Transplant Med (IJOTM) 12(1): 33–43

Izadi MR, Habibi A, Khodabandeh Z, Nikbakht M (2021b) Synergistic effect of high-intensity interval training and stem cell transplantation with amniotic membrane scaffold on repair and rehabilitation after volumetric muscle loss injury. Cell Tissue Res 383(2):765–779. https://doi.org/10.1007/s00441-020-03304-8

Järvinen TA, Józsa L, Kannus P, Järvinen TL, Järvinen M (2002) Organization and distribution of intramuscular connective tissue in normal and immobilized skeletal muscles. An immunohistochemical, polarization and scanning electron microscopic study. J Muscle Res Cell Motil 23(3):245–254. https://doi.org/10.1023/a:1020904518336

Kamelger FS, Marksteiner R, Margreiter E, Klima G, Wechselberger G, Hering S, Piza H (2004) A comparative study of three different biomaterials in the engineering of skeletal muscle using a rat animal model. Biomaterials 25(9):1649–1655. https://doi.org/10.1016/s0142-9612(03)00520-9

Kasukonis B, Kim J, Brown L, Jones J, Ahmadi S, Washington T, Wolchok J (2016) Codelivery of infusion decellularized skeletal muscle with minced muscle autografts improved recovery from volumetric muscle loss injury in a rat model. Tissue Eng Part A 22(19–20):1151–1163. https://doi.org/10.1089/ten.TEA.2016.0134

Kelley DE, Goodpaster B, Wing RR, Simoneau JA (1999) Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Phys 277(6):E1130–E1141. https://doi.org/10.1152/ajpendo.1999.277.6.E1130

Kim JH, Ko IK, Atala A, Yoo JJ (2016) Progressive muscle cell delivery as a solution for volumetric muscle defect repair. Sci Rep 6:38754. https://doi.org/10.1038/srep38754

Kin S, Hagiwara A, Nakase Y, Kuriu Y, Nakashima S, Yoshikawa T, Sakakura C, Otsuji E, Nakamura T, Yamagishi H (2007) Regeneration of skeletal muscle using in situ tissue engineering on an acellular collagen sponge scaffold in a rabbit model. ASAIO J 53(4):506–513. https://doi.org/10.1097/MAT.0b013e3180d09d81

Krueger CA, Wenke JC, Ficke JR (2012) Ten years at war: comprehensive analysis of amputation trends. J Trauma Acute Care Surg 73(6 Suppl 5):S438–S444. https://doi.org/10.1097/TA.0b013e318275469c

Krumschnabel G, Eigentler A, Fasching M, Gnaiger E (2014) Use of safranin for the assessment of mitochondrial membrane potential by high-resolution respirometry and fluorometry. Methods Enzymol 542:163–181. https://doi.org/10.1016/b978-0-12-416618-9.00009-1

Larouche J, Greising SM, Corona BT, Aguilar CA (2018) Robust inflammatory and fibrotic signaling following volumetric muscle loss: a barrier to muscle regeneration. Cell Death Dis 9(3):409. https://doi.org/10.1038/s41419-018-0455-7

Li Y, Foster W, Deasy BM, Chan Y, Prisk V, Tang Y, Cummins J, Huard J (2004) Transforming growth factor-beta1 induces the differentiation of myogenic cells into fibrotic cells in injured skeletal muscle: a key event in muscle fibrogenesis. Am J Pathol 164(3):1007–1019. https://doi.org/10.1016/s0002-9440(10)63188-4

Li MT, Willett NJ, Uhrig BA, Guldberg RE, Warren GL (2014) Functional analysis of limb recovery following autograft treatment of volumetric muscle loss in the quadriceps femoris. J Biomech 47(9):2013–2021. https://doi.org/10.1016/j.jbiomech.2013.10.057

Li MT, Ruehle MA, Stevens HY, Servies N, Willett NJ, Karthikeyakannan S, Warren GL, Guldberg RE, Krishnan L (2017) (*) skeletal myoblast-seeded vascularized tissue scaffolds in the treatment of a large volumetric muscle defect in the rat biceps femoris muscle. Tissue Eng Part A 23(17–18):989–1000. https://doi.org/10.1089/ten.TEA.2016.0523

Ma J, Baker AR, Calabro A, Derwin KA (2017) Exploratory study on the effect of osteoactivin on muscle regeneration in a rat volumetric muscle loss model. PLoS One 12(4):e0175853. https://doi.org/10.1371/journal.pone.0175853

Machingal MA, Corona BT, Walters TJ, Kesireddy V, Koval CN, Dannahower A, Zhao W, Yoo JJ, Christ GJ (2011) A tissue-engineered muscle repair construct for functional restoration of an irrecoverable muscle injury in a murine model. Tissue Eng Part A 17(17–18):2291–2303. https://doi.org/10.1089/ten.TEA.2010.0682

Maggio AB, Martin XE, Ceroni D (2017) Activity-related energy expenditure during lower limb cast immobilization in children. J Pediatr Orthop B 26(3):217–221. https://doi.org/10.1097/bpb.0000000000000389

Mase VJ Jr, Hsu JR, Wolf SE, Wenke JC, Baer DG, Owens J, Badylak SF, Walters TJ (2010) Clinical application of an acellular biologic scaffold for surgical repair of a large, traumatic quadriceps femoris muscle defect. Orthopedics 33(7):511. https://doi.org/10.3928/01477447-20100526-24

McGonigle P, Ruggeri B (2014) Animal models of human disease: challenges in enabling translation. Biochem Pharmacol 87(1):162–171. https://doi.org/10.1016/j.bcp.2013.08.006

McLoughlin TJ, Mylona E, Hornberger TA, Esser KA, Pizza FX (2003) Inflammatory cells in rat skeletal muscle are elevated after electrically stimulated contractions. J Appl Physiol (1985) 94(3):876–882. https://doi.org/10.1152/japplphysiol.00766.2002

Mendias CL, Schwartz AJ, Grekin JA, Gumucio JP, Sugg KB (2017) Changes in muscle fiber contractility and extracellular matrix production during skeletal muscle hypertrophy. J Appl Physiol (1985) 122(3):571–579. https://doi.org/10.1152/japplphysiol.00719.2016

Merritt EK, Cannon MV, Hammers DW, Le LN, Gokhale R, Sarathy A, Song TJ, Tierney MT, Suggs LJ, Walters TJ, Farrar RP (2010) Repair of traumatic skeletal muscle injury with bone-

marrow-derived mesenchymal stem cells seeded on extracellular matrix. Tissue Eng Part A 16(9):2871–2881. https://doi.org/10.1089/ten.TEA.2009.0826

Migonney V (2014) History of biomaterials. In: Biomaterials. Wiley, Hoboken, pp 1–10

Moran CJ, Ramesh A, Brama PA, O'Byrne JM, O'Brien FJ, Levingstone TJ (2016) The benefits and limitations of animal models for translational research in cartilage repair. J Exp Orthop 3(1): 1. https://doi.org/10.1186/s40634-015-0037-x

Mori R, Kamei N, Okawa S, Nakabayashi A, Yokota K, Higashi Y, Ochi M (2015) Promotion of skeletal muscle repair in a rat skeletal muscle injury model by local injection of human adipose tissue-derived regenerative cells. J Tissue Eng Regen Med 9(10):1150–1160. https://doi.org/10.1002/term.1659

Musther H, Olivares-Morales A, Hatley OJD, Liu B, Rostami Hodjegan A (2014) Animal versus human oral drug bioavailability: do they correlate? Eur J Pharm Sci 57:280–291. https://doi.org/10.1016/j.ejps.2013.08.018

Narayanan N, Jiang C, Wang C, Uzunalli G, Whittern N, Chen D, Jones OG, Kuang S, Deng M (2020) Harnessing fiber diameter-dependent effects of myoblasts toward biomimetic scaffold-based skeletal muscle regeneration. Front Bioeng Biotechnol 8:203. https://doi.org/10.3389/fbioe.2020.00203

Nuutila K, Sakthivel D, Kruse C, Tran P, Giatsidis G, Sinha I (2017) Gene expression profiling of skeletal muscle after volumetric muscle loss. Wound Repair Regen 25(3):408–413. https://doi.org/10.1111/wrr.12547

Oshima S, Kamei N, Nakasa T, Yasunaga Y, Ochi M (2014) Enhancement of muscle repair using human mesenchymal stem cells with a magnetic targeting system in a subchronic muscle injury model. J Orthop Sci 19(3):478–488. https://doi.org/10.1007/s00776-014-0548-9

Owens BD, Kragh JF Jr, Macaitis J, Svoboda SJ, Wenke JC (2007) Characterization of extremity wounds in operation Iraqi freedom and operation enduring freedom. J Orthop Trauma 21(4): 254–257. https://doi.org/10.1097/BOT.0b013e31802f78fb

Owens BD, Kragh JF Jr, Wenke JC, Macaitis J, Wade CE, Holcomb JB (2008) Combat wounds in operation Iraqi freedom and operation enduring freedom. J Trauma 64(2):295–299. https://doi.org/10.1097/TA.0b013e318163b875

Page RL, Malcuit C, Vilner L, Vojtic I, Shaw S, Hedblom E, Hu J, Pins GD, Rolle MW, Dominko T (2011) Restoration of skeletal muscle defects with adult human cells delivered on fibrin microthreads. Tissue Eng Part A 17(21–22):2629–2640. https://doi.org/10.1089/ten.TEA.2011.0024

Passipieri JA, Baker HB, Siriwardane M, Ellenburg MD, Vadhavkar M, Saul JM, Tomblyn S, Burnett L, Christ GJ (2017) Keratin hydrogel enhances in vivo skeletal muscle function in a rat model of volumetric muscle loss. Tissue Eng Part A 23(11–12):556–571. https://doi.org/10.1089/ten.TEA.2016.0458

Patel KH, Dunn AJ, Talovic M, Haas GJ, Marcinczyk M, Elmashhady H, Kalaf EG, Sell SA, Garg K (2019) Aligned nanofibers of decellularized muscle ECM support myogenic activity in primary satellite cells in vitro. Biomed Mater 14(3):035010. https://doi.org/10.1088/1748-605X/ab0b06

Perniconi B, Costa A, Aulino P, Teodori L, Adamo S, Coletti D (2011) The pro-myogenic environment provided by whole organ scale acellular scaffolds from skeletal muscle. Biomaterials 32(31):7870–7882. https://doi.org/10.1016/j.biomaterials.2011.07.016

Piñol-Jurado P, Suárez-Calvet X, Fernández-Simón E, Gallardo E, de la Oliva N, Martínez-Muriana A, Gómez-Gálvez P, Escudero LM, Pérez-Peiró M, Wollin L, de Luna N, Navarro X, Illa I, Díaz-Manera J (2018) Nintedanib decreases muscle fibrosis and improves muscle function in a murine model of dystrophinopathy. Cell Death Dis 9(7):776. https://doi.org/10.1038/s41419-018-0792-6

Purslow PP, Trotter JA (1994) The morphology and mechanical properties of endomysium in series-fibred muscles: variations with muscle length. J Muscle Res Cell Motil 15(3):299–308. https://doi.org/10.1007/bf00123482

Qiu X, Liu S, Zhang H, Zhu B, Su Y, Zheng C, Tian R, Wang M, Kuang H, Zhao X, Jin Y (2018) Mesenchymal stem cells and extracellular matrix scaffold promote muscle regeneration by synergistically regulating macrophage polarization toward the M2 phenotype. Stem Cell Res Ther 9(1):88. https://doi.org/10.1186/s13287-018-0821-5

Quarta M, Cromie M, Chacon R, Blonigan J, Garcia V, Akimenko I, Hamer M, Paine P, Stok M, Shrager JB, Rando TA (2017) Bioengineered constructs combined with exercise enhance stem cell-mediated treatment of volumetric muscle loss. Nat Commun 8:15613. https://doi.org/10.1038/ncomms15613

Rahmati M, Silva EA, Reseland JE, Heyward CA, Haugen HJ (2020) Biological responses to physicochemical properties of biomaterial surface. Chem Soc Rev 49(15):5178–5224. https://doi.org/10.1039/d0cs00103a

Ramaswamy KS, Palmer ML, van der Meulen JH, Renoux A, Kostrominova TY, Michele DE, Faulkner JA (2011) Lateral transmission of force is impaired in skeletal muscles of dystrophic mice and very old rats. J Physiol 589(Pt 5):1195–1208. https://doi.org/10.1113/jphysiol.2010.201921

Ratner BD (2019) Biomaterials: been there, done that, and evolving into the future. Annu Rev Biomed Eng 21:171–191. https://doi.org/10.1146/annurev-bioeng-062117-120940

Ratner BD, Zhang G (2020) A history of biomaterials. In: Biomaterials science. Elsevier, Amsterdam, pp 21–34

Rhrissorrakrai K, Belcastro V, Bilal E, Norel R, Poussin C, Mathis C, Dulize RH, Ivanov NV, Alexopoulos L, Rice JJ, Peitsch MC, Stolovitzky G, Meyer P, Hoeng J (2015) Understanding the limits of animal models as predictors of human biology: lessons learned from the sbv IMPROVER species translation challenge. Bioinformatics 31(4):471–483. https://doi.org/10.1093/bioinformatics/btu611

Rivera JC, Corona BT (2016) Muscle-related disability following combat injury increases with time. US Army Med Dep J Jan–Mar:30–34

Rodriguez BL, Nguyen MH, Armstrong RE, Vega-Soto EE, Polkowski PM, Larkin LM (2020) A comparison of ovine facial and limb muscle as a primary cell source for engineered skeletal muscle. Tissue Eng Part A 26(3–4):167–177. https://doi.org/10.1089/ten.TEA.2019.0087

Rouse JG, Van Dyke ME (2010) A review of keratin-based biomaterials for biomedical applications. Materials 3(2):999–1014. https://doi.org/10.3390/ma3020999

Rynders CA, Blanc S, DeJong N, Bessesen DH, Bergouignan A (2018) Sedentary behaviour is a key determinant of metabolic inflexibility. J Physiol 596(8):1319–1330. https://doi.org/10.1113/jp273282

Sarkar K, Xue Y, Sant S (2017) Host response to synthetic versus natural biomaterials. In: The immune response to implanted materials and devices. Springer International Publishing, Cham, pp 81–105. https://doi.org/10.1007/978-3-319-45433-7_5

Saunders D, Rose L (2021) Regenerative rehabilitation of catastrophic extremity injury in military conflicts and a review of recent developmental efforts. Connect Tissue Res 62(1):83–98. https://doi.org/10.1080/03008207.2020.1776707

Saxena AK, Willital GH, Vacanti JP (2001) Vascularized three-dimensional skeletal muscle tissue-engineering. Biomed Mater Eng 11(4):275–281

Seifert J (2007) Incidence and economic burden of injuries in the United States. J Epidemiol Community Health 61(10):926–926. https://doi.org/10.1136/jech.2007.059717

Shayan M, Huang NF (2020) Pre-clinical cell therapeutic approaches for repair of volumetric muscle loss. Bioengineering (Basel) 7(3):97. https://doi.org/10.3390/bioengineering7030097

Shi M, Ishikawa M, Kamei N, Nakasa T, Adachi N, Deie M, Asahara T, Ochi M (2009) Acceleration of skeletal muscle regeneration in a rat skeletal muscle injury model by local injection of human peripheral blood-derived CD133-positive cells. Stem Cells 27(4):949–960. https://doi.org/10.1002/stem.4

Sicari BM, Agrawal V, Siu BF, Medberry CJ, Dearth CL, Turner NJ, Badylak SF (2012) A murine model of volumetric muscle loss and a regenerative medicine approach for tissue replacement. Tissue Eng Part A 18(19–20):1941–1948. https://doi.org/10.1089/ten.TEA.2012.0475

Sicari BM, Rubin JP, Dearth CL, Wolf MT, Ambrosio F, Boninger M, Turner NJ, Weber DJ, Simpson TW, Wyse A, Brown EH, Dziki JL, Fisher LE, Brown S, Badylak SF (2014) An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss. Sci Transl Med 6(234):234ra258. https://doi.org/10.1126/scitranslmed.3008085

Silva Garcia JM, Panitch A, Calve S (2019) Functionalization of hyaluronic acid hydrogels with ECM-derived peptides to control myoblast behavior. Acta Biomater 84:169–179. https://doi.org/10.1016/j.actbio.2018.11.030

Smith LR, Barton ER (2014) Collagen content does not alter the passive mechanical properties of fibrotic skeletal muscle in mdx mice. Am J Physiol Cell Physiol 306(10):C889–C898. https://doi.org/10.1152/ajpcell.00383.2013

Sorensen JR, Skousen C, Holland A, Williams K, Hyldahl RD (2018) Acute extracellular matrix, inflammatory and MAPK response to lengthening contractions in elderly human skeletal muscle. Exp Gerontol 106:28–38. https://doi.org/10.1016/j.exger.2018.02.013

Sorensen JR, Hoffman DB, Corona BT, Greising SM (2021) Secondary denervation is a chronic pathophysiologic sequela of volumetric muscle loss. J Appl Physiol (1985) 130(5):1614–1625. https://doi.org/10.1152/japplphysiol.00049.2021

Southern WM, Nichenko AS, Tehrani KF, McGranahan MJ, Krishnan L, Qualls AE, Jenkins NT, Mortensen LJ, Yin H, Yin A, Guldberg RE, Greising SM, Call JA (2019) PGC-1alpha overexpression partially rescues impaired oxidative and contractile pathophysiology following volumetric muscle loss injury. Sci Rep 9(1):4079. https://doi.org/10.1038/s41598-019-40606-6

Stinner DJ (2016) Improving outcomes following extremity trauma: the need for a multidisciplinary approach. Mil Med 181(S4):26–29. https://doi.org/10.7205/milmed-d-15-00511

Stinner DJ, Burns TC, Kirk KL, Scoville CR, Ficke JR, Hsu JR (2010) Prevalence of late amputations during the current conflicts in Afghanistan and Iraq. Mil Med 175(12):1027–1029. https://doi.org/10.7205/milmed-d-10-00102

Tanaka A, Yoshimura Y, Aoki K, Okamoto M, Kito M, Suzuki S, Takazawa A, Ishida T, Kato H (2017) Prediction of muscle strength and postoperative function after knee flexor muscle resection for soft tissue sarcoma of the lower limbs. Orthop Traumatol Surg Res 103(7):1081–1085. https://doi.org/10.1016/j.otsr.2017.07.005

Tehrani KF, Latchoumane CV, Southern WM, Pendleton EG, Maslesa A, Karumbaiah L, Call JA, Mortensen LJ (2019) Five-dimensional two-photon volumetric microscopy of in-vivo dynamic activities using liquid lens remote focusing. Biomed Opt Express 10(7):3591–3604. https://doi.org/10.1364/boe.10.003591

Tomblyn S, Pettit Kneller EL, Walker SJ, Ellenburg MD, Kowalczewski CJ, Van Dyke M, Burnett L, Saul JM (2016) Keratin hydrogel carrier system for simultaneous delivery of exogenous growth factors and muscle progenitor cells. J Biomed Mater Res B Appl Biomater 104(5):864–879. https://doi.org/10.1002/jbm.b.33438

Turner NJ, Yates AJ Jr, Weber DJ, Qureshi IR, Stolz DB, Gilbert TW, Badylak SF (2010) Xenogeneic extracellular matrix as an inductive scaffold for regeneration of a functioning musculotendinous junction. Tissue Eng Part A 16(11):3309–3317. https://doi.org/10.1089/ten.TEA.2010.0169

VanDusen KW, Syverud BC, Williams ML, Lee JD, Larkin LM (2014) Engineered skeletal muscle units for repair of volumetric muscle loss in the tibialis anterior muscle of a rat. Tissue Eng Part A 20(21–22):2920–2930. https://doi.org/10.1089/ten.TEA.2014.0060

Ward CL, Ji L, Corona BT (2015) An autologous muscle tissue expansion approach for the treatment of volumetric muscle loss. Biores Open Access 4(1):198–208. https://doi.org/10.1089/biores.2015.0009

Ward CL, Pollot BE, Goldman SM, Greising SM, Wenke JC, Corona BT (2016) Autologous minced muscle grafts improve muscle strength in a porcine model of volumetric muscle loss injury. J Orthop Trauma 30(12):e396–e403. https://doi.org/10.1097/BOT.0000000000000673

Washington TA, Perry RA Jr, Kim JT, Haynie WS, Greene NP, Wolchok JC (2021) The effect of autologous repair and voluntary wheel running on force recovery in a rat model of volumetric muscle loss. Exp Physiol 106(4):994–1004. https://doi.org/10.1113/ep089207

Wolf MT, Dearth CL, Sonnenberg SB, Loboa EG, Badylak SF (2015) Naturally derived and synthetic scaffolds for skeletal muscle reconstruction. Adv Drug Deliv Rev 84:208–221. https://doi.org/10.1016/j.addr.2014.08.011

Wosczyna MN, Rando TA (2018) A muscle stem cell support group: coordinated cellular responses in muscle regeneration. Dev Cell 46(2):135–143. https://doi.org/10.1016/j.devcel.2018.06.018

Zanotti S, Negri T, Cappelletti C, Bernasconi P, Canioni E, Di Blasi C, Pegoraro E, Angelini C, Ciscato P, Prelle A, Mantegazza R, Morandi L, Mora M (2005) Decorin and biglycan expression is differentially altered in several muscular dystrophies. Brain 128(Pt 11):2546–2555. https://doi.org/10.1093/brain/awh635

Zou P, Yu Y, Zheng N, Yang Y, Paholak HJ, Yu LX, Sun D (2012) Applications of human pharmacokinetic prediction in first-in-human dose estimation. AAPS J 14(2):262–281. https://doi.org/10.1208/s12248-012-9332-y

Chapter 7
Novel Cell-Based Therapeutics for Diseases of the Heart and Skeletal Muscle

Russell G. Rogers and Eduardo Marbán

Abstract Diseases of the heart and skeletal muscle represent a major cause of mortality and morbidity in the United States. Many, if not most, are refractory to contemporary medical approaches, which rarely address the root pathogenesis. Regenerative medicine is a specialized field focused on replacing or repairing damaged tissue, which is otherwise incapable of self-healing. For more than four decades, stem and progenitor cells have been recognized for their potential as therapeutic products for regenerative medicine. Despite the long history of scientific inquiry, no cell therapy product has received regulatory approval for regenerative medicine applications in the United States. Recent initiatives focused on understanding the mechanistic basis of cell therapy have fundamentally redirected the trajectory of their development. In doing so, a class of extracellular vesicles called exosomes have emerged as next-generation therapeutic candidates for regenerative medicine. In this chapter, we discuss the difficulties with the commercial development of cell therapy products and the promise of exosomes as next-generation therapeutics. The field of regenerative medicine is ever-changing, and the latest technological advances continue to define our path forward.

Keywords Cell therapy · Exosomes · Heart disease · Regenerative medicine · Skeletal muscle disease

7.1 Diseases of the Heart and Skeletal Muscle

All vertebrates have specialized muscle cells called myocytes which form the functional apparatus of the heart and skeletal muscle. Although myocytes from both organs share similarities such as contracting to generate force to perform their functional duties, crucial differences distinguish the two. Cardiomyocytes beat spontaneously and are electromechanically coupled to form the myocardium—the

R. G. Rogers · E. Marbán (✉)
Smidt Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA
e-mail: Eduardo.Marban@csmc.edu

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_7

contractile tissue of the heart. In contrast, skeletal myocytes do not beat spontaneously (motor neurons trigger contraction in response to efferent input) and skeletal myocytes are electrically isolated from each other. Another distinguishing feature of heart and skeletal muscle is the intrinsic ability to repair in response to injury or disease. Unlike the adult heart, which lacks meaningful regenerative capabilities, skeletal muscle exhibits a robust regenerative response primarily driven by resident stem cells. Notwithstanding such ability, certain injuries overwhelm the innate regenerative capacity, and some chronic skeletal muscle conditions impair the reparative machinery, which consequently manifests as muscle dysfunction. Diseases of the heart and skeletal muscle, for which a paucity of effective therapies often exist, can lead to long-lasting physical inability and poor quality of life. In this chapter, we discuss the most prevalent forms of degenerative or traumatic heart and skeletal muscle disease (see Fig. 7.1 for a schematic overview), avenues for regenerative cell therapy, challenges in the translation of therapeutic products to the clinic, and the promise of novel cell-based approaches. We conclude with a look at future perspectives in regenerative medicine.

7.1.1 Heart Failure with Reduced Ejection Fraction

Heart failure (HF) is a disorder in which exercise tolerance is reduced and pulmonary congestion develops. HF affects more than five million people in the United States, with cases expected to climb to eight million within the next decade (Mozaffarian et al. 2016). Based on ejection fraction (EF), that is, a gross measure of the heart's pumping efficiency, HF can be broadly classified into two groups: (1) HF with reduced EF (HFrEF) and (2) HF with preserved EF (HFpEF) (Ponikowski et al. 2016; Savarese and Lund 2017). Approximately half of HF cases in the USA are due to HFrEF and the remainder to HFpEF. In this section, we summarize the etiology of HFrEF and provide an outlook of clinical outcomes in patients with HFrEF.

HFrEF is often an evolution of the heart's failed adaptation to ischemic injury: thrombosis of a coronary artery reduces blood and oxygen supply to the myocardium resulting in necrosis—a process commonly known as myocardial infarction (Marbán 2018a). Prompt restoration of blood flow is crucial to minimize infarct size and thus has become a staple in the first-line treatment of myocardial ischemia. However, despite successful recanalization of the blocked artery, persistent ischemic regions in the myocardium can remain, leading to HF and poor clinical outcomes (Basalay et al. 2020). In humans, clear evidence of irreversible myocardial damage can be seen in as little as 24 h post-infarction. The damaged myocardium undergoes a dynamic remodeling process, which unfavorably reshapes the heart (Heusch et al. 2014), manifesting as ventricular dilation and infarct expansion (Pfeffer and Braunwald 1990). Independent of the death of living heart tissue, myocardial remodeling contributes to the development of ischemic heart disease and progression to HF, a stage at which prognosis is generally considered poor. The Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure

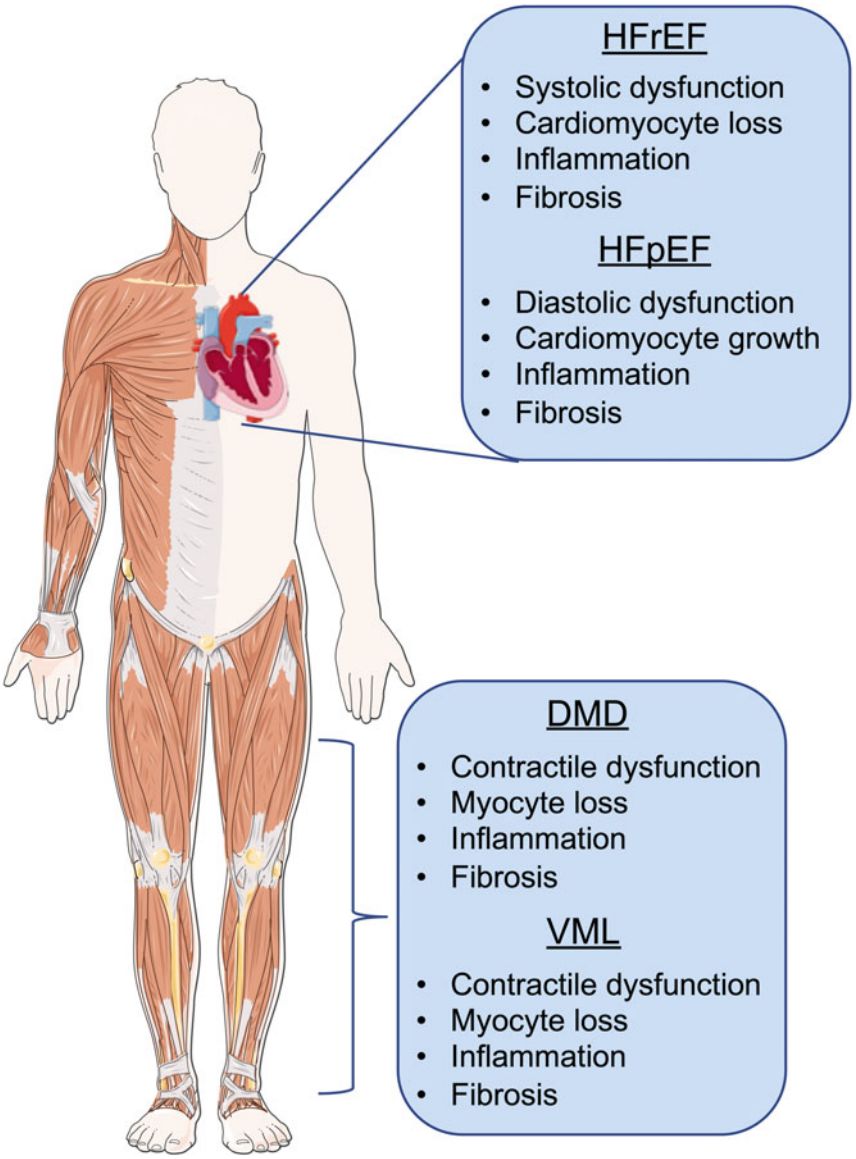

Fig. 7.1 Diseases of the heart and skeletal muscle. Illustration of shared pathology in the heart and skeletal muscle, and targets for cell-based regenerative therapies. Some schematics were adapted from Servier Medical Art

(OPTIMIZE-HF) study which enrolled 20,118 patients with HFrEF reported three dismal statistics: (1) a 60–90 day mortality of 9.8%, (2) a rehospitalization rate of 29.9%, and (3) an in-hospital mortality of 3.9% (Savarese and Lund 2017). Despite significant advancements in the field, standard-of-care for HFrEF mostly relies on

symptom relief and slowing of disease progression by a variety of neurohormonal blockers and other agents acting indirectly on the heart.

7.1.1.1 Current Clinical Practice in HFrEF

In HFrEF, both pharmacological and non-pharmacological interventions are in current clinical practice and have been demonstrated to extend life. Pharmacological agents such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), beta-adrenergic blockers, and angiotensin receptor-neprilysin inhibitors (ARNI) have been associated with improvements in clinically meaningful outcomes in patients with HFrEF (Yancy et al. 2013). Thus, a patient with HFrEF is typically on a cocktail of various types of agents, titrated to maximal doses tolerated (Yancy et al. 2017). Non-pharmacologic interventions include: (a) implantable cardioverter defibrillator to prevent sudden death due to ventricular tachyarrhythmias, (b) cardiac resynchronization therapy to improve myocardial mechanics, and, in the most extreme cases, (c) organ transplantation (Yancy et al. 2013). Despite pharmacological and non-pharmacological interventions, the 5-year mortality rate for symptomatic HFrEF patients approaches 50–75% (Shah et al. 2017). This gloomy statistic highlights the desperate need for the development of novel therapies.

A major limitation of therapeutic approaches to HFrEF is that none addresses the root physiologic cause of the HF: loss of functional myocardium. This is where regenerative approaches may, in principle, prove helpful.

7.1.2 Heart Failure with Preserved Ejection Fraction

The other form of HF, HFpEF, already constitutes ~50% of all HF admissions and is increasing in prevalence (Gladden et al. 2018). Despite having a normal EF, HFpEF patients exhibit classical HF symptoms including breathlessness and exertional intolerance. In HFpEF, the myocardium tends to be thicker than normal, rather than dilating and thinning as seen in HFrEF (Borlaug 2014). Clinical diagnosis of HFpEF requires a patient to present with normal EF and diastolic dysfunction. However, recent research suggests HFpEF is a multifactorial systemic disease involving hypertension, metabolic disorders, and inflammation (Soni et al. 2020).

Regardless of comorbidities, the main cause of functional deterioration is diastolic dysfunction, which is clinically defined as an impaired ability of the ventricle to fill with blood to an appropriate preload volume at a physiological pulmonary venous pressure (Borlaug et al. 2013). When other heart conditions such as endocardial or pericardial disease can be ruled out, diastolic dysfunction is the direct result of increased myocardial stiffness which can be regulated by both the extracellular matrix and the cardiomyocyte (Borlaug and Paulus 2011). In the most fundamental sense, deposition of matricellular proteins such as collagen contributes

to increases in extracellular matrix stiffness. However, changes inside the cardiomyocyte can be a little more complex. The sarcomeric protein titin has been identified as the primary culprit: up to 80% of passive stiffness can be explained by this protein alone—especially when the sarcomere length is within the physiological range (Borlaug and Paulus 2011). Mechanistically, the more compliant titin isoform, N2BA, shifts to the less compliant, that is, more stiff, isoform N2B (Borlaug and Paulus 2011). Additionally, passive tension of the cardiomyocyte can be increased by posttranslational phosphorylation of titin by protein kinases A and G (Borlaug and Paulus 2011). Clinical outcomes from the OPTIMIZE-HF study, which enrolled 21,149 HFpEF patients, reported nearly identical statistics compared to HFrEF: (1) a 60–90 day mortality of 9.5%, (2) a rehospitalization rate of 29.2%, and (3) in-hospital mortality of 2.9% (Savarese and Lund 2017). Thus, while HFpEF is fundamentally distinct from HFrEF, HFpEF patients experience a correspondingly poor prognosis.

7.1.2.1 Current Clinical Practice in HFpEF

Given the principal distinction between HFpEF and HFrEF, it is not surprising the management of HFpEF *should* differ from that of HFrEF. In support of this notion, clinical trials testing the efficacy of agents commonly used for HFrEF, such as ACE inhibitors and ARBs, failed to decrease morbidity and mortality in HFpEF (Ma et al. 2020). Given the lack of clinical trials reporting positive results, HFpEF treatment remains largely focused on managing associated conditions such as hypertension and acute decompensations due to pulmonary edema. Like HFrEF, the 5-year mortality rate for HFpEF is 50–75% (Shah et al. 2017), further driving the need for medical interventions to meet dire demand.

In terms of therapeutic options, the situation in HFpEF is worse than that in HFrEF, in that no drugs or devices have been shown to increase survival. Our best current understanding implicates fibrosis and inflammation in the pathogenesis of HFpEF, so targeting those processes may eventually turn out to be more fruitful. Here, cell therapies may have a niche, not by replacing lost myocardium but rather by virtue of anti-fibrotic and anti-inflammatory effects. Such pleiotropic effects of cell therapy are discussed further below.

7.1.3 Duchenne Muscular Dystrophy

For an overview of Duchenne muscular dystrophy (DMD), we turn the reader to Chap. 4 where DMD has been reviewed in detail; here we cover regenerative approaches only. Initially identified as a disease of skeletal muscle, DMD is now recognized also as a disease of the heart. As heart function deteriorates in DMD patients, clinical signs of HF develop. Improved respiratory care has prolonged life such that HF is now often the terminal process in DMD (Buddhe et al. 2018). As part of disease management for the DMD patient, regular heart evaluations have become

standard (Birnkrant et al. 2018), but little can be done clinically in terms of improving heart function in DMD. The goal is to identify early myocardial changes and initiate therapy to favorably affect adverse myocardial remodeling, and thus improving clinical outcomes and quality of life (Buddhe et al. 2018). Given a lack of therapies for DMD-related cardiomyopathy, the 2018 DMD Care Considerations have endorsed following traditional HF treatment strategies, such as those highlighted in Sect. 7.1.1.1. Unfortunately, with the lack of information regarding the use of these therapies in patients with DMD, specific recommendations for their use in DMD-related cardiomyopathy remains challenging. And, in most cases, the degree of respiratory insufficiency and muscular weakness commonly observed in patients with DMD, who have developed severe myocardial dysfunction, is thought to be a contraindication to transplantation (Connuck et al. 2008; Buddhe et al. 2018). Therefore, novel interventions that improve both heart and skeletal muscle health are desperately needed.

Other than exon-skipping therapies and corticosteroids, which appear not to improve the heart in DMD, no therapies address the root pathophysiological causes of the muscle dysfunction.

7.1.4 Volumetric Muscle Loss

In the field of regenerative medicine, much of the clinical and basic research experience to date has been focused on diseases of the heart. Owing to its ability to self-repair after nontraumatic injury, skeletal muscle has received much less attention in the realm of regenerative medicine. Nevertheless, certain disorders of skeletal muscle may represent viable targets. One such condition is volumetric muscle loss (VML). Because VML has been covered in detail in Chap. 6, the discussion here will be focused on regenerative approaches in the context of cell-based therapy. In VML, suboptimal, if any, endogenous muscle regeneration occurs, which is thought to be the result of three principal defects to the skeletal muscle repair machinery (Greising et al. 2019). First, resident muscle stem cells (satellite cells) no longer populate the affected area, which are strictly required for skeletal myogenesis (Caldwell et al. 1990; Lepper et al. 2011). Second, the muscle extracellular matrix, which provides a three-dimensional scaffold for cell migration and tissue organization, becomes disrupted (Caldwell et al. 1990). And third, a prolonged wave of inflammation stimulates the deposition of fibrotic scar tissue (Greising et al. 2017; Aguilar et al. 2018). Without successful regenerative medicine interventions, the VML-injured limb, even if salvaged, may have little function and thus is later amputated.

7.2 Cell Therapy for Regenerative Medicine

Regenerative medicine is a specialized branch of medicine which seeks to repair or replace tissues and organs damaged by trauma or disease. The recognition that stem or progenitor cells can be instructed to differentiate into numerous cell types quickly garnered the attention of researchers and has since become a conceptually attractive approach to repair damaged tissue. In principle, cell therapy can be used for a broad spectrum of acquired and genetic disorders by targeting the primary and/or secondary disease features. Nevertheless, the amount of hype in this area far exceeds that of translationally viable approaches to date, such that, despite four decades of preclinical and clinical efforts, no cell therapy products have been approved for regenerative indications.

7.2.1 From Discovery to Clinical Translation

The process of translating findings, beginning from discovery-level science to preclinical development, to clinical evaluation is instrumental in the effort to advance medical science. In this section, we discuss the dedication of decades of scientific inquiry that has led to what is now an era of superlative possibilities (but generally disappointing realities).

7.2.1.1 Heart Failure with Reduced Ejection Fraction

The first efforts in regenerative cardiology were founded on the earlier finding that autologous skeletal muscle-derived myoblasts could engraft and proliferate when transplanted directly into the heart (Voronov 1975). As a reminder, skeletal myocytes (unlike cardiomyocytes) do not couple in syncytium, nor do they spontaneously contract. Even so, the hypothesis was that the transplanted cells would stimulate the generation of new contractile tissue within the host myocardium. The development of skeletal myoblasts for cardiac regeneration started with small animal models (Koh et al. 1993), advanced to more clinically relevant models (Taylor et al. 1998), and ultimately finished with in-human clinical trials (Hare et al. 2008). Although signs of efficacy were evident, efforts were later abandoned after it was discovered that transplanted myoblasts were arrhythmogenic in patients with HFrEF (Marbán 2018a).

The next cell type evaluated for cardiac regeneration was unselected bone marrow-derived cells, which were shown to drive repair in preclinical studies (Orlic et al. 2001; Jackson et al. 2001). While early phase clinical testing demonstrated improvements in EF (Meyer et al. 2006), the study of bone marrow cells was short-lived insofar as larger randomized, placebo-controlled clinical trials were unable to reproduce earlier findings (Traverse et al. 2012; Sürder et al. 2013). A

second-generation of cell products began experimental evaluation as the trials of bone marrow cells were concluding. Notably, bone marrow-derived mesenchymal stem cells (MSCs) (Wang et al. 2016) were tested for cardiac regeneration owing to success in other disease models, in which they had already been evaluated previously. More specifically, antigen-selected mesenchymal precursor cells (Psaltis et al. 2010) and cardiopoietic cells (autologous MSCs reinforced in vitro) (Behfar et al. 2008) have reached clinical testing for heart failure, but have been met with a modest level of improvement. In the search for alternative strategies, recruiting and augmenting innate regenerative responses in the heart by cardiac progenitor cells seemed particularly attractive. In this context, cardiac progenitor cells were evaluated due to their potential to engraft, proliferate, and differentiate into new viable myocardium. Cells isolated from hearts by the selection of the c-kit surface antigen were proposed to regenerate the myocardium by these canonical mechanisms (Beltrami et al. 2003), which eventually motivated the SCIPIO trial using autologous c-kit-selected heart cells (Bolli et al. 2011). Unfortunately, the background science supporting this trial was later called into question (Van Berlo et al. 2014), numerous basic studies were deemed fraudulent, and the SCIPIO trial itself was later retracted (Lancet 2019).

At about the same time many researchers were evaluating the efficacy of bone marrow cells and autologous MSCs (circ. 2006), a population of cardiac progenitor cells, called cardiosphere-derived cells (CDCs) was first described (Smith et al. 2007). CDCs do not require antigenic selection and were confirmed to be of intrinsic cardiac origin Marbán and Liao (2022). Early preclinical work in animal models of HFrEF demonstrated CDCs exhibit profound regenerative bioactivity in mice, rats, and pigs, and in some experiments CDCs outperformed MSCs (Malliaras and Marbán 2011). In light of the remarkable preclinical safety and efficacy data demonstrated by CDCs, a first in-human clinical trial (CADUCEUS) of autologous CDCs began in 2009. The results from CADUCEUS showed a reduction in infarct size and an increase in viable myocardium, as evidenced by cardiac MRI at 6 and 12 months following treatment (Makkar et al. 2012). While CADUCEUS was in process, basic studies made it clear that transplanted CDCs left behind lasting structural and functional benefits but did not linger in the heart for more than 3–4 weeks, leading to a shift from the autologous paradigm (cells grown from a given patient and transplanted back into that same patient) to an allogeneic (unrelated donor) paradigm. Following the CADUCEUS trial, two additional trials tested the efficacy of allogeneic CDCs—such that a cell product can be manufactured and stored for on-demand access. The first trial (ALLSTAR), while showing a favorable safety profile, did not meet the primary efficacy endpoint. Nevertheless, reductions in left ventricular volumes and circulating natriuretic peptides demonstrated disease-modifying bioactivity (Makkar et al. 2020). The second trial (DYNAMIC) tested CDCs in patients with advanced HFrEF, which reported improvements in EF, end-systolic volume, and quality of life score at 6 months. The improvements in EF and quality of life score remained significant at the 12-month follow-up (Chakravarty et al. 2020).

At the time of writing, only two cell types appear to be on a path for active commercial development as cell-based therapies with the ultimate goal of product registration for cardiac indications: allogeneic mesenchymal precursor cells are being evaluated in a phase III study for HFrEF (DREAM HF-1 trial), and allogeneic CDCs are being developed for the cardiomyopathy (and the skeletal myopathy) associated with DMD (HOPE-2 trial). More and more, the realization that cell therapy exerts indirect benefits has led to changes in treatment strategies, with a shift toward systemic (typically intravenous) delivery and repeat dosing in the case of chronic illnesses such as DMD (Marbán 2018a). Both of these innovations are first reflected in the design of the HOPE-2 trial, which is discussed more extensively below.

7.2.1.2 Heart Failure with Preserved Ejection Fraction

Given the bleak outlook in the search of effective therapeutic strategies for HFpEF, and recent mechanistic insights implicating inflammation, fibrosis, and vascular dysfunction, some researchers have turned to cell therapy for answers. To date, only a limited number of cell therapy studies have been performed in preclinical models of HFpEF—most notably CDCs—for which the evidence at present indicates favorable therapeutic bioactivity. In the first published study of cell therapy in HFpEF, CDCs normalized myocardial relaxation and diastolic pressure while improving survival in a preclinical rat model (Gallet et al. 2016). These benefits occurred despite the continued presence of hypertension and myocardial hypertrophy. Serendipitously, CDCs reversed myocardial inflammation and fibrosis—two critical pathological features of HFpEF. Subsequent preclinical studies revealed cardiac electrical abnormalities underlie sudden death in HFpEF (Cho et al. 2017, 2018b) which could be reversed by CDC treatment (Cho et al. 2018a).

With the favorable safety profile and hints of efficacy of CDCs in human patients with HFrEF, and preclinical data supporting efficacy in HFpEF, the REGRESS-HFpEF trial was initiated. The trial is currently testing allogenic CDCs using catheter-based intracoronary infusions in patients with HFpEF. The only other clinical trial using cell therapy for HFpEF is CELLpEF, which is assessing the safety and efficacy of transendocardial CD34+ cells. If the preliminary evidence from either of these two clinical trials is promising, it may lay the foundation for future studies exploring the efficacy of novel cell therapies for HFpEF. Nevertheless, the conceptual innovations of systemic delivery and repeat dosing (Rogers et al. 2019b, 2020b; Aminzadeh et al. 2018; Reich et al. 2016) have not yet reached clinical testing in the case of HFpEF, such that any benefit seen in the ongoing trials may be a lower limit estimate of likely therapeutic value.

7.2.1.3 Duchenne Muscular Dystrophy

As in the initial efforts of cell therapy for cardiac regeneration, transplantation of skeletal myoblasts was also evaluated as a therapeutic solution in DMD. Here, the underlying hypothesis was that transplanted myoblasts with a non-mutated dystrophin gene would engraft, proliferate, differentiate, and form new myofibers, which would also express functional dystrophin. This notion was supported by pilot studies in the *mdx* mouse, a widely studied preclinical model of DMD, which showed transplanted myoblasts (with a non-mutated copy of the dystrophin gene) sufficed to regenerate dystrophic skeletal muscle (Law et al. 1988; Partridge et al. 1989). Success of these early myoblast transfer studies motivated in-human clinical testing in DMD patients. However, despite preclinical efficacy, the majority of early phase clinical trials failed to demonstrate any functional improvements nor any evidence of dystrophin-positive myofibers (Mendell et al. 1995; Gussoni et al. 1997; Karpati et al. 1993). Poor survival and insufficient migration of the transplanted myoblasts, and the possibility of immune rejection were offered as explanations for the lack of efficacy (Skuk and Tremblay 2014). However, nearly a decade of technical and conceptual innovations in trial design, transplantation techniques, and immunosuppression strategies have provided insight to improve outcomes. Consequently, the next-generation of clinical trials demonstrated donor-derived dystrophin-positive myofibers (Skuk et al. 2004, 2006; 2007). Still, there remains much room for improvement. These latest trials illustrate the importance of trial design based on foundations supported by basic studies.

Other cell types have also been evaluated, based on known mechanism of action, for efficacy in DMD. One such example is mesangioblasts, which not only have a demonstrated ability to differentiate into several mesodermal lineages including skeletal muscle but also associate with the vascular endothelium to enable extravasation from the circulation after systemic infusion (Sampaolesi et al. 2003; Minasi et al. 2002). In preclinical studies, mesoangioblasts exert therapeutic bioactivity in murine models of muscular dystrophy, including DMD (Sampaolesi et al. 2006; Berry et al. 2007; Diaz-Manera et al. 2010). Moreover, genetically corrected mesoangioblasts, delivered systemically to the golden retriever muscular dystrophy (GRMD) dog model, stimulated dystrophin expression in up to 50% of myofibers from studied hind-limb muscles, leading to improved muscle structure and function (Sampaolesi et al. 2006). As a result, a phase I/IIa trial (EudraCT) delivering HLA-matched mesoangioblasts to five DMD patients was launched (Cossu et al. 2015). Unfortunately, the hope for mesoangioblasts as a therapeutic candidate for DMD has faded due to poor donor cell engraftment and dystrophin expression (Biressi et al. 2020).

The potential of MSCs to give rise to myogenic stem cells (a population distinct from satellite cells) (Liu et al. 2007; De Bari et al. 2003) supported their evaluation in DMD. MSCs from diverse sources such as human adipose tissue (Vieira et al. 2012) and human dental pulp (Kerkis et al. 2008) engraft and express dystrophin to varying degrees in GRMD dogs. In one pediatric and two adult Becker muscular dystrophy

patients, intravenous administration of human umbilical cord-derived MSCs (UC-MSCs) were studied (Li et al. 2015). No improvements in the histology of muscle biopsies were reported; however, gait improvements were observed during the clinical examination of the pediatric patient. A subsequent in-human study (NCT02484560) recruited nine DMD patients to receive allogeneic UC-MSCs delivered in a combination of intramuscular and systemic injections. Despite pulmonary function being improved in all patients, variable dystrophin expression was reported, and limb muscle strength was not different between pre- and post-treatment assessments, though most treated patients had a reduction in circulating creatine kinase levels (Dai et al. 2018).

Nevertheless, the daunting search for novel therapeutic candidates for DMD continues. Because of their ability to favorably target inflammation, fibrosis, and cardiomyogenesis, CDCs represent a logical candidate to antagonize the pathophysiology of DMD. We first evaluated the therapeutic bioactivity of intramyocardially delivered CDCs to impact on cardiomyopathy in *mdx* mice (Aminzadeh et al. 2018). Based on insights discovered from earlier work in HFrEF, the hope was that CDCs would target the secondary pathology in the hearts of *mdx* mice—that is, inflammation, fibrosis, and necrosis. Indeed, not only did CDCs reduce myocardial inflammation and fibrosis they also stimulated cardiomyogenesis by way of increased cardiomyocyte proliferation. A salient finding from this study was the discovery that CDCs were also bioactive in the skeletal muscle of *mdx* mice—a finding that has paved the way for their use in other skeletal muscle indications. Consequently, this work motivated a phase I/IIa clinical trial (HOPE-Duchenne) to deliver CDCs into the coronary circulation, and a follow-on preclinical study to deliver CDCs intravenously to *mdx* mice (Rogers et al. 2019b). The HOPE-Duchenne trial demonstrated a favorable safety profile for CDCs with indications of disease-modifying bioactivity, while the preclinical study demonstrated profound disease-modifying bioactivity of CDCs when delivered intravenously to *mdx* mice. The results from both preclinical and clinical studies motivated a second phase II clinical trial (HOPE-2) to repeatedly deliver CDCs intravenously to patients with advanced-stage DMD. Data reported from this clinical trial showed significant improvements in both cardiac and skeletal muscle parameters (Marbán et al. 2020; McDonald et al. 2022), supporting both the insights discovered from the preclinical work and the adoption of a systemic delivery with repeat dosing paradigm.

7.2.1.4 Volumetric Muscle Loss

Despite decades of research, VML has remained largely refractory to many experimental strategies—all designed to recover lost muscle tissue and function. Although skeletal myoblasts were initially considered a promising cell source for VML treatment, several limitations preclude their clinical translation. Such limitations include low abundance within muscle, challenges related to purification, poor engraftment posttransplantation, and a meager self-renewal and differentiation capacity (Ding et al. 2017; Pantelic and Larkin 2018). Because of these limitations,

MSCs represent an alternative cell type with a demonstrated ability to accelerate the repair of injured animal skeletal muscle. Compared to skeletal myoblasts, MSCs can be found in greater abundance and can be harvested by minimally invasive processes such as bone marrow extraction or lipoaspiration (Mori et al. 2015; Berebichez-Fridman and Montero-Olvera 2018). By themselves, MSCs may be a suitable cell type for the treatment of VML. Unfortunately, MSCs are also susceptible to low engraftment potential and a lack of lineage-specific differentiation. The discovery of microenvironmental cues that direct MSCs toward a myogenic phenotype will be instrumental to harness the therapeutic potential of MSCs. Future studies should delineate whether MSCs work canonically or by noncanonical mechanisms such as paracrine signaling to repair damaged skeletal muscle (Shayan and Huang 2020).

We have learned a great deal regarding the appropriateness of CDCs in skeletal muscle regeneration from our experience with *mdx* mice. Our basic studies have followed a logical sequence starting with the discovery of regenerative bioactivity in *mdx* skeletal muscle, to the exploration of the regenerative mechanisms, and the adoption to pathologically similar skeletal muscle injuries such as VML. For example, in dystrophic skeletal muscle, CDCs (by way of paracrine actions) regulate inflammation by modulating the macrophage phenotype, reducing fibrotic scar size, and coaxing dysfunctional satellite cells into forming de novo myofibers (Rogers et al. 2019a, b). The congruency of these actions in DMD and VML reasonably motivated us to test the efficacy of CDCs for the VML indication. Our initial experience with CDCs in a mouse model of VML has been quite promising (Rogers et al. 2021), where CDCs: (1) improve recovery of muscle function, (2) partially restore lost muscle volume and mass, (3) increase the number of innervated myofibers, and (4) stimulate the endogenous repair machinery. If proven successful, CDCs may be a therapeutic candidate for in-human clinical testing for trauma patients with associated VML.

7.3 Current Challenges and Novel Approaches

The field of cell therapy has not been short of novel ideas, some of which have been substantiated by considerable preclinical testing to establish concepts that define the current state of regenerative medicine. Unfortunately, to date, no cell product has received FDA approval for regenerative applications. How is this possible, despite developments produced from decades of preclinical and clinical research? Several obstacles to the translation of cell products are discussed in detail below (Marbán 2018a).

7.3.1 Barriers to Product Approval

Manufacturing Challenges Nearly four decades of preclinical research have shown ample evidence for the efficacy of cell therapy in regenerative applications. Despite this fact, cells are fragile living entities that can be often difficult to manufacture and handle, which precludes the implementation of conventional manufacturing protocols (Dodson and Levine 2015). In academic settings, where cellular therapeutic candidates generally originate, little consideration is given to regulatory compliance or scalability. Early phase in-human studies generally require only a modest degree of manufacturing process optimization. Further, reproducibility of the cell product, scalability, and cost are secondary concerns as these studies are primarily designed to treat only a small cohort of patients. The progress beyond phase I clinical trials of cell therapies is dismal, in large part owing to pitfalls that lie in the progression that follows. When therapeutic products advance to further development, manufacturing priorities shift toward the cost of goods sold, process development, and quality control. Herein lies a significant barrier for cell products: in contrast to small molecules, cells are living entities with dynamic and adapting responses to environmental conditions. Therefore, a continuous, yet fundamental, challenge is maintaining therapeutic efficacy, especially for cell types that are susceptible to senescence after several cycles of cell division (Campbell et al. 2015). Another important consideration is the optimization of manufacturing processes. For example, seemingly subtle changes such as a switch from monolayer culture to suspension culture can introduce phenotypic changes that can unfavorably skew the final product (Karnieli 2015). It is easy to envision, in extreme cases, therapeutic candidates may lose their disease-modifying properties as they drift further away from the intended formulation (Szymczak et al. 2011; Marbán 2018a).

Reproducibility and Efficacy Product consistency is a major concern for commercialization. In order to be compliant with regulatory standards, a commercial product must be comparable in identity, potency, and composition from year to year. Quality control measures must be employed at integral steps in the manufacturing process to ensure product stability of master cell banks and those destined for product manufacturing. Since no allogeneic cell therapy products for regenerative medicine have been approved in the United States, no standard operating procedures exist detailing how this should be done. An ever-present concern in commercial product development is diminished efficacy, which warrants the development and validation of assays that reliably measure product potency. In the context of cell therapy, this can be quite challenging as cell therapy is often multifactorial with diverse targets, making it difficult to identify one molecule or pathway as being strictly required for a global view of therapeutic efficacy. Both in vivo and in vitro models are met with unique challenges that often preclude their utility as potency assays. Mechanistic dissection is a seemingly obvious route for identifying key factors of efficacy which, in turn, may reveal markers for potency assay development (Marbán 2018a).

Safety One critical concern in cell therapy is safety. Generally speaking, such concerns can be categorized as either: (a) related to how the cells are delivered or (b) as intrinsic to the cells themselves. Cell delivery methods can vary from being relatively harmless to exceptionally invasive, which (of course) tracks accordingly with an escalating risk. Although intravenous infusion is the least invasive method used clinically, it has been considered to be inferior if the goal is targeting specific tissues such as the heart or skeletal muscle—due to the absence of direct administration to the organ itself. In light of mounting evidence that cells can exert long-distance effects by way of secreted exosomes (as reviewed in the next section), intravenous delivery is poised for a resurgence.

Naturally, the risk of neoplasm formation is pervasive with any proliferating transplanted cell. In the extreme, pluripotent cells are known to form tumors in vivo. As such, pre-differentiation and removal of residual pluripotent cells are critical precautions one must consider prior to clinical application (Fox et al. 2014). However, allogeneic adult stem and progenitor cells are likely cleared by the patient's immune system over time (Malliaras et al. 2012), which may fortuitously confer an added level of safety. On the other hand, expedient immune clearance may undermine the ability of the allogeneic cells to exert their paracrine effects, potentially limiting their efficacy (Schu et al. 2012). A related concern with allogeneic cells is the risk for sensitization to foreign antigens, which may subvert the efficacy of repeat dosing protocols (Schu et al. 2012). In cases of progressive underlying pathology such as DMD, wherein multiple sequential doses over time may be needed to sustain efficacy, sensitization to allogeneic cells may be problematic. In theory, however, crossmatching donor compatibility with the recipient may mitigate the potential concern of sensitization, if such a concern proves to be true (Al-Daccak and Charron 2015; Marbán 2018a). Another approach involves premedication before infusion to avoid immune sensitization and/or infusion-related hypersensitivity reactions.

Lastly, and unique to cardiac applications of cell therapy, is the possibility of elevated electrical disturbances, that is, the potential for arrhythmogenesis. As previously discussed, the heart functions as an electromechanical syncytium where efficient coupling is required to facilitate the conduction of electrical impulses and the subsequent transmission of mechanical force. Transplanted cells may disrupt the propagation of electrical impulses in two ways: (1) by creating uncoupled or even poorly coupled clusters of heart tissue which create roadblocks for conduction and (2) by electrically coupling to the adjacent myocardium and eliciting local activation and repolarization (Smith et al. 2008). Using skeletal myoblasts as an example, their transplantation into the myocardium worsens arrhythmia by the first mechanism (Miyagawa et al. 2017). Alternatively, transplanting immature cardiomyocytes derived from pluripotent cells triggers arrhythmias by the second mechanism, creating regional substrates that facilitate reentrant circuits (Smith et al. 2008). Because of this, non-integrating adult heart cells, which typically work by secreting paracrine factors (such as exosomes), may offer a safety advantage over engrafting cell types (Marbán 2018a).

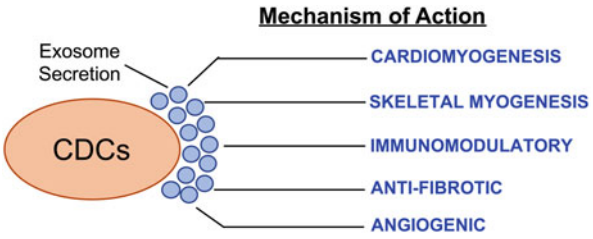

Fig. 7.2 Mechanisms of action underlying CDCs' bioactivity. CDCs secrete exosomes which target key pathological mechanisms of heart and skeletal muscle disease. Adapted from Akhmerov and Marbán (2020)

7.3.2 A Novel Paradigm in Regenerative Medicine

The development of cell therapies, to date, has not been without its challenges. To succeed in the clinic, their development must be focused on the underlying biological mechanisms that govern therapeutic efficacy. In this context, deep mechanistic insights are now placing cells themselves in the backseat and shifting attention to the cells' paracrine functions. For example, the development of CDCs for HF (and now skeletal muscle disease and injury) illustrates how mechanistic discoveries can influence the translational trajectory of cell therapies. Such paradigm shifts are only possible by questioning accepted dogma and following the data (Marbán 2018a).

The Promise of Exosomes It is widely known now that many adult stem and progenitor cells, such as MSCs and CDCs, function primarily by paracrine mechanisms. One such paracrine function is the secretion of extracellular vesicles, including exosomes, which are lipid bilayer nanoparticles laden with rich repertoires of bioactive molecules such as nucleic acids, proteins, and lipids. Exosomes are synthesized by the ceramide-requiring endolysosomal pathway, where cell membrane invaginations (endosomes) fuse with products of the Golgi apparatus to create multivesicular bodies (Ibrahim and Marbán 2016). When multivesicular bodies fuse to the intracellular face of the cell membrane, they release their contents (including exosomes) into the extracellular space. Neighboring or distant cells internalize the exosomes which can reshape the transcriptomic landscape and greatly influence cell behavior.

Several lines of evidence (in both heart and skeletal muscle disease) now demonstrate blocking exosome production disrupts CDC bioactivity, whereas CDC-derived exosomes (CDC_{EXO}) suffice to reproduce the benefits of the parent CDCs (Ibrahim et al. 2014; Rogers et al. 2019b). Thus, exosomes secretion is a necessary step for CDC efficacy (Fig. 7.2). Many of the effects of exosomes can be explained by their RNA cargo, specifically non-coding RNAs (ncRNAs) (Rogers et al. 2020a). Among the several species of ncRNAs, miRs and Y RNA fragments are abundantly present in CDC_{EXO} and (generally speaking) regulate gene

expression in the recipient cell (Rogers et al. 2020a). These discoveries have shaped the emerging concept that the mechanism of action of CDC_{EXO} requires the parent cells to secrete exosomes which act as vehicles to transfer bioactive cargo into recipient cells; thus, inducing the transcriptomic and phenotypic changes that underlie the benefits of cell therapy. Given the fact exosomes are bioactive themselves, they may be an attractive next-generation cell-free therapeutic candidate (Marbán 2018a).

The question then becomes: will exosomes entirely replace cells as therapeutic products? The answer is not so trivial, but with all things considered—probably not. Therapeutic cells have been in development for decades, in contrast to exosomes, which have been tested in only a handful of trials in humans (Marbán 2018b). This may be in large part due to the fact exogenously delivered exosomes are still poorly understood in terms of optimal delivery methods, their tissue distribution profile, and mechanisms of bioactivity. The challenge is identifying situations where cells would be preferable to exosomes. Acute myocardial infarction, for example, may be one indication where cells may outperform exosomes. Here, the rationale is when both products are directly infused into the coronary circulation (Gallet et al. 2017), cells become lodged within the capillaries and may function as sustained-release factories for exosomes, whereas exosomes (being up to 1000 times smaller) quickly pass through the coronary vessels to the systemic circulation thus diluting their bioactivity (Marbán 2018a).

Notwithstanding, therapeutic exosomes offer the potential to circumvent the main limitations of cell therapy. For example, some advantages of exosomes include product stability (Akers et al. 2016) and immune tolerance (i.e., human CDC_{EXO} are therapeutic in non-immunosuppressed mice (Rogers et al. 2019b), rats (Cambier et al. 2017), and pigs (Gallet et al. 2017; Dawkins et al. 2022), even after multiple doses (Aminzadeh et al. 2018)). Further, exosome dosing strategies are not limited by microvascular plugging, and their efficacy can be enhanced by multiple approaches such as engineering of the producer cells (Conlan et al. 2017). Given the role of exosomes is clear, focus has now shifted to identifying the factors within exosomes that are responsible for mediating their disease-modifying bioactivity in any given indication. For example, miR-146a, miR-181b, and a Y RNA fragment have been shown to be central mediators of cardioprotection in acute myocardial infarction (Ibrahim et al. 2014; de Couto et al. 2017; Cambier et al. 2017); and miR-148a is required for CDC_{EXO}-mediated myogenesis in *mdx* skeletal muscle (Rogers et al. 2019b). Two important implications can be borne from the identification of defined factors themselves: (1) the factors themselves, individually or in combination, may be developed as new therapeutic candidates and (2) the potential to use mechanistic discoveries to aid the development of cell therapies toward the goal of enhanced efficacy. For example, the development of potency assays based on mechanistic insights may offer the quantification of the defined factors in exosomes secreted by prospective cell candidate. Thus, the search for mechanistic discoveries, instead of simply drifting science away from cells completely and toward exosomes and their defined factors, should lead to the development of approaches to improve the quality, reproducibility, and efficacy of cell therapy (Marbán 2018a).

7.4 Outlook and Future Perspectives

Within the last decade, the deconstruction of cell therapy into its mechanistic underpinnings has shifted focus away from autologous cell therapy to allogeneic applications and onwards, to exosomes and their contents as next-generation therapies. The discovery that exosomes are key mediators stemmed from the recognition that both autologous and allogeneic cell sources resulted in equivalent benefits that persisted long after the original cells were no longer detectable. Mining of the exosomal cargo, in turn, has implicated specific ncRNA species as defined factors worthy of scientific inquiry. The commercialization of therapeutic exosome products is in its infancy, and their trajectory toward clinical use remains to be determined. Despite the shift away from cells toward exosomes and defined factors, cell therapies still hold potential for application in well-defined indications.

References

Aguilar CA, Greising SM, Watts A, Goldman SM, Peragallo C, Zook C, Larouche J, Corona BT (2018) Multiscale analysis of a regenerative therapy for treatment of volumetric muscle loss injury. Cell Death Dis 4(1):1–11

Akers JC, Ramakrishnan V, Yang I, Hua W, Mao Y, Carter BS, Chen CC (2016) Optimizing preservation of extracellular vesicular miRNAs derived from clinical cerebrospinal fluid. Cancer Biomark 17(2):125–132

Akhmerov A, Marbán E (2020) COVID-19 and the heart. Circ Res 126(10):1443–1455

Al-Daccak R, Charron D (2015) Allogenic benefit in stem cell therapy: cardiac repair and regeneration. Tissue Antigens 86(3):155–162

Aminzadeh MA, Rogers RG, Fournier M, Tobin RE, Guan X, Childers MK, Andres AM, Taylor DJ, Ibrahim A, Ding X, Torrente A, Goldhaber JI, Lewis MI, Gottlieb RA, Victor RA, Marbán E (2018) Exosome-mediated benefits of cell therapy in mouse and human models of Duchenne muscular dystrophy. Stem Cell Rep 10(3):942–955

Basalay M, Yellon D, Davidson S (2020) Targeting myocardial ischaemic injury in the absence of reperfusion. Basic Res Cardiol 115(6):1–16

Behfar A, Faustino RS, Arrell DK, Dzeja PP, Perez-Terzic C, Terzic A (2008) Guided stem cell cardiopoiesis: discovery and translation. J Mol Cell Cardiol 45(4):523–529

Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K (2003) Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 114(6):763–776

Berebichez-Fridman R, Montero-Olvera PR (2018) Sources and clinical applications of mesenchymal stem cells: state-of-the-art review. Sultan Qaboos Univ Med J 18(3):e264

Berry SE, Liu J, Chaney EJ, Kaufman SJ (2007) Multipotential mesoangioblast stem cell therapy in the mdx/utrn-/-mouse model for Duchenne muscular dystrophy. Regen Med 2(3):275–288

Biressi S, Filareto A, Rando TA (2020) Stem cell therapy for muscular dystrophies. J Clin Invest 130(11):5652–5664

Birnkrant DJ, Bushby K, Bann CM, Alman BA, Apkon SD, Blackwell A, Case LE, Cripe L, Hadjiyannakis S, Olson AK (2018) Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. Lancet Neurol 17(4):347–361

Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T (2011) Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. The Lancet 378(9806):1847–1857

Borlaug BA (2014) The pathophysiology of heart failure with preserved ejection fraction. Nat Rev Cardiol 11(9):507–515

Borlaug BA, Paulus WJ (2011) Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. Eur Heart J 32(6):670–679

Borlaug BA, Redfield MM, Melenovsky V, Kane GC, Karon BL, Jacobsen SJ, Rodeheffer RJ (2013) Longitudinal changes in left ventricular stiffness: a community-based study. Circ Heart Fail 6(5):944–952

Buddhe S, Cripe L, Friedland-Little J, Kertesz N, Eghtesady P, Finder J, Hor K, Judge DP, Kinnett K, McNally EM (2018) Cardiac management of the patient with Duchenne muscular dystrophy. Pediatrics 142(suppl 2):S72–S81

Caldwell C, Mattey D, Weller R (1990) Role of the basement membrane in the regeneration of skeletal muscle. Neuropathol Appl Neurobiol 16(3):225–238

Cambier L, de Couto G, Ibrahim A, Echavez AK, Valle J, Liu W, Kreke M, Smith RR, Marbán L, Marbán E (2017) Y RNA fragment in extracellular vesicles confers cardioprotection via modulation of IL-10 expression and secretion. EMBO Mol Med 9(3):337–352

Campbell A, Brieva T, Raviv L, Rowley J, Niss K, Brandwein H, Oh S, Karnieli O (2015) Concise review: process development considerations for cell therapy. Stem Cells Transl Med 4(10): 1155–1163

Chakravarty T, Henry TD, Kittleson M, Lima J, Siegel RJ, Slipczuk L, Pogoda JM, Smith RR, Malliaras K, Marbán L, Ascheim DD, Marbán E, Makkar RR (2020) Allogeneic cardiosphere-derived cells for the treatment of heart failure with reduced ejection fraction: results of the Dilated cardiomYopathy iNtervention with Allogeneic Myocardially-regeneratIve Cells (DYNAMIC) trial. EuroIntervention 16:e293–e300

Cho JH, Zhang R, Kilfoil PJ, Gallet R, de Couto G, Bresee C, Goldhaber JI, Marbán E, Cingolani E (2017) Delayed repolarization underlies ventricular arrhythmias in rats with heart failure and preserved ejection fraction. Circulation 136(21):2037–2050

Cho JH, Kilfoil PJ, Zhang R, Solymani RE, Bresee C, Kang EM, Luther K, Rogers RG, de Couto G, Goldhaber JI, Marbán E, Cingolani E (2018a) Reverse electrical remodeling in rats with heart failure and preserved ejection fraction. JCI Insight 3(19)

Cho JH, Zhang R, Aynaszyan S, Holm K, Goldhaber JI, Marbán E, Cingolani E (2018b) Ventricular arrhythmias underlie sudden death in rats with heart failure and preserved ejection fraction. Circ Arrhythm Electrophysiol 11(8):e006452

Conlan RS, Pisano S, Oliveira MI, Ferrari M, Pinto IM (2017) Exosomes as reconfigurable therapeutic systems. Trends Mol Med 23(7):636–650

Connuck DM, Sleeper LA, Colan SD, Cox GF, Towbin JA, Lowe AM, Wilkinson JD, Orav EJ, Cuniberti L, Salbert BA (2008) Characteristics and outcomes of cardiomyopathy in children with Duchenne or Becker muscular dystrophy: a comparative study from the Pediatric Cardiomyopathy Registry. Am Heart J 155(6):998–1005

Cossu G, Previtali SC, Napolitano S, Cicalese MP, Tedesco FS, Nicastro F, Noviello M, Roostalu U, Natali Sora MG, Scarlato M (2015) Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy. EMBO Mol Med 7(12):1513–1528

Dai A, Baspinar O, Yeşilyurt A, Sun E, Aydemir Çİ, Öztel ON, Capkan DU, Pinarli F, Agar A, Karaöz E (2018) Efficacy of stem cell therapy in ambulatory and nonambulatory children with Duchenne muscular dystrophy—phase I–II. Degener Neurol Neuromuscul Dis 8:63

Dawkins JF, Ehdaie A, Rogers R, Soetkamp D, Valle J, Holm K et al (2022) Biological substrate modification suppresses ventricular arrhythmias in a porcine model of chronic ischaemic cardiomyopathy. Eur Heart J

De Bari C, Dell'Accio F, Vandenabeele F, Vermeesch JR, Raymackers J-M, Luyten FP (2003) Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. J Cell Biol 160(6):909–918

de Couto G, Gallet R, Cambier L, Jaghatspanyan E, Makkar N, Dawkins JF, Berman BP, Marbán E (2017) Exosomal microRNA transfer into macrophages mediates cellular postconditioning. Circulation 136(2):200–214

Diaz-Manera J, Touvier T, Dellavalle A, Tonlorenzi R, Tedesco F, Messina G, Meregalli M, Navarro C, Perani L, Bonfanti C (2010) Partial dysferlin reconstitution by adult murine mesoangioblasts is sufficient for full functional recovery in a murine model of dysferlinopathy. Cell Death Dis 1(8):e61–e61

Ding S, Wang F, Liu Y, Li S, Zhou G, Hu P (2017) Characterization and isolation of highly purified porcine satellite cells. Cell Death Dis 3(1):1–11

Dodson BP, Levine AD (2015) Challenges in the translation and commercialization of cell therapies. BMC Biotechnol 15(1):1–15

Fox IJ, Daley GQ, Goldman SA, Huard J, Kamp TJ, Trucco M (2014) Use of differentiated pluripotent stem cells in replacement therapy for treating disease. Science 345(6199)

Gallet R, de Couto G, Simsolo E, Valle J, Sun B, Liu W, Tseliou E, Zile MR, Marbán E (2016) Cardiosphere-derived cells reverse heart failure with preserved ejection fraction in rats by decreasing fibrosis and inflammation. JACC Basic Transl Sci 1(1):14–28

Gallet R, Dawkins J, Valle J, Simsolo E, De Couto G, Middleton R, Tseliou E, Luthringer D, Kreke M, Smith RR, Marbán L, Ghaleb B, Marbán E (2017) Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction. Eur Heart J 38(3):201–211

Gladden JD, Chaanine AH, Redfield MM (2018) Heart failure with preserved ejection fraction. Annu Rev Med 69:65–79

Greising SM, Rivera JC, Goldman SM, Watts A, Aguilar CA, Corona BT (2017) Unwavering pathobiology of volumetric muscle loss injury. Sci Rep 7(1):1–14

Greising SM, Corona BT, McGann C, Frankum JK, Warren GL (2019) Therapeutic approaches for volumetric muscle loss injury: a systematic review and meta-analysis. Tissue Eng Part B Rev 25(6):510–525

Gussoni E, Blau HM, Kunkel LM (1997) The fate of individual myoblasts after transplantation into muscles of DMD patients. Nat Med 3(9):970–977

Hare J, Traverse J, Henry T (2008) The myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) trial: first randomized placebo controlled study of myoblast transplantation. Circulation 117:1189–1200

Heusch G, Libby P, Gersh B, Yellon D, Böhm M, Lopaschuk G, Opie L (2014) Cardiovascular remodelling in coronary artery disease and heart failure. Lancet 383(9932):1933–1943

Ibrahim A, Marbán E (2016) Exosomes: fundamental biology and roles in cardiovascular physiology. Annu Rev Physiol 78:67–83

Ibrahim A, Cheng K, Marbán E (2014) Exosomes as critical agents of cardiac regeneration triggered by cell therapy. Stem Cell Rep 2(5):606–619

Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA (2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 107(11):1395–1402

Karnieli O (2015) Cell therapy: early process development and optimization of the manufacturing process are critical to ensure viability of the product, quality, consistency and cost efficiency. J Commerc Biotechnol 21(1)

Karpati G, Ajdukovic D, Arnold D, Gledhill RB, Guttmann R, Holland P, Koch PA, Shoubridge E, Spence D, Vanasse M (1993) Myoblast transfer in Duchenne muscular dystrophy. Ann Neurol 34(1):8–17

Kerkis I, Ambrosio CE, Kerkis A, Martins DS, Zucconi E, Fonseca SA, Cabral RM, Maranduba CM, Gaiad TP, Morini AC (2008) Early transplantation of human immature dental pulp stem

cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: local or systemic? J Transl Med 6(1):1–13

Koh GY, Klug MG, Soonpaa MH, Field LJ (1993) Differentiation and long-term survival of C2C12 myoblast grafts in heart. J Clin Invest 92(3):1548–1554

Lancet T (2019) Retraction—Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet (London, England) 393(10176): 1084

Law PK, Goodwin TG, Wang MG (1988) Normal myoblast injections provide genetic treatment for murine dystrophy. Muscle Nerve 11(6):525–533

Lepper C, Partridge TA, Fan C-M (2011) An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. Development 138(17):3639–3646

Li P, Cui K, Zhang B, Wang Z, Shen Y, Wang X, Zhang J, Tong F, Li S (2015) Transplantation of human umbilical cord-derived mesenchymal stems cells for the treatment of Becker muscular dystrophy in affected pedigree members. Int J Mol Med 35(4):1051–1057

Liu Y, Yan X, Sun Z, Chen B, Han Q, Li J, Zhao RC (2007) Flk-1+ adipose-derived mesenchymal stem cells differentiate into skeletal muscle satellite cells and ameliorate muscular dystrophy in mdx mice. Stem Cells Dev 16(5):695–706

Ma C, Luo H, Fan L, Liu X, Gao C (2020) Heart failure with preserved ejection fraction: an update on pathophysiology, diagnosis, treatment, and prognosis. Braz J Med Biol Res 53(7)

Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marbán L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marbán E (2012) Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. The Lancet 379(9819):895–904

Marbán E, Liao K (2022) On the cellular origin of cardiosphere-derived cells (CDCs). Basic Res Cardiol 117(1):1–4

Makkar RR, Kereiakes DJ, Aguirre F, Kowalchuk G, Chakravarty T, Malliaras K, Francis GS, Povsic TJ, Schatz R, Traverse JH, Pogoda JM, Smith RR, Marbán L, Ascheim DD, Ostovaneh MR, Lima J, Demaria A, Marbán E, Henry TD (2020) Intracoronary ALLogeneic heart STem cells to Achieve myocardial Regeneration (ALLSTAR): a randomized, placebo-controlled, double-blinded trial. Eur Heart J 41(36):3451–3458

Malliaras K, Marbán E (2011) Cardiac cell therapy: where we've been, where we are, and where we should be headed. Br Med Bull 98(1):161–185

Malliaras K, Li T-S, Luthringer D, Terrovitis J, Cheng K, Chakravarty T, Galang G, Zhang Y, Schoenhoff F, Van Eyk J, Marbán L, Marbán E (2012) Safety and efficacy of allogeneic cell therapy in infarcted rats transplanted with mismatched cardiosphere-derived cells. Circulation 125(1):100–112

Marbán E (2018a) A mechanistic roadmap for the clinical application of cardiac cell therapies. Nature biomedical engineering 2(6):353–361

Marbán E (2018b) The secret life of exosomes: what bees can teach us about next-generation therapeutics. J Am Coll Cardiol 71(2):193–200

Marbán L, Rogy S, McDonald C, Eagle M, Finkel R, Tian C, Taylor M, Janas J, Harmelink M, Varadhachary A, Hor K, Mayer OH, Furlong P, Committee H-S (2020) HOPE-2 one-year results show clinically relevant improvements in upper limb & cardiac function in patients with later stage Duchenne muscular dystrophy. Neuromuscul Disord 30:S168–S169

McDonald CM, Marbán E, Hendrix S, Hogan N, Smith RR, Eagle M et al (2022) Repeated intravenous cardiosphere-derived cell therapy in late-stage Duchenne muscular dystrophy (HOPE-2): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 399(10329):1049–1058

Mendell JR, Kissel JT, Amato AA, King W, Signore L, Prior TW, Sahenk Z, Benson S, McAndrew PE, Rice R (1995) Myoblast transfer in the treatment of Duchenne's muscular dystrophy. N Engl J Med 333(13):832–838

Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L, Hertenstein B (2006) Intracoronary bone marrow cell transfer after myocardial infarction. Circulation 113(10):1287–1294

Minasi MG, Riminucci M, De Angelis L, Borello U, Berarducci B, Innocenzi A, Caprioli A, Sirabella D, Baiocchi M, De Maria R (2002) The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. Development 129(11):2773–2783

Miyagawa S, Domae K, Yoshikawa Y, Fukushima S, Nakamura T, Saito A, Sakata Y, Hamada S, Toda K, Pak K (2017) Phase I clinical trial of autologous stem cell–sheet transplantation therapy for treating cardiomyopathy. J Am Heart Assoc 6(4):e003918

Mori R, Kamei N, Okawa S, Nakabayashi A, Yokota K, Higashi Y, Ochi M (2015) Promotion of skeletal muscle repair in a rat skeletal muscle injury model by local injection of human adipose tissue-derived regenerative cells. J Tissue Eng Regen Med 9(10):1150–1160

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, De Ferranti S, Després J-P, Fullerton HJ (2016) Heart disease and stroke statistics—2016 update: a report from the American Heart Association. Circulation 133(4):e38–e360

Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM (2001) Bone marrow cells regenerate infarcted myocardium. Nature 410(6829):701–705

Pantelic MN, Larkin LM (2018) Stem cells for skeletal muscle tissue engineering. Tissue Eng Part B Rev 24(5):373–391

Partridge TA, Morgan J, Coulton G, Hoffman E, Kunkel L (1989) Conversion of mdx myofibres from dystrophin-negative to-positive by injection of normal myoblasts. Nature 337(6203): 176–179

Pfeffer MA, Braunwald E (1990) Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation 81(4):1161–1172

Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, González-Juanatey JR, Harjola V-P, Jankowska EA (2016) 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 37(27):2129–2200

Psaltis P, Paton S, See F, Arthur A, Martin S, Itescu S, Worthley S, Gronthos S, Zannettino A (2010) Enrichment for STRO-1 expression enhances the cardiovascular paracrine activity of human bone marrow-derived mesenchymal cell populations. J Cell Physiol 223(2):530–540

Reich H, Tseliou E, de Couto G, Angert D, Valle J, Kubota Y, Luthringer D, Mirocha J, Sun B, Smith RR (2016) Repeated transplantation of allogeneic cardiosphere-derived cells boosts therapeutic benefits without immune sensitization in a rat model of myocardial infarction. J Heart Lung Transplant 35(11):1348–1357

Rogers RG, de Couto G, Liu W, Sanchez L, Marbán E (2019a) Cardiosphere-derived cell exosomes modulate mdx macrophage phenotype and alter their secretome. FASEB J 33(S1):lb611–lb611

Rogers RG, Fournier M, Sanchez L, Ibrahim AG, Aminzadeh MA, Lewis MI, Marbán E (2019b) Disease-modifying bioactivity of intravenous cardiosphere-derived cells and exosomes in mdx mice. JCI Insight 4(7)

Rogers RG, Ciullo A, Marbán E, Ibrahim AG (2020a) Extracellular vesicles as therapeutic agents for cardiac fibrosis. Front Physiol 11:479

Rogers RG, Fournier M, Lee Y, Jones K, Sanchez L, Marbán E (2020b) Repeated administration of cardiosphere-derived cells preserves exercise capacity and muscle function for at least one year in mdx mice. Neuromuscul Disord 30:S107–S108

Rogers RG, Li L, Peck K, Sanchez L, Liu W, Ciullo A, Alfaro J, Rannou A, Fournier M, Lee Y, Marbán E (2021) Cardiosphere-derived cells, with and without a biological scaffold, stimulate myogenesis and recovery of muscle function in mice with volumetric muscle loss. Biomaterials 274:120852

Sampaolesi M, Torrente Y, Innocenzi A, Tonlorenzi R, D'Antona G, Pellegrino MA, Barresi R, Bresolin N, De Angelis MGC, Campbell KP (2003) Cell therapy of α-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. Science 301(5632):487–492

Sampaolesi M, Blot S, D'Antona G, Granger N, Tonlorenzi R, Innocenzi A, Mognol P, Thibaud J-L, Galvez BG, Barthélémy I (2006) Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. Nature 444(7119):574–579

Savarese G, Lund LH (2017) Global public health burden of heart failure. Card Fail Rev 3(1):7

Schu S, Nosov M, O'Flynn L, Shaw G, Treacy O, Barry F, Murphy M, O'Brien T, Ritter T (2012) Immunogenicity of allogeneic mesenchymal stem cells. J Cell Mol Med 16(9):2094–2103

Shah KS, Xu H, Matsouaka RA, Bhatt DL, Heidenreich PA, Hernandez AF, Devore AD, Yancy CW, Fonarow GC (2017) Heart failure with preserved, borderline, and reduced ejection fraction: 5-year outcomes. J Am Coll Cardiol 70(20):2476–2486

Shayan M, Huang NF (2020) Pre-clinical cell therapeutic approaches for repair of volumetric muscle loss. Bioengineering 7(3):97

Skuk D, Tremblay JP (2014) Clarifying misconceptions about myoblast transplantation in myology. Mol Ther 22(5):897–898

Skuk D, Roy B, Goulet M, Chapdelaine P, Bouchard J-P, Roy R, Dugré FJ, Lachance J-G, Deschênes L, Senay H (2004) Dystrophin expression in myofibers of Duchenne muscular dystrophy patients following intramuscular injections of normal myogenic cells. Mol Ther 9(3):475–482

Skuk D, Goulet M, Roy B, Chapdelaine P, Bouchard J-P, Roy R, Dugré FJ, Sylvain M, Lachance J-G, Deschênes L (2006) Dystrophin expression in muscles of Duchenne muscular dystrophy patients after high-density injections of normal myogenic cells. J Neuropathol Exp Neurol 65(4):371–386

Skuk D, Goulet M, Roy B, Piette V, Côté CH, Chapdelaine P, Hogrel J-Y, Paradis M, Bouchard J-P, Sylvain M (2007) First test of a "high-density injection" protocol for myogenic cell transplantation throughout large volumes of muscles in a Duchenne muscular dystrophy patient: eighteen months follow-up. Neuromuscul Disord 17(1):38–46

Smith R, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, Giacomello A, Abraham MR, Marbán E (2007) Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation 115(7):896–908

Smith RR, Barile L, Messina E, Marbán E (2008) Stem cells in the heart: what's the buzz all about? Part 2: Arrhythmic risks and clinical studies. Heart Rhythm 5(6):880–887

Soni M, Ferrell B, Wikholm C, Wilson LT (2020) Stem cell therapies and treatment advances for heart failure with preserved ejection fraction. Georgetown Med Rev 4(1):12344

Sürder D, Manka R, Lo Cicero V, Moccetti T, Rufibach K, Soncin S, Turchetto L, Radrizzani M, Astori G, Schwitter J (2013) Intracoronary injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: effects on global left ventricular function. Circulation 127(19):1968–1979

Szymczak MM, Friedman RL, Uppoor R, Yacobi A (2011) Detection, measurement, and control in pharma manufacturing. Pharm Technol 35:70–76

Taylor DA, Atkins BZ, Hungspreugs P, Jones TR, Reedy MC, Hutcheson KA, Glower DD, Kraus WE (1998) Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. Nat Med 4(8):929–933

Traverse JH, Henry TD, Pepine CJ, Willerson JT, Zhao DX, Ellis SG, Forder JR, Anderson RD, Hatzopoulos AK, Penn MS (2012) Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial. JAMA 308(22):2380–2389

Van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin S-CJ, Middleton RC, Marbán E, Molkentin JD (2014) C-kit+ cells minimally contribute cardiomyocytes to the heart. Nature 509(7500):337–341

Vieira N, Valadares M, Zucconi E, Secco M, Junior CB, Brandalise V, Assoni A, Gomes J, Landini V, Andrade T (2012) Human adipose-derived mesenchymal stromal cells injected

systemically into GRMD dogs without immunosuppression are able to reach the host muscle and express human dystrophin. Cell Transplant 21(7):1407–1417

Voronov R (1975) Experimental study of the regenerative potentialities of the cardiac and somatic musculatures. Arkh Anat Gistol Embriol 69(9):35–40

Wang L-T, Ting C-H, Yen M-L, Liu K-J, Sytwu H-K, Wu KK, Yen BL (2016) Human mesenchymal stem cells (MSCs) for treatment towards immune-and inflammation-mediated diseases: review of current clinical trials. J Biomed Sci 23(1):1–13

Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL (2013) 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 62(16):e147–e239

Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Colvin MM, Drazner MH, Filippatos GS, Fonarow GC, Givertz MM (2017) 2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. J Am Coll Cardiol 70(6):776–803

Chapter 8
Regenerative Rehabilitation Strategies for Complex Bone Injuries

Kylie E. Nash, Keat Ghee Ong, Eyerusalem A. Gebreyesus, Steven A. LaBelle, Jeffrey A. Weiss, Julia A. Harrer, Nick J. Willett, Philipp Leucht, and Robert E. Guldberg

Abstract Complex bone injuries often impact several types of tissue and experience healing complications due to injury severity, infection, instability, or other comorbidities such as diabetes. The intricacies and poor functional outcomes of these injuries merit the consideration of regenerative medicine in concert with rehabilitation strategies to improve healing. The field of regenerative rehabilitation is emerging with promising technologies, since bone is a dynamic tissue that is highly sensitive to mechanical stimulus and its regeneration is accomplished by a cascade of biological processes that are strongly influenced by local mechanical loading (Carter et al., Clin Orthop Relat Res (1976–2007) 355:S41, 1998; Kenwright and Goodship, Clin Orthop Relat Res 241:36–47, 1989). Enabling technologies to investigate these injuries include regenerative strategies such as protein or cell delivery to augment the endogenous biological environment and promote healing; implantable sensors and wireless technologies to longitudinally quantify the in vivo mechanical environment of the defect in a real-time, patient-specific manner; as well as computational models of tissue differentiation and healing to provide predictive mechanical boundary conditions that optimize healing. Ultimately, integration of

K. E. Nash · K. G. Ong · E. A. Gebreyesus · N. J. Willett · R. E. Guldberg (✉)
Phil and Penny Knight Campus for Accelerating Scientific Impact, University of Oregon, Eugene, OR, USA
e-mail: guldberg@uoregon.edu

S. A. LaBelle · J. A. Weiss
Scientific Computing and Imaging Institute and Department of Biomedical Engineering, University of Utah, Salt Lake City, UT, USA

J. A. Harrer
Phil and Penny Knight Campus for Accelerating Scientific Impact, University of Oregon, Eugene, OR, USA

Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, USA, Atlanta, GA

P. Leucht
Department of Orthopedic Surgery and Cell Biology, NYU Grossman School of Medicine, New York, NY, USA

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_8

these emerging innovations with advancements in understanding the fundamental biological principles of mechanotransduction may enable optimizing personalized feedback-controlled rehabilitation strategies that promote functional restoration of complex bone injuries.

Keywords Regenerative medicine · Rehabilitation · Mechanical loading · Sensors · Patient-specific · Real-time

8.1 Introduction and Current Clinical Practice

This chapter will discuss the intersection of regenerative medicine and rehabilitative strategies to address complex bone injuries that often experience healing complications due to injury severity, infection, instability, or other comorbidities such as type I and II diabetes. These injuries are intricate and prone to poor functional outcomes, which merits the consideration of bone regeneration in concert with rehabilitation strategies to improve healing. Regenerative medicine concentrates on regrowing or replacing injured, diseased, or defective tissues, while the purpose of rehabilitation is to facilitate functional recovery after an injury, illness, or disease. The process of bone healing involves regeneration of bone tissue that continually responds to the biological and mechanical environment. Therefore, the field of regenerative rehabilitation can provide potential therapeutic strategies for complex injuries, because rehabilitation can provide a beneficial mechanical environment while regenerative medicine promotes a beneficial biologic environment to optimize functional healing. The following sections will contextualize the intersection of regenerative medicine and rehabilitation strategies for complex bone injuries to ultimately identify some of the current best practices, limitations, and areas for continued investigation. This chapter is organized into emerging technologies and translatable knowledge gained from multivariate computational models, implantable orthopedic sensors, and preclinical studies that combine regenerative and rehabilitation strategies.

8.1.1 Pathophysiology of Complex Bone Injuries

Complex bone injuries often leave patients devastated with high complication rates and poor functional restoration due to diminished coordination between the cellular activity within the healing niche and the surrounding tissue (Fig. 8.1). In fact, 5–10% of the 12 million annual fractures in the United States do not heal in a timely fashion or lack restoration of function entirely (Klosterhoff et al. 2017a). Large or complex bone injuries often stem from accident or combat traumas, tumor resection surgeries, or congenital defects (Uhrig et al. 2013). Open fractures can be classified using the Gustilo-Anderson classification which includes type I, II, or III where type III can be further described as grade A, B, or C (Egol et al. 2010). The Gustilo-Anderson classification design ranks injury severity on factors such as wound size, level of

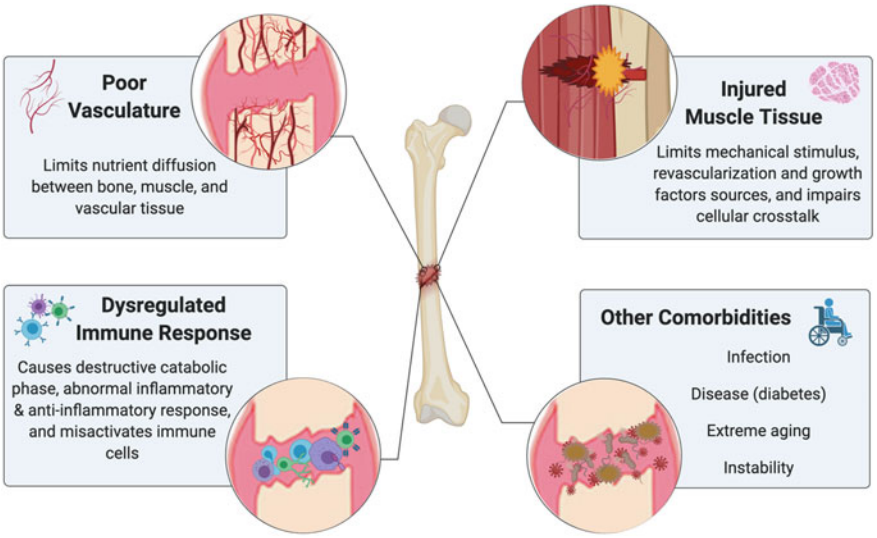

Fig. 8.1 Factors that contribute to bone nonunion or healing complications following complex bone injuries. Created with BioRender.com

contamination, and damage to soft tissue and bone (Egol et al. 2010). Injuries are ranked more severe for increasing injury types, so type IIIC is the most severe with a wound size greater than 10 cm in length, high level of contamination, severe loss of soft tissue and bone coverage, plus vascular damage that requires repair (Egol et al. 2010). In preclinical research, large bone injuries can also be described as critical-size defects, which do not heal despite surgical stabilization. Bone healing can result in union, delayed union, or nonunion. Following injury, bone union is achieved when the bone is healed and strong enough to resume normal activity, delayed union is when the bone takes longer than usual to heal but the function is eventually restored, and nonunion is when the bone does not bridge and mechanical function is not restored following a severe injury.

In preclinical research, complex bone injuries that result in nonunion can be modeled by inducing a critical-size bone defect in animal subjects (Boerckel et al. 2011). To examine the functional ability of the regenerated bone tissue, defects are made in a weight-bearing bone and ex vivo mechanical tests are performed to quantify functional restoration (Boerckel et al. 2012). Radiography and microcomputed tomography may also be performed to longitudinally examine the defect throughout healing. These injury models provide crucial preclinical platforms to evaluate potential regenerative strategies in vivo to address clinically observed complications associated with delayed or nonunion bone healing.

8.1.1.1 Stages of Bone Healing

Following injury, bone healing consists of distinct, yet overlapping stages, including the early inflammatory stage, the repair stage, and the late remodeling stage (Kalfas 2001). Within a few hours of injury, the early inflammatory stage is initiated with hematoma formation to trigger infiltration of inflammatory cells and fibroblasts. The infiltration of these cells results in the formation of granulation tissue, ingrowth of vascular tissue, and migration of mesenchymal cells. Nonunion or poor healing complications can arise during this early stage if the inflammatory response is altered by the use of anti-inflammatory or cytotoxic medications (Kalfas 2001).

Next, the repair stage consists of fibroblasts building a stroma to support continual vascular ingrowth and infiltration of osteoprogenitor cells. As vascular ingrowth progresses, mesenchymal stem cells (MSCs) can differentiate directly into osteoblasts to form bone via intramembranous ossification or into chondrocytes to promote the formation of cartilage which serves as a template to be completely replaced by bone via endochondral ossification. The transition from cartilage to bone tissue begins with the differentiation of cartilage-producing chondrocytes into terminally differentiated hypertrophic chondrocytes which is associated with the subsequent invasion of blood vessels. Then MSCs from the periosteum (the enveloping connective tissue for cartilage) or vascular pericytes differentiate into osteoblasts and begin to form woven bone in the mineralized callus that is later remodeled into mature lamellar bone. The newly formed callus and the surrounding blood supply is sensitive to mechanical stimulus and thus requires adequate protection and stability in the form of external fixation via brace or internal fixation via a plate or nail (Elliott et al. 2016; McKibbin 1978). Stabilizing the defect at this stage is crucial to preserve the newly formed tissue because instability can prevent ossification of the callus and result in nonunion or poor healing (Elliott et al. 2016; Kalfas 2001).

If proper stability is maintained, the callus ossifies to form woven bone across the defect region. At this point, the defect will enter the remodeling stage where the shape, structure, and mechanical strength of the bone is restored. Remodeling occurs over a longer period of time (months to years) and is facilitated by mechanical stress, which activates osteogenic cells to promote bone formation in regions where bone is needed and resorption where bone is not needed (Kalfas 2001). Mechanical stimulation is necessary to promote bone modeling and remodeling during later stages of bone healing, but too much too early, can impair healing (Kenwright and Goodship 1989; Klosterhoff et al. 2017a).

8.1.1.2 Role of Skeletal Muscle

Several studies have found skeletal muscle to be a key player in functional bone regeneration following complex bone injury (Elliott et al. 2016; Uhrig et al. 2013). Before injury, muscle and functional weight-bearing, provide the mechanical stimulus necessary to trigger bone modeling and remodeling, as well as proper

morphogenesis, maintenance, and repair of other tissue types (Guder et al. 2020). Beyond biomechanical stimuli, muscle is also a critical source for vascularization, progenitor cells, and osteogenic myokines to support bone repair after injury (Uhrig et al. 2013). In vivo research that utilized a preclinical model of segmental bone and volumetric muscle loss injury further established the relationship between bone and muscle tissue because injury to both tissues resulted in compromised bone healing (Willett et al. 2013). They found that a concomitant muscle injury decreased bone healing by approximately 50% compared to segmental bone injury alone for the same treatment (Willett et al. 2013). Muscle cells also help bone healing by secreting several osteogenic factors such as insulin-like growth factor (IGF-1), fibroblast growth factor (FGF-2), and transforming growth factor (TGT-β) (Ruehle et al. 2019). Additionally, muscle progenitor cells were shown to differentiate into osteogenic cell lineages when tracked in an open bone fracture and found incorporated into regenerated bone (Willett et al. 2013). The relationship between muscle and bone tissue is particularly relevant to regenerative rehabilitation because activation of muscle during rehabilitation can help guide bone regeneration and healing outcomes. Further, regenerative rehabilitation strategies can emulate the relationship between muscle and bone by modulating the mechanobiology via internal and external fixation devices that play a major role in determining the mechanical environment and subsequent pattern of healing (Kenwright and Goodship 1989).

8.1.1.3 Role of Vasculature

Vasculature is another key aspect of bone healing. In fact, vascular integrity is a key clinical indicator of injury severity, since revascularization is a critical early step for functional bone healing and is needed to stimulate bone repair (Boerckel et al. 2011; Klosterhoff et al. 2017a). However, little is known about the process of revascularization in the context of loading-induced bone regeneration. To address this gap in knowledge, a preclinical in vivo study done by Boerckel et al. quantified the effects of early and delayed functional loading on new vascular growth using their large bone defect model (Boerckel et al. 2011). The defects were stabilized with compliant internal fixation plates that were unlocked to allow ambulatory load transfer either at the time of implantation or after 4 weeks of the stiff (locked) internal fixation plate. They found that early mechanical loading inhibited vascular invasion into the defect and reduced bone formation compared to the stiff plate controls. In contrast, the delayed loading significantly enhanced bone formation and stimulated vascular remodeling (Boerckel et al. 2011). Their results demonstrate that neovascular networks are mechanosensitive and biomechanical stimulations have the capacity to modulate postnatal vascular growth and remodeling.

To expand the exploration of mechanobiology for vascular and bone tissue healing, Klosterhoff et al. engineered an implantable strain sensor platform to longitudinally measure strain across the defect stabilized with internal fixation plates in real-time and throughout rehabilitation. This in vivo study found an initial increase in deformation magnitude and a subsequent increase in bone formation

for the compliant fixator that permitted load-sharing (Klosterhoff et al. 2020). Further load-shielding from the stiff fixation plate increased the number of relatively small vessels in the defect, while the load-sharing from the compliant fixation plate had a similar vessel number and size as the naïve contralateral femur (Klosterhoff et al. 2020). Therefore, although the increased strain magnitudes from the compliant plates impaired angiogenesis, the loading still supported sufficient tissue revascularization to enhance bone repair. Their work revealed distinct magnitude-dependent mechanobiological thresholds that differentially impair either bone of neovascular growth when exceeded.

To further analyze vasculature in the context of mechanical loading, an in vitro study by Ruehle et al. studied the effect of two load initiation times, three strain magnitudes, and two modes of compressive deformation on microvascular network growth within microvascular fragment-containing gels. They found that immediate loading inhibited angiogenesis and expression of vascular-related genes, while delayed loading enhanced microvascular network formation and upstream mechanotransduction signaling pathways. From this, they demonstrated that the magnitude, mode, and initiation time of the extracellular matrix (ECM) loading were all critical regulatory parameters for angiogenesis. Vasculature is a potential target for regenerative rehabilitation strategies because the blood supply is crucial for tissue regeneration and preclinical studies found vascular tissue to respond to varying magnitudes of mechanical stimulation (Boerckel et al. 2011; Klosterhoff et al. 2020; Ruehle et al. 2020). The knowledge gained from these studies provides the potential to enhance revascularization during tissue regeneration to optimize regenerative rehabilitation strategies for complex bone injuries.

8.1.1.4 Impact of Immune Dysregulation

Healing complications after complex bone injuries can also stem from a dysregulated immune response. Past work has characterized a detrimental dysregulated immune response which consists of chronic immunosuppression and immune paralysis. Trauma-induced immune dysregulation occurs in multiple stages, including an initial systemic inflammatory response syndrome (SIRS) and a compensatory anti-inflammatory response syndrome (CARS), each with unique cytokine profiles (Lord et al. 2014). The prolonged exposure to high levels of inflammatory factors and reactive oxygen species generated during SIRS is damaging to the surrounding tissues, so the CARS compensatory response immediately follows SIRS (Rosenthal and Moore 2016). In physiological healing, the SIRS and CARS responses resolve themselves within a couple of weeks; however, failure to restore homeostasis can lead to a destructive catabolic phase, otherwise known as systemic immune dysregulation and immune suppression (SIDIS) (Vantucci et al. 2018). Patients with symptoms of SIDIS are prone to complications and greater healthcare cost (Lord et al. 2014). A recent study using a preclinical model of orthopedic trauma demonstrated that distinct systemic immune profiles correlate with this long-term immune dysregulation and impaired bone regeneration (Ruehle et al. 2020). Some of

the primary cellular mediators include T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs). These mediators contribute to the secretion of inflammatory cytokines and activation of other immune cells. Their work further supports the relationship between early systemic immune responses to trauma and local bone regeneration, with the potential to help predict poor functional outcomes and provide novel targets for immunotherapeutic interventions.

8.1.1.5 Conclusion

Overall, complex bone injuries are prone to nonunion or healing complications because of several factors including limited cellular communication and instability due to a large defect size, injury to neighboring vascular and muscular tissue, dysregulation of the systemic immune response, infection, or other comorbidities such as disease (Fig. 8.1). The restoration of bone tissue is crucial for functional recovery after complex injuries, but recent literature has found that restoring the vascular and muscular tissue, as well as controlling the immune response can help optimize necessary bone regeneration (Cheng et al. 2019; Ruehle et al. 2020). Research has also motivated the emerging field of mechanobiology because of the observed cellular response to mechanical stimulation throughout all stages of healing (Boerckel et al. 2011; Carter et al. 1998; Guldberg et al. 1997; Kalfas 2001; Klosterhoff et al. 2017a; Olesen et al. 2015). Therefore, modulating the mechanobiology of the healing niche via internal factors such as fixation stiffness or external factors such as rehabilitation has the potential to help address complex bone injuries that would otherwise face nonunion or healing complications. Additionally, the field of regenerative rehabilitation is emerging with promising strategies for complex bone injuries because the bone is a dynamic tissue that is highly sensitive to mechanical stimulus and its regeneration is accomplished by a cascade of biological processes that are strongly influenced by local tissue mechanical loading (Carter et al. 1998; Kenwright and Goodship 1989).

8.1.2 Clinical Treatment Strategies

8.1.2.1 Grafting: Masquelet Technique

There is currently no accepted medical standard to treat complex bone injuries, though the most common include debridement of necrotic tissue, prevention of infection, muscle flap coverage, bone grafting, and amputation as the last resort (Masquelet 2003; Nauth et al. 2011; Yazar et al. 2004). Within the realm of bone grafting, clinicians utilize allografts, autografts, as well as ceramic and polymeric bone graft substitutes. Allografts use bone from a deceased donor, so this practice is challenged with limited graft material as well as lower osteoinductive and osteogenic properties due to the sterilization process. Autografts use bone from the patient, so

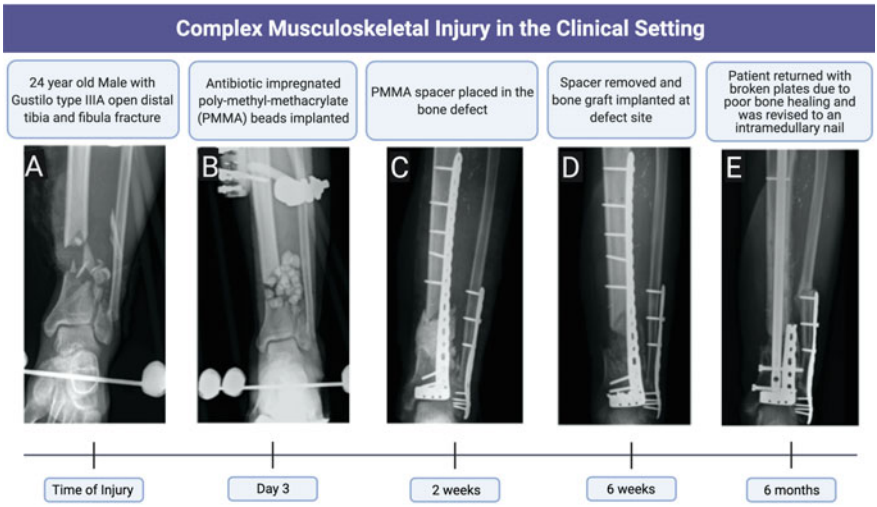

Fig. 8.2 Clinical example of a complex musculoskeletal injury that required revision surgery due to lack of bone healing after initial treatment. Courtesy of Dr. Philipp Leucht from NYU Langone. Created with BioRender.com

challenges include limited graft materials and surgical complications such as donor site morbidity. At the same time, ceramic or polymeric grafts have limited bioactivity, so they often rely on osteogenic cells or bone material to accompany the graft. Complications for this strategy include insufficient bioactivity or rejection of synthetic material altogether. The interplay between muscle and bone tissue has also motivated the clinical use of muscle flaps to help revascularize bone and promote healing of large range fractures. However, muscle flaps exhibit complications such as infection, partial or total flap loss, seroma or hematoma, necrosis, and wound dehiscence (Deramo and Rose 2020).

Historically, clinicians have tried using grafts in surgical treatments such as the Masquelet technique (Fig. 8.2) which uses a temporary cement spacer followed by staged bone grafting to manage posttraumatic bone defects (Wong et al. 2014). This strategy involves a two-stage surgical technique; the first operation includes debridement of necrotic bone, skeletal stabilization, and the insertion of polymethylmethacrylate (PMMA) cement spacer to envelope the bone ends (Olesen et al. 2015). Then after several weeks, the second operation involves the removal of the spacer without disrupting the induced biomembrane and filling the defect with bone graft (Deramo and Rose 2020). For this strategy, the cement spacer is vital for increasing stability, fighting infection, hindering fibrous ingrowth into the defect, and inducing a membrane that harbors and secretes growth factors such as vascular and osteoinductive factors to promote regeneration (Deramo and Rose 2020; Vantucci et al. 2018). Patients who underwent the Masquelet technique experienced pain-free weight-bearing after an average recovery time of 9 months (Deramo and

Rose 2020). However, major differences were seen in consolidation time between patients and the correlation between consolidation time and bone defect size (Deramo and Rose 2020). Therefore, the healing timeframe for this technique is unpredictable (Aronson 1994; Deramo and Rose 2020).

8.1.2.2 Mechanical Loading: Distraction Osteogenesis

To avoid surgical intervention, scientists and clinicians have also utilized mechanical loading to promote bone union because interfragmentary movement, determined by the applied load and stability, has a strong impact on bone healing (Claes and Heigele 1999). This was evident in the study by Claes and Heigele that relates tissue formation in a fracture gap to local stress and strain (1999). Claes and Heigele found that varying levels of stress and strain along existing calcified surfaces in the fracture callus controlled whether the callus tissue differentiated into cortical bone, connective tissue, or fibrous cartilage (Claes and Heigele 1999). They knew mechanical loading influenced the healing process, but in vivo studies to determine the stress and strain of the cells in a fracture callus were not possible. To overcome this limitation, they employed the finite element method (FEM) to estimate the local stress and strain at the cellular level in callus tissue. This method allowed them to quantify the relationship between the ossification pattern and the loading treatment for the first time. They found the critical values that guided cell differentiation into either an osteoblast or a chondrocyte to further direct either intramembranous differentiation or endochondral ossification. However, the FEM poses limitations because it depends on set parameters that only correlate to simple oblique fractures and their study only described bone healing as three distinct stages (Wong et al. 2014). Future work that examines the cellular mechanisms of bone healing may help explain the pathology of delayed or nonunion defects. Longitudinal analysis of fixation techniques is also needed to understand how the healing progresses in response to loading. The amount of interfragmentary movement to optimize healing and avoid nonunion complications remains unknown, but work continues to highlight the potential for mechanical loading to control and promote bone regeneration.

Utilizing mechanical loading, surgeons have tried distraction osteogenesis to address large bone defects. This strategy includes an initial osteotomy followed by gradual distraction to induce the integration of cells, growth factors, and extracellular matrix to form bone (Compton et al. 2015). Bone formation using distraction osteogenesis relies on control of mechanical tension by altering the rate and rhythm of distraction to influence cell proliferation, angiogenesis, and genetic expression within the distraction gap (Olesen et al. 2015). The exact mechanism in which strain stimulates bone formation remains unclear, but research suggests that slow and steady traction can metabolically activate living tissue through mechanotransduction, which stimulates proliferation and biosynthetic cellular function (Ai-Aql et al. 2008). Similarly, other investigations have found molecular signaling cascades to play an important role in strain-induced bone regeneration (Olesen et al. 2015). After, initial success, this technique was modified to shorten the

consolidation time. From this, the rate of distraction was seen as an important clinical consideration because high-speed distraction exhibited painful neuropathy and soft tissue complications, while slow distraction diminished the osteogenic potential (Olesen et al. 2015). Although this technique has seen promising results, robust investigations are still underway because of the delicate physiological balance required for all stages of healing. Such investigations involve applying systemic and local factors that may improve healing, as well as other mechanical loading strategies to promote regeneration. Distraction osteogenesis also illuminates the need for personalized medicine because the healthy population may require faster distraction rates than the elderly or ill to maintain osteogenesis.

8.1.3 Current Clinical Rehabilitation Practices and Limitations

Rehabilitation is a critical component of the healing process—in terms of both tissue repair and functional recovery—and is commonly prescribed to patients after regenerative or reparative intervention for complex bone injuries in the lower extremities. Key factors that influence the rehabilitation regimen include the severity, type, and location of the injury along with the fixation method. Typically, clinicians prescribe relatively conservative regimens that begin with non-weight-bearing for up to 12 weeks before progressing to the next stages of active rehabilitation for severe injuries. Once patients are permitted to use their injured limb, rehabilitation incorporates a variety of loading, strengthening, and pain management strategies.

Clinical rehabilitation can start with non-loading practices for a recommended length of time, and varies based on injury severity, type, and fixation method. The major methods of fixation include internal and external fixation. External fixation methods are commonly used for traumatic injuries that often result in open fractures and severe damage to the surrounding soft tissue (Hoyt et al. 2015). Since external fixation can shield the injured region from excessive axial loading, immediate weight-bearing can be permitted as long as the patient is not experiencing unusual pain (Bacon et al. 2008; Joslin et al. 2008; Kershaw et al. 1993; Taljanovic et al. 2003) However, for more severe injuries, patients are often prescribed a 6-week non-weight-bearing period following surgery because the open reduction and internal fixation (ORIF) method often used for these more severe fractures is most effective when the injured region is completely stable (Joslin et al. 2008; Kershaw et al. 1993; Mitkovic et al. 2002; Sato et al. 1999; Zlowodzki et al. 2007). If the internal fixation is coupled with intramedullary fixation, immediate weight-bearing may be permitted, again providing the patient is free of pain (Arazi et al. 2001; Lin et al. 2014; Brumback et al. 1999). Many of these regimens are based on the severity of the bone injury and guidelines often do not account for the severity of soft tissue injury or volumetric muscle loss which can accompany complex injuries. Research implementing rehabilitation for the treatment of volumetric muscle loss has shown

significant challenges in restoring function and general guidelines do not currently exist (Greising et al. 2020).

The most common form of rehabilitation following the non-loading period for complex bone injuries is progressive touchdown weight-bearing (Hoyt et al. 2015; Hurkmans et al. 2007). This process begins with toe-touch weight-bearing, during which only the toes of the injured limb should touch the ground. This is followed by partial weight-bearing, where the clinician may prescribe 10%, 25%, or 50% weight-bearing following injury. The percentage increases on a patient-by-patient basis until the patient is able to place full weight on the injured limb (Inverarity 2020). Patients may be provided scales or body weight supports to control forces in the lower limb. One of the major issues with restricted weight-bearing is poor patient compliance; even when patients attempt to follow the clinician's recommendations, they frequently exceed the weight permitted. Alternatively, other techniques such as hydrotherapy and lower body positive pressure (LBPP), which allow for better control of the pressure on the injury, may be used (Stucky et al. 2018).

In addition to direct loading on the bone, rehabilitation regimens also focus on muscle strengthening and pain management. Serious leg injuries, especially those which require several weeks of non-loading, lead to significant muscle loss. Studies have found that patients prescribed with muscle strengthening exercises improve strength, balance, mobility, and function (Latham et al. 2014). Exercises include standing from a chair, walking up steps, and other functional-based exercises. Pain following injury is typically managed through analgesia and the use of peripheral nerve blocks, which have been shown to aid in functional use of the limb and reduce falls (Ilfeld et al. 2010; Long et al. 2006). Rehabilitation regimens have been shown to help patients with pain management and as a result reduce the doses of analgesia prescribed (Cheville et al. 2018). Such rehabilitation strategies often incorporate stretching and strengthening with the goal of restoring basic functions and activities (Osterweis et al. 1987).

Preclinical studies have shown that early loading improved healing outcomes in both bone growth and bone strength compared to those with extended periods of non-loading after injury. However, current rehabilitation strategies still prescribe extended periods of non-loading after injury. Section 8.2.3 will further detail the results of preclinical trials regarding the effects of early loading on tissue regeneration and functional restoration.

8.2 Effects of Mechanical Environment on Vascular Growth, Tissue Differentiation, and Bone Regeneration

The notion that bone adapts to mechanical stress during rehabilitation via remodeling processes is often attributed to Julius Wolff in the late nineteenth century. Wolff observed that trabeculae of fractured bone remodeled over time due to changes in bone shape or mechanical stresses (Brand 2010; Wolff 1892).

Mechanosensitivity has since been demonstrated in numerous physiological processes in most tissues and throughout the organ to intracellular levels. Near the turn of the century, researchers began formalizing approaches to predict patterns of tissue differentiation, mineralization, osteogenesis, chondrogenesis, and vascular growth based on mechanical loading environments. These approaches are beginning to guide patient and etiology-specific rehabilitation and regenerative strategies. In this section, we review computational methods, wireless strain sensor developments, and preclinical models that have advanced our understanding of tissue adaptation in response to mechanobiological stimuli in fracture healing and remodeling. These approaches may guide regenerative and rehabilitation strategies by encouraging proper tissue differentiation and ultimate union after fracture in long bones.

8.2.1 Computational Models of Tissue Differentiation and Healing

Nearly a century after Wolff's law was established, researchers still disagreed on how stress mechanistically controlled bone morphology. Experimental models have delineated links between strain and patterns of tissue differentiation during healing, though these pathways are more complex in vivo. This section overviews computational approaches to incorporate mechanical and biological regulators of osteogenesis, chondrogenesis, and angiogenesis into computational models. We first review seminal computational advances in simulating tissue differentiation and healing in response to fracture mechanical environments, and then we demonstrate their capability of probing and designing regenerative rehabilitation protocols.

8.2.1.1 Approaches to Modeling Osteogenesis, Chondrogenesis, and Angiogenesis

Computational models of osteogenesis and tissue differentiation first attempted to predict long bone mineral distribution and tissue density in response to mechanical loading. Some of the initial research needed to support these computational aims focused on developing material models to explain bone in the context of fracture healing. A robust theory relating stress to bone orientation and density presented by Fyhrie and Carter proposed that cancellous bone is an anisotropic material that simultaneously maximizes structural integrity and minimizes bone density (Fyhrie and Carter 1986). In addition to developing material models, they also developed a fundamental approach to simulate the effects of cyclic load histories without explicitly modeling each individual load, a task that may have been computationally cumbersome at the time (Carter et al. 1987). Once the material model and approach to simulating cyclic load history were formalized, they were applied in one of the first computational models that predicted a physiological distribution of bone

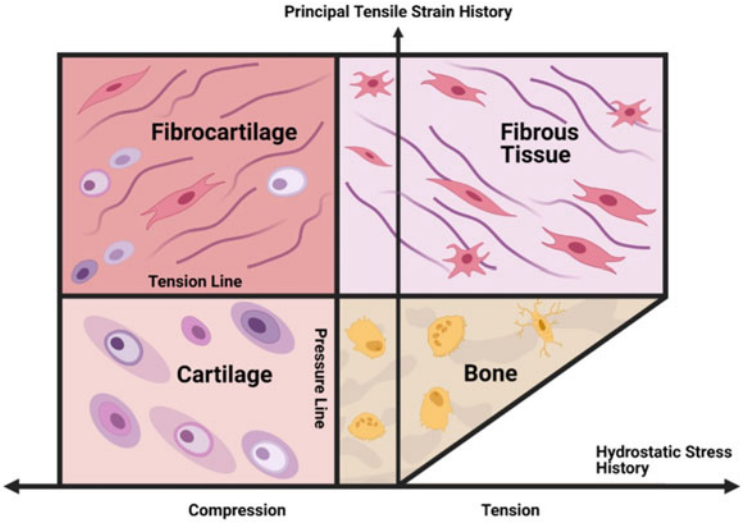

Fig. 8.3 Reproduction of the mechanoregulatory model proposed by Carter et al. (1998) created with BioRender.com. Mechanoregulatory models typically predict differentiation based on state variables such as stress, strain, and fluid velocity. Further, the distribution of tissue types can change over time to reflect the dynamic nature of healing

mineral density in response to mechanical loads. Importantly, the authors noted that the degree of tissue stress stimulus determined the degree of bone resorption or apposition (Beaupré et al. 1990). Further studies found that physiological bone density patterns can be predicted in numerous bones as long as the simulated load histories are comprehensive and reflective of multiple activities of daily living (Levenston et al. 1993).

While investigating the effects of a micromotion implant in canine condyles, Prendergast et al. developed a theory of a mechanoregulatory pathway. Their theory stated that biophysical stimuli determine the trajectory of cells during tissue differentiation (Huiskes et al. 1997). The differentiated interface tissue took on either a bony, fibro-cartilaginous, or fibrous phenotype depending on the distortional strain and relative fluid velocity (Huiskes et al. 1997). Subsequent models studied the adaptation of segmental defects to mechanical loads (Beaupré et al. 1990; Fyhrie and Carter 1986). They created FE models of the callus, medullary tissue, and diaphyseal bone at the defect and applied simplified loading histories of compression or tension to six models with unique initial boundary conditions representing different mechanobiological environments. Notably, a single modeling approach was able to predict experimental tissue differentiation patterns of fracture healing and distraction osteogenesis. This model of tissue differentiation was refined and applied numerous times in different contexts (Fig. 8.3). Claes et al. found that low magnitude strains encourage intramembranous healing while high magnitude strains and pressures lead to connective and fibrous tissue differentiation (Claes and Heigele 1999). Loboa et al. focused on the continuous change of material properties, allowing their

material parameters to continuously adapt to local mechanical stimuli, rather than at fixed time points. Proteoglycan synthesis, collagen fibrillogenesis, crosslinking, and material moduli were regulated by fluid pressure and tensile strain. Further, materials were allowed to adapt into any phenotype unlike prior models which assumed differentiation into one of a few predefined phenotypes (Loboa et al. 2003; Wren et al. 2000). Indeed, these modeling approaches were a promising platform to study mechanoregulation of tissue differentiation during fracture healing; however, a few studies demonstrated that mechanoregulation alone was insufficient to account for biological factors and subject variation (Beaupré et al. 1990; Stevens et al. 1999). Thus, models around the turn of the century began to incorporate both mechanical and biological variables to better predict bone adaptation to mechanical stimuli.

In addition to mechanical activity, in vivo bone healing outcomes can be influenced by oxygen, growth factor, and metabolite transport and production. Investigators of previous studies began introducing cell-based modeling approaches which simulate differentiation of cell populations rather than only tissue types in response to mechanobiological stimuli. These cells transformed the tissue around them through reactions representing bone apposition and resorption (Beaupré et al. 1990; Hernandez 2000). Ament and colleagues adapted the models of Carter et al. (1998) by introducing a fuzzy logic controller to determine tissue differentiation and composition (Ament and Hofer 2000). Previous models had only allowed tissues to differentiate into a single phenotype; fuzzy set theory however allowed various degrees of membership to multiple tissue types depending on mechanical and biological stimuli (Zadeh 1965). A subsequent study used fuzzy logic, which included local vascularity as a biological variable to predict trabecular bone fracture healing and remodeling as distinct events (Shefelbine et al. 2005). At this time, most models had only represented the influence of biological variables in an abstract manner rather than evaluating the transport of oxygen, growth factors, or metabolites. Bailón-Plaza and van der Meulen were perhaps the first to develop a bioregulatory model that simulated the transport, production, and inductive role of growth factors in the context of tissue differentiation during fracture healing (Bailón-Plaza and van der Meulen 2001). In their model, mesenchyme was allowed to take on chondrocyte, osteoblast, and osteocyte lineages as well as undergo mitosis and produce matrix and other cellular products in response to BMP-2, BMP-4, and TGF-β1. In a follow-up study, a combined mechanobioregulatory modeling approach was used to simulate growth across a fracture while varying the onset and magnitude of loading (Bailón-Plaza and van der Meulen 2003). In both experimental and computational studies, delayed onset of loading led to low-stiffness tissue properties and nonunion or poor healing. Simulations indicated that by the time of load onset, the concentration of osteoblasts had greatly declined, leading to insufficient endochondral ossification. Early, moderate loads however were associated with stiff gap tissue and successful bridging. Bailón-Plaza et al.'s approach and observations became common in successive studies that demonstrated the ability of experimentally based computer simulations to guide regenerative rehabilitation therapies. However, these models still took simplified approaches to represent

transport wherein cells, biophysical species, and growth factors were assumed to move only via diffusion.

Geris and colleagues acknowledged that the simplifications of previous models had limited the models' ability to study compromised healing cases. To address this, they extended the bioregulatory models of Bailón-Plaza et al. by simulating endothelial cells that migrated from preexisting vasculature (Geris et al. 2008). Endothelial cell movement was guided by both a random component and an angiogenic factor gradient. Osteoblasts and hypertrophic chondrocytes generated the angiogenic factor, attracting endothelial cells to the callus after the formation of the initial fibrous and cartilaginous tissue in the soft callus. The model was sensitive to the production and consumption of the angiogenic factor—overproduction or saturation of the angiogenic factor did not permit gradient formation, resulting in poor healing. Further, reduced production of angiogenic factor led to slow healing and nonunion. The mechanoregulatory mechanism was reintroduced in a later study where Geris et al. demonstrated that overload-induced nonunion could only be predicted when both angiogenesis and osteogenesis were governed by mechanoregulatory and bioregulatory mechanisms (Geris et al. 2010). A similar approach included mechanoregulatory elements to study osteogenesis and angiogenesis in the bone-implant interface (Checa and Prendergast 2009). Notably, they predicted dendritic growth of new vasculature similar to angiogenesis, as well as decreased angiogenesis and osteogenesis when high shear loads were introduced between the implant and bone. The basic concepts developed thus far built a basis that at first reproduced experimental results of healing and nonhealing based on mechanical and biological stimuli. More recently, these results have been applied to patient-specific and etiology-specific cases to guide regenerative rehabilitation strategies for the clinic.

8.2.1.2 Applied Models of Angiogenesis and Osteogenesis

With the knowledge that interfragmentary movement strongly relates to bone adaptation and healing outcomes (Claes and Heigele 1999), investigators began to study how fracture stabilization protocols in both the operating room and rehabilitation clinic influence interfragmentary movement and long-term fracture healing. Prior simulations of healing relied on idealized fracture site geometry and loading conditions which may smooth over local hotspots of stress and strain introduced by irregular fracture geometries. One of the first patient-specific models of interfragmentary movement was performed while a patient was simultaneously recovering with a stabilized fracture (Gardner et al. 2000). The patient's fracture geometry and gait-cycle loads during recovery were used as inputs. The patient experienced delayed healing, which based on predicted distributions of strain throughout the callus, the authors believe may stem from insufficient interfragmentary support by the fixation device during the early course of healing. In addition to patient-specific studies, some began to evaluate fixation procedures themselves. One group found that significant non-axial movement can occur during

axial dynamization depending on fixator joint location and the ability for joints to slide (Liu et al. 2005).

In addition to studying fixator design, recent interest has emerged pertaining to when fixator dynamization or removal can begin. Byrne et al. modeled a fixation device which was automatically removed once the callus had reached a threshold stiffness in an approach that may be useful for patient-specific preoperative treatment planning (Byrne et al. 2011). Alierta et al. studied the effects of combined compressive and shear loads during healing across a fixated fracture, which had scarcely been studied experimentally at that time (Alierta et al. 2014). Further, they studied the effect of fixation on comminuted fractures (in this case an oblique fracture with three fragments) and found that comminuted fractures may require stiffer fixation than simple transverse fractures. Wilson et al., modeled inverse dynamization—a recently proposed fixation strategy where some interfragmentary movement is initially allowed in order to promote a large callus to form, at which point, dynamization is restricted (Wilson et al. 2017). They found that inverse dynamization led to quicker callus formation but ultimately did not provide significant additional stiffness to the fracture during healing. A similar study by Ganadhiepan et al. investigated dynamization with the treatment of Ilizarov circular fixators (Ganadhiepan et al. 2019). They found that early dynamic physiological loads may encourage advective transport and improve secondary healing. Simultaneously, their simulations predicted changes in cell population distributions and matrix deposition associated with different fixator material properties. While much attention has been given to external fixators, simulations are also used to study internal stabilization methods. For example, Mehboob and colleagues used simulations to select composite materials for intramedullary rods that best supported callus formation and healing in transverse or oblique fractures at multiple locations along the length of the femur (Mehboob and Chang 2018). Indeed, simulations have enabled a deeper understanding of how fixation techniques and fracture etiology alter the mechanical environment and biological adaptation.

8.2.2 Implantable Sensors for In Vivo Load Monitoring

The healing outcomes from orthopedic injuries that require the use of temporary or permanent implants depend on the surgical technique, rehabilitation approach, patient's health, and physical activities, as well as the mechanical environments at the implant sites (O'Connor and Kiourti 2017). Today, assessments of the mechanical environment at a musculoskeletal injury site are still typically done using mechanical loading/measurement instruments and/or numerical simulations based on assumed boundary conditions (Klosterhoff et al. 2017a). These methods may be inaccurate in many instances because they are usually performed under ideal and controlled conditions with assumptions that may not capture the actual mechanical loading experienced during the patient's physical activities. Direct measurement of the mechanical loading outside of a laboratory or clinical setting would provide a

better assessment of how mechanical forces can promote or impair functional healing of an orthopedic injury (Klosterhoff et al. 2017a).

In 1960s, researchers started to integrate sensors to orthopedic implants through percutaneous wires, so measurements could communicate to external data processing electronics for monitoring in vivo mechanical forces in the shoulder, spine, hip, and knee implants (Karipott et al. 2018a). These sensors were mostly used as investigative tools to study the healing process and shed light on the physical environment in the musculoskeletal system (Karipott et al. 2018a). Since then, rapid innovations in electronics, computing, battery, and wireless communications have significantly increased the integration of sensors into orthopedic implants. Modern wireless sensors, which are smaller, safer, and more convenient to use, have been incorporated into various orthopedic implants to further improve their performance. These sensor-integrated orthopedic implants can allow efficient measurement of clinical data that was not possible before, providing new therapeutic and diagnostic capabilities to enable personalized medicine, which leads to better treatment outcomes for orthopedic injuries (Ledet et al. 2018). Specifically, sensor-integrated orthopedic implants generate useful information characterizing the environment inside the body, giving an opportunity to tailor treatment regimens, trigger transition in care, and detect adverse events earlier (Ledet et al. 2018). They also minimize complications, reduce recovery time, and decrease readmission and revision procedures (Ledet et al. 2018). Moreover, the use of sensors in orthopedic implants gives a greater understanding of the healing processes, tissue-implant interactions, and biomechanics of the injury hence providing knowledge for the development of improved implants and surgical techniques.

8.2.2.1 Sensor and Wireless Technologies

Stress and strain are critical parameters in orthopedic care, providing useful information on the healing conditions after an injury. These parameters can be used to determine the effectiveness of the treatment and predict patient outcomes. While many sensors are available, strain gauges are still the dominant technology for measuring stress and strain (Karipott et al. 2018a). In fact, strain gauges continue to be used in orthopedic implants since the 1960s due to their robustness, sensitivity to applied strain, and convenience to integrate on or within the implants (Ledet et al. 2018).

Strain gauges, as shown in Fig. 8.4a, are electronic components that vary their electrical resistance in response to applied mechanical strains. A Wheatstone bridge circuit, illustrated in Fig. 8.4b, is typically implemented to change an input resistance of a strain gauge to a voltage output that can then be processed to a digital output. Typically, strain gauges are a thin layer of electrically conducting pattern deposited onto a polymeric backing substrate (Ledet et al. 2018). A strain gauge is usually directly attached to the surface of an implant. The deformation of the implant alters the dimension of the strain gauge, creating a proportional change in the gauge

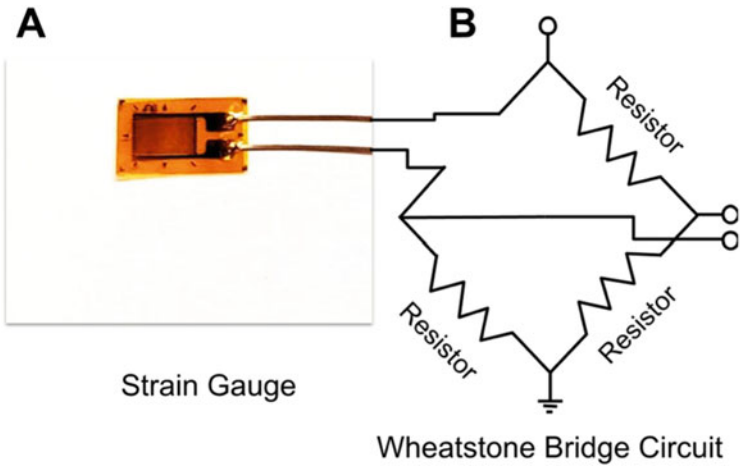

Fig. 8.4 (**a**) A resistive foil strain gauge is connected to (**b**) a Wheatstone bridge circuit to convert the change in the gauge resistance into a voltage output

resistance. The resistance change is then converted to a digital voltage output as a record of change in strain (Ledet et al. 2018).

Traditional strain gauge sensors require transcutaneous wired connections to supply power and collect in vivo data from patients or test subjects. For example, Sato and coworkers developed a piezoresistive strain gauge pressure sensor that was percutaneously wired to external electronics to measure intradiscal pressure (Sato et al. 1999). Another group developed a similar strain gauge embedded inside a femoral head prosthesis via percutaneous wires to monitor forces on a hip implant (Rydell 1966). In addition, Burny and coworkers have also demonstrated the application of strain gauges for orthopedic monitoring (Burny et al. 2000) by integrating resistive strain gauges into a modified nail plate implant to perform in vitro and in vivo evaluations on the effectiveness and safety of the implant system (Burny et al. 2000).

Wired sensors, while able to provide continuous and high-resolution measurements on orthopedic conditions, are limited to laboratory and/or clinical settings and have an increased risk of infection to the test subjects. Advancements in telemetry systems have allowed for the development of sensors that can wirelessly communicate and transfer data without wires (Nelson et al. 2020), which alleviates these issues. As depicted in Fig. 8.5, a telemetry system commonly consists of two components: a transmitter that is implanted along with the strain gauge (or other types of strain/pressure sensors) and a receiver that is typically connected to a signal processor such as a computer or a handheld electronic recording/displaying device. An example of a wireless orthopedic sensor was developed by Ledet and coworkers for direct in vivo measurements of spinal loads on the lumbar spine of baboons (Ledet et al. 2012). Other strain gauge implants include those developed by Rohlman and coworkers to study loads on the vertebral body (Rohlmann et al. 2014), and the

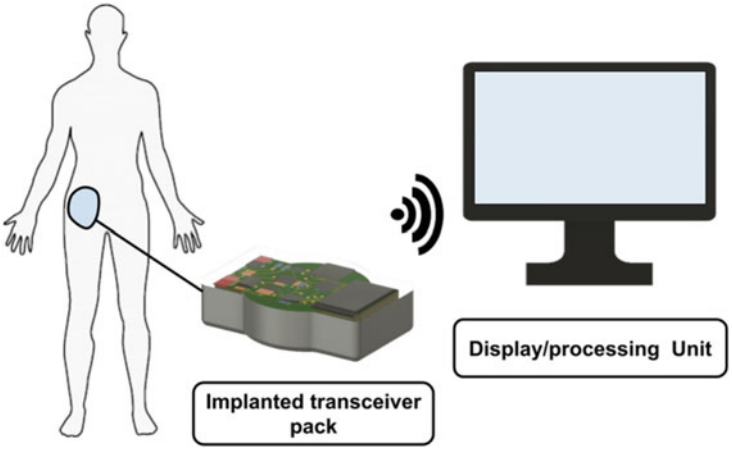

Fig. 8.5 An implantable telemetry system consisting of a transmitter that is implanted along with the sensor(s), and an external receiver that interfaces with a signal processor such as a computer or a handheld electronic recording/displaying device

calcium phosphate ceramic-coated strain gauges by Szivek and coworkers (Szivek et al. 2005) that exhibited improved osteointegration.

Although wireless sensors possess clear advantages over wired sensors in terms of safety and convenience, a limitation of their usage in orthopedic implants is the power requirement at the implanted part of the sensor system. They are also bulky due to the size of the battery. Therefore, wireless sensors typically have a limited operation lifetime and require a carefully planned power budget. To extend the performance period of wireless sensors, some sensor-integrated implants employ batteries that can be charged remotely, or use an energy harvesting system that can generate power from mechanical forces (Nelson et al. 2020). For example, Santos and coworkers generated energy needed by a hip implantable sensor by utilizing the motion around it (Soares dos Santos et al. 2013). Another approach to prolong operation time is to reduce the power consumption rate. Integrated circuit technology has enabled the fabrication of implantable sensors (Soares dos Santos et al. 2013) featuring power-saving circuit designs such as the bulk-driven technique, the floating gates, and the subthreshold design (Borchani et al. 2016).

Besides batteries, some sensors are powered by an external device through wireless energy coupling and communicate through electromagnetic fields or acoustic waves. These sensors have been used to measure various parameters in hip prostheses, as well as loads in the knee and spine. The most common wirelessly powered systems are inductively coupled systems, which receive power through electromagnetic energy (Ledet et al. 2018). For example, Graichen and coworkers developed a system that consisted of six strain gauges and a telemetry system that utilized radio frequency (RF) to inductively power the electronics and transmit data (Graichen et al. 2007). It was implemented on three patients with shoulder endoprostheses to monitor in vivo load (Graichen et al. 2007).

Another type of battery-free sensor is comprised of only energy-passive elements such as magnetic materials or passive electronic components (capacitors, inductors, and resistors) forming a combined sensing unit and wireless transceiver. They have no active circuitry and do not require an internal power source, making them small, robust, reliable, and ideal for long-term, in vivo monitoring. One example of passive sensors is the magnetoelastic sensor, made of magnetostrictive materials that can convert magnetic energy to mechanical energy and vice versa. When under the excitation of a magnetic AC field, the sensor undergoes a mechanical resonance, generating a secondary magnetic flux that can be remotely captured (Pacella et al. 2014). These sensors, which are wireless, battery-free, and sensitive to stress/strain, are used to measure forces at bone fixation plates or medical sutures (DeRouin et al. 2016; Oess et al. 2009). They are easily integrated into existing implants because of their simple designs and have demonstrated application to monitor cell adhesion on an implant and bone tissue integration to implant (Vlaisavljevich et al. 2013). Another passive sensor is the inductive-capacitive sensor which consists of an electrical resistor, inductor, and capacitor (RLC) that exhibit an electrical resonance when interrogated with an RF wave. By sensitizing the RLC element to stress/strain, these sensors have been used to monitor pulling force and deformation at orthopedic implants (Karipott et al. 2018b). Karipott and coworkers also developed a wireless RLC-based sensor that was inductively powered to detect infection at an orthopedic implant site by analyzing the change in temperature, measured through the shift in resonance frequency due to the temperature-induced variation in the resistor value (Karipott et al. 2018b). Although these battery-free wireless sensors have a much longer operational life compared to their battery-powered counterparts, they need to be passively powered to function thus preventing them from applications where continuous, uninterrupted monitoring is needed (Karipott et al. 2018b). Moreover, metallic orthopedic implants may limit the ability to use electromagnetic energy, and result in signal distortion, loss of energy, and performance reduction (Karipott et al. 2018b).

With these technologies, sensors have been incorporated into orthopedic implants to monitor physical parameters (force, loading, pressure, strain, etc.) in bones at the knees, hips, shoulders, etc., to study healing outcomes and monitor conditions of implants after surgery. Most of these sensors are based on strain gauges and rely on wireless transmission protocols (with custom communication schemes or standard protocols such as Bluetooth). For example, Klosterhoff and coworkers developed a bone fixation device for rats with an embedded wireless strain gauge sensor to quantify the mechanical environment experienced by healing tissues during physical activities (Klosterhoff et al. 2020). A digital transceiver based on Bluetooth low energy (BLE) was used for remote-controlled circuit calibration (Klosterhoff et al. 2020). Although Klosterhoff's sensor was limited as an investigational tool to study changes in mechanobiological cues in vivo for an extended period following surgery, other sensors are already in the process or at the early stage of clinical adoption (Bergmann et al. 2007; Karipott et al. 2018a; Ledet et al. 2005), and a few of them are commercially employed to assist orthopedic surgeries (Gustke et al. 2017).

In conclusion, implantable sensors have become a key component in orthopedic regenerative medicine. These sensors, when integrated within existing orthopedic implants, have allowed new understandings of the mechanics of bone and tissue regeneration, leading to the development of better implants and treatment procedures. While implantable sensors will continue to play an increasing role in orthopedic care, they still face challenges in terms of their efficacy, cost, and biocompatibility for long-term monitoring. Furthermore, the lack of standardization in implantable sensors still causes confusion and miscommunications between sensor developers and physicians (Clausen and Glott 2014), slowing down the development process. Section 8.3.2 provides further discussions on the future of implantable sensors for orthopedic regenerative medicine, including new technologies, specific challenges, and innovative solutions to overcome the barrier to successful clinical translation.

8.2.3 Preclinical Regenerative Rehabilitation Studies

Revascularization is a primary limiting factor in tissue regeneration and in vitro work has shown that endothelial cells and vascular networks are mechanosensitive thus supporting mechanical loading as a potential strategy to address vascular limitations and help tissue regeneration (Kannan et al. 2005; Koike et al. 2004). To explore this potential, Boerckel et al. analyzed the effects of functional loading on neovascular growth and subsequent tissue regeneration in large bone defects (2011). Their preclinical study utilized the rat segmental bone defect model stabilized with a compliant plate that was either unlocked at the time of implantation (early) or 4 weeks after surgery (delayed). Microcomputed tomography and histology analysis found robust angiogenesis and collateral vessel formation after the initial vascular response to bone injury. However, early loading significantly inhibited vascular invasion into the defect by 66% and reduced bone formation by 75% compared to the stiff plate controls (Boerckel et al. 2011). In contrast, delayed loading enhanced bone formation by 20% and stimulated vascular remodeling by increasing the number of large vessels while decreasing the number of small vessels (Boerckel et al. 2011). Although early mechanical loading inhibited vascular growth into the defect, it did not change the overall quantified angiogenic response to the injury, which suggests that loading had a localizing effect. Ultimately, delayed loading exhibited an accelerated maturation and remodeling of new vessels which thereby enhanced bone tissue regeneration, while early loading disrupted neovascular ingrowth and impaired bone formation. Their results indicate that neovascular network formation and growth are regulated by mechanical conditions. Further, the timing and magnitude of loading were suggested as key variables and warrant further research to determine the optimal window for therapeutic effect.

Shortly after this study, Boerckel et al. further analyzed the effect of mechanical loading on BMP-2-induced bone regeneration (Boerckel et al. 2012). Following a similar design as their previous work, this study analyzed 6 mm segmental bone

defects in rat femurs that were treated with BMP-2 and stabilized by either a stiff or compliant fixation plate, where the compliant plate allowed for compressive ambulatory load transfers 4 weeks after surgery (Boerckel et al. 2012). Radiography and microcomputed tomography found that loading significantly increased the regenerated bone volume, as well as the amount and distribution of bone formation within the defect. Mechanical testing also found loading to result in bone that was torsional more stiff than that of the unoperated limb (Boerckel et al. 2012). However, new bone distribution was limited to the proximal defect region of the femur, which could stem from the less favorable vascular environment for progenitor differentiation. The lack of progenitor cells means a limited presence of mechanosensitive cells to respond to local stimulus. Histology also found that loading prolonged the presence of woven bone, based on the collagen organization. Overall, the load magnitude resulted in modulated bone maturity, which was previously observed and speculated to result from irregular osteoblast and osteoclast activity (Guldberg et al. 1997). As a whole, this preclinical study demonstrates that altering the fixation plate stiffness to modulate functional load transfers significantly impacted BMP-mediated bone repair. Further, more transfer of axial loads increased bone formation and distribution and modulated the tissue organization and cellular differentiation within the defect. Their work motivates further investigation into mechanical loading as a potential clinical treatment for challenging segmental bone defects. The lack of new bone formation at the distal end also warrants evaluation of the suboptimal vascular environment.

These studies reassured mechanical loading as a bone healing strategy, but work was still needed for clinical translation. Research with more complicated models, such as the composite bone and muscle injury model established by Willett et al., was needed because complex bone injuries often impact neighboring tissues (Willett et al. 2013). This model combines a critically sized segmental bone defect with an adjacent volumetric muscle loss injury and was used to quantitatively assess BMP-mediated tissue regeneration and restoration of limb function. Their analysis looked at three groups: muscle injury, bone injury, and composite injury of the femur and quadricep. Treatment included pre-gelled alginate injected into a cylindrical perforated nanofiber mesh. Their assessment included microcomputed tomography to assess bone regeneration, as well as gait analysis and muscle strength measurements to assess limb function. By week 12, the bone injury subjects were consistently bridged, but the composite injury group exhibited bone volume and mechanical strength that were attenuated by 45% and 58%, respectively. The injured muscle strength, normalized to the contralateral intact muscle, was also reduced by 51% in the composite injury group. Gait function was inhibited by all groups, but the composite group displayed the greatest functional deficit. The deleterious effects of concomitant muscle injury on bone regeneration may be attributed to a diminished blood supply. Altered blood supply may result in changes to nutrient and waste exchange, inflammation, circulating stem cell recruitment, and thus revascularization of the defect. The loss of muscle volume could diminish bone regeneration because the source of resident muscle stem cells and myokines is limited, especially since these cells have displayed osteogenic capabilities. Overall, the BMP levels

which consistently healed large segmental bone defects failed to promote functional regeneration when challenged with a concomitant muscle injury, indicating the need for further intervention. After the effect of mechanical loading on bone, muscle, and vasculature tissue is better understood, future work should implement mechanical loading in concert with BMP treatment to promote healing in multi-tissue injury models.

Motivated to quantitatively assess the effect of mechanical loading on bone repair and revascularization, Klosterhoff et al. deployed a strain sensor platform to examine the evolution of biomechanical cues within the regenerative niche following injury (Klosterhoff et al. 2020). The functional loads consisted of two different loading magnitudes similar to previous work with either a stiff or compliant fixation plate (Boerckel et al. 2011, 2012). Previously the dynamic biomechanical signals were difficult to measure inside the body, so they engineered a wireless implantable sensor that integrates into the internal fixation plates to perturb and remotely quantify the mechanical environment in real-time during ambulation. The data from the strain sensors found an initial two-fold increase in deformation magnitude for the load-sharing compliant fixation plate, subsequent three-fold increase in mineralized bridging, and over 60% increase in bone formation (Klosterhoff et al. 2020). Additionally, their work formed strong implications that early mechanical cues play a critical role in predicting long-term healing response because the strain magnitude at week 1 had a significant, positive correlation with long-term bone healing outcomes. They also found that defects stabilized with the compliant load-sharing plate exhibited vessel size distribution that more closely matched naïve vasculature. Ultimately, the compliant plate allowed for increased strain across the defect during gait relative to the stiff plate, and the increased strain enhanced bone repair. The sensor readings also correlated with the status of healing, suggesting an X-ray-free healing assessment platform. The wireless sensor could provide a non-invasive readout of the progression of healing in a personalized, real-time manner, which represents a notable shift in the ability to prescribe and monitor regenerative rehabilitation therapies. This study further motivates inquiry into mechanical loading as a clinical therapeutic, with emphasis on the mechanosensitive thresholds critical to both angiogenesis and osteogenesis to leverage and augment tissue repair.

8.3 Future Opportunities in Bone Regenerative Rehabilitation

8.3.1 Advances in Computational Modeling

In addition to the mechanical roles of patient geometry and fixation design, there are often nonmechanical factors that lead to fracture nonunion which can be included in computational studies of fracture healing. As noted by Carlier and colleagues, much of the current hesitation to bring computational models to the clinic results from the

over-idealization and over-simplification of models which assume the patient is healthy and typical; in reality, patients may experience a number of factors that impair the fracture repair process not limited to "age…, malnutrition, immune compromise, genetic disorders, osteoporosis, anticoagulants, smoking, and anti-inflammatory agents" (Carlier et al. 2015).

Future advances in computational modeling will likely rely on guidelines developed from patient-specific comorbidities and injury composition and geometry. For example, it has been shown that genetic deficiencies in MMP9—a matrix metalloproteinase involved in skeletal healing, inflammation, and release of matrix-bound VEGF—led to altered patterns of hard callus cartilage remodeling, callus mechanical properties, and angiogenesis (Colnot et al. 2003). Peiffer et al. studied the effect of VEGF injections (either as a daily bolus or a slow-release carrier) on mechanically stimulated fractures in MMP9 deficient individuals (Peiffer et al. 2011). They found that injection of slow-release VEGF carriers, but not bolus injections, led to improved vascularization and healing (although not quite to levels seen in wild-type MMP9 models). Carlier and colleagues later demonstrated that nonunion could be reduced by addressing patient and injury-specific factors (Carlier et al. 2015). Using mechanoregulatory and mechanobioregulatory approaches, they studied four etiologies that lead to nonunion: damage to the marrow canal, large interfragmentary gap, genetic disorder, and mechanical overload during healing. They showed that the success of various therapeutic interventions depended on the etiology of fracture—highlighting the ability of personalized or etiology-based simulations to guide treatment regimens and clinical guidelines.

The inclusion of novel wireless sensors in preclinical and clinical studies will also allow better refinement of mechanobioregulatory models. Strain sensor measurements can now be used as inputs for image-based simulations to more accurately predict local strains and tissue differentiation. Biologic sensors and temperature sensors may also be used to better simulate etiology-specific healing environments. Guidelines formed from these simulations may also guide patient rehabilitation programs and identify when strategy interventions may be required based on individual comorbidities and sensor feedback.

8.3.2 Next Generation Wireless Implantable Orthopedic Devices

Section 8.2.2 has provided a review on the evolution and current state of implantable orthopedic sensors. This section will further describe the underlying technologies that enable the continuous development of current and future orthopedic sensors. This section will also highlight the various challenges for further clinical adoption of implantable orthopedic sensors in terms of their wireless communication, power source, long-term compatibility, etc.

8.3.2.1 Wireless Technologies for Orthopedic Monitoring

Although percutaneous wires were used in the earlier implementations of orthopedic sensors, they had been largely replaced by wireless sensors. The major advantage of wireless implantable sensors is that they can be employed without significant alteration to the standard orthopedic care while providing continuous, long-term monitoring for the study, treatment, and prevention of future orthopedic injuries and diseases (Karipott et al. 2018a). Today, most of the wireless implantable sensors are clinical tools for preclinical studies, but some of them have been clinically implemented. For example, implantable wireless sensors have been incorporated into orthopedic prostheses to characterize forces and design better prosthetics (Ledet et al. 2012). They are also used to measure flow and pressure in blood vessels as well as implemented as neurostimulators to treat muscular and neurological damages (Loeb et al. 2006; Potkay 2008). However, implantable sensors have limitations especially in their operational lifetime, safety, and potential interference with other medical devices; thus, careful design considerations regarding communication and power for wireless sensors are needed (Nelson et al. 2020).

Wireless sensors can be *passive*, which are powered through an external device, or *active*, which have an internal battery that can operate independently without an external energy source (Nelson et al. 2020). Passive sensors are typically simpler in design and smaller but limited in functionality, and only operate when being remotely powered. On the other hand, the main advantage of passive sensors is that they have a much longer operation lifetime since they are not constrained by battery capacity. Active sensors typically contain electrical components like microcontrollers and battery-powered transducers that need continuous power. Compared to passive sensors, the complexity of active sensors allows customization of its operation, simultaneous measurements of multiple analytes, and improved performance. However, the operation lifetime and footprint of active sensors are limited by their batteries (Nelson et al. 2020).

Both active and passive sensors have been employed for orthopedic monitoring. For example, Karipott and coworkers embedded a passive temperature sensor in an implantable screw to detect early infection of a wound site by monitoring the change in its local temperature. The sensor was an inductive-capacitive-resistive (LCR) sensor integrated into an interference screw and could be monitored wirelessly by a detection coil. Similarly, Melik and coworkers (Melik et al. 2008), and Burton and coworkers (Burton et al. 2019) developed LCR-type sensors for monitoring forces on lower body orthopedic implants as well as bone growth in osseointegrated implants, respectively. While these sensors provided real-time monitoring, they only operated when they were powered by an external device. Therefore, they were not suitable for continuous monitoring applications.

Compared to active sensors that can simultaneously measure multiple parameters and potentially provide comprehensive data on the in vivo physiological environments, passive sensors have relatively limited functionalities. However, passive sensors are still prevalent and have a significant place in the future of orthopedics.

Passive sensors are simple, inexpensive, reliable, robust, and can be fabricated to be very small. In addition, the interest in biodegradable sensors that can be implemented for either short-term or long-term in vivo monitoring in orthopedic implants makes passive sensors crucial for orthopedic care since they are made from biodegradable materials.

For active implantable orthopedic sensors, the methods of wireless communication are paramount for their applicability and performance. Existing communication technologies such as Wi-Fi networks, Bluetooth, and Zigbee are frequently used by researchers (Baker 2005; Chakole et al. 2017). These wireless communication methods have their advantages and disadvantages, and the choice of modality depends on the distance of transmission, location of the device in the body as well as safety and security considerations. However, these communication methods will be critical for the development of future implantable orthopedic sensors because they allow flexibility for standardized transmission of in vivo physiological parameters, which coincides with the future trend of a more remote and personalized approach to healthcare.

8.3.2.2 New Innovations in Power Technologies

For active implantable devices, the method of powering them depends on the electronic circuit's energy consumption rate, operational period, size constraints, and safety considerations (Nelson et al. 2020). For most implantable sensors, wireless communication is one of the major, if not the major, consumer of power (Mathúna et al. 2008). Communication power consumption depends on the microcontroller, antenna design, signal processing, and voltage levels. Compared to other commonly used communication protocols, Bluetooth is more optimized for power consumption since it can use a relayed transmission approach to maintain a linked connection between the implant and external devices (Nelson et al. 2020). Specifically, Bluetooth can form a network of devices, which is termed as the piconet, to link two or more devices together. Once on the network, the connected devices can serve as signal relays for one another to shorten the transmission distance for each device (Frenzel 2018). Since transmission power increases exponentially with distance, the shortening of transmission distance can result in lowering the overall power for a Bluetooth network. Aside from the choice of wireless communication method, power consumption by wireless communication can be reduced by minimizing the overhead of the microcontroller by dynamically adjusting the gain of the antenna and/or managing the transmission time (Nelson et al. 2020). These energy efficient communications are important for long-term monitoring of orthopedic regeneration, which may need to function for months to years inside the body.

Batteries are a crucial source of consistent power especially for implants that need to function continuously. However, they have a limited lifetime and their size prevents miniaturization of the implant (Mond and Proclemer 2011). To overcome these challenges, some wireless implants adopt rechargeable batteries that can be charged through various remote power methods (Nelson et al. 2020). Remote power

can be transferred through RF or magnetic induction, ultrasound, and infrared (IR) light methods. RF or magnetic induction uses a coil antenna as a transmitter, where it generates electromagnetic (EM) waves that are received by a secondary coil that converts the EM energy into an electrical current (Nelson et al. 2020). Ultrasound is another mode of energy source that can remotely power the implant or charge its battery. It has higher energy transfer efficiency through greater distances and can be used in smaller implants. Although more complex to implement, ultrasound power has decreased tissue damage and lowered tissue heating compared to induction, making it a safer choice (Nelson et al. 2020). However, there are limitations with EM and acoustic remote power charging because the alignment between the implant and the external power source can significantly affect the power transfer efficiency. Nevertheless, advancements in charging systems can reliably resolve the time limitation for many orthopedic sensors.

IR-based remote power is a newer concept that is being explored to power or recharge implantable sensors. This method uses superficial photodetectors to receive near-infrared lights which are transmitted through the skin with relatively high efficiency (Goto et al. 2001). Another approach is the use of photovoltaic power because near-infrared light exhibits low attenuation in tissues. However, the effectiveness of these methods might vary from person to person and requires more research before it can be implemented clinically (Bereuter et al. 2017).

Energy harvesting is another promising research area. Some of the more common techniques include using solar energy or the motion of the body to generate power. Furthermore, utilizing the body's thermal energy is being proposed (Stark and Stordeur 1999). Although thermal energy harvesting techniques had been previously reported in wearable devices, the production cost of the thermoelectric components was very high, making the sensors expensive (Stark and Stordeur 1999). Another energy harvesting scheme involves the use of piezoelectric (PZT) elements, which convert mechanical energy to produce electricity in vivo (Holmberg et al. 2013; Platt et al. 2005). These energy harvesting techniques will be able to address limitations in battery power and allow for more reliable implantable sensors that can be applied for long-term monitoring of orthopedic regeneration.

8.3.2.3 Development of Biocompatible and Absorbable Implantable Sensors

A critical requirement for implantable sensors is their ability to be functional and stable for a prolonged amount of time in the human body without inducing severe foreign body response. Foreign body responses such as fibrous encapsulation and inflammation are some of the limitations of implantable sensors based on Micro-Electro-Mechanical Systems (MEMS) due to the materials used to make these sensors (Bashir 2004; Gilleo 2005). Therefore, using similar biocompatible materials as those used in fabricating the prosthesis is the first step to minimize the host response from the implantable sensors themselves (O'Connor and Kiourti 2017). Approaches to make sensors more biocompatible include changes to packaging and

polymer encapsulation, as well as using materials that are flexible or tissue mimicking. Flexible implants and biological materials that closely resemble tissue can reduce local damage, leading to reduced inflammation and minimized chance of a foreign body response (Pang et al. 2013). Parylene and polyimide are some of the most biocompatible polymers used to fabricate implantable MEMS (Pang et al. 2013).

Another approach to reducing host response is the development of biodegradable implantable sensors that break down into nontoxic components after a specified functional lifetime. The duration of function for biodegradable sensors can be modified through the choice of the encapsulating material and the surface area of the exposed electrodes (Klosterhoff et al. 2017a). Aside from their biocompatibility, biodegradable sensors are ideal for use because they remove the need for secondary surgery. In recent years, there have been encouraging new developments in biodegradable implantable sensors. One example of a biodegradable sensor is the passive wireless pressure sensor developed by Luo and coworkers (Luo et al. 2014), which was consisted of Poly (l-lactic acid) (PLLA) and polycaprolactone (PCL) for its structural and dielectric material while the conductive portion was made of Zinc/Iron bilayers.

While biodegradable implantable sensors are promising, they are all passive sensors due to the challenges in developing biodegradable batteries and biocompatible active electronic components. However, new advancements in degradable batteries and electronic components have made the development of biodegradable *active* sensors a real possibility. For example, Tsang and coworkers developed a magnesium/iron battery packaged in PCL, which had six times the energy density while being 1–2 orders smaller in volume (Tsang et al. 2015). Another similar battery technology consisted of sodium chloride and PCL as the electrochemical cell and used the fluid that entered the cell as the electrolyte (She et al. 2015). However, these batteries have only been benchtop tested and demonstration for in vivo application has yet to be performed.

Biodegradable electronic components have also been developed to realize a fully biodegradable implantable sensor. Zhang and coworkers developed conductive polymer-based biodegradable interconnects that were made with iron microparticles as a conductor and PCL as the insulating material (Yin et al. 2014). Furthermore, Kim and coworkers fabricated an electronic system consisting of biodegradable silk fibroin as a supporting substrate (Kim et al. 2010). The development of biodegradable electronics and batteries allows monitoring of orthopedic injuries for a controlled timeframe while avoiding the need for an additional surgery for the sensor removal after the monitoring period.

8.3.2.4 Conclusion

Extensive research and development have been conducted to apply wireless implantable sensors to orthopedics. However, wireless implantable sensors have not been widely adopted clinically due to various issues and are mostly limited for research

with few products available commercially. The two notable wireless implantable sensors in the market are the VERASENSE by Orthosensor used in intraoperative monitoring in TKA procedure, and the open eDisk developed by Theken Disc for measuring motions and loads experienced by a spinal disc implant. Some of the challenges associated with implementing wireless sensors include regulatory and cost issues, lack of research on long-term effectiveness, biocompatibility as well as privacy and security concerns from the wireless signals (Karipott et al. 2018a). Besides these challenges, the future is promising for implantable wireless sensors due to the rapid developments in wireless technologies, electronics, biodegradable materials, and energy harvesting techniques, which can help address the current issues with power, biocompatibility, reliability, and size of the sensors.

8.3.3 Current Gaps in Knowledge and Technologies

8.3.3.1 Utilizing more Complex Trauma Models

The field of regenerative rehabilitation works to minimize complications seen with severe injuries that often effects bone, skeletal muscle, nerve, and vascular tissue. However, preclinical research lacks knowledge and utility of multi-tissue models and thus therapeutics. Well-characterized preclinical models with quantified outcomes for functional restoration are crucial testbeds for evaluating emerging technologies and rehabilitation. However, most musculoskeletal trauma models consist of single-tissue defects, which have an undeniable place in the field of regenerative medicine but have limited utility for investigating regenerative strategies for complex, multi-tissue defects. In summary, research laboratories have developed composite bone and vascular injury models, bone and nerve injury models, as well as bone and muscle injury models (Uhrig et al. 2013). However, preclinical research that utilizes these complex models are limited because of their added complexity. In order to advance functional interventions and address multi-tissue injuries, a greater understanding of the biological interactions between damaged tissue is required upon injury and throughout healing. Although certain therapeutics will exhibit promising results in one tissue, unwanted negative consequences could arise in other damaged tissues, as seen with early mechanical loading that affects bone and vascular tissue differently (Klosterhoff et al. 2020). Selection of the model type is paramount to in vivo research, and work with composite models could motivate multistage and multifaceted therapeutic strategies to better address more complicated injuries, with hopes of optimizing therapeutics for clinical use (Uhrig et al. 2013).

8.3.3.2 Optimizing Regeneration of all Tissue Types

Another common limitation in the field of regenerative medicine is reestablishing the correct cellular environment for all tissue types involved. In the context of complex

bone injuries, this often means an environment to promote adequate osteogenesis and angiogenesis. Further, when utilizing mechanical loading as a rehabilitative strategy, biological control is extremely important because progenitor cells for bone, muscle, and vascular tissue are highly mechanosensitive (Claes and Heigele 1999; Kalfas 2001). Past work found the time of loading to be crucial for bone healing, and strain sensors found that different tissue types may require specific timing and magnitude of loading to optimize tissue regeneration (Klosterhoff et al. 2020). The biological intricacies involved in regenerative medicine is extremely complex, and utilizing loading strategies adds to the complexity. Preclinical research needs to continue in vitro and in vivo work to identify the mechanical thresholds needed to promote functional tissue healing in all tissue types as well as composite models. Further, research should continue to investigate the mechanical boundary conditions that optimize the therapeutic effects of rehabilitation. Additionally, the continued development of wireless strain sensor that ensure loading remains within the boundary conditions can allow for dynamic, patient-specific adjustments to the rehabilitation regimen with hopes of accelerating the functional regeneration of musculoskeletal tissue.

8.3.3.3 The Immune System's Role in Bone Healing

Another emerging aspect of bone healing is the dysregulation of the systemic immune response following some severe bone injuries. This detrimental response involves dysregulation of the systemic inflammatory response and compensatory anti-inflammatory response following injury and is not well understood. The complications that arise from immune dysregulation often result in poor healing outcomes, which are devastating to patients. Novel research is investigating a potential link between systemic immune health and bone healing. This preclinical work is identifying cellular markers and cytokine levels that correlated to compromised bone healing (Ruehle et al. 2020). Future work could use these predictive markers as therapeutic targets. Additionally, research should consider how mechanical loading might modulate the immune response, as well as how the patients' immune profile might impact the therapeutic effect of mechanical loading. The immune system plays a key role in tissue healing and regeneration, so future work should continue assessing immune cells at the time of injury and throughout the healing process.

8.3.3.4 Real-Time, Patient-Specific Monitoring for Clinical Application

One of the challenges in orthopedic regenerative medicine is the ability to accurately and continuously track the progression of key physiological parameters (Klosterhoff et al. 2017b). Therefore, technologies that enable real-time measurement of in vivo environmental conditions such as pH, temperature, and oxygen tension in orthopedic wound sites would provide great benefits to patient outcomes, as well as translatable knowledge to improve treatment approaches (Klosterhoff et al. 2017b). Today,

preclinical and clinical methods used to quantify in vivo parameters in orthopedic regeneration are highly dependent on animal or human injury models (Klosterhoff et al. 2017b). Thus, platforms for longitudinal tracking of physiological cues in vivo can significantly increase the resolution and quantity of data acquisition from individual test subjects, which can lower the number of animals and/or human subjects needed for an adequately powered study (Klosterhoff et al. 2017b). Imaging modalities such as ultrasound strain imaging, shear wave elastography, and magnetic resonance imaging are also used in preclinical and clinical studies. However, these methods do not provide continuous measurement, are generally more expensive, and require a larger patient/animal sample size to be effective. On the other hand, FE analysis and computational fluid dynamic analysis are used to gather information such as the surface distribution of stress-strain and oxygen tension, but they are limited by assumed boundary conditions that are difficult to be validated without direct measurement with sensors (Klosterhoff et al. 2017b).

8.3.3.5 Miniaturizing Wireless Technologies

Advancements in MEMS and wireless technologies have allowed for the fabrication of small, flexible, and low-power implantable sensors that can be used in preclinical in vivo models and clinical settings. Miniaturized sensors fabricated for small animal models can wirelessly transmit quantitative measurement in real-time, eliminating the need to anesthetize the animal and disrupt normal activities (Klosterhoff et al. 2017b). Implementing minimally invasive implantable sensors in clinical settings has also improved the outcomes and safety of orthopedic regenerative therapies (Klosterhoff et al. 2017b).

8.3.3.6 Integration of Regenerative and Rehabilitation Strategies

The field of regenerative rehabilitation has suffered from a lack of integrated multidisciplinary research support to advance technology and current clinical standards. As a result, there is tremendous variability in treatment approaches and post-injury rehabilitation regimens. To help progress, multidisciplinary projects should focus on evidence-based guidelines to synergize regenerative therapies and rehabilitation protocols to functionally restore musculoskeletal tissue (Fig. 8.6).

In the long term, combining both regenerative medicines related to stem cells, orthobiologics, biomaterials, and tissue organoids with rehabilitation protocols related to functional imaging, assistive technologies, biophysical stimuli, and biosensors could optimize functional recovery and performance. Future integrative work should advance technologies and rehabilitative strategies that proactively avoid injury by promoting pre-injury reversal of tissue degeneration; improve post-injury rate and quality of healing; and decrease long-term pain and disability. In preclinical studies, research should focus on rigorous evaluations of regenerative rehabilitation strategies, predictive multivariate models of functional recovery,

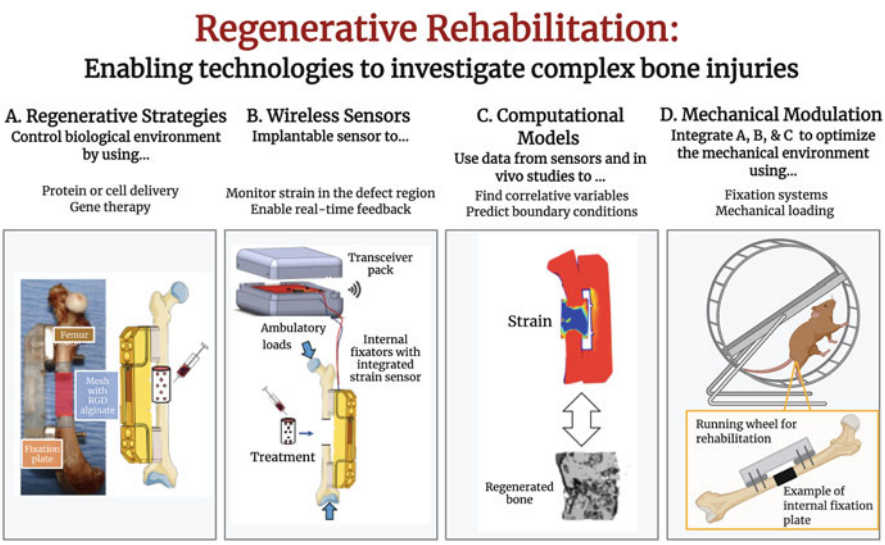

Fig. 8.6 Regenerative rehabilitation enabling technologies to invest complex bone injuries including (**a**) regenerative strategies such as BMP-2 delivery for bone regeneration (Kolambkar et al. 2011), (**b**) wireless strain sensors to monitor the strain in the defect region of segmental bone injuries (Klosterhoff et al. 2017a), (**c**) computational models to build predictive models related to optimal strain boundary conditions, and (**d**) integrating all of these strategies to optimize rehabilitation regimen for accelerated bone healing of complex bone injuries. Created with BioRender.com

combined treatment related to regenerative medicine and rehabilitation, as well as evidence-based guidance that allows patient-specific treatment (Fig. 8.6). Focusing on these emerging aspects of regenerative rehabilitation will help patient outcomes because the rehabilitative protocol will rely on patient-specific factors to accelerated functional regeneration and overall recovery.

References

Ai-Aql ZS, Alagl AS, Graves DT, Gerstenfeld LC, Einhorn TA (2008) Molecular mechanisms controlling bone formation during fracture healing and distraction osteogenesis. J Dent Res 87: 107–118. https://doi.org/10.1177/154405910808700215

Alierta JA, Pérez MA, García-Aznar JM (2014) An interface finite element model can be used to predict healing outcome of bone fractures. J Mech Behav Biomed Mater 29:328–338. https://doi.org/10.1016/j.jmbbm.2013.09.023

Ament C, Hofer EP (2000) A fuzzy logic model of fracture healing. J Biomech 33:961–968. https://doi.org/10.1016/s0021-9290(00)00049-x

Arazi M, Oğün TC, Oktar MN, Memik R, Kutlu A (2001) Early weight-bearing after statically locked reamed intramedullary nailing of comminuted femoral fractures: is it a safe procedure? J Trauma 50:711–716. https://doi.org/10.1097/00005373-200104000-00019

Aronson JMD (1994) Experimental and clinical experience with distraction osteogenesis. Cleft Palate Craniofac J 31:473–482

Bacon S, Smith WR, Morgan SJ, Hasenboehler E, Philips G, Williams A, Ziran BH, Stahel PF (2008) A retrospective analysis of comminuted intra-articular fractures of the tibial plafond: open reduction and internal fixation versus external ilizarov fixation. Injury 39:196–202. https://doi.org/10.1016/j.injury.2007.09.003

Bailón-Plaza A, van der Meulen MC (2001) A mathematical framework to study the effects of growth factor influences on fracture healing. J Theor Biol 212:191–209. https://doi.org/10.1006/jtbi.2001.2372

Bailón-Plaza A, van der Meulen MCH (2003) Beneficial effects of moderate, early loading and adverse effects of delayed or excessive loading on bone healing. J Biomech 36:1069–1077. https://doi.org/10.1016/S0021-9290(03)00117-9

Baker N (2005) ZigBee and Bluetooth: strengths and weaknesses for industrial applications. Comput Control Eng 16:20–25. https://doi.org/10.1049/cce:20050204

Bashir R (2004) BioMEMS: state-of-the-art in detection, opportunities and prospects. Adv Drug Deliv Rev 56:1565–1586. https://doi.org/10.1016/j.addr.2004.03.002

Beaupré GS, Orr TE, Carter DR (1990) An approach for time-dependent bone modeling and remodeling—theoretical development. J Orthop Res 8:651–661. https://doi.org/10.1002/jor.1100080506

Bereuter L, Williner S, Pianezzi F, Bissig B, Buecheler S, Burger J, Vogel R, Zurbuchen A, Haeberlin A (2017) Energy harvesting by subcutaneous solar cells: a long-term study on achievable energy output. Ann Biomed Eng 45:1172–1180. https://doi.org/10.1007/s10439-016-1774-4

Bergmann G, Graichen F, Bender A, Kääb M, Rohlmann A, Westerhoff P (2007) In vivo glenohumeral contact forces--measurements in the first patient 7 months postoperatively. J Biomech 40:2139–2149. https://doi.org/10.1016/j.jbiomech.2006.10.037

Boerckel JD, Uhrig BA, Willett NJ, Huebsch N, Guldberg RE (2011) Mechanical regulation of vascular growth and tissue regeneration in vivo. Proc Natl Acad Sci 108:E674–E680

Boerckel JD, Kolambkar YM, Stevens HY, Lin AS, Dupont KM, Guldberg RE (2012) Effects of in vivo mechanical loading on large bone defect regeneration. J Orthop Res 30:1067–1075

Borchani W, Aono K, Lajnef N, Chakrabartty S (2016) Monitoring of postoperative bone healing using smart trauma-fixation device with integrated self-powered piezo-floating-gate sensors. IEEE Trans Biomed Eng 63:1463–1472. https://doi.org/10.1109/TBME.2015.2496237

Brand RA (2010) Biographical sketch: Julius Wolff, 1836–1902. Clin Orthop Relat Res 468:1047–1049. https://doi.org/10.1007/s11999-010-1258-z

Brumback RJ, Toal TR Jr, Murphy-Zane MS, Novak VP, Belkoff SM (1999) Immediate weight-bearing after treatment of a comminuted fracture of the femoral shaft with a statically locked intramedullary nail. J Bone Joint Surg Am 81:1538–1544. https://doi.org/10.2106/00004623-199911000-00005

Burny F, Donkerwolcke M, Moulart F, Bourgois R, Puers R, Van Schuylenbergh K, Barbosa M, Paiva O, Rodes F, Bégueret JB, Lawes P (2000) Concept, design and fabrication of smart orthopedic implants. Med Eng Phys 22:469–479. https://doi.org/10.1016/s1350-4533(00)00062-x

Burton AR, Sun P, Lynch JP (2019) Bio-compatible wireless inductive thin-film strain sensor for monitoring the growth and strain response of bone in osseointegrated prostheses. Struct Health Monit 1475921719831452. https://doi.org/10.1177/1475921719831452

Byrne DP, Lacroix D, Prendergast PJ (2011) Simulation of fracture healing in the tibia: mechanoregulation of cell activity using a lattice modeling approach. J Orthop Res 29:1496–1503. https://doi.org/10.1002/jor.21362

Carlier A, Geris L, Lammens J, Oosterwyck HV (2015) Bringing computational models of bone regeneration to the clinic. WIREs Syst Biol Med 7:183–194. https://doi.org/10.1002/wsbm.1299

Carter DR, Fyhrie DP, Whalen RT (1987) Trabecular bone density and loading history: regulation of connective tissue biology by mechanical energy. J Biomech 20:785–794. https://doi.org/10.1016/0021-9290(87)90058-3

Carter DR, Beaupré GS, Giori NJ, Helms JA (1998) Mechanobiology of skeletal regeneration. Clin Orthop Relat Res (1976–2007) 355:S41

Chakole MS, Jibhkate RR, Choudhari AV, Gawali SR, Tule PR (2017) A healthcare monitoring system using. WiFi Module 04:5

Checa S, Prendergast PJ (2009) A mechanobiological model for tissue differentiation that includes angiogenesis: a lattice-based modeling approach. Ann Biomed Eng 37:129–145. https://doi.org/10.1007/s10439-008-9594-9

Cheng A, Krishnan L, Pradhan P, Weinstock LD, Wood LB, Roy K, Guldberg RE (2019) Impaired bone healing following treatment of established nonunion correlates with serum cytokine expression: cytokines correlate with nonunion healing. J Orthop Res 37:299–307. https://doi.org/10.1002/jor.24186

Cheville AL, Smith SR, Basford JR (2018) Rehabilitation medicine approaches to pain management. Hematology/Oncology Clinics of North America, Pain Control 32:469–482. https://doi.org/10.1016/j.hoc.2018.02.001

Claes LE, Heigele CA (1999) Magnitudes of local stress and strain along bony surfaces predict the course and type of fracture healing. J Biomech 32:255–266. https://doi.org/10.1016/s0021-9290(98)00153-5

Clausen I, Glott T (2014) Development of clinically relevant implantable pressure sensors: perspectives and challenges. Sensors (Basel) 14:17686–17702. https://doi.org/10.3390/s140917686

Colnot C, Thompson Z, Miclau T, Werb Z, Helms JA (2003) Altered fracture repair in the absence of MMP9. Development 130:4123–4133. https://doi.org/10.1242/dev.00559

Compton J, Fragomen A, Rozbruch SR (2015) Skeletal repair in distraction osteogenesis: mechanisms and enhancements. JBJS Rev 3:1. https://doi.org/10.2106/JBJS.RVW.N.00107

Deramo P, Rose J (2020) Flaps, muscle and musculocutaneous. StatPearls, Treasure Island (FL)

DeRouin AJ, Pacella N, Zhao C, An K, Ong KG (2016) A wireless sensor for real-time monitoring of tensile force on sutured wound sites. IEEE Trans Biomed Eng. https://doi.org/10.1109/TBME.2015.2470248

Egol KA, Koval KJ, Zuckerman JD (2010) Handbook of fractures, 4th edn. Lippincott Williams & Wilkins, Philadelphia, PA

Elliott DS, Newman KJH, Forward DP, Hahn DM, Ollivere B, Kojima K, Handley R, Rossiter ND, Wixted JJ, Smith RM, Moran CG (2016) A unified theory of bone healing and nonunion: BHN theory. Bone Joint J 98-B:884–891. https://doi.org/10.1302/0301-620X.98B7.36061

Frenzel LE (2018) Chapter 9 - networking: wired and wireless: all devices talking to one another. In: Electronics explained, 2nd edn. Newnes, London

Fyhrie DP, Carter DR (1986) A unifying principle relating stress to trabecular bone morphology. J Orthop Res 4:304–317. https://doi.org/10.1002/jor.1100040307

Ganadhiepan G, Zhang L, Miramini S, Mendis P, Patel M, Ebeling P, Wang Y (2019) The effects of dynamic loading on bone fracture healing under ilizarov circular fixators. J Biomech Eng 141:051005. https://doi.org/10.1115/1.4043037

Gardner TN, Stoll T, Marks L, Mishra S, Knothe Tate M (2000) The influence of mechanical stimulus on the pattern of tissue differentiation in a long bone fracture — an FEM study. J Biomech 33:415–425. https://doi.org/10.1016/S0021-9290(99)00189-X

Geris L, Gerisch A, Sloten JV, Weiner R, Oosterwyck HV (2008) Angiogenesis in bone fracture healing: a bioregulatory model. J Theor Biol 251:137–158. https://doi.org/10.1016/j.jtbi.2007.11.008

Geris L, Sloten JV, Oosterwyck HV (2010) Connecting biology and mechanics in fracture healing: an integrated mathematical modeling framework for the study of nonunions. Biomech Model Mechanobiol 9:713–724. https://doi.org/10.1007/s10237-010-0208-8

Gilleo K (2005) MEMS in medicine. Circuit. Assembly 16:32–33

Goto K, Nakagawa T, Nakamura O, Kawata S (2001) An implantable power supply with an optically rechargeable lithium battery. IEEE Trans Biomed Eng 48:830–833. https://doi.org/10.1109/10.930908

Graichen F, Arnold R, Rohlmann A, Bergmann G (2007) Implantable 9-channel telemetry system for in vivo load measurements with orthopedic implants. IEEE Trans Biomed Eng 54:253–261. https://doi.org/10.1109/TBME.2006.886857

Greising SM, Corona BT, Call JA (2020) Musculoskeletal regeneration, rehabilitation, and plasticity following traumatic injury. Int J Sports Med 41:495–504. https://doi.org/10.1055/a-1128-7128

Guder C, Gravius S, Burger C, Wirtz DC, Schildberg FA (2020) Osteoimmunology: a current update of the interplay between bone and the immune system. Front Immunol 11:58

Guldberg RE, Caldwell NJ, Guo XE, Goulet RW, Hollister SJ, Goldstein SA (1997) Mechanical stimulation of tissue repair in the hydraulic bone chamber. J Bone Miner Res 12:1295–1302. https://doi.org/10.1359/jbmr.1997.12.8.1295

Gustke KA, Golladay GJ, Roche MW, Elson LC, Anderson CR (2017) A targeted approach to ligament balancing using kinetic sensors. J Arthroplast 32:2127–2132. https://doi.org/10.1016/j.arth.2017.02.021

Hernandez CJ (2000) A model of mechanobiologic and metabolic influences on bone adaptation. J Rehabil Res Dev 37(2):235–244. PMID: 10850830

Holmberg J, Alexander L, Rajamani R, Bechtold JE (2013) Battery-less wireless instrumented knee implant. J Med Devices 7:011006. https://doi.org/10.1115/1.4023412

Hoyt BW, Pavey GJ, Pasquina PF, Potter BK (2015) Rehabilitation of lower extremity trauma: a review of principles and military perspective on future directions. Curr Trauma Rep 1:50–60. https://doi.org/10.1007/s40719-014-0004-5

Huiskes R, Driel WDV, Prendergast PJ, Søballe K (1997) A biomechanical regulatory model for periprosthetic fibrous-tissue differentiation. J Mater Sci Mater Med 8:785–788. https://doi.org/10.1023/A:1018520914512

Hurkmans HL, Bussmann JB, Selles RW, Benda E, Stam HJ, Verhaar JA (2007) The difference between actual and prescribed weight bearing of total hip patients with a trochanteric osteotomy: long-term vertical force measurements inside and outside the hospital. Arch Phys Med Rehabil 88:200–206. https://doi.org/10.1016/j.apmr.2006.11.005

Ilfeld BM, Duke KB, Donohue MC (2010) The association between lower extremity continuous peripheral nerve blocks and patient falls after knee and hip arthroplasty. Anesth Analg 111:1552–1554. https://doi.org/10.1213/ANE.0b013e3181fb9507

Inverarity L (2020) Weight-bearing restrictions and activity after surgery [WWW document]. Verywell Health. URL https://www.verywellhealth.com/weight-bearing-restrictions-2696668 (accessed 2.26.21)

Joslin CC, Eastaugh-Waring SJ, Hardy JRW, Cunningham JL (2008) Weight bearing after tibial fracture as a guide to healing. Clin Biomech (Bristol, Avon) 23:329–333. https://doi.org/10.1016/j.clinbiomech.2007.09.013

Kalfas IH (2001) Principles of bone healing. Neurosurg Focus 10:1–4. https://doi.org/10.3171/foc.2001.10.4.2

Kannan RY, Salacinski HJ, Sales K, Butler P, Seifalian AM (2005) The roles of tissue engineering and vascularisation in the development of micro-vascular networks: a review. Biomaterials 26:1857–1875

Karipott SS, Nelson BD, Guldberg RE, Ong KG (2018a) Clinical potential of implantable wireless sensors for orthopedic treatments. Expert Rev Med Devices 15:255–264. https://doi.org/10.1080/17434440.2018.1454310

Karipott SS, Veetil PM, Nelson BD, Guldberg RE, Ong KG (2018b) An embedded wireless temperature sensor for orthopedic implants. IEEE Sensors J 18:1265–1272. https://doi.org/10.1109/JSEN.2017.2780226

Kenwright J, Goodship AE (1989) Controlled mechanical stimulation in the treatment of tibial fractures. Clin Orthop Relat Res 241:36–47

Kershaw CJ, Cunningham JL, Kenwright J (1993) Tibial external fixation, weight bearing, and fracture movement. Clin Orthop Relat Res (293):28–36

Kim D-H, Viventi J, Amsden JJ, Xiao J, Vigeland L, Kim Y-S, Blanco JA, Panilaitis B, Frechette ES, Contreras D, Kaplan DL, Omenetto FG, Huang Y, Hwang K-C, Zakin MR, Litt B, Rogers JA (2010) Dissolvable films of silk fibroin for ultrathin conformal bio-integrated electronics. Nat Mater 9:511–517. https://doi.org/10.1038/nmat2745

Klosterhoff BS, Tsang MM, She D, Ong KG, Allen MG, Willett NJ, Guldberg RE (2017a) Implantable sensors for regenerative medicine. J Biomech Eng 139(2). https://doi.org/10.1115/1.4035436

Klosterhoff BS, Ghee Ong K, Krishnan L, Hetzendorfer KM, Chang Y-H, Allen MG, Guldberg RE, Willett NJ (2017b) Wireless implantable sensor for noninvasive, longitudinal quantification of axial strain across rodent long bone defects. J Biomech Eng 139(11):111004-8. https://doi.org/10.1115/1.4037937

Klosterhoff BS, Kaiser J, Nelson BD, Karipott SS, Ruehle MA, Hollister SJ, Weiss JA, Ong KG, Willett NJ, Guldberg RE (2020) Wireless sensor enables longitudinal monitoring of regenerative niche mechanics during rehabilitation that enhance bone repair. Bone 135:115311. https://doi.org/10.1016/j.bone.2020.115311

Koike N, Fukumura D, Gralla O, Au P, Schechner JS, Jain RK (2004) Creation of long-lasting blood vessels. Nature 428:138–139

Kolambkar YM, Dupont KM, Boerckel JD, Huebsch N, Mooney DJ, Hutmacher DW, Guldberg RE (2011) An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects. Biomaterials 32:65–74

Latham NK, Harris BA, Bean JF, Heeren T, Goodyear C, Zawacki S, Heislein DM, Mustafa J, Pardasaney P, Giorgetti M, Holt N, Goehring L, Jette AM (2014) Effect of a home-based exercise program on functional recovery following rehabilitation after hip fracture a randomized clinical trial. JAMA 311:700–708. https://doi.org/10.1001/jama.2014.469

Ledet EH, Tymeson MP, Dirisio DJ, Cohen B, Uhl RL (2005) Direct real-time measurement of in vivo forces in the lumbar spine. Spine J 5:85–94

Ledet EH, D'Lima D, Westerhoff P, Szivek JA, Wachs RA, Bergmann G (2012) Implantable sensor technology: from research to clinical practice. J Am Acad Orthop Surg 20:383–392. https://doi.org/10.5435/JAAOS-20-06-383

Ledet EH, Liddle B, Kradinova K, Harper S (2018) Smart implants in orthopedic surgery, improving patient outcomes: a review. Innov Entrep Health 5:41–51. https://doi.org/10.2147/IEH.S133518

Levenston ME, Beaupré GS, Schurman DJ, Carter DR (1993) Computer simulations of stress-related bone remodeling around noncemented acetabular components. J Arthroplast 8:595–605. https://doi.org/10.1016/0883-5403(93)90006-P

Lin S-J, Chen C-L, Peng K-T, Hsu W-H (2014) Effect of fragmentary displacement and morphology in the treatment of comminuted femoral shaft fractures with an intramedullary nail. Injury 45:752–756. https://doi.org/10.1016/j.injury.2013.10.015

Liu RW, Kim YH, Lee DC, Inoue N, Koo TK, Chao EYS (2005) Computational simulation of axial dynamization on long bone fractures. Clin Biomech 20:83–90. https://doi.org/10.1016/j.clinbiomech.2004.08.009

Loboa EG, Wren TAL, Beaupré GS, Carter DR (2003) Mechanobiology of soft skeletal tissue differentiation—a computational approach of a fiber-reinforced poroelastic model based on homogeneous and isotropic simplifications. Biomech Model Mechanobiol 2:83–96. https://doi.org/10.1007/s10237-003-0030-7

Loeb GE, Richmond FJR, Baker LL (2006) The BION devices: injectable interfaces with peripheral nerves and muscles. Neurosurg Focus 20:E2. https://doi.org/10.3171/foc.2006.20.5.3

Long WT, Ward SR, Dorr LD, Raya J, Boutary M, Sirianni LE (2006) Postoperative pain management following total knee arthroplasty: a randomized comparison of continuous epidural versus femoral nerve infusion. J Knee Surg 19:137–143. https://doi.org/10.1055/s-0030-1248096

Lord JM, Midwinter MJ, Chen Y-F, Belli A, Brohi K, Kovacs EJ, Koenderman L, Kubes P, Lilford RJ (2014) The systemic immune response to trauma: an overview of pathophysiology and treatment. Lancet 384:1455–1465. https://doi.org/10.1016/S0140-6736(14)60687-5

Luo M, Martinez AW, Song C, Herrault F, Allen MG (2014) A microfabricated wireless rf pressure sensor made completely of biodegradable materials. J Microelectromech Syst 23:4–13. https://doi.org/10.1109/JMEMS.2013.2290111

Masquelet AC (2003) Muscle reconstruction in reconstructive surgery: soft tissue repair and long bone reconstruction. Langenbeck's Arch Surg 388:344–346. https://doi.org/10.1007/s00423-003-0379-1

Mathúna CÓ, O'Donnell T, Martinez-Catala RV, Rohan J, O'Flynn B (2008) Energy scavenging for long-term deployable wireless sensor networks. Talanta, Special Section: Remote Sensing 75:613–623. https://doi.org/10.1016/j.talanta.2007.12.021

McKibbin B (1978) The biology of fracture healing in long bones. J Bone Joint Surg Br 60-B:150–162. https://doi.org/10.1302/0301-620X.60B2.350882

Mehboob A, Chang S-H (2018) Biomechanical simulation of healing process of fractured femoral shaft applied by composite intramedullary nails according to fracture configuration. Compos Struct 185:81–93. https://doi.org/10.1016/j.compstruct.2017.11.002

Melik R, Perkgoz NK, Unal E, Puttlitz C, Demir HV (2008) Bio-implantable passive on-chip RF-MEMS strain sensing resonators for orthopaedic applications. J Micromech Microeng 18:115017. https://doi.org/10.1088/0960-1317/18/11/115017

Mitkovic MB, Bumbasirevic MZ, Lesic A, Golubovic Z (2002) Dynamic external fixation of comminuted intra-articular fractures of the distal tibia (type C pilon fractures). Acta Orthop Belg 68:508–514

Mond HG, Proclemer A (2011) The 11th world survey of cardiac pacing and implantable cardioverter-defibrillators: calendar year 2009--a World Society of Arrhythmia's project. Pacing Clin Electrophysiol 34:1013–1027. https://doi.org/10.1111/j.1540-8159.2011.03150.x

Nauth A, McKee MD, Einhorn TA, Watson JT, Li R, Schemitsch EH (2011) Managing bone defects. J Orthop Trauma 25:462–466. https://doi.org/10.1097/BOT.0b013e318224caf0

Nelson BD, Karipott SS, Wang Y, Ong KG (2020) Wireless technologies for implantable devices. Sensors 20:4604. https://doi.org/10.3390/s20164604

O'Connor C, Kiourti A (2017) Wireless sensors for smart orthopedic implants. J Bio Tribo Corros 3:20. https://doi.org/10.1007/s40735-017-0078-z

Oess NP, Weisse B, Nelson B (2009) Magnetoelastic strain sensor for optimized assessment of bone fracture fixation. IEEE Sensors J. https://doi.org/10.1109/JSEN.2009.2025575

Olesen UK, Eckardt H, Bosemark P, Paulsen AW, Dahl B, Hede A (2015) The Masquelet technique of induced membrane for healing of bone defects. A review of 8 cases. Injury. https://doi.org/10.1016/S0020-1383(15)30054-1

Osterweis M, Kleinman A, Mechanic D (1987) Rehabilitation approaches and issues in chronic pain, pain and disability: clinical, behavioral, and public policy perspectives. National Academies Press (US), Washington, DC

Pacella N, DeRouin A, Pereles B, Ong KG (2014) Geometrical modification of magnetoelastic sensors to enhance sensitivity. Smart Mater Struct 24:025018. https://doi.org/10.1088/0964-1726/24/2/025018

Pang C, Lee C, Suh K-Y (2013) Recent advances in flexible sensors for wearable and implantable devices. J Appl Polym Sci 130:1429–1441. https://doi.org/10.1002/app.39461

Peiffer V, Gerisch A, Vandepitte D, Van Oosterwyck H, Geris L (2011) A hybrid bioregulatory model of angiogenesis during bone fracture healing. Biomech Model Mechanobiol 10:383–395. https://doi.org/10.1007/s10237-010-0241-7

Platt SR, Farritor S, Garvin K, Haider H (2005) The use of piezoelectric ceramics for electric power generation within orthopedic implants. IEEE/ASME Transactions on Mechatronics 10:455–461. https://doi.org/10.1109/TMECH.2005.852482

Potkay JA (2008) Long term, implantable blood pressure monitoring systems. Biomed Microdevices 10:379–392. https://doi.org/10.1007/s10544-007-9146-3

Rohlmann A, Schwachmeyer V, Graichen F, Bergmann G (2014) Spinal loads during post-operative physiotherapeutic exercises. PLoS One 9:e102005. https://doi.org/10.1371/journal.pone.0102005

Rosenthal MD, Moore FA (2016) Persistent inflammation, immunosuppression, and catabolism: evolution of multiple organ dysfunction. Surg Infect 17:167–172. https://doi.org/10.1089/sur.2015.184

Ruehle MA, Li M-TA, Cheng A, Krishnan L, Willett NJ, Guldberg RE (2019) Decorin-supplemented collagen hydrogels for the co-delivery of bone morphogenetic protein-2 and microvascular fragments to a composite bone-muscle injury model with impaired vascularization. Acta Biomater 93:210–221. https://doi.org/10.1016/j.actbio.2019.01.045

Ruehle MA, Eastburn EA, LaBelle SA, Krishnan L, Weiss JA, Boerckel JD, Wood LB, Guldberg RE, Willett NJ (2020) Extracellular matrix compression temporally regulates microvascular angiogenesis. Sci Adv 6:eabb6351. https://doi.org/10.1126/sciadv.abb6351

Rydell NW (1966) Forces acting on the femoral head-prosthesis: a study on strain gauge supplied prostheses in living persons. Acta Orthop Scand 37:1–132. https://doi.org/10.3109/ort.1966.37.suppl-88.01

Sato K, Kikuchi S, Yonezawa T (1999) In vivo intradiscal pressure measurement in healthy individuals and in patients with ongoing back problems. Spine (Phila Pa 1976) 24:2468–2474. https://doi.org/10.1097/00007632-199912010-00008

She D, Tsang M, Kim J, Allen M (2015) Immobilized electrolyte biodegradable batteries for implantable MEMS. 2015 Transducers – 2015 18th International Conference on Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS). https://doi.org/10.1109/TRANSDUCERS.2015.7180968

Shefelbine SJ, Augat P, Claes L, Simon U (2005) Trabecular bone fracture healing simulation with finite element analysis and fuzzy logic. J Biomech 38:2440–2450. https://doi.org/10.1016/j.jbiomech.2004.10.019

Soares dos Santos MP, Ferreira JAF, Ramos A, Simões JAO, Morais R, Silva NM, Santos PM, Reis MJCS, Oliveira T (2013) Instrumented hip implants: electric supply systems. J Biomech 46:2561–2571. https://doi.org/10.1016/j.jbiomech.2013.08.002

Stark I, Stordeur M (1999) New micro thermoelectric devices based on bismuth telluride-type thin solid films. In: Eighteenth International Conference on Thermoelectrics Proceedings, ICT'99 (Cat. No.99TH8407). IEEE, Piscataway. https://doi.org/10.1109/ICT.1999.843432

Stevens SS, Beaupré GS, Carter DR (1999) Computer model of endochondral growth and ossification in long bones: biological and mechanobiological influences. J Orthop Res 17:646–653. https://doi.org/10.1002/jor.1100170505

Stucky F, Vesin J-M, Kayser B, Uva B (2018) The effect of lower-body positive pressure on the cardiorespiratory response at rest and during submaximal running exercise. Front Physiol 9:34. https://doi.org/10.3389/fphys.2018.00034

Szivek JA, Roberto RF, Margolis DS (2005) In vivo strain measurements from hardware and lamina during spine fusion. J Biomed Mater Res B Appl Biomater 75:243–250. https://doi.org/10.1002/jbm.b.30262

Taljanovic MS, Jones MD, Ruth JT, Benjamin JB, Sheppard JE, Hunter TB (2003) Fracture fixation. Radiographics 23:1569–1590. https://doi.org/10.1148/rg.236035159

Tsang M, Armutlulu A, Martinez AW, Allen SA, Allen M (2015) Biodegradable magnesium/iron batteries with polycaprolactone encapsulation: a microfabricated power source for transient implantable devices. Microsystems Nanoeng. https://doi.org/10.1038/MICRONANO.2015.24

Uhrig BA, Li M-TA, Willett NJ, Guldberg RE (2013) Models of composite bone and soft-tissue limb trauma. In: Ma PX (ed) Biomaterials and regenerative medicine. Cambridge University Press, Cambridge, pp 534–554. https://doi.org/10.1017/CBO9780511997839.035

Vantucci CE, Roy K, Guldberg RE (2018) Immunomodulatory strategies for immune dysregulation following severe musculoskeletal trauma. J Immunol Regen Med 2:21–35. https://doi.org/10.1016/j.regen.2018.07.001

Vlaisavljevich E, Holmes HR, Tan EL, Qian Z, Trierweiler S, Ong KG, Rajachar RM (2013) Magnetoelastic vibrational biomaterials for real-time monitoring and modulation of the host response. J Mater Sci Mater Med 24:1093–1104. https://doi.org/10.1007/s10856-013-4854-0

Willett NJ, Li M-TA, Uhrig BA, Boerckel JD, Huebsch N, Lundgren TS, Warren GL, Guldberg RE (2013) Attenuated human bone morphogenetic protein-2–mediated bone regeneration in a rat model of composite bone and muscle injury. Tissue Eng Part C Methods 19:316–325. https://doi.org/10.1089/ten.tec.2012.0290

Wilson CJ, Schütz MA, Epari DR (2017) Computational simulation of bone fracture healing under inverse dynamization. Biomech Model Mechanobiol 16:5–14. https://doi.org/10.1007/s10237-016-0798-x

Wolff J (1892) Das gesetz der transformation der knochen. A Hirshwald 1:1–152

Wong TM, Lau TW, Li X, Fang C, Yeung K, Leung F (2014) Masquelet technique for treatment of posttraumatic bone defects. ScientificWorldJournal 2014:710302. https://doi.org/10.1155/2014/710302

Wren TA, Beaupré GS, Carter DR (2000) Tendon and ligament adaptation to exercise, immobilization, and remobilization. J Rehabil Res Dev 37:217–224

Yazar S, Lin C-H, Wei F-C (2004) One-stage reconstruction of composite bone and soft-tissue defects in traumatic lower extremities. Plast Reconstr Surg 114:1457–1466. https://doi.org/10.1097/01.PRS.0000138811.88807.65

Yin L, Huang X, Xu H, Zhang Y, Lam J, Cheng J, Rogers JA (2014) Materials, designs, and operational characteristics for fully biodegradable primary batteries. Adv Mater 26:3879–3884. https://doi.org/10.1002/adma.201306304

Zadeh LA (1965) Fuzzy sets. Inf Control 8:338–353. https://doi.org/10.1016/S0019-9958(65)90241-X

Zlowodzki M, Vogt D, Cole PA, Kregor PJ (2007) Plating of femoral shaft fractures: open reduction and internal fixation versus submuscular fixation. J Trauma Acute Care Surg 63:1061–1065. https://doi.org/10.1097/TA.0b013e318154c0b4

Chapter 9
Biomaterials in Connective Tissue Regeneration and Rehabilitation

Michael Kowalski, Maddie Hasson, Kyle Hammond, Sameh Labib, and Jay Milan Patel

Abstract Connective tissue encompasses a wide range of properties, including cell types, multi-scale structure, and mechanical properties, and thus, treatment modalities of each require distinct criteria for successful regeneration or rehabilitation. As a result, the role of biomaterials in treating injuries to these tissues has broadened to study a multitude of factors that may impact each individual's ability to rehabilitate injured tissue and heal. This chapter focuses on the types of connective tissue, their unique properties, and their associated injuries and treatments; the structural, mechanical, and biological considerations for biomaterial application in each of these tissues are then highlighted. The chapter then discusses the role of these considerations on connective tissues to promote improved healing, tissue regeneration, and rehabilitation.

Keywords Connective tissue · Biomaterials · Tissue engineering · Tissue interfaces · Regenerative medicine

9.1 Introduction

Connective tissues provide a wide array of functions to the human body, including support, separation, and attachment. Examples of connective tissues include cartilage, ligament and tendon, bone, adipose, and blood, all of which have specific structural, mechanical, and biological attributes that drive their function. Unfortunately, these tissues are often injured or experience pathologies, and thus require interventions to restore function and alleviate patient symptoms. While some repair

M. Kowalski · M. Hasson · J. M. Patel (✉)
Department of Orthopaedics, Emory University School of Medicine, Atlanta, GA, USA

Atlanta VA Medical Center, Department of Veterans Affairs, Decatur, GA, USA
e-mail: jay.milan.patel@emory.edu

K. Hammond · S. Labib
Department of Orthopaedics, Emory University School of Medicine, Atlanta, GA, USA

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_9

techniques and replacement grafts exist, the field of biomaterials has made significant strides in recent decades to improve the regeneration of connective tissues, and ultimately, the rehabilitation and recovery of patients.

The distinct composition and organization of each type of connective tissue gives it specific functions in the body (Fig. 9.1). The soft, spongy adipose tissue is not only an energy storage unit for the body, but also serves a role in thermal insulation and cushioning of organs. On the other end of the spectrum is bone, a rigid, mineralized tissue that constitutes the skeleton, providing mechanical support and enabling functional mobility. Other connective tissues include ligaments and tendons, which are fibrous aligned tissues that connect bones to either bones or muscles, and cartilaginous tissues, which are durable and resilient tissues that provide padding, shock adsorption, and load distribution in our joints. The extracellular matrices (ECM) of these tissues are all unique; the ECM consists of a particular milieu of fibers (mostly collagen) and ground substance (proteoglycans). Specific properties of each tissue are discussed further in their respective subsections.

Throughout this chapter, we will discuss the use of biomaterials to regenerate and rehabilitate each of these tissues with respect to three major categories: structure, mechanics, and biology. Each of these is influential in how biomaterials and

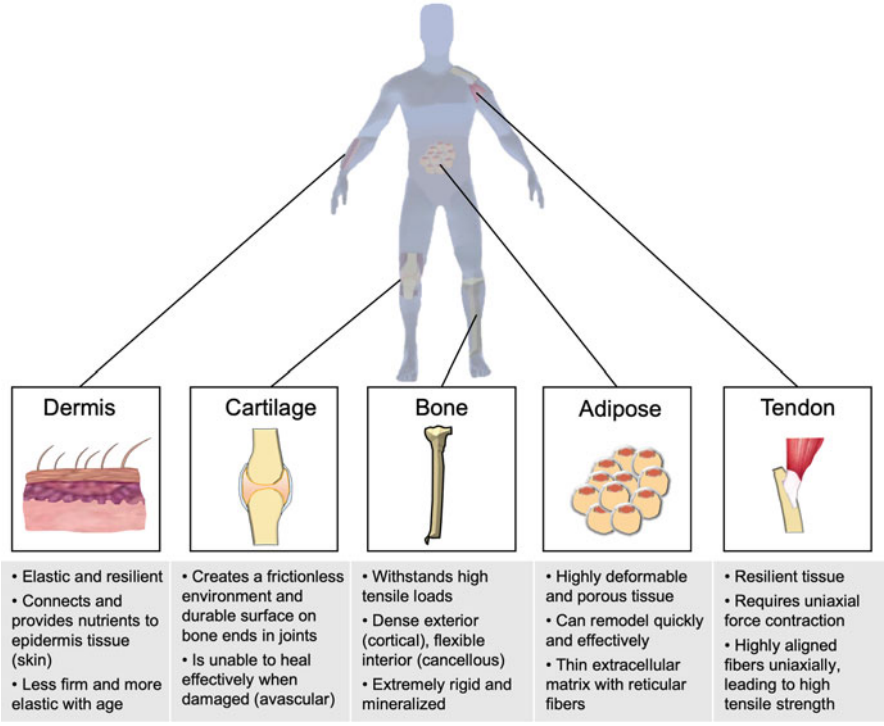

Fig. 9.1 Types of Connective Tissues. Connective tissues include dermis, cartilage, bone, adipose, and tendons/ligaments, with a broad array of functions

regenerated tissue will function, both in the short and long term. We will highlight recent advances in biomaterial design, synthesis, fabrication, and application, first detailing biomaterial types (biologic vs synthetic) and overall approaches (e.g., 3D/bioprinting, drug delivery), followed by a discussion of each connective tissue type.

9.2 Biological and Synthetic Polymers

Perhaps, the most used classification of biomaterials is biological versus synthetic. Biological materials are mostly derived from ECM components; collagen- and polysaccharide-based scaffolds are perhaps the most frequent. As the major constituent of the ECM, collagen is a substrate that cells can readily attach to and remodel, providing a template for tissue regeneration (K. Lin et al. 2019; Parenteau-Bareil et al. 2010). Polysaccharide-based scaffold materials (e.g., glycosaminoglycans) include hyaluronic acid, agarose, alginate, heparin sulfate, chondroitin sulfate, and chitosan, and their biocompatible and hydrophilic nature is typically supportive of cell and tissue growth (Dinoro et al. 2019; Tiwari et al. 2018). These materials are relatively biocompatible due to their presence in the existing ECM, and they can be combined and tuned to provide cues that influence cellular and tissue response. For this reason, decellularized tissue matrices are becoming increasingly popular (Cheng et al. 2014; Rowland et al. 2016; Shimomura et al. 2017), as they present a tailored set of ECM cues for each tissue that infiltrating cells sense, leading to increased tissue-specific gene expression and ECM deposition. These biological materials are usually fabricated into sponges or gels, which provide environments that allow cellular infiltration or encapsulation. However, the soft nature of these scaffolds limits their direct use in terms of functional restoration of many connective tissues, and oftentimes a pre-culture period with cells and bioactive factors is required to develop the requisite mechanical properties (e.g., stiffness, yield stress). Other limitations or concerns of biologic materials include production variability and relatively quick degradation/resorption.

Synthetic materials can help to combat many of these concerns, especially those related to inadequate mechanical properties. A variety of synthetic polymers (polylactic acid, polyglycolic acid, polyethylene glycol, polyvinyl acid, polycaprolactone) present a broad range of utilizable chemistries. By further altering properties such as polymer concentration, molecular weight, and copolymer ratios, a library of materials, each with controlled fabrication and precise mechanics and degradation, can be achieved. Thus, by using both biological and synthetic polymers, a broad range of properties that resemble native connective tissues can be achieved (Fig. 9.2). The main drawbacks of synthetic polymers are their relative inability to be remodeled and possibility of inflammatory or encapsulation response, due to their lack of presence in native tissues. However, functionalizing synthetic polymers or combining them with biological polymers can improve biocompatibility, engraftment and remodeling of neo-tissue, and ultimately long-term function.

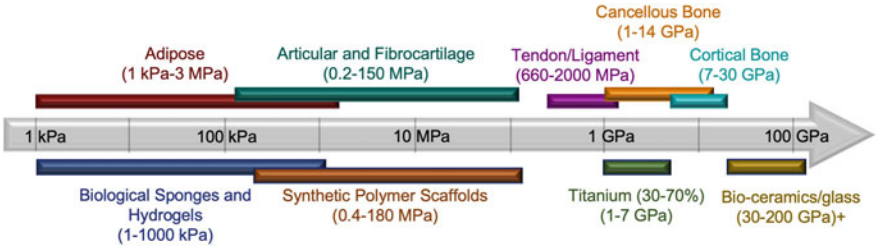

Fig. 9.2 Mechanical Properties of Connective Tissues. Elastic modulus values of tissues (top) and materials (bottom) on a spectrum from 1 kPa to 100GPa. Modulus values include bulk, compressive, tensile, and torsional values

9.3 Functional Biomaterial Fabrication

While many of the aforementioned biomaterial types have been utilized for decades, the field of biomaterials has seen rapid development of material fabrication approaches. The traditional use of isotropic sponges and gels has progressed to guided techniques to recreate the spatial structure, mechanics, and biology of native tissues.

9.3.1 3D Printing and Bioprinting

The field of 3D printing for tissue engineering has made significant strides in the past decade. While tissue-scale anatomy was previously recreated with anatomical molds, 3D printing offers the ability to recreate spatial organization and orientations of a variety of tissues (Fig. 9.3). Traditionally, the technology has used synthetic polymers, such as poly(L-lactic acid) (PLLA) and polycaprolactone (PCL), but biological polymers, including decellularized ECM, have been printed recently

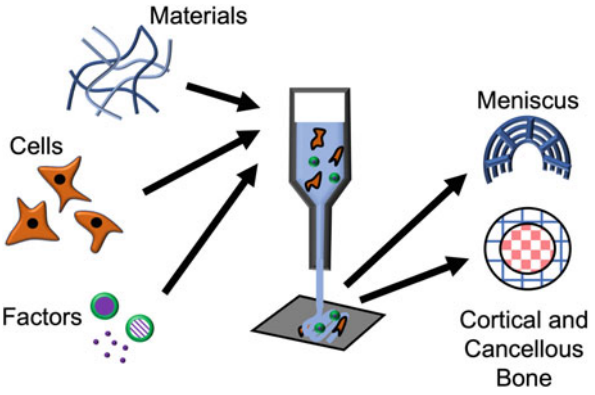

Fig. 9.3 Three-dimensional printing. Materials, cells, and factors (bioprinting) can be combined and printed with spatial resolution to reconstruct complex, multiphasic tissues, such as the meniscus and bone, respectively

(Dzobo et al. 2019; Pati et al. 2014). By incorporating aligned polymer fibers, the complex anisotropic structure and mechanics of tissues such as the meniscus (Ghodbane et al. 2019) and bone (Jariwala et al. 2015) can be recapitulated. These printers are also well-suited to incorporate two or more dispensers, allowing for simultaneous deposition of multiple polymer types in an organized spatial arrangement. For instance, cortical and trabecular bone, which are composed of distinct matrix components with vastly different mechanical properties, can be printed side by side with a stiff cortical shell surrounding a spongier core (Almela et al. 2017). While the majority of fabricated scaffolds have been acellular, a subset of 3D printing called bioprinting has emerged that allows for the incorporation of cells (M. Zhang et al. 2020) and/or factors (Lee et al. 2014) within printed fibers in a specific spatial organization. For multicomponent tissues and tissue interfaces, this is especially important, as multiphasic or gradient properties with one continuous scaffold can be developed; for example, Sun et al. bioprinted an intervertebral disk with dual growth-factor release in the annulus fibrosus and nucleus pulposus regions (Sun et al. 2021). Hundreds of papers have been published with connective tissue 3D or bioprinting, all of which have contributed new techniques to improve print fidelity and resolution, fiber size and spacing, biocompatibility, fabrication conditions, etc. In fact, these properties have become so refined that printing vascular networks or nutrient channels has become quite common (Skylar-Scott et al. 2019). Altogether, 3D printing can allow for control of tissue structure and anatomy through printing organization, tissue mechanics with polymer selection and fiber arrangement, and biology through the incorporation of specific cell types and bioactive factors.

9.3.2 Biomaterial-Mediated Drug Delivery

Biomaterials are not only utilized as a scaffold material to replace tissues, but can also aid in rehabilitation by controlling the release of bioactive factors. Often times, these bioactive factors, such as growth factors, easily diffuse out of scaffolds if the factors are not covalently linked to the material, creating a burst release in the first or two days post-implantation, rather than a gradual release over a lengthy rehabilitation timeline. To extend the release of factors, a variety of micro- and nano-carriers have been used (Fig. 9.4a); both particles (solid spheres) and capsules (polymer shells, aqueous cores) can be utilized with encapsulated or functionalized bioactive factors, extending the release of factors to weeks, or even months (Fig. 9.4b) (J.M. Patel et al. 2019b). Furthermore, these biomaterials can be modified in order to tune their application and release according to various stimuli. For example, enzyme-degradable biomaterials can be utilized to respond to inflammatory or matrix-degrading cytokines, allowing for on-demand factor release. Other approaches have developed materials responsive to pH (Cummings and Nordby 1966) and temperature (Tsai et al. 2003) changes, for example in the knee joint, or to external stimuli such as ultraviolet light (Karimi et al. 2015), magnetism (Wang et al. 2014), or ultrasound (Nieminen et al. 2015), for controlled release at specific points

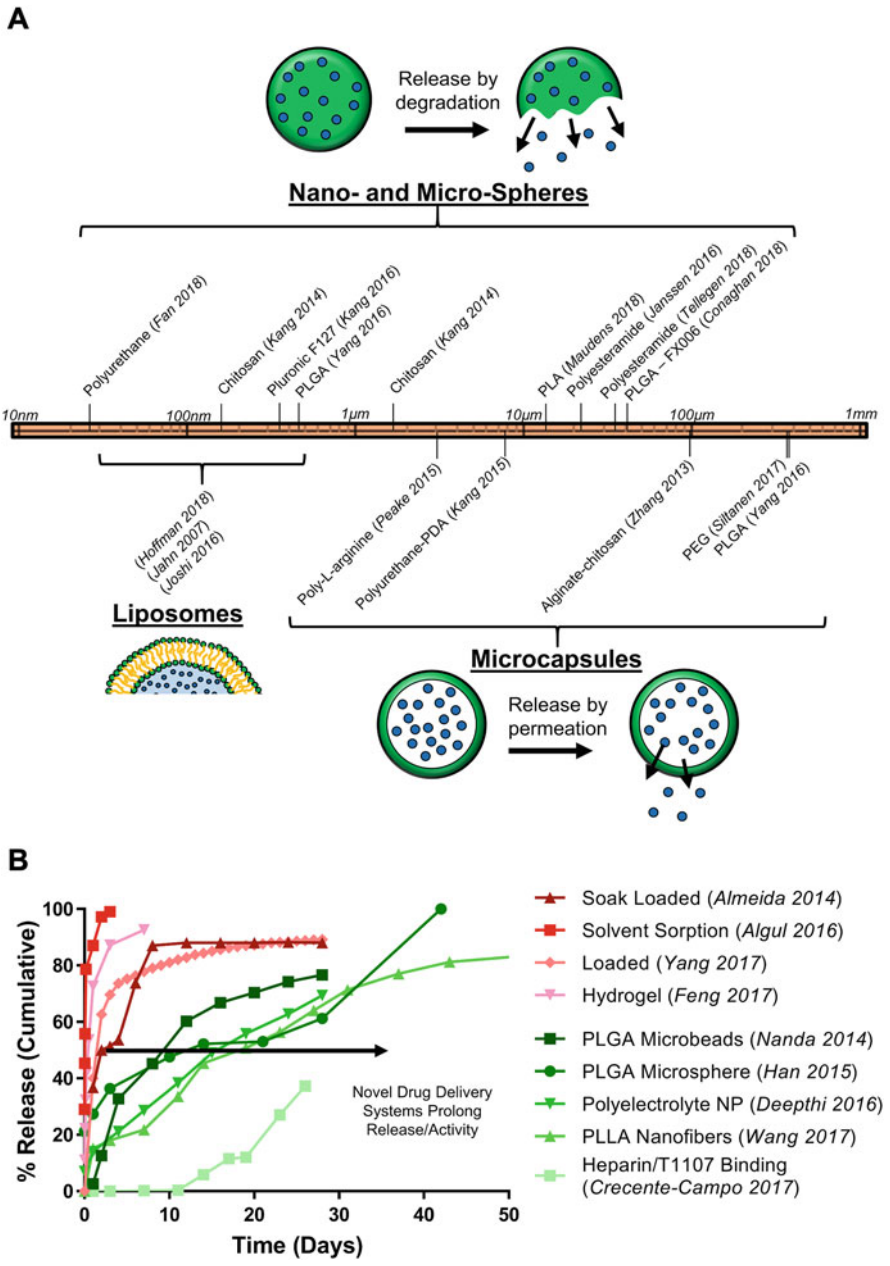

Fig. 9.4 Biomaterials to deliver bioactive factors. (**a**) Relative sizes of spheres, capsules, and liposome. (**b**) Release profiles of standard encapsulation (red) versus biomaterial encapsulation (green) show extended release. Figure from (Jay M Patel et al. 2019c), reprinted with permission from Elsevier

during rehabilitation. One final technique that is particularly useful for load-bearing connective tissues is the use of mechanically activated microcapsules (Mohanraj et al. 2019); upon application of force, for example during weight-bearing recovery, factors can be released to promote enhanced tissue growth and regeneration.

9.3.3 *Tissue Engineering: Degradation Vs Growth*

A common paradigm in the field of tissue engineering revolves around the degradation of materials, and that the resorption of materials and decrease in mechanical properties should mirror the increase in properties from regenerative tissue growth. Biological polymers are typically resorbed within months, whereas synthetics can last for years, so depending on tissue type, a more intermediate degradation profile may better match neo-tissue formation (weeks to months). Furthermore, composite devices, consisting of both biological and synthetic components, are promising; the synthetic component initially bears load and provides structure, while the biological component can be remodeled into the desired tissue type. For example, a composite meniscus replacement device, consisting of a synthetic polymer fiber surrounded by a collagen sponge, enabled successful meniscus reconstruction, as neo-tissue formed between synthetic fibers (Merriam et al. 2015). If the degradation of the biomaterial occurs too quickly, as patients begin to bear load and stress the material during rehabilitation, failure and rupture are almost certain. Thus, this tissue engineering balance (degradation vs growth) is an important consideration (Fig. 9.5).

9.3.4 *Implantation Considerations*

Another consideration for biomaterial usage in connective tissue repair and regeneration is the mode of implantation and retention. Certainly, the less invasive an approach to implant materials, the better the recovery of the patient. Injectable

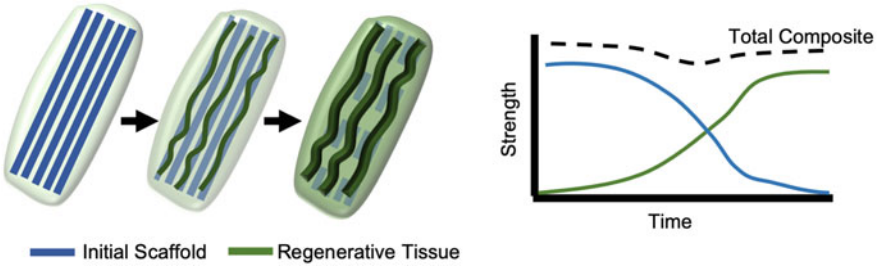

Fig. 9.5 Temporal tissue engineering. Initial scaffold materials degrade over time and give way to regenerative tissue, giving total composite mechanics that are maintained with time

treatments are perhaps the most ideal, especially solutions that can be injected and form solids within the body. Certain materials are thermo-responsive, solidifying at body temperature. Shear-thinning hydrogels have also garnered a lot of recent interest, as they lose viscosity under shear, allowing for injection and subsequent solidification once in place, typically best suited for soft-tissue applications. Stiffer materials and scaffolds are best implanted through small portals, such as arthroscopic portals or small arthrotomies for joints, minimizing bleeding and the amount of tissue that needs to be cut through for access. By minimizing bleeding at the site of injury, the inflammatory cascade can be reduced, improving the regenerative potential of the biomaterial system. Retention of biomaterials at the desired site is also very important to its function and to patient rehabilitation. For softer substances, like adipose fillers, the surrounding capsule needs to be secured tightly to prevent leakage. For load-bearing tissues, biomaterial implants are often fixed with screws, pins, or rods to maintain position, and thereby function. The choice of fixation is important, as to provide sufficient fixation strength but not prevent tissue regeneration or integration with surrounding tissues.

9.4 Biomaterial Considerations for Connective Tissues

9.4.1 Bone

Bone is categorized into two distinct types that are differentiated by function: cortical and cancellous. Cortical bone is responsible for supporting the body's weight, protecting organs, and acting as a lever for movement. As a result, cortical bone is extremely stiff and dense, in order to maintain its shape and withstand routine loading (Viguet-Carrin et al. 2006). Cancellous bone is less stiff and dense, with a more porous structure that has a higher surface area. Primarily found near joints and at the end of long bones, cancellous bone is adaptable to stresses (Gibson 1985), aiding in the absorption of sudden stresses. Cancellous bone is also highly vascular; the bone marrow within is responsible for hematopoiesis, or the production of blood cells. In long bones, the hollow cylindrical cortical bone encompasses the cancellous bone and marrow. As a result of these varying functions, clinicians have used a plethora of methods to restore the proper function of bone.

The primary treatments for severe bone injuries include casting and splinting, fixation with rigid metals, or bone grafts (Schlickewei et al. 2019; Somford et al. 2013). Casting and splinting of bone fractures in the body's smaller extremities have favorable outcomes (Kollitz et al. 2014); however, compact injuries, load-bearing injuries, and other severe/complex injuries require additional treatment. Often times, these injuries are treated with metallic hardware, including plates, screws, and/or rods. While these methods provide a mode of rigid fixation to provide load support to the initial healing environment, the regenerated bone is variable between patients and locations, is often weaker than the surrounding tissue, and is susceptible to

re-fracture (Olmstead 1991). Thus, replacing the bone with functional and pro-regenerative materials is advantageous.

Bone grafts provide an alternative to fracture fixation, providing a template for regeneration. The two main types of bone grafts include autografts, usually derived from the patient's iliac crest, and allografts, derived from cadaveric sources (Finkemeier 2002). While autografts from the iliac crest are a good source of graft material for smaller injuries, their limitations include donor site morbidity and infection risk, neurological risk, vascular injuries, and deep hematomas. (Arrington et al. 1996). For larger injuries and to avoid harvest site morbidity, allografts are more readily available, but present challenges with patient immune response and size matching (Burchardt 1983). One clinically utilized source of material, derived from allograft sources, is demineralized bone matrix (DMB), which is ground bone matrix that provides key proteins and growth factors from the ECM to promote osteoinduction (Finkemeier 2002); however, this "putty" may not provide the initial structural and mechanical support needed to treat bone injuries. Due to these limitations, researchers have investigated bone graft substitutes that meet both the mechanical and biological needs of bone to promote improved clinical outcomes in patients.

9.4.1.1 Structural Considerations

The structure of cortical and cancellous bone provides researchers with key properties to mimic, as they design various biomaterials to improve rehabilitation of the tissue. Some researchers have considered bone tissue a "two-phased porous structure" with cancellous bone within the cortical bone; however, others consider these as two morphologically different types of bone as a result of the different structure (Choi et al. 1990; Wall and Board 2014). Regardless, modeling bone tissue through the nano- (mineralization, bone crystal formation, and collagen fibril alignment), micro- (lamellae and osteon development), and macro- (cortical and cancellous) levels is an effective way to design bone biomaterials (Fig. 9.6) (Rho et al. 1998). These factors have led to synthetic polymers, like PLA, PGA, PCL, PVA, or PPF, which can be fabricated into scaffolds that simultaneously mimic macro-scale structure and mechanics, as well as micro-scale collagen fibril alignment and porosity (H. Qu et al. 2019). Furthermore, while natural materials are too soft to use on their own, they can be incorporated to present biological cues (Sheikh et al. 2017). Mineralization of the new tissue is also important in the structural development of the bone tissue. Biomaterials can be augmented with simulated body fluids that have similar ion concentrations of human plasma to induce mineralization of newly forming tissue (Kokubo and Takadama 2006). Perhaps, more popular is functionalizing calcium and phosphate ($CaCl_2$ and Na_2HPO_4) or by using biomaterials that contain minerals (hydroxyapatite) to promote osteogenesis of recruited cells (T. Gao et al. 2015; Hutchens et al. 2009). Finally, the macro-scale biomaterial structure can exhibit anisotropy to be similar in structure to native cortical and cancellous bone tissue, either with biphasic or gradient scaffolds.

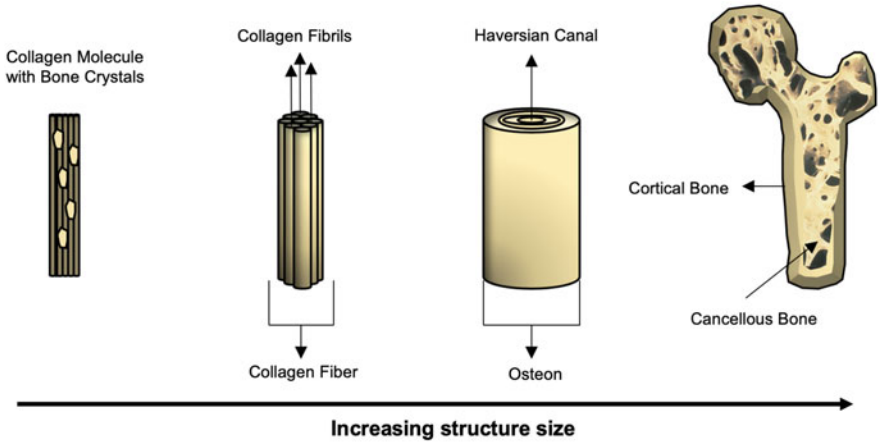

Fig. 9.6 Multi-scale bone structure. Bone is composed of collagen molecules with bone crystals at the micro-scale, which form collagen fibrils, osteons, and the entire bone structure as the size scale increases

9.4.1.2 Mechanical Considerations

Bone tissue mechanics are extremely important to maintain the proper movement and shape of the body, as well as to protect vital organs. Cortical bone has a much higher Young's modulus of 7–30 GPa compared to a modulus of 1–14 GPa for cancellous bone (Rho et al. 1993; Wu et al. 2018). This allows the cancellous bone to be more adaptable to extreme stresses (Rho et al. 1993). Clinicians and researchers have focused on meeting this specific mechanical need of bones when treating trauma with a variety of biomaterials that are able to withstand high loads without significant deformation. These materials have primarily consisted of metals (e.g., aluminum) that are not biologically active, but provide the mechanical support for the trauma site (Zaffe 2005). Titanium and titanium alloys are other examples of metals that can withstand extremely high loads without undergoing much deformation. Titanium provides up to two times the tensile strength, compressive strength, and elastic modulus of cortical bone (Chen et al. 2012; Niinomi 1998). Bioinert ceramics, like zirconium, and bioactive glasses can also provide the key mechanical properties to restore the function and are long-term solutions for severe bone injuries because they typically do not cause any adverse reactions. Both metals and ceramics can allow for loading soon after implantation, since a period of bone regeneration may not be required to achieve adequate mechanical properties.

Another mechanical consideration for bone biomaterials is to provide environmental cues that promote ossification rather than using biomaterials that permanently replace the injured tissue. Mechanical stimuli, biophysical environments, and cellular mechano-transduction have all shown to have a significant effect in the bone tissue healing process (Rosa et al. 2015). For example, a strain environment of 2.5–9% can contribute to intramembranous bone formation; however, apoptosis

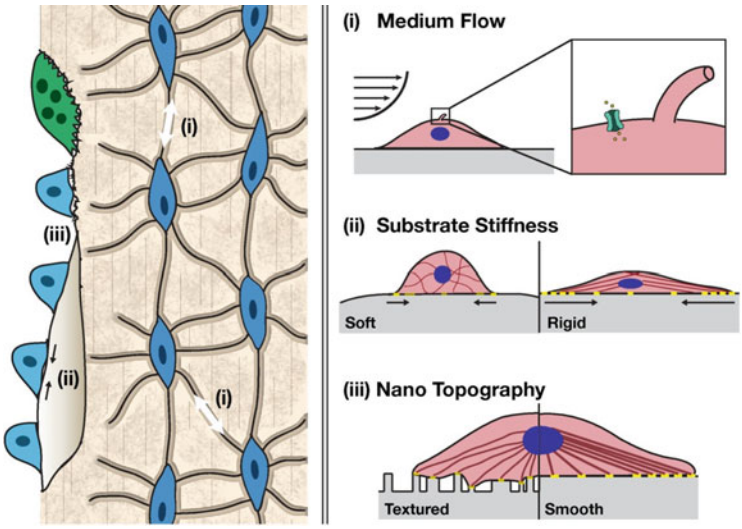

Fig. 9.7 Mechanical cues on osteogenic differentiation. (Left): native bone. (i) Physiological loading of bone induces fluid flow within the canaliculi, resulting in shear stress on the osteocytes, which transmit these signals to osteoclasts and osteoblasts to remodel the bone. (ii) Stiffness and (iii) topography of native bone matrix also impact new bone deposition by osteoblastic cells. (Right): tissue engineers apply mechanical stimuli to enhance the osteogenic response of stem and progenitor cells in vitro, via (i) fluid shear, (ii) substrate stiffness, (iii) nanotopography. Figure from (Hung et al. 2013), reprinted with permission from Springer Nature

occurs at strains greater than 10% (Carter et al. 1998; Claes et al. 1998; Kearney et al. 2010), which should be considered during rehabilitation protocols. Additionally, higher shear strain and fluid flow directly lead to more granular tissue formation, while lower strain and fluid flow contribute to ossification (Lacroix and Prendergast 2002; Prendergast et al. 1997), influential both during the pre-culture period of bone tissue engineering scaffolds and during the recovery phase post-implantation. The biophysical environment around recruited or exogenously added cells is also highly influential in new bone formation, as the specific perception and transduction of signals can be highly influential in the osteogenic potential of biomaterials. Tuning the substrate stiffness, nanotopography, and the aforementioned mechanical loading (Fig. 9.7) can precisely control progenitor cells and osteoblasts to deposit new functional bone tissue (Hung et al. 2013; McDermott et al. 2019).

9.4.1.3 Biological Considerations

As mentioned previously, while the structure and mechanics of bone are influential to successful regeneration and rehabilitation, the formation of new osseous tissue is paramount to long-term function and prevention of re-fractures. Thus, the biological activity of materials can be guided toward healing. Components of native healthy

bone present a method to do so, for example, tricalcium phosphate and calcium silicate ceramics. Ni et al. showed calcium silicate promoted higher proliferation, differentiation, and attachment of osteoblast-like cells in comparison to calcium phosphate (Ni et al. 2007). Similarly, calcium phosphate improves collagen production, cell proliferation, and osteogenic marker expression (Park et al. 2011). As the primary ECM protein of bone, type 1 collagen provides an environment that can enhance cell interaction and direct tissue formation (Ferreira et al. 2012). Alternatively, cells and growth factors can be incorporated into scaffolds to improve osteogenesis. For example, Kim et al. utilized hyaluronic acid hydrogels with mesenchymal stem cells and the growth factor bone morphogenic protein-2 (BMP-2) to promote bone tissue growth (Kim et al. 2007). This class of growth factors (BMPs) has long been shown to improve bone formation, and the aforementioned drug delivery techniques can help to prolong release over the healing and rehabilitation timeline, which can last months. Another biological consideration includes integration of biomaterials with the native tissue. Generally, biologically inert compounds had been used clinically, until recently. Synthetics and ceramics can undergo surface modifications using other bioactive ceramics such as tricalcium phosphate (TCP) or hydroxyapatite to provide sites for cell attachment and tissue growth (Niinomi 2008), potentially enhancing tissue deposition at the interface to improve integration. Also, bioactive glasses are known to rapidly produce a hydroxycarbonate layer that bonds to tissue in biological fluid (Stevens 2008). These modifications can lead to increased tissue integration of native and newly forming tissue.

9.4.1.4 Rehabilitation

These biomaterial strategies have the potential to improve the current treatment modalities. Biomaterials can provide a structural scaffold, with the requisite mechanical properties to provide initial support and biological activity to provide a long-term replacement to injured bone. Tuning the biomaterial resorption profile (Fig. 9.5) provides a unique avenue to ensure that the structure of the original scaffold is preserved until new osseous tissue growth replaces it. Furthermore, different resorption rates have been developed to address the varying recovery times of bone tissue injuries, ranging from 4 to 24 weeks. For example, femur fractures typically require recovery times of about six months, but can last up to one year depending on the severity of injury and age of the patient (Dyer et al. 2016; Fischer et al. 2019). At a minimum, bone regeneration requires 12 weeks of healing from a clinical perspective, indicating that the biomaterial should maintain most mechanical properties up until this time point (Dado and Levenberg 2009). Many common synthetic materials, such as PLA and PLGA, can exhibit considerable loss in mass and molecular weight by 10 weeks in vivo (H. Zhang et al. 2014), indicating degradation of the biomaterial and a compromise in the mechanics of the biomaterial-injury site due to increased strains. Therefore, the degradation rate of the biomaterials should be matched to the length of rehabilitation for the fracture in question, as to avoid re-fractures and to

utilize rehabilitation weight bearing as a mechanical stimulus for improved osteogenesis.

9.4.2 *Cartilage and Fibrocartilage*

Three main types of cartilage exist: hyaline (i.e., articular), fibro- (e.g., meniscus, intervertebral disk), and elastic (e.g., nose, ears) (Fig. 9.8a) (Krishnan and Grodzinsky 2018), each with a unique composition and organization for their specific functions (Fig. 9.8b). Articular cartilage lines the ends of long bones in our joints, creating a near-frictionless environment to distribute loads and allow smooth motion in the synovial joint (Sophia Fox et al. 2009). Due to this, articular cartilage has strong compressive and shear properties, as well as lubricating capabilities to enable frictionless motion. Fibrocartilage is a mix of fibrous and cartilaginous tissue, and its function is also to distribute loads within our joints. The primary forms of fibrocartilage include the meniscus, intervertebral disk (IVD), and temporomandibular joint (TMJ), and these tissues typically act in compression, shear, and tension, where aligned circumferential fibers prevent extrusion of these tissues. Elastic cartilage is the most flexible and is not load-bearing. Since elastic cartilage is typically not damaged as severely as fibrocartilage or hyaline cartilage (Hutmacher et al. 2003), and when damaged, does not present as many long-term complications, this section focuses on biomaterial approaches for fibrocartilage and articular cartilage (Horner et al. 2016).

Cartilage injuries lack an inherent capacity to heal because the tissue can be relatively avascular. As a result, interventions are almost always required. A host of injections have been utilized as palliative measures to combat these injuries, including nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, hyaluronic

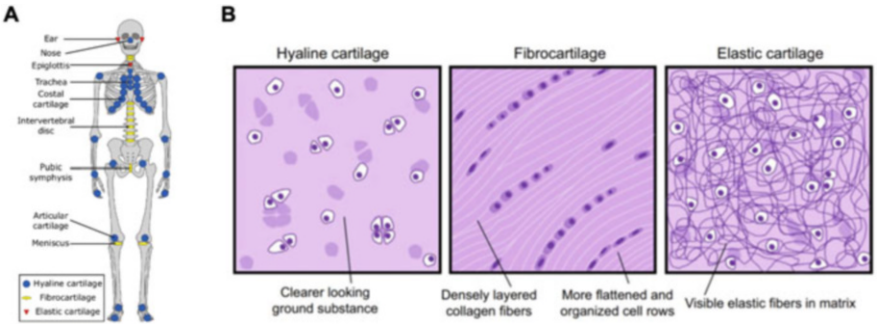

Fig. 9.8 Cartilage types and structure. (**a**) Human skeleton with hyaline, fibro-, and elastic cartilage highlighted. Figure from (Krishnan and Grodzinsky 2018), permission granted to reproduce. (**b**) Structure of the three types of cartilage. Figure obtained from (Teixeira et al. 2020); MDPI grants open permission privileges

acid, as well as biologics like platelet-rich plasma, bone marrow aspirate, and stem cells. However, surgical treatment is often needed to address symptoms, and physicians typically debride (remove) the damaged tissue to create a smoother surface, reducing mechanical irritation and providing symptom relief. However, removal of the tissue (e.g., chondroplasty, meniscectomy, discectomy; G. Li et al. 2008) alters the biomechanics on the entire unit, whether the joint or spine, and leaves the remaining cartilage more susceptible for deterioration. For articular cartilage, perforating the subchondral bone in a process called microfracture can promote healing via progenitor cell recruitment from the underlying marrow that fills the defect (Buckwalter 1998); while microfracture is easy to perform and typically leads to short-term symptomatic relief, the repair tissue is mechanically inferior and wears with time (Buckwalter 2002). More recently, modified microfracture techniques, autologous chondrocyte implantation (ACI), and modified ACI techniques have been used clinically, with some improvement in patient outcomes, but challenges relating to ACI include inconsistent long-term outcomes, donor site morbidity, cost of harvest, and the need for a second procedure.

For fibrocartilage, when injured, the optimal mode of treatment is suture repair, generally used to reattach the two loose ends of tissue. However, depending on the location of the tear, removal may be the only possible treatment to provide relief of mechanical symptoms, and pain may return as a result of progressive degeneration, of the tissue itself and the surrounding environment. For example, meniscal tears, especially when treated with meniscectomy, can lead to accelerated articular cartilage degeneration (Chambers and Chambers 2019). For both hyaline and fibrocartilage, while autologous (for hyaline) and allogenic (for hyaline and fibrous) grafts serve as a mode of replacement, they are limited in their long-term efficacy, and present challenges with regard to availability, size and surface matching, and disease transmission. This has led researchers to investigate how biomaterials can regenerate tissues and recapitulate structure, mechanics, and biology to restore articular cartilage and fibrocartilage function.

9.4.2.1 Structural Considerations

To best rehabilitate and restore the function of cartilage tissue, it is necessary to provide specific compositional and structural characteristics specialized to articular cartilage and fibrocartilage. Since water content is generally high throughout cartilage, hydrogels that mimic the solid–liquid ratio are popular, including agarose and hyaluronic acid (Armiento et al. 2018). These hydrogels, including synthetic ones like polyethylene glycol, provide tunable characteristics like porosity and density to promote cartilaginous ECM deposition while still preserving the biomaterial's high water content (Bryant and Anseth 2002, 2003). However, hydrogels may not best mimic the bulk mechanical properties and specific collagen fiber alignment of cartilage tissues. For articular cartilage strategies should aim to recapitulate the superficial alignment of smaller collagen fibers parallel to the surface (for friction and shear properties), the randomly oriented in the transitional zone (Thompson

et al. 2014), and the perpendicularly oriented, larger fibers in the deep zone (Fig. 9.9a), altogether arranged in structures called the Benninghoff arcades. These characteristics have led researchers to study the effects of fiber alignment on the cells in these biomaterial scaffolds. Alignment can be achieved via 3D printing and electrospinning, which show a positive effect on chondrogenesis when the fiber alignment is modeled to native tissue in each zone (Accardi et al. 2013; Guo et al. 2018; Reboredo et al. 2016). Additionally, the micro-scale structure of scaffolds is also important; the morphology of chondrocytes becomes more elongated (fibroblast like) in micro-fibrous scaffolds, but remains relatively spherical on nanofibrous substrates (W. J. Li et al. 2006), allowing cells to preferentially orient themselves and maintain chondrogenic capacity, while recapitulating articular cartilage's native structure.

Fibrocartilage has a slightly different structure than hyaline cartilage, with a mixture of dense fibrous connective tissue with fibroblasts and hyaline-like cartilage with chondrocytes. Fibrocartilaginous tissues have a specific tissue-scale morphology that enable them to effectively distribute loads. To maintain this specific structure, 3D reconstructions, for example of the meniscus, can be obtained from medical imaging scans, and be used to fabricate scaffolds that maintain this shape (Ghodbane et al. 2019; Szojka et al. 2017). Furthermore, a large portion of the collagen fibers in fibrocartilage are organized in dense bundles arranged parallel to each other, along a circumferential axis to resist extrusion and "hoop stresses" (Fig. 9.9b) (Watkins 2014). As previously discussed, methods to orient the multi-scale structure of scaffolds can simultaneously recreate fibrocartilage mechanics and guide cells toward aligned tissue deposition, re-establishing the native circumferential alignment of the meniscus (Lee et al. 2014; Jay M Patel et al. 2018) and IVD tissues (Gullbrand et al. 2018). Novel methods, like bioprinting, provide viable routes for the proper spatial incorporation of cells and factors to enhance regeneration (Costa et al. 2020). These approaches to make custom, anisotropic scaffolds

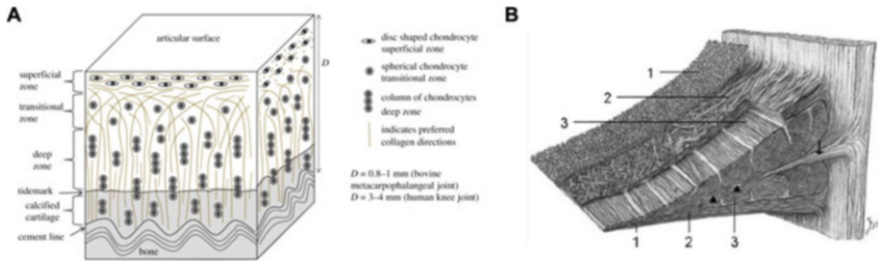

Fig. 9.9 Collagen fiber alignment in cartilage and fibrocartilage. (**a**) Benninghoff arcades of cartilage, with fibers parallel to the surface in the superficial zone, randomly oriented in the transitional zone, and perpendicularly oriented in the deep zone. Figure from (Mansfield et al. 2015), available to be reproduced by the Creative Commons Attribution License. (**b**) Alignment of collagen fibers in fibrocartilaginous meniscus. [1] Superficial fibers aligned randomly on surface for shear properties. [2] Radial tie fibers hold together the [3] densely packed circumferential fiber network. Figure from (Petersen and Tillmann 1998), reproduced with permission

present the opportunity to improve the structural organization and ultimately the long-term function of these techniques.

9.4.2.2 Mechanical Considerations

Cartilage tissue has unique mechanical properties that allow the tissue to mediate the motility of joints and prevent bones from grinding against one another. When damaged, the macro- and micro-scale cartilage mechanics are jeopardized and can lead to progressive degeneration. One of the most important mechanical considerations for both tissue types is the compressive properties of the tissue. The tensile Young's modulus of articular cartilage is 5–25 MPa, while fibrocartilage by comparison is much higher; however, the compressive modulus of articular cartilage (0.2–0.8 MPa) is usually higher than that of fibrocartilage (Jin and Lewis 2004; Watkins 2014). Simple isotropic sponges or hydrogels may provide a template for regenerating these tissues, but they are usually a few orders of magnitude lower in mechanical properties than healthy cartilage tissue (Pillai et al. 2018), leaving them susceptible to failure post-implantation. To better approximate native tissue mechanics, the density and porosity can be tuned to increase the stiffness of biomaterial scaffolds (Pillai et al. 2018). Reinforced composite scaffolds are perhaps the best route to provide the necessary support, at the time of implantation, and beyond during rehabilitation (Moutos and Guilak 2008). Also, important to the compressive properties of all cartilage tissues is their multiphasic response; the retention of water due to high proteoglycan content allows for stress absorption and time-dependent mechanics that can withstand repetitive loading. Polymer chemistry and functionalization can improve these biphasic properties and better mimic native tissue. Additionally, when fluid pressurization increases, the friction within the joint drops dramatically, offering a very slick surface for articulation (Ateshian 2009). Methods to restore the near-frictionless environment in the joint are of great interest, for example, lipid-based boundary-lubricated hydrogels that decrease the friction and wear by over eighty percent (W. Lin et al. 2020). Finally, as discussed previously, fibrocartilage structural properties are often recapitulated with aligned fibers via printing or extrusion; this alignment and reinforcement of scaffolds can also provide the necessary tensile properties that these tissues need to resist rupture (J. Patel et al. 2019a).

9.4.2.3 Biological Considerations

Cartilage tissue engineering has long combined materials, cells, and bioactive factors in order to regenerate functional cartilage tissue (Crecente-Campo et al. 2017; J.M. Patel et al. 2019b). While great strides have been made with various cell types (induced pluripotent stem cells, re-differentiated chondrocytes, guided stem cells) and growth factors (e.g., TGF-β3, BMP-2), the material environment is highly influential in the formation of tissue, as well as the delivery of chondrogenic factors.

Decellularized cartilage ECM scaffolds have garnered much recent attention, as they present cues from the native tissue itself that promote cellular differentiation (Benders et al. 2013) and production of cartilaginous ECM components. Increasing production of these ECM components, mainly type II collagen and proteoglycans, would improve the biphasic mechanical properties of the repair tissue, enabling it to absorb stresses more effectively. Chitosan (Lahiji et al. 2000; Nettles et al. 2002) and hyaluronic acid (Antoine et al. 2014; Bian et al. 2013) may similarly improve chondrogenesis, supporting aggrecan and collagen II production. However, these approaches have not made it to the clinic, and thus require further preclinical evaluation and improvement over current standards of care (microfracture, ACI).

To enhance the regeneration and rehabilitation of these tissues, biomaterials can be used facilitate the chondrogenesis of encapsulated or recruited cells. First, microparticles or nanoparticles have been used to incorporate both cells and factors into scaffolds (Fig. 9.4a), prolonging their retention and activity during the regeneration timeline (Fig. 9.4b). One study used PLGA-based nanoparticles to slowly release kartogenin, a bioactive factor known for improving chondrogenesis, into their scaffold, driving cell behavior (Shi et al. 2016). The release of the drug was modeled for 60 days and showed prolonged drug exposure that drove type II collagen growth up to 12 weeks. Other studies have corroborated these findings by using similar synthetic nanoparticles as a drug delivery mechanism for various bioactive factors to prevent degeneration of cartilage tissue (Pi et al. 2015; Rothenfluh et al. 2008). Since the clinical cartilage repair setting typically takes place in an environment with greater inflammation (due to injury or surgical procedure), successful cartilage regeneration is not often achieved as the inflammation leads to catabolic activities that prevent deposition of cartilage ECM. Matrix metalloproteinase (MMP)-degradable systems of drug delivery would be a highly effective route to combat this, since these systems can quell inflammation within the regenerating tissue, allowing chondrogenesis to occur (Lan et al. 2020). By enhancing the regeneration of new cartilage tissue, whether by adding chondrogenic factors or preventing tissue catabolism/inflammation, tissue properties and mechanics can be maximized, limiting long-term deterioration and improving rehabilitation.

In order for these biomaterial strategies to be effective during and beyond the rehabilitation process, they must integrate well with the surrounding tissues. The inability to do so can result in tissue failure, symptomatic discomfort and irritation, and pain for patients. To ensure that the newly regenerated tissue integrates with the native extracellular matrix, researchers have investigated biomaterials that bind to native tissue, encourage cell migration to and from the native tissue, or promote biological responses that cause cells to interact with another. Composite multilayered grafts have shown excellent integration with the subchondral bone (for both articular and fibrocartilage), but relatively low integration of the cartilaginous layers (J. Gao et al. 2001; Miot et al. 2012; Schaefer et al. 2002). Enzymes have been incorporated into various scaffold-based approaches, loosening the surrounding matrix to allow cells to remodel the interface zone and improve integration. Most notably, in vitro culture of core-ring constructs, of both articular cartilage and meniscus tissue, in the presence of enzymes has resulted in improved interfacial

matrix quality and integration strength (F. Qu et al. 2017; Zanotto et al. 2019). This improved integration reduces shear stress and stress concentrations at these interfacial zones, lowering the risk of re-injury and enhancing the success rate and efficacy of the biomaterial approach.

9.4.2.4 Rehabilitation

The rehabilitation timeline for cartilage injuries is also especially crucial to the selection of biomaterial type. Specifically, cartilage and meniscal injuries that do not require surgery are often followed by one to three months of rest, ice, and anti-inflammatories (Simon and Jackson 2018). More severe injuries requiring surgery generally take up to three months of restricted weight bearing, and subsequent rehabilitation focused on range of motion (Giuliani et al. 2011). Fibrocartilage spinal injuries of the IVD have varying timelines for rehab that can range from weeks to months (Vangelder et al. 2013). Articular cartilage injuries have extremely long recovery times divided into distinct rehabilitation phases. The first phase allows healing for the injury at four weeks post-surgery followed by partial to full weight bearing in the second phase (Reinold et al. 2006). The last two phases involve continued rehabilitation and incorporating low impact activities to high impact activities for 12–18 months post-surgery (Hambly et al. 2012; Korpershoek et al. 2020). Groups have now postulated load-bearing earlier in recovery, as dynamic compressive and/or tensile loading has been showing to improve the anabolic activity and matrix production of cells within these biomaterials. Thus, the rehabilitation process itself can be used to enhance regeneration, while simultaneously preventing further degradation of the surrounding tissue through the inhibition of catabolic pathways (Leong et al. 2011). Tuning biomaterial selection and activity to account for this rehabilitation gives a timeline for functional regeneration of cartilage and fibrocartilage tissues.

9.4.3 Ligament and Tendon Biomaterials

Ligament and tendon tissues, similar in composition and behavior, are composed of fibrous, uniaxially aligned collagen bands. The dense ECM consists of a hierarchical composition in which collagen molecules aggregate to form crimped collagen fibrils, which assemble in a parallel arrangement with other fibrils to form a fascicle (Santos et al. 2017). These fascicles are arranged longitudinally alongside other fascicles, forming the ligament/tendon unit. The multi-scale fibrous and aligned nature (Fig. 9.10a) of ligaments and tendons accounts for their anisotropic properties. Due to this specific organization, ligaments exhibit remarkable tensile strength, stiffness, and viscoelasticity, all of which allow them to resist failure and reassume shape after experiencing high amounts of repetitive mechanical loading (No et al. 2020).

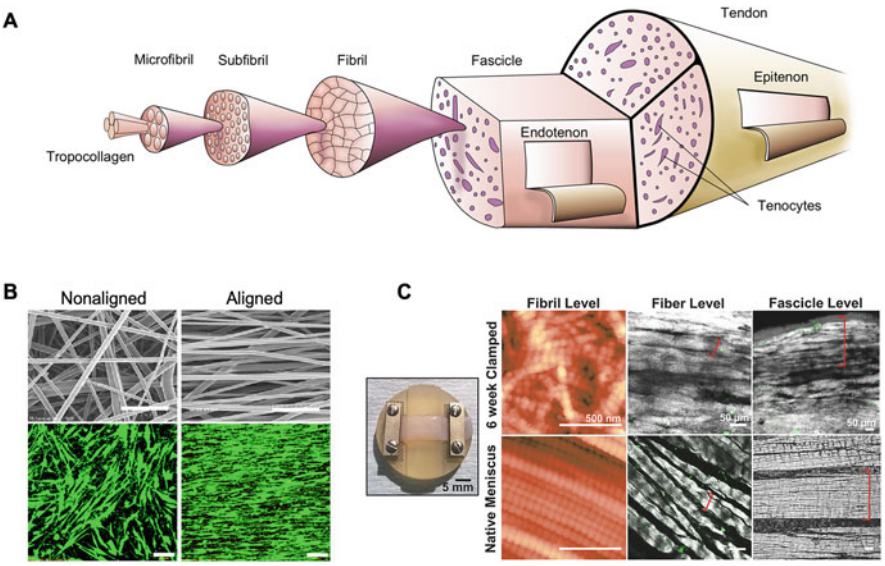

Fig. 9.10 Tendon and ligament structural reconstruction. (**a**) Tendons and ligaments have an aligned multi-scale structure, from tropocollagen molecules, to fibrils, to fascicles, to the entire tissue unit. Figure reproduced via Creative Commons License from (Lozano et al. 2019) (**b**) Scaffold alignment can be achieved via electrospinning, causing cells to align along an axis. Figure reproduced with permission from (Moffat et al. 2009) (**c**) Clamped cultures can allow multi-scale structural organization of collagen to better reconstruct tendons and ligaments. Figure reproduced via Creative Commons License from (Puetzer et al. 2021)

Treatment of injured ligament and tendon can be challenging, and often depends on the location and severity of the injury. The first mode of treatment includes rest, ice, compression, and elevation (a.k.a., RICE) for mild and/or overuse injuries, as well as the use of ibuprofen or other anti-inflammatory drugs. However, in many cases, surgical intervention is required, either suture repair of the two ends of the torn tissue or graft transplantation. Since tears are often perpendicular to the oriented structure of the tissue, suture repair often does not restore this hierarchical organization, and thus graft implantation is required. However, the harvesting of autografts can result in donor site morbidity (Santos et al. 2017), causing pain, instability, and even degeneration of the harvest site. Autograft transfers also require additional operation time and the rehabilitation of two separate injury sites. For this reason, allograft tissues have also been used as replacements, but are often expensive and not readily available (Kew et al. 2011). Moreover, there is also a small risk of disease transmission associated with implanting donor tissues, and the biomechanical properties of the graft may become compromised by the sterilization and decellularization procedures used in preparation. Adverse immune reactions, high rates of graft failure, and ineffective remodeling of the dense tissue are all concerns in using grafts for ligament and tendon replacement. Thus, biomaterials to engineer ligament and tendon tissue could improve treatment of these injuries.

9.4.3.1 Structural Considerations

The unique, fibrous structure of ligament and tendon tissue has led researchers to explore various engineering approaches to mimic the multi-scale alignment of native tissues. Nanofibrous scaffolds fabricated from a range of synthetic and naturally derived biomaterials are typically used, as the fibers are similar in size and strength to native collagen fibers (Cross et al. 2016). Furthermore, electrospinning, which is commonly used for nanofibrous scaffold fabrication, allows for control over the spatial geometry, porosity, and fiber width. Many of these techniques allow for the deposition of aligned nanofibers (Fig. 9.10b), recreating both fiber structure and organization (Kew et al. 2011; Moffat et al. 2009; G. Yang et al. 2016). Additionally, any seeded or recruited cells can respond to these aligned microenvironments, enabling cellular alignment along the oriented microstructure, eventually leading to uniaxial tissue deposition and remodeling.

Ligaments and tendons also exhibit collagen fiber crimping in the form of a sinusoidal waveform created by the arrangement of collagen fibers throughout the tissue. When the tissue is strained, crimped fibers begin to straighten with increased load; thus, extensive research has been conducted to engineer biomaterials that mimic these qualities. Recent studies using aligned nanofibrous scaffolds with the inclusion of a sacrificial fiber fraction recreated the crimped microstructure and mechanics of ACL tissue (Szczesny et al. 2017). To recapitulate this structure–function relationship, certain textile grafts have been constructed using methods such as weaving, braiding, and knitting (Kimura et al. 2008; Lu et al. 2005). Some approaches achieve the advantages of multiple methods by creating hybrid constructs; for example, one group developed a scaffold for ligament regeneration that was composed of braided bundles of electrospun PLLA nanofibers (Narayanan et al. 2016). The specific geometries of these structures afford the engineered tissues different properties. For example, knitted grafts have very high porosity, which promotes cell seeding and migration throughout the scaffold, and is optimal for tissue ingrowth, whereas braided grafts offer more stability but have limited porosity in comparison (Ge et al. 2006). These interwoven techniques provide further mechanical support to the injury site due to increased frictional forces between the fibers and help recreate the complex bundles of tissue seen in various ligaments like the ACL (Kimura et al. 2008; Lu et al. 2005; Merriam et al. 2015). The structural properties of ligament and tendon biomaterials are essential to their success in restoring native tissues and are an important contributor to related qualities of the biomaterial, especially mechanical properties.

9.4.3.2 Mechanical Considerations

To meet the mechanical requirements of damaged ligament and tendon tissues, researchers focus on architecture and composition. Collagen, silk, and alginate have been used as materials for ligament and tendon scaffolds (Cornwell et al.

2004; No et al. 2020), but with the significant loading that ligament and tendon tissues face, these natural polymers are often unable to provide the required tensile mechanics. Synthetic polymers, such as poly(lactic-co-glycolic acid), polyglycolic acid, and poly(lactic) acid, can provide these higher mechanical properties (G. Yang et al. 2016). Recreating the aligned arrangement of nanofibers can help to recreate uniaxial tensile mechanics, at least initially, but tissue deposition and organization must be guided in order to maintain long-term tissue functionality as the initial synthetic polymer degrades. Surgical repair of ligaments and tendons often results in scar tissue formation, leading to disorganized structure and loss of mechanical function (Connizzo et al. 2013), and thus, these biomaterial strategies, and their associate rehabilitative protocols, must aim to prevent disorganized tissue formation.

Much work has been performed in inducing the hierarchical organization of collagen, even by routes like clamping ends of a scaffold during culture, which can promote the formation of aligned collagen bundles (Fig. 9.10c) (Puetzer et al. 2015, 2021). Robust fixation of scaffolds in ligament and tendon applications may similarly fix the ends of the tissue, providing an axis of stress that cells can use to remodel biomaterials and lay down new aligned tissue. Moreover, the use of highly aligned cross-linked fibers stimulates cells to deposit and organize neo-tissue formation, promoting the preservation of the properties imparted by the original structure of ligament and tendon tissues. Finally, dynamic loading of biomaterials and the cells within can be beneficial to mechanically superior tissue deposition; the magnitude and frequency of loading can be varied to tune the biological healing response (No et al. 2020). Doroski et al. found that the employment of one Hz of tensile strain over three hours in a tensile culture bioreactor induced differentiation of hMSCs toward a tendon/ligament fibroblast phenotype (Doroski et al. 2010), improving the mechanical properties of cultured constructs. These loading protocols during culture could theoretically be incorporated into the rehabilitation timeline, discussed later.

9.4.3.3 Biological Considerations

To extend the capabilities of ligament and tendon biomaterials, the biological components that contribute to regeneration and differentiation must be considered. The low cellularity of ligaments and tendons leads to its limited regenerative ability, leading researchers to augment biomaterials with exogenous cells and factors to improve tissue regeneration. Bone marrow-derived mesenchymal stromal cells (MSCs), tenocytes, and fibroblasts (Kew et al. 2011; Santos et al. 2017) are commonly utilized, as are growth factors such as fibroblastic growth factor (FGF; (Kuo et al. 2010)), connective tissue growth factor (CCN2/CTGF; (Lee et al. 2010)); and transforming growth factor-β (TGF-β; (Ohba et al. 2012)). Hydrogel systems, micro-, and nanoparticles can be tuned to control the release of these factors (No et al. 2020), and thus, combining some of these softer delivery systems with more rigid polymeric structures presents a composite approach to enhance the healing environment while providing suitable mechanical properties to avoid

rupture. In addition to exogenous cells and factors, aligned biomaterial structures provide biological adhesion sites for cells to align along (Fig. 9.10b), and may need to be combined with bioactive factors to stimulate robust and aligned tissue deposition. Finally, many ligament and tendon scaffolds have been augmented with orthobiologics, such as platelet-rich plasma or bone marrow aspirate concentrate (BMAC), as they are easily isolated at the time of surgery, and contain many of the factors that enhance healing outcomes (T. Yuan et al. 2013).

9.4.3.4 Rehabilitation

The application of ligament and tendon biomaterials has demonstrated the potential to facilitate healing following the repair or replacement of damaged tissues, mostly in preclinical models. However, although biomaterials may enhance cell growth and proliferation, engineered ligament and tendon tissues often do not entirely fulfill the biomechanical properties of native tissues, presenting a barrier to clinical implementation (T. W. Lin et al. 2004). This can lead to a high rate of re-rupture as load-bearing and activity is reintroduced. ACL injuries usually require 9–12 months of postoperative rehab in order to make a complete return to sports, while athletes with Achilles injuries take 12–24 months to achieve pre-injury level (Kruse et al. 2012; X. Yang et al. 2018). These timelines present the opportunity to couple biomaterials designed to promote neo-tissue growth with gradual loading in rehabilitation to mimic the in vitro dynamic loading studies to improve tissue formation. For example, early timepoints of physiological loading to the tissue in the rehabilitation provided increased Achilles and ACL strength after rehab concluded (Kruse et al. 2012; X. Yang et al. 2018). Furthermore, in a rabbit MCL model of ligament healing, immobilization was shown to actually upregulate catabolic activity and decrease strength (Thornton et al. 2005), so balancing re-rupture rates and catabolic activity during early loading is paramount toward ensuring proper rehabilitation. Another major challenge in the rehabilitation process of ligament and tendon injuries is failure at the interface, such as within bone tunnels following ligament reconstruction. Thus, recapitulation of the interface between tendon or ligament and other tissues, such as bone, is important, and rehabilitation protocols need to carefully balance loading to improve tendon regeneration while maintaining fixation to the surrounding tissues.

9.4.4 Adipose Biomaterials

Adipose tissue is a loose connective tissue that is often categorized into two main types: brown and white adipose tissue. White adipose tissue plays a role in metabolism, endocrine function, insulation, tissue support, and energy storage (Symonds 2012), and is thus utilized in many cellular therapies and regenerative medicine. Recently, research has focused on regenerating adipose tissue, as it can be useful in

treating patients with traumas or defects like third-degree burns, congenital defects, or tissue damage or loss resulting from surgeries. The general treatment for adipose injuries is grafting, where surgeons remove areas of extra fat in the body, called liposuction, and inject it back into the deformation site. Specifically, the gold standard treatment to injuries that damage the subcutaneous fat layer and require reconstruction is an autologous fat transfer with vascularized flaps; however, the technique can cause donor site morbidity and requires surgical and hospital time to recover (Beahm et al. 2003). Depending on the severity of the trauma and availability of autologous tissue, surgeons are required to use donor tissue, which unfortunately runs the risk of failing to integrate with the host tissue from immune rejection, allergic reactions, or even implant resorption (Patrick 2001). These weaknesses in the current techniques used to treat adipose tissue injuries have led researchers to investigate the use of varying biomaterials to replicate the structural, mechanical, and biological properties of healthy adipose tissue to improve rehabilitation.

9.4.4.1 Structural and Mechanical Considerations

The structure of adipose biomaterials is a key property that researchers are investigating to maintain the shape, volume, and organization of the regenerated tissue. Adipose tissue is known to be highly porous and have high lipid content. Biomaterial porosity has been shown to have a significant impact on the healing process and can be tuned to avoid fibrous tissue deposition (Salzmann et al. 1997), which is problematic in adipose applications. Decellularized adipose scaffolds and sponges can create this porous structure while incorporating key biological factors necessary for cell differentiation and tissue growth (Jaipan et al. 2017; Takemoto et al. 2008). Since adipose replacement is often a cosmetic feature and needs to fit a certain shape, personalized scaffolds that can fit the desired defect site can be fabricated via molding and 3D printing to customize their shape (Asmatulu and Khan 2019; Tibbitt and Anseth 2009). As a soft and deformable tissue, adipose biomaterials should similarly exhibit these properties and be able to bounce back and retain their shape. At the lower end of the mechanical property range, hydrogels can be tuned to have similar mechanical properties to adipose that allow them to be a viable biomaterial for scaffold tissue engineering (O'Halloran et al. 2018). If biomaterials are too stiff, the cells within likely generate cytoskeletal tension (Y. Yuan et al. 2016), which can lead to a state of fibrosis and lower adipogenic regeneration, further motivating softer materials. Finally, due to the ability for fat to dissipate energy (Alkhouli et al. 2013), biomaterials should strive to mimic these stress-relaxation properties, to avoid residual stresses within the tissue.

9.4.4.2 Biological Considerations

The biologic properties of adipose biomaterials can also help promote fatty regeneration and reduce immune rejection. Natural materials like collagen, albumin, and

fibronectin are a part of healthy adipose tissue and provide regenerative cues to help direct adipogenic cell behavior (Kral and Crandall 1999). Decellularized fat matrices have shown to promote improved biocompatibility, adipose-derived stem cell differentiation, and fat deposition in comparison to synthetic scaffolds (Ghiasi et al. 2016), as far as exogenous mesenchymal stem cells are able to differentiate into adipose tissue when cultured with vascular endothelial, hepatocyte, platelet-derived, and insulin-like growth factor (De Ugarte et al. 2003; Ogawa 2008). Other growth factors that can be used in conjunction with scaffolds to promote adipogenesis are biotin, pantothenate, insulin, and rosiglitazone (Xie et al. 2013).

Regenerative rehabilitation typically focuses on the regrowth of new tissue as a result of tissue damage; however, rehabilitation of adipose tissue is unique because it can also focus on reducing excess tissue growth in overweight patients. Colloidal biomaterials, biomaterials that have microscopic particles dispersed throughout, are one such option to alter the metabolism in patients. These biomaterials typically act through binding to extracellular signaling peptides or to induce intracellular signaling pathways through cellular uptake (Joyce et al. 2020; Mao et al. 2013). Specifically, these biomaterials have been studied to induce weight loss by acting extracellularly through absorbing lipase enzymes, lipid interfaces, and lipid digestion products to limit fat digestion and promote increased excretion (Joyce et al. 2019). These biomaterials could also be mixed with microscopic particles that promote adipogenesis in the future to improve adipose tissue formation in regenerative medicine; however, studies involving regeneration in colloidal biomaterials are a relatively new and expanding field. Thus, these biological augmentations can enhance the structural and mechanical attributes of biomaterials to promote rehabilitation through fat formation or limiting excess formation.

9.4.4.3 Rehabilitation

Due to the soft nature of adipose tissue, injectable biomaterials are used regularly by tissue engineers because they offer control over the composition and structure of the material, while conforming to the desired volume and shape of the defect. Furthermore, injectable materials greatly reduce the invasiveness of application over surgical implantation, improving rehabilitation post-injury. Synthetic injectables, like derivatives of polyethylene terephthalate, polylactic acid, and polyethylene glycol, take on a fluid-like initial state, filling the defect site, and harden over time (Alhadlaq et al. 2005; Shanti et al. 2008). This provides a template for tissue regeneration and support to the surrounding tissue. However, these synthetics are not bioactive and the by-products can be toxic when combined with in situ gelation (Young and Christman 2012). To remedy these complications with synthetics, injectable biopolymers have been extensively researched and used to avoid the negative immune response. The biopolymers provide specific structural components of the original tissue to provide improved integration into the surrounding tissue; however, collagen

and fibrin have shown rapid resorption after injected alone (Cho et al. 2006; Orbay et al. 2011). These rapid resorption rates do not provide long-term volumetric filling of the defect site and have led to failure. *Combinations of biopolymers and synthetics can be used in order to provide longer resorption times until new tissue fills the entire defect site.*

When fat tissue is damaged, monocytes and macrophages are recruited to the injury site, reduce adipogenesis, increase inflammation, and form fibrous scar tissue (Fig. 9.11) (Liu et al. 2020; Wynn and Vannella 2016). By limiting this response via the use of biomaterials that limit inflammation and hypertrophy, adipogenic healing can occur more quickly and fibrosis can be prevented. Moreover, fibrosis can complicate the rehabilitation protocol as it can cause pain, discomfort, and irritation of the normally compliant tissue. Adipogenesis and the prevention of scarring are especially important in patients undergoing grafting procedures for congenital defects, breast deformities from mastectomies, and burns. As a result, these biomaterials may be useful and translatable to clinical settings because they are able to help prevent further damage, abscess formation, and fibrosis from occurring and maintain the desired function of the tissue. Finally, biomaterials can not only help accelerate the regeneration of new adipose tissue, but also provide a rehabilitative process of limiting the metabolism of triglycerides and lipids to promote weight loss in patients suffering from obesity.

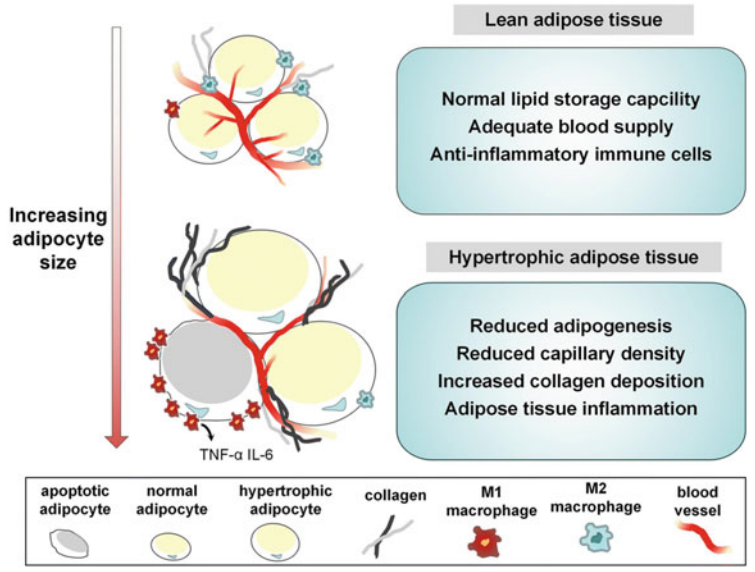

Fig. 9.11 Adipose hypertrophy and fibrosis. Adipocytes in lean adipose tissue exhibit normal lipid storage and vascularity, but hypertrophy and inflammation lead to reduced adipogenesis and collagen deposition. Figure reproduced via Creative Commons License, from (Liu et al. 2020)

9.5 Interfaces of Connective Tissues and Biomaterials

The clinical presentation of connective tissue injuries often involves multiple types of tissues (Fig. 9.12); the interfaces include but are not limited to osteochondral, enthesis (meniscus or tendon to bone), myotendinous (muscle to tendon), vertebral endplate (disc to bone), temporomandibular, and dermal epidermal. Clinicians are presented with cases in which multiple tissues are impacted and the interfaces between these tissues must be regenerated in order to restore healthy functioning. These traumas are extremely difficult to heal resulting from the varied characteristics of each tissue, and also from the unique transition zone properties between the different tissue types (Lu and Jiang 2006). In this section, we will focus on various biomaterials that promote regeneration of multiple tissue types, as well as integrate within these tissue and transition regions to restore proper motion and function of the inter-reliant tissues.

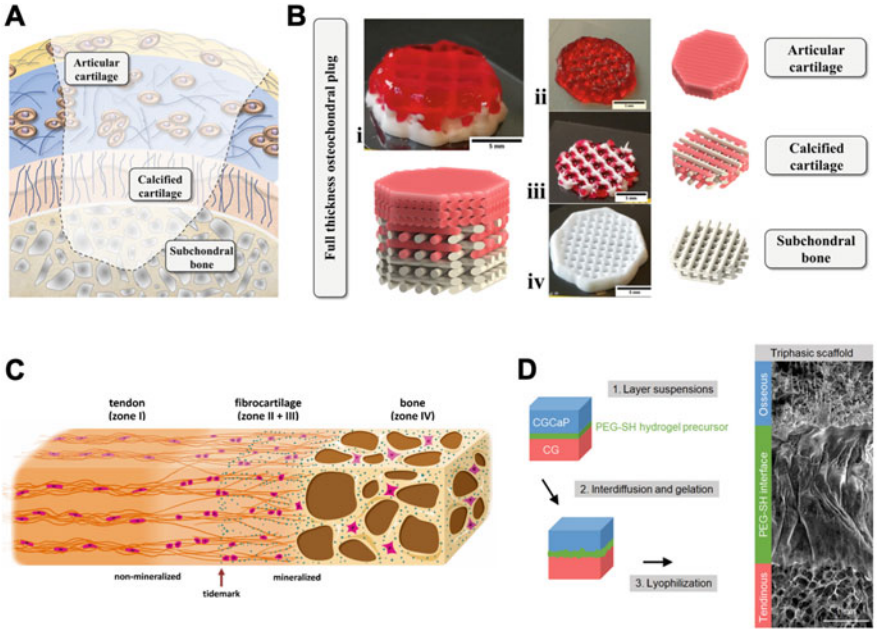

Fig. 9.12 Connective tissue interfaces. (**a**) Osteochondral interface includes both articular cartilage and the underlying subchondral bone, which can be recapitulated with (**b**) a multiphasic osteochondral scaffold. Figures **a** and **b** reproduced via the Creative Commons license, from (Kilian et al. 2020). (**c**) Tendon-to-bone interface, known as the enthesis. Figure reproduced via the Creative Commons license, from (Friese et al. 2020) (**d**) Multiphasic scaffold with osseous, tendinous, and interface zones. Figure reproduced via the Create Commons License, from (Sun Han Chang et al. 2020)

9.5.1 Osteochondral Interface

The osteochondral interface is the region encompassing articular cartilage and the underlying subchondral bone (Fig. 9.12a). These two tissue types have a significant impact on one another, especially in an injured state. Articular cartilage defects can lead to osteoarthritis development and create osteophytes due to altered loading or catabolism. The interface mechanics are quite integral to restoring the function of both tissues locally, which have vastly different mechanical, structural, and biological properties. The bulk stiffness of cartilage is in the low megapascal range, while the much stiffer subchondral bone is at a few gigapascals (Arvidson et al. 2011; P. J. Yang and Temenoff 2009). Also, bone tissue comprises collagen I and hydroxyapatite, while articular cartilage has much more collagen II and proteoglycan. Bi-layered or multiphasic scaffolds provide the ability to incorporate layers to mimic the upper cartilage region and underlying bone region. The use of two separate compartments in a bioreactor scaffold allows osteogenic cells and chondrogenic cells to be cultured in different environments helping establish a transition region from cartilage to bone (Frenkel et al. 2005; Ghosh et al. 2008; Lien et al. 2009; Wendt et al. 2005). Sharp transition between regions of scaffolds may lead to stress concentrations and high shear rates, so gradient or triphasic scaffolds have also garnered much attention to prevent these issues (Sun Han Chang et al. 2019, 2020; Xiao et al. 2019). These scaffolds provide an engineered design to recapitulate the osteochondral interface prior to implantation, or a mix of cartilage and bone properties (Fig. 9.12b). This could result in improvement of the rehabilitation process by ensuring proper formation of the neo-osteochondral interface.

9.5.2 Enthesis Interface

The enthesis interface consists of the connective tissue interface that anchors softer load-bearing tissues, such as ligament/tendon and fibrocartilages, to the rigid bone (Thomopoulos et al. 2013). The enthesis mimics cartilaginous properties such as avascularity, dense collagen fibrils, and mineralization close to the bone (Apostolakos et al. 2014; Benjamin and McGonagle 2009), and this transition zone is highly compliant, toughens under tensile loads, and anchors the soft tissue (tendon, ligament, meniscus) to bone (Deymier et al. 2017). To mimic this transition zone, researchers have used multiphasic scaffolds for the enthesis, similar to those in the osteochondral interface. Specifically, a multiphasic scaffold with PCL, tricalcium phosphate (TCP), and a PCL/TCP porous scaffold was used to mimic the highly compliant tendon, the fibrocartilage-like interface zone, and the bone (Cao et al. 2020). Other multiphasic scaffolds have been used as well; however, many biomaterials are unable to completely recapitulate the mechanics in the transition zone or still present sharp transitions between scaffold zones, leading to failure of the

overall repair approach (Caliari et al. 2015; Caliari and Harley 2014; D. Qu et al. 2015). To reduce stress concentrations between zones, gradient scaffolds can provide a gradual transition between stiffer osseus regions and softer tissue regions to better represent the gradient mechanics of the native enthesis (Seidi et al. 2011). These biomaterials ensure that there are no increased strain concentrations and thus prevent cell death or re-tears of the enthesis.

9.5.3 Myotendinous Interface

The myotendinous junction (MTJ) is the interfacial region between muscle and tendon, two vastly different tissues. Tendon is composed of dense ECM and is relatively acellular, while the muscle tissue is comparatively cellular and vascularized (Nukavarapu et al. 2015). Mechanically, muscle is more compliant than tendon and the myotendinous interface allows for force transmission between the tissues. As a result, the greatest strain is often found at the thinnest region of the entire tissue unit, which is typically the MTJ. At this interface, the muscle interdigitates the tendon to increase the contact surface area, reducing strain on the tissue. The rehabilitation of this interface is often unsuccessful due to its complex structure. Multiphasic scaffolds have shown promise in matching the mechanical properties of each tissue individually, but researchers have faced difficulty in creating an effective MTJ interface (Ladd et al. 2011; Mertens et al. 2014; Nukavarapu et al. 2015; Shandalov et al. 2014). In contrast to multiphasic scaffolds, gradient scaffolds provide a transition with a region of adequate strength and compliance that gradually transitions between the two properties (Sensini et al. 2021), making it a suitable MTJ replacement. In order to successfully improve the rehabilitation timeline for myotendinous injuries like rotator cuff tears and Achilles tendon tears, biomaterials must successfully match native mechanical and structural characteristics throughout the entire interface region, as it is a common site of re-rupture.

9.5.4 Dermal Epidermal Interface

Skin is a connective tissue that plays an important role in the human body as a protective barrier and mediator between the body and its surroundings. Its complex, multilayered structure serves to facilitate this interaction. Skin has three distinct layers: the epidermis, dermis, and hypodermis. The deepest layer of skin, the hypodermis, is highly vascularized and attached to underlying structures such as fat and muscle (Desaix 2018). Whereas skin exhibits certain self-healing mechanisms, many large defects that penetrate the dermis and hypodermis do not functionally heal without intervention. The current standard replacement therapy for skin defects is skin grafting; a split thickness skin graft (STSG) consists of an epidermal layer and a partial dermal layer, and a full thickness skin graft (FTSG) consists of

both complete layers (Bell Jr. 2020). Because these grafts do not have their own blood supply, they depend on a well-vascularized wound bed to promote vascular ingrowth of recipient tissues. However, grafts, whether autologous or allogenic, may not be relatively available. Therefore, biomaterials have been utilized to better promote the regeneration of the dermal epidermal interface. These materials should stimulate the growth of blood vessels to encourage the integration of skin with underlying connective tissues. Naturally derived scaffolds like collagen or hyaluronic acid can be modified to include amino acid sequences or growth factors that promote angiogenesis (Moon et al. 2010). Vascular endothelial cell growth factor (VEGF) is one factor that promotes vascularization of skin grafts, previously delivered via subfascial injection (Richter et al. 2006). Peptide amphiphiles (PAs), able to self-assemble into bioactive nanofibers, can develop supramolecular nanostructures that mimic VEGF (Webber et al. 2011). Heparin-binding PAs (HBPAs), when combined with heparin sulfate-like glycosaminoglycans, produce nanofiber gels that are shown to induce formation of vascularized connective tissue following subcutaneous implantation into host tissue (Ghanaati et al. 2009; Wells et al. 2013). Vascular ingrowth of grafted skin tissue is crucial to properly integrate skin grafts and to ensure the viability of the graft. Thus, materials can be used to improve regeneration and angiogenesis of the interface between skin and connective tissue, improving healing and rehabilitation following skin defects and grafting.

9.6 Conclusions

Recently, there have been major breakthroughs in the field of connective tissue biomaterials for rehabilitation. These breakthroughs have primarily led to successful products including Infuse™ bone grafts, MACI™ cartilage grafts, and the Bear ACL™. All of these products have observed some degree of clinical success, indicative of new tissue engineering strategies that provide biomimetic characteristics, whether structural, mechanical, or biological, of native tissues. However, these techniques are relatively new and require long-term investigation. The combination of these biomaterial scaffolds with various biologics may serve to enhance outcomes by accelerating the body's regenerative responses and suppressing inflammatory or degenerative processes. Future research will certainly evaluate the augmentation of acellular strategies with biologics and factors, including improved drug delivery systems, as well as research into the consistency of drug response between patients via clinical trials. Furthermore, with recent breakthroughs in cell-based therapies, such as CAR-T, the incorporation of pro-regenerative cells, such as induced pluripotent stem cells, could significantly enhance outcomes over previously acellular approaches. Other exciting advances that may revolutionize the field are novel material design and synthesis, innovative biomaterial fabrication techniques, spatial micro-scale bioprinting, and precision/personalized medicine, all of which can better recreate the structural and mechanical properties of these biomaterial strategies. Altogether, the ability to improve and accelerate tissue-specific regeneration can

expedite the required rehabilitation post-injury, and delay or prevent the need for subsequent long-term care.

Funding Department of Veterans Affairs (IK1 RX003208, IK2 RX003928), Emory Department of Orthopaedics.

References

Accardi MA, McCullen SD, Callanan A, Chung S, Cann PM, Stevens MM, Dini D (2013) Effects of fiber orientation on the frictional properties and damage of regenerative articular cartilage surfaces. Tissue Eng A 19(19–20):2300–2310. https://doi.org/10.1089/ten.tea.2012.0580

Alhadlaq A, Tang M, Mao JJ (2005) Engineered adipose tissue from human mesenchymal stem cells maintains predefined shape and dimension: implications in soft tissue augmentation and reconstruction. Tissue Eng. https://doi.org/10.1089/ten.2005.11.556

Alkhouli N, Mansfield J, Green E, Bell J, Knight B, Liversedge N, Tham JC, Welbourn R, Shore AC, Kos K, Winlove CP (2013) The mechanical properties of human adipose tissues and their relationships to the structure and composition of the extracellular matrix. Am J Physiol-Endocrinol Metabolism 305(12):E1427–E1435. https://doi.org/10.1152/ajpendo.00111.2013

Almela T, Brook I, Khoshroo K, Rasoulianboroujeni M, Fahimipour F, Tahriri M, Dashtimoghadam E, El-Awa A, Tayebi L, Moharamzadeh K (2017) Simulation of cortico-cancellous bone structure by 3D printing of bilayer calcium phosphate-based scaffolds. Bioprinting 6:1–7. https://doi.org/10.1016/j.bprint.2017.04.001

Antoine EE, Vlachos PP, Rylander MN (2014) Review of collagen i hydrogels for bioengineered tissue microenvironments: characterization of mechanics, structure, and transport. Tissue Eng Part B Rev. https://doi.org/10.1089/ten.teb.2014.0086

Apostolakos J, Durant TJS, Dwyer CR, Russell RP, Weinreb JH, Alaee F, Beitzel K, McCarthy MB, Cote MP, Mazzocca AD (2014) The enthesis: a review of the tendon-to-bone insertion. Muscles Ligaments Tendons J. https://doi.org/10.11138/mltj/2014.4.3.333

Armiento AR, Stoddart MJ, Alini M, Eglin D (2018) Biomaterials for articular cartilage tissue engineering: learning from biology. Acta Biomaterialia. https://doi.org/10.1016/j.actbio.2017.11.021

Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA (1996) Complications of iliac crest bone graft harvesting. Clin Orthop Relat Res. https://doi.org/10.1097/00003086-199608000-00037

Arvidson K, Abdallah BM, Applegate LA, Baldini N, Cenni E, Gomez-Barrena E, Granchi D, Kassem M, Konttinen YT, Mustafa K, Pioletti DP, Sillat T, Finne-Wistrand A (2011) Bone regeneration and stem cells. J Cell Mol Med. https://doi.org/10.1111/j.1582-4934.2010.01224.x

Asmatulu R, Khan WS (2019) Introduction to electrospun nanofibers. Synth Appl Electrospun Nanofibers. https://doi.org/10.1016/b978-0-12-813914-1.00001-8

Ateshian GA (2009) The role of interstitial fluid pressurization in articular cartilage lubrication. J Biomech 42(9):1163–1176. https://doi.org/10.1016/j.jbiomech.2009.04.040

Beahm EK, Walton RL, Patrick CW (2003) Progress in adipose tissue construct development. Clinics Plastic Surg. https://doi.org/10.1016/S0094-1298(03)00072-5

Bell RH Jr (2020) Split-thickness skin grafts. Northwestern Handbook of Surgical Procedures. https://doi.org/10.1201/b17659-109

Benders KEM, van Weeren PR, Badylak SF, Saris DBF, Dhert WJA, Malda J (2013) Extracellular matrix scaffolds for cartilage and bone regeneration. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2012.12.004

Benjamin M, McGonagle D (2009) Entheses: tendon and ligament attachment sites. Scand J Med Sci Sports. https://doi.org/10.1111/j.1600-0838.2009.00906.x

Bian L, Guvendiren M, Mauck RL, Burdick JA (2013) Hydrogels that mimic developmentally relevant matrix and N-cadherin interactions enhance MSC chondrogenesis. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1214100110

Bryant SJ, Anseth KS (2002) Hydrogel properties influence ECM production by chondrocytes photoencapsulated in poly(ethylene glycol) hydrogels. J Biomed Mater Res. https://doi.org/10.1002/jbm.1217

Bryant SJ, Anseth KS (2003) Controlling the spatial distribution of ECM components in degradable PEG hydrogels for tissue engineering cartilage. J Biomed Mater Res Part A. https://doi.org/10.1002/jbm.a.10319

Buckwalter JA (1998) Articular cartilage: injuries and potential for healing. J Orthop Sports Phy Ther. https://doi.org/10.2519/jospt.1998.28.4.192

Buckwalter JA (2002) Articular cartilage injuries. Clin Orthop Relat Res. https://doi.org/10.1097/00003086-200209000-00004

Burchardt H (1983) The biology of bone graft repair. Clin Orthop Relat Res. https://doi.org/10.1097/00003086-198304000-00005

Caliari SR, Harley BAC (2014) Structural and biochemical modification of a collagen scaffold to selectively enhance MSC tenogenic, chondrogenic, and osteogenic differentiation. Adv Healthc Mater. https://doi.org/10.1002/adhm.201300646

Caliari SR, Weisgerber DW, Grier WK, Mahmassani Z, Boppart MD, Harley BAC (2015) Collagen scaffolds incorporating coincident gradations of instructive structural and biochemical cues for osteotendinous junction engineering. Adv Healthc Mater. https://doi.org/10.1002/adhm.201400809

Cao Y, Yang S, Zhao D, Li Y, Cheong SS, Han D, Li Q (2020) Three-dimensional printed multiphasic scaffolds with stratified cell-laden gelatin methacrylate hydrogels for biomimetic tendon-to-bone interface engineering. J Orthop Translat. https://doi.org/10.1016/j.jot.2020.01.004

Carter DR, Beaupré GS, Giori NJ, Helms JA (1998) Mechanobiology of skeletal regeneration. Clin Orthop Relat Res. https://doi.org/10.1097/00003086-199810001-00006

Chambers HG, Chambers RC (2019) The natural history of meniscus tears. J Pediatr Orthop. https://doi.org/10.1097/BPO.0000000000001386

Chen Q, Zhu C, Thouas GA (2012) Progress and challenges in biomaterials used for bone tissue engineering: bioactive glasses and elastomeric composites. Prog Biomat. https://doi.org/10.1186/2194-0517-1-2

Cheng CW, Solorio LD, Alsberg E (2014) Decellularized tissue and cell-derived extracellular matrices as scaffolds for orthopaedic tissue engineering. Biotechnol Adv 32(2):462–484. https://doi.org/10.1016/j.biotechadv.2013.12.012

Cho SW, Kim I, Kim SH, Rhie JW, Choi CY, Kim BS (2006) Enhancement of adipose tissue formation by implantation of adipogenic-differentiated preadipocytes. Biochem Biophys Res Commun. https://doi.org/10.1016/j.bbrc.2006.04.089

Choi K, Kuhn JL, Ciarelli MJ, Goldstein SA (1990) The elastic moduli of human subchondral, trabecular, and cortical bone tissue and the size-dependency of cortical bone modulus. J Biomech. https://doi.org/10.1016/0021-9290(90)90003-L

Claes LE, Heigele CA, Neidlinger-Wilke C, Kaspar D, Seidl W, Margevicius KJ, Augat P (1998) Effects of mechanical factors on the fracture healing process. Clin Orthop Relat Res. https://doi.org/10.1097/00003086-199810001-00015

Connizzo BK, Yannascoli SM, Soslowsky LJ (2013) Structure-function relationships of postnatal tendon development: a parallel to healing. Matrix Biol. https://doi.org/10.1016/j.matbio.2013.01.007

Cornwell KG, Downing BR, Pins GD (2004) Characterizing fibroblast migration on discrete collagen threads for applications in tissue regeneration. J Biomed Mater Res Part A. https://doi.org/10.1002/jbm.a.30132

Costa JB, Park J, Jorgensen AM, Silva-Correia J, Reis RL, Oliveira JM, Atala A, Yoo JJ, Lee SJ (2020) 3D bioprinted highly elastic hybrid constructs for advanced fibrocartilaginous tissue regeneration. Chem Mater. https://doi.org/10.1021/acs.chemmater.0c03556

Crecente-Campo J, Borrajo E, Vidal A, Garcia-Fuentes M (2017) New scaffolds encapsulating TGF-β3/BMP-7 combinations driving strong chondrogenic differentiation. Eur J Pharm Biopharm 114:69–78. https://doi.org/10.1016/j.ejpb.2016.12.021

Cross LM, Thakur A, Jalili NA, Detamore M, Gaharwar AK (2016) Nanoengineered biomaterials for repair and regeneration of orthopedic tissue interfaces. Acta Biomaterialia. https://doi.org/10.1016/j.actbio.2016.06.023

Cummings NA, Nordby GL (1966) Measurement of synovial fluid pH in normal and arthritic knees. Arthritis Rheum 9(1):47–56. https://doi.org/10.1002/art.1780090106

Dado D, Levenberg S (2009) Cell-scaffold mechanical interplay within engineered tissue. Semin Cell Dev Biol. https://doi.org/10.1016/j.semcdb.2009.02.001

De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, Dragoo JL, Ashjian P, Thomas B, Benhaim P, Chen I, Fraser J, Hedrick MH (2003) Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs. https://doi.org/10.1159/000071150

Desaix P (2018) Layers of the skin – anatomy and physiology. OpenStax

Deymier AC, An Y, Boyle JJ, Schwartz AG, Birman V, Genin GM, Thomopoulos S, Barber AH (2017) Micro-mechanical properties of the tendon-to-bone attachment. Acta Biomater. https://doi.org/10.1016/j.actbio.2017.01.037

Dinoro J, Maher M, Talebian S, Jafarkhani M, Mehrali M, Orive G, Foroughi J, Lord MS, Dolatshahi-Pirouz A (2019) Sulfated polysaccharide-based scaffolds for orthopaedic tissue engineering. In: Biomaterials, vol 214. Elsevier, Amsterdam, p 119214. https://doi.org/10.1016/j.biomaterials.2019.05.025

Doroski DM, Levenston ME, Temenoff JS (2010) Cyclic tensile culture promotes fibroblastic differentiation of marrow stromal cells encapsulated in poly(ethylene glycol)-based hydrogels. Tissue Eng Part A. https://doi.org/10.1089/ten.tea.2010.0233

Dyer SM, Crotty M, Fairhall N, Magaziner J, Beaupre LA, Cameron ID, Sherrington C (2016) A critical review of the long-term disability outcomes following hip fracture. BMC Geriatrics. https://doi.org/10.1186/s12877-016-0332-0

Dzobo K, Motaung KSCM, Adesida A (2019) Recent trends in Decellularized extracellular matrix bioinks for 3D printing: An updated review. Int J Mol Sci 20(18):4628. https://doi.org/10.3390/ijms20184628

Ferreira AM, Gentile P, Chiono V, Ciardelli G (2012) Collagen for bone tissue regeneration. Acta Biomaterialia. https://doi.org/10.1016/j.actbio.2012.06.014

Finkemeier CG (2002) Bone-grafting and bone-graft substitutes. J Bone Joint Surg Series A. https://doi.org/10.2106/00004623-200203000-00020

Fischer K, Trombik M, Freystätter G, Egli A, Theiler R, Bischoff-Ferrari HA (2019) Timeline of functional recovery after hip fracture in seniors aged 65 and older: a prospective observational analysis. Osteoporos Int. https://doi.org/10.1007/s00198-019-04944-5

Frenkel SR, Bradica G, Brekke JH, Goldman SM, Ieska K, Issack P, Bong MR, Tian H, Gokhale J, Coutts RD, Kronengold RT (2005) Regeneration of articular cartilage - evaluation of osteochondral defect repair in the rabbit using multiphasic implants. Osteoarthr Cartil. https://doi.org/10.1016/j.joca.2005.04.018

Friese N, Gierschner MB, Schadzek P, Roger Y, Hoffmann A (2020) Regeneration of damaged tendon-bone junctions (Entheses)—TAK1 as a potential node factor. Int J Mol Sci 21(15). https://doi.org/10.3390/ijms21155177

Gao J, Dennis JE, Solchaga LA, Awadallah AS, Goldberg VM, Caplan AI (2001) Tissue-engineered fabrication of an osteochondral composite graft using rat bone marrow-derived mesenchymal stem cells. Tissue Eng. https://doi.org/10.1089/10763270152436427

Gao T, Zhang N, Wang Z, Wang Y, Liu Y, Ito Y, Zhang P (2015) Biodegradable microcarriers of poly(Lactide-co-Glycolide) and Nano-hydroxyapatite decorated with IGF-1 via Polydopamine coating for enhancing cell proliferation and osteogenic differentiation. Macromol Biosci. https://doi.org/10.1002/mabi.201500069

Ge Z, Yang F, Goh JCH, Ramakrishna S, Lee EH (2006) Biomaterials and scaffolds for ligament tissue engineering. J Biomed Mater Res Part A. https://doi.org/10.1002/jbm.a.30578

Ghanaati S, Webber MJ, Unger RE, Orth C, Hulvat JF, Kiehna SE, Barbeck M, Rasic A, Stupp SI, Kirkpatrick CJ (2009) Dynamic in vivo biocompatibility of angiogenic peptide amphiphile nanofibers. Biomaterials. https://doi.org/10.1016/j.biomaterials.2009.07.063

Ghiasi M, Kalhor N, Tabatabaei Qomi R, Sheykhhasan M (2016) The effects of synthetic and natural scaffolds on viability and proliferation of adipose-derived stem cells. Front Life Sci. https://doi.org/10.1080/21553769.2015.1077477

Ghodbane SA, Patel JM, Brzezinski A, Lu TM, Gatt CJ, Dunn MG (2019) Biomechanical characterization of a novel collagen-hyaluronan infused 3D-printed polymeric device for partial meniscus replacement. J Biomed Mater Res Part B Appl Biomater. https://doi.org/10.1002/jbm.b.34336

Ghosh S, Viana JC, Reis RL, Mano JF (2008) Bi-layered constructs based on poly(l-lactic acid) and starch for tissue engineering of osteochondral defects. Mater Sci Eng C. https://doi.org/10.1016/j.msec.2006.12.012

Gibson LJ (1985) The mechanical behaviour of cancellous bone. J Biomech. https://doi.org/10.1016/0021-9290(85)90287-8

Giuliani JR, Burns TC, Svoboda SJ, Cameron KL, Owens BD (2011) Treatment of meniscal injuries in young athletes. J Knee Surg. https://doi.org/10.1055/s-0031-1280877

Gullbrand SE, Ashinsky BG, Bonnevie ED, Kim DH, Engiles JB, Smith LJ, Elliott DM, Schaer TP, Smith HE, Mauck RL (2018) Long-term mechanical function and integration of an implanted tissue-engineered intervertebral disc. Sci Transl Med. https://doi.org/10.1126/scitranslmed.aau0670

Guo T, Ringel JP, Lim CG, Bracaglia LG, Noshin M, Baker HB, Powell DA, Fisher JP (2018) Three dimensional extrusion printing induces polymer molecule alignment and cell organization within engineered cartilage. J Biomed Mater Res Part A. https://doi.org/10.1002/jbm.a.36426

Hambly K, Silvers HJ, Steinwachs M (2012) Rehabilitation after articular cartilage repair of the knee in the football (soccer) player. Cartilage 3(1 Suppl):50S–56S. https://doi.org/10.1177/1947603511413569

Horner CB, Low K, Nam J (2016) Electrospun scaffolds for cartilage regeneration. Nanocomposites for Musculoskeletal Tissue Regeneration. https://doi.org/10.1016/B978-1-78242-452-9.00010-8

Hung BP, Hutton DL, Grayson WL (2013) Mechanical control of tissue-engineered bone. Stem Cell Res Ther 4(1):10. https://doi.org/10.1186/scrt158

Hutchens SA, Benson RS, Evans BR, Rawn CJ, O'Neill H (2009) A resorbable calcium-deficient hydroxyapatite hydrogel composite for osseous regeneration. Cellulose. https://doi.org/10.1007/s10570-009-9300-6

Hutmacher DW, Ng KW, Kaps C, Sittinger M, Kläring S (2003) Elastic cartilage engineering using novel scaffold architectures in combination with a biomimetic cell carrier. Biomaterials. https://doi.org/10.1016/S0142-9612(03)00350-8

Jaipan P, Nguyen A, Narayan RJ (2017) Gelatin-based hydrogels for biomedical applications. MRS Communications. https://doi.org/10.1557/mrc.2017.92

Jariwala SH, Lewis GS, Bushman ZJ, Adair JH, Donahue HJ (2015) 3D printing of personalized artificial bone scaffolds. 3D Print Addit Manuf 2(2):56–64. https://doi.org/10.1089/3dp.2015.0001

Jin H, Lewis JL (2004) Determination of Poisson's ratio of articular cartilage by indentation using different-sized indenters. J Biomech Eng. https://doi.org/10.1115/1.1688772

Joyce P, Dening TJ, Meola TR, Gustafsson H, Kovalainen M, Prestidge CA (2019) Nanostructured clay particles supplement orlistat action in inhibiting lipid digestion: An in vitro evaluation for the treatment of obesity. Eur J Pharm Sci. https://doi.org/10.1016/j.ejps.2019.05.001

Joyce P, Meola TR, Schultz HB, Prestidge CA (2020) Biomaterials that regulate fat digestion for the treatment of obesity. Trends Food Sci Technol. https://doi.org/10.1016/j.tifs.2020.04.011

Karimi M, Zangabad PS, Ghasemi A, Hamblin MR (2015) Smart external stimulus-responsive Nanocarriers for drug and gene delivery. Morgan & Claypool Publishers, San Rafael, CA. https://doi.org/10.1088/978-1-6817-4202-1

Kearney EM, Farrell E, Prendergast PJ, Campbell VA (2010) Tensile strain as a regulator of mesenchymal stem cell osteogenesis. Ann Biomed Eng. https://doi.org/10.1007/s10439-010-9979-4

Kew SJ, Gwynne JH, Enea D, Abu-Rub M, Pandit A, Zeugolis D, Brooks RA, Rushton N, Best SM, Cameron RE (2011) Regeneration and repair of tendon and ligament tissue using collagen fibre biomaterials. Acta Biomaterialia. https://doi.org/10.1016/j.actbio.2011.06.002

Kilian D, Ahlfeld T, Akkineni AR, Bernhardt A, Gelinsky M, Lode A (2020) 3D bioprinting of osteochondral tissue substitutes – in vitro-chondrogenesis in multi-layered mineralized constructs. Sci Rep 10(1):8277. https://doi.org/10.1038/s41598-020-65050-9

Kim J, Kim IS, Cho TH, Lee KB, Hwang SJ, Tae G, Noh I, Lee SH, Park Y, Sun K (2007) Bone regeneration using hyaluronic acid-based hydrogel with bone morphogenic protein-2 and human mesenchymal stem cells. Biomaterials. https://doi.org/10.1016/j.biomaterials.2006.11.050

Kimura Y, Hokugo A, Takamoto T, Tabata Y, Kurosawa H (2008) Regeneration of anterior cruciate ligament by biodegradable scaffold combined with local controlled release of basic fibroblast growth factor and collagen wrapping. Tissue Eng Part C Methods. https://doi.org/10.1089/tec.2007.0286

Kokubo T, Takadama H (2006) How useful is SBF in predicting in vivo bone bioactivity? Biomaterials. https://doi.org/10.1016/j.biomaterials.2006.01.017

Kollitz KM, Hammert WC, Vedder NB, Huang JI (2014) Metacarpal fractures: treatment and complications. Hand (New York, N.Y.) 9(1):16–23. https://doi.org/10.1007/s11552-013-9562-1

Korpershoek JV, Vonk LA, Kester EC, Creemers LB, de Windt TS, Kip MMA, Saris DBF, Custers RJH (2020) Efficacy of one-stage cartilage repair using allogeneic mesenchymal stromal cells and autologous chondron transplantation (IMPACT) compared to nonsurgical treatment for focal articular cartilage lesions of the knee: study protocol for a crossover randomized controlled trial. Trials 21(1):842. https://doi.org/10.1186/s13063-020-04771-8

Kral JG, Crandall DL (1999) Development of a human adipocyte synthetic polymer scaffold. Plast Reconstr Surg. https://doi.org/10.1097/00006534-199911000-00018

Krishnan Y, Grodzinsky A (2018) Cartilage diseases. Matrix Biol: Journal of the International Society for Matrix Biology 71–72:51–69

Kruse LM, Gray B, Wright RW (2012) Rehabilitation after anterior cruciate ligament reconstruction: a systematic review. J Bone Joint Surg Series A. https://doi.org/10.2106/JBJS.K.01246

Kuo CK, Marturano JE, Tuan RS (2010) Novel strategies in tendon and ligament tissue engineering: advanced biomaterials and regeneration motifs. BMC Sports Sci Med Rehabil. https://doi.org/10.1186/1758-2555-2-20

Lacroix D, Prendergast PJ (2002) A mechano-regulation model for tissue differentiation during fracture healing: analysis of gap size and loading. J Biomech. https://doi.org/10.1016/S0021-9290(02)00086-6

Ladd MR, Lee SJ, Stitzel JD, Atala A, Yoo JJ (2011) Co-electrospun dual scaffolding system with potential for muscle-tendon junction tissue engineering. Biomaterials. https://doi.org/10.1016/j.biomaterials.2010.10.038

Lahiji A, Sohrabi A, Hungerford DS, Frondoza CG (2000) Chitosan supports the expression of extracellular matrix proteins in human osteoblasts and chondrocytes. J Biomed Mater Res. https://doi.org/10.1002/1097-4636(20000915)51:4<586::AID-JBM6>3.0.CO;2-S

Lan Q, Lu R, Chen H, Pang Y, Xiong F, Shen C, Qin Z, Zheng L, Xu G, Zhao J (2020) MMP-13 enzyme and pH responsive theranostic nanoplatform for osteoarthritis. J Nanobiotechnol. https://doi.org/10.1186/s12951-020-00666-7

Lee CH, Shah B, Moioli EK, Mao JJ (2010) CTGF directs fibroblast differentiation from human mesenchymal stem/stromal cells and defines connective tissue healing in a rodent injury model. J Clin Investig 120(9):3340–3349. https://doi.org/10.1172/JCI43230

Lee CH, Rodeo SA, Fortier LA, Lu C, Erisken C, Mao JJ (2014) Protein-releasing polymeric scaffolds induce fibrochondrocytic differentiation of endogenous cells for knee meniscus regeneration in sheep. Sci Transl Med 6(266). https://doi.org/10.1126/scitranslmed.3009696

Leong DJ, Hardin JA, Cobelli NJ, Sun HB (2011) Mechanotransduction and cartilage integrity. Ann N Y Acad Sci. https://doi.org/10.1111/j.1749-6632.2011.06301.x

Li WJ, Jiang YJ, Tuan RS (2006) Chondrocyte phenotype in engineered fibrous matrix is regulated by fiber size. Tissue Eng. https://doi.org/10.1089/ten.2006.12.1775

Li G, Patil CG, Lad SP, Ho C, Tian W, Boakye M (2008) Effects of age and comorbidities on complication rates and adverse outcomes after lumbar laminectomy in elderly patients. Spine. https://doi.org/10.1097/BRS.0b013e3181714a44

Lien SM, Chien CH, Huang TJ (2009) A novel osteochondral scaffold of ceramic-gelatin assembly for articular cartilage repair. Mater Sci Eng C. https://doi.org/10.1016/j.msec.2008.07.017

Lin TW, Cardenas L, Soslowsky LJ (2004) Biomechanics of tendon injury and repair. J Biomech. https://doi.org/10.1016/j.jbiomech.2003.11.005

Lin K, Zhang D, Macedo MH, Cui W, Sarmento B, Shen G (2019) Advanced collagen-based biomaterials for regenerative biomedicine. Adv Funct Mater 29(3):1804943. https://doi.org/10.1002/adfm.201804943

Lin W, Kluzek M, Iuster N, Shimoni E, Kampf N, Goldberg R, Klein J (2020) Cartilage-inspired, lipid-based boundary-lubricated hydrogels. Science. https://doi.org/10.1126/science.aay8276

Liu F, He J, Wang H, Zhu D, Bi Y (2020) Adipose morphology: a critical factor in regulation of human metabolic diseases and adipose tissue dysfunction. Obes Surg 30(12):5086–5100. https://doi.org/10.1007/s11695-020-04983-6

Lozano PF, Scholze M, Babian C, Scheidt H, Vielmuth F, Waschke J, Ondruschka B, Hammer N (2019) Water-content related alterations in macro and micro scale tendon biomechanics. Sci Rep 9(1):7887. https://doi.org/10.1038/s41598-019-44306-z

Lu HH, Jiang J (2006) Interface tissue engineering and the formulation of multiple-tissue systems. Adv Biochem Eng/Biotechnol. https://doi.org/10.1007/b138509

Lu HH, Cooper JA, Manuel S, Freeman JW, Attawia MA, Ko FK, Laurencin CT (2005) Anterior cruciate ligament regeneration using braided biodegradable scaffolds: in vitro optimization studies. Biomaterials. https://doi.org/10.1016/j.biomaterials.2004.11.050

Mansfield JC, Bell JS, Winlove CP (2015) The micromechanics of the superficial zone of articular cartilage. Osteoarthr Cartil 23(10):1806–1816. https://doi.org/10.1016/j.joca.2015.05.030

Mao Z, Zhou X, Gao C (2013) Influence of structure and properties of colloidal biomaterials on cellular uptake and cell functions. Biomater Sci. https://doi.org/10.1039/c3bm00137g

McDermott AM, Herberg S, Mason DE, Collins JM, Pearson HB, Dawahare JH, Tang R, Patwa AN, Grinstaff MW, Kelly DJ, Alsberg E, Boerckel JD (2019) Recapitulating bone development through engineered mesenchymal condensations and mechanical cues for tissue regeneration. Sci Transl Med 11(495):eaav7756. https://doi.org/10.1126/scitranslmed.aav7756

Merriam AR, Patel JM, Culp BM, Gatt CJJ, Dunn MG (2015) Successful Total meniscus reconstruction using a novel fiber-reinforced scaffold: A 16- and 32-week study in an ovine model. Am J Sports Med 43(10):2528–2537. https://doi.org/10.1177/0363546515595065

Mertens JP, Sugg KB, Lee JD, Larkin LM (2014) Engineering muscle constructs for the creation of functional engineered musculoskeletal tissue. Regen Med. https://doi.org/10.2217/rme.13.81

Miot S, Brehm W, Dickinson S, Sims T, Wixmerten A, Longinotti C, Hollander AP, Mainil-Varlet P, Martin I (2012) Influence of in vitro maturation of engineered cartilage on the outcome of osteochondral repair in a goat model. Eur Cells Mater. https://doi.org/10.22203/ecm.v023a17

Moffat KL, Wang I-NE, Rodeo SA, Lu HH (2009) Orthopedic interface tissue engineering for the biological fixation of soft tissue grafts. Clin Sports Med 28(1):157–176. https://doi.org/10.1016/j.csm.2008.08.006

Mohanraj B, Duan G, Peredo A, Kim M, Tu F, Lee D, Dodge GR, Mauck R (2019) Mechanically-activated microcapsules for "on-demand" drug delivery in dynamically loaded musculoskeletal tissues. Adv Funct Mater. https://doi.org/10.1002/Adfm.201807909

Moon JJ, Saik JE, Poché RA, Leslie-Barbick JE, Lee SH, Smith AA, Dickinson ME, West JL (2010) Biomimetic hydrogels with pro-angiogenic properties. Biomaterials. https://doi.org/10.1016/j.biomaterials.2010.01.104

Moutos FT, Guilak F (2008) Composite scaffolds for cartilage tissue engineering. Biorheology. https://doi.org/10.3233/BIR-2008-0491

Narayanan G, Vernekar VN, Kuyinu EL, Laurencin CT (2016) Poly (lactic acid)-based biomaterials for orthopaedic regenerative engineering. Adv Drug Delivery Rev. https://doi.org/10.1016/j.addr.2016.04.015

Nettles DL, Elder SH, Gilbert JA (2002) Potential use of chitosan as a cell scaffold material for cartilage tissue engineering. Tissue Eng. https://doi.org/10.1089/107632702320934100

Ni S, Chang J, Chou L, Zhai W (2007) Comparison of osteoblast-like cell responses to calcium silicate and tricalcium phosphate ceramics in vitro. J Biomed Mater Res Part B Appl Biomater. https://doi.org/10.1002/jbm.b.30582

Nieminen HJ, Ylitalo T, Suuronen J-P, Rahunen K, Salmi A, Saarakkala S, Serimaa R, Hæggström E (2015) Delivering agents locally into articular cartilage by intense MHz ultrasound. Ultrasound Med Biol 41(8):2259–2265. https://doi.org/10.1016/j.ultrasmedbio.2015.03.025

Niinomi M (1998) Mechanical properties of biomedical titanium alloys. Mater Sci Eng A. https://doi.org/10.1016/s0921-5093(97)00806-x

Niinomi M (2008) Biologically and mechanically biocompatible titanium alloys. Mater Trans. https://doi.org/10.2320/matertrans.L-MRA2008828

No YJ, Castilho M, Ramaswamy Y, Zreiqat H (2020) Role of biomaterials and controlled architecture on tendon/ligament repair and regeneration. Adv Mater. https://doi.org/10.1002/adma.201904511

Nukavarapu SP, Freeman JW, Laurencin CT (2015) Regenerative engineering of musculoskeletal tissues and interfaces. Elsevier, Amsterdam. https://doi.org/10.1016/C2014-0-02826-2

O'Halloran NA, Dolan EB, Kerin MJ, Lowery AJ, Duffy GP (2018) Hydrogels in adipose tissue engineering—potential application in post-mastectomy breast regeneration. J Tissue Eng Regen Med. https://doi.org/10.1002/term.2753

Ogawa R (2008) The importance of adipose-derived stem cells and vascularized tissue regeneration in the field of tissue transplantation. Curr Stem Cell Res Ther. https://doi.org/10.2174/157488806775269043

Ohba S, Hojo H, Chung U-I (2012) Bioactive factors for tissue regeneration: state of the art. Muscles Ligaments Tendons J 2(3):193–203. https://www.ncbi.nlm.nih.gov/pubmed/23738297

Olmstead ML (1991) Complications of fractures repaired with plates and screws. Vet Clin North Am Small Anim Pract. https://doi.org/10.1016/S0195-5616(91)50078-4

Orbay H, Takami Y, Hyakusoku H, Mizuno H (2011) Acellular dermal matrix seeded with adipose-derived stem cells as a subcutaneous implant. Aesthet Plast Surg. https://doi.org/10.1007/s00266-011-9683-2

Parenteau-Bareil R, Gauvin R, Berthod F (2010) Collagen-based biomaterials for tissue engineering applications. Materials 3(3):1863–1887. https://doi.org/10.3390/ma3031863

Park SH, Tofighi A, Wang X, Strunk M, Ricketts T, Chang J, Kaplan DL (2011) Calcium phosphate combination biomaterials as human mesenchymal stem cell delivery vehicles for bone repair. J Biomed Mater Res Part B Appl Biomater. https://doi.org/10.1002/jbm.b.31805

Patel JM, Ghodbane SA, Brzezinski A, Gatt CJ, Dunn MG (2018) Tissue-engineered total meniscal replacement using a fiber-reinforced scaffold in a two-year ovine model. Am J Sports Med 46(8):1844–1856. Accepted

Patel J, Brzezinski A, Ghodbane S, Tarapore R, Lu T, Gatt C, Dunn M (2019a) Personalized fiber-reinforcement networks FOR meniscus reconstruction. J Biomech Eng 142(5):051008. https://doi.org/10.1115/1.4045402

Patel JM, Saleh KS, Burdick JA, Mauck RL (2019b) Bioactive factors for cartilage repair and regeneration: improving delivery, retention, and activity. Acta Biomater 93:222–238. https://doi.org/10.1016/j.actbio.2019.01.061

Patel JM, Saleh KS, Burdick JA, Mauck RL (2019c) Bioactive factors for cartilage repair and regeneration: improving delivery, retention, and activity. Acta Biomaterialia. https://doi.org/10.1016/j.actbio.2019.01.061

Pati F, Jang J, Ha D-H, Won Kim S, Rhie J-W, Shim J-H, Kim D-H, Cho D-W (2014) Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun 5(1):3935. https://doi.org/10.1038/ncomms4935

Patrick CW (2001) Tissue engineering strategies for adipose tissue repair. Anat Rec. https://doi.org/10.1002/ar.1113

Petersen W, Tillmann B (1998) Collagenous fibril texture of the human knee joint menisci. Anat Embryol 197(4):317–324. https://doi.org/10.1007/s004290050141

Pi Y, Zhang X, Shao Z, Zhao F, Hu X, Ao Y (2015) Intra-articular delivery of anti-Hif-2α siRNA by chondrocyte-homing nanoparticles to prevent cartilage degeneration in arthritic mice. Gene Ther 22:439. https://doi.org/10.1038/gt.2015.16

Pillai MM, Gopinathan J, Senthil Kumar R, Sathish Kumar G, Shanthakumari S, Sahanand KS, Bhattacharyya A, Selvakumar R (2018) Tissue engineering of human knee meniscus using functionalized and reinforced silk-polyvinyl alcohol composite three-dimensional scaffolds: understanding the in vitro and in vivo behavior. J Biomed Mater Res Part A. https://doi.org/10.1002/jbm.a.36372

Prendergast PJ, Huiskes R, Søballe K (1997) Biophysical stimuli on cells during tissue differentiation at implant interfaces. J Biomech. https://doi.org/10.1016/S0021-9290(96)00140-6

Puetzer JL, Koo E, Bonassar LJ (2015) Induction of fiber alignment and mechanical anisotropy in tissue engineered menisci with mechanical anchoring. J Biomech. https://doi.org/10.1016/j.jbiomech.2015.02.033

Puetzer JL, Ma T, Sallent I, Gelmi A, Stevens MM (2021) Driving hierarchical collagen fiber formation for functional tendon, ligament, and meniscus replacement. Biomaterials 269:120527. https://doi.org/10.1016/j.biomaterials.2020.120527

Qu D, Mosher CZ, Boushell MK, Lu HH (2015) Engineering complex Orthopaedic tissues via strategic biomimicry. Ann Biomed Eng. https://doi.org/10.1007/s10439-014-1190-6

Qu F, Holloway JL, Esterhai JL, Burdick JA, Mauck RL (2017) Programmed biomolecule delivery to enable and direct cell migration for connective tissue repair. Nat Commun. https://doi.org/10.1038/s41467-017-01955-w

Qu H, Fu H, Han Z, Sun Y (2019) Biomaterials for bone tissue engineering scaffolds: a review. RSC Adv. https://doi.org/10.1039/c9ra05214c

Reboredo JW, Weigel T, Steinert A, Rackwitz L, Rudert M, Walles H (2016) Cartilage tissue engineering: investigation of migration and differentiation of human mesenchymal stem cells on five-layered collagenous electrospun sjcaffold mimicking native cartilage structure (Adv. Healthcare Mater. 17/2016). Adv Healthc Mater. https://doi.org/10.1002/adhm.201670095

Reinold MM, Wilk KE, Macrina LC, Dugas JR, Cain EL (2006) Current concepts in the rehabilitation following articular cartilage repair procedures in the knee. J Orthop Sports Phys Ther. https://doi.org/10.2519/jospt.2006.2228

Rho JY, Ashman RB, Turner CH (1993) Young's modulus of trabecular and cortical bone material: ultrasonic and microtensile measurements. J Biomech. https://doi.org/10.1016/0021-9290(93)90042-D

Rho JY, Kuhn-Spearing L, Zioupos P (1998) Mechanical properties and the hierarchical structure of bone. Med Eng Phys. https://doi.org/10.1016/S1350-4533(98)00007-1

Richter GT, Fan CY, Ozgursoy O, McCoy J, Vural E (2006) Effect of vascular endothelial growth factor on skin graft survival in Sprague-Dawley rats. Arch Otolaryngol Head Neck Surg. https://doi.org/10.1001/archotol.132.6.637

Rosa N, Simoes R, Magalhães FD, Marques AT (2015) From mechanical stimulus to bone formation: a review. Med Eng Phys. https://doi.org/10.1016/j.medengphy.2015.05.015

Rothenfluh DA, Bermudez H, O'Neil CP, Hubbell JA (2008) Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage. Nat Mater 7:248. https://doi.org/10.1038/nmat2116

Rowland CR, Colucci LA, Guilak F (2016) Fabrication of anatomically-shaped cartilage constructs using decellularized cartilage-derived matrix scaffolds. Biomaterials 91:57–72. https://doi.org/10.1016/j.biomaterials.2016.03.012

Salzmann DL, Kleinert LB, Berman SS, Williams SK (1997) The effects of porosity on endothelialization of ePTFE implanted in subcutaneous and adipose tissue. J Biomed Mater Res. https://doi.org/10.1002/(SICI)1097-4636(19970315)34:4<463::AID-JBM7>3.0.CO;2-I

Santos ML, Rodrigues MT, Domingues RMA, Reis RL, Gomes ME (2017) Biomaterials as tendon and ligament substitutes: current developments. Studies Mechanobiol Tissue Eng Biomater. https://doi.org/10.1007/978-3-319-44785-8_17

Schaefer D, Martin I, Jundt G, Seidel J, Heberer M, Grodzinsky A, Bergin I, Vunjak-Novakovic G, Freed LE (2002) Tissue-engineered composites for the repair of large osteochondral defects. Arthritis Rheum. https://doi.org/10.1002/art.10493

Schlickewei CW, Kleinertz H, Thiesen DM, Mader K, Priemel M, Frosch K-H, Keller J (2019) Current and future concepts for the treatment of impaired fracture healing. Int J Mol Sci 20(22): 5805. https://doi.org/10.3390/ijms20225805

Seidi A, Ramalingam M, Elloumi-Hannachi I, Ostrovidov S, Khademhosseini A (2011) Gradient biomaterials for soft-to-hard interface tissue engineering. Acta Biomaterialia. https://doi.org/10.1016/j.actbio.2011.01.011

Sensini A, Massafra G, Gotti C, Zucchelli A, Cristofolini L (2021) Tissue engineering for the insertions of tendons and ligaments: An overview of electrospun biomaterials and structures. Front Bioeng Biotechnol 9:645544. https://doi.org/10.3389/fbioe.2021.645544

Shandalov Y, Egozi D, Koffler J, Dado-Rosenfeld D, Ben-Shimol D, Freiman A, Shor E, Kabala A, Levenberg S (2014) An engineered muscle flap for reconstruction of large soft tissue defects. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1402679111

Shanti RM, Janjanin S, Li WJ, Nesti LJ, Mueller MB, Tzeng MB, Tuan RS (2008) In vitro adipose tissue engineering using an electrospun nanofibrous scaffold. Ann Plast Surg. https://doi.org/10.1097/SAP.0b013e31816d9579

Sheikh Z, Hamdan N, Ikeda Y, Grynpas M, Ganss B, Glogauer M (2017) Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: a review. Biomaters Res. https://doi.org/10.1186/s40824-017-0095-5

Shi D, Xu X, Ye Y, Song K, Cheng Y, Di J, Hu Q, Li J, Ju H, Jiang Q, Gu Z (2016) Photo-cross-linked scaffold with kartogenin-encapsulated nanoparticles for cartilage regeneration. ACS Nano. https://doi.org/10.1021/acsnano.5b06663

Shimomura K, Rothrauff BB, Tuan RS (2017) Region-specific effect of the Decellularized meniscus extracellular matrix on mesenchymal stem cell-based meniscus tissue engineering. Am J Sports Med. https://doi.org/10.1177/0363546516674184

Simon TM, Jackson DW (2018) Articular cartilage: injury pathways and treatment options. Sports Med Arthrosc Rev. https://doi.org/10.1097/JSA.0000000000000182

Skylar-Scott MA, Uzel SGM, Nam LL, Ahrens JH, Truby RL, Damaraju S, Lewis JA (2019) Biomanufacturing of organ-specific tissues with high cellular density and embedded vascular channels. Sci Adv 5(9):eaaw2459. https://doi.org/10.1126/sciadv.aaw2459

Somford MP, van den Bekerom MPJ, Kloen P (2013) Operative treatment for femoral shaft nonunions, a systematic review of the literature. Strategies Trauma Limb Reconstr 8(2): 77–88. https://doi.org/10.1007/s11751-013-0168-5

Sophia Fox AJ, Bedi A, Rodeo SA (2009) The basic science of articular cartilage: structure, composition, and function. Sports Health 1(6):461–468. https://doi.org/10.1177/1941738109350438

Stevens MM (2008) Biomaterials for bone tissue engineering. Materials Today 11(5):18–25. https://doi.org/10.1016/S1369-7021(08)70086-5

Sun Han Chang RA, Lee CW, Rogers S, Harley BAC (2019) Biomimetic triphasic scaffolds for osteotendinous junction regeneration. In: Transactions of the annual meeting of the society for biomaterials and the annual international biomaterials symposium. Society for Biomaterials, Seattle

Sun Han Chang RA, Kersh ME, Harley BAC (2020) Tough and tunable scaffold-hydrogel composite biomaterial for soft-to-hard musculoskeletal tissue interfaces. bioRxiv. https://doi.org/10.1101/2020.01.22.915850

Sun B, Lian M, Han Y, Mo X, Jiang W, Qiao Z, Dai K (2021) A 3D-bioprinted dual growth factor-releasing intervertebral disc scaffold induces nucleus pulposus and annulus fibrosus reconstruction. Bioact Mater 6(1):179–190. https://doi.org/10.1016/j.bioactmat.2020.06.022

Symonds ME (2012) Adipose tissue biology. Adipose Tissue Biol. https://doi.org/10.1007/978-1-4614-0965-6

Szczesny SE, Driscoll TP, Tseng HY, Liu PC, Heo SJ, Mauck RL, Chao PHG (2017) Crimped Nanofibrous biomaterials mimic microstructure and mechanics of native tissue and Alter strain transfer to cells. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.6b00646

Szojka A, Lalh K, Andrews SHJ, Jomha NM, Osswald M, Adesida AB (2017) Biomimetic 3D printed scaffolds for meniscus tissue engineering. Bioprinting 8:1–7. https://doi.org/10.1016/j.bprint.2017.08.001

Takemoto S, Morimoto N, Kimura Y, Taira T, Kitagawa T, Tomihata K, Tabata Y, Suzuki S (2008) Preparation of collagen/gelatin sponge scaffold for sustained release of bFGF. Tissue Engineering - Part A. https://doi.org/10.1089/ten.tea.2007.0215

Teixeira MA, Amorim MTP, Felgueiras HP (2020) Poly(vinyl alcohol)-based Nanofibrous electrospun scaffolds for tissue engineering applications. Polymers 12(1):7. https://doi.org/10.3390/polym12010007

Thomopoulos S, Birman V, Genin GM (2013) Structural interfaces and attachments in biology. Struct Interfaces Attach Biol. https://doi.org/10.1007/978-1-4614-3317-0

Thompson WR, Gottardi R, Stearns KM, Rubin J, Ambrosio F, Tuan RS (2014) Biologics in cartilage, bone repair, and regeneration. In: Applications of regenerative medicine to orthopaedic physical therapy. https://doi.org/10.17832/isc.2014.23.2.5

Thornton GM, Johnson JC, Maser RV, Marchuk LL, Shrive NG, Frank CB (2005) Strength of medial structures of the knee joint are decreased by isolated injury to the medial collateral ligament and subsequent joint immobilization. J Orthop Res 23(5):1191–1198. https://doi.org/10.1016/j.orthres.2005.03.002

Tibbitt MW, Anseth KS (2009) Hydrogels as extracellular matrix mimics for 3D cell culture. Biotechnol Bioeng. https://doi.org/10.1002/bit.22361

Tiwari S, Patil R, Bahadur P (2018) Polysaccharide based scaffolds for soft tissue engineering applications. Polymers 11(1):1. https://doi.org/10.3390/polym11010001

Tsai PF, Richards K, Tatom I (2003) The association between knee temperature and pain in elders with osteoarthritis of the knee: a pilot study. J Adv Nurs 42(4):373–381. https://doi.org/10.1046/j.1365-2648.2003.02629.x

Vangelder LH, Hoogenboom BJ, Vaughn DW (2013) A phased rehabilitation protocol for athletes with lumbar intervertebral disc herniation. Int J Sports Phys Ther 8(4):482–516

Viguet-Carrin S, Garnero P, Delmas PD (2006) The role of collagen in bone strength. Osteoporos Int. https://doi.org/10.1007/s00198-005-2035-9

Wall A, Board T (2014) The compressive behavior of bone as a two-phase porous structure. In: Classic papers in orthopaedics. Springer, London. https://doi.org/10.1007/978-1-4471-5451-8_116

Wang H, Yi J, Mukherjee S, Banerjee P, Zhou S (2014) Magnetic/NIR-thermally responsive hybrid nanogels for optical temperature sensing, tumor cell imaging and triggered drug release. Nanoscale 6(21):13001–13011. https://doi.org/10.1039/C4NR03748K

Watkins J (2014) Biomechanics of musculoskeletal adaptation. Comprehensive Biomed Phys. https://doi.org/10.1016/B978-0-444-53632-7.01002-9

Webber MJ, Tongers J, Newcomb CJ, Marquardt KT, Bauersachs J, Losordo DW, Stupp SI (2011) Supramolecular nanostructures that mimic VEGF as a strategy for ischemic tissue repair. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1016546108

Wells LA, Valic MS, Lisovsky A, Sefton MV (2013) Angiogenic biomaterials to promote tissue vascularization and integration. Isr J Chem. https://doi.org/10.1002/ijch.201300053

Wendt D, Jakob M, Martin I (2005) Bioreactor-based engineering of osteochondral grafts: from model systems to tissue manufacturing. J Biosci Bioeng. https://doi.org/10.1263/jbb.100.489

Wu D, Isaksson P, Ferguson SJ, Persson C (2018) Young's modulus of trabecular bone at the tissue level: a review. Acta Biomaterialia. https://doi.org/10.1016/j.actbio.2018.08.001

Wynn TA, Vannella KM (2016) Macrophages in tissue repair, regeneration, and fibrosis. Immunity. https://doi.org/10.1016/j.immuni.2016.02.015

Xiao H, Huang W, Xiong K, Ruan S, Yuan C, Mo G, Tian R, Zhou S, She R, Ye P, Liu B, Deng J (2019) Osteochondral repair using scaffolds with gradient pore sizes constructed with silk fibroin, chitosan, and nano-hydroxyapatite. Int J Nanomedicine 14:2011–2027. https://doi.org/10.2147/IJN.S191627

Xie L, Zhang N, Marsano A, Vunjak-Novakovic G, Zhang Y, Lopez MJ (2013) In vitro mesenchymal trilineage differentiation and extracellular matrix production by adipose and bone marrow derived adult equine multipotent stromal cells on a collagen scaffold. Stem Cell Rev Rep. https://doi.org/10.1007/s12015-013-9456-1

Yang PJ, Temenoff JS (2009) Engineering orthopedic tissue interfaces. Tissue Eng Part B Rev. https://doi.org/10.1089/ten.teb.2008.0371

Yang G, Lin H, Rothrauff BB, Yu S, Tuan RS (2016) Multilayered polycaprolactone/gelatin fiber-hydrogel composite for tendon tissue engineering. Acta Biomater. https://doi.org/10.1016/j.actbio.2016.03.004

Yang X, Meng H, Quan Q, Peng J, Lu S, Wang A (2018) Management of acute Achilles tendon ruptures. Bone Joint Res. https://doi.org/10.1302/2046-3758.710.BJR-2018-0004.R2

Young DA, Christman KL (2012) Injectable biomaterials for adipose tissue engineering. Biomed Mater 7(2):024104. https://doi.org/10.1088/1748-6041/7/2/024104

Yuan T, Zhang CQ, Wang JHC (2013) Augmenting tendon and ligament repair with platelet-rich plasma (PRP). Muscles Ligaments Tendons J 3:139–149. https://doi.org/10.11138/mltj/2013.3.3.139

Yuan Y, Gao J, Ogawa R (2016) Mechanobiology and Mechanotherapy of adipose tissue-effect of mechanical force on fat tissue engineering. Plast Reconstr Surg Glob Open 3(12):e578–e578. https://doi.org/10.1097/GOX.0000000000000564

Zaffe D (2005) Some considerations on biomaterials and bone. Micron 36:583–592. https://doi.org/10.1016/j.micron.2005.05.008

Zanotto G, Liebesny P, Barrett M, Zlotnick H, Grodzinsky A, Frisbie D (2019) Trypsin pre-treatment combined with growth factor functionalized self-assembling peptide hydrogel improves cartilage repair in rabbit model. J Orthop Res 37(11):2307–2315. https://doi.org/10.1002/jor.24414

Zhang H, Zhou L, Zhang W (2014) Control of scaffold degradation in tissue engineering: a review. Tissue Eng Part B Rev 20(5):492–502. https://doi.org/10.1089/ten.teb.2013.0452

Zhang M, Lin R, Wang X, Xue J, Deng C, Feng C, Zhuang H, Ma J, Qin C, Wan L, Chang J, Wu C (2020) 3D printing of Haversian bone–mimicking scaffolds for multicellular delivery in bone regeneration. Sci Adv 6(12):eaaz6725. https://doi.org/10.1126/sciadv.aaz6725

Chapter 10
Ultrasound Stimulation of Tendon Healing: Current Strategies and Opportunities for Novel Therapeutic Approaches

Chitra Meduri, Eli Vlaisavljevich, P. Gunnar Brolinson, and Vincent M. Wang

Abstract Tendons are mechanosensitive tissues that are critical to musculoskeletal mobility. An in-depth understanding of the mechanisms of tendon injury and healing is crucial to the development of new therapeutic strategies for tendon healing. Tendons do not possess a robust, intrinsic healing response, and conservative and surgical treatments have shown limited efficacy. For chronic tendon injuries (tendinopathies), the principal treatment of choice is exercise-based rehabilitation, which confers improvements in clinical symptoms and function. Therapeutic Ultrasound (TUS) is commonly incorporated within physiotherapy applications and provides pain relief, likely via a thermal modality, to soft skeletal tissues. While numerous animal studies have examined the efficacy of TUS in treating acute tendon injuries, few clinical studies have examined this treatment for chronic tendinopathies. Recently, focused ultrasound (FUS) methods have shown great promise for noninvasive tissue ablation and stimulation of tissue healing but have been minimally explored for musculoskeletal ailments. Precise and customizable therapeutic FUS methods offer the potential to achieve effective, functional tissue healing via thermal and/or mechanical stimulation pathways. This chapter explores the potential of FUS therapies as customizable, noninvasive treatment options for tendon injuries and offers insights into the current state and potential advancements of ultrasound stimulation for tendon healing.

Keywords Tendinopathy · Rehabilitation · Mechanotherapy · Therapeutic ultrasound · Focused ultrasound · Acoustic parameters

C. Meduri · E. Vlaisavljevich · V. M. Wang (✉)
Department of Biomedical Engineering and Mechanics, Virginia Tech, Blacksburg, VA, USA
e-mail: vmwang@vt.edu

P. G. Brolinson
Edward Via College of Osteopathic Medicine, Blacksburg, VA, USA

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_10

10.1 Tendon: Anatomy, Injury, and Healing

10.1.1 Function and Anatomy

Tendons are dense, fibrous connective tissues that link muscle to bone and are critical to the overall function of the musculoskeletal system. While the primary function of tendons is to efficiently transmit tensile forces from muscle to bone, they are also subjected to compression and shear forces. Mature tendon is characterized by its hierarchical structure, and its mechanical function is dependent upon the biochemical composition and organization of its extracellular matrix (ECM) (Voleti et al. 2012; Screen et al. 2015; Snedeker and Foolen 2017). Tendon ECM is primarily composed of fibrillar collagens, proteoglycans, glycosaminoglycans, glycoproteins, and elastin (Sharma and Maffulli 2006) (Fig. 10.1). Collagen is the primary constituent of the tendon ECM, accounting for 60 to 85% of the dry weight of the tissue (Screen et al. 2015). Type I collagen is the dominant structural component, constituting nearly 95% of the total collagen content (Screen et al. 2015), while collagen types III, V, XI, XII, and XIV are present in much smaller proportions (Benjamin et al. 2008; Screen et al. 2015). The typical, highly organized collagen matrix can become disrupted in chronic injuries such as tendinopathy,

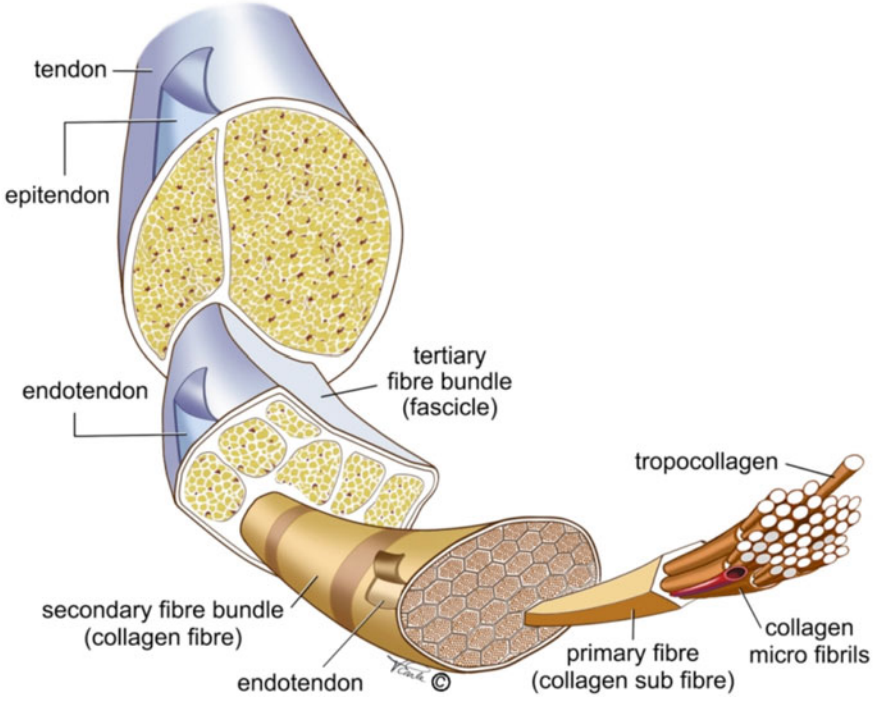

Fig. 10.1 Tendon hierarchical structure (reproduced with permission from Marqueti et al. (2019))

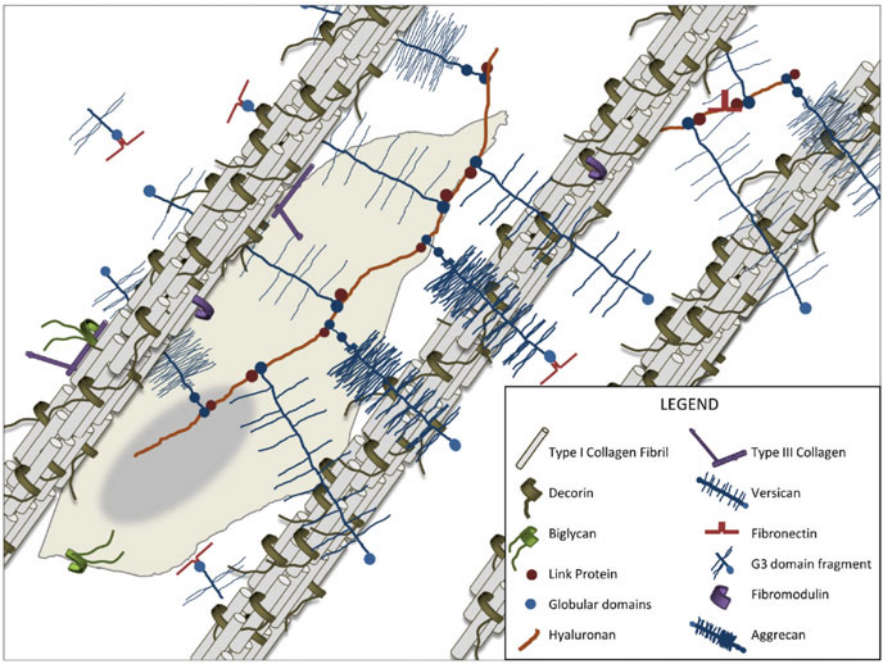

Fig. 10.2 Interactions of proteoglycans and other matrix macromolecules within the extracellular matrix of the tendon proper (reproduced with permission from Parkinson et al. (2011))

while the presence of collagen III, which typically amounts to 3–5% of the total collagen in a healthy tendon, may be elevated in tendinopathic tissue (Snedeker and Foolen 2017). Non-collagenous ECM components include: proteoglycans (whose hydrophilic nature allows for rapid diffusion of water-soluble molecules and cell migration), glycoproteins such as fibronectin (which contributes to tendon repair and regeneration processes), and elastic proteins such as Tenascin-C (which is associated with collagen fiber alignment and orientation, and is upregulated by mechanical strain) (Mackie and Ramsey 1996; Mehr et al. 2000) (Fig. 10.2).

Tendon cell populations are heterogeneous and contribute to ECM dynamics and homeostasis (Costa-Almeida et al. 2019; Zhang et al. 2019). Tenoytes, also referred to as resident cells, are fibroblast-like cells that are mainly responsible for ECM turnover, collagen production and assembly. They are arranged in longitudinal rows proximal to the collagen fibrils (Benjamin et al. 2008). Additionally, tendon stem/progenitor cells (TPSCs) replenish tendon cells by undergoing self-renewal and differentiation (Bi et al. 2007; Zhang and Wang 2010). Other cell populations include endothelial and synovial cells of the tendon sheaths as well as chondrocytes which are present within tendon-bone insertion sites and in tendon regions subjected to compressive forces during physiologic loading (Benjamin and Ralphs 1998; Kannus 2000; Zelzer et al. 2014). Tenocytes are *mechanosensitive*, and upon experiencing mechanical load, they stretch along the collagen fibrils longitudinally,

signaling collagen production. By modulating their alignment and signaling in accordance with their mechanical environment they alter ECM composition and structure (Humphrey et al. 2014; Muller et al. 2015; Popov et al. 2015). While little is known regarding the optimal loading conditions that may positively influence tendon healing, understanding how biophysical stimulation influences healing is critical to developing therapeutic treatments to restore pre-injury function.

10.1.2 Tendon Injuries and Healing

As tendons are frequently subjected to continuous or intermittent high magnitude forces, these tissues are prone to both acute and chronic injuries. Such injuries are debilitating, and are associated with ineffective healing, long-term pain, and loss of function (Nourissat et al. 2015). The type, severity, and prevalence of tendon injuries are dependent on multiple factors such as age, sex, activity levels, and genetic disposition (Sharma and Maffulli 2006; Thomopoulos et al. 2015). While tendon injuries are predominant in the elderly and athletic populations, they are becoming increasingly prevalent in the general population, due to increasing life expectancy, manual labor, and the popularity of strenuous loading activities such as exercise. Worldwide, more than half of the sport-related injuries involve tendons, and tendon damage is the most common orthopedic soft tissue injury (Walden et al. 2017). Apart from being highly prone to injury, tendons generally have a poor intrinsic capacity for healing, although the latter is dependent on the anatomic location and local environment (Thomopoulos et al. 2015). Intrasynovial tendon injuries do not exhibit spontaneous healing, while extrasynovial tendon injuries often result in fibrous tissue formation owing to a robust, scar-mediated healing response post-injury (Shen et al. 2021). Following its intrinsic repair response, tendon exhibits material properties which are inferior to those of native, uninjured tissue (Muller et al. 2015; Nourissat et al. 2015). Surgical and conservative medical interventions have limited, short-term efficacy and thus, there is significant motivation for the development of alternative approaches to improve tendon healing. Tendon injuries can be broadly categorized as acute or chronic, the former being the result of a "macro-trauma" such as a sudden mechanical overload, leading to a partial or complete rupture of the tendon. Chronic injuries typically present with an absence of inflammatory cells due to multiple stressors, including metabolic, biomechanical, genetic, and hypoxic factors; the latter may induce cellular responses that lead to disruption of matrix organization, loss of tissue material properties, and disrupted cell-matrix mechanotransduction (Nourissat et al. 2015; Sayegh et al. 2015). The etiology of chronic tendon disorders is multifaceted, and the subsequent degenerative pathway is triggered by dysregulated cell-cell and cell-matrix communication.

A primary etiologic factor in *tendinopathies* is repeated mechanical loading which exceeds the tendon's ability to heal (Steinmann et al. 2020; Millar et al. 2021). Although a biomechanical loading event individually may be of a magnitude within physiological limits, cumulative microtrauma from repetitive loading often

leads to localized collagen fiber damage (Herod and Veres 2018; Leek et al. 2020; Steinmann et al. 2020). When subjected to these stresses, a tendon may experience either inflammation of its sheath or degeneration of its body, or a combination of both (Sharma and Maffulli 2006). Recent evidence suggests the role of an inflammatory response in mediating tendon pathophysiology (Millar et al. 2017; Sunwoo et al. 2020; Millar et al. 2021). Specifically, various immune cell types (mast cells, macrophages, T cells) and inflammatory cytokines (interleukin (IL)-6, IL-5, IL-17, IL-18, IL-33, tumor necrosis factor alpha (TNF-α)) have been identified to play a critical role in the initiation and progression (early stages) of chronic tendon injuries (Millar et al. 2010; Garcia-Melchor et al. 2021). Interactions between resident and infiltrating immune cells and resident tenocytes are important in directing the inflammatory response phase of tendon healing, via secretion of cytokines and chemokines that regulate extracellular matrix remodeling. Pro-inflammatory cytokines (including members of the IL- and TNF-families) have been implicated in tendinopathy and are associated with immune cell recruitment, increased collagen type III and reduced collagen type I production, and reduced tendon biomechanical strength (Lin et al. 2006; Legerlotz et al. 2012; Dakin et al. 2014; Millar et al. 2017). Very recently, Garcia-Melchor et al. (2021) reported that tenocytes upregulate the genes involved in inflammation and T cell recruitment in vitro. T cell–tenocyte interactions, in turn, resulted in the upregulation of inflammatory cytokine expression and an increased expression of collagen III. It has been proposed that this autoregulated feedback loop plays a key role in chronicity and long-term complications of tendinopathy.

Largely due to the challenges presented in studying human tendinopathy, including the difficulty in identifying the onset of the disease as well as in procuring injured tissues at different stages of the post-injury response (Dirks and Warden 2011; Hast et al. 2014), our understanding of the precise mechanisms of tendon injury and healing remains incomplete. Tendon healing has primarily been studied using animal models of acute tendon injury (e.g., transection) or other experimentally induced tendon damage/injury models (Sharma and Maffulli 2005; Docheva et al. 2015). Tendon healing consists of sequential and overlapping phases (Docheva et al. 2015; Nourissat et al. 2015) including *inflammation, cell proliferation, migration, and remodeling* (Voleti et al. 2012; Docheva et al. 2015); however, it is common for incomplete healing to result in fibrovascular scar tissue which does not recapitulate native composition and material properties (Nourissat et al. 2015).

In order to study mechanisms of mechanical "overload" on the development of tendinopathy, researchers have developed a variety of preclinical approaches (Thomopoulos et al. 2015; Theodossiou and Schiele 2019). Some of these methods include uphill and downhill treadmill running in rats or mice (Heinemeier et al. 2012; Pingel et al. 2013; Zhang et al. 2020) and application of controlled, in vivo fatigue loading to rat or mouse tendons (Fung et al. 2010; Andarawis-Puri et al. 2012; Sereysky et al. 2012). Furthermore, biochemical induction of tendon injury has been studied using collagenase injections in various models including rabbits, sheep, and rats (Chen et al. 2014; Lacitignola et al. 2014; Solchaga et al. 2014; Urdzikova et al. 2014). Surgical repair following tendon transection has been

particularly useful in studying acute injury healing mechanisms (Yoshida et al. 2016; Moser et al. 2018). In vitro cell culture and tendon explant models (Goodman et al. 2004; Wunderli et al. 2020), as well as ex vivo rodent and equine tendon models, have been used to examine the effects of repetitive mechanical loading (e.g., cyclic strain or fatigue) on tendons (Arnoczky et al. 2007; Fung et al. 2009; Spiesz et al. 2015). Alteration of the tendon mechanical loading environment (e.g., by transection) can be effectively used to study biochemical responses (Maeda et al. 2011). An in vivo tendinopathy model was developed (Bell et al. 2013a) by injecting TGFβ-1 into adult mouse Achilles tendons. This injury model induces tendinopathic changes consistent with human histopathology (Bell et al. 2013a, b) and is amenable to therapeutic mechanical interventions (Bell et al. 2013a; Rezvani et al. 2021) simulating human treatments (Heinemeier et al. 2012; Dirks et al. 2013; Pingel et al. 2013; Reuther et al. 2013).

10.1.3 Mechanotherapy for Treatment of Chronic Tendon Disease

There exist several common, conservative, and surgical approaches for the treatment of chronic tendinopathies, such as rest and immobilization, anti-inflammatory drugs, growth factor injections (i.e., platelet-rich plasma), and surgical repair (Lim et al. 2019; Tsai et al. 2021) (Table 10.1). However, there is limited evidence of their long-term efficacy (Maffulli et al. 2010; Cardoso et al. 2019). The molecular mechanisms of disease initiation and progression, as well as the reasons for a failed healing response in lieu of restoration of tissue, are not well understood (Tsai et al. 2021). However, it is hypothesized that dysregulated and/or missing cues underlie the deficient healing response of a tendon; hence, a detailed understanding of such cues and mechanisms will greatly assist in the identification and design of novel therapeutic strategies to augment existing strategies to heal tendons. Rehabilitation protocols aim to robustly repair injured tissues in a manner that reduces their risk for reinjury (Gray and Brolinson 2001). This strategy involves the design of therapeutic modalities and rehabilitative exercises that address the type of injury (acute vs. chronic), symptoms, and tissue performance, via an in-depth understanding of tissue biomechanics and pathophysiology of injury (Gray and Brolinson 2001). Rehabilitation protocols generally utilize "*mechanotherapy*" to induce adaptation of the musculoskeletal tissues to mechanical forces and/or strain by directing cellular and molecular responses to achieve healing and/or regeneration. Identifying optimal mechanical loading regimes defined by transcriptional, molecular, and cellular responses is crucial in designing strategies for tendon repair and healing. For example, eccentric exercise (lengthening of the muscle and tendon while under load) is commonly used as a therapeutic modality to manage tendinopathy. These exercises have been shown to improve tendon structure and mechanical properties with corresponding improvements in clinical outcomes (Mafi et al. 2001; Fahlstrom

Table 10.1 Advantages and limitations of existing treatments for chronic tendon injuries

	Method	Advantages	Limitations
1	Topical/systemic anti-inflammatory drugs for pain relief	Effectively relieve pain and inflammation short-term; accessible and inexpensive	Insufficient evidence to support use in chronic injuries; may negatively alter natural tendon healing process; long-term use may cause adverse renal and gastrointestinal effects
2	Exercise-based rehabilitation	Principal treatment of choice across all tendinopathies; may tendon structural and biomechanical properties with corresponding improvements in clinical outcomes	Precise mechanisms of action are unknown; often require long periods of rehabilitation; customizing loading protocols to specific degrees of pathology is challenging; chronic pain may deter participation; requires patient compliance with protocols
3	Growth factor, i.e., platelet-rich plasma (PRP) treatment	Inexpensive; ease of administration (injections); low risk with autologous treatment; potentially beneficial in combination with therapeutic exercise	Variable and conflicting outcomes, potentially due to variability among PRP components
4	Therapeutic Ultrasound (TUS)	Widely accessible, noninvasive, painless	Few existing clinical trial data; conflicting data from preclinical studies of efficacy for treatment of acute injuries; no data on efficacy for chronic injuries
5	Low energy laser therapy (LLLT)	Evidence of reduction in inflammatory markers; provides pain relief when used in conjunction with exercise	Unclear mechanism of action; lack of standardized/reliable protocols (parameters) for administration; lack of homogeneous efficacy data
6	Extracorporeal Shockwave Therapy (ESWT)	Ease of administration; proven efficacy for treatment of specific types of tendinopathy	Unclear mechanism of action, lack of optimized treatment parameters for different types of tendon injuries

et al. 2003; Yu et al. 2013) and have emerged as the most efficacious therapy across numerous tendinopathies (Kingma et al. 2007; Murphy et al. 2018; Irby et al. 2020; Vander Doelen and Jelley 2020). Given that the goal of tendinopathy treatments is to restore normal tendon function, controlling the mechanical cues directed to the injured tendon can potentially promote healing via mechanotransduction mechanisms (Özer Kaya 2020). Regenerative rehabilitation approaches are, thus, central to translational tendon healing research (Gottardi and Stoddart 2018; Rando and Ambrosio 2018; Willett et al. 2020).

10.2 Therapeutic Ultrasound and Tendon Healing

10.2.1 Physical Principles, Characteristics, and Modalities

Therapeutic Ultrasound (TUS) utilizes acoustic pressures and/or intensities higher than those of diagnostic ultrasound to elicit biological responses in tissues. The ultrasound beam is directed into a specific area or a region within the tissue of interest to avoid damage to surrounding tissues. Existing TUS methods (e.g., in physiotherapy to provide deep heating to tissues such as tendons, ligaments, and skeletal muscles (Watson 2008) deliver low-intensity energy through the targeted tissue via the propagation of sound waves applied by an external source. The most common musculoskeletal application for TUS to date remains for pain and physiotherapy in conditions such as osteoarthritis-related knee pain, chronic back pain, lateral epicondylitis, and myofascial pain (Dedes et al. 2020; Gulati and Ottestad 2020; Petterson et al. 2020). Within the categorization of TUS, multiple ultrasound modalities have been developed and examined with various delivery modes, acoustic pressures, and duty cycles, to elicit different biological mechanisms in tissues (Fig. 10.3). Several examples include low-intensity pulsed ultrasound (LIPUS) (Warden et al. 2008; Hsu and Holmes 2016; Tanaka et al. 2020), low-intensity continuous ultrasound (cLIUS) (Lucchetti et al. 2020; Mittelstein et al. 2020), and pulsed focused ultrasound (pFUS) for soft tissue healing (Burks et al. 2011; Poliachik et al. 2014), nanoparticle delivery (O'Neill et al. 2009; Tharkar et al. 2019), physiotherapy for pain relief by providing deep heating to soft tissues (typically combined with physical therapy) (Brown et al. 2015; Papadopoulos and Mani 2020) and high-intensity focused ultrasound (HIFU) for thermal (tHIFU)

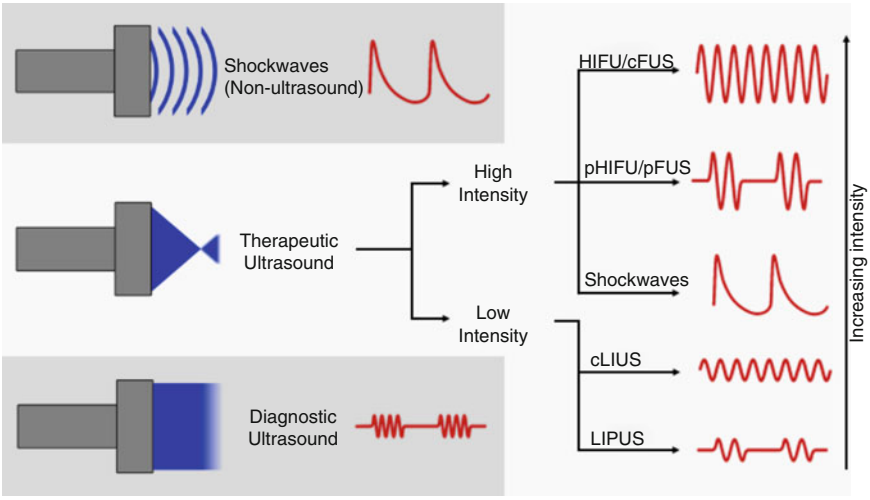

Fig. 10.3 Schematic of waveforms (different amplitudes) illustrating different modalities of TUS (Modified with permission from Liu et al. (2020))

ablation (Dubinsky et al. 2008; Vidal-Jove et al. 2015; Mauri et al. 2018) and non-thermal (histotripsy) tissue ablation (Vlaisavljevich et al. 2013; Bader et al. 2019; Xu et al. 2021).

A variety of labels have been utilized in published literature to describe different types of therapeutic ultrasound, with some overlap between commonly used terms. In general, these groups are broadly categorized based on the ultrasound intensity (high vs. low) and exposure mode (continuous vs. pulsed) (Liu et al. 2020). For clarity, in this chapter, we use the acronym *HIFU* to describe High Intensity Focused Ultrasound exposures that induce thermal and/or non-thermal irreversible changes within a short time frame (typically, it requires microseconds for direct effects during the pulse or histotripsy cavitation, milliseconds to elicit physiological responses such as in neuromodulation, to seconds or minutes for thermal changes). *FUS* refers to focused ultrasound methods (see Sect. 10.3 below), while *TUS* is used to describe low-intensity ultrasound exposures used currently for musculoskeletal physiotherapy and pain relief applications, as noted above. Different forms of TUS can be further categorized by understanding its basic physical parameters. A brief description of ultrasound parameters critical to the generated bioeffects and safety is given in Table 10.2.

Thermal bioeffects of ultrasound result from absorption of the applied ultrasonic energy. The amount of absorption and the accompanying heat generated depend upon ultrasound acoustic parameters as well as tissue properties. The primary determinants of thermal effects in tissues include tissue absorption coefficients and ultrasound exposure conditions, such as duration, intensity, beam width, and frequency. Importantly, there exists a direct relationship between the absorption capacity of a tissue and its protein content. Highly collagenous tissues such as tendon and ligament are known to absorb ultrasound energy more efficiently (Watson 2008). Other determinants of tissue temperature changes include ultrasound pulse repetition frequency and pulse duration, along with tissue characteristics such as density, acoustic impedance, and thermal conductivity (Dalecki 2004; Shankar and Pagel 2011).

Research suggests that an increase of temperature between 1 °C and 4 °C from baseline can provide therapeutic effects in tendons; however, higher elevations could potentially result in harmful effects such as thermal denaturation of collagen (Vlaisavljevich et al. 2015). An in vivo study investigated the ability of ultrasound to heat human patellar tendon and found that ultrasound frequency, intensity, duration of treatment, and size of the treatment area influenced heat production in tendon (Chan et al. 1998). The rate of temperature rise was found to be higher in the tendon compared to the adjoining muscle. Using a 3-MHz continuous ultrasound treatment lasting 4 minutes, temperature rises in the range of 8 °C to 10 °C were achieved using templates measuring two times the effective irradiation area of the transducer head. Such controlled thermal effects via TUS application are desirable to achieve pain relief, increased blood flow, decreased joint stiffness, and hyperdynamic tissue metabolism (Watson 2008; Papadopoulos and Mani 2020). Typically, physiotherapists utilize thermal effects of TUS to treat injuries such as tendonitis, joint pain, low back and neck pain, muscle strains, plantar fasciitis,

Table 10.2 Ultrasound parameters

	Parameter	Definition
1	Frequency (Hertz, Hz)	Number of US waves per second, or, number of times per second a particle experiences a complete compression and rarefaction cycle.
2	Duty Cycle (%)	The ratio of the time the transducer is "on" to the total exposure time (time "on" plus time "off")
3	Pulse Repetition Frequency (Hz)	Number of pulses transmitted per second
4	Intensity (W/cm^2)	A measure of ultrasound exposure that can be calculated based on maximum pressure measured in the field (Spatial Peak) or based on pressure averaged over a specific area (Spatial Average). When describing pulsed exposures, intensity may be applicable only while the pulse is "ON" (Pulse Average) or may be averaged over total time (Temporal Average). The most commonly reported intensity is Spatial Average Temporal Average (I_{SATA}); other reported indices include Spatial Average Pulse Average (I_{SAPA}), Spatial Average Temporal Peak (I_{SATP}), Spatial Peak Temporal Average (I_{SPTA}), and Spatial Peak Pulse Average (I_{SPPA}) (ter Haar 2007)
5	Acoustic power (Watts)	Total energy passing through a surface per unit time (measure of strength of ultrasound wave)
6	Mechanical index	Peak negative pressure of US waves divided by peak frequency; measures the likelihood of occurrence of a mechanical bioeffect due to cavitation.
7	Acoustic pressure (Pascal)	Changes in the local pressure of the medium, recorded as acoustic compression and rarefaction, typically measured as peak to peak pressure, peak negative pressure, or peak positive pressure. Defined as the difference between maximum or minimum pressure of the wave and average pressure of the medium in the absence of the wave
8	Attenuation (dB/cm/MHz)	Decrease in acoustic wave intensity per unit distance due to interactions between the wave and medium. It is usually expressed as a ratio of wave amplitudes in decibel notation, commonly per centimeter depth of tissue, per unit frequency, or at a specified frequency (e.g., dB/cm/MHz or dB/cm at 1 MHz)

ligament sprains, and arthritis pain (Brown et al. 2015; Papadopoulos and Mani 2020).

A combination of *non-thermal effects* such as acoustic streaming, acoustic cavitation, and radiation force displacement (Dalecki 2004; Izadifar et al. 2017) can also be produced by TUS or FUS application. Acoustic cavitation is the stable oscillation (inertial cavitation) or collapse (non-inertial cavitation) of a gas bubble in the presence of an acoustic field (Holland and Apfel 1990; Bader et al. 2019). Acoustic radiation force is that which results from momentum transfer from the sound field to the tissue of interest (Nightingale 2011; Urban 2018; Wang 2018). This effect itself is a consequence of radiation torque and acoustic streaming. The acoustic streaming phenomenon occurs when acoustic field propagation induces an increased rate of fluid flow (Dalecki 2004).

TUS is an appealing method to safely transfer mechanical energy to tendon tissue and elicit thermal and mechanical stimulation pathways in a prescribed manner. Although the therapeutic efficacy of TUS has been demonstrated in multiple studies, there is a substantial knowledge gap in understanding relationships between ultrasound dose (acoustic parameters) and the bioeffects elicited in these tissues. Optimal identification of TUS parameters and their correlation with molecular and tissue-level responses will largely benefit future research in ultrasound-induced tissue regeneration and rehabilitation.

10.2.2 TUS in Tendon Healing

The most widely studied form of TUS for musculoskeletal tissue repair and regeneration is low-intensity pulsed ultrasound (LIPUS), with applications in osteoporosis, fracture healing, mesenchymal stem cell recruitment and homing, and tendon–bone junction healing (Warden et al. 2008; Khanna et al. 2009; Zhang et al. 2017; Tanaka et al. 2020). Targeted application of TUS during different phases of tissue repair can produce a synergistic effect on healing (Saber and Saber 2017). During the inflammatory phase, TUS can stimulate mast cells, platelets, and macrophages, activating inflammatory mediators (Maxwell 1992; Leung et al. 2004). Efficiency of the proliferation phase of healing is also enhanced by TUS, by increasing collagen production and scar tissue formation (Zhou et al. 2004; Watson 2008). Lastly, TUS has also been shown to enhance the remodeling of scar tissue by improving collagen fiber orientation and increasing tensile strength (Nussbaum 1998; Maan et al. 2014).

The therapeutic potential of TUS, specifically low-intensity ultrasound (LIUS), in stimulating healing of acute tendon injuries has been investigated predominantly in animal studies, which enable the concurrent evaluation of tissue biomechanics and physiological responses to LIUS application (Table 10.3). Biomechanical metrics such as ultimate load, tensile strength and energy absorption, and structural metrics such as collagen organization and aggregation have been commonly characterized after applying treatments to assess the efficacy of TUS on healing (Ng et al. 2003; Yeung et al. 2006; Jeremias Junior et al. 2011). A 2016 review by Best et al. summarized the effects of LIUS on tendon, tendon–bone junction, muscle and ligament injuries (Best et al. 2016). The authors concluded that LIUS improves tendon strength and accelerates collagen formation after acute injury in preclinical models. Tensile strength and collagen expression (types I and III) were found to be greater in LIUS treated tendons compared to untreated controls (Jackson et al. 1991; da Cunha et al. 2001; Fu et al. 2008, 2010; Jeremias Junior et al. 2011; Kosaka et al. 2011). Regarding treatment time and duration (Fu et al. 2008, 2010), ultrasound treatment in the earlier (relative to later) stages of healing appears to improve tensile strength and matrix synthesis. While the available literature generally indicates that LIUS enhances biomechanical and structural properties of injured tendons, ultrasound parameters (e.g., intensity, stimulation frequency, and mode) and animal models (species, tendon of interest, and injury type) have varied across studies

Table 10.3 Results of studies investigating therapeutic ultrasound (TUS) treatment of tendon injuries

Author, Year	Species	Injury (or Injury Model)	Treatment paradigm	Key takeaways
da Cunha et al. (2001)	Rat	Achilles tenotomy	1 MHz, 0.5 W/cm^2, 5 min/day for 14 days	Improved collagen organization and aggregation when applied during early stages of healing in pulsed mode
Ng et al. (2003)	Rat	Achilles hemitransection	1 MHz, 1.0 or 2 W/cm^2, 4 min/session, for 22 sessions	Both treatment groups showed improved ultimate tensile strength compared to controls
Demir et al. (2004)	Rat	Achilles tenotomy	1 MHz, 0.5 W/cm^2, 5 min/day, 9 days	Increased tendon breaking strength following either TUS or laser therapy; treatment using combined modalities did not show additional positive effects.
Yeung et al. (2006)	Rat	Achilles tenotomy	1 MHz, 0.5 W/cm^2, 5 min/day, 3 times/week, for 2 or 4 weeks	Increased ultimate tensile strength and improved collagen bundle alignment.
Larsen et al. (2005)	Rabbit	Achilles tenotomy	3 MHz, varying intensities from 50 to 2000 mW/cm^2, 5 min/session, 10 sessions	No improvement in mechanical properties of healing tendons; mild decline in stiffness with increasing treatment intensity
Ng and Fung (2007)	Rat	Achilles tenotomy	1 MHz, varying intensities from 0.5 W/cm^2 to 2 W/cm^2 daily starting from day 5 after injury for 4 min/session for 22 sessions	Collagen fibril size increased with treatment, independent of intensity level.
Fu et al. (2008)	Rat	Patellar tendon mid-portion window defect	1.0 MHz, 30 mW/cm^2, 20 min/day, 5 days/week, for 2, 4, or 6 weeks	Beneficial effects of LIPUS (2-week treatment group) included improved ultimate tensile strength and collagen fiber alignment. 4 or 6 weeks of treatment was found to be detrimental to collagen remodeling.
Fu et al. (2010)	Rat	Central third patellar tendon removal	1.5 MHz, 150 mW/cm^2, 20 min/day for 14 or 28 days	Enhanced collagen synthesis during the granulation phase of healing.

(continued)

Table 10.3 (continued)

Author, Year	Species	Injury (or Injury Model)	Treatment paradigm	Key takeaways
Wood et al. (2010)	Rat	Achilles tendon partial rupture by direct trauma	3 MHz, 0.2 W/cm^2, 5 min/day, 5 days	Improved collagen organization; increased collagen type 1 in laser- and US-treated groups.
Jeremias Junior et al. (2011)	Rat	Achilles tenotomy	1 MHz, 0.1 W/cm^2, 5 min/day for 28 days	Increased ultimate load and tensile strength compared to controls.
Kosaka et al. (2011)	Rat	Achilles tenotomy	1.5 MHz, 45 mW/cm^2, 20 min/day	During the inflammatory phase, COX-2 and EP4 were overexpressed with LIPUS treatment, hence exaggerating inflammation. *TGF-β1*, *Col I*, and *Col III* expression levels were elevated in treated groups, encouraging tissue remodeling.
Farcic et al. (2013)	Rat	Achilles tenotomy	1 MHz, 0.5 W/cm^2, pulsed application for 1, 2, or 3 minutes per transducer area for 10 sessions	Collagen fibers showed better aggregation and organization in 3-minute treatment group
Farcic et al. (2018)	Rat	Achilles tenotomy	1 MHz, 0.5 W/cm^2, for 6 min/8 min/10 min for 10 days with 2 days of interval after fifth treatment day	10-minute treatment group showed the best collagen fiber aggregation and organization.
D'Vaz et al. (2006)	Human	Lateral epicondylitis	1.5 MHz, 30 mW/cm^2, 20 min/day for 12 weeks	Low-intensity ultrasound was not very effective compared to placebo.
Warden et al. (2008)	Human	Patellar tendinopathy	1.0 MHz, 100 mW/cm^2, 20 min/day for 12 weeks	Visual Analog Scale scores improved in both placebo and LIPUS treated groups; LIPUS provided no additional benefits over placebo.
Hsu and Holmes (2016)	Human	Achilles tendinopathy	Exogen® LIPUS device used for 20 min/day for 8 weeks over an area of maximum tenderness	Of 14 participants, 7 had excellent clinical outcomes with complete resolution of pain and other symptoms. 2 patients had good outcomes with mild tendon irritation and stiffness and 5 patients had minimal benefit with continued pain, swelling, and tenderness.

(continued)

Table 10.3 (continued)

Author, Year	Species	Injury (or Injury Model)	Treatment paradigm	Key takeaways
de Jesus et al. (2019)	Human	Patellar tendinopathy	1.0 MHz, 1.2 W/cm^2, 8 min/day, 2×/week for 8 weeks in combination with regimented exercise program	TUS enhanced the results obtained with rehabilitative exercise including pain and lower limb motor function.

COX-2 cyclooxygenase-2 enzyme, *EP4* Prostaglandin E_2 receptor 4, *TGF-β1* Transforming growth factor beta 1, *Col I* Collagen type I, *Col III* Collagen type III

(Tsai et al. 2011). In consideration of these inconsistencies, direct comparison of results is likely not warranted and researchers should exercise caution in deducing the cellular and molecular mechanisms attributable to treatments. Hence, there is not only an urgent need for standardization of TUS parameters and experimental conditions (treatment time, injury models) in future investigations, but also clinically relevant animal models whose tendon injury characteristics mirror those observed in human injuries.

Clinical studies have examined the influence of TUS on lateral epicondylitis (D'Vaz et al. 2006; Dedes et al. 2020), patellar tendinopathy (Warden et al. 2008; de Jesus et al. 2019) and Achilles tendinopathy (Hsu and Holmes 2016). A 2015 study on the short-term effectiveness of LIPUS on human Achilles tendinopathy revealed that all participants who had undergone traditional, nonsurgical treatment modalities prior to using LIPUS, showed good to excellent improvements in pain relief and function post-treatment (Hsu and Holmes 2016). In three studies that examined the influence of TUS on epicondylitis and patellar tendinopathy, a significant decrease in tendon pain was observed after daily treatments with continuous or pulsed LIUS treatment for 6 weeks (Best et al. 2016). However, two randomized controlled trials were used to assess the utility of LIPUS to treat chronic tendinopathies and reported that LIPUS provided no additional benefit to physical therapy for chronic tendinopathies (D'Vaz et al. 2006; Warden et al. 2008). Thus, while acute injury animal models demonstrate moderate effectiveness of LIUS in tendon healing, human studies specifically investigating chronic tendon injuries do not demonstrate promise as a noninvasive treatment option. Human studies investigating TUS effects on acute tendon injuries would provide further evidence to guide clinical practice, particularly if treatment paradigms across such studies were standardized, as noted above.

Extracorporeal Shockwave Therapy (ESWT) utilizes rapid, short-duration pressure waves ("shockwaves") intended to elicit physicochemical and cellular reparative responses and has shown promise in treating a variety of musculoskeletal conditions (Simplicio et al. 2020). ESWT can be regarded as high-intensity therapeutic ultrasound, since shockwaves can be generated under both continuous and pulsed modes via transducers that induce nonlinear propagation effects resulting in shock formation. Alternatively, shockwaves can also be generated by non-ultrasonic

sources such as electrohydraulic and electromagnetic systems (Simplicio et al. 2020). To date, the US FDA has only approved the use of ESWT for plantar fasciitis and lateral epicondylitis (Wang 2012). Studies have shown that ESWT acts as a mechanical stimulus and promotes healing via mechanotransduction (Moya et al. 2018; Simplicio et al. 2020) and may also alleviate pain (Hausdorf 2008). In humans, ESWT may aid tendon healing by providing mechanical stimulation to aid inflammatory and catabolic processes that are associated with damaged matrix constituents (Waugh et al. 2015). A recent study compared the effectiveness of ESWT and LIUS on pain, return to functionality, and quality of life in patients with Achilles tendinopathy (Dedes et al. 2020). Although both interventions resulted in significant improvements in pain and functionality immediately and 4 weeks post-treatment, the effects of ESWT were more pronounced compared to LIUS. Another recent, randomized controlled trial evaluated the effectiveness of point-focused (small treatment volume focused on the point of maximum pain) and line-focused (larger treatment volume with equally distributed energy density but smaller maximum pressure compared to point-focused) ESWT on patients with confirmed Achilles tendinopathy and concluded that both modalities showed superior outcomes in terms of pain relief compared to placebo treatment (Gatz et al. 2021). Previous in vitro studies have shown the positive effects of ESWT on tenocyte viability and proliferation, collagen fiber synthesis and organization, expression of TGF-β1 and IGF-1, and decreased expression of MMPs and pro-inflammatory interleukins (ILs) (Banes et al. 1999; Chen et al. 2004; Notarnicola and Moretti 2012). While ESWT is currently deemed as a safe and effective "mechanotherapy" to treat many musculoskeletal pathologies including chronic tendon injuries, unfortunately, there exists very little guidance with regard to parameter selection to ensure repeatability and effectiveness for degenerative tendinopathy (d'Agostino et al. 2017; Fan et al. 2020).

10.3 Focused Ultrasound: A Novel Therapeutic for Tendon Healing?

Recently, Focused Ultrasound (FUS) methods have shown great promise for non-invasive tissue ablation, neuromodulation, and drug delivery (Daoudi et al. 2017; Miller and O'Callaghan 2017; Chua and Faigel 2019). FUS treatments are currently approved for numerous applications including treatment of painful bone metastases, essential tremor, uterine fibroids, and prostate cancer (Duc and Keserci 2019). FUS is also gaining considerable interest as a musculoskeletal treatment option, with clinical research in applications such as osteoarthritis, bone and desmoid tumors, epicondylitis, rotator cuff injury, and plantar fasciitis (Liberman et al. 2009; Weeks et al. 2012; Masashi Izumi et al. 2013; Chan et al. 2017). Another emerging application of FUS is pain relief. Although the precise mechanisms of FUS-induced analgesia are not clear, localized denervation of tissue targets and neuromodulatory effects have been presumed (Brown et al. 2015).

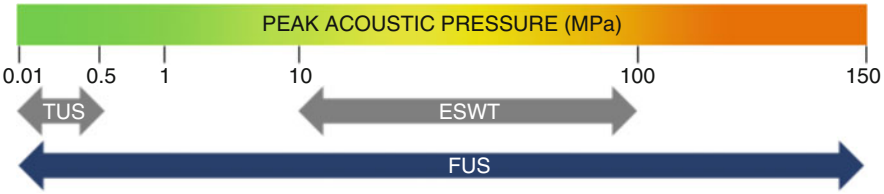

Fig. 10.4 Comparison of an approximate range of peak acoustic pressures delivered by FUS, ESWT, and Low-Intensity TUS

The multitude of diverse applications of FUS are largely dependent on the exposure conditions and the manner in which they are delivered (Fishman and Frenkel 2017). In contrast to HIFU (which is primarily used for tissue ablation), *therapeutic FUS* can be leveraged to achieve effective, functional tissue healing via thermal and/or mechanical stimulation pathways, without inducing irreversible tissue damage. Furthermore, FUS can achieve higher precision with a wide range of acoustic exposures that can encapsulate those utilized in LIUS and ESWT (Fig. 10.4). Additionally, the pulsing parameters can be modulated in real-time during treatment across a wide parameter space. With the advent of state-of-the-art, customizable, reliable, and safe FUS systems, researchers and clinicians are beginning to leverage the potential of image-guided, therapeutic FUS for the treatment of many debilitating conditions.

Focusing on the ultrasound beams prevents them from being applied to other regions, and minimizes the potential for thermal or mechanical damage to tissues located outside the focal zone, allowing for precise treatments of targeted tissues or tissue regions. To achieve localized biological effects, FUS transducers are designed such that focused ultrasound beams converge at a single focal point, using techniques such as geometric focusing (concave transducers that cause waves to arrive at a single focal point), electronic focusing (using phased array transducers composed of multiple piezoelectric elements), or by using acoustic lenses (mimicking a concave transducer surface) (Elhelf et al. 2018).

Ultrasound waves interact with tissues to produce thermal and non-thermal bioeffects (Sect. 10.2.1). Acoustic cavitation, which is one of the most widely studied non-thermal mechanisms, is not significant at lower intensities and is often associated with "high" acoustic pressures (Bader et al. 2019). Typically, at high-pressure amplitudes, microscopic gas bubbles form and oscillate (non-inertial cavitation) or steadily grow in size and collapse above certain pressure thresholds (inertial cavitation). Stable or non-inertial cavitation may induce reversible tissue effects such as sonoporation, whereas inertial cavitation induces large stresses and strains on the tissue, ultimately leading to irreversible tissue damage, i.e., histotripsy ablation. Cavitation can also enhance thermal effects by increasing energy absorption at the focal point. Thus, acoustic amplitudes can directly alter the threshold for inertial cavitation as they can change bubble response from non-inertial to inertial cavitation. Mechanical Index (MI) is another parameter that is commonly used to

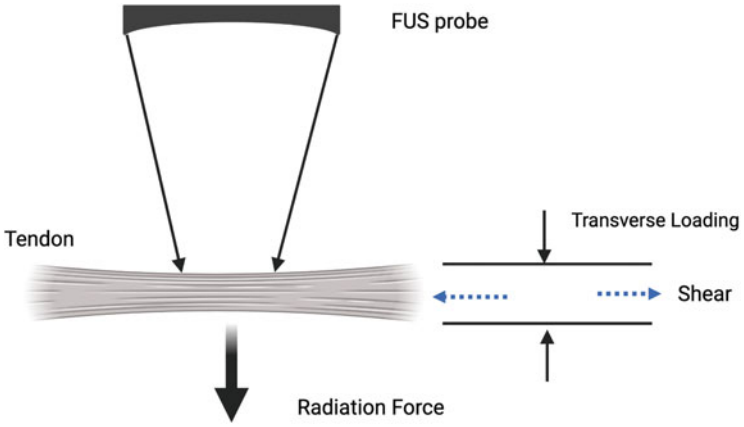

Fig. 10.5 An FUS transducer producing Acoustic Radiation Force to generate tissue displacements and/or deformation

determine the likelihood of cavitation. It is defined as the maximum value of negative peak pressure divided by the root square of the acoustic center frequency. The MI is frequently used to determine the exposures below which cavitation and related bioeffects would not be observed.

In the absence of cavitation, tissue displacement due to FUS application typically occurs as a result of radiation forces. Acoustic Radiation Force (ARF) is defined as the time-averaged force exerted by acoustic waves on the tissue (Urban 2018). As a result of these forces, localized tissue displacement and deformation can be observed, due to transfer of momentum from the sound field to the tissue. Depending on variables such as probe orientation, ultrasound can induce mechanical loading of the extracellular matrix, which in turn provides a biophysical stimulus to the resident cells. ARFs are known to influence cellular proliferation and protein synthesis as evidenced by augmented wound healing and bone remodeling and healing (Curra and Crum 2003; Zhang et al. 2012; Tang et al. 2015). Figure 10.5 depicts the application of radial forces, which deform the tendon transversely while, simultaneously, shear loading of the tendon occurs longitudinally (i.e., along the tendon's long axis). Irrespective of the mode of mechanical loading, tissue deformation can be spatially and temporally quantified using speckle-tracking methods in conjunction with high-frequency imaging (Bercoff et al. 2004; Liu and Ebbini 2010; Ebbini and ter Haar 2015). Perhaps the most prominent biomedical application utilizing ARFs is in conjunction with imaging, to determine the mechanical properties of tissue by utilizing radiation forces (Wells and Liang 2011; Doherty et al. 2013; Urban 2018). Acoustic Radiation Force Impulse (ARFI) imaging utilizes short-duration acoustic radiation forces to generate localized, quantifiable tissue deformation, thereby providing a noninvasive method to quantify tissue biomechanical properties. Recently, the utility of ARFI imaging has been recognized for multiple tissue types including tendon, thyroid, breast, kidney, and pancreas (Bojunga et al. 2012; Wang 2016; Kaya et al. 2018).

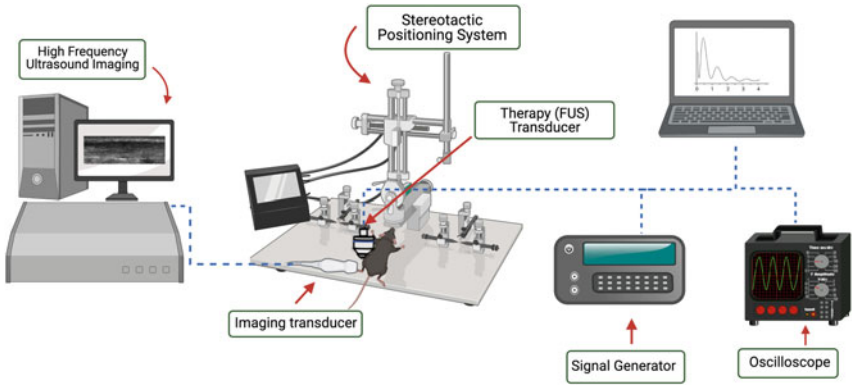

Fig. 10.6 Schematic of a custom-built small animal FUS system allowing interchangeable transducers and driving systems (for different FUS exposures) and a high-frequency imaging system for real-time treatment monitoring and guidance

Currently, FUS methods are being explored to achieve effective tendon healing. One approach is to utilize "low intensity" FUS methods to promote tendon healing via radiation forces (predominantly mechanical stimulation) without producing thermal damage and cavitation-like bioeffects (Meduri et al. 2020). Higher amplitude pulses (compared to traditional physiotherapy applications) are expected to induce larger tendon matrix strains, and this mechanical effect, similar to existing mechanical loading (exercise) therapies typically used for the treatment of chronic tendon injuries (Mafi et al. 2001; Kingma et al. 2007; Irby et al. 2020), in turn, can effectively stimulate tendon repair. Researchers are also exploring histotripsy, a cavitation-based therapy that utilizes short, high-intensity pulses to mechanically homogenize tissue with negligible heating in the tissue (Smallcomb and Simon 2019). Such cavitation effects can induce targeted microdamage within the tendon and promote a healing response, thus serving as an improved, noninvasive alternative to traditional dry needling approaches (Khandare et al. 2021). Initial studies have shown that this approach preserves the mechanical properties of tendons better than dry needling, without damaging the surrounding tissue.

The wide range of FUS applications utilizing both thermal and non-thermal bioeffects indicate the need for robust, real-time image guidance systems with high sensitivity and specificity, for target visualization, beam focusing, and accuracy verification. An ideal, innovative FUS system for tendon healing applications can precisely target tendons, accurately measure the resulting thermal and mechanical bioeffects and facilitate the investigation of cell/tissue responses. Given the paucity of published data on acoustic parameters and mechanisms of action associated with focused ultrasound therapies for tendon injuries, a thorough investigation of such methods in suitable preclinical models is necessary. Controlled studies in relevant animal models will provide a rigorous basis for optimizing acoustic pulsing parameters for FUS tendon treatments, strengthen the rationale for using FUS as a noninvasive treatment method of stimulating tendon healing and will establish a

modular, scalable experimental platform upon which further studies of different species (e.g., rabbit, equine, and human) can readily be undertaken. Figure 10.6 depicts a modular, custom designed system that can apply controlled mechanical, thermal, and mechanical-thermal (dual) stimulation to murine Achilles tendons, under image guidance (Meduri et al. 2020). To establish the feasibility and efficacy of applying different pulsing schemes to injured tendons, investigators may utilize reliable preclinical tendon injury models such as tenotomy for acute injuries or a previously established murine Achilles model (Bell et al. 2013a; Rezvani et al. 2021) of degenerative tendinopathy. The concurrent utilization of real-time, high-frequency ultrasound imaging (as depicted in Fig. 10.6), high field magnetic resonance imaging (MRI) methods, or laser vibrometry enables the quantification of mechanical effects.

Noninvasive (MRI thermometry) or invasive (thermocouple) assessment of dynamic temperature changes accompanying FUS treatment of tendons is a crucial component in analyzing thermal effects of pulsing. Experimental, regional measurements of temperature may further be used to validate computational simulations of predicted heating effects from FUS fields. The effect of predominantly thermal and predominantly mechanical stimulation on the healing profiles of injured tendons can then be established using biomechanical, geometric, cellular, and histologic analyses.

10.4 Future Advancements in Ultrasound-Based Stimulation of Tendon Healing

Although therapeutic ultrasound approaches are widely used for physiotherapy applications, *the mechanism of action and optimal acoustic parameters are poorly understood* and have not been systematically investigated in comparative studies utilizing in vivo tendon injury models. Novel in vivo data from small animal FUS studies will serve as a foundation upon which the methodology can be readily adapted to larger species in order to explore a wider range of FUS treatments in more clinically relevant animal models. Future studies aimed at identifying optimal TUS modalities (e.g., FUS and ESWT) and the corresponding acoustic parameter sets for the treatment of specific tendon injuries (acute and chronic) will strengthen the rationale for using ultrasound modalities for noninvasive stimulation of tendon healing and regeneration.

Specifically, for the treatment of chronic tendon injuries, it is widely known that mechanical loading-based treatments such as exercise-based rehabilitation can effectively treat symptomatic tendinopathy; however, the mechanism of this healing response is not well understood. Furthermore, physiotherapy requires patient compliance, frequently causes discomfort, and may require lengthy treatment periods (e.g., up to 5 years) for symptomatic relief and restored functionality. Successful development of US-based treatments for chronic tendon injuries may provide further

insights into the aforementioned healing pathways in response to mechanical loading. Finally, FUS approaches for treatment of tendon injuries alone or in combination with other therapies could represent an attractive alternative for individuals who are unable or unwilling (e.g., due to pain or injury severity) to pursue physical therapy. In turn, prompt and effective treatment of injured tendons is expected to halt the progression of long-term, degenerative changes that may lead to chronic mobility issues.

References

Andarawis-Puri N, Sereysky JB, Sun HB, Jepsen KJ, Flatow EL (2012) Molecular response of the patellar tendon to fatigue loading explained in the context of the initial induced damage and number of fatigue loading cycles. J Orthop Res 30(8):1327–1334

Arnoczky SP, Lavagnino M, Egerbacher M (2007) The mechanobiological aetiopathogenesis of tendinopathy: is it the over-stimulation or the under-stimulation of tendon cells? Int J Exp Pathol 88(4):217–226

Bader KB, Vlaisavljevich E, Maxwell AD (2019) For whom the bubble grows: physical principles of bubble nucleation and dynamics in histotripsy ultrasound therapy. Ultrasound Med Biol 45(5):1056–1080

Banes AJ, Horesovsky G, Larson C, Tsuzaki M, Judex S, Archambault J, Zernicke R, Herzog W, Kelley S, Miller L (1999) Mechanical load stimulates expression of novel genes in vivo and in vitro in avian flexor tendon cells. Osteo Cart 7(1):141–153

Bell R, Li J, Gorski DJ, Bartels AK, Shewman EF, Wysocki RW, Cole BJ, Bach BR Jr, Mikecz K, Sandy JD, Plaas AH, Wang VM (2013a) Controlled treadmill exercise eliminates Chondroid deposits and restores tensile properties in a new murine tendinopathy model. J Biomech 46(3): 498–505

Bell R, Li J, Shewman EF, Galante JO, Cole BJ, Bach BR Jr, Troy KL, Mikecz K, Sandy JD, Plaas AH, Wang VM (2013b) ADAMTS5 is required for biomechanically-stimulated healing of murine tendinopathy. J Orthop Res 31(10):1540–1548

Benjamin M, Ralphs JR (1998) Fibrocartilage in tendons and ligaments-an adaptation to compressive load. JAnat 193:481–494

Benjamin M, Kaiser E, Milz S (2008) Structure-function relationships in tendons: a review. J Anat 212(3):211–228

Bercoff J, Tanter M, Fink M (2004) Supersonic shear imaging: a new technique for soft tissue elasticity mapping. IEEE Trans Ultrason Ferroelectr Freq Control 51(4):396–409

Best TM, Wilk KE, Moorman CT, Draper DO (2016) Low intensity ultrasound for promoting soft tissue healing: a systematic review of the literature and medical technology. Intern Med Rev (Wash D C) 2(11):271

Bi Y, Ehirchiou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, Li L, Leet AI, Seo BM, Zhang L, Shi S, Young MF (2007) Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 13(10):1219–1227

Bojunga J, Dauth N, Berner C, Meyer G, Holzer K, Voelkl L, Herrmann E, Schroeter H, Zeuzem S, Friedrich-Rust M (2012) Acoustic radiation force impulse imaging for differentiation of thyroid nodules. PLoS One 7(8):e42735

Brown MR, Farquhar-Smith P, Williams JE, ter Haar G, deSouza NM (2015) The use of high-intensity focused ultrasound as a novel treatment for painful conditions-a description and narrative review of the literature. Br J Anaesth 115(4):520–530

Burks SR, Ziadloo A, Hancock HA, Chaudhry A, Dean DD, Lewis BK, Frenkel V, Frank JA (2011) Investigation of cellular and molecular responses to pulsed focused ultrasound in a mouse model. PLoS One 6(9):e24730

Cardoso TB, Pizzari T, Kinsella R, Hope D, Cook JL (2019) Current trends in tendinopathy management. Best Pract Res Clin Rheumatol 33:122–140

Chan AK, Myrer JW, Measom GJ, Draper DO (1998) Temperature changes in human patellar tendon in response to therapeutic ultrasound. J Atthl Train 33(2):130–135

Chan M, Dennis K, Huang Y, Mougenot C, Chow E, DeAngelis C, Coccagna J, Sahgal A, Hynynen K, Czarnota G, Chu W (2017) Magnetic resonance-guided high-intensity-focused ultrasound for palliation of painful skeletal metastases: a pilot study. Technol Cancer Res Treat 16(5):570–576

Chen YJ, Wang CJ, Yang KD, Kuo YR, Huang HC, Huang YT, Sun YC, Wang FS (2004) Extracorporeal shock waves promote healing of collagenase-induced Achilles tendinitis and increase TGF- p 1 and IGF-I expression. J Ortho Res 22:854–861

Chen L, Liu JP, Tang KL, Wang Q, Wang GD, Cai XH, Liu XM (2014) Tendon derived stem cells promote platelet-rich plasma healing in collagenase-induced rat achilles tendinopathy. Cell Physiol Biochem 34(6):2153–2168

Chua T, Faigel D (2019) Endoscopic ultrasound-guided ablation of liver tumors. Gastrointest Endosc Clin N Am 29(2):369–379

Costa-Almeida R, Calejo I, Gomes ME (2019) Mesenchymal stem cells empowering tendon regenerative therapies. Int J Mol Sci 20(12):3002

Curra FP, Crum LA (2003) Therapeutic ultrasound: surgery and drug delivery. Acoust Sci Technol 24(6):343–348

d'Agostino MC, Tibalt E, Craig KV, Respizzi S (2017) Shock wave therapy for tendinopathies. In: Muscle and tendon injuries. Springer, Berlin, pp 421–439

da Cunha A, Parizotto NA, Vidal B d C (2001) The effect of therapeutic US on Achilles tendon repair of the rat. Ultrasound Med Biol 27(12):1691–1696

Dakin SG, Smith RK, Heinegard D, Onnerfjord P, Khabut A, Dudhia J (2014) Proteomic analysis of tendon extracellular matrix reveals disease stage-specific fragmentation and differential cleavage of COMP (cartilage oligomeric matrix protein). J Biol Chem 289(8):4919–4927

Dalecki D (2004) Mechanical bioeffects of ultrasound. Annu Rev Biomed Eng 6:229–248

Daoudi K, Hoogenboom M, den Brok M, Eikelenboom D, Adema GJ, Futterer JJ, de Korte CL (2017) In vivo photoacoustics and high frequency ultrasound imaging of mechanical high intensity focused ultrasound (HIFU) ablation. Biomed Opt Express 8(4):2235–2244

de Jesus JF, de Albuquerque TAB, Shimba LG, Bryk FF, Cook J, Pinfildi CE (2019) High-energy dose of therapeutic ultrasound in the treatment of patellar tendinopathy: protocol of a randomized placebo-controlled clinical trial. BMC Musculoskelet Disord 20(1):624

Dedes V, Tzirogiannis K, Polikandrioti M, Dede AM, Mitseas A, Panoutsopoulos GI (2020) Comparison of radial extracorporeal shockwave therapy with ultrasound therapy in patients with lateral epicondylitis. J Med Ultrason (2001) 47(2):319–325

Demir H, Menku P, Kirnap M, Calis M, Ikizceli I (2004) Comparison of the effects of laser, ultrasound, and combined laser + ultrasound treatments in experimental tendon healing. Lasers Surg Med 35(1):84–89

Dirks RC, Warden SJ (2011) Models for the study of tendinopathy. J Musculoskelet Neuronal Interact 11(2):141–149

Dirks RC, Richard JS, Fearon AM, Scott A, Koch LG, Britton SL, Warden SJ (2013) Uphill treadmill running does not induce histopathological changes in the rat Achilles tendon. BMC Musculoskelet Disord 14:90

Docheva D, Muller SA, Majewski M, Evans CH (2015) Biologics for tendon repair. Adv Drug Deliv Rev 84:222–239

Doherty JR, Trahey GE, Nightingale KR, Palmeri ML (2013) Acoustic radiation force elasticity imaging in diagnostic ultrasound. IEEE Trans Ultrason Ferroelectr Freq Control 60(4):685–701

Dubinsky TJ, Cuevas C, Dighe MK, Kolokythas O, Hwang JH (2008) High-intensity focused ultrasound: current potential and oncologic applications. AJR Am J Roentgenol 190(1):191–199
Duc NM, Keserci B (2019) Emerging clinical applications of high-intensity focused ultrasound. Diagn Interv Radiol 25(5):398–409
D'Vaz AP, Ostor AJ, Speed CA, Jenner JR, Bradley M, Prevost AT, Hazleman BL (2006) Pulsed low-intensity ultrasound therapy for chronic lateral epicondylitis: a randomized controlled trial. Rheumatology (Oxford) 45(5):566–570
Ebbini ES, ter Haar G (2015) Ultrasound-guided therapeutic focused ultrasound: current status and future directions. Int J Hyperth 31(2):77–89
Elhelf IAS, Albahar H, Shah U, Oto A, Cressman E, Almekkawy M (2018) High intensity focused ultrasound: the fundamentals, clinical applications and research trends. Diagn Interv Imaging 99(6):349–359
Fahlstrom M, Jonsson P, Lorentzon R, Alfredson H (2003) Chronic Achilles tendon pain treated with eccentric calf-muscle training. Knee Surg Sports Traumatol Arthrosc 11(5):327–333
Fan Y, Feng Z, Cao J, Fu W (2020) Efficacy of extracorporeal shock wave therapy for Achilles tendinopathy: a meta-analysis. Orthop J Sports Med 8(2):2325967120903430
Farcic TS, Baldan CS, Cattapan CG, Parizotto NA, Joao SM, Casarotto RA (2013) Treatment time of ultrasound therapy interferes with the organization of collagen fibers in rat tendons. Braz J Phys Ther 17(3):263–271
Farcic TS, Baldan CS, Machado AFP, Caffaro LAM, Masson IFB, Casarotto RA (2018) Collagen fibers in the healing process of rat Achilles tendon rupture using different times of ultrasound therapy. Adv Wound Care (New Rochelle) 7(4):114–120
Fishman PS, Frenkel V (2017) Treatment of movement disorders with focused ultrasound. J Cent Nerv Syst Dis 9:1179573517705670
Fu SC, Shum WT, Hung LK, Wong MW, Qin L, Chan KM (2008) Low-intensity pulsed ultrasound on tendon healing: a study of the effect of treatment duration and treatment initiation. Am J Sports Med 36(9):1742–1749
Fu SC, Hung LK, Shum WT, Lee YW, Chan LS, Ho G, Chan KM (2010) In vivo low-intensity pulsed ultrasound (LIPUS) following tendon injury promotes repair during granulation but suppresses decorin and biglycan expression during remodeling. J Orthop Sports Phys Ther 40(7):422–429
Fung DT, Wang VM, Laudier DM, Shine JH, Basta-Pljakic J, Jepsen KJ, Schaffler MB, Flatow EL (2009) Subrupture tendon fatigue damage. J Orthop Res 27(2):264–273
Fung DT, Wang VM, Andarawis-Puri N, Basta-Pljakic J, Li Y, Laudier DM, Sun HB, Jepsen KJ, Schaffler MB, Flatow EL (2010) Early response to tendon fatigue damage accumulation in a novel in vivo model. J Biomech 43(2):274–279
Garcia-Melchor E, Cafaro G, MacDonald L, Crowe LAN, Sood S, McLean M, Fazzi UG, McInnes IB, Akbar M, Millar NL (2021) Novel self-amplificatory loop between T cells and tenocytes as a driver of chronicity in tendon disease. Ann Rheum Dis 80(8):1075–1085
Gatz M, Schweda S, Betsch M, Dirrichs T, de la Fuente M, Reinhardt N, Quack V (2021) Line- and point-focused extracorporeal shock wave therapy for Achilles tendinopathy: a placebo-controlled RCT study. Sports Health 13(5):511–518
Goodman SA, May SA, Heinegard D, Smith RKW (2004) Tenocyte response to cyclical strain and transforming growth factor beta is dependent upon age and site of origin. Biorheology 41(5): 613–628
Gottardi R, Stoddart MJ (2018) Regenerative rehabilitation of the musculoskeletal system. J Am Acad Orthop Surg 26(15):e321–e323
Gray G, Brolinson PG (2001) Principle-centered rehabilitation. In: Principles and practice of primary care sports medicine. Lippincott Williams & Wilkins, Philadelphia, PA, pp 645–652
Gulati A, Ottestad E (2020) Hearing the call for therapeutic ultrasound. Pain Med 21(7):1317–1318
Hast MW, Zuskov A, Soslowsky LJ (2014) The role of animal models in tendon research. Bone Joint Res 3:193–202

Hausdorf J (2008) Selective loss of unmyelinated nerve fibers after extracorporeal shockwave application to the musculoskeletal system. J Neuroscience 155(1):138–144
Heinemeier KM, Skovgaard D, Bayer ML, Qvortrup K, Kjaer A, Kjaer M, Magnusson SP, Kongsgaard M (2012) Uphill running improves rat Achilles tendon tissue mechanical properties and alters gene expression without inducing pathological changes. J Appl Physiol (1985) 113(5):827–836
Herod TW, Veres SP (2018) Development of overuse tendinopathy: a new descriptive model for the initiation of tendon damage during cyclic loading. J Orthop Res 36(1):467–476
Holland CK, Apfel RE (1990) Thresholds for transient cavitation produced by pulsed ultrasound in a controlled nuclei environment. J Acoust Soc Am 88(5):2059–2069
Hsu AR, Holmes GB (2016) Preliminary treatment of Achilles tendinopathy using low-intensity pulsed ultrasound. Foot Ankle Spec 9(1):52–57
Humphrey JD, Dufresne ER, Schwartz MA (2014) Mechanotransduction and extracellular matrix homeostasis. Nat Rev Mol Cell Biol 15(12):802–812
Irby A, Gutierrez J, Chamberlin C, Thomas SJ, Rosen AB (2020) Clinical management of tendinopathy: a systematic review of systematic reviews evaluating the effectiveness of tendinopathy treatments. Scand J Med Sci Sports 30(10):1810–1826
Izadifar Z, Babyn P, Chapman D (2017) Mechanical and biological effects of ultrasound: a review of present knowledge. Ultrasound Med Biol 43(6):1085–1104
Jackson BA, Schwane JA, Starcher BC (1991) Effect of ultrasound therapy on the repair of Achilles tendon injuries in rats. Med Sci Sport Exer 23(2):271–176
Jeremias Junior SL, Camanho GL, Bassit AC, Forgas A, Ingham SJ, Abdalla RJ (2011) Low-intensity pulsed ultrasound accelerates healing in rat calcaneus tendon injuries. J Orthop Sports Phys Ther 41(7):526–531
Kannus P (2000) Structure of the tendon connective tissue. Scand J Med Sci Sports 10:312–320
Kaya M, Degirmenci S, Goya C, Tuncel ET, Ucmak F, Kaplan MA (2018) The importance of acoustic radiation force impulse (ARFI) elastography in the diagnosis and clinical course of acute pancreatitis. Turk J Gastroenterol 29(3):342–347
Khandare S, Smallcomb M, Klein B, Geary C, Simon JC, Vidt ME (2021) Comparison between dry needling and focused ultrasound on the mechanical properties of the rat Achilles tendon: a pilot study. J Biomech 120:110384
Khanna A, Nelmes RT, Gougoulias N, Maffulli N, Gray J (2009) The effects of LIPUS on soft-tissue healing: a review of literature. Br Med Bull 89:169–182
Kingma JJ, de Knikker R, Wittink HM, Takken T (2007) Eccentric overload training in patients with chronic Achilles tendinopathy: a systematic review. Br J Sports Med 41(6):e3
Kosaka T, Masaoka T, Yamamoto K (2011) Possible molecular mechanism of promotion of repair of acute Achilles tendon rupture by low intensity-pulsed ultrasound treatment in a rat model. West Indian Med J 60(3):263–268
Lacitignola L, Staffieri F, Rossi G, Francioso E, Crovace A (2014) Survival of bone marrow mesenchymal stem cells labelled with red fluorescent protein in an ovine model of collagenase-induced tendinitis. Vet Comp Orthop Traumatol 27(3):204–209
Larsen A, Kristensen G, Thorlacius-Ussing O, Oxlund H (2005) The influence of ultrasound on the mechanical properties of healing tendons in rabbits. Acta Orthop 76(2):225–230
Leek CC, Soulas JM, Sullivan AL, Killian ML (2020) Using tools in mechanobiology to repair tendons. Curr Tissue Microenviron Rep 1(2):31–40
Legerlotz K, Jones ER, Screen HR, Riley GP (2012) Increased expression of IL-6 family members in tendon pathology. Rheumatology (Oxford) 51(7):1161–1165
Leung MC, Ng GY, Yip KK (2004) Effect of ultrasound on acute inflammation of transected medial collateral ligaments. Arch Phys Med Rehabil 85(6):963–966
Liberman B, Gianfelice D, Inbar Y, Beck A, Rabin T, Shabshin N, Chander G, Hengst S, Pfeffer R, Chechick A, Hanannel A, Dogadkin O, Catane R (2009) Pain palliation in patients with bone metastases using MR-guided focused ultrasound surgery: a multicenter study. Ann Surg Oncol 16(1):140–146

Lim WL, Liau LL, Ng MH, Chowdhury SR, Law JX (2019) Current Progress in tendon and ligament tissue engineering. Tissue Eng Regen Med 16(6):549–571
Lin TW, Cardenas L, Glaser DL, Soslowsky LJ (2006) Tendon healing in interleukin-4 and interleukin-6 knockout mice. J Biomech 39(1):61–69
Liu D, Ebbini ES (2010) Real-time 2-D temperature imaging using ultrasound. IEEE Trans Biomed Eng 57(1):12–16
Liu DD, Ullah M, Concepcion W, Dahl JJ, Thakor AS (2020) The role of ultrasound in enhancing mesenchymal stromal cell-based therapies. Stem Cells Transl Med 9:850–866
Lucchetti D, Perelli L, Colella F, Ricciardi-Tenore C, Scoarughi GL, Barbato G, Boninsegna A, De Maria R, Sgambato A (2020) Low-intensity pulsed ultrasound affects growth, differentiation, migration, and epithelial-to-mesenchymal transition of colorectal cancer cells. J Cell Physiol 235(6):5363–5377
Maan ZN, Januszyk M, Rennert RC, Duscher D, Rodrigues M, Fujiwara T, Ho N, Whitmore A, Hu MS, Longaker MT, Gurtner GC (2014) Noncontact, low-frequency ultrasound therapy enhances neovascularization and wound healing in diabetic mice. Plast Reconstr Surg 134(3):402e–411e
Mackie EJ, Ramsey S (1996) Expression of tenascin in joint-associated tissues during development and postnatal growth. J Anat 188:157–165
Maeda T, Sakabe T, Sunaga A, Sakai K, Rivera AL, Keene DR, Sasaki T, Stavnezer E, Iannotti J, Schweitzer R, Ilic D, Baskaran H, Sakai T (2011) Conversion of mechanical force into TGF-beta-mediated biochemical signals. Curr Biol 21(11):933–941
Maffulli N, Longo UG, Denaro V (2010) Novel approaches for the management of tendinopathy. J Bone Joint Surg Am 92(15):2604–2613
Mafi N, Lorentzon R, Alfredson H (2001) Superior short-term results with eccentric calf muscle training compared to concentric training in a randomized prospective multicenter study on patients with chronic Achilles tendinosis. Knee Surg Sports Traumatol Arthrosc 9(1):42–47
Marqueti R, Neto I, Barin F, Ramos G (2019) Exercise and tendon remodeling mechanism. In: Sozen H (ed) Tendons. IntechOpen Publishing, pp 37–56
Masashi Izumi MI, Kawasaki M, Ushida T, Morio K, Namba H, Graven-Nielsen T, Ogawa Y, Tani T (2013) MR-guided focused ultrasound for the novel and innovative management of osteoarthritic knee pain. BMC Musculoskelet Disord 14:267
Mauri G, Nicosia L, Xu Z, Di Pietro S, Monfardini L, Bonomo G, Varano GM, Prada F, Vigna PD, Orsi F (2018) Focused ultrasound: tumour ablation and its potential to enhance immunological therapy to cancer. Br J Radiol 91(1083):20170641
Maxwell L (1992) Therapeutic ultrasound: its effects on the cellular and molecular mechanisms of inflammation and repair. Physiotherapy 78(6):421–426
Meduri C, Simon, A, De Konick L, Coffey M, Claros E, Velazquez M, Brolinson PG, McCrady B, Vlaisavljevich E, Wang VM (2020) Design of a Focused Ultrasound System for the non-invasive treatment of injured tendons. In: 7th International Symposium on Focused Ultrasound
Mehr D, Pardubsky PD, Martin JA, Buckwalter JA (2000) Tenascin-C in tendon regions subjected to compression. J Orthop Res 18:537–545
Millar NL, Hueber AJ, Reilly JH, Xu Y, Fazzi UG, Murrell GA, McInnes IB (2010) Inflammation is present in early human tendinopathy. Am J Sports Med 38(10):2085–2091
Millar NL, Murrell GA, McInnes IB (2017) Inflammatory mechanisms in tendinopathy - towards translation. Nat Rev Rheumatol 13(2):110–122
Millar NL, Silbernagel KG, Thorborg K, Kirwan PD, Galatz LM, Abrams GD, Murrell GAC, McInnes IB, Rodeo SA (2021) Tendinopathy. Nat Rev Dis Primers 7(1):1
Miller DB, O'Callaghan JP (2017) New horizons for focused ultrasound (FUS) - therapeutic applications in neurodegenerative diseases. Metabolism 69S:S3–S7
Mittelstein DR, Ye J, Schibber EF, Roychoudhury A, Martinez LT, Fekrazad MH, Ortiz M, Lee PP, Shapiro MG, Gharib M (2020) Selective ablation of cancer cells with low intensity pulsed ultrasound. Appl Phys Lett 116(1):013701

Moser HL, Doe AP, Meier K, Garnier S, Laudier D, Akiyama H, Zumstein MA, Galatz LM, Huang AH (2018) Genetic lineage tracing of targeted cell populations during enthesis healing. J Orthop Res 36(12):3275–3284

Moya D, Ramon S, Schaden W, Wang CJ, Guiloff L, Cheng JH (2018) The role of extracorporeal shockwave treatment in musculoskeletal disorders. J Bone Joint Surg Am 100(3):251–263

Muller SA, Todorov A, Heisterbach PE, Martin I, Majewski M (2015) Tendon healing: an overview of physiology, biology, and pathology of tendon healing and systematic review of state of the art in tendon bioengineering. Knee Surg Sports Traumatol Arthrosc 23(7):2097–2105

Murphy M, Travers M, Gibson W (2018) Is heavy eccentric calf training superior to wait-and-see, sham rehabilitation, traditional physiotherapy and other exercise interventions for pain and function in mid-portion Achilles tendinopathy? Syst Rev 7(1):58

Ng GY, Fung DT (2007) The effect of therapeutic ultrasound intensity on the ultrastructural morphology of tendon repair. Ultrasound Med Biol 33(11):1750–1754

Ng C, Ng G, See E, Leung M (2003) Therapeutic ultrasound improves strength of achilles tendon repair in rats. Ultrasound Med Biol 29(10):1501–1506

Nightingale K (2011) Acoustic radiation force impulse (ARFI) imaging: a review. Curr Med Imaging Rev 7(4):328–339

Notarnicola A, Moretti B (2012) The biological effects of extracorporeal shock wave therapy (ESWT) on tendon tissue. MLTJ 2(1):33–37

Nourissat G, Berenbaum F, Duprez D (2015) Tendon injury: from biology to tendon repair. Nat Rev Rheumatol 11:223–233

Nussbaum E (1998) The influence of ultrasound on healing tissues. J Hand Ther 11(2):140–147

O'Neill BE, Vo H, Angstadt M, Li KP, Quinn T, Frenkel V (2009) Pulsed high intensity focused ultrasound mediated nanoparticle delivery: mechanisms and efficacy in murine muscle. Ultrasound Med Biol 35(3):416–424

Özer Kaya D (2020) Architecture of tendon and ligament and their adaptation to pathological conditions. In: Angin S, Simsek I (eds) Comparative kinesiology of the human body. Elsevier, pp 115–147

Papadopoulos ES, Mani R (2020) The role of ultrasound therapy in the management of musculoskeletal soft tissue pain. Int J Low Extrem Wounds 19(4):350–358

Parkinson J, Samiric T, Ilic MZ, Cook J, Handley CJ (2011) Involvement of proteoglycans in tendinopathy. J Musculoskelet Neuronal Interact 11(2):86–93

Petterson S, Plancher K, Klyve D, Draper D, Ortiz R (2020) Low-intensity continuous ultrasound for the symptomatic treatment of upper shoulder and neck pain: a randomized, double-blind placebo-controlled clinical trial. J Pain Res 13:1277–1287

Pingel J, Wienecke J, Kongsgaard M, Behzad H, Abraham T, Langberg H, Scott A (2013) Increased mast cell numbers in a calcaneal tendon overuse model. Scand J Med Sci Sports 23(6):e353–e360

Poliachik SL, Khokhlova TD, Wang YN, Simon JC, Bailey MR (2014) Pulsed focused ultrasound treatment of muscle mitigates paralysis-induced bone loss in the adjacent bone: a study in a mouse model. Ultrasound Med Biol 40(9):2113–2124

Popov C, Burggraf M, Kreja L, Ignatius A, Schieker M, Docheva D (2015) Mechanical stimulation of human tendon stem/progenitor cells results in upregulation of matrix proteins, integrins and MMPs, and activation of p38 and ERK1/2 kinases. BMC Mol Biol 16:6

Rando TA, Ambrosio F (2018) Regenerative rehabilitation: applied biophysics meets stem cell therapeutics. Cell Stem Cell 22(3):306–309

Reuther KE, Thomas SJ, Sarver JJ, Tucker JJ, Lee CS, Gray CF, Glaser DL, Soslowsky LJ (2013) Effect of return to overuse activity following an isolated supraspinatus tendon tear on adjacent intact tendons and glenoid cartilage in a rat model. J Orthop Res 31(5):710–715

Rezvani SN, Nichols AEC, Grange RW, Dahlgren LA, Brolinson PG, Wang VM (2021) A novel murine muscle loading model to investigate Achilles musculotendinous adaptation. J Appl Physiol (1985) 130(4):1043–1051

Saber AA, Saber A (2017) Therapeutic ultrasound: physiological role, clinical applications and precautions. J Surg 5(3–1):61–69
Sayegh ET, Sandy JD, Virk MS, Romeo AA, Wysocki RW, Galante JO, Trella KJ, Plaas A, Wang VM (2015) Recent scientific advances towards the development of tendon healing strategies. Curr Tissue Eng 4(2):128–143
Screen HR, Berk DE, Kadler KE, Ramirez F, Young MF (2015) Tendon functional extracellular matrix. J Orthop Res 33(6):793–799
Sereysky JB, Andarawis-Puri N, Jepsen KJ, Flatow EL (2012) Structural and mechanical effects of in vivo fatigue damage induction on murine tendon. J Orthop Res 30(6):965–972
Shankar H, Pagel PS (2011) Potential adverse ultrasound-related biological effects. Am S Anes 115(5):1109–1124
Sharma P, Maffulli N (2005) Tendon injury and tendinopathy: healing and repair. J Bone Joint Surg 87-A(1):187–202
Sharma P, Maffulli N (2006) Biology of tendon injury-healing, modeling and remodeling. J Musculoskelet Neuronal Interact 6(2):181–190
Shen H, Yoneda S, Sakiyama-Elbert SE, Zhang Q, Thomopoulos S, Gelberman RH (2021) Flexor tendon injury and repair: the influence of synovial environment on the early healing response in a canine mode. J Bone Joint Surg Am 103(9):e36
Simplicio CL, Purita J, Murrell W, Santos GS, Dos Santos RG, Lana J (2020) Extracorporeal shock wave therapy mechanisms in musculoskeletal regenerative medicine. J Clin Orthop Trauma 11 (Suppl 3):S309–S318
Smallcomb M, Simon J (2019) Histotripsy in collagenous tendons. In: 178th Meeting of the Acoustical Society of America
Snedeker JG, Foolen J (2017) Tendon injury and repair - a perspective on the basic mechanisms of tendon disease and future clinical therapy. Acta Biomater 63:18–36
Solchaga LA, Bendele A, Shah V, Snel LB, Kestler HK, Dines JS, Hee CK (2014) Comparison of the effect of intra-tendon applications of recombinant human platelet-derived growth factor-BB, platelet-rich plasma, steroids in a rat achilles tendon collagenase model. J Orthop Res 32(1): 145–150
Spiesz EM, Thorpe CT, Chaudhry S, Riley GP, Birch HL, Clegg PD, Screen HR (2015) Tendon extracellular matrix damage, degradation and inflammation in response to in vitro overload exercise. J Orthop Res 33(6):889–897
Steinmann S, Pfeifer CG, Brochhausen C, Docheva D (2020) Spectrum of tendon pathologies: triggers, trails and end-state. Int J Mol Sci 21(3):844
Sunwoo JY, Eliasberg CD, Carballo CB, Rodeo SA (2020) The role of the macrophage in tendinopathy and tendon healing. J Orthop Res 38(8):1666–1675
Tanaka E, Liu Y, Xia L, Ogasawara N, Sakamaki T, Kano F, Hashimoto N, Feng X, Yamamoto A (2020) Effectiveness of low-intensity pulsed ultrasound on osteoarthritis of the temporomandibular joint: a review. Ann Biomed Eng 48(8):2158–2170
Tang J, Guha C, Tome WA (2015) Biological effects induced by non-thermal ultrasound and implications for cancer therapy: a review of the current literature. Technol Cancer Res Treat 14(2):221–235
ter Haar G (2007) Therapeutic applications of ultrasound. Prog Biophys Mol Biol 93(1–3):111–129
Tharkar P, Varanasi R, Wong WSF, Jin CT, Chrzanowski W (2019) Nano-enhanced drug delivery and therapeutic ultrasound for cancer treatment and beyond. Front Bioeng Biotechnol 7:324
Theodossiou SK, Schiele NR (2019) Models of tendon development and injury. BMC Biomed Eng 1:32
Thomopoulos S, Parks WC, Rifkin DB, Derwin KA (2015) Mechanisms of tendon injury and repair. J Orthop Res 33(6):832–839
Tsai WC, Tang ST, Liang FC (2011) Effect of therapeutic ultrasound on tendons. Am J Phys Med Rehabil 90(12):1068–1073

Tsai SL, Nodl MT, Galloway JL (2021) Bringing tendon biology to heel: leveraging mechanisms of tendon development, healing, and regeneration to advance therapeutic strategies. Dev Dyn 250(3):393–413

Urban MW (2018) Production of acoustic radiation force using ultrasound: methods and applications. Expert Rev Med Devices 15(11):819–834

Urdzikova LM, Sedlacek R, Suchy T, Amemori T, Ruzicka J, Lesny P, Havlas V, Sykova E, Jendelova P (2014) Human multipotent mesenchymal stem cells improve healing after collagenase tendon injury in the rat. BioMed Eng OnLine 13:42

Vander Doelen T, Jelley W (2020) Non-surgical treatment of patellar tendinopathy: a systematic review of randomized controlled trials. J Sci Med Sport 23(2):118–124

Vidal-Jove J, Perich E, Del Castillo MA (2015) Ultrasound guided high intensity focused ultrasound for malignant tumors: the Spanish experience of survival advantage in stage III and IV pancreatic cancer. Ultrason Sonochem 27:703–706

Vlaisavljevich E, Kim Y, Allen S, Owens G, Pelletier S, Cain C, Ives K, Xu Z (2013) Image-guided non-invasive ultrasound liver ablation using histotripsy: feasibility study in an in vivo porcine model. Ultrasound Med Biol 39(8):1398–1409

Vlaisavljevich E, Xu Z, Arvidson A, Jin L, Roberts W, Cain C (2015) Effects of thermal preconditioning on tissue susceptibility to histotripsy. Ultrasound Med Biol 41(11):2938–2954

Voleti PB, Buckley MR, Soslowsky LJ (2012) Tendon healing: repair and regeneration. Annu Rev Biomed Eng 14:47–71

Walden G, Liao X, Donell S, Raxworthy MJ, Riley GP, Saeed A (2017) A clinical, biological, and biomaterials perspective into tendon injuries and regeneration. Tissue Eng Part B Rev 23(1): 44–58

Wang CJ (2012) Extracorporeal shockwave therapy in musculoskeletal disorders. J Ortho Surg Res 7:11

Wang L (2016) Applications of acoustic radiation force impulse quantification in chronic kidney disease: a review. Ultrasonography 35(4):302–308

Wang L (2018) Acoustic radiation force based ultrasound elasticity imaging for biomedical applications. Sensors (Basel) 18(7):E2252

Warden SJ, Metcalf BR, Kiss ZS, Cook JL, Purdam CR, Bennell KL, Crossley KM (2008) Low-intensity pulsed ultrasound for chronic patellar tendinopathy: a randomized, double-blind, placebo-controlled trial. Rheumatology (Oxford) 47(4):467–471

Watson T (2008) Ultrasound in contemporary physiotherapy practice. Ultrasonics 48(4):321–329

Waugh CM, Morrissey D, Jones E, Riley GP, Langberg H, Screen HR (2015) In vivo biological response to extracorporeal shockwave therapy in human tendinopathy. Eur Cell Mater 29:268–280. discussion 280

Weeks EM, Platt MW, Gedroyc W (2012) MRI-guided focused ultrasound (MRgFUS) to treat facet joint osteoarthritis low back pain--case series of an innovative new technique. Eur Radiol 22(12):2822–2835

Wells PN, Liang HD (2011) Medical ultrasound: imaging of soft tissue strain and elasticity. J R Soc Interface 8(64):1521–1549

Willett NJ, Boninger ML, Miller LJ, Alvarez L, Aoyama T, Bedoni M, Brix KA, Chisari C, Christ G, Dearth CL, Dyson-Hudson TA, Evans CH, Goldman SM, Gregory K, Gualerzi A, Hart J, Ito A, Kuroki H, Loghmani MT, Mack DL, Malanga GA, Noble-Haeusslein L, Pasquina P, Roche JA, Rose L, Stoddart MJ, Tajino J, Terzic C, Topp KS, Wagner WR, Warden SJ, Wolf SL, Xie H, Rando TA, Ambrosio F (2020) Taking the next steps in regenerative rehabilitation: establishment of a new interdisciplinary field. Arch Phys Med Rehabil 101(5):917–923

Wood VT, Pinfildi CE, Neves MA, Parizoto NA, Hochman B, Ferreira LM (2010) Collagen changes and realignment induced by low-level laser therapy and low-intensity ultrasound in the calcaneal tendon. Lasers Surg Med 42(6):559–565

Wunderli SL, Blache U, Snedeker JG (2020) Tendon explant models for physiologically relevant in vitro study of tissue biology - a perspective. Connect Tissue Res 61(3–4):262–277

Xu Z, Hall TL, Vlaisavljevich E, Lee FT Jr (2021) Histotripsy: the first noninvasive, non-ionizing, non-thermal ablation technique based on ultrasound. Int J Hyperth 38(1):561–575

Yeung CK, Guo X, Ng YF (2006) Pulsed ultrasound treatment accelerates the repair of Achilles tendon rupture in rats. J Orthop Res 24(2):193–201

Yoshida R, Alaee F, Dyrna F, Kronenberg MS, Maye P, Kalajzic I, Rowe DW, Mazzocca AD, Dyment NA (2016) Murine supraspinatus tendon injury model to identify the cellular origins of rotator cuff healing. Connect Tissue Res 57(6):507–515

Yu J, Park D, Lee G (2013) Effect of eccentric strengthening on pain, muscle strength, endurance, and functional fitness factors in male patients with achilles tendinopathy. Am J Phys Med Rehabil 92(1):68–76

Zelzer E, Blitz E, Killian ML, Thomopoulos S (2014) Tendon-to-bone attachment: from development to maturity. Birth Defects Res C Embryo Today 102(1):101–112

Zhang J, Wang JH-C (2010) Characterization of differential properties of rabbit tendon stem cells and tenocytes. BMC Musculoskelet Disord 11:10

Zhang S, Cheng J, Qin YX (2012) Mechanobiological modulation of cytoskeleton and calcium influx in osteoblastic cells by short-term focused acoustic radiation force. PLoS One 7(6): e38343

Zhang N, Chow SK, Leung KS, Cheung WH (2017) Ultrasound as a stimulus for musculoskeletal disorders. J Orthop Translat 9:52–59

Zhang C, Zhu J, Zhou Y, Thampatty BP, Wang JH (2019) Tendon stem/progenitor cells and their interactions with extracellular matrix and mechanical loading. Stem Cells Int 2019:3674647

Zhang J, Nie D, Williamson K, McDowell A, Hogan MV, Wang JH (2020) Moderate and intensive mechanical loading differentially modulate the phenotype of tendon stem/progenitor cells in vivo. PLoS One 15(12):e0242640

Zhou S, Schmelz A, Seufferlein T, Li Y, Zhao J, Bachem MG (2004) Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts. J Biol Chem 279(52):54463–54469

Chapter 11
Mechanical Stimulation as Both the Cause and the Cure of Tendon and Ligament Injuries

Lauren Paschall, Krishna Pedaprolu, Sabrina Carrozzi, Aman Dhawan, and Spencer Szczesny

Abstract Tendon and ligament injuries are common orthopedic disorders whose current treatment fails to restore native tissue structure and function. The resulting structural and mechanical deficits often lead to pain, dysfunction, and an increased risk of reinjury. To improve both injury prevention and treatment, it is necessary to better understand the etiology of tendon/ligament injury and the factors that inhibit recovery and healing. Given that the primary function of tendons and ligaments is to transmit loads, it is not surprising that mechanics play an important role in both the cause and cure of tendon and ligament injuries. The objective of this chapter is to review the existing data demonstrating the importance of mechanical stimuli (proper and improper) on tendon/ligament injury and repair, with a focus on degenerative tendinopathy and anterior cruciate ligament tears/reconstruction. Additionally, we identify the most critical unanswered questions preventing progress in the field as well as the potential opportunities for regenerative rehabilitation to prevent injury and improve structural, functional, and patient-reported outcomes. We hope that this review serves as a valuable reference for those new to the field of regenerative rehabilitation and provides insight into its application to tendon and ligament injuries.

L. Paschall · K. Pedaprolu · S. Carrozzi
Department of Biomedical Engineering, Pennsylvania State University, University Park, PA, USA

A. Dhawan
Department of Orthopaedics and Rehabilitation, Pennsylvania State University, Hershey, PA, USA

S. Szczesny (✉)
Department of Biomedical Engineering, Pennsylvania State University, University Park, PA, USA

Department of Orthopaedics and Rehabilitation, Pennsylvania State University, Hershey, PA, USA
e-mail: ses297@psu.edu

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_11

Keywords Tendon · Ligament · Mechanical loading · Anterior cruciate ligament · Allograft · Autograft · Tendinopathy

11.1 Introduction

Current estimates suggest that tendon and ligament injuries account for 20–30% of all musculoskeletal disorders and are the most common form of non-fatal occupational injury resulting in over 420,000 days away from work each year in the USA (Bureau of Labor Statistics 2016; Badley and Tennant 1993; Fleming et al. 2005). Tears are most common in the tendons of the rotator cuff (e.g., supraspinatus), the Achilles tendon, and the anterior cruciate ligament (ACL) (Sanders et al. 2016; Millar et al. 2021). In fact, ACL reconstruction is one of the most common procedures performed in orthopedic surgery (Garrett et al. 2006).

There are a number of operative and non-operative treatment options for tendon and ligament injuries. In the case of tendinopathy, pain may be manageable with non-surgical options such as nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and eccentric loading (Andres and Murrell 2008). However, pain recurrence is common and, particularly in the case of anti-inflammatory treatments, the structural deficits of the tendon remain or even worsen, which increases the risk of subsequent tissue rupture (Shrier et al. 1996; Virchenko et al. 2004; Su and O'Connor 2013; Bedi et al. 2020). Surgical repair of torn tendons has varied success with rates ranging from 98% (for Achilles' tendons) to 56% (for supraspinatus tendons) (Rashid et al. 2017; Ochen et al. 2019). Even in the case of successful repairs, structural and functional deficits remain, which pose a risk for reinjury (Freedman et al. 2014; Collin et al. 2019). For ACL tears, surgical reconstruction is the gold-standard treatment given the limited intrinsic repair capacity of the ACL (Hefti et al. 1991; Dunn et al. 2004). Reconstruction involves replacing the ACL with a tendon either from the patient (i.e., autograft) or from a cadaver (i.e., allograft). While the success rates can be satisfactory depending upon patient and graft specifics, reconstruction failure occurs in up to 30% of young active patients, who are three times more likely to tear their ACL and account for nearly 70% of all ACL reconstructions (Barrett et al. 2010; Buller et al. 2015; Sanders et al. 2016). Therefore, there is significant interest in preventing tendon/ligament injuries and improving treatment success.

The objective of this chapter is to summarize the data regarding the role of mechanics in the etiology and treatment of tendinopathy and ligament tears, specifically involving the ACL. Given that the primary function of tendons and ligaments is to transmit loads, it is not surprising that mechanics play an important role in both the cause and cure of tendon and ligament injuries. In the following sections, we will demonstrate that, while tendon and ligament ruptures may appear to be acute injuries, they are often preceded by the accumulation of tissue damage, microtrauma, and degeneration, which further weaken the tissue and result in injury. Additionally, we will examine the evidence suggesting that, in the case of tendinopathy, proper physical therapy (i.e., mechanical loading) can help reduce pain and potentially help

restore tissue structure and function. Similarly, evidence suggests that proper mechanical loading of ACL reconstructions is critical for successful surgical outcomes, especially for allografts. At the end of this chapter, we hope that this information regarding the role of mechanics in tendinopathy and ACL injury will demonstrate the potential of using regenerative rehabilitation for developing better methods of injury prevention and treatment.

11.2 Tendinopathy

11.2.1 Mechanical Loading is a Primary Risk Factor for Tendinopathy

Tendinopathy is a broad term that can include a variety of symptoms, including chronic pain, inflammation, matrix disorganization, and tendon rupture (Millar et al. 2021). Tendinopathic tissues are often marked by tissue degeneration in the form of disorganized collagen fibers, increased activity of matrix metalloproteinases (MMPs), synthesis of inflammatory markers such as interleukins and cyclooxygenases, production of abnormal matrix deposits (i.e., calcium, lipid, cartilage), and inferior mechanical properties (Kannus and Józsa 1991; Tallon et al. 2001; Magra and Maffulli 2005; Jones et al. 2006; Arya and Kulig 2010; Dakin et al. 2014; Wiesinger et al. 2020). While the cause of tendinopathy and the associated tissue degeneration is unclear, risk factors include age, obesity, and chronic disease (e.g., diabetes) (Kannus et al. 2005; Scott et al. 2015; Ranger et al. 2016; Svensson et al. 2016; Macchi et al. 2020; Millar et al. 2021). Tendons in aged individuals exhibit changes in matrix composition, such as the accumulation of atypical deposits containing calcium, lipids, and glycosaminoglycans (GAGs), as well as many cellular and vascular changes (Kannus et al. 2005). Additionally, the incidence of tendon rupture increases with age (Yamaguchi et al. 2006). While there is data to suggest that these structural changes and higher failure rates are due to biological changes associated with aging (Zhou et al. 2010; McBeath et al. 2019), it is also possible that they are simply a result of the accumulation of tissue damage over time.

Obesity is another factor associated with a greater risk of tendinopathy. Obese patients have a higher incidence of surgical treatments for tendon tears, more complications after surgeries, and inferior tendon mechanical properties (Macchi et al. 2020). Furthermore, obese patients have a higher incidence rate of low-grade chronic inflammation similar to that seen in chronic diseases that are also risk factors of musculoskeletal disease (Abate et al. 2013). For example, diabetic patients have a higher incidence of tendon injuries (Ranger et al. 2016; Lui 2017) resulting from reduced biomechanical properties and an altered tissue structure, (Snedeker 2016; Nichols et al. 2020; Lu et al. 2020) which is possibly due to abnormal behavior of tenocytes and tendon stem/progenitor cells in response to high glucose levels (Tsai et al. 2013; Ueda et al. 2018; Nichols et al. 2020; Lu et al. 2020).

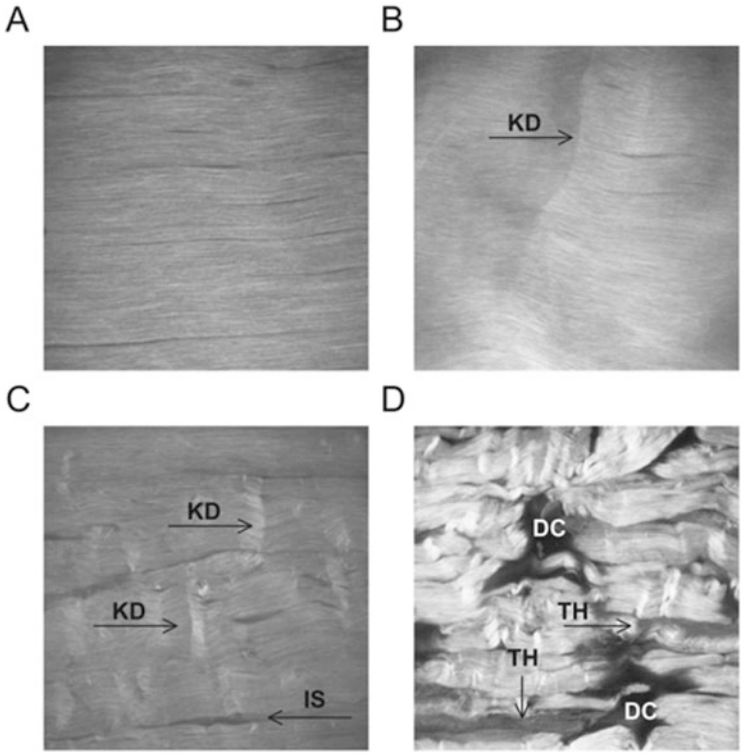

Fig. 11.1 Disorganization of collagen fibers with fatigue loading. (**a**) Highly aligned collagen fibers in the unloaded tendon. (**b**) Isolated kinked fiber deformations (KD) in a low fatigue tendon. (**c**) Density of kinked fiber deformations increases in moderately fatigued tendons. Widening of inter-fiber space (IS) is also observed. (**d**) Significant matrix disruption, fiber disorganization, and greater widening of inter-fiber space are observed. FOV = 400 mm. Adapted with permission from Fung et al. (2010)

While biologic factors likely contribute to tendon injuries, overuse (i.e., fatigue loading) is the most well-established cause of tendinopathy. Cases are commonly seen in people who use the same muscles repeatedly, such as athletes and physical workers (Werner et al. 2005; Forde et al. 2005; Kujala et al. 2005). Fatigue loading has been shown to directly cause the accumulation of tissue damage and collagen fiber disorganization (Fig. 11.1), which is dependent on the loading magnitude, cycle number, and tendon type/function (Wang et al. 1995; Gibbon et al. 1999; Schechtman and Bader 2002; Nakama et al. 2005; Cook and Purdam 2009; Fung et al. 2010; Herod et al. 2016; Szczesny et al. 2018; Zitnay et al. 2020). In addition to direct mechanical damage, fatigue loading has also been shown to induce many of the biological changes associated with tendinopathy, including the synthesis of catabolic proteases and inflammatory cytokines as well as the formation of abnormal (e.g., calcium, lipid, and cartilaginous) matrix deposits. Specifically, tendons in animals subjected to intense treadmill running show increased expression of

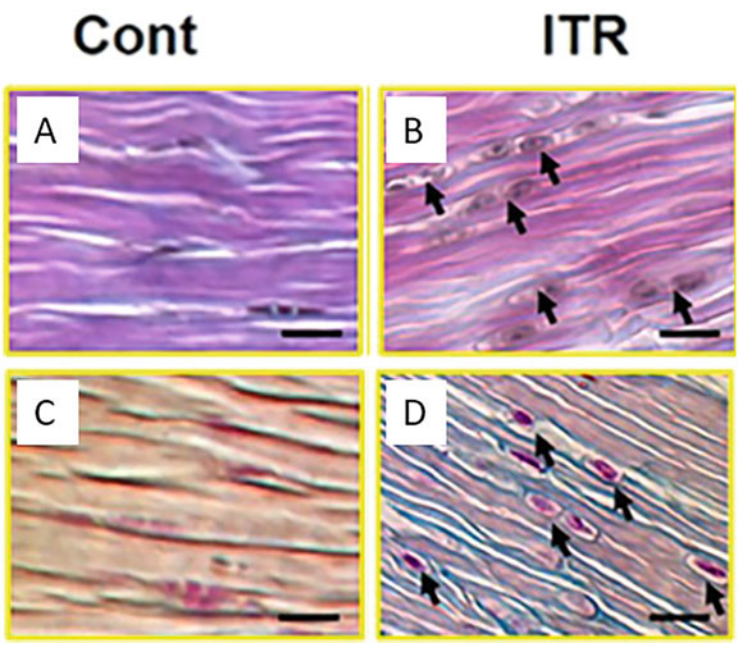

Fig. 11.2 Degenerative changes in mouse Achilles tendons with excessive treadmill running. (**a**) H & E staining of tendons from cage control mice does not show round cells with cavities. (**b**) Intensive treadmill running (ITR) creates numerous chondrocyte-like cells with cavities. (**c**) Alcian blue staining shows minimal GAG presence in tendons from cage control mice (**d**) Strong Alcian blue staining for GAGs and presence of chondrocyte-like cells in intensive treadmill running group. Bar: 25 μm. Adapted with permission from Zhao et al. (2019)

chondrogenic (collagen II, aggrecan, SOX9), adipogenic (LPL, PPARγ), and osteogenic (RUNX2, Osterix) genes, the appearance of round chondrocyte-like cells, GAG deposition, hypercellularity, increased vascularity, and reduced mechanical properties (Fig. 11.2) (Archambault et al. 2007; Glazebrook et al. 2008; Ng et al. 2011; Zhang and Wang 2013; Zhao et al. 2019). Increases in gene expression and protein synthesis of matrix metalloproteinases (MMP13) and inflammatory cytokines (IL1-β) were also observed in an *in vivo* fatigue loading model of the rat patellar tendon (Sun et al. 2008). While these animal studies clearly link fatigue loading and abnormal mechanical stimuli with tendon degeneration, it is unclear whether the degenerative cellular response to fatigue loading is due to excessive or reduced mechanical stimuli (Arnoczky et al. 2007a).

In vitro cell stretching experiments provide the clearest demonstration of the effect of mechanical over-stimulation on tendon cells. Typically, these studies involve isolating tenocytes or tendon/stem progenitor cells, seeding them onto flexible 2D substrates, and stretching the substrates at various strain levels (Robbins et al. 1997; Banes et al. 1999; Skutek et al. 2001, 2003; Archambault et al. 2002; Wang et al. 2003; Yang et al. 2005; Zhang and Wang 2013; Jones et al. 2013). In

response to mechanical stimulation, these studies found that tendon cells increase expression and synthesis of inflammatory mediators, like PGE2, COX2, IL1-β, and IL-6, as well as matrix metalloproteinases MMP1 and MMP3 (Almekinders et al. 1993; Skutek et al. 2001; Wang et al. 2003; Tsuzaki et al. 2003; Yang et al. 2005). Furthermore, excessive stretching of tendon stem/progenitor cells leads to an increase in the production of non-tenogenic (i.e., osteogenic, adipogenic, and chondrogenic) genes, which supports the hypothesis that excessive loading of tendon cells causes the accumulation of abnormal tissue deposits (Zhang and Wang 2013). However, these in vitro conditions do not replicate the mechanical stimulation, cell-matrix interactions, and cell-cell communication that is present in the native tendon microenvironment. In contrast to a dense cell monolayer, tendons are sparsely populated with cells that are enveloped by a pericellular matrix, which physically separates the cells from the surrounding collagen fibrils (Bray et al. 1993; Senga et al. 1995; Keene et al. 1998; Ritty et al. 2003). This pericellular matrix not only reduces the mechanical stimuli that cells experience *in situ* (Puxkandl et al. 2002; Screen et al. 2004; Rigozzi et al. 2011; Szczesny and Elliott 2014) but also influences the biological behavior of tendon cells (Bi et al. 2007). To overcome these artificial in vitro conditions, tissue explant models have been used to study the response of tendons to fatigue loading. These studies also found an increase in levels of proteinases (MMP1, MMP3, MMP13) and inflammatory markers (COX2, IL-6, PGE2) with fatigue loading (Devkota and Weinhold 2010; Spiesz et al. 2015; Thorpe et al. 2015). This agreement between 2D in vitro and tissue explant models suggests that excessive mechanical loading may indeed be responsible for the degenerative response of tendon cells observed with tendon overuse.

However, there is also substantial data suggesting that the degenerative changes associated with tendon overuse are due to a lack of mechanical stimulation. Fatigue damage to the extracellular matrix likely reduces the strains transmitted to tendon cells (Arnoczky et al. 2007a; Ros et al. 2019). This cellular unloading increases the production of catabolic proteases (Lavagnino and Arnoczky 2005; Lavagnino et al. 2006) that induce further deterioration of the pericellular matrix, thereby increasing cellular unloading and leading to progressive tendon degeneration. This hypothesis is supported by in vitro stress deprivation experiments of tendons, which causes an upregulation of MMP3 and MMP13 (Arnoczky et al. 2007b, 2008; Leigh et al. 2008), a decrease in the level of tissue inhibitors of metalloproteinases (TIMPs) (Gardner et al. 2008), and reduced collagen synthesis (Dideriksen et al. 2017). This overall increase in catabolic activity leads to a decrease in the tissue mechanical properties by approximately 50% in rat tail tendon fascicles and canine flexor digitorum profundus tendons (Hannafin et al. 1995; Arnoczky et al. 2007b). Furthermore, stress deprivation leads to degenerative changes, including tendon cell apoptosis (Egerbacher et al. 2008) and production of large, GAG-rich proteoglycans (Egerbacher et al. 2021). Very similar results have also been found from *in vivo* animal and clinical studies of tendon unloading or immobilization, including large reductions in tissue mechanical properties (Almeida-Silveira et al. 2000; Kubo et al. 2004; Reeves et al. 2005; de Boer et al. 2007), tendon cell apoptosis (Kawabata et al. 2009), and increased expression of inflammatory cytokines (TNF-α) and

chondrogenic factors (TGF-β) (Uchida et al. 2005). Finally, an adaptive mechanobiological model incorporating these data has shown that cellular unloading due to fatigue-induced tissue damage can explain the degenerative changes associated with tendinopathy (Mehdizadeh et al. 2017). Therefore, it is still unclear whether mechanical over-stimulation, under-stimulation, or potentially both, is responsible for fatigue-induced tendon degeneration. Identifying the underlying mechanisms driving tendon degeneration is essential for preventing tendon injury and developing novel therapies for restoring tissue structure and function.

11.2.2 Treatment Opportunities Via Regenerative Rehabilitation

Many treatment options exist for tendinopathy, including NSAIDs, corticosteroids, platelet-rich plasma, glyceryl trinitrate, extracorporeal shockwave therapy, low-level laser therapy, and surgery (Millar et al. 2021). In general, NSAIDs and corticosteroids are effective for short-term pain relief (Riley 2008), and surgery has mixed success rates (Paavola et al. 2000), whereas most other treatments are unproven. For the purpose of this chapter, we will focus on exercise, specifically eccentric exercise, since it is the most relevant to regenerative rehabilitation and is the most effective conservative approach for treating tendinopathy (Alfredson et al. 1998; Cannell et al. 2001; Fahlström et al. 2003; Ohberg et al. 2004; Jonsson and Alfredson 2005; Kongsgaard et al. 2006; Riley 2008; Girgis and Duarte 2020; Millar et al. 2021).

For several decades, eccentric exercise (i.e., lengthening of active/force-generating muscle) has been a popular first-line treatment for tendinopathy given that it is noninvasive and has no major adverse side effects. Furthermore, eccentric exercise has been shown to significantly reduce pain and improve the recovery of functional strength in symptomatic Achilles tendons to preinjury levels in athletes (Alfredson et al. 1998). This initial study was replicated in a larger group of patients (Fahlström et al. 2003), and a comparison of different rehabilitation techniques demonstrated that eccentric exercise is better than concentric exercise (Mafi et al. 2001). Importantly, not only does eccentric exercise reduce pain, but it also restores the tissue structure (Ohberg et al. 2004). Finally, similar success with slow eccentric exercises has also been observed for patellar tendinopathy (Jonsson and Alfredson 2005; Lee et al. 2020).

Despite the popularity and established clinical success of eccentric exercise, the mechanism underlying its effectiveness has not been clearly established (Maganaris et al. 2017). One hypothesis for the beneficial effects of eccentric exercise is that the larger tensile forces produced by eccentric muscle contraction may lead to higher tendon stiffness (Malliaras et al. 2013a). However, another study found no significant difference in peak tendon force or changes in tendon length between eccentric and concentric exercises (Rees et al. 2008). Instead, the authors discovered high-frequency oscillations in tendon force during eccentric exercise that were absent in

concentric exercises, suggesting that these force fluctuations provide an important remodeling stimulus to tendon cells. Another hypothesis is that eccentric exercise increases tendon cell metabolism and blood flow. Specifically, eccentric exercise produces greater oxyhemoglobin, total hemoglobin, and saturated oxygen in Achilles' tendons compared to concentric exercises (Kubo 2015). One final hypothesis is that eccentric exercise creates greater water exudation compared to concentric exercise due to a larger decrease in tendon volume and that this might also be an important stimulus responsible for healing (Grigg et al. 2009). However, it is unknown whether any of these differences observed between eccentric and concentric exercise are responsible for the differential clinical benefits.

More recent studies suggest that the benefits of eccentric exercise are not universal and that individual factors like pain, patient compliance, and personal performance should be considered to optimize treatment protocols (Millar et al. 2021). In fact, progressive tendon loading [succession of isometric (static), isotonic (dynamic), energy-storage (explosive), and sport-specific exercises] might be more effective than eccentric exercises for treating patellar tendinopathy (Breda et al. 2020). Additionally, heavy slow resistance training was equivalent to (if not better than) eccentric exercise in terms of neuromuscular performance for both Achilles and patellar tendons (Malliaras et al. 2013b). Moreover, it was associated with reduced vascularity, reduced tendon diameter, and greater collagen turnover. Finally, isometric loading was also effective in reducing pain in patients with patellar tendinopathy (Malliaras et al. 2013a). However, most of this evidence is qualitative, and personalization of exercise protocols is complicated by the lack of knowledge regarding the association between exercise therapy outcomes and individual patient factors, like BMI, sex, and baseline pain levels (Färnqvist et al. 2020).

It is also important to note that mechanical loading can not only treat tendinopathy but is also essential for maintaining healthy tendons and, therefore, can prevent disease development. Moderate tendon loading is beneficial for anabolic gene production, collagen turnover, and maintaining tendon mechanical properties (Langberg et al. 2001; Wang et al. 2012). Even short-duration exercise upregulates key tenogenic genes, such as scleraxis, tenomodulin, collagen I, and mechano-growth factor (Mendias et al. 2012; Zhang and Wang 2013; Thampatty and Wang 2018). Moderate loading also increases tendon tensile strength (Viidik 1967) and offsets the decline in tendon material properties observed with aging (Narici and Maganaris 2006). Interestingly, similar to the treatment of degenerated tendons, eccentric loading may also provide greater preventative benefits in healthy tendons. Specifically, in rats subjected to both concentric and eccentric loading, the triceps, patellar, and Achilles tendons accumulated more collagen in the eccentric group (Kaux et al. 2013). Therefore, while inappropriate mechanical stimuli can induce tissue degeneration and ultimately tendon injury, proper tendon loading is essential for maintaining tissue health and treating tendinopathy.

11.3 ACL Reconstructions

11.3.1 Repetitive Mechanical Loading as Potential Cause of ACL Rupture

The prevailing medical and scientific perspective is that noncontact ACL injuries are predominantly a "single traumatic event" caused by an acute overload of an otherwise healthy ligament (Boden et al. 2000). However, a couple recent studies suggest that repetitive subfailure loading creates microtrauma and structural tissue damage at multiple length scales and predisposes the ACL to injury (Lipps et al. 2013; Chen et al. 2019). Specifically, repetitive loading of cadaveric knees under conditions simulating single-legged pivot landings leads to rapid (i.e., low cycle number) fatigue failure of human ACLs (Lipps et al. 2013). Additionally, infrared spectroscopy of the fatigue-loaded ligaments revealed a strong 1740 cm^{-1} feature that is indicative of disrupted collagen molecules (Chen et al. 2019). This interpretation was confirmed by increased labeling of loaded ACLs (compared to controls) with a collagen hybridizing peptide that targets denatured collagen molecules. Furthermore, the same study found a significant increase in disruption of collagen fibrils and fibers in ACLs from tested knees using atomic force microscopy and second harmonic generation imaging. Consistent with these data, abnormal mechanical loading of the ACL is also suggested to induce mucoid tissue degeneration within the ACL (Yigman and Toprak 2020). Specifically, mucoid degeneration of the ACL is prevalent in patients with trochlear dysplasia (TD), which is a developmental condition characterized by an abnormally shallow femoral trochlea. A normal trochlear groove is essential for the extensor mechanism function of the knee (Botchu et al. 2013). Therefore, it is hypothesized that knee instability in patients with TD may increase ACL loading which leads to structural damage and inadequate repair (Yigman and Toprak 2020). Together, these data suggest that noncontact ACL injuries may be due to fatigue damage and the accumulation of microtrauma and progressive tissue degeneration, similar to the onset of tendon injuries.

11.3.2 Role of Mechanical Loading in Success of Allograft ACL Reconstruction

Reconstruction of the ACL is the gold-standard treatment following rupture given the lack of spontaneous tissue repair and risk of secondary knee damage (Dunn et al. 2004; Cristiani et al. 2020; Mortazavi et al. 2021). Graft options for reconstructing the ACL come in the form of autogenous or allogeneic tendon. Across the general patient population, allografts exhibit higher failure rates than autografts (Kaeding et al. 2011); however, this is likely due to the detrimental effect of irradiation that is sometimes used to sterilize allografts (Schwartz et al. 2006). Indeed, when irradiated grafts are excluded from large-scale analyses, no significant difference is observed

between autografts and non-irradiated allografts (Krych et al. 2008; Zeng et al. 2016; Kan et al. 2016). Nevertheless, rerupture rates of non-irradiated allografts are 3–4 times higher than autografts in young (<35 years) active individuals (Singhal et al. 2007; Barrett et al. 2010; Bottoni et al. 2015), who account for nearly 70% of all ACL reconstructions (Buller et al. 2015; Sanders et al. 2016). While the reason for increased allograft failure in young active patients is unclear, there is evidence suggesting that it may be due to impaired post-surgical graft remodeling.

Following surgical implantation, all tendon grafts undergo a process termed "ligamentization" in which the graft structure is substantially remodeled toward a tissue resembling the native ACL (Amiel et al. 1986; Scheffler et al. 2008b; Claes et al. 2011; Giordano et al. 2015). This remodeling process can be described in three key phases: an early healing phase, a proliferation phase, and a final ligamentization phase. In animal models, the early healing phase occurs from the time of the ACL reconstruction up until around the 4th postoperative week (Scheffler et al. 2008b). This period is marked by graft necrosis, hypocellularity, structural degradation, and a drop in mechanical properties (Bosch and Kasperczyk 1992; Papageorgiou et al. 2001). Additionally, the grafts slowly become revascularized and infiltrated by host cells (Kleiner et al. 1986; Bosch and Kasperczyk 1992). In the proliferation phase, which occurs roughly between the 4th and 12th postoperative week in animals, the graft mechanical properties stop declining and begin to recover due to an increase in cellular activity and remodeling of the extracellular matrix (Curtis et al. 1985; Grana et al. 1994; Scheffler et al. 2008b; Dong et al. 2015). Specifically, the proliferation phase exhibits increased cellularity, increased collagen synthesis, and the formation of a more organized tissue structure (Kleiner et al. 1989; Spindler et al. 1996; Goradia et al. 2000; Giordano et al. 2015). The proliferation phase gradually transitions into the ligamentization phase, which encompasses the long-term process of remodeling the graft into a tissue that more closely resembles the structure and function of the native ACL. In particular, the reconstructed tissue obtains moderate amounts of collagen III fibrils, GAGs, and collagen crosslinks that are comparable to levels observed in ACLs and distinct from the original tendon graft (Amiel et al. 1986; Lane et al. 1993; Ng et al. 1995). Additionally, the cellularity and vascularization of the graft return to normative values (Arnoczky et al. 1982). Nevertheless, even after several years, the tissue structure and function never completely replicate that of the native ACL. In both allografts and autografts, the reconstruction contains an altered collagen crimp pattern (Weiler et al. 2002) and a high percentage of small-diameter fibrils (Jackson et al. 1993), which is consistent with inferior mechanical properties compared to the ACL (Shino et al. 1984; Bosch and Kasperczyk 1992; Jackson et al. 1993; Ng et al. 1995; Weiler et al. 2001). The same basic process is believed to occur in humans, albeit with less robust tissue remodeling that occurs over a longer timeframe (Scheffler et al. 2008b; Claes et al. 2011).

While both graft types undergo the same post-surgical remodeling process, allografts recover their mechanical properties more slowly, which leaves lasting structural and mechanical deficits. As mentioned above, one of the initial effects of graft remodeling is the breakdown of the collagen fibrils into small diameter structures, which is less pronounced in allograft reconstructions (Jackson et al.

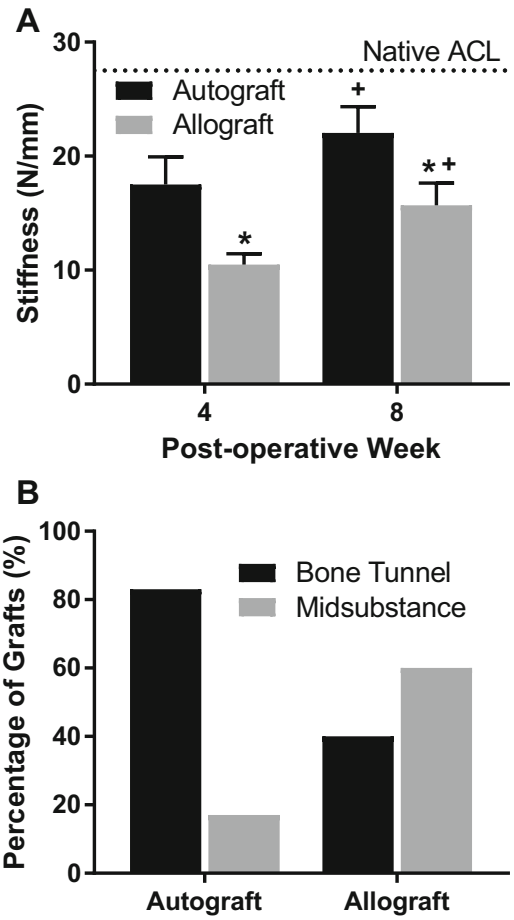

Fig. 11.3 Impaired allograft remodeling. (**a**) Allografts have inferior mechanical properties compared to autografts throughout the post-surgical remodeling period. * $p<0.05$ compared to autograft, + $p<0.05$ compared to 4 weeks. Data adapted from Wang et al. (2017a). (**b**) Allografts often fail within the graft tissue midsubstance, which rarely occurs in autograft reconstructions. Data adapted from Magnussen et al. (2012)

1993). Additionally, reorganization of the tissue structure during later phases of remodeling is delayed in allografts (Scheffler et al. 2008a), which also exhibit higher levels of MMP activity that stretch into the proliferation phase (Wang et al. 2017a). These structural deficits are consistent with the inferior stiffness and strength of allografts compared to autografts throughout the remodeling period (Fig. 11.3) (Jackson et al. 1993; Dustmann et al. 2008; Wang et al. 2017a). The impaired remodeling of allografts is also consistent with clinical data demonstrating that allografts tend to fail within the tissue midsubstance whereas autografts fail almost exclusively at the bone tunnel insertions, indicating that the tissue midsubstance is the weak point for allograft reconstructions (Magnussen et al. 2012). Together, these data suggest that impaired graft remodeling is responsible for increased rerupture of allograft ACL reconstructions.

One explanation for impaired allograft remodeling is a heightened inflammatory response to the exogenous tissue. While this may seem obvious, evidence for the

hypothesis that allografts generate an increased inflammatory response that leads to impaired graft remodeling is mixed. Research suggests that a single freeze–thaw cycle is sufficient to nearly eliminate the antigenicity of tendon allografts (Minami et al. 1982). This is because the freezing process kills the cells within the graft (likely disrupting the cell surface proteins responsible for immune cell activation) and because collagen itself within the extracellular matrix is weakly antigenic (Steffen et al. 1968). While some studies still demonstrate a measurably increased immune response with allografts compared to autografts (Jackson et al. 1993; Yang et al. 2012), much of this difference could be due to graft type (bone-tendon-bone versus free tendon grafts) (Biau et al. 2006) and sterilization (i.e., ethylene oxide) (Schwartz et al. 2006; Bottoni et al. 2015) rather than an intrinsic property of allografts. Indeed, several studies demonstrate that there is no heightened immunological response with allografts (Arnoczky et al. 1982; Shino et al. 1984; Curtis et al. 1985; Fromm et al. 1996; Scheffler et al. 2008a). Nevertheless, ACL reconstruction failure is correlated with increased numbers of pro-inflammatory cells (e.g., M1 macrophages and Th17 cells) (Hays et al. 2008; Yang et al. 2012; Song et al. 2017). Therefore, while more research is necessary to conclusively demonstrate immunological differences between graft types, it is possible that a heightened immune response to allograft reconstructions is responsible for impaired remodeling. However, this still does not explain why allograft failure is increased compared to autografts particularly in young active patients (Singhal et al. 2007; Barrett et al. 2010; Bottoni et al. 2015).

Another possible reason for poor allograft outcomes is that allografts have a deficient or abnormal response to increased mechanical stimuli present during rehabilitation and post-surgical remodeling. In general, ACL reconstruction remodeling is highly sensitive to mechanical loading. Animal studies demonstrate that stress shielding ACL reconstructions significantly impair structural remodeling and reduces the tissue mechanical properties in a dose-dependent manner (Ohno et al. 1993). Additionally, excessive mechanical loading (induced by reducing the cross-section of the grafts) also leads to inferior mechanical properties (Tohyama and Yasuda 2002). These data suggest that there is an optimal amount of mechanical loading during graft remodeling and that too much or too little loading can be detrimental to the overall success of the graft. These animal studies are also supported by clinical data indicating that high graft bending angles, which put greater stress on the reconstruction, impairs tissue remodeling (Tashiro et al. 2017). Furthermore, increased patient activity (and presumably greater graft loading) amplifies outcome differences between allograft and autograft reconstructions. Specifically, highly active patients (e.g., athletes, military service members) with allograft reconstructions are almost seven times more likely to rupture their reconstruction (controlling for age and gender) than low activity patients, whereas there was no effect of patient activity with autografts (Borchers et al. 2009; Pallis et al. 2012). Given that allografts and autografts have similar mechanical properties at the time of implantation (Jackson et al. 1988; Jung et al. 2011; Suto et al. 2012; Arnout et al. 2013), these clinical data strongly suggest that allograft remodeling is negatively affected by mechanical stimuli and that this is responsible for their increased failure. Consistent with these findings, animal studies demonstrate that

allograft reconstructions contain proportionally fewer myofibroblasts compared to autografts (Dustmann et al. 2008; Scheffler et al. 2008b), which are highly productive cells responsible for the remodeling of fibrous tissues (Bell et al. 2018). Since myofibroblast differentiation is induced by mechanical loading (Tomasek et al. 2002), these data further suggest that impaired allograft remodeling is due to a deficient response to mechanical stimuli.

11.3.3 Opportunities for Improving Allograft Reconstruction Via Regenerative Rehabilitation

Understanding the importance of mechanical stimuli on allograft remodeling and failure offers many new opportunities for improving their performance. Of course, rehabilitation protocols can be tailored to meet the patient's goals while also protecting the reconstruction. However, the ideal option would be to augment allograft reconstructions such that they have a positive (rather than negative) response to increased mechanical stimuli. Intriguingly, the interconnection between fibroblast mechanobiology, immune cell polarization, and mechanical loading suggests that these may be effective targets for allograft augmentation strategies. For example, in vitro co-culture experiments show that macrophages influence myofibroblast differentiation and survival (Ullm et al. 2020; Sapudom et al. 2020). Specifically, co-culture of fibroblasts with anti-inflammatory M2 macrophages significantly activates myofibroblast differentiation and the secretion of extracellular proteins (Sapudom et al. 2020). Furthermore, M2 macrophages themselves can differentiate into myofibroblasts through a process called "macrophage to myofibroblast transition" (Wang et al. 2016, 2017b), suggesting that M2 macrophages may improve graft remodeling. In contrast, pro-inflammatory M1 macrophages are associated with reconstruction failure (Song et al. 2017). Consistent with poor allograft outcomes with increased mechanical stimuli, mechanical loading of ACL reconstructions leads to increased macrophage recruitment to the graft (Dagher et al. 2009; Brophy et al. 2011), which is associated with impaired graft-to-bone healing (Hays et al. 2008). Therefore, these data collectively suggest that efforts to enhance macrophage polarization to anti-inflammatory phenotypes may increase myofibroblast density within allografts and improve their remodeling response to mechanical loading.

Additional data linking tissue structure with immune cell polarization also suggest that impaired allograft remodeling with increased mechanical loading may be due to a runaway biological feedback loop (Fig. 11.4a). Specifically, increased recruitment of pro-inflammatory (i.e., Th17) immune cells to allografts (Yang et al. 2012) may delay graft remodeling and increase tissue disorganization (Scheffler et al. 2008a). Based on in vitro studies, this disorganized tissue environment could promote pro-inflammatory M1 macrophage polarization, which would be further enhanced by increased mechanical loading (Schoenenberger et al. 2020).

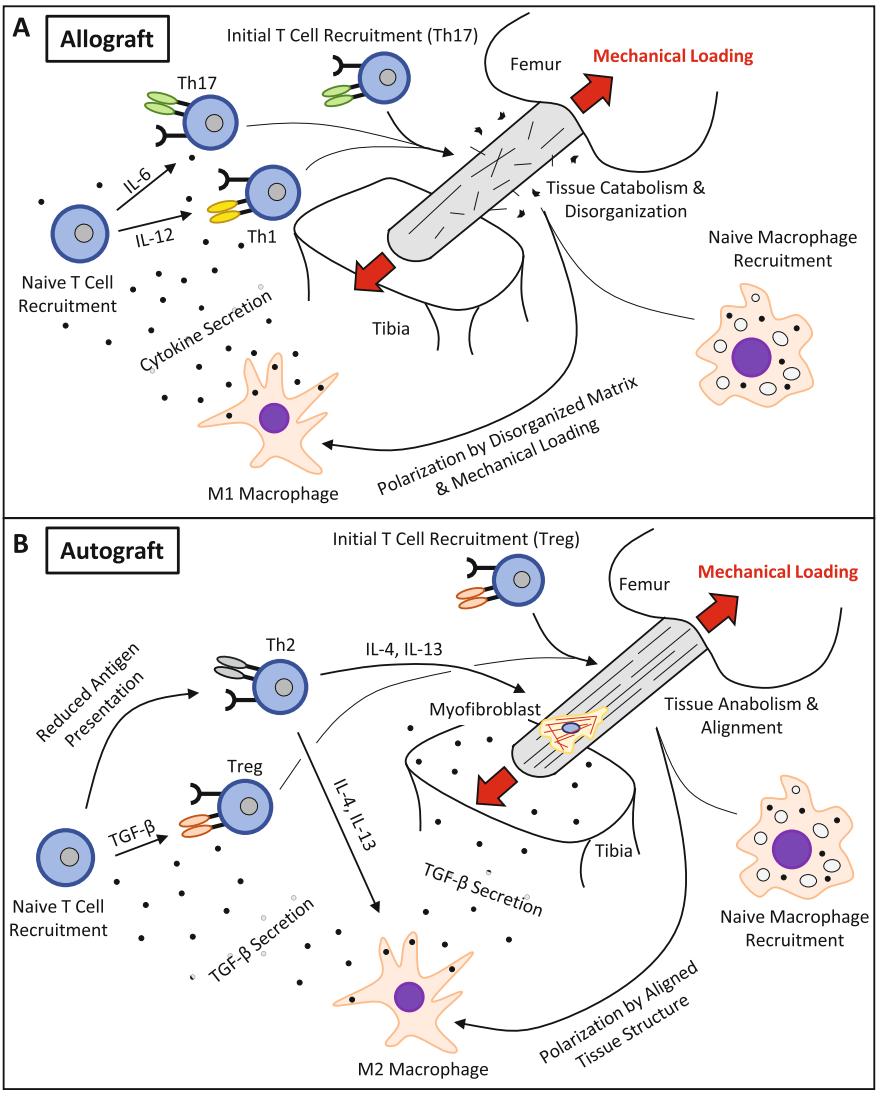

Fig. 11.4 Biological feedback loops potentially driving differential remodeling of allograft and autograft ACL reconstructions. (**a**) In allografts, an initial increased recruitment of pro-inflammatory immune cells may delay graft remodeling and increase tissue disorganization through a catabolic feedback loop. (**b**) In autografts, an increased recruitment of anti-inflammatory cells may promote the formation of a more aligned tissue structure during initial graft remodeling through an anabolic feedback loop

Macrophage secretion of IL-12 and IL-6 will in turn cause newly recruited T cells to differentiate into pro-inflammatory Th1 and Th17 cells, respectively (Luckheeram et al. 2012; Italiani and Boraschi 2014), thereby completing the vicious cycle and

leading to impaired allograft remodeling with increased mechanical loading. In contrast, a beneficial feedback loop may explain why autograft remodeling remains productive with increased mechanical loading (Fig. 11.4b). Specifically, an increased recruitment of anti-inflammatory immune cells [i.e., regulatory T cells (Treg)] to autografts (Yang et al. 2012) will promote the formation of a more aligned tissue structure during initial graft remodeling (Scheffler et al. 2008a), which could enhance M2 macrophage polarization (Schoenenberger et al. 2020). The resulting increase in TGF-β secretion would increase myofibroblast and Treg differentiation (Hays et al. 2008; Luckheeram et al. 2012; Sapudom et al. 2020). This overall anti-inflammatory environment would serve to reduce antigen presentation and T cell receptor strength, thereby promoting the differentiation of arriving naive T cells into anti-inflammatory Th2 phenotypes (Bhattacharyya and Feng 2020). Secretion of IL-4 and IL-13 from these Th2 cells would then reinforce M2 macrophage polarization and amplify collagen synthesis by myofibroblasts (Barron and Wynn 2011), which would be further amplified by mechanical loading (Lee et al. 2005; Schoenenberger et al. 2020). Whether these feedback loops actually operate during ACL reconstruction remodeling is unknown; however, they may potentially offer new insight into the differential response of allografts and autografts to mechanical stimuli.

Another potential therapeutic strategy is the inhibition or management of MMP activity. In general, MMPs are necessary for reconstruction remodeling since they break down the initial tendon graft structure so that a more ligamentous tissue can be formed (Bramono et al. 2004). Indeed, both autografts and allografts exhibit increased MMP synthesis compared to the native ACLs (Wang et al. 2017a). However, excessive MMP activity impairs graft integration within the bone tunnels (Demirag et al. 2005) and could also prevent the recovery of the tissue mechanical properties during the proliferation and ligamentization remodeling phases. As mentioned in the previous section on tendinopathy, tendons increase their production of MMPs in response to fatigue loading (Tsuzaki et al. 2003; Yang et al. 2005; Wiesinger et al. 2020). Consistent with the hypothesis that allografts have an abnormal (i.e., catabolic) response to increased mechanical stimuli, allograft reconstructions exhibit increased synthesis of MMPs compared to autografts throughout the remodeling period (Wang et al. 2017a). One reason for this could be an increased concentration of pro-inflammatory cytokines (e.g., IL1-β, PGE2), which are upregulated with excessive tendon loading and activate MMP expression (Tsuzaki et al. 2003; Liu et al. 2010). This would fit with the hypothesis of a counterproductive feedback loop between mechanical loading and the immune response to allografts proposed in the preceding paragraph (Fig. 11.4a). However, MMP expression is also downstream of Wnt signaling (Wu et al. 2007), and allograft reconstructions have been shown to contain elevated levels of Wnt and reduced levels of the Wnt pathway inhibitor adenomatous polyposis coli (APC) (Wang et al. 2017a). These data suggest that Wnt/β-catenin may be another potential target for improving allograft remodeling in young active patients.

One final consideration is the effect of sex and sex hormones on ACL reconstruction remodeling. It is well known that the incidence of ACL injury is strongly

dependent on sex; females who participate in sports that require cutting and/or jumping have a four to six times greater chance of tearing their ACL when compared to males (Lee et al. 2015). While this could be potentially explained by the fact that female ACLs have inferior mechanical properties (Jackson et al. 1993), there is also substantial evidence that the increased risk of ACL rupture is due to levels of sex hormones. Specifically, 70% of ACL injuries in women occur within the first two days of the menstrual cycle (Slauterbeck et al. 2002). Furthermore, ACL stiffness is significantly correlated with levels of estradiol, estriol, estrogen, and progesterone (Romani et al. 2003), which is consistent with data from in vitro constructs demonstrating that estrogen inhibits lysyl oxidase and impairs mechanical function (Lee et al. 2015). Therefore, it is highly likely that levels of sex hormones also strongly affect ACL reconstruction remodeling. Additionally, sex is an important factor to consider when choosing the appropriate donor for allograft reconstructions; ACL reconstructions using allografts harvested from females over 50 years old were seven times more likely to fail compared to allografts from younger women or men of any age (Shumborski et al. 2020). The authors of this study hypothesized that this could be explained by the loss of estrogen in older women leading to sex-related changes in elastin and collagen metabolism. Therefore, levels of sex hormones in the patient and the donor are important considerations for optimizing allograft reconstruction remodeling.

11.4 Conclusions

As presented in this chapter, there are many parallels between tendon and ligament injuries, which is not surprising given their similar structure and function. Interestingly, by considering both tissues in a single review, it is possible to fill in the gaps of our understanding and offer novel hypotheses regarding the role of mechanical stimuli on their injury and repair. The depth of information regarding the effect of abnormal mechanical stimuli on tendon degeneration provides context and support to the relatively recent idea that ACL tears are a result of subfailure damage accumulation and degeneration. Additionally, the decades of study on post-surgical remodeling of ACL reconstructions help inform the role of mechanical loading (e.g., eccentric loading) on the remodeling of degenerative tendons. In both cases, the mechanisms underlying the effects of mechanical stimuli on tissue injury and repair need to be elucidated so that injury prevention and treatment programs can be optimized. However, the data clearly indicate that there is a strong potential for the use of regenerative rehabilitation to improve clinical outcomes by enhancing constructive cellular activity and tissue remodeling through the manipulation of mechanical stimuli. Future research coupling clinical and animal studies with novel imaging and characterization techniques will hopefully lay the mechanistic foundation for future therapies.

References

Abate M, Schiavone C, Salini V, Andia I (2013) Occurrence of tendon pathologies in metabolic disorders. Rheumatol Oxf Engl 52:599–608. https://doi.org/10.1093/rheumatology/kes395

Alfredson H, Pietilä T, Jonsson P, Lorentzon R (1998) Heavy-load eccentric calf muscle training for the treatment of chronic Achilles tendinosis. Am J Sports Med 26:360–366. https://doi.org/10.1177/03635465980260030301

Almeida-Silveira MI, Lambertz D, Pérot C, Goubel F (2000) Changes in stiffness induced by hindlimb suspension in rat Achilles tendon. Eur J Appl Physiol 81:252–257. https://doi.org/10.1007/s004210050039

Almekinders LC, Banes AJ, Ballenger CA (1993) Effects of repetitive motion on human fibroblasts. Med Sci Sports Exerc 25:603–607

Amiel D, Kleiner JB, Roux RD et al (1986) The phenomenon of "ligamentization": anterior cruciate ligament reconstruction with autogenous patellar tendon. J Orthop Res Off Publ Orthop Res Soc 4:162–172. https://doi.org/10.1002/jor.1100040204

Andres BM, Murrell GAC (2008) Treatment of tendinopathy: what works, what does not, and what is on the horizon. Clin Orthop 466:1539–1554. https://doi.org/10.1007/s11999-008-0260-1

Archambault J, Tsuzaki M, Herzog W, Banes AJ (2002) Stretch and interleukin-1beta induce matrix metalloproteinases in rabbit tendon cells in vitro. J Orthop Res Off Publ Orthop Res Soc 20:36–39. https://doi.org/10.1016/S0736-0266(01)00075-4

Archambault JM, Jelinsky SA, Lake SP et al (2007) Rat supraspinatus tendon expresses cartilage markers with overuse. J Orthop Res Off Publ Orthop Res Soc 25:617–624. https://doi.org/10.1002/jor.20347

Arnoczky SP, Tarvin GB, Marshall JL (1982) Anterior cruciate ligament replacement using patellar tendon. An evaluation of graft revascularization in the dog. J Bone Joint Surg Am 64:217–224

Arnoczky SP, Lavagnino M, Egerbacher M (2007a) The mechanobiological aetiopathogenesis of tendinopathy: is it the over-stimulation or the under-stimulation of tendon cells? Int J Exp Pathol 88:217–226. https://doi.org/10.1111/j.1365-2613.2007.00548.x

Arnoczky SP, Lavagnino M, Egerbacher M et al (2007b) Matrix metalloproteinase inhibitors prevent a decrease in the mechanical properties of stress-deprived tendons: an in vitro experimental study. Am J Sports Med 35:763–769. https://doi.org/10.1177/0363546506296043

Arnoczky SP, Lavagnino M, Egerbacher M et al (2008) Loss of homeostatic strain alters mechanostat "set point" of tendon cells in vitro. Clin Orthop 466:1583–1591. https://doi.org/10.1007/s11999-008-0264-x

Arnout N, Myncke J, Vanlauwe J et al (2013) The influence of freezing on the tensile strength of tendon grafts : a biomechanical study. Acta Orthop Belg 79:435–443

Arya S, Kulig K (2010) Tendinopathy alters mechanical and material properties of the Achilles tendon. J Appl Physiol Bethesda Md 1985 108:670–675. https://doi.org/10.1152/japplphysiol.00259.2009

Badley EM, Tennant A (1993) Impact of disablement due to rheumatic disorders in a British population: estimates of severity and prevalence from the Calderdale rheumatic disablement survey. Ann Rheum Dis 52:6–13. https://doi.org/10.1136/ard.52.1.6

Banes AJ, Horesovsky G, Larson C et al (1999) Mechanical load stimulates expression of novel genes in vivo and in vitro in avian flexor tendon cells. Osteoarthritis Cartilage 7:141–153. https://doi.org/10.1053/joca.1998.0169

Barrett GR, Luber K, Replogle WH, Manley JL (2010) Allograft anterior cruciate ligament reconstruction in the young, active patient: tegner activity level and failure rate. Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc N Am Int Arthrosc Assoc 26:1593–1601. https://doi.org/10.1016/j.arthro.2010.05.014

Barron L, Wynn TA (2011) Fibrosis is regulated by Th2 and Th17 responses and by dynamic interactions between fibroblasts and macrophages. Am J Physiol Gastrointest Liver Physiol 300:G723–G728. https://doi.org/10.1152/ajpgi.00414.2010

Bedi A, Trinh TQ, Olszewski AM et al (2020) Nonbiologic injections in sports medicine. JBJS Rev 8:e0052. https://doi.org/10.2106/JBJS.RVW.19.00052

Bell R, Gendron NR, Anderson M et al (2018) A potential new role for myofibroblasts in remodeling of sub-rupture fatigue tendon injuries by exercise. Sci Rep 8:8933. https://doi.org/10.1038/s41598-018-27196-5

Bhattacharyya ND, Feng CG (2020) Regulation of T helper cell fate by TCR signal strength. Front Immunol 11:624. https://doi.org/10.3389/fimmu.2020.00624

Bi Y, Ehirchiou D, Kilts TM et al (2007) Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 13:1219–1227. https://doi.org/10.1038/nm1630

Biau DJ, Tournoux C, Katsahian S et al (2006) Bone-patellar tendon-bone autografts versus hamstring autografts for reconstruction of anterior cruciate ligament: meta-analysis. BMJ 332:995–1001. https://doi.org/10.1136/bmj.38784.384109.2F

Boden BP, Dean GS, Feagin JA, Garrett WE (2000) Mechanisms of anterior cruciate ligament injury. Orthopedics 23:573–578

Borchers JR, Pedroza A, Kaeding C (2009) Activity level and graft type as risk factors for anterior cruciate ligament graft failure: a case-control study. Am J Sports Med 37:2362–2367. https://doi.org/10.1177/0363546509340633

Bosch U, Kasperczyk WJ (1992) Healing of the patellar tendon autograft after posterior cruciate ligament reconstruction--a process of ligamentization? An experimental study in a sheep model. Am J Sports Med 20:558–566. https://doi.org/10.1177/036354659202000513

Botchu R, Obaid H, Rennie WJ (2013) Correlation between trochlear dysplasia and the notch index. J Orthop Surg Hong Kong 21:290–293. https://doi.org/10.1177/230949901302100305

Bottoni CR, Smith EL, Shaha J et al (2015) Autograft versus allograft anterior cruciate ligament reconstruction: a prospective, randomized clinical study with a minimum 10-year follow-up. Am J Sports Med 43:2501–2509. https://doi.org/10.1177/0363546515596406

Bramono DS, Richmond JC, Weitzel PP et al (2004) Matrix metalloproteinases and their clinical applications in orthopaedics. Clin Orthop 428:272–285. https://doi.org/10.1097/01.blo.0000144166.66737.3a

Bray DF, Bray RC, Frank CB (1993) Ultrastructural immunolocalization of type-VI collagen and chondroitin sulphate in ligament. J Orthop Res Off Publ Orthop Res Soc 11:677–685. https://doi.org/10.1002/jor.1100110510

Breda SJ, Oei EHG, Zwerver J et al (2020) Effectiveness of progressive tendon-loading exercise therapy in patients with patellar tendinopathy: a randomised clinical trial. Br J Sports Med. https://doi.org/10.1136/bjsports-2020-103403

Brophy RH, Kovacevic D, Imhauser CW et al (2011) Effect of short-duration low-magnitude cyclic loading versus immobilization on tendon-bone healing after ACL reconstruction in a rat model. J Bone Joint Surg Am 93:381–393. https://doi.org/10.2106/JBJS.I.00933

Buller LT, Best MJ, Baraga MG, Kaplan LD (2015) Trends in anterior cruciate ligament reconstruction in the United States. Orthop J Sports Med 3:2325967114563664. https://doi.org/10.1177/2325967114563664

Bureau of Labor and Statistics, U.S. Department of Labor. Nonfatal occupational injuries and illnesses requiring days away from work 2015. News Release. 2016

Cannell LJ, Taunton JE, Clement DB et al (2001) A randomised clinical trial of the efficacy of drop squats or leg extension/leg curl exercises to treat clinically diagnosed jumper's knee in athletes: pilot study. Br J Sports Med 35:60–64. https://doi.org/10.1136/bjsm.35.1.60

Chen J, Kim J, Shao W et al (2019) An anterior cruciate ligament failure mechanism. Am J Sports Med 47:2067–2076. https://doi.org/10.1177/0363546519854450

Claes S, Verdonk P, Forsyth R, Bellemans J (2011) The "ligamentization" process in anterior cruciate ligament reconstruction: what happens to the human graft? A systematic review of the literature. Am J Sports Med 39:2476–2483. https://doi.org/10.1177/0363546511402662

Collin P, Thomazeau H, Walch G et al (2019) Clinical and structural outcome twenty years after repair of isolated supraspinatus tendon tears. J Shoulder Elbow Surg 28:196–202. https://doi.org/10.1016/j.jse.2018.07.023

Cook JL, Purdam CR (2009) Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. Br J Sports Med 43:409–416. https://doi.org/10.1136/bjsm.2008.051193

Cristiani R, Janarv P-M, EngstrÖm B et al (2020) Delayed anterior cruciate ligament reconstruction increases the risk of abnormal pre-reconstruction laxity, cartilage and medial meniscus injuries. Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc N Am Int Arthrosc Assoc. https://doi.org/10.1016/j.arthro.2020.11.030

Curtis RJ, Delee JC, Drez DJ (1985) Reconstruction of the anterior cruciate ligament with freeze dried fascia lata allografts in dogs. A preliminary report. Am J Sports Med 13:408–414. https://doi.org/10.1177/036354658501300608

Dagher E, Hays PL, Kawamura S et al (2009) Immobilization modulates macrophage accumulation in tendon-bone healing. Clin Orthop 467:281–287. https://doi.org/10.1007/s11999-008-0512-0

Dakin SG, Dudhia J, Smith RKW (2014) Resolving an inflammatory concept: the importance of inflammation and resolution in tendinopathy. Vet Immunol Immunopathol 158:121–127. https://doi.org/10.1016/j.vetimm.2014.01.007

de Boer MD, Maganaris CN, Seynnes OR et al (2007) Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. J Physiol 583:1079–1091. https://doi.org/10.1113/jphysiol.2007.135392

Demirag B, Sarisozen B, Ozer O et al (2005) Enhancement of tendon-bone healing of anterior cruciate ligament grafts by blockage of matrix metalloproteinases. J Bone Joint Surg Am 87:2401–2410. https://doi.org/10.2106/JBJS.D.01952

Devkota AC, Weinhold PS (2010) Prostaglandin E(2), collagenase, and cell death responses depend on cyclical load magnitude in an explant model of tendinopathy. Connect Tissue Res 51:306–313. https://doi.org/10.3109/03008200903318261

Dideriksen K, Boesen AP, Reitelseder S et al (2017) Tendon collagen synthesis declines with immobilization in elderly humans: no effect of anti-inflammatory medication. J Appl Physiol Bethesda Md 1985 122:273–282. https://doi.org/10.1152/japplphysiol.00809.2015

Dong S, Huangfu X, Xie G et al (2015) Decellularized versus fresh-frozen allografts in anterior cruciate ligament reconstruction: an in vitro study in a rabbit model. Am J Sports Med 43:1924–1934. https://doi.org/10.1177/0363546515585314

Dunn WR, Lyman S, Lincoln AE et al (2004) The effect of anterior cruciate ligament reconstruction on the risk of knee reinjury. Am J Sports Med 32:1906–1914. https://doi.org/10.1177/0363546504265006

Dustmann M, Schmidt T, Gangey I et al (2008) The extracellular remodeling of free-soft-tissue autografts and allografts for reconstruction of the anterior cruciate ligament: a comparison study in a sheep model. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 16:360–369. https://doi.org/10.1007/s00167-007-0471-0

Egerbacher M, Arnoczky SP, Caballero O et al (2008) Loss of homeostatic tension induces apoptosis in tendon cells: an in vitro study. Clin Orthop 466:1562–1568. https://doi.org/10.1007/s11999-008-0274-8

Egerbacher M, Gardner K, Caballero O et al (2021) Stress-deprivation induces an up-regulation of versican and connexin-43 mRNA and protein synthesis and increased ADAMTS-1 production in tendon cells in situ. Connect Tissue Res 63(1):43–52. https://doi.org/10.1080/03008207.2021.1873302

Fahlström M, Jonsson P, Lorentzon R, Alfredson H (2003) Chronic Achilles tendon pain treated with eccentric calf-muscle training. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 11:327–333. https://doi.org/10.1007/s00167-003-0418-z

Färnqvist K, Morrissey D, Malliaras P (2020) Factors associated with outcome following exercise interventions for Achilles tendinopathy: a systematic review. Physiother Res Int J Res Clin Phys Ther 26:e1889. https://doi.org/10.1002/pri.1889

Fleming DM, Cross KW, Barley MA (2005) Recent changes in the prevalence of diseases presenting for health care. Br J Gen Pract J R Coll Gen Pract 55:589–595

Forde MS, Punnett L, Wegman DH (2005) Prevalence of musculoskeletal disorders in union ironworkers. J Occup Environ Hyg 2:203–212. https://doi.org/10.1080/15459620590929635

Freedman BR, Gordon JA, Soslowsky LJ (2014) The Achilles tendon: fundamental properties and mechanisms governing healing. Muscles Ligaments Tendons J 4:245–255

Fromm B, Schäfer B, Parsch D, Kummer W (1996) Reconstruction of the anterior cruciate ligament with a cyropreserved ACL allograft. A microangiographic and immunohistochemical study in rabbits. Int Orthop 20:378–382. https://doi.org/10.1007/s002640050102

Fung DT, Wang VM, Andarawis-Puri N et al (2010) Early response to tendon fatigue damage accumulation in a novel in vivo model. J Biomech 43:274–279. https://doi.org/10.1016/j.jbiomech.2009.08.039

Gardner K, Arnoczky SP, Caballero O, Lavagnino M (2008) The effect of stress-deprivation and cyclic loading on the TIMP/MMP ratio in tendon cells: an in vitro experimental study. Disabil Rehabil 30:1523–1529. https://doi.org/10.1080/09638280701785395

Garrett WE, Swiontkowski MF, Weinstein JN et al (2006) American board of Orthopaedic surgery practice of the Orthopaedic surgeon: part-II, certification examination case mix. J Bone Joint Surg Am 88:660–667. https://doi.org/10.2106/JBJS.E.01208

Gibbon WW, Cooper JR, Radcliffe GS (1999) Sonographic incidence of tendon microtears in athletes with chronic Achilles tendinosis. Br J Sports Med 33:129–130. https://doi.org/10.1136/bjsm.33.2.129

Giordano M, Falciglia F, Poggiaroni A et al (2015) Histological changes of semitendinosus autograft after anterior cruciate ligament reconstruction in an immature rabbit model. J Exp Orthop 2:17. https://doi.org/10.1186/s40634-015-0033-1

Girgis B, Duarte JA (2020) Physical therapy for tendinopathy: an umbrella review of systematic reviews and meta-analyses. Phys Ther Sport Off J Assoc Chart Physiother Sports Med 46:30–46. https://doi.org/10.1016/j.ptsp.2020.08.002

Glazebrook MA, Wright JR, Langman M et al (2008) Histological analysis of achilles tendons in an overuse rat model. J Orthop Res Off Publ Orthop Res Soc 26:840–846. https://doi.org/10.1002/jor.20546

Goradia VK, Rochat MC, Kida M, Grana WA (2000) Natural history of a hamstring tendon autograft used for anterior cruciate ligament reconstruction in a sheep model. Am J Sports Med 28:40–46. https://doi.org/10.1177/03635465000280011901

Grana WA, Egle DM, Mahnken R, Goodhart CW (1994) An analysis of autograft fixation after anterior cruciate ligament reconstruction in a rabbit model. Am J Sports Med 22:344–351. https://doi.org/10.1177/036354659402200309

Grigg NL, Wearing SC, Smeathers JE (2009) Eccentric calf muscle exercise produces a greater acute reduction in Achilles tendon thickness than concentric exercise. Br J Sports Med 43:280–283. https://doi.org/10.1136/bjsm.2008.053165

Hannafin JA, Arnoczky SP, Hoonjan A, Torzilli PA (1995) Effect of stress deprivation and cyclic tensile loading on the material and morphologic properties of canine flexor digitorum profundus tendon: an in vitro study. J Orthop Res Off Publ Orthop Res Soc 13:907–914. https://doi.org/10.1002/jor.1100130615

Hays PL, Kawamura S, Deng X-H et al (2008) The role of macrophages in early healing of a tendon graft in a bone tunnel. J Bone Joint Surg Am 90:565–579. https://doi.org/10.2106/JBJS.F.00531

Hefti FL, Kress A, Fasel J, Morscher EW (1991) Healing of the transected anterior cruciate ligament in the rabbit. J Bone Joint Surg Am 73:373–383

Herod TW, Chambers NC, Veres SP (2016) Collagen fibrils in functionally distinct tendons have differing structural responses to tendon rupture and fatigue loading. Acta Biomater 42:296–307. https://doi.org/10.1016/j.actbio.2016.06.017

Italiani P, Boraschi D (2014) From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. Front Immunol 5:514. https://doi.org/10.3389/fimmu.2014.00514

Jackson DW, Grood ES, Wilcox P et al (1988) The effects of processing techniques on the mechanical properties of bone-anterior cruciate ligament-bone allografts. An experimental study in goats. Am J Sports Med 16:101–105. https://doi.org/10.1177/036354658801600203

Jackson DW, Grood ES, Goldstein JD et al (1993) A comparison of patellar tendon autograft and allograft used for anterior cruciate ligament reconstruction in the goat model. Am J Sports Med 21:176–185. https://doi.org/10.1177/036354659302100203

Jones GC, Corps AN, Pennington CJ et al (2006) Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human achilles tendon. Arthritis Rheum 54:832–842. https://doi.org/10.1002/art.21672

Jones ER, Jones GC, Legerlotz K, Riley GP (2013) Cyclical strain modulates metalloprotease and matrix gene expression in human tenocytes via activation of TGFβ. Biochim Biophys Acta 1833:2596–2607. https://doi.org/10.1016/j.bbamcr.2013.06.019

Jonsson P, Alfredson H (2005) Superior results with eccentric compared to concentric quadriceps training in patients with jumper's knee: a prospective randomised study. Br J Sports Med 39: 847–850. https://doi.org/10.1136/bjsm.2005.018630

Jung H-J, Vangipuram G, Fisher MB et al (2011) The effects of multiple freeze-thaw cycles on the biomechanical properties of the human bone-patellar tendon-bone allograft. J Orthop Res Off Publ Orthop Res Soc 29:1193–1198. https://doi.org/10.1002/jor.21373

Kaeding CC, Aros B, Pedroza A et al (2011) Allograft versus autograft anterior cruciate ligament reconstruction: predictors of failure from a MOON prospective longitudinal cohort. Sports Health 3:73–81. https://doi.org/10.1177/1941738110386185

Kan S-L, Yuan Z-F, Ning G-Z et al (2016) Autograft versus allograft in anterior cruciate ligament reconstruction: a meta-analysis with trial sequential analysis. Medicine (Baltimore) 95:e4936. https://doi.org/10.1097/MD.0000000000004936

Kannus P, Józsa L (1991) Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. J Bone Joint Surg Am 73:1507–1525

Kannus P, Paavola M, Józsa L (2005) Aging and degeneration of tendons. In: Maffulli N, Renström P, Leadbetter WB (eds) Tendon injuries. Springer-Verlag, London, pp 25–31

Kaux J-F, Drion P, Libertiaux V et al (2013) Eccentric training improves tendon biomechanical properties: a rat model. J Orthop Res Off Publ Orthop Res Soc 31:119–124. https://doi.org/10.1002/jor.22202

Kawabata H, Katsura T, Kondo E et al (2009) Stress deprivation from the patellar tendon induces apoptosis of fibroblasts in vivo with activation of mitogen-activated protein kinases. J Biomech 42:2611–2615. https://doi.org/10.1016/j.jbiomech.2009.07.027

Keene DR, Ridgway CC, Iozzo RV (1998) Type VI microfilaments interact with a specific region of banded collagen fibrils in skin. J Histochem Cytochem Off J Histochem Soc 46:215–220. https://doi.org/10.1177/002215549804600210

Kleiner JB, Amiel D, Roux RD, Akeson WH (1986) Origin of replacement cells for the anterior cruciate ligament autograft. J Orthop Res Off Publ Orthop Res Soc 4:466–474. https://doi.org/10.1002/jor.1100040410

Kleiner JB, Amiel D, Harwood FL, Akeson WH (1989) Early histologic, metabolic, and vascular assessment of anterior cruciate ligament autografts. J Orthop Res Off Publ Orthop Res Soc 7: 235–242. https://doi.org/10.1002/jor.1100070211

Kongsgaard M, Aagaard P, Roikjaer S et al (2006) Decline eccentric squats increases patellar tendon loading compared to standard eccentric squats. Clin Biomech Bristol Avon 21:748–754. https://doi.org/10.1016/j.clinbiomech.2006.03.004

Krych AJ, Jackson JD, Hoskin TL, Dahm DL (2008) A meta-analysis of patellar tendon autograft versus patellar tendon allograft in anterior cruciate ligament reconstruction. Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc N Am Int Arthrosc Assoc 24:292–298. https://doi.org/10.1016/j.arthro.2007.08.029

Kubo K (2015) Effects of repeated concentric and eccentric contractions on tendon blood circulation. Int J Sports Med 36:481–484. https://doi.org/10.1055/s-0034-1398649

Kubo K, Akima H, Ushiyama J et al (2004) Effects of 20 days of bed rest on the viscoelastic properties of tendon structures in lower limb muscles. Br J Sports Med 38:324–330. https://doi.org/10.1136/bjsm.2003.005595

Kujala UM, Sarna S, Kaprio J (2005) Cumulative incidence of achilles tendon rupture and tendinopathy in male former elite athletes. Clin J Sport Med Off J Can Acad Sport Med 15:133–135. https://doi.org/10.1097/01.jsm.0000165347.55638.23

Lane JG, McFadden P, Bowden K, Amiel D (1993) The ligamentization process: a 4 year case study following ACL reconstruction with a semitendinosis graft. Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc N Am Int Arthrosc Assoc 9:149–153. https://doi.org/10.1016/s0749-8063(05)80364-0

Langberg H, Rosendal L, Kjaer M (2001) Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. J Physiol 534:297–302. https://doi.org/10.1111/j.1469-7793.2001.00297.x

Lavagnino M, Arnoczky SP (2005) In vitro alterations in cytoskeletal tensional homeostasis control gene expression in tendon cells. J Orthop Res Off Publ Orthop Res Soc 23:1211–1218. https://doi.org/10.1016/j.orthres.2005.04.001

Lavagnino M, Arnoczky SP, Egerbacher M et al (2006) Isolated fibrillar damage in tendons stimulates local collagenase mRNA expression and protein synthesis. J Biomech 39:2355–2362. https://doi.org/10.1016/j.jbiomech.2005.08.008

Lee CH, Shin HJ, Cho IH et al (2005) Nanofiber alignment and direction of mechanical strain affect the ECM production of human ACL fibroblast. Biomaterials 26:1261–1270. https://doi.org/10.1016/j.biomaterials.2004.04.037

Lee CA, Lee-Barthel A, Marquino L et al (2015) Estrogen inhibits lysyl oxidase and decreases mechanical function in engineered ligaments. J Appl Physiol Bethesda Md 1985 118:1250–1257. https://doi.org/10.1152/japplphysiol.00823.2014

Lee W-C, Ng GY-F, Zhang Z-J et al (2020) Changes on tendon stiffness and clinical outcomes in athletes are associated with patellar tendinopathy after eccentric exercise. Clin J Sport Med Off J Can Acad Sport Med 30:25–32. https://doi.org/10.1097/JSM.0000000000000562

Leigh DR, Abreu EL, Derwin KA (2008) Changes in gene expression of individual matrix metalloproteinases differ in response to mechanical unloading of tendon fascicles in explant culture. J Orthop Res Off Publ Orthop Res Soc 26:1306–1312. https://doi.org/10.1002/jor.20650

Lipps DB, Wojtys EM, Ashton-Miller JA (2013) Anterior cruciate ligament fatigue failures in knees subjected to repeated simulated pivot landings. Am J Sports Med 41:1058–1066. https://doi.org/10.1177/0363546513477836

Liu F, Mih JD, Shea BS et al (2010) Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. J Cell Biol 190:693–706. https://doi.org/10.1083/jcb.201004082

Lu P-P, Chen M-H, Dai G-C et al (2020) Understanding cellular and molecular mechanisms of pathogenesis of diabetic tendinopathy. World J Stem Cells 12:1255–1275. https://doi.org/10.4252/wjsc.v12.i11.1255

Luckheeram RV, Zhou R, Verma AD, Xia B (2012) $CD4^+$T cells: differentiation and functions. Clin Dev Immunol 2012:925135. https://doi.org/10.1155/2012/925135

Lui PPY (2017) Tendinopathy in diabetes mellitus patients-Epidemiology, pathogenesis, and management. Scand J Med Sci Sports 27:776–787. https://doi.org/10.1111/sms.12824

Macchi M, Spezia M, Elli S et al (2020) Obesity increases the risk of tendinopathy, tendon tear and rupture, and postoperative complications: a systematic review of clinical studies. Clin Orthop 478:1839–1847. https://doi.org/10.1097/CORR.0000000000001261

Mafi N, Lorentzon R, Alfredson H (2001) Superior short-term results with eccentric calf muscle training compared to concentric training in a randomized prospective multicenter study on patients with chronic Achilles tendinosis. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 9:42–47. https://doi.org/10.1007/s001670000148

Maganaris CN, Chatzistergos P, Reeves ND, Narici MV (2017) Quantification of internal stress-strain fields in human tendon: unraveling the mechanisms that underlie regional tendon

adaptations and mal-adaptations to mechanical loading and the effectiveness of therapeutic eccentric exercise. Front Physiol 8:91. https://doi.org/10.3389/fphys.2017.00091

Magnussen RA, Taylor DC, Toth AP, Garrett WE (2012) ACL graft failure location differs between allografts and autografts. Sports Med Arthrosc Rehabil Ther Technol SMARTT 4:22. https://doi.org/10.1186/1758-2555-4-22

Magra M, Maffulli N (2005) Matrix metalloproteases: a role in overuse tendinopathies. Br J Sports Med 39:789–791. https://doi.org/10.1136/bjsm.2005.017855

Malliaras P, Barton CJ, Reeves ND, Langberg H (2013a) Achilles and patellar tendinopathy loading programmes : a systematic review comparing clinical outcomes and identifying potential mechanisms for effectiveness. Sports Med Auckl NZ 43:267–286. https://doi.org/10.1007/s40279-013-0019-z

Malliaras P, Kamal B, Nowell A et al (2013b) Patellar tendon adaptation in relation to load-intensity and contraction type. J Biomech 46:1893–1899. https://doi.org/10.1016/j.jbiomech.2013.04.022

McBeath R, Edwards RW, O'Hara BJ et al (2019) Tendinosis develops from age- and oxygen tension-dependent modulation of Rac1 activity. Aging Cell 18:e12934. https://doi.org/10.1111/acel.12934

Mehdizadeh A, Gardiner BS, Lavagnino M, Smith DW (2017) Predicting tenocyte expression profiles and average molecular concentrations in Achilles tendon ECM from tissue strain and fiber damage. Biomech Model Mechanobiol 16:1329–1348. https://doi.org/10.1007/s10237-017-0890-x

Mendias CL, Gumucio JP, Bakhurin KI et al (2012) Physiological loading of tendons induces scleraxis expression in epitenon fibroblasts. J Orthop Res Off Publ Orthop Res Soc 30:606–612. https://doi.org/10.1002/jor.21550

Millar NL, Silbernagel KG, Thorborg K et al (2021) Tendinopathy. Nat Rev Dis Primer 7:1. https://doi.org/10.1038/s41572-020-00234-1

Minami A, Ishii S, Ogino T et al (1982) Effect of the immunological antigenicity of the allogeneic tendons on tendon grafting. The Hand 14:111–119. https://doi.org/10.1016/s0072-968x(82)80001-6

Mortazavi SMJ, Moharrami A, Tamhri SS et al (2021) Time from injury is the key predictor of meniscal injury in ACL-deficient knees. J Knee Surg. https://doi.org/10.1055/s-0040-1722324

Nakama LH, King KB, Abrahamsson S, Rempel DM (2005) Evidence of tendon microtears due to cyclical loading in an in vivo tendinopathy model. J Orthop Res Off Publ Orthop Res Soc 23:1199–1205. https://doi.org/10.1016/j.orthres.2005.03.006

Narici MV, Maganaris CN (2006) Adaptability of elderly human muscles and tendons to increased loading. J Anat 208:433–443. https://doi.org/10.1111/j.1469-7580.2006.00548.x

Ng GY, Oakes BW, Deacon OW et al (1995) Biomechanics of patellar tendon autograft for reconstruction of the anterior cruciate ligament in the goat: three-year study. J Orthop Res Off Publ Orthop Res Soc 13:602–608. https://doi.org/10.1002/jor.1100130416

Ng GY-F, Chung PY-M, Wang JS, Cheung RT-H (2011) Enforced bipedal downhill running induces Achilles tendinosis in rats. Connect Tissue Res 52:466–471. https://doi.org/10.3109/03008207.2011.562334

Nichols AEC, Oh I, Loiselle AE (2020) Effects of type II diabetes mellitus on tendon homeostasis and healing. J Orthop Res Off Publ Orthop Res Soc 38:13–22. https://doi.org/10.1002/jor.24388

Ochen Y, Beks RB, van Heijl M et al (2019) Operative treatment versus nonoperative treatment of Achilles tendon ruptures: systematic review and meta-analysis. BMJ 364:k5120. https://doi.org/10.1136/bmj.k5120

Ohberg L, Lorentzon R, Alfredson H (2004) Eccentric training in patients with chronic Achilles tendinosis: normalised tendon structure and decreased thickness at follow up. Br J Sports Med 38:8–11.; discussion 11. https://doi.org/10.1136/bjsm.2001.000284

Ohno K, Yasuda K, Yamamoto N et al (1993) Effects of complete stress-shielding on the mechanical properties and histology of in situ frozen patellar tendon. J Orthop Res Off Publ Orthop Res Soc 11:592–602. https://doi.org/10.1002/jor.1100110414

Paavola M, Orava S, Leppilahti J et al (2000) Chronic Achilles tendon overuse injury: complications after surgical treatment. An analysis of 432 consecutive patients. Am J Sports Med 28:77–82. https://doi.org/10.1177/03635465000280012501

Pallis M, Svoboda SJ, Cameron KL, Owens BD (2012) Survival comparison of allograft and autograft anterior cruciate ligament reconstruction at the United States Military Academy. Am J Sports Med 40:1242–1246. https://doi.org/10.1177/0363546512443945

Papageorgiou CD, Ma CB, Abramowitch SD et al (2001) A multidisciplinary study of the healing of an intraarticular anterior cruciate ligament graft in a goat model. Am J Sports Med 29:620–626. https://doi.org/10.1177/03635465010290051501

Puxkandl R, Zizak I, Paris O et al (2002) Viscoelastic properties of collagen: synchrotron radiation investigations and structural model. Philos Trans R Soc Lond B Biol Sci 357:191–197. https://doi.org/10.1098/rstb.2001.1033

Ranger TA, Wong AMY, Cook JL, Gaida JE (2016) Is there an association between tendinopathy and diabetes mellitus? A systematic review with meta-analysis. Br J Sports Med 50:982–989. https://doi.org/10.1136/bjsports-2015-094735

Rashid MS, Cooper C, Cook J et al (2017) Increasing age and tear size reduce rotator cuff repair healing rate at 1 year. Acta Orthop 88:606–611. https://doi.org/10.1080/17453674.2017.1370844

Rees JD, Lichtwark GA, Wolman RL, Wilson AM (2008) The mechanism for efficacy of eccentric loading in Achilles tendon injury; an in vivo study in humans. Rheumatol Oxf Engl 47:1493–1497. https://doi.org/10.1093/rheumatology/ken262

Reeves ND, Maganaris CN, Ferretti G, Narici MV (2005) Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures. J Appl Physiol Bethesda Md 1985 98:2278–2286. https://doi.org/10.1152/japplphysiol.01266.2004

Rigozzi S, Stemmer A, Müller R, Snedeker JG (2011) Mechanical response of individual collagen fibrils in loaded tendon as measured by atomic force microscopy. J Struct Biol 176:9–15. https://doi.org/10.1016/j.jsb.2011.07.002

Riley G (2008) Tendinopathy--from basic science to treatment. Nat Clin Pract Rheumatol 4:82–89. https://doi.org/10.1038/ncprheum0700

Ritty TM, Roth R, Heuser JE (2003) Tendon cell array isolation reveals a previously unknown fibrillin-2-containing macromolecular assembly. Struct Lond Engl 1993 11:1179–1188. https://doi.org/10.1016/s0969-2126(03)00181-3

Robbins JR, Evanko SP, Vogel KG (1997) Mechanical loading and TGF-beta regulate proteoglycan synthesis in tendon. Arch Biochem Biophys 342:203–211. https://doi.org/10.1006/abbi.1997.0102

Romani W, Patrie J, Curl LA, Flaws JA (2003) The correlations between estradiol, estrone, estriol, progesterone, and sex hormone-binding globulin and anterior cruciate ligament stiffness in healthy, active females. J Womens Health 2002 12:287–298. https://doi.org/10.1089/154099903321667627

Ros SJ, Muljadi PM, Flatow EL, Andarawis-Puri N (2019) Multiscale mechanisms of tendon fatigue damage progression and severity are strain and cycle dependent. J Biomech 85:148–156. https://doi.org/10.1016/j.jbiomech.2019.01.026

Sanders TL, Maradit Kremers H, Bryan AJ et al (2016) Incidence of anterior cruciate ligament tears and reconstruction: a 21-year population-based study. Am J Sports Med 44:1502–1507. https://doi.org/10.1177/0363546516629944

Sapudom J, Mohamed WKE, Garcia-Sabaté A et al (2020) Collagen fibril density modulates macrophage activation and cellular functions during tissue repair. Bioeng Basel Switz 7:E33. https://doi.org/10.3390/bioengineering7020033

Schechtman H, Bader DL (2002) Fatigue damage of human tendons. J Biomech 35:347–353. https://doi.org/10.1016/s0021-9290(01)00177-4

Scheffler SU, Schmidt T, Gangéy I et al (2008a) Fresh-frozen free-tendon allografts versus autografts in anterior cruciate ligament reconstruction: delayed remodeling and inferior

mechanical function during long-term healing in sheep. Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc N Am Int Arthrosc Assoc 24:448–458. https://doi.org/10.1016/j.arthro.2007.10.011

Scheffler SU, Unterhauser FN, Weiler A (2008b) Graft remodeling and ligamentization after cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 16: 834–842. https://doi.org/10.1007/s00167-008-0560-8

Schoenenberger AD, Tempfer H, Lehner C et al (2020) Macromechanics and polycaprolactone fiber organization drive macrophage polarization and regulate inflammatory activation of tendon in vitro and in vivo. Biomaterials 249:120034. https://doi.org/10.1016/j.biomaterials.2020.120034

Schwartz HE, Matava MJ, Proch FS et al (2006) The effect of gamma irradiation on anterior cruciate ligament allograft biomechanical and biochemical properties in the caprine model at time zero and at 6 months after surgery. Am J Sports Med 34:1747–1755. https://doi.org/10.1177/0363546506288851

Scott A, Zwerver J, Grewal N et al (2015) Lipids, adiposity and tendinopathy: is there a mechanistic link? Critical review. Br J Sports Med 49:984–988. https://doi.org/10.1136/bjsports-2014-093989

Screen HRC, Lee DA, Bader DL, Shelton JC (2004) An investigation into the effects of the hierarchical structure of tendon fascicles on micromechanical properties. Proc Inst Mech Eng [H] 218:109–119. https://doi.org/10.1243/095441104322984004

Senga K, Kobayashi M, Hattori H et al (1995) Type VI collagen in mouse masseter tendon, from osseous attachment to myotendinous junction. Anat Rec 243:294–302. https://doi.org/10.1002/ar.1092430303

Shino K, Kawasaki T, Hirose H et al (1984) Replacement of the anterior cruciate ligament by an allogeneic tendon graft. An experimental study in the dog. J Bone Joint Surg Br 66:672–681. https://doi.org/10.1302/0301-620X.66B5.6501359

Shrier I, Matheson GO, Kohl HW (1996) Achilles tendonitis: are corticosteroid injections useful or harmful? Clin J Sport Med Off J Can Acad Sport Med 6:245–250

Shumborski S, Salmon LJ, Monk C et al (2020) Allograft donor characteristics significantly influence graft rupture after anterior cruciate ligament reconstruction in a young active population. Am J Sports Med 48:2401–2407. https://doi.org/10.1177/0363546520938777

Singhal MC, Gardiner JR, Johnson DL (2007) Failure of primary anterior cruciate ligament surgery using anterior tibialis allograft. Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc N Am Int Arthrosc Assoc 23:469–475. https://doi.org/10.1016/j.arthro.2006.12.010

Skutek M, van Griensven M, Zeichen J et al (2001) Cyclic mechanical stretching enhances secretion of Interleukin 6 in human tendon fibroblasts. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 9:322–326. https://doi.org/10.1007/s001670100217

Skutek M, van Griensven M, Zeichen J et al (2003) Cyclic mechanical stretching of human patellar tendon fibroblasts: activation of JNK and modulation of apoptosis. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 11:122–129. https://doi.org/10.1007/s00167-002-0322-y

Slauterbeck JR, Fuzie SF, Smith MP et al (2002) The menstrual cycle, sex hormones, and anterior cruciate ligament injury. J Athl Train 37:275–278

Snedeker JG (2016) How high glucose levels affect tendon homeostasis. Adv Exp Med Biol 920: 191–198. https://doi.org/10.1007/978-3-319-33943-6_18

Song B, Jiang C, Luo H et al (2017) Macrophage M1 plays a positive role in aseptic inflammation-related graft loosening after anterior cruciate ligament reconstruction surgery. Inflammation 40: 1815–1824. https://doi.org/10.1007/s10753-017-0616-3

Spiesz EM, Thorpe CT, Chaudhry S et al (2015) Tendon extracellular matrix damage, degradation and inflammation in response to in vitro overload exercise. J Orthop Res Off Publ Orthop Res Soc 33:889–897. https://doi.org/10.1002/jor.22879

Spindler KP, Andrish JT, Miller RR et al (1996) Distribution of cellular repopulation and collagen synthesis in a canine anterior cruciate ligament autograft. J Orthop Res Off Publ Orthop Res Soc 14:384–389. https://doi.org/10.1002/jor.1100140307

Steffen C, Timpl R, Wolff I (1968) Immunogenicity and specificity of collagen. Immunology 15: 135–144

Su B, O'Connor JP (2013) NSAID therapy effects on healing of bone, tendon, and the enthesis. J Appl Physiol Bethesda Md 1985 115:892–899. https://doi.org/10.1152/japplphysiol.00053.2013

Sun HB, Li Y, Fung DT et al (2008) Coordinate regulation of IL-1beta and MMP-13 in rat tendons following subrupture fatigue damage. Clin Orthop 466:1555–1561. https://doi.org/10.1007/s11999-008-0278-4

Suto K, Urabe K, Naruse K et al (2012) Repeated freeze-thaw cycles reduce the survival rate of osteocytes in bone-tendon constructs without affecting the mechanical properties of tendons. Cell Tissue Bank 13:71–80. https://doi.org/10.1007/s10561-010-9234-0

Svensson RB, Heinemeier KM, Couppé C et al (2016) Effect of aging and exercise on the tendon. J Appl Physiol Bethesda Md 1985 121:1237–1246. https://doi.org/10.1152/japplphysiol.00328.2016

Szczesny SE, Elliott DM (2014) Interfibrillar shear stress is the loading mechanism of collagen fibrils in tendon. Acta Biomater 10:2582–2590. https://doi.org/10.1016/j.actbio.2014.01.032

Szczesny SE, Aeppli C, David A, Mauck RL (2018) Fatigue loading of tendon results in collagen kinking and denaturation but does not change local tissue mechanics. J Biomech 71:251–256. https://doi.org/10.1016/j.jbiomech.2018.02.014

Tallon C, Maffulli N, Ewen SW (2001) Ruptured Achilles tendons are significantly more degenerated than tendinopathic tendons. Med Sci Sports Exerc 33:1983–1990. https://doi.org/10.1097/00005768-200112000-00002

Tashiro Y, Gale T, Sundaram V et al (2017) The graft bending angle can affect early graft healing after anterior cruciate ligament reconstruction: in vivo analysis with 2 years' follow-up. Am J Sports Med 45:1829–1836. https://doi.org/10.1177/0363546517698676

Thampatty BP, Wang JH-C (2018) Mechanobiology of young and aging tendons: In vivo studies with treadmill running. J Orthop Res Off Publ Orthop Res Soc 36:557–565. https://doi.org/10.1002/jor.23761

Thorpe CT, Chaudhry S, Lei II et al (2015) Tendon overload results in alterations in cell shape and increased markers of inflammation and matrix degradation. Scand J Med Sci Sports 25:e381–e391. https://doi.org/10.1111/sms.12333

Tohyama H, Yasuda K (2002) The effect of increased stress on the patellar tendon. J Bone Joint Surg Br 84:440–446. https://doi.org/10.1302/0301-620x.84b3.11107

Tomasek JJ, Gabbiani G, Hinz B et al (2002) Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 3:349–363. https://doi.org/10.1038/nrm809

Tsai W-C, Liang F-C, Cheng J-W et al (2013) High glucose concentration up-regulates the expression of matrix metalloproteinase-9 and -13 in tendon cells. BMC Musculoskelet Disord 14:255. https://doi.org/10.1186/1471-2474-14-255

Tsuzaki M, Bynum D, Almekinders L et al (2003) ATP modulates load-inducible IL-1beta, COX 2, and MMP-3 gene expression in human tendon cells. J Cell Biochem 89:556–562. https://doi.org/10.1002/jcb.10534

Uchida H, Tohyama H, Nagashima K et al (2005) Stress deprivation simultaneously induces over-expression of interleukin-1beta, tumor necrosis factor-alpha, and transforming growth factor-beta in fibroblasts and mechanical deterioration of the tissue in the patellar tendon. J Biomech 38:791–798. https://doi.org/10.1016/j.jbiomech.2004.05.009

Ueda Y, Inui A, Mifune Y et al (2018) The effects of high glucose condition on rat tenocytes in vitro and rat Achilles tendon in vivo. Bone Jt Res 7:362–372. https://doi.org/10.1302/2046-3758.75.BJR-2017-0126.R2

Ullm F, Riedl P, Machado de Amorim A et al (2020) 3D Scaffold-Based Macrophage Fibroblast Coculture Model Reveals IL-10 Dependence of Wound Resolution Phase. Adv Biosyst 4: e1900220. https://doi.org/10.1002/adbi.201900220

Viidik A (1967) The effect of training on the tensile strength of isolated rabbit tendons. Scand J Plast Reconstr Surg 1:141–147. https://doi.org/10.3109/02844316709022844

Virchenko O, Skoglund B, Aspenberg P (2004) Parecoxib impairs early tendon repair but improves later remodeling. Am J Sports Med 32:1743–1747. https://doi.org/10.1177/0363546504263403

Wang XT, Ker RF, Alexander RM (1995) Fatigue rupture of wallaby tail tendons. J Exp Biol 198: 847–852

Wang JH-C, Jia F, Yang G et al (2003) Cyclic mechanical stretching of human tendon fibroblasts increases the production of prostaglandin E2 and levels of cyclooxygenase expression: a novel in vitro model study. Connect Tissue Res 44:128–133. https://doi.org/10.1080/03008200390223909

Wang JH-C, Guo Q, Li B (2012) Tendon biomechanics and mechanobiology--a minireview of basic concepts and recent advancements. J Hand Ther Off J Am Soc Hand Ther 25:133–140.; quiz 141. https://doi.org/10.1016/j.jht.2011.07.004

Wang S, Meng X-M, Ng Y-Y et al (2016) TGF-β/Smad3 signalling regulates the transition of bone marrow-derived macrophages into myofibroblasts during tissue fibrosis. Oncotarget 7:8809–8822. https://doi.org/10.18632/oncotarget.6604

Wang W-M, Ma X-J, Huang S-B et al (2017a) A comparative study of effect of autograft compared with allograft anterior cruciate ligament reconstruction on expressions of LOXs and MMPs. Biosci Rep 37(2):BSR20160533. https://doi.org/10.1042/BSR20160533

Wang Y-Y, Jiang H, Pan J et al (2017b) Macrophage-to-myofibroblast transition contributes to interstitial fibrosis in chronic renal allograft injury. J Am Soc Nephrol JASN 28:2053–2067. https://doi.org/10.1681/ASN.2016050573

Weiler A, Peters G, Mäurer J et al (2001) Biomechanical properties and vascularity of an anterior cruciate ligament graft can be predicted by contrast-enhanced magnetic resonance imaging. A two-year study in sheep. Am J Sports Med 29:751–761. https://doi.org/10.1177/03635465010290061401

Weiler A, Unterhauser FN, Bail HJ et al (2002) Alpha-smooth muscle actin is expressed by fibroblastic cells of the ovine anterior cruciate ligament and its free tendon graft during remodeling. J Orthop Res Off Publ Orthop Res Soc 20:310–317. https://doi.org/10.1016/S0736-0266(01)00109-7

Werner RA, Franzblau A, Gell N et al (2005) A longitudinal study of industrial and clerical workers: predictors of upper extremity tendonitis. J Occup Rehabil 15:37–46. https://doi.org/10.1007/s10926-005-0872-1

Wiesinger H-P, Seynnes OR, Kösters A et al (2020) Mechanical and material tendon properties in patients with proximal patellar tendinopathy. Front Physiol 11:704. https://doi.org/10.3389/fphys.2020.00704

Wu B, Crampton SP, Hughes CCW (2007) Wnt signaling induces matrix metalloproteinase expression and regulates T cell transmigration. Immunity 26:227–239. https://doi.org/10.1016/j.immuni.2006.12.007

Yamaguchi K, Ditsios K, Middleton WD et al (2006) The demographic and morphological features of rotator cuff disease. A comparison of asymptomatic and symptomatic shoulders. J Bone Joint Surg Am 88:1699–1704. https://doi.org/10.2106/JBJS.E.00835

Yang G, Im H-J, Wang JH-C (2005) Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. Gene 363:166–172. https://doi.org/10.1016/j.gene.2005.08.006

Yang R, Zhang Z, Song B et al (2012) Ratio of T helper to regulatory T cells in synovial fluid and postoperative joint laxity after allograft anterior cruciate ligament reconstruction. Transplantation 94:1160–1166. https://doi.org/10.1097/TP.0b013e31826dddeb

Yigman GT, Toprak H (2020) Coexistence of trochlear dysplasia and anterior cruciate ligament mucoid degeneration and relationship between dysplasia degree and mucoid degeneration. Acad Radiol. https://doi.org/10.1016/j.acra.2020.07.007

Zeng C, Gao S, Li H et al (2016) Autograft versus allograft in anterior cruciate ligament reconstruction: a meta-analysis of randomized controlled trials and systematic review of overlapping systematic reviews. Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc N Am Int Arthrosc Assoc 32:153–163.e18. https://doi.org/10.1016/j.arthro.2015.07.027

Zhang J, Wang JH-C (2013) The effects of mechanical loading on tendons--an in vivo and in vitro model study. PloS One 8:e71740. https://doi.org/10.1371/journal.pone.0071740

Zhao G, Zhang J, Nie D et al (2019) HMGB1 mediates the development of tendinopathy due to mechanical overloading. PloS One 14:e0222369. https://doi.org/10.1371/journal.pone.0222369

Zhou Z, Akinbiyi T, Xu L et al (2010) Tendon-derived stem/progenitor cell aging: defective self-renewal and altered fate. Aging Cell 9:911–915. https://doi.org/10.1111/j.1474-9726.2010.00598.x

Zitnay JL, Jung GS, Lin AH et al (2020) Accumulation of collagen molecular unfolding is the mechanism of cyclic fatigue damage and failure in collagenous tissues. Sci Adv 6:eaba2795. https://doi.org/10.1126/sciadv.aba2795

Chapter 12
Methods to Enhance the Beneficial Effects of Exercise in Individuals with Spinal Cord Injuries

Matthew R. Ely, Brett R. Ely, Ryan J. Solinsky, and J. Andrew Taylor

Abstract Physical deconditioning commonly occurs following spinal cord injury (SCI) due to loss of voluntary functional movement and resultant increased sedentary behavior. This lesser energy expenditure leads to increased fat mass, decreased lean tissue mass, increased body mass index, declines in cardiac structure and function, reduced insulin sensitivity, and lower cardiorespiratory fitness. Collectively these physiological changes increase the risk of morbidity and mortality from cardiovascular diseases. Exercise as a therapy after an SCI may mitigate these negative health effects and improve quality and longevity of life. However, current exercise interventions for individuals with SCI may not be sufficient to prevent the elevations in risk factors for cardiovascular disease. Therefore, interventions to enhance the effectiveness of exercise therapy may be needed in this population in order to experience the same benefits seen by the uninjured population. Further, adjunctive therapies that mimic exercise may induce health benefits to combat cardiovascular disease. This chapter highlights novel interventions that may enhance function, increase exercise capacity, and decrease disease risk in individuals following an SCI. An effort was made to concentrate this chapter on human investigations of SCI but, where appropriate, investigations using animal models of SCI are referenced and specifically stated. Although this chapter highlights novel interventions to enhance the positive health benefits of exercise, combinations of these interventions may be necessary to improve the health of these individuals and warrants future investigation.

Keywords Spinal cord injury · Rehabilitation · Aerobic exercise · Cardiovascular disease · Regenerative medicine

M. R. Ely · R. J. Solinsky · J. A. Taylor (✉)
Harvard Medical School, Department of Physical Medicine and Rehabilitation, Cardiovascular Research Laboratory, Spaulding Rehabilitation Hospital, Cambridge, MA, USA
e-mail: Jandrew_Taylor@hms.harvard.edu

B. R. Ely
Department of Sport Movement Science, Salem State University, Salem, MA, USA

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_12

12.1 Introduction

Spinal cord injuries (SCI) occur in approximately 18,000 individuals in the United States each year, in 250,000–500,000 individuals globally, and are among the most catastrophic injuries that a person can experience (Jain et al. 2015; McDonald and Sadowsky 2002; Roberts et al. 2017; WHO 2013). An SCI results in lifelong disability in those who are typically young adults, predominantly men aged 16–44 years, and are primarily caused by vehicular accidents (DeVivo et al. 1999; Jain et al. 2015). An SCI disrupts neural connections below the level of injury that affect almost every major organ system, resulting in muscle paralysis, loss of sensation, and autonomic dysfunction. An SCI can occur anywhere along the 24 vertebrae and vary in severity from complete (American Spinal Injury Association Impairment Scale; AIS A) to incomplete impairment (AIS B, C, D). Complete impairments occur when there is an absence of all motor and sensory functions, including the sacral roots, distal to the site of the injury. Incomplete impairments occur when some voluntary movement or sensation is preserved. Additionally, SCIs may occur where normal motor and sensory function is retained but there may be abnormalities in reflex control (AIS E) (Roberts et al. 2017). Beyond neural disruption, SCI results in damage to vertebral bones, intervertebral disks, spinal ligaments, and blood vessels (McDonald and Sadowsky 2002).

Regardless of the neurological level of injury, there is rapid physical deconditioning secondary to the loss of voluntary motor control and increased sedentary activity following the injury (Pelletier and Hicks 2013). The reduced physical activity leads to a myriad of health issues associated with increased fat mass, decreased lean tissue mass, lipid disorders, blood pressure irregularities, abnormal glycemic control, and chronic inflammation (Bigford et al. 2017; Cragg et al. 2012; LaVela et al. 2006; Myers et al. 2007; Pelletier and Hicks 2013; Warburton et al. 2007; Weaver et al. 2007; Whiteneck et al. 1992). In addition, lesser activity induces structural and functional cardiovascular maladaptations (Ely et al. 2021; Williams et al. 2019). Together, these broad systemic physiological and structural changes increase the risk for cardiovascular diseases (Mercier and Taylor 2016; Myers et al. 2007). In fact, there is an increased prevalence and earlier onset of cardiovascular diseases, including heart failure, atrial fibrillation, atherosclerosis, and ischemic heart disease which are the leading causes of morbidity and mortality in individuals with chronic SCI (Cragg et al. 2012; Myers et al. 2007; Roger et al. 2012; Whiteneck et al. 1992).

12.2 Exercise Rehabilitation

Regular physical exercise is preventative for a broad range of diseases in uninjured individuals. Similarly, aerobic exercise in individuals with SCI is important to slow the progression or reverse negative health risks and minimize the incidence of

cardiovascular disease (Mercier and Taylor 2016; Myers et al. 2007; Qiu and Taylor 2016). However, exercise must meet certain intensity and volume criteria to create cardiovascular and metabolic demands sufficient to induce significant benefits across multiple physiological systems (Kikkinos et al. 2014; Qiu and Taylor 2016). These demands include increased oxygen consumption to elevate cardiac output and respiration, skeletal muscle blood flow demands to redistribute blood flow and increase vascular shear stress, and metabolic heat production sufficient to increase core temperature, all of which are implicated in the cardiometabolic benefits of exercise and are reduced in individuals with SCI (Green 2009; Joyner and Green 2009; Laughlin 1999; Laughlin et al. 2008). Muscle paralysis, as a consequence of SCI, reduces the amount of skeletal muscle that can voluntarily contribute to exercise, and therefore lessens the exercise intensity and duration that can be attained, and therefore the associated health benefits. Additionally, with an increased duration of time since injury, there are parallel declines in whole-body cardiorespiratory fitness and non-paralyzed muscle strength such that these individuals rank near the bottom of the physical fitness spectrum (Dearwater et al. 1986; Qiu and Taylor 2016). To maintain or increase cardiorespiratory fitness, it is currently recommended that individuals with SCI complete at least 30 min of moderate aerobic exercise 3 or more days per week or 20 min of vigorous aerobic exercise more than 3 days per week (Martin Ginis et al. 2018; Tweedy et al. 2017). Although these exercise guidelines are based on improvements in cardiorespiratory fitness, an increase in fitness occurs in parallel with reductions of many factors related to risk for cardiovascular disease (Franklin and McCullough 2009). However, the ability simply to participate in exercise, much less meet exercise intensity and duration guidelines is a challenge. Therefore, interventions to enhance exercise capacity or supplemental therapy to mimic exercise may be beneficial for those with SCI to confer the protective benefits of exercise.

12.3 Restoration of Function

Many types and modes of therapy are available for SCI rehabilitation but most do not fully restore the motor function of paralyzed limbs (Harvey et al. 2016). The lack of recovery is due to limited plasticity and regenerative capacity of the nervous system (Ashammakhi et al. 2019). In the past 10 years, there have been substantial advances in cell-based therapies, biomaterials, and biomolecules that aid in neuroregeneration but these techniques have not advanced to a point to restore functional recovery in humans (Ashammakhi et al. 2019). In general, treatment for SCI is focused on stabilization of the injury site, prevention of complications, and physical rehabilitation. Physical therapies are pragmatic and intended to improve quality of life (McDonald and Sadowsky 2002). Therefore, therapies for SCI are not "regenerative" but focus on maintaining or enhancing remaining function. In this context, exercise has become an important and quantifiable means for functional recovery (Fu et al. 2016; Sandrow-Feinberg et al. 2009; Warburton et al. 2007). Exercise not

only strengthens non-paralyzed and potentially paralyzed muscle but may also increase functional recovery through promoting brain remodeling, improving spinal microenvironments, and maintaining distal motor neuron function (Fu et al. 2016).

12.3.1 Spinal Stimulation

Motor deficits are considered the most significant barrier to functional recovery. Currently, some rehabilitative strategies attempt to activate and enhance remaining functional neurons in individuals with partially preserved motor function. Spinal stimulation (epidural or transcutaneous) requires a neural computer interfaced with an electrode to apply electrical impulses onto dorsal root spinal neurons (Ozpinar et al. 2016; Wagner et al. 2018). The impulses are synchronized with voluntary repetitive muscle contractions or joint movements (i.e., exercise) (Ievins and Moritz 2017; McPherson et al. 2015). Following the cessation of stimulation, there can be improvements in muscle activation and limb movement/mobility. While used primarily in rodents, this technique has resulted in large improvements of joint movements that aid walking, reaching, and grasping (Mushahwar et al. 2002; Sunshine et al. 2013; Zimmermann et al. 2011). In humans, smaller improvements in joint movement have occurred with spinal stimulation. Importantly, spinal stimulation in combination with physical therapy has shown greater recovery of movement compared to physical training alone and has improved lower limb flexion/extension, leg strength, sit-to-stand tasks, ankle mobility during walking, as well as hand control and grip strength (Al'joboori et al. 2020; Donovan et al. 2021; Jilge et al. 2004; Lu et al. 2016; Meyer et al. 2020; Minassian et al. 2016; Sayenko et al. 2019; Wagner et al. 2018). Although the precise mechanisms leading to the improved function are unknown, it is believed that the stimulation drives neural plasticity by increasing neural output, increasing neural activation, recruiting peripheral undamaged neurons, or increasing the sensitivity of proprioceptive pathways (Ievins and Moritz 2017; Wagner et al. 2018). The improved mobility and muscle activation may enhance exercise options for those with SCI. Unfortunately, continuous stimulation appears to lose its effect after an extended period of time, and the improvements in motor control gained from stimulation are often lost or significantly reduced in the hours to days following the cessation of stimulation.

12.3.2 Functional Electrical Stimulation (FES)

Most aerobic exercise options for those with SCI are limited to the volitional movement of the upper body given the loss of motor function in the legs. Unfortunately, the small muscle mass of the upper body is insufficient to produce sustainable high levels of aerobic work (Jacobs et al. 2001; Qiu and Taylor 2016). For example, peak oxygen consumption during arms-only exercise can reach 25 ml/kg/min at

workloads (~34 Watts) that can only be maintained for a few minutes (Glaser et al. 1980; Sawka et al. 1980). Hence, arms-only exercise is limited as a therapy to fulfill exercise intensity and duration requirements for cardiovascular health. This is likely the reason for the modest cardiovascular and respiratory improvements with arms-only training (Taylor et al. 1986). To overcome the limitations of arm-only exercise, external stimulation of paralyzed muscle, specifically the lower body, has been promoted as a practical and effective intervention to increase active muscle mass and whole-body oxygen consumption (Mutton et al. 1997).

Contraction of paralyzed skeletal muscle is accomplished by using an electrical stimulus applied through muscle/nerve implantable probes or skin electrodes. The electrical stimuli initiate action potentials within motor neurons resulting in muscle contraction (Peckham and Knutson 2005). When the electrically elicited muscle contractions are coordinated in a manner that provides functional movement, the technique is termed functional electrical stimulation (FES) (Peckham and Knutson 2005). The purpose of FES is to generate muscular contractions and produce useful movements such as leg flexion/extension for cycling or rowing exercises, and in some cases walking.

For example, FES-evoked cycling uses bilateral stimulation of the quadriceps, hamstrings, and gluteal muscles to perform cyclical pedaling movements of the legs (Deley et al. 2014; Qiu and Taylor 2016). This commonly used FES modality activates a relatively small amount of muscle mass and causes modest increases in oxygen consumption, heart rate, and cardiac output (Fornusek and Davis 2008; Hunt et al. 2007). FES activation of lower limb muscles has been shown to be important to the exercise response as it engages the skeletal muscle pump during rhythmic contractions to aid in venous return to the heart. Repeat sessions of FES have been shown to increase quadriceps torque, glucose transport, citrate synthase activity, capillary number, fatigue resistance, and muscle fiber cross sectional area (Chilibeck et al. 1999a, 1999b; Rodgers et al. 1991; Sabatier et al. 2006). This technique has been promoted as an effective way to increased exercise tolerance and improves overall cardiovascular health by mimicking moderate-to-vigorous intensity exercise training (Warburton et al. 2007). However, FES should not be considered synonymous with voluntary exercise. Externally activating skeletal muscle bypasses feedforward input to the cardiorespiratory system from the central nervous system (i.e., central command) and the paralyzed muscle provides minimal or no feedback from the periphery (i.e., group III/IV muscle afferents) to the cardiovascular and pulmonary centers in the brain stem Ely and Taylor 2021. Additionally, due to the nature of electrical stimulation, muscle fibers are activated in reverse physiological order. This reverse recruitment induces a high rate of muscle fatigue and a potentially altered metabolism (Binder-Macleod and Snyder-Mackler 1993; Gregory and Bickel 2005; Peckham and Knutson 2005). FES also results in low levels of systemic vascular shear stress and small elevations of body core temperature. These factors are not trivial and have causative connections to improving cardiovascular health.

12.3.3 Hybrid Functional Electrical Stimulation

To induce greater exercise benefits and better mimic exercise in uninjured individuals, a combination of FES of paralyzed leg muscle with voluntary arm exercise has been implemented for cycling and rowing (Laskin et al. 1993). This hybrid mode of exercise increases the mass of active musculature and produces simultaneous training of the upper and lower extremities (Qiu and Taylor 2016). Hybrid FES-exercise results in greater cardiovascular responses and higher oxygen consumption than either upper or lower body exercise alone (Brurok et al. 2011; Mutton et al. 1997). Importantly, exercise training using hybrid methods produce between 10 and 60% larger increases in cardiorespiratory fitness and cardiac function compared to arms-only or FES lower body exercise training (Brurok et al. 2011; Gibbons et al. 2016; Hettinga and Andrews 2008; Taylor et al. 2011). Importantly, studies have shown that hybrid exercise is well-tolerated by individuals with SCI and can be maintained at sufficient submaximal exercise intensities for long durations to meet exercise guidelines for cardiovascular health (Hettinga and Andrews 2008; Qiu and Taylor 2016; Taylor et al. 2011). In fact, hybrid FES-exercise has been shown to decrease blood pressure, insulin resistance, blood glucose, systemic inflammation, and improve overall cardiovascular health (Bakkum et al. 2015; Griffin et al. 2009; Warburton et al. 2007).

12.4 Ventilatory Limitations to Exercise in SCI

To meet the higher oxidative needs of muscle as exercise workload increases, pulmonary ventilation is normally increased in parallel by increases in tidal volume and breathing frequency. In most uninjured individuals ventilatory capacity is more than adequate to meet metabolic demands for all exercise intensities, even following large increases in muscle oxygen demand after strenuous training programs (Casaburi et al. 1992; McParland et al. 1992). Individuals with SCI can have impaired respiratory muscle control proportional to the level of injury. For example, individuals with SCI above the third thoracic vertebra (<T3) have profound motor loss/spasticity to accessory muscles of respiration, atrophy of respiratory muscles, and reduced compliance of the lungs and chest wall. These factors not only reduce the total amount of air an individual can move in and out of their lungs with each breath but also contribute to an increased oxygen cost of breathing (Shields 2002). A larger recruited skeletal muscle mass during exercise via FES creates a mismatch between the oxygen demand of the muscle and the ventilatory capacity of the lungs, especially in those with high-level injuries (Taylor et al. 2014). Therefore, when large amounts of muscle mass are active (i.e., hybrid FES-exercise), exercise intensity can be limited by pulmonary capacity (Qiu et al. 2016).

12.4.1 Non-Invasive Ventilation (NIV)

A novel approach to potentially improve exercise capacity would be a support of ventilation during exercise. Non-invasive ventilation (NIV) does not require intubation and provides external ventilatory support via positive air pressure through a facemask during inhalation. Current NIV machines use bi-level positive pressure to assist with lung expansion during inhalation and a reduced positive pressure upon exhalation to limit airflow back to the machine. This technique has been demonstrated to reduce the work of breathing, enhance exercise tolerance, and improve exercise capacity in individuals with obstructive and resistive pulmonary diseases (Borel et al. 2008; Dreher et al. 2010; van't Hul et al. 2006; Vila et al. 2007). Additionally, in individuals with chronic obstructive pulmonary disease (COPD), NIV during a single bout of exercise reduced dyspnea, improved breathing patterns, and enhanced oxygen and carbon dioxide exchange (Dreher et al. 2007; Maltais et al. 1995; van't Hul et al. 2004). One study examining maximal exercise capacity in individuals with SCI noted that NIV increased oxygen consumption only in individuals with high-level injuries and shorter time since injury. The improvement in this population likely reflects the greater amount of respiratory motor control loss due to the high injury and remaining muscle strength due to lesser atrophy from the shorter time since injury (Vivodtzev et al. 2020). Therefore, targeted use of NIV to support exercise could be an effective approach to overcome ventilatory limits.

12.4.2 Buspirone (Serotonergic Receptor Agonist)

Although paralyzed pulmonary musculature is partly responsible for the reduced function, spinal and supraspinal neural control of respiration are reduced after SCI and may also contribute to the reduced ventilation (De Troyer et al. 1986; De Troyer and Heilporn 1980; Schilero et al. 2014; Zimmer and Goshgarian 2007). In addition to damaged descending neurons, impaired ascending neuronal feedback contributes to dysregulation during inspiratory and expiratory phases of breathing (Bezdudnaya et al. 2017). One important neurotransmitter in both descending and ascending pathways is serotonin. As a result, the serotonin 5HT1A receptor agonist buspirone may increase the excitability of pulmonary neurons that survived the injury (Choi et al. 2005; Kheck et al. 1995). Although buspirone is commonly prescribed as an anxiolytic, it has been shown to increase respiratory responses to carbon dioxide in an animal model of SCI (Choi et al. 2005; Teng et al. 2003), and in human case studies, it has been found to improve chemosensitivity and respiratory rhythms in individuals with apneustic syndromes/injuries (El-Khatib et al. 2003; Saito et al. 1999). In patients with COPD, 14 days of buspirone treatment reduced symptoms of dyspnea and increased exercise tolerance (Argyropoulou et al. 1993). Interestingly, a retrospective analysis of individuals with SCI taking buspirone displayed greater increases in peak oxygen consumption and ventilation following 6 months of

FES-rowing compared to a matched group of individuals in the same training program not taking buspirone (Vivodtzev et al. 2021). Hence, this anxiolytic may have the potential to improve exercise respiration, exercise capacity, and health outcomes in those with SCI.

12.4.3 Drug Therapy that May Improve Locomotor Function

There are a number of medications that have displayed the potential to be neurorestorative or improve motor function following a SCI. Some of these medications include metformin (Afshari et al. 2018; Zhang et al. 2017), riluzole (Srinivas et al. 2019), dalfampridine (Hansebout et al. 1993), and antiNOGO (Zörner and Schwab 2010). These medications have various targets on motor neurons including modulating glutamine, potassium channels, and the myelin sheath. Individually, these medications have varying levels of efficacy at improving function in animal models of SCI. A medication that is showing some promise is spinalon. Spinalon, currently in Phase I/II trials, is an investigational drug that is a combination of monoamine receptor agonists, noradrenaline/dopamine precursors, and decarboxylase inhibitors (buspirone, levodopa, and carbodopa). This drug combination has enhanced walking coordination in mice, turtles, and humans with SCI. These drugs appear to stimulate the spinal walking reflex, allowing for up to 60 minutes of walking motions to occur after administration (Guertin and Guertin 2012; Guertin et al. 2010; Ung et al. 2012). Interestingly, this drug combination has initiated walking motions in individuals with motor incomplete (AIS B) and complete (AIS A) injuries. This combinational therapy may greatly improve exercise options, including bipedal exercise therapy, and improve the health of individuals with SCI. Unfortunately, early outcomes from transected mice models show that the long duration sessions of walking may not reach an exercise intensity to improve health outcomes in all body systems. In these mice, the walking attenuated loss of muscle mass but did not slow the rate of reduction in bone density (Guertin et al. 2011). These investigations suggest that there is promise in improving motor function through medications, and that combinational medications may be most efficacious. Additionally, combinational drug therapies may be an important avenue of enhancing exercise capabilities in those with SCI.

12.4.4 Intermittent Hypoxia

Intermittent hypoxia is a non-pharmacological intervention that may also increase respiratory responses to exercise. This technique exposes individuals to short (60 s to 5 min) bouts of air with reduced oxygen content (~5% O_2) which results in increased ventilation. This practice appears to strengthen synaptic pathways to respiratory motor neurons by a mechanism known as phrenic long-term facilitation (Ling et al.

2001). Long-term facilitation is a serotonin-dependent change in spinal plasticity that is characterized as a progressive increase in phrenic motor output during hypoxia which remains elevated upon return to normal arterial oxygen levels (Fuller et al. 2001, 2003). Only a small number of hypoxic events are required for a lasting increase in respiratory motor output. In rats, the increased motor output lasts approximately 90 min after three 90 sec hypoxic exposures, and it is reported that 10 exposures over 7 days could produce an effect that lasts 24 hours. In humans, there is elevated ventilation immediately after hypoxic exposure and the effect is larger in those with higher level injuries (Sankari et al. 2015). A case report in an individual with a chronic C4 injury showed that 10 days of intermittent hypoxia improved inspiratory capacity (Jaiswal et al. 2016). Therefore, longer duration hypoxic exposure may enhance the magnitude of ventilatory long-term facilitation in those with SCI (Jaiswal et al. 2016; Tester et al. 2014).

Intermittent hypoxia may also have positive effects on motor output, as hypoxia increased the size of the motor action potentials of finger muscles by 20% (Christiansen et al. 2021). In individuals with incomplete SCI, 15 90 s sessions of intermittent hypoxia in combination with overground walking improved speed of walking during 10-meter walk tests after 1 day and walking distance in a 6 min walk test after 2 weeks of exposure (Hayes et al. 2014). Hence, intermittent hypoxia may have therapeutic potential to enhance respiratory and motor function and may improve exercise tolerance and capacity in individuals with SCI (Fuller et al. 2003). However, it should be noted that, potentially counterproductive to decreasing risk factors for cardiovascular disease, intermittent hypoxia has been shown to increase serum levels of cholesterol, phospholipids, and triglycerides in lean mice (Li et al. 2005) and increase pro-inflammatory pathways in individuals with sleep apnea (Ryan et al. 2005).

12.5 Heat Stress

Individuals with SCI are unlikely to experience large increases in body core temperature during regular exercise therapies. Lesser whole-body metabolism from relatively low exercise intensities, short duration, and smaller total skeletal mass recruited to perform the exercise result in small elevations in core temperature. Some of the cardiovascular benefits of exercise training are related to repeated intermittent increases in body core temperature (Locke et al. 1990; Rhind et al. 2004) and resultant alterations in vascular shear stress (Laughlin et al. 2008). The reduced influence of this potentially important signaling pathway could limit cardiovascular and metabolic adaptations. Therefore, heat therapy, or repeated exposure to passive heat stress, has been proposed as a means to improve cardiovascular and metabolic health in individuals with SCI (Ely et al. 2018; Hooper and Hooper 2009; Neff et al. 2016). Exercise and heat stress elicit many common physiological responses, in addition to increasing core temperature, there are increases in cardiac chronotropy and inotropy, redistribution of blood flow, and increased endothelial shear stress, all

of which impact cardiovascular health (Johnson and Proppe 2011). Passive heat therapy, using either sauna or hot water immersion, has been shown to improve cardiovascular health in healthy, uninjured humans and in patients with elevated cardiovascular disease risk. Passive heating increases cardiac function (Tei et al. 1995), decreases systemic vascular resistance (Tei et al. 1995), improves autonomic profile (Ely et al., 2019b), augments brachial artery flow-mediated vasodilation (Brunt et al. 2016b; Ely et al. 2019b; Imamura et al. 2001; Kihara et al. 2002), elicits protection from ischemia-reperfusion injury (Brunt et al. 2016c; Ely et al. 2019b; Engelland et al. 2020), and improves microvascular function (Brunt et al. 2016a; Romero et al. 2017). Improvements have also been observed in cardiometabolic variables including fasting blood glucose (Ely et al. 2019a; Hooper 1992), blood lipid profile (Ely et al. 2019b), and markers of inflammation (Ely et al. 2019a). Additionally, repeated heat exposure leads to the induction of cytoprotective pathways which are associated with protection from cardiovascular and metabolic disease (Horowitz and Assadi 2010; Krause et al. 2015; Kurucz et al. 2002; Maloyan et al. 2005). Heat therapy research in individuals with SCI is currently limited to single sessions studies, but importantly, these studies indicate heat therapy is safe and well-tolerated. The single session studies indicate that passive heating interventions such as lower limb or whole-body hot water immersion lead to altered inflammatory profiles (Leicht et al. 2015) and endothelial cell activation (Coombs et al. 2019), similar to what is observed following acute exercise. These initial first studies indicate that heat therapy may be a novel and important approach to restore cardiometabolic function in individuals with SCI.

12.6 Exoskeleton/Body Weight Supported Treadmill Exercise

Robotic exoskeletons, limb orthoses, or bionic suits can allow individuals with varying levels of SCI to safely and functionally walk for mobility or exercise (Kandilakis and Sasso-Lance 2019). These orthoses increase walking/exercise time by increasing the number of steps individuals can take (Gorgey et al. 2017). A limitation often associated with exoskeletons is that they slow the movements of the individual and often require greater oxygen consumption than normal walking (Asselin et al. 2015; Evans et al. 2015; Massucci et al. 1998; Waters and Mulroy 1999). The greater oxygen consumption or metabolic load of movement may actually be a benefit, as this indicates a greater exercise intensity (Kandilakis and Sasso-Lance 2019). Previous individual sessions of exercise using exoskeletons in conjunction with bodyweight supported treadmill training have shown a prolonged exercise time, decreased ratings of fatigue, and improved muscle strength and endurance (Wu et al. 2012). Using exoskeletons for up to 6 months of exercise training has resulted in global changes in body composition such as an increased bone density and decreased intramuscular and subcutaneous adipose tissue (Gorgey

et al. 2017; Karelis et al. 2017). Six months of exoskeleton training also resulted in improved blood glucose regulation (Phillips et al. 2004). Cardiac improvements with exoskeleton exercise training include increased ejection fraction, increased heart mass, decreased end-systolic and increased end-diastolic volumes, and reduced isovolumetric relaxation times (Turiel et al. 2011). The positive changes in body composition, cardiac structure and function, and glucose regulation ultimately reduce the cardiovascular disease risk in those with SCI.

12.7 Combined Therapies

Exercise training in combination with other treatments that enhance or mimic exercise may be important to realize the benefits of exercise. Combination therapies may better mimic the stress of exercise upon the body than singular therapies and may be more effective at improving the cardiovascular health of individuals with SCI. For example, the combination of intermittent hypoxia with transcranial magnetic stimulation of the motor cortex produced larger motor evoked potentials of finger muscles than repeated stimulation alone (Christiansen et al. 2021). Similarly, the pairing of serotonin agonists drugs with electrical stimulation produced enhanced motor function and greater muscle movements in mice than stimulation alone (Gerasimenko et al. 2007, 2015; Van Den Brand et al. 2012). Recently, one study on individuals with incomplete SCI paired peripheral nerve stimulation and magnetic transcortical stimulation with exercise. The combination of exercise and stimulation contributed to a lasting retention of muscle strength and a decreased time in a 10-minute walk test (Jo and Perez 2020). Incredibly, this combination produced improvements in motor function that remained 6 months after the therapy. These combinational therapies are showing great promise at increasing mobility and exercise capacity. Therefore, combinational therapies with exercise may further decrease cardiovascular health risk in those with SCI. Moreover, SCI can be heterogenous; individuals with the same injury level and AIS scale may have a very different loss of respiratory or autonomic function (Draghici and Taylor 2018). Hence, some patients may respond better to some adjunctive therapies than others, and so it may be wise to apply combination therapies to ensure the greatest response across the spectrum of SCI.

12.8 Limitations

Many of the therapies to increase exercise tolerance may not be practical options for all individuals. For example, to complete FES-exercise, electrical stimulation units must be integrated with modified exercise equipment (e.g., bicycle or rowing ergometer). In general, a basic electrical stimulation unit is not cost prohibitive (<$200) but units that coordinate antagonistic muscle firing may be a few thousand

dollars and are not commonly covered by insurance. Additionally, exoskeletons for exercise rehabilitation are cost prohibitive for personal use (>$80,000) and require additional trained personnel to set up and operate. Therefore, these therapies are generally limited to clinical outpatient settings at hospitals or rehabilitation clinics where equipment and trained staff are available for guidance. Similarly, heat therapy using lower leg hot water immersion is a feasible home-based option using a bathtub or heated leg bath (<$200), but supervision may be recommended for individuals with higher level injuries (T6 and above) due to challenges with thermoregulation and blood pressure regulation during heat stress, in addition to potential burning of insensate skin (Schmidt and Chan 1992). Additionally, there are home-based units that are able to produce hypoxic environments (e.g., HYPO2XICO) which can be used in conjunction with exercise equipment, but these are often cost prohibitive at a few thousand dollars per unit. Finally, animal models do not approximate the effects of SCI in humans across all systems (Akhtar et al. 2008; Seok et al. 2013). Therefore, many animal studies should be interpreted with caution as they were conducted in murine species. Murine species are often used as surrogates for understanding human physiology, but genomic differences often result in divergent findings between the species (Seok et al. 2013).

12.9 Concluding Remarks and Future Directions

Individuals with SCIs are 2–6 times more likely to experience cardiovascular disease than uninjured individuals (Cragg et al. 2012, 2013). The increased incidence of CVD is due to amplified risk including increased physical inactivity, dyslipidemia, uncontrolled blood pressure, and uncontrolled blood glucose (Cragg et al. 2013). Currently, the best therapy to improve these cardiovascular disease risk factors is exercise. However, benefits to health and wellness may not be available to those with SCI since they may not be able to attain necessary exercise intensity or duration thresholds. Therefore, a combination of approaches including drug and adjunctive therapy in addition to exercise may be needed for this population to obtain reductions in cardiovascular disease risk. Future research focusing on combining exercise with other treatments to maximize benefits will further elucidate the potential for these adjunctive treatments to improve health and reduce morbidity and mortality from metabolic and cardiovascular diseases in individuals with SCI.

References

Afshari K, Dehdashtian A, Haddadi NS, Haj-Mirzaian A, Iranmehr A, Ebrahimi MA, Tavangar SM, Faghir-Ghanesefat H, Mohammadi F, Rahimi N, Javidan AN, Dehpour AR (2018) Anti-inflammatory effects of metformin improve the neuropathic pain and locomotor activity in spinal cord injured rats: introduction of an alternative therapy. Spinal Cord 56:1032–1041

Akhtar AZ, Pippin JJ, Sandusky CB (2008) Animal models in spinal cord injury: a review. Rev Neurosci 19:47–60

Al'joboori Y, Massey SJ, Knight SL, Donaldson N d N, Duffell LD (2020) The effects of adding transcutaneous spinal cord stimulation (tSCS) to sit-to-stand training in people with spinal cord injury: a pilot study. J Clin Med 9:2765

Argyropoulou P, Patakas D, Koukou A, Vasiliadis P, Georgopoulos D (1993) Buspirone effect on breathlessness and exercise performance in patients with chronic obstructive pulmonary disease. Respiration 60:216–220

Ashammakhi N, Kim H-J, Ehsanipour A, Bierman RD, Kaarela O, Xue C, Khademhosseini A, Seidlits SK (2019) Regenerative therapies for spinal cord injury. Tissue Eng Rev 25:471–491

Asselin P, Knezevic S, Kornfeld S, Cirnigliaro C, Agranova-Breyter I, Bauman WA, Spungen AM (2015) Heart rate and oxygen demand of powered exoskeleton-assisted walking in persons with paraplegia. J Rehabil Res Dev 52:147–158

Bakkum A, Paulson T, Bishop N, Goosey-Tolfrey V, Stolwijk-Swüste J, Kuppevelt D, Groot S, Janssen T (2015) Effects of hybrid cycle and handcycle exercise on cardiovascular disease risk factors in people with spinal cord injury: a randomized controlled trial. J Rehabil Med 47:523–530

Bezdudnaya T, Marchenko V, Zholudeva LV, Spruance VM, Lane MA (2017) Supraspinal respiratory plasticity following acute cervical spinal cord injury. Exp Neurol 293:181–189

Bigford GE, Mendez AJ, Betancourt L, Burns-Drecq P, Backus D, Nash MS (2017) A lifestyle intervention program for successfully addressing major cardiometabolic risks in persons with SCI: a three-subject case series. Spinal Cord Ser Cases 3:1–8

Binder-Macleod SA, Snyder-Mackler L (1993) Muscle fatigue: clinical implications for fatigue assessment and neuromuscular electrical stimulation. Phys Ther 73:902–910

Borel JC, Wuyam B, Chouri-Pontarollo N, Deschaux C, Levy P, Pépin JL (2008) During exercise non-invasive ventilation in chronic restrictive respiratory failure. Respir Med 102:711–719

Brunt VE, Eymann TM, Francisco MA, Howard MJ, Minson CT (2016a) Passive heat therapy improves cutaneous microvascular function in sedentary humans via improved nitric oxide-dependent dilation. J Appl Physiol 121:716–723

Brunt VE, Howard MJ, Francisco MA, Ely BR, Minson CT (2016b) Passive heat therapy improves endothelial function, arterial stiffness and blood pressure in sedentary humans. J Physiol 594: 5329–5342

Brunt VE, Jeckell AT, Ely BR, Howard MJ, Thijssen DHJ, Minson CT (2016c) Acute hot water immersion is protective against impaired vascular function following forearm ischemia-reperfusion in young healthy humans. Am J Physiol - Regul Integr Comp Physiol 311: R1060–R1067

Brurok B, Helgerud J, Karlsen T, Leivseth G, Hoff J (2011) Effect of aerobic high-intensity hybrid training on stroke volume and peak oxygen consumption in men with spinal cord injury. Am J Phys Med Rehabil 90:407–414

Casaburi R, Barstow TJ, Robinson T, Wasserman K (1992) Dynamic and steady-state ventilatory and gas exchange responses to arm exercise. Med Sci Sports Exerc 24:1365–1374

Chilibeck PD, Bell G, Jeon J, Weiss CB, Murdoch G, MacLean I, Ryan E, Burnham R (1999a) Functional electrical stimulation exercise increases GLUT-1 and GLUT-4 in paralyzed skeletal muscle. Metabolism 48:1409–1413

Chilibeck PD, Jeon J, Weiss C, Bell G, Burnham R (1999b) Histochemical changes in muscle of individuals with spinal cord injury following functional electrical stimulated exercise training. Spinal Cord 37:264–268

Choi H, Liao WL, Newton KM, Onario RC, King AM, Desilets FC, Woodard EJ, Eichler ME, Frontera WR, Sabharwal S, Teng YD (2005) Respiratory abnormalities resulting from midcervical spinal cord injury and their reversal by serotonin 1A agonists in conscious rats. J Neurosci 25:4550–4559

Christiansen L, Chen B, Lei Y, Urbin MA, Richardson MSA, Oudega M, Sandhu M, Rymer WZ, Trumbower RD, Mitchell GS, Perez MA (2021) Acute intermittent hypoxia boosts spinal plasticity in humans with tetraplegia. Exp Neurol 335:113483

Coombs GB, Barak OF, Phillips AA, Mijacika T, Sarafis ZK, Lee AHX, Squair JW, Bammert TD, DeSouza NM, Gagnon D, Krassioukov AV, Dujic Z, DeSouza CA, Ainslie PN (2019) Acute heat stress reduces biomarkers of endothelial activation but not macro- or microvascular dysfunction in cervical spinal cord injury. Am J Physiol Circ Physiol 316:H722–H733

Cragg JJ, Stone JA, Krassioukov AV (2012) Management of Cardiovascular Disease Risk Factors in individuals with chronic spinal cord injury: an evidence-based review. J Neurotrauma 29: 1999–2012

Cragg JJ, Noonan VK, Krassioukov A, Borisoff J (2013) Cardiovascular disease and spinal cord injury results from a national population health survey. Neurology 81:723–728

De Troyer A, Heilporn A (1980) Respiratory mechanics in quadriplegia. The respiratory function of the intercostal muscles. Am Rev Respir Dis 122:591–600

De Troyer A, Estenne M, Heilporn A (1986) Mechanism of active expiration in tetraplegic subjects. N Engl J Med 314:740–744

Dearwater SR, Laporte RE, Robertson RJ, Brenes G, Adams LL, Becker D (1986) Activity in the spinal cord-injured patient: an epidemiologic analysis of metabolic parameters. Med Sci Sports Exerc 18:541–544

Deley G, Denuziller J, Babault N (2014) Functional electrical stimulation: cardiorespiratory adaptations and applications for training in paraplegia. Sport Med 45:71–82

DeVivo MJ, Krause JS, Lammertse DP (1999) Recent trends in mortality and causes of death among persons with spinal cord injury. Arch Phys Med Rehabil 80:1411–1419

Donovan J, Forrest G, Linsenmeyer T, Kirshblum S (2021) Spinal cord stimulation after spinal cord injury: promising multisystem effects. Curr Phys Med Rehabil Reports 9:23–31

Draghici AE, Taylor JA (2018) Baroreflex autonomic control in human spinal cord injury: physiology, measurement, and potential alterations. Auton Neurosci Basic Clin 209:37–42

Dreher M, Storre JH, Windisch W (2007) Noninvasive ventilation during walking in patients with severe COPD: a randomised cross-over trial. Eur Respir J 29:930–936

Dreher M, Kabitz H-J, Burgardt V, Walterspacher S, Windisch W (2010) Proportional assist ventilation improves exercise capacity in patients with obesity. Respiration 80:106–111

El-Khatib MF, Kiwan RA, Jamaleddine GW (2003) Buspirone treatment for Apneustic breathing in brain stem infarct. Respir Care 48:956–958

Ely BR, Clayton ZS, McCurdy CE, Pfeiffer J, Minson CT (2018) Meta-inflammation and cardiometabolic disease in obesity: can heat therapy help? Temperature 5:9–21

Ely BR, Clayton ZS, McCurdy CE, Pfeiffer J, Needham KW, Comrada LN, Minson CT, Wiedenfeld-Needham K, Comrada LN, Minson CT, Needham KW, Comrada LN, Minson CT (2019a) Heat therapy improves glucose tolerance and adipose tissue insulin signaling in polycystic ovary syndrome 317:E172–E182

Ely BR, Francisco MA, Halliwill JR, Bryan SD, Comrada LN, Larson EA, Brunt VE, Minson CT (2019b) Heat therapy reduces sympathetic activity and improves cardiovascular risk profile in women who are obese with polycystic ovary syndrome. Am J Physiol Integr Comp Physiol 317: R630–R640

Ely MR, Taylor JA (2021) The practical utility of functional electrical stimulation exercise for cardiovascular health in individuals with spinal cord injury. Curr Phys Med Rehabil Rep 9 (3):154–162

Ely MR, Singh TK, Baggish AL, Taylor JA (2021) Reductions in cardiac structure and function 24 months after spinal cord injury: a cross-sectional study. Arch Phys Med Rehabil 102(8): 1490–1498

Engelland RE, Hemingway HW, Tomasco OG, Olivencia-Yurvati AH, Romero SA (2020) Acute lower leg hot water immersion protects macrovascular dilator function following ischaemia–reperfusion injury in humans. Exp Physiol 105:302–311

Evans N, Hartigan C, Kandilakis C, Pharo E, Clesson I (2015) Acute cardiorespiratory and metabolic responses during exoskeleton-assisted walking overground among persons with chronic spinal cord injury. Top Spinal Cord Inj Rehabil 21:122–132

Fornusek C, Davis GM (2008) Cardiovascular and metabolic responses during functional electric stimulation cycling at different cadences. Arch Phys Med Rehabil 89:719–725

Franklin BA, McCullough PA (2009) Cardiorespiratory fitness: an independent and additive marker of risk stratification and health outcomes. Mayo Clin Proc 84:776–779

Fu J, Wang H, Deng L, Li J (2016) Exercise training promotes functional recovery after spinal cord injury. Neural Plast 2016:1–7

Fuller DD, Zabka AG, Baker TL, Mitchell GS (2001) Physiological and genomic consequences of intermittent hypoxia selected contribution: phrenic long-term facilitation requires 5-HT receptor activation during but not following episodic hypoxia. J Appl Physiol 90:2001–2006

Fuller DD, Johnson SM, Olson EB, Mitchell GS (2003) Synaptic pathways to phrenic motoneurons are enhanced by chronic intermittent hypoxia after cervical spinal cord injury. J Neurosci 23: 2993–3000

Gerasimenko YP, Ichiyama RM, Lavrov IA, Courtine G, Cai L, Zhong H, Roy RR, Edgerton VR (2007) Epidural spinal cord stimulation plus quipazine administration enable stepping in complete spinal adult rats. J Neurophysiol 98:2525–2536

Gerasimenko YP, Lu DC, Modaber M, Zdunowski S, Gad P, Sayenko DG, Morikawa E, Haakana P, Ferguson AR, Roy RR, Edgerton VR (2015) Noninvasive reactivation of motor descending control after paralysis. J Neurotrauma 32:1968–1980

Gibbons RS, Stock CG, Andrews BJ, Gall A, Shave RE (2016) The effect of FES-rowing training on cardiac structure and function: pilot studies in people with spinal cord injury. Spinal Cord 54: 822–829

Glaser RM, Sawka MN, Brune MF, Wilde SW (1980) Physiological responses to maximal effort wheelchair and arm crank ergometry. J Appl Physiol Respir Environ Exerc Physiol 48:1060–1064

Gorgey AS, Wade R, Sumrell R, Villadelgado L, Khalil RE, Lavis T (2017) Exoskeleton training may improve level of physical activity after spinal cord injury: a case series. Top. Spinal Cord Inj. Rehabil. 23:245–255

Green DJ (2009) Exercise training as vascular medicine: direct impacts on the vasculature in humans. Exerc Sport Sci Rev 37:196–202

Gregory CM, Bickel CS (2005) Recruitment patterns in human skeletal muscle during electrical stimulation. Phys Ther 85:358–364

Griffin L, Decker MJ, Hwang JY, Wang B, Kitchen K, Ding Z, Ivy JL (2009) Functional electrical stimulation cycling improves body composition, metabolic and neural factors in persons with spinal cord injury. J Electromyogr Kinesiol 19:614–622

Guertin P, Guertin PA (2012) Two promising novel classes of drug treatment in clinical development for acute and chronic spinal cord injury. J Neurol Neurophysiol 3:107

Guertin PA, Ung R-V, Rouleau P (2010) Oral administration of a tri-therapy for central pattern generator activation in paraplegic mice: proof-of-concept of efficacy. Biotechnol J 5:421–426

Guertin PA, Ung RV, Rouleau P, Steuer I (2011) Effects on locomotion, muscle, bone, and blood induced by a combination therapy eliciting weight-bearing stepping in nonassisted spinal cord-transected mice. Neurorehabil Neural Repair 25:234–242

Hansebout RR, Blight AR, Fawcett S, Reddy K (1993) 4-Aminopyridine in chronic spinal cord injury: a controlled, double-blind, crossover study in eight patients. J Neurotrauma 10:1–18

Harvey LA, Glinsky JV, Bowden JL (2016) The effectiveness of 22 commonly administered physiotherapy interventions for people with spinal cord injury: a systematic review. Spinal Cord 54:914–923

Hayes HB, Jayaraman A, Herrmann M, Mitchell GS, Rymer WZ, Trumbower RD (2014) Daily intermittent hypoxia enhances walking after chronic spinal cord injury a randomized trial. Neurology 82:104–113

Hettinga DM, Andrews BJ (2008) Oxygen consumption during functional electrical stimulation-assisted exercise in persons with spinal cord injury: implications for fitness and health. Sport. Med. 38:825–838

Hooper PL (1992) Hot-tub therapy for type 2 diabetes mellitus. N Engl J Med 327:742–747

Hooper PL, Hooper PL (2009) Inflammation, heat shock proteins, and type 2 diabetes. Cell Stress Chaperones 14:113–115

Horowitz M, Assadi H (2010) Heat acclimation-mediated cross-tolerance in cardioprotection: do HSP70 and HIF1α play a role? Ann N Y Acad Sci 1188:199–206

Hunt KJ, Saunders BA, Perret C, Berry H, Allan DB, Donaldson N, Kakebeeke TH (2007) Energetics of paraplegic cycling: a new theoretical framework and efficiency characterisation for untrained subjects. Eur J Appl Physiol 101:277–285

Ievins A, Moritz CT (2017) Therapeutic stimulation for restoration of function after spinal cord injury. Physiology 32:391–398

Imamura M, Biro S, Kihara T, Yoshifuku S (2001) Repeated thermal therapy improves impaired vascular endothelial function in patients with coronary risk factors. J Am Coll Cardiol 38:1083–1088

Jacobs P, Nash M, Rusinowski JW (2001) Circuit training provides cardiorespiratory and strength benefits in persons with paraplegia. Med Sci Sports Exerc 33:711–717

Jain NB, Ayers GD, Peterson EN, Harris MB, Morse L, O'Connor KC, Garshick E (2015) Traumatic spinal cord injury in the United States, 1993-2012. JAMA - J Am Med Assoc 313: 2236–2243

Jaiswal PB, Tester NJ, Davenport PW (2016) Effect of acute intermittent hypoxia treatment on ventilatory load compensation and magnitude estimation of inspiratory resistive loads in an individual with chronic incomplete cervical spinal cord injury. J Spinal Cord Med 39:103–110

Jilge B, Minassian K, Rattay F, Pinter MM, Gerstenbrand F, Binder H, Dimitrijevic MR (2004) Initiating extension of the lower limbs in subjects with complete spinal cord injury by epidural lumbar cord stimulation. Exp Brain Res 154:308–326

Jo HJ, Perez MA (2020) Corticospinal-motor neuronal plasticity promotes exercise-mediated recovery in humans with spinal cord injury. Brain 143:1368–1382

Johnson JM, Proppe DW (2011) Cardiovascular adjustments to heat stress. In: Handbook of physiology. Environmental Physiology, Bethesda, MD

Joyner MJ, Green DJ (2009) Exercise protects the cardiovascular system: effects beyond traditional risk factors. J Physiol 587:5551–5558

Kandilakis C, Sasso-Lance E (2019) Exoskeletons for personal use after spinal cord injury. Arch Phys Med Rehabil 102:331–337

Karelis AD, Carvalho LP, Castillo MJE, Gagnon DH, Aubertin-Leheudre M (2017) Effect on body composition and bone mineral density of walking with a robotic exoskeleton in adults with chronic spinal cord injury. J Rehabil Med 49:84–87

Kheck NM, Gannon PJ, Azmitia EC (1995) 5-HT1A receptor localization on the axon hillock of cervical spinal motoneurons in primates. J Comp Neurol 355:211–220

Kihara T, Biro S, Imamura M, Yoshifuku S (2002) Repeated sauna treatment improves vascular endothelial and cardiac function in patients with chronic heart failure. J Am Coll Cardiol 39: 754–759

Kikkinos P, Faselis C, Meyers J, Sui X, Zhang J, Blair SN (2014) Age-specific exercise capacity threshold for mortality risk assessment in male veterans. Circulation 130:653–658

Krause M, Ludwig MS, Heck TG, Takahashi HK (2015) Heat shock proteins and heat therapy for type 2 diabetes. Curr Opin Clin Nutr Metab Care 18:374–380

Kurucz I, Morva A, Vaag A, Eriksson K, Huang X, Groop L, Koranyi L (2002) Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance. Diabetes 51:1102–1109

Laskin JJ, Ashley EA, Olenik LM, Burnham R, Cumming DC, Steadward RD, Wheeler GD (1993) Electrical stimulation-assisted rowing exercise in spinal cord injured people. A pilot study. Paraplegia 31:534–541

Laughlin MH (1999) Cardiovascular response to exercise. Adv. Physiol. Educ 22(1):S244–S259
Laughlin MHH, Newcomer SCSC, Bender SBSB (2008) Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. J Appl Physiol 104:588–600
LaVela SL, Weaver FM, Goldstein B, Chen K, Miskevics S, Rajan S, Gater DR (2006) Diabetes mellitus in individuals with spinal cord injury or disorder. J Spinal Cord Med 29:387–395
Leicht CA, Kouda K, Umemoto Y, Banno M, Kinoshita T, Moriki T, Nakamura T, Bishop NC, Goosey-Tolfrey VL, Tajima F (2015) Hot water immersion induces an acute cytokine response in cervical spinal cord injury. Eur J Appl Physiol 115:2243–2252
Li J, Thorne LN, Punjabi NM, Sun CK, Schwartz AR, Smith PL, Marino RL, Rodriguez A, Hubbard WC, O'Donnell CP, Polotsky VY (2005) Intermittent hypoxia induces hyperlipidemia in lean mice. Circ Res 97:698–706
Ling L, Fuller DD, Bach KB, Kinkead R, Olson EB, Mitchell GS (2001) Chronic intermittent hypoxia elicits serotonin-dependent plasticity in the central neural control of breathing. J Neurosci 21:5381–5388
Locke M, Noble EG, Atkinson BG (1990) Exercising mammals synthesize stress proteins. Am J Physiol - Cell Physiol 258:C723–C1246
Lu DC, Edgerton VR, Modaber M, Auyong N, Morikawa E, Zdunowski S, Sarino ME, Sarrafzadeh M, Nuwer MR, Roy RR, Gerasimenko Y (2016) Engaging cervical spinal cord networks to Reenable volitional control of hand function in tetraplegic patients. Neurorehabil Neural Repair 30:951–962
Maloyan A, Eli-Berchoer L, Semenza GL, Gerstenblith G, Stern MD, Horowitz M (2005) HIF-1alpha-targeted pathways are activated by heat acclimation and contribute to acclimation-ischemic cross-tolerance in the heart. Physiol Genomics 23:79–88
Maltais F, Reissmann H, Gottfried SB (1995) Pressure support reduces inspiratory effort and dyspnea during exercise in chronic airflow obstruction. Am J Respir Crit Care Med 151: 1027–1033
Martin Ginis KA, Van Der Scheer JW, Latimer-Cheung AE, Barrow A, Bourne C, Carruthers P, Bernardi M, Ditor DS, Gaudet S, De Groot S, Hayes KC, Hicks AL, Leicht CA, Lexell J, MacAluso S, Manns PJ, McBride CB, Noonan VK, Pomerleau P, Rimmer JH, Shaw RB, Smith B, Smith KM, Steeves JD, Tussler D, West CR, Wolfe DL, Goosey-Tolfrey VL (2018) Evidence-based scientific exercise guidelines for adults with spinal cord injury: an update and a new guideline. Spinal Cord 56:308–321
Massucci M, Brunetti G, Piperno R, Betti L, Franceschini M (1998) Walking with the advanced reciprocating gait orthosis (ARGO) in thoracic paraplegic patients: energy expenditure and cardiorespiratory performance. Spinal Cord 36:223–227
McDonald JW, Sadowsky C (2002) Spinal-cord injury. Elsevier Limited, Lancet, pp 417–425
McParland C, Krishnan B, Lobo J, Gallagher CG (1992) Effect of physical training on breathing pattern during progressive exercise. Respir Physiol 90:311–323
McPherson JG, Miller RR, Perlmutter SI, Poo MM (2015) Targeted, activity-dependent spinal stimulation produces long-lasting motor recovery in chronic cervical spinal cord injury. Proc Natl Acad Sci U S A 112:12193–12198
Mercier HW, Taylor JA (2016) The physiology of exercise in Spinal Cord Injury (SCI): an overview of the limitations and adaptations. In: The physiology of exercise in spinal cord injury. Springer US, Berlin, pp 1–11
Meyer C, Hofstoetter US, Hubli M, Hassani RH, Rinaldo C, Curt A, Bolliger M (2020) Immediate effects of transcutaneous spinal cord stimulation on motor function in chronic, sensorimotor incomplete spinal cord injury. J Clin Med 9:3541
Minassian K, Hofstoetter US, Danner SM, Mayr W, Bruce JA, McKay WB, Tansey KE (2016) Spinal rhythm generation by step-induced feedback and transcutaneous posterior root stimulation in complete spinal cord-injured individuals. Neurorehabil Neural Repair 30:233–243
Mushahwar VK, Gillard DM, Gauthier MJA, Prochazka A (2002) Intraspinal microstimulation generates locomotor-like and feedback-controlled movements. IEEE Trans Neural Syst Rehabil Eng 10:68–81

Mutton DL, Scremin AME, Barstow TJ, Scott MD, Kunkel CF, Cagle TG (1997) Physiologic responses during functional electrical stimulation leg cycling and hybrid exercise in spinal cord injured subjects. Arch Phys Med Rehabil 78:712–718

Myers J, Lee M, Kiratli J (2007) Cardiovascular disease in spinal cord injury: an overview of prevalence, risk, evaluation, and management. Am. J. Phys. Med. Rehabil 86:142–152

Neff D, Kuhlenhoelter AM, Lin C, Wong BJ, Motaganahalli RL, Roseguini BT (2016) Thermotherapy reduces blood pressure and circulating endothelin-1 concentration and enhances leg blood flow in patients with symptomatic peripheral artery disease. Am. J. Physiol. Integr. Comp. Physiol. 311:R392–R400

Ozpinar A, Tempel ZJ, Monaco EA (2016) Targeted, activity-dependent spinal stimulation produces long-lasting motor recovery in chronic cervical spinal cord injury. Neurosurgery 78:N18–N19

Peckham PH, Knutson JS (2005) Functional electrical stimulation for neuromuscular applications. Annu Rev Biomed Eng 7:327–360

Pelletier CA, Hicks AL (2013) Importance of exercise in the rehabilitation process after spinal cord injury. Crit Rev Phys Rehabil Med 25:143–158

Phillips SM, Stewart BG, Mahoney DJ, Hicks AL, McCartney N, Tang JE, Wilkinson SB, Armstrong D, Tarnopolsky MA (2004) Body-weight-support treadmill training improves blood glucose regulation in persons with incomplete spinal cord injury. J Appl Physiol 97: 716–724

Qiu S, Taylor JA (2016) Hybrid functional electrical stimulation exercise for improved cardiorespiratory fitness in SCI. In: The physiology of exercise in spinal cord injury. Springer US, New York, pp 269–286

Qiu S, Alzhab S, Picard G, Taylor JA (2016) Ventilation limits aerobic capacity after functional electrical stimulation row training in high spinal cord injury. Med Sci Sports Exerc 48:1111–1118

Rhind SG, Gannon GA, Shephard RJ, Buguet A, Shek PN, Radomski MW (2004) Cytokine induction during exertional hyperthermia is abolished by core temperature clamping: neuroendocrine regulatory mechanisms. Int J Hyperth 20:503–516

Roberts TT, Leonard GR, Cepela DJ (2017) Classifications in brief: American spinal injury association (ASIA) impairment scale. Clin Orthop Relat Res 475:1499–1504

Rodgers MM, Figoni S, Hooker S, Glaser RM, Figoni SI, Rkt P, Hooker SP, Ezenwa BN, Collins SR, Nlathews T, Gupta SC (1991) Musculoskeletal responses of spinal cord injured individuals to functional neuromuscular stimulation-induced knee extension exercise training REGARDS-physical activity view project impacts of encouraging dog walking on returns of new adopted dogs to a shelter view project Department of Veterans Affairs Musculoskeletal responses of spinal cord injured individuals to functional neuromuscular stimulation-induced knee extension exercise. Artic J Rehabil Res Dev 28:19–26

Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB (2012) Heart disease and stroke statistics-2012 update: a report from the American heart association. Circulation 125:e2

Romero SA, Gagnon D, Adams AN, Cramer MN, Kouda K, Crandall CG (2017) Acute limb heating improves macro- and microvascular dilator function in the leg of aged humans. Am. J. Physiol. Circ. Physiol. 312:H89–H97

Ryan S, Taylor CT, McNicholas WT (2005) Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. Circulation 112:2660–2667

Sabatier MJ, Stoner L, Mahoney ET, Black C, Elder C, Dudley GA, McCully K (2006) Electrically stimulated resistance training in SCI individuals increases muscle fatigue resistance but not femoral artery size or blood flow. Spinal Cord 44:227–233

Saito Y, Hashimoto T, Iwata H, Takahashi K, Fukumizu M, Sasaki M, Hanaoka S, Sugai K (1999) Apneustic breathing in children with brainstem damage due to hypoxic-ischemic encephalopathy. Dev Med Child Neurol 41:560–567

Sandrow-Feinberg HR, Izzi J, Shumsky JS, Zhukareva V, Houle JD (2009) Forced exercise as a rehabilitation strategy after unilateral cervical spinal cord contusion injury. J Neurotrauma 26: 721–731

Sankari A, Bascom AT, Riehani A, Badr MS (2015) Tetraplegia is associated with enhanced peripheral chemoreflex sensitivity and ventilatory long-term facilitation. J Appl Physiol 119: 1183–1193

Sawka MN, Glaser RM, Wilde SW, Von Luhrte TC (1980) Metabolic and circulatory responses to wheelchair and arm crank exercise. J Appl Physiol Respir Environ Exerc Physiol 49:784–788

Sayenko DG, Rath M, Ferguson AR, Burdick JW, Havton LA, Edgerton VR, Gerasimenko YP (2019) Self-assisted standing enabled by non-invasive spinal stimulation after spinal cord injury. J Neurotrauma 36:1435–1450

Schilero GJ, Radulovic M, Wecht JM, Spungen AM, Bauman WA, Lesser M (2014) A center's experience: pulmonary function in spinal cord injury. Lung 192:339–346

Schmidt KD, Chan CW (1992) Thermoregulation and fever in Normal persons and in those with spinal cord injuries. Mayo Clin Proc 67:469–475

Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, Mcdonald-Smith GP, Gao H, Hennessy L, Finnerty CC, López CM, Honari S, Moore EE, Minei JP, Cuschieri J, Bankey PE, Johnson JL, Sperry J, Nathens AB, Billiar TR, West MA, Jeschke MG, Klein MB, Gamelli RL, Gibran NS, Brownstein BH, Miller-Graziano C, Calvano SE, Mason PH, Cobb JP, Rahme LG, Lowry SF, Maier RV, Moldawer LL, Herndon DN, Davis RW, Xiao W, Tompkins RG (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 110:3507–3512

Shields RK (2002) Muscular, skeletal, and neural adaptations following spinal cord injury. J Orthop Sports Phys Ther 32:65–74

Srinivas S, Wali AR, Pham MH (2019) Efficacy of riluzole in the treatment of spinal cord injury: a systematic review of the literature. Neurosurg Focus 46:1–18

Sunshine MD, Cho FS, Lockwood DR, Fechko AS, Kasten MR, Moritz CT (2013) Cervical intraspinal microstimulation evokes robust forelimb movements before and after injury. J Neural Eng 10:036001

Taylor AW, McDonell E, Brassard L (1986) The effects of an arm ergometer training programme on wheelchair subjects. Paraplegia 24:105–114

Taylor JA, Picard G, Widrick JJ (2011) Aerobic capacity with hybrid FES rowing in spinal cord injury: comparison with arms-only exercise and preliminary findings with regular training. PM R 3:817–824

Taylor JA, Picard G, Porter A, Morse LR, Pronovost MF, Deley G (2014) Hybrid functional electrical stimulation exercise training alters the relationship between spinal cord injury level and aerobic capacity. Arch Phys Med Rehabil 95:2172–2179

Tei C, Horikiri Y, Park JC, Jeong JW, Chang KS, Toyama Y, Tanaka N (1995) Acute hemodynamic improvement by thermal vasodilation in congestive heart failure. Circulation 91:2582–2590

Teng YD, Bingaman M, Taveira-DaSilva AM, Pace PP, Gillis RA, Wrathall JR (2003) Serotonin 1A receptor agonists reverse respiratory abnormalities in spinal cord-injured rats. J Neurosci 23: 4182–4189

Tester NJ, Fuller DD, Fromm JS, Spiess MR, Behrman AL, Mateika JH (2014) Long-term facilitation of ventilation in humans with chronic spinal cord injury. Am J Respir Crit Care Med 189:57–65

Turiel M, Sitia S, Cicala S, Magagnin V, Bo I, Porta A, Caiani E, Ricci C, Licari V, De Gennaro Colonna V, Tomasoni L (2011) Robotic treadmill training improves cardiovascular function in spinal cord injury patients. Int J Cardiol 149:323–329

Tweedy SM, Beckman EM, Geraghty TJ, Theisen D, Perret C, Harvey LA, Vanlandewijck YC (2017) Exercise and sports science Australia (ESSA) position statement on exercise and spinal cord injury. J Sci Med Sport 20:108–115

Ung RV, Rouleau P, Guertin PA (2012) Functional and physiological effects of treadmill training induced by buspirone, carbidopa, and L-DOPA in clenbuterol-treated paraplegic mice. Neurorehabil Neural Repair 26:385–394

Van Den Brand R, Heutschi J, Barraud Q, DiGiovanna J, Bartholdi K, Huerlimann M, Friedli L, Vollenweider I, Moraud EM, Duis S, Dominici N, Micera S, Musienko P, Courtine G (2012) Restoring voluntary control of locomotion after paralyzing spinal cord injury. Science (80-) 336: 1182–1185

van't Hul A, Gosselink R, Hollander P, Postmus P, Kwakkel G (2004) Acute effects of inspiratory pressure support during exercise in patients with COPD. Eur Respir J 23:34–40

van't Hul A, Gosselink R, Hollander P, Postmus P, Kwakkel G (2006) Training with inspiratory pressure support in patients with severe COPD. Eur Respir J 27:65–72

Vila B, Servera E, Marín J, Díaz J, Giménez M, Komaroff E, Bach J (2007) Noninvasive Ventilatory assistance during exercise for patients with kyphoscoliosis. Am J Phys Med Rehabil 86:672–677

Vivodtzev I, Picard G, Cepeda FX, Taylor JA (2020) Acute Ventilatory support during whole-body hybrid rowing in patients with high-level spinal cord injury: a randomized controlled crossover trial. In: Chest. Elsevier Inc, Amsterdam, pp 1230–1240

Vivodtzev I, Picard G, O'Connor K, Taylor JA (2021) Serotonin 1A agonist and cardiopulmonary improvements with whole-body exercise in acute, high-level spinal cord injury: a retrospective analysis. Eur J Appl Physiol 121:453–463

Wagner FB, Mignardot JB, Le Goff-Mignardot CG, Demesmaeker R, Komi S, Capogrosso M, Rowald A, Seáñez I, Caban M, Pirondini E, Vat M, McCracken LA, Heimgartner R, Fodor I, Watrin A, Seguin P, Paoles E, Van Den Keybus K, Eberle G, Schurch B, Pralong E, Becce F, Prior J, Buse N, Buschman R, Neufeld E, Kuster N, Carda S, von Zitzewitz J, Delattre V, Denison T, Lambert H, Minassian K, Bloch J, Courtine G (2018) Targeted neurotechnology restores walking in humans with spinal cord injury. Nature 563:65–93

Warburton DER, Eng JJ, Krassioukov A, Sproule S (2007) Cardiovascular health and exercise rehabilitation in spinal cord injury. Top Spinal Cord Inj Rehabil 13:98–122

Waters RL, Mulroy S (1999) The energy expenditure of normal and pathologic gait. Gait Posture 9(3):207–231

Weaver FM, Collins EG, Kurichi J, Miskevics S, Smith B, Rajan S, Gater D (2007) Prevalence of obesity and high blood pressure in veterans with spinal cord injuries and disorders. Am J Phys Med Rehabil 86:22–29

Whiteneck GG, Charlifue SW, Frankel HL, Fraser MH, Gardner BP, Gerhart KA, Krishnan KR, Menter RR, Nuseibeh I, Short DJ, Silver JR (1992) Mortality, morbidity, and psychosocial outcomes of persons spinal cord injured more than 20 years ago. Paraplegia 30:617–630

WHO (2013) World Health Organization and international spinal cord society. International perspectives on spinal cord injury. [WWW Document]

Williams AM, Gee CM, Voss C, West CR (2019) Cardiac consequences of spinal cord injury: systematic review and meta-analysis. Heart 105:217–225

Wu M, Landry JM, Schmit BD, Hornby TG, Yen SC (2012) Robotic resistance treadmill training improves locomotor function in human spinal cord injury: a pilot study. Arch Phys Med Rehabil 93:782–789

Zhang D, Xuan J, Zheng BB, Zhou YL, Lin Y, Wu YS, Zhou YF, Huang YX, Wang Q, Shen LY, Mao C, Wu Y, Wang XY, Tian NF, Xu HZ, Zhang XL (2017) Metformin improves functional recovery after spinal cord injury via autophagy flux stimulation. Mol Neurobiol 54:3327–3341

Zimmer MB, Goshgarian HG (2007) Spinal cord injury in neonates alters respiratory motor output via supraspinal mechanisms. Exp Neurol 206:137–145

Zimmermann JB, Seki K, Jackson A (2011) Reanimating the arm and hand with intraspinal microstimulation. J Neural Eng 8:054001

Zörner B, Schwab ME (2010) Anti-Nogo on the go: from animal models to a clinical trial. Ann N Y Acad Sci 1198:E22–E34

Chapter 13
Emerging Approaches for Regenerative Rehabilitation Following Traumatic Brain Injury

Regenerative Rehabilitation in TBI

John C. O'Donnell, Randel L. Swanson, Kathryn L. Wofford, Michael R. Grovola, Erin M. Purvis, Dmitriy Petrov, and D. Kacy Cullen

J. C. O'Donnell · K. L. Wofford · M. R. Grovola · D. Petrov
Center for Brain Injury & Repair, Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Center for Neurotrauma, Neurodegeneration & Restoration, Corporal Michael J. Crescenz Veterans Affairs Medical Center, Philadelphia, PA, USA

R. L. Swanson
Center for Brain Injury & Repair, Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Center for Neurotrauma, Neurodegeneration & Restoration, Corporal Michael J. Crescenz Veterans Affairs Medical Center, Philadelphia, PA, USA

Department of Physical Medicine & Rehabilitation, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

E. M. Purvis
Center for Brain Injury & Repair, Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Center for Neurotrauma, Neurodegeneration & Restoration, Corporal Michael J. Crescenz Veterans Affairs Medical Center, Philadelphia, PA, USA

Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

D. K. Cullen (✉)
Center for Brain Injury & Repair, Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Center for Neurotrauma, Neurodegeneration & Restoration, Corporal Michael J. Crescenz Veterans Affairs Medical Center, Philadelphia, PA, USA

Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA, USA
e-mail: dkacy@pennmedicine.upenn.edu

S. M. Greising, J. A. Call (eds.), *Regenerative Rehabilitation*, Physiology in Health and Disease, https://doi.org/10.1007/978-3-030-95884-8_13

Abstract The potential for rehabilitation to improve recovery after traumatic brain injury (TBI) is limited by a lack of inherent regenerative capacity in the brain as well as the chronic disabilities and ongoing pathologies of various injury endophenotypes. A large body of previous work has shown that traditional rehabilitative therapies in combination with dietary modifications and regular exercise can enhance brain plasticity and, in some cases, neurogenesis, but prolonged secondary injury and limits to plasticity and regeneration significantly limit the impact of rehabilitation. Therefore, there is an urgent need for therapeutic strategies to promote regeneration and complement rehabilitation efforts to maximize recovery from TBI. In the following chapter, we discuss the unique translational challenges for developing TBI therapeutics, existing approaches to rehabilitation, promising therapeutic targets for enhancing regeneration and plasticity, and emerging regenerative medicine approaches that could significantly expand attainable levels of functional recovery following TBI.

Keywords Traumatic brain injury · Regenerative medicine · Rehabilitation

13.1 Introduction

Traumatic Brain Injury (TBI) is a surprisingly common injury that can have devastating health consequences. According to a recent report from the Centers for Disease Control and Prevention, there were approximately 2.87 million TBI Emergency Department visits or hospitalizations in the USA in 2014—a 53% increase from 2006—of which 56,800 resulted in death (CDC 2019). Incidence has also increased in the modern military, with 22% of all combat casualties from Iraq and Afghanistan estimated to be TBIs compared to 12% from Vietnam (VA Office of R & D). While TBI is among the leading causes of death, survival can also be devastating as roughly 2% of the U.S. population currently lives with chronic TBI-related disabilities (Langlois et al. 2006; Wilson et al. 2017). Long-term disability along with increased risk for neurodegenerative disease, stroke, and other maladies have led many to view TBI as a chronic health condition (Wilson et al. 2017; Edlow et al. 2018). Thus, TBI has a tremendous impact on health and the overall economy, resulting from both direct medical expenditures and indirect costs totaling over $60 billion annually (Langlois et al. 2006). This underscores the fact that there are currently no targeted medical therapeutic agents to attenuate TBI-induced neural degeneration or to promote effective regeneration.

While TBI creates a chronic health condition, it begins with a physical event that generates injurious forces in the brain. More detailed descriptions of the mechanical forces of TBI and their translation to brain pathology can be found in several in-depth reviews (LaPlaca et al. 2007; Meaney and Smith 2015; Meaney and Cullen 2016; Keating and Cullen 2020). For the convenience of the reader, we are providing illustrations from the recent review by Keating and Cullen in Fig. 13.1. Mechanical loading in TBI can occur via direct impact, impulsive motion, blast pressure waves from an explosion, or a combination thereof (Fig. 13.1a). For example, while

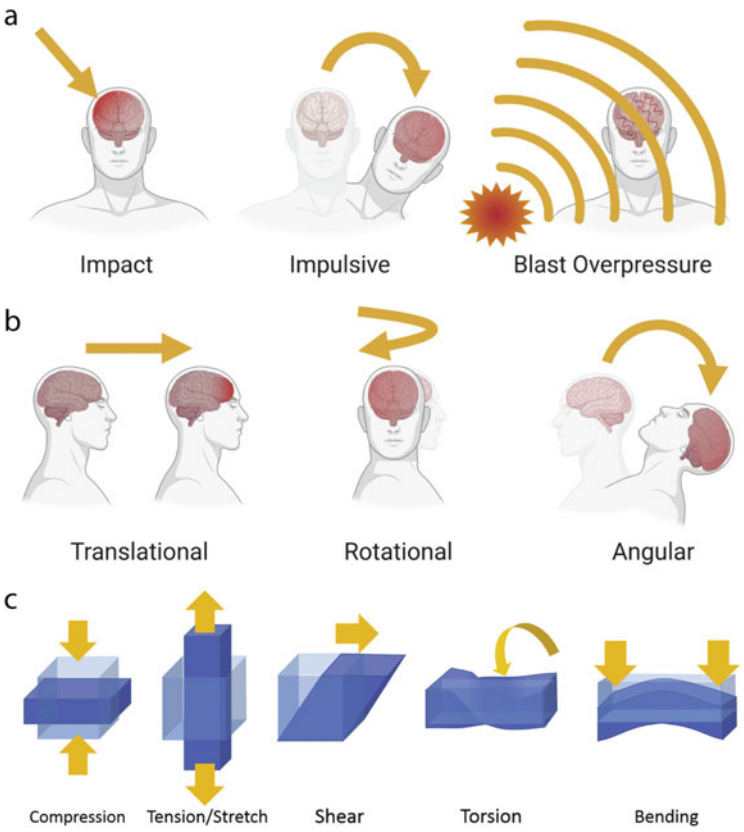

Fig. 13.1 Mechanical loading and deformation in TBI. Impact, impulse, and blast loading mechanisms of TBI are depicted (**a**). Head motion relative to the brain's center of mass affects impulse loading (**b**). Mechanical loading produces a variety of brain tissue deformations (**c**). Figure adapted with permission from Keating and Cullen (2020) (http://creativecommons.org/licenses/by-nc-nd/4.0/)

impulsive loading can be initiated without direct impact to the head (e.g., car accidents rapidly accelerating/decelerating the entire body), it most often occurs due to a direct impact to the head that sets the head in motion. Conversely, in the case of impulsive loading initiated by rapid body movement, the resulting head motion can also lead to impact loading due to the moving head encountering an object. Therefore impact-to-impulsive and impulsive-to-impact injuries are common (also impact-to-impulsive-to-impact). While primary impulsive loading with no focal impact lesion is common in mild TBI, impact to an immobilized head with no impulsive component is rare. The centrality of impulse loading to human TBI presents a challenge in preclinical studies, since the injurious forces are dependent on acceleration and brain mass, requiring large gyrencephalic animal models like non-human primates or swine to study mechanisms of injury and novel therapeutics in the context of impulse-generated TBI (Cullen et al. 2016; O'Donnell et al. 2019).

Table 13.1 Classification of TBI severity

(If a patient meets criteria in more than one category of severity, the higher severity level is assigned)			
Criteria	Mild	Moderate	Severe
Structural imaging	Normal	Normal or abnormal	Normal or abnormal
Loss of consciousness (LOC)	0–30 min	>30 min and <24 h	>24 h
Alterations of consciousness/mental state (AOC)[a]	Up to 24 h	>24 h; severity based on other criteria	
Posttraumatic amnesia (PTA)	0–1 day	>1 day and <7 days	>7 days
Glasgow Coma Scale (GCS) (best available score in first 24 h)[b]	13–15	9–12	<9

[a]Alteration of mental status must be immediately related to the trauma to the head. Typical symptoms would be looking and feeling dazed and uncertain of what is happening, confusion, and difficulty thinking clearly or responding appropriately to mental status questions, and being unable to describe events immediately before or after the trauma event.
[b]In April 2015, the DoD released a memorandum recommending against the use of GCS scores to diagnose TBI. See the memorandum for additional information
Table reproduced from VA/DoD Clinical Practice Guideline for the Management of Concussion-Mild Traumatic Brain Injury

Despite the impractical accelerations necessary to account for the small brain mass of rodents, there have been attempts to apply impulse loading in small animal TBI models, but unfortunately, it does not appear possible to reach scaled thresholds with such disparate masses (Meaney et al. 2001; Sauerbeck et al. 2018). Blast TBI often also includes impulsive or impact loading components, contributing to the heterogeneity of the injury. Adding to this heterogeneity, the magnitude, distribution, and consequences of injurious forces generated by impulse loading vary based on the way the head moves relative to the brain's center of mass (Fig. 13.1b). Translational (a.k.a. linear) impulse loading occurs when the center of mass moves without rotation, and may be associated with brain surface injury that can occur due to the brain impacting the skull (sometimes referred to as contrecoup injury). Rotational loading involves head swivel around the brain's center of mass, and angular rotational loading involves rotational acceleration affecting the brain's center of mass. While injury at the surface of the brain from translational/linear loading can be a concern, there are far more injurious forces generated by angular rotational loading. Foundational work in non-human primates revealed that angular rotational loading (not translational/linear) was necessary and sufficient to produce extended loss of consciousness, a key element for clinical classification of TBI severity as shown in Table 13.1 (Denny-Brown and Russell 1941; Ommaya and Gennarelli 1974). Later work in the swine model utilizing pure impulse loading demonstrated that angular rotational head acceleration in the axial plane produced diffuse injury throughout the brain and prolonged coma, with lesions in the pons associated with coma duration (Smith et al. 2000; Cullen et al. 2016). The pons is a key branch point of the

Ascending Reticular Activating System (ARAS), and trauma to deep brain structures of the ARAS are associated with coma and prolonged Disorders of Consciousness (DoC) in humans following TBI (Edlow et al. 2012, 2013; Snider et al. 2019, 2020).

Direction of head rotation was a key determinant of outcomes such as duration of unconsciousness in a piglet model of TBI, and a more recent study suggests that properties of individual kinematic elements of head rotation (e.g., maximum negative velocity and peak-to-minimum acceleration time, both associated with more abrupt deceleration) influence recovery parameters in adult swine (Eucker et al. 2011; Wofford et al. 2021). These various mechanical loading parameters result in a variety of distinct types of tissue deformation determined by the type of loading as well as the physical properties and relative orientation of the material (in this case the brain) being deformed (Fig. 13.1c). Forces generated by mechanical loading will result in a combination of these deformations, with impact generally associated with direct compression at the brain's surface, and impulse generally associated with shear deformation throughout the brain. These deformation patterns may result in gross damage to vasculature and diffuse axonal injury (DAI), and at a sub-cellular level may cause cytoskeletal damage, loss of membrane potential due to opening mechanically-sensitive ion channels, plasma and organelle membrane permeabilization, disruption of the extracellular matrix and cell contacts, and other primary pathologies (Meaney and Smith 2015; Keating et al. 2020; Keating and Cullen 2020). The mechanical loading and tissue deformation of TBI is a rapid, high-energy event that typically lasts only milliseconds, but can produce pathological consequences that last a lifetime.

Following the initial mechanical TBI, secondary injury cascades produce waves of additional pathology and dysfunction over days, weeks, months, and even years. There are currently no approved therapeutics to mitigate this ongoing secondary injury. The varied consequences of the initial mechanical injury and the resulting multi-faceted secondary sequelae have been termed "endophenotypes." The concept of endophenotypes recognizes the heterogeneity of TBI and its consequences and allows a focus on treatment strategies and targeted therapeutics based on affecting specific phenomena. The heterogeneity of the primary injuries of TBI along with that of the patient population (e.g., age, sex, genetic background, medical history) contribute to the emergence of distinct endophenotypes—like microvascular injury and post-traumatic epilepsy (PTE)—with varying levels of contribution to the overall patient outcome (Diaz-Arrastia et al. 2009; Sandsmark et al. 2019). Endophenotypes that make major contributions to secondary injury cascades include inflammation, excitotoxicity, mitochondrial dysfunction, and oxidative stress at a cell/molecular level, and blood–brain barrier (BBB) disruption, microvascular injury, and PTE at the organ/systems level. Other endophenotypes of TBI exist as disabilities that impair rehabilitation and limit recovery but are not typically categorized as secondary injuries due to less-than-direct contributions to ongoing pathology. These include consequences such as sleep disturbances, confusion, and fatigue. Just as characteristics of the acute physical trauma are associated with—and indeed directly initiate—the subacute secondary injury cascades, the presence of various endophenotypes of secondary injury have been associated with increased

likelihood of chronic sequelae, such as pathological protein aggregation and neuroinflammation, potentially adding an additional layer of chronic neurodegenerative disease and disability. The line between injury and recovery is blurred following TBI due to prolonged secondary injury processes and ongoing neurodegeneration. Therefore, regenerative rehabilitation approaches to TBI must address both recovery and ongoing sequelae to be effective.

13.2 Current Approaches to TBI Rehabilitation

As highlighted above, there is significant heterogeneity inherent with a clinical diagnosis of TBI (Zasler et al. 2007). This includes variations in the number and severity of possible TBI-induced endophenotypes (e.g., DAI, cerebral microvascular injury, intraparenchymal contusion, intracranial hemorrhage, cerebral edema) within one or more regions of the brain (e.g., focal, multifocal, or diffuse injury), depending on the mechanism of injury and direction of the applied mechanical force(s). Further, patients exposed to TBI often have a wide variety of pre-existing medical comorbidities, many of which further contribute to compromised neurocognitive function and/or predict a worse outcome. In an effort to parse this heterogeneity, current diagnostic guidelines classify TBI as "mild," "moderate," or "severe" based on specific diagnostic criteria at the time of injury, including the presence and duration of any loss of consciousness, the duration of an alteration in consciousness or mental state, the duration of post-traumatic amnesia, the presence or absence of pathological findings on traditional structural neuroimaging, and the best available Glasgow Coma Scale (GCS) within 24-h of the TBI exposure, as shown in Table 13.1 (VA/DoD 2016).

As a result of the considerable heterogeneity and the non-specific nature of TBI diagnoses, current neurorehabilitation interventions are not designed to target or mitigate specific neuropathological component(s) (e.g., DAI, microvascular injury, or persistent neuroinflammation), whether present in discrete regions of the brain or diffusely throughout the brain parenchyma. Rather, an individualized treatment/neurorehabilitation plan is developed for each patient by a Physiatrist (physician specializing in Physical Medicine and Rehabilitation, often sub-specializing in Brain Injury Medicine) centered around common physical, cognitive, and neurobehavioral symptoms and/or functional deficits, which occur across the spectrum of TBI severity (Zasler et al. 2007; VA/DoD 2016). The resulting individualized neurorehabilitation plan is generally implemented through collaboration with a multidisciplinary team, including Physical Therapy, Occupational Therapy, Speech-Language Pathology, Neuropsychology, and Neuro-Optometry, amongst others. For each component of the overall neurorehabilitation prescription (e.g., Vestibular Physical Therapy), there is an emphasis on goal-based interventions to improve specific neurological symptoms or functional deficits, which are continually

reevaluated to ensure the prescribed neurorehabilitation interventions are leading to symptomatic and/or functional improvement. Thus, neurorehabilitation interventions selected for each patient are modified throughout the rehabilitation course, based on their individual therapeutic response. Three of the most common neurological deficits seen throughout all severities of TBI for which neurorehabilitation interventions are prescribed include cognitive, oculomotor, and vestibular deficits.

13.2.1 Cognitive Deficits

Cognitive complaints are often the most concerning symptom for patients following TBI exposure, and the extent of objective cognitive deficits vary widely during the acute and sub-acute phase of TBI recovery, throughout the spectrum of TBI severity (Esslinger et al. 2007; VA/DoD 2016). Initial clinical evaluation includes screening for orientation, executive, visuospatial, naming, memory, attention, and language deficits. Depending on the findings and the overall clinical presentation, the cognitive rehabilitation plan may be to (1) monitor for natural recovery and re-assess at a future date; (2) prescribe specific cognitive rehabilitation with Speech-Language Pathology and/or Occupational Therapy; or (3) refer to Neuropsychology for in-depth cognitive evaluation. The decision for referral for comprehensive neuropsychological testing depends on both the severity of TBI and the time post-TBI exposure. When performed, neuropsychological testing involves extensive objective testing of multiple cognitive domains, such as memory, working memory, attention, executive and academic functioning, language, reasoning, processing speed, visual-spatial perception, visual-motor construction, and motor function (Lezak et al. 2004; Esslinger et al. 2007). Neuropsychological testing can be used to (1) identify the specific cognitive domains that demonstrate an impairment, weakness, or strength; (2) help guide the cognitive rehabilitation provided by Speech-Language Pathology and/or Occupational Therapy; (3) generate specific accommodation request(s) for school or work; and (4) perform longitudinal evaluations to evaluate for cognitive improvement or secondary cognitive decline. This last point is becoming especially important given the increasing numbers of epidemiological studies demonstrating an association between TBI exposure and an increased risk of age-related cognitive decline or dementia (Fleminger et al. 2003; Gardner and Yaffe 2014, 2015; Gardner et al. 2014), including a recent large cohort study involving over 350,000 Veterans which documented a dose–response relationship between the severity of TBI (from "mild" TBI without a loss of consciousness through "moderate-to-severe" TBI) and the cumulative incidence of dementia diagnosis as illustrated in Fig. 13.2 (Barnes et al. 2018).

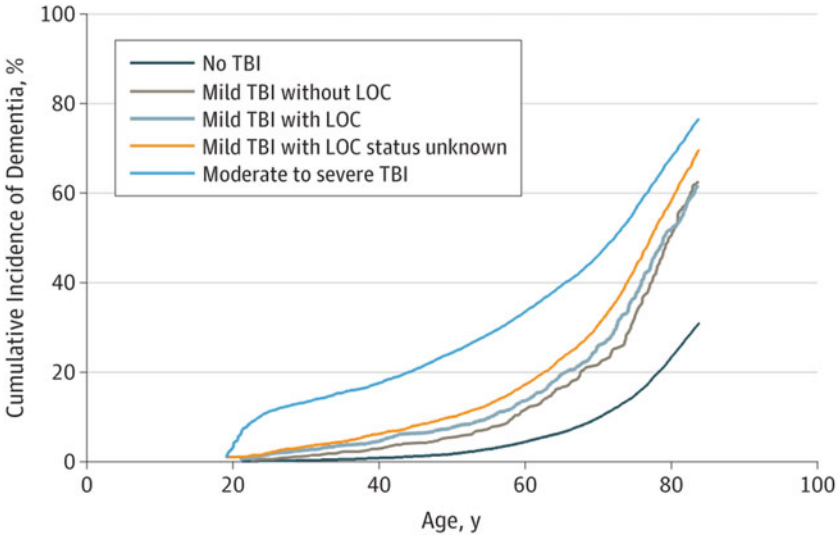

Fig. 13.2 Cumulative Incidence of Dementia by TBI Severity. The unadjusted cumulative incidence of dementia (age at dementia diagnosis) is shown as a function of TBI severity. After adjustment for demographics, medical conditions, and psychiatric disorders, there was a dose–response relationship between TBI severity and dementia diagnosis with hazard ratios of 2.36 (95% CI, 2.10-2.66) for mild TBI without loss of consciousness (LOC); 2.51 (95% CI, 2.29-2.76) for mild TBI with LOC; 3.19 (95% CI, 3.05-3.33) for mild TBI with LOC status unknown, and 3.77 (95% CI, 3.63-3.91) for moderate to severe TBI. Reproduced with permission from Barnes et al. (2018)

13.2.2 Oculomotor Deficits

Given that approximately half of the brain's neural networks are involved in binocular vision, abnormalities of oculomotor function are very sensitive to neurological insult and are commonly diagnosed throughout the spectrum of TBI severity (Padula et al. 2007; Ventura et al. 2014; VA/DoD 2016). Initial clinical assessment of oculomotor function often utilizes the validated Vestibular/Ocular Motor Screening (VOMS) assessment, which documents a patient's symptomatic response (headache, dizziness, nausea, and mental fogginess) when testing smooth pursuits, horizontal and vertical saccades, near point of convergence, the vestibulo-ocular reflex (VOR), and visual motion sensitivity (Mucha et al. 2014). When abnormalities are identified, patients are referred to Neuro-Optometry and/or Vestibular Physical Therapy for further sub-specialty evaluation and treatment (Padula et al. 2007; Scheiman and Wick 2008). Convergence insufficiency is then treated with either neuro-optometric rehabilitation or custom prism glasses when neuro-optometric rehabilitation is not indicated or available, while abnormalities of saccadic and smooth pursuit eye movements are treated by varied combinations of Vestibular Therapy, Occupational Therapy, and Neuro-Optometric Rehabilitation, depending

on the overall clinical presentation (Padula et al. 2007; Shepard et al. 2007; Scheiman and Wick 2008; Gallaway et al. 2017).

13.2.3 Vestibular Deficits

Vestibular deficits are commonly present throughout all severities of TBI and can be central and/or peripheral in origin (Shepard et al. 2007). Initial clinical evaluation often includes the validated Balance Error Scoring System (BESS) for static balance assessment, and components of a Functional Gait Assessment (FGA) for evaluation of dynamic balance (Wrisley et al. 2004; Bell et al. 2011; Iverson and Koehle 2013). Static balance screening with the BESS is a standardized assessment that evaluates a patient's stability during double limb stance, non-dominant single limb stance, and tandem stance, on both firm ground and a soft foam pad (Bell et al. 2011; Iverson and Koehle 2013). In contrast, dynamic balance screening with the FGA is a standardized gait assessment under ten conditions, including ambulation on level surfaces with changes in gait speed, horizontal and vertical head movements, eyes open versus closed, normal versus narrow base of support, pivot turns, stepping over obstacles, and ambulating backward (Wrisley et al. 2004). When abnormalities of static and/or dynamic balance are identified on initial clinical screening, referral is made to vestibular physical therapy for formal vestibular evaluation and treatment, where a more thorough assessment is conducted (Nashner 1993; Powell and Myers 1995; Shepard et al. 2007; Alahmari et al. 2014; Horn et al. 2015; VA/DoD 2016). Vestibular physical therapy evaluation commonly includes computerized dynamic posturography, which quantifies the ability to maintain postural stability through the use of visual, proprioceptive, and/or vestibular cues (Nashner 1993; Alahmari et al. 2014). TBI-induced static and/or dynamic vestibular deficits are then treated with a course of vestibular physical therapy, with the goal of retraining the vestibular system to maintain both static and dynamic postural control, utilizing a combination of visual and somatosensory substitution techniques, gaze stabilization exercises, saccadic and smooth pursuit eye movement exercises during both static stance and ambulation, and techniques to habituate the patient to chronic vestibular deficits which may remain despite targeted rehabilitation interventions (Shepard et al. 2007).

13.2.4 Developing the Evidence-Base for Rehabilitation Interventions

Apparent in the above overview of neurorehabilitation following TBI is the fact that there is substantial heterogeneity not only in TBI exposure and diagnosis but also in the subsequent neurorehabilitation prescribed. Further, the current neurorehabilitation interventions prescribed are targeting resulting neurological

symptoms and functional deficits, rather than targeting specific underlying neuropathology. Perhaps that is one reason why—despite numerous clinical studies—it is not currently possible to distinguish the effectiveness of different rehabilitation approaches after TBI (Injury et al. 2012; Brasure et al. 2013; Oberholzer and Müri 2019). Available neurorehabilitation interventions, while better than doing nothing, have indistinguishable efficacy and are limited to mechanistically vague, minimally invasive interventions due to a lack of a clear translatable preclinical pipeline for research and development of novel therapeutics. The inability to distinguish between the efficacy of neurorehabilitation strategies also stems from a disconnect between the "active ingredients" and the "therapeutic targets" within the overall strategies (Whyte et al. 2014). New frameworks like the Rehabilitation Treatment Specification System (RTSS) seek to improve the design, reporting, replication, and synthesis of rehabilitation research by providing guidance on forming specific hypotheses based on clearly identified "ingredients" and "targets" being tested, enabling refinement of the underlying theories that comprise neurorehabilitation strategies by elucidating their mechanism of action (Van Stan et al. 2019). This precise and rigorous approach to evaluating the efficacy of neurorehabilitation treatments will be vital for measuring and comparing the efficacy of novel regenerative treatments when integrated into neurorehabilitation, as will establishing a viable translational pipeline for developing those novel regenerative treatments.

13.2.5 Exercise and Diet

Currently, various forms of exercise and diet constitute key "ingredients" of TBI rehabilitation strategies. Exercise is generally believed to enhance the expression of brain-derived neurotrophic factor (BDNF), reduce reactive oxygen species, and improve hemodynamics leading to improved cognitive recovery in humans and animals (Devine and Zafonte 2009; Lojovich 2010). Exercise-induced growth factor cascades enhance synaptic plasticity by instructing a change in synaptic structure and potentiation of synaptic strength (Cotman et al. 2007). Exercise has been shown to increase neural stem cell proliferation (i.e., neurogenesis) in the injured brain parenchyma, prevent neurodegeneration, and improve cognition following experimental TBI (Itoh et al. 2011a, b). Additionally, exercise was found to augment hippocampal neurogenesis and was associated with improved neurobehavioral recovery in a preclinical model of TBI (Karelina et al. 2021). Exercise-induced neurogenesis following TBI may therefore contribute to cognitive recovery by enhancing plasticity and compensatory rewiring, increasing neurogenesis, and/or by increasing resistance to insult via indirect improvements to learning and memory. While we still have much to learn and refine, the benefits of exercise during recovery from TBI are widely accepted and it is an active ingredient in most rehabilitation therapies. Dietary factors have been found to improve recovery from TBI through similar mechanisms, and have even been found to complement exercise during rehabilitation (Gomez-Pinilla and Gomez 2011; Wu et al. 2013). Omega-3 fatty

acids—particularly the essential fatty acid and neural membrane component docosahexaenoic acid (DHA)—have been shown to promote the restoration of energy homeostasis, reduce reactive oxygen species, and increase BDNF after brain injury (Wu et al. 2004; Gomez-Pinilla and Gomez 2011). Interestingly, unlike the benefits for improving recovery after injury, neuroprotective effects were not observed in rats administered a prophylactic diet rich in fish oil (high in omega-3s) prior to FPI, while a diet high in saturated fatty acids and cholesterol—associated with reduced plasticity and negative impact on recovery—was protective against acute permeabilization of neuronal plasma membranes and reduced lesion size; thus highlighting the importance of considering injury mechanism and phase when developing therapeutic strategies (Keating et al. 2021). Dietary administration of branched-chain amino acids (BCAAs) has enhanced cognitive recovery after TBI in several rodent studies, due in part to correcting neurotransmitter synthesis deficiencies (Cole et al. 2010; Elliott et al. 2018; Paterno et al. 2018). Lateral FPI in mice led to a significant reduction in brain BCAA concentrations that were corrected with dietary BCAA administration, as were deficits in contextual fear conditioning (Cole et al. 2010) and spatial memory (Paterno et al. 2018). Dietary BCAAs and physical exercise share mechanisms of action, such as PGC1α-mediated increases in BDNF expression (Blomstrand 2001; Samuelsson et al. 2016; Nasrallah et al. 2019). They have also both been shown to improve the sleep and cognitive deficits associated with damage to the ARAS after TBI (Devine and Zafonte 2009; Cole et al. 2010; Lojovich 2010; Lim et al. 2013; Elliott et al. 2018; Paterno et al. 2018). Furthermore, there is evidence that dietary BCAAs may reduce exercise-induced cognitive fatigue by competitively inhibiting increased tryptophan transport into the brain that typically occurs in response to exercise (Blomstrand 2001). Due to the limitations of small animal models, exercise and diet have not been thoroughly investigated in the context of neuronal loss associated with their therapeutic targets in humans. However, there appears to be some potential for local plasticity changes (i.e., new or strengthened local connections that can form and/or reinforce certain neural networks) and this plasticity may underlie improved recovery with contemporary rehabilitation strategies.

Developing effective regenerative therapeutics to pair with traditional rehabilitation approaches offers the greatest potential for improving outcomes. There are a variety of pathological TBI endophenotypes that can impair recovery, as well as secondary injury mechanisms that remain active during the rehabilitation/recovery phase of TBI. Different treatments and therapies should be based on the goals of the patient and adequately address underlying issues at the time via (1) neuroprotection: reduce ongoing sequelae to prevent ongoing cell death and axon loss, (2) plasticity: new or strengthened local connections/synapses that can form and/or reinforce neural networks involved in certain behaviors, and/or (3) regeneration: new neural cells and/or new long-distance connections. Traditional rehabilitation techniques and emerging regenerative rehabilitative techniques could be targeted to address one or more of these areas. In the following sections, we will discuss some of these pathological endophenotypes and how to target them, demonstrating the variety of potential strategies for providing regenerative rehabilitation following TBI, as well

as the urgent need for developing therapeutic approaches to target these mechanisms.

13.3 Removing Anti-regenerative Barriers

The brain's inherent lack of regenerative capacity along with dysfunction and ongoing pathology after TBI create an anti-regenerative environment. Therefore, any treatment that removes these barriers after injury is pro-regenerative, and mitigating dysfunction and secondary pathology following TBI is necessary to facilitate strategies intended to directly enhance underlying regenerative capacity. Beyond the broad categories of neuronal loss and inflammation (discussed in more detail in later sections), there are other endophenotypes present during the chronic phase of TBI that could present attractive targets for regenerative rehabilitation.

13.3.1 Post-Traumatic Epilepsy (PTE)

PTE has been reviewed elsewhere in much greater detail than what would fit within the scope of this chapter (Diaz-Arrastia et al. 2009), but we will provide a general summary of the etiology and need for regenerative treatments for this important endophenotype of TBI. Functional brain signaling is a product of coordinated and balanced excitatory and inhibitory signaling, but damage to brain circuitry due to the mechanical insult and secondary pathologies of TBI can result in disruption of excitatory/inhibitory coordination (Cohen et al. 2007; Wolf and Koch 2016; Wolf et al. 2017; Ulyanova et al. 2018, 2019; Koch et al. 2020). Ongoing secondary injury cascades along with aberrant neuroregeneration and reorganization can produce a discordant signaling imbalance that can in turn result in seizures that cause additional excitotoxic cell death and further exacerbate inflammation, metabolic distress, and other mechanisms of secondary pathology following TBI. Beyond secondary pathology, there also appears to be a connection to chronic neurodegenerative disease, as an association between seizure activity and tauopathy has been suggested by the increased prevalence of seizures in Alzheimer's disease patients and animal models (Yan et al. 2012; Sánchez et al. 2018). A recent study utilizing a model of blast TBI in tauopathy reporter zebrafish found that seizure-like activity was associated with increased accumulation of human tau in the brain, and blocking seizure activity after injury prevented that accumulation (Alyenbaawi et al. 2021). Inflammation-focused therapeutic approaches could be effective at mitigating PTE, as inflammation has been implicated in several studies investigating the mechanistic underpinnings of PTE (Webster et al. 2017; Sharma et al. 2019; Therajaran et al. 2020). Although surgical interventions can be effective (Hitti et al. 2020), many common anticonvulsants are ineffective against trauma-induced epilepsy, indicating a need to improve our mechanistic understanding of this unique condition via translational

modeling and data collection modalities (Diaz-Arrastia et al. 2009). PTE can significantly impair patients' ability to engage in exercise and cognitive rehabilitation activities. Therefore, it is imperative to develop regenerative rehabilitation strategies focused on managing post-traumatic epilepsy to maximize the effect of rehabilitation on recovery.

13.3.2 Vascular Injury

A recent study found that chronic BBB dysfunction and inflammation after TBI in rats and humans is associated with increased seizure susceptibility, suggesting that targeting these chronic endophenotypes may be effective for treating PTE (van Vliet et al. 2020). BBB disruption from mechanical and secondary injury is a persistent endophenotype of TBI that contributes to neuroinflammation and limits rehabilitation and recovery (Hay et al. 2015). In addition to BBB disruption, TBI also results in diffuse damage to microvasculature throughout the brain and in focal contusions. Beyond the direct consequences of disrupted circulation (e.g., ischemia), microvascular injury is also associated with inflammation and thrombosis via mechanisms that warrant further study (Hubbard et al. 2021). Microvascular injury, particularly in areas like the dorsal pons, can be predictive of long-term outcome (Izzy et al. 2017; Griffin et al. 2019). A recent review from Sandsmark and colleagues presents an in-depth discussion of the mechanisms and consequences of TBI-induced microvascular injury as well as the potential for therapeutic intervention (Sandsmark et al. 2019). Among treatments under investigation for addressing neurovascular dysfunction, there are several focused on the chronic phase of TBI that could be relevant for enhancing the impact of rehabilitation. For example, a study in mice 1 year after TBI found that administering the aminopropyl carbazole P7C3-A20 for 30 days restored BBB integrity, arrested axonal degeneration, and improved cognitive recovery (Vázquez-Rosa et al. 2020). Cerebrovascular reactivity—the change in cerebral blood flow in response to a stimulus—is commonly used as a measure of microvascular health, and a recent clinical study utilizing the phosphodiesterase-5 inhibitor sildenafil restored cerebrovascular reactivity in patients in the chronic phase of TBI (Kenney et al. 2018). As BBB and microvascular dysfunction are intricately linked to other endophenotypes of chronic TBI, these results suggesting that they are viable targets for therapy bode well for future research into regenerative rehabilitation.

13.3.3 Mitochondrial Dysfunction

Dysregulated metabolism and energy deficits are prominent characteristics of TBI and a critical component of the secondary injury cascade. Mitochondria are fundamental to cellular bioenergetics, and in addition to providing energy substrates, mitochondria also buffer Ca^{2+} and provide antioxidant support. Axons and astrocytic

processes are full of mitochondria moving to and from the soma, pausing at nodes of Ranvier or glutamate transporters servicing synapses, and engaging in constant dynamic fission and fusion to maintain mitochondrial health (Ohno et al. 2011; Genda et al. 2011; Youle and Bliek 2012; Schwarz 2013; Jackson et al. 2014). Axons and astrocytic processes are highly vulnerable to the diffuse shearing forces of TBI, producing cytoskeletal damage that disrupts or eliminates mitochondrial dynamics, and also leading to increased cytosolic Ca^{2+} that can exceed mitochondrial buffering capacities (Wang et al. 2021; Nguyen et al. 2021). The resultant mitochondrial dysfunction and energy failure lead to a collapse of ion gradients causing exacerbation of excitotoxicity due to reduced Ca^{2+} buffering capacity, a switch from providing protective antioxidants to producing damaging reactive oxygen species and, potentially, culmination in mitochondrial permeability transition that triggers programmed cell death. Cells that survive are often left with dysfunctional mitochondria, resulting in prolonged foundational impairments to energy production, neurotransmitter synthesis, Ca^{2+} signaling/buffering, and oxidative stress that negatively affect all downstream aspects of cell function and exacerbate the inflammatory extracellular environment. In addition to the central role of mitochondria during secondary injury after TBI leading to an anti-regenerative environment, they are also essential for cellular regeneration, making them a very attractive target for developing new neurotherapeutics (Wang et al. 2021).

There are numerous pharmacological approaches under investigation to mitigate mitochondrial dysfunction after TBI. A recent study utilizing focal TBI in swine found that a new lipid emulsion formulation of cyclosporine—a drug that functions in part via inhibiting the formation of the mitochondrial permeability transition pore and has significant preclinical evidence for mitigating TBI pathology—preserved fractional anisotropy as measured by diffusion tensor imaging (DTI) and reduced concentrations of neurofilament light (NF-L) in cerebrospinal fluid (Karlsson et al. 2020). These results are significant not only for providing evidence that this cyclosporine formulation reduces white matter pathology from TBI in a large animal model but also for validating DTI and NF-L as translational endpoints for future neurotherapeutic studies. A more unconventional and early-stage regenerative strategy involves the transfer of healthy mitochondria into cells with damaged mitochondria (McCully et al. 2016; Chang et al. 2019; Chen et al. 2020). Preliminary clinical studies in cardiac arrest have yielded encouraging results (Emani and McCully 2018). While models of ischemia/reperfusion injury have produced mitochondrial damage and autophagic degradation in astrocytes, other brain injury models have demonstrated that astrocytes transfer healthy mitochondria to neurons in distress (O'Donnell et al. 2016; Hayakawa et al. 2016; Quintana et al. 2019; English et al. 2020). Seeking to emulate this endogenous phenomenon on a larger scale, therapeutic vehicles for mitochondrial transfer currently under investigation include synaptosomes and mesenchymal stem cell-derived exosomes (Zhang et al. 2020; Lu et al. 2020; Picone et al. 2021). Mitochondria are both essential for cellular function and central to mechanisms of cell death, making them an ideal therapeutic target for reducing secondary injury after TBI and for facilitating plasticity and regeneration to maximize the effect of rehabilitation on recovery.

13.3.4 Astrocytic Dysfunction

Astrocytes are the most abundant cell type in the brain, where they are responsible for ion gradient homeostasis, facilitating anabolic and catabolic metabolism, providing antioxidant protection, fixing NH_4, incorporating nitrogen into biological molecules, preventing edema, removing glutamate from the extracellular space, coupling neuronal activity to changes in blood flow and glucose uptake, regulating breathing in response to changes in brain oxygenation, directly participating in signaling and plasticity, and many other vital functions. Not surprisingly, disruptions of each of these functions, often in combination, have been implicated in acute trauma and neurodegenerative disease (for reviews, see (Chen and Swanson 2003; Rossi et al. 2007; Sheldon and Robinson 2007; Barreto et al. 2011; Lange et al. 2012; Brambilla et al. 2013; Stary and Giffard 2015; Nguyen et al. 2021)). Historically, the vast heterogeneity of astrocytes and their wide variety of responses to pathological conditions have been inappropriately classified into a single "reactive" phenotype, and a recent consensus statement drawing attention to this oversimplification emphasized the need to move away from a cursory quantification of "astrogliosis" to study pathological responses of astrocytes in vivo in the context of multiple molecular and functional endpoints (Escartin et al. 2021).

Astrocytes are vital for brain metabolism, a myriad of essential homeostatic functions, stemming the perpetual threat of excitotoxicity, and communicating between brain and body. As such, they provide an excellent therapeutic target for rescuing distressed neurons and directly facilitating regeneration. One particular endophenotype following TBI—elevated intracranial pressure—has been mechanistically linked to the disrupted homeostatic function of astrocytes leading to cerebral edema, and as a result, the astrocytic water channel aquaporin 4 has emerged as a potential therapeutic target, at least in the acute/subacute phase of injury and recovery (Shields et al. 2011). Indeed, loss of aquaporin 4 and other astrocytic responses to TBI such as clasmatodendrosis were recently described in an in-depth histological and transcriptomic analysis in mice and were also found to be exacerbated with age (Early et al. 2020). Although astrocytes possess significant glycolytic capacity that contributes to their ability to survive pathological conditions, their mitochondria are involved in nearly all of the essential functions that astrocytes provide to the rest of the brain. As described in the previous section, these astrocytic compartments and the mitochondria therein appear to be uniquely susceptible to pathological conditions. Studies examining astrocytic mitochondria in primary culture have revealed depolarization and dysfunction in response to various pathological conditions as well as a few techniques to prevent that dysfunction (Stary and Giffard 2015). Heat shock proteins involved in mitochondrial Ca^{2+} handling have been implicated in mitochondrial dysfunction in primary astrocytes, and pharmacological or genetic induction is neuroprotective in in vitro and in vivo models of ischemia (Ouyang et al. 2005, 2006; Sun et al. 2006; Xu et al. 2010; Li et al. 2021). Astrocyte-targeted reduction of microRNAs that have been implicated in mitochondrial homeostatic mechanisms is neuroprotective in in vivo models of ischemic

stroke (Ouyang et al. 2011, 2012a, b, 2013; Xu et al. 2015b). Purinergic signaling plays a prominent role in astrocytic communication during health and disease (Franke et al. 2012). Calcium-mediated stimulation of mitochondrial metabolism in astrocytes via activation of purinergic P2Y1 receptors provides neuroprotection against oxidative stress in primary co-cultures (Wu et al. 2007) and reduces edema and infarct size in an in vivo photothrombotic stroke model in mice (Zheng et al. 2010, 2013). Compared to the often-fatal consequences in neurons, mitochondrial dysfunction and other secondary injury mechanisms are far less severe and very rarely fatal for astrocytes. During the chronic phase of TBI, astrocytes are intimately involved in angiogenesis and BBB repair, as well as neurogenesis, synaptogenesis, and synaptic remodeling (plasticity), and they simultaneously perform pro- and anti-regenerative functions that should be specifically targeted to improve recovery (Zhou et al. 2020). Since astrocytes are capable of rescuing neurons from a multitude of pathways simultaneously, a therapeutic approach that targets the less-severe dysfunction in astrocytes may offer greater chances of success compared to therapies focused on a single neuronal target. Astrocytes are also entangled in the processes of neuroinflammation, and anti-inflammatory strategies are therefore also likely to affect astrocytic dysfunction, providing additional benefits for enhancing regeneration during rehabilitation.

13.4 Current and Future Approaches to Mitigate Inflammation

Neuroinflammation encompasses myriad mechanisms by which the immune system responds to events in the central nervous system (CNS). These complex mechanisms involve central and peripheral cellular activity such as resident microglial activation and peripheral recruitment of neutrophils, lymphocytes, and monocyte-derived macrophages, as well as molecular components such as cytokine and chemokine signaling. Under normal conditions, neuroinflammation provides vital physiological functions, but in the case of TBI, this response is often pushed beyond homeostatic parameters to become pathological and can contribute to a lifetime of disability and neurodegenerative disease. This critical need for inflammation is exemplified by the numerous failed anti-inflammatory therapy clinical trials. Therefore, a new framework has been proposed to optimize targeted interventions and the immune response to TBI: acute proinflammatory response should be limited to levels needed for debris clearance and danger signaling; anti-inflammatory and pro-regenerative immune cell phenotypes should be promoted; and the development of chronic neuroinflammation should be prevented (Simon et al. 2017). This framework addresses the critical role inflammation plays in neuroprotection, fostering plasticity (synaptic remodeling), and facilitating regeneration. In the sections below, we will outline current and future therapeutic approaches that can be utilized within these framework guidelines to modify the neuroinflammatory response to TBI.

13.4.1 Complement Activation

The complement system is a vital part of the innate immunological response and plays a key role in various functions of the immune system. Classically described as having three distinct activation patterns—the classical, alternative, and lectin pathways—all leading to a cascade-like enzymatic process that converges on common end products as depicted in the schematic in Fig. 13.3 from a recent review by Dalakas, Alexopoulos, and Spaeth (Dalakas et al. 2020). Unlike the regulated complement activation that occurs in response to infection and autoimmune processes, an exaggerated complement response follows a traumatic injury. The cleavage of complement components initiates the subsequent steps and produces activated complement cleavage products that act as anaphylatoxins both locally and systemically. As a result, complement components comprise a large proportion of circulating blood proteins and play an important role in a multitude of processes.

While the CNS is generally considered to be immune-privileged due to the BBB, emerging evidence has demonstrated that the innate immune system functions

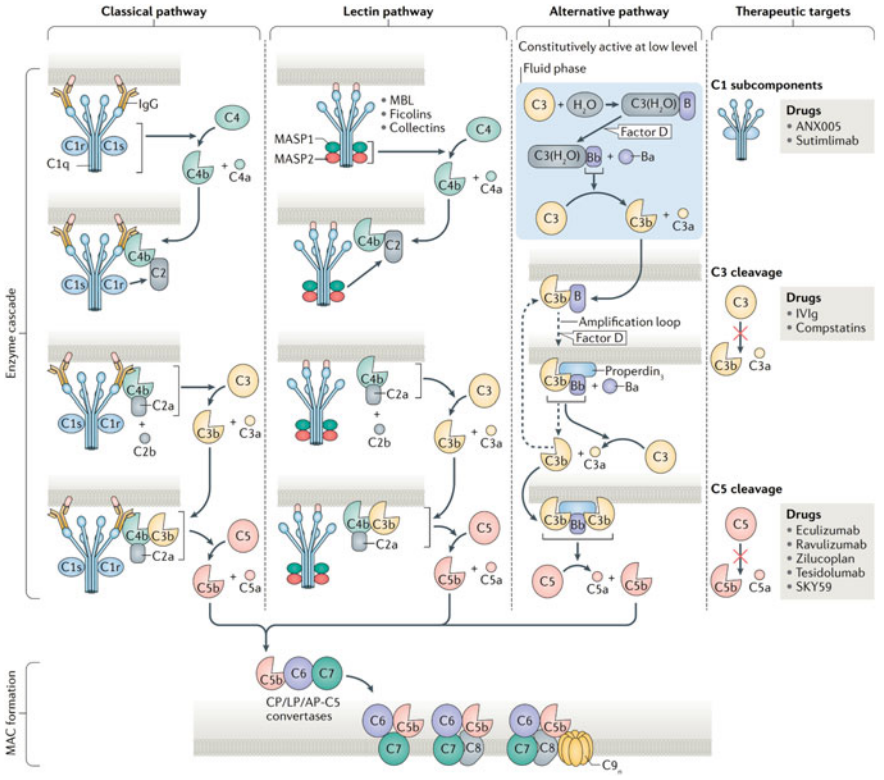

Fig. 13.3 Complement activation pathways and emerging therapeutic targets. Schematic reprinted with permission from Springer Nature (Dalakas et al. 2020)

within CNS. Cells of the brain can indeed produce complement components. Likewise, neurons, astrocytes, oligodendrocytes, and especially microglia express complement receptors (Orsini et al. 2014). Complement plays an important function in homeostasis, helping to clear protein debris and damaged cells as well as neuronal pruning during development (Veerhuis et al. 2011). Consequently, complement has been implicated in neurodegenerative diseases such as Alzheimer's and autoimmune diseases like Multiple Sclerosis. Indeed, increased complement component deposition has been found in hippocampi of aged mice, an indicator of the function of complement in senescence (Krukowski et al. 2018).

In addition to its role in homeostasis, complement is activated in times of injury and cerebral distress. TBI is often devastating due to the multifactorial nature of injury mechanisms leading to acute and long-lasting damage. While several processes contribute to the clinical manifestations of TBI, neuroinflammation and complement activation specifically play a critical role. Following TBI, the immune reaction is vast, with both systemic and localized activation, changes in epigenetic transcription, and enzymatic expression (Orsini et al. 2014). Activated complement components act as anaphylatoxins and lead to immune cell activation and recruitment in the injured brain, endothelial damage, and BBB breakdown. In addition to exogenous cell recruitment, an important function of complement anaphylatoxins is the activation of glial cells and recruitment of microglia specifically to the site of injury. This in turn can lead to localized cytokine release with pathologic consequences. Breakdown of the BBB results in a further influx of systemic complement components in addition to locally produced proteins. The complement cascade has been shown to contribute to both acute and subacute secondary injury following TBI through anaphylatoxin release, immune cell recruitment and activation, and directly causing neuronal death. Both the classical and the lectin pathways have been implicated in TBI-related secondary injury (Ciechanowska et al. 2020). In addition, the interplay between neuroinflammation and platelet activation has pointed to the role of complement in post-TBI hypercoagulability and microthrmobosis (Fletcher-Sandersjöö et al. 2020). Complement has likewise been linked with long-term disability and cognitive decline following TBI (Alawieh et al. 2021).

The vast majority of research into the role of complement in TBI has been performed in rodent models using gene knockout techniques and small molecule complement inhibitors. Not surprisingly, complement blockade has emerged as an attractive target for TBI therapy in preclinical rodent models with studies investigating the efficacy of complement inhibition as a therapeutic strategy following TBI (Leinhase et al. 2007; Rostami et al. 2013; Fluiter et al. 2014; Ruseva et al. 2015; Bambakidis et al. 2016; Alawieh et al. 2018; Rowe et al. 2018; De Blasio et al. 2019; Weiss et al. 2020). C1 inhibition has shown improved motor function at 4 weeks following TBI, as well as significant behavioral improvement and decreases in injury volume (Longhi et al. 2009). C3 knockout mice demonstrated decreased edema and microglial activation after TBI. Likewise, neutrophil recruitment was significantly reduced following TBI in the knockout animals (You et al. 2007). In addition, C3 knockout animals show decreased proinflammatory gene expression, lesion size, and vascular damage (Sewell et al. 2004). Studies show that inhibiting C3 cleavage

reduces post-injury activation of microglia and astrocytes, C3 deposition, and neuronal cell death, leading to improvements in cognitive and functional recovery (Rich et al. 2016; Alawieh et al. 2018). Likewise, evidence points to C3 convertase inhibition as a potent inhibitor of blood–brain barrier breakdown and neutrophil recruitment to the CNS, mirroring evidence in knockout experiments (Kaczorowski et al. 1995). Furthermore, overexpression of C3 convertase inhibitor demonstrated improvements in behavioral outcomes following TBI, both acutely at 4 and 24 hours after injury, as well histopathological evidence of less neuronal loss in key anatomic areas (Rancan et al. 2003). In longer-term studies, C3 activation promotes a sustained degenerative state through microglial and astrocyte activation for several weeks following injury, resulting in long-term effects (Alawieh et al. 2018). The resultant neuroinflammation is longer lasting and seems to be a key driver of negative outcomes. Mice deficient in C4 also demonstrated attenuated damage and improved recovery following TBI (You et al. 2007).

Further down the complement cascade, C5 has also emerged as a potential target for intervention, although less potent due to the downstream position in the activation sequence (Sewell et al. 2004). The administration of a C5-binding protein inhibitor was shown to reduce neurologic deficits after TBI (Fluiter et al. 2014). Direct C5a blockade has also been shown to improve outcomes in mice (Sewell et al. 2004; Yang et al. 2006). Inhibiting the formation of the membrane attack complex (MAC, a.k.a. C5b-9), the end product of the complement cascade and downstream of C5 cleavage, has likewise been studied as a potential intervention strategy with results suggesting a reduction in the accumulation of microglia and macrophages as well as reduced neuronal death and axonal pathology (Fluiter et al. 2014). Downregulation of CD59 in knockout animals resulted in increased neurologic deficits and neuronal damage, further evidence that the role CD59 plays in MAC inhibition is potentially impactful for attenuating traumatic injury (Stahel et al. 2009). C3a and C5a receptor antagonists (C3aRA and C5aRA respectively) have been investigated in rodent models of TBI and stroke and found to be effective in suppressing the complement response, decreasing the expression of complement receptor, and decreasing secondary brain injury (Fattouch et al. 2007; Ducruet et al. 2008; Kim et al. 2008; Rynkowski et al. 2009; Széplaki et al. 2009; Garrett et al. 2009; Banz and Rieben 2012).

While the majority of evidence supporting the critical role of complement in TBI is from rodent studies, a significant body of work has shown complement up-regulation in humans following TBI. Studies examining the CSF of TBI patients identified a significant increase of activated C3 (Kossmann et al. 1997; Morganti-Kossmann et al. 2001a). Brain injured patients have increased levels of complement activation products in CSF (Lindsberg et al. 1996; Mocco et al. 2006; Széplaki et al. 2009; Elvington et al. 2012; Manek et al. 2018; Si et al. 2019). Pathologic studies have also demonstrated expression of complement receptor and deposition of complement components on injured brain tissue including the MAC, the convergent end-product of all three complement pathways, and an effector of direct cellular damage (Rostami et al. 2013). Likewise, pathological studies demonstrated increased deposition of complement in perilesional and peri-vascular regions. This

is supported with rodent evidence of increased mannose-binding lectin (MBL) deposition in perivascular and perilesional space in injured mice resulting in activation of the lectin pathway (Longhi et al. 2014). In longer-term studies, increased expression of complement protein has been described up to 6 months post-injury with implications for amyloid homeostasis and chronic sequela (Bao et al. 2018).

With significant animal model evidence indicating therapeutic efficacy, attention is turning to complement inhibition as a therapy in human neurological diseases including TBI. Unfortunately, the TBI field has failed to translate any therapies strongly supported by rodent preclinical data despite over 30 clinical trials (Loane and Faden 2010; Xiong et al. 2013; Kabadi and Faden 2014; Vink 2018). To address the weaknesses in the translational pipeline and bridge the gap between rodents and humans, swine appears to be a viable preclinical model for complement research. Swine studies of severe TBI resuscitation with valproic acid led to a down-regulation of complement activation, and a resultant decrease in injury severity (Dekker et al. 2014; Bambakidis et al. 2016). Complement-directed therapeutics such as eculizumab (Soliris, Alexion, USA) have gained significant traction with a well-established safety profile and have been proposed as a potential therapy in TBI (Roselli et al. 2018). Likewise, small molecule complement receptor antagonists, specifically for C3a and C5a, are entering human trials and will likely move on to TBI as a disease target (Ducruet et al. 2009; Garrett et al. 2009). The complement system appears to be integral to the acute and chronic inflammatory response following TBI, offering promising therapeutic targets for neuroprotection, improved plasticity, and increased regenerative potential to enhance rehabilitation and improve outcomes.

13.4.2 Microglial Activation

One of the earliest inflammatory cellular responses after TBI is the activation of microglia, the primary immune cells of the CNS. Microglia in the healthy adult brain survey the local environment and monitor synapses, while microglia after TBI (and in other disease pathogenesis) engulf cellular debris and promote both regeneration and inflammatory cytokine release (Salter and Stevens 2017; Wofford et al. 2017). This activity may have both beneficial and detrimental effects, as prolonged or uncontrolled activation may contribute to more serve cognitive impairments and neurodegenerative disorders (Simon et al. 2017). Indeed, microglial activation can persist for weeks, years, or decades after injury as demonstrated in human TBI and preclinical animal models of injury (Gentleman et al. 2004; Johnson et al. 2013a; Loane et al. 2014; Lafrenaye et al. 2015; Grovola et al. 2020, 2021).

Recent therapeutic advances have attempted to target the mechanisms responsible for neuroimmune dysregulation using a variety of approaches. Unfortunately, many clinical trials targeting inflammation TBI have failed to demonstrate beneficial effects on neurological outcomes. These failed trials include corticosteroids, such as hydrocortisone and methylprednisolone, hypothermia therapy, and hypertonic

saline infusion (Roberts et al. 2004; Hutchison et al. 2008; Bulger et al. 2010; Asehnoune et al. 2014). Other anti-inflammatory drugs, such as minocycline, have had mixed results; in a clinical trial of 15 patients, minocycline administered for 12 weeks reduced chronic microglial activation after TBI but increased neurodegeneration (Scott et al. 2018). These minocycline trial results suggest that microglia play a reparative role in the chronic phase of TBI and drug treatment may need to be employed at specific time points post-injury.

One promising method that allows finer control over microglia involves inhibiting the microglia colony-stimulating factor 1 receptor (CSF1R). CSF1R is expressed by microglia, macrophages, and osteoclasts, and knocking out the CSF1R gene eliminates the brain's microglia population (Patel and Player 2009; Erblich et al. 2011). To determine the role of CSF1R signaling in microglial homeostasis, Elmore et al. (2014) tested the effectiveness of CSF1R inhibitors in adult mice. After initial compound selection experiments, PLX3397 displayed the greatest decrease in brain microglia by demonstrating a 50% reduction after just three days of administration in standard rodent chow and greater than 90% reduction after 7 days of administration. Furthermore, remaining microglia stained for active caspase-3, a marker for apoptosis, indicating that CSF1R inhibition initiates microglial cell death. Researchers then withdrew drug administration and made two remarkable discoveries. First, microglia began to repopulate the brain within 3 days, though these microglia were hypertrophied with short stubby processes compared to sham. Second, microglia density and morphology mirrored sham specimens 14 days after drug withdrawal. Therefore, microglia repopulation occurs through rapid increase in cell number followed by stabilization of their morphology. Finally, profiling of 86 immune-related genes lead to a reduction of these genes after microglial depletion (Elmore et al. 2014). Overall, CSF1R inhibitors allow for highly-selective microglial depletion through non-invasive administration and lacks a cytokine inflammatory response.

Recently, Spangenberg et al. (2019) developed the next generation of CSF1R inhibitors for microglial elimination (Spangenberg et al. 2019). These researchers sought to create a CSF1R inhibitor that is orally bioavailable, brain-penetrant, and depletes microglia for an extended time period. After several key changes to the chemical structure of PLX3397, PLX5622 was synthesized. Thorough pharmacokinetic investigation in mice, rats, dogs, and monkeys revealed a 20% brain penetrance for PLX5622 compared to 5% for PLX3397. This improved penetrance can be attributed to PLX5622's lower molecular weight, higher lipophilicity, and better cell permeability, thus allowing PLX5622 to cross the BBB more easily. Additionally, PLX5622 caused a 90% reduction in microglia within 5 days of administration in standard rodent chow at doses as low as 1200 ppm. Importantly, withdrawal of PLX5622 also allows for microglial repopulation, potentially offering a means to reset a predominantly pathological microglial phenotype after injury.

While Spangenberg et al. initially applied PLX5622 to plaque formation in preclinical models of Alzheimer's disease, Henry et al. (2020) investigated the elimination of microglia utilizing a controlled cortical impact injury in rodents (Henry et al. 2020). At 28 days after injury, Henry et al. administered PLX5622 to

mice for 1 week to potentially mitigate posttraumatic neurodegeneration and neurological dysfunction. This delayed depletion of microglia improved motor function recovery in beam walk and rotarod tests, as well as improved cognitive function recovery in Y-maze and Morris Water maze tasks. Additionally, PLX5622 treated mice had decreased lesion volume and attenuated cortical and dentate gyrus neuron loss. Histological examination of microglia in PLX5622 treated animals showed an increase in resting, ramified microglia in the injured cortex compared to TBI + vehicle-treated animals. Finally, PLX5622 altered cortical transcription patterns of oxidative stress, neuroinflammation, neuroplasticity, and apoptosis (Henry et al. 2020). These findings suggest that functional recovery after TBI may occur at chronic time points, thus expanding the therapeutic window for post-TBI interventions. Furthermore, CSF1R inhibitors did not cause cognitive or motor impairments despite the critical role of microglia in brain surveillance and synapse monitoring (Salter and Stevens 2017).

While CSF1R inhibitors are showing increasing potential as a therapy for TBI and a range of other neurological disorders, it should be noted that CSF1R inhibition by PLX5622 also affects peripheral immune cells. Lei et al. (2020) administered PLX5622 to adult mice for 3 weeks, then ceased treatment for 3 weeks before assessing bone marrow, spleen, and blood for immunological changes (Lei et al. 2020). PLX5622 administration resulted in the suppression of select monocyte progenitor cells, bone marrow-derived macrophages, hematopoietic stem cells, and hematopoietic progenitor cells. Importantly, these cell populations did not recover by this 3-week post-treatment experimental timepoint (Lei et al. 2020). Therefore, research focusing on peripheral and circulating macrophages in addition to microglia is necessary to understand the consequences—positive and/or negative—of administering PLX5622 following TBI.

Despite the need for further investigation into the impact of depleting peripheral immune cells, PLX5622 remains the best available tool to deplete microglia in vivo—both to investigate their functions in TBI and a myriad of other conditions, and also for proof-of-concept TBI therapeutics studies. Thorough characterization of all immune cell types at extended time points should be monitored across various factors, such as subject age, sex, mechanism and degree of injury, and secondary insults to determine the full effectiveness of CSF1R inhibitors as a potential therapy for TBI. Targeted modulation of microglia continues to garner significant research interest due to their potential to mitigate specific drivers of neurodegeneration and dysfunction following TBI, and though it may seem extreme to some, short-term depletion of microglia may yet prove to be a powerful non-invasive therapy to mitigate neurodegeneration and dysfunction following TBI, providing a more regenerative environment for rehabilitation. Future studies will need to discern if temporary microglia removal/repopulation or a more sustained and targeted modulation of detrimental microglia behavior will yield superior therapeutic benefits.

13.4.3 Macrophage Infiltration

In addition to resident cells of the CNS, the peripheral immune system can also have profound effects on the extent of regeneration and recovery following TBI. In healthy conditions the BBB limits interactions between the CNS and the peripheral immune system. However, following trauma, the BBB can become mechanically and chemically altered, thereby reducing the impedance between the peripheral whole blood and the CNS. Of course, penetrating TBIs and focal TBIs result in mechanical trauma to cerebral vasculature, affecting the neurovascular unit integrity. Furthermore, changes in signaling molecules can affect the stability of astrocytic endfeet, endothelial cell tight junctions, and pericyte support (Cash and Theus 2020), all of which can affect vasculature integrity after trauma. These changes to BBB integrity have been described in focal, diffuse, and closed-head TBI and have been noted in clinical TBI cases (Hay et al. 2015; Li et al. 2016; Johnson et al. 2018; van Vliet et al. 2020).

It is therefore unsurprising that peripheral immune cells, which are primed to identify tissue damage, can infiltrate into the brain when the BBB becomes leaky. Within hours of the injury, neutrophils, the first responders of the peripheral immune system, infiltrate into the brain tissue (Liu et al. 2018). Once inside the brain, they can contribute to pathological progression by secreting neutrophil extracellular traps (NETs) and altering the cerebral blood flow rate (Vaibhav et al. 2020). Following the neutrophils, monocyte-derived macrophage numbers in the brain begin to significantly increase approximately three days after trauma (Alam et al. 2020; Hazy et al. 2020). Lastly, adaptive immune cells, including B cells and T cells have been reported to infiltrate brain tissue several days after TBI (Morganti-Kossmann et al. 2001b; Ling et al. 2006; Alam et al. 2020).

Research efforts aimed at mitigating pathology and behavioral deficits after TBI have turned to controlling monocyte-derived macrophages because of the convenient timing, magnitude of infiltration, and association of monocyte-derived macrophage infiltration into the brain with neurotoxicity and neurological deficits in animal models (Hsieh et al. 2014; Gyoneva et al. 2015; Morganti et al. 2015). Over the last couple decades, much attention has been garnered to attempt to understand, remove, or reprogram monocyte-derived macrophages in the CNS after trauma (Lee et al. 2016; Chan and Viswanathan 2019). Here, we will briefly review the contributions of monocyte-derived macrophages to TBI and some therapeutic approaches that employ these cells to provide a more regenerative environment for rehabilitation.

Monocyte-derived macrophages are unique because they can exhibit a wide range of behavioral phenotypes that can amplify inflammation, promote angiogenesis, remodel extracellular matrices, or stimulate phagocytosis (Mosser and Edwards 2008; Wynn et al. 2013; Brown et al. 2014; De Paoli et al. 2014; Graney et al. 2020). As a result of the environmental cues in the injured brain, infiltrating monocyte-derived macrophages generally promote a chronic inflammatory phenotype and exacerbate neuroinflammation (Wofford et al. 2019b; Hazy et al. 2020).

This preserved inflammatory phenotype is distinct from the phenotype progression that monocyte-derived macrophages typically present in other models of healthy tissue regeneration (Kim et al. 2016). Typically, monocyte-derived macrophages will exhibit a transient inflammatory phenotype followed by a tissue remodeling phenotype (Snyder et al. 2016; Spiller and Koh 2017). It is theorized that this temporal sequence encourages clearance of necrotic and infectious material, wound closure, and tissue remodeling. To avoid or overcome the chronic inflammatory processes in the brain after TBI, several strategies have emerged to control monocyte-derived macrophages that broadly fall into three categories: (1) keeping monocytes and macrophages out of the CNS, (2) employing monocyte-derived macrophages to deliver therapeutics to the CNS, or (3) controlling monocyte-derived macrophage phenotype in the CNS.

Preventing infiltration of monocyte-derived macrophages in animal models of TBI lessened neuropathology and behavioral deficits (Hsieh et al. 2014; Makinde et al. 2017). These studies suggest that preventing monocyte-derived macrophage infiltration into the injured brain may be a logical treatment strategy. In line with this premise, researchers developed a drug-free microparticle-based treatment strategy that directs monocyte homing toward the spleen instead of sites of damage or disease (Getts et al. 2014). This team found that intravenous infusion of negatively charged microparticles would be rapidly phagocytosed by circulating monocytes. Thereafter, the particle-loaded cells would preferentially home to the spleen, where they subsequently undergo apoptosis, rather than traffic to the sites of inflammation (the brain, peritoneum, bowel, or heart in several models of disease or damage). Building on this work, researchers have administered similar negatively charged particles intravenously to mice following a closed head or a focal TBI (Sharma et al. 2020). Particles were administered 2–3 h, 24 h, and 48 h after a TBI and resulted in reduced myeloid cell infiltration, decreased lesion volume, decreased GFAP intensity, attenuated edema, and preserved long-term motor behavior (Sharma et al. 2020). These studies utilized FDA-approved materials and a reasonable treatment timeline that is promising for the field of neurotrauma. This strategy is a novel way to selectively suppress detrimental immune functions and suggests that the depletion of peripheral macrophages along with microglia via CSF1R inhibition may provide additive therapeutic benefits. Indeed, other attempts to broadly suppress the peripheral immune system have resulted in poor long-term neurological outcomes and also increase the risk of secondary infections (Lim and Smith 2007; Hazeldine et al. 2015). Targeting circulating monocytes without broadly suppressing immune function could have utility as a TBI treatment, although this needs to be tested in other species and in clinical situations.

In contrast to preventing monocyte homing to the injured brain, other researchers are attempting to leverage the convenient homing behavior of monocytes to enhance the delivery of therapeutics into the CNS. Indeed, delivery of therapeutics to the injured brain is notoriously challenging. Employing monocytes to carry and deliver therapeutics to injured brain could be a strategic way to locally increase the concentration of beneficial therapeutics in the CNS after TBI. For example, reactive oxygen species are especially detrimental to neuronal health and are a major driver of

secondary injury progression after TBI. Indeed, because the brain's baseline oxygen consumption is much higher than other organs it is especially vulnerable to reactive oxygen species and free radicals. Administration of antioxidants has emerged as a potentially promising therapeutic strategy (Corps et al. 2015). However, these treatment strategies typically require antioxidant administration prior to the injury, within minutes of the injury, or direct injection into the CNS. A more translational approach was employed by the Batrakova lab where they loaded the redox enzyme, catalase, into phagocytosable nanoparticles (Klyachko et al. 2014). These "nanozymes" were taken up by macrophages, were stable intracellularly, and preserved catalase activity. When nanozyme-loaded macrophages were administered intravenously 48 hours after a CNS injury, they reduced neuroinflammation and increased neuronal survival. Additionally, other groups have attempted to use monocytes to deliver other types of therapeutics to the injured brain, including viral vectors (Tong et al. 2016). Other researchers are working to enhance monocyte delivery of therapeutics to peripheral tissues and organs. For example, recent studies suggest that monocytes can carry hypoxia-activated pro-drugs to sites of hypoxia (Evans et al. 2019). Additionally, others are working to develop monocyte "backpacks" that adhere to the surface of homing cells (Anselmo and Mitragotri 2014; Anselmo et al. 2015). These loaded monocytes can home to sites of inflammation and reduce off-target delivery. While these results are promising, much work still remains to determine if the homing potential of loaded monocytes is conserved and the therapeutic efficacy can reduce secondary injury cascades following TBI.

Finally, new strategies are emerging that attempt to control macrophage phenotype as a therapeutic strategy for TBI treatment. As previously mentioned, when not assuming an inflammatory phenotype macrophages can perform a variety of functions that are required for tissue regeneration and stability including angiogenesis, extracellular matrix remodeling, and clearing damaged tissue. Indeed, there are a number of problems in TBI-induced secondary injury that monocyte-derived macrophages are uniquely poised to remedy. Macrophages cultured with CNS slice cultures subjected to oxygen-glucose deprivation rescued hypoxic neurons (Desestret et al. 2013). Macrophages can secrete essential neuronal growth factors including brain-derived neurotrophic factor (BDNF) (Kerschensteiner et al. 1999). Additionally, macrophages may be more capable of clearing toxic components including myelin debris and erythrocytes compared to other brain cells (Hikawa and Takenaka 1996; Kroner et al. 2014; Nairz et al. 2017). Indeed, a number of clinical trials have attempted to deliver monocytes or macrophages to the brain, spinal cord, and other peripheral organs (Chan and Viswanathan 2019). However, methods to control an ideal phenotype over time are necessary for these approaches to reach their full potential.

To address this need, our group has co-developed a strategy to exogenously reprogram monocytes with drug-loaded microparticles. Phagocytosed microparticles degrade over time releasing immunomodulatory drugs into the cytosol of the monocyte-derived macrophages, thus controlling their phenotype over time (Wofford et al. 2019a, 2020). Intracellular microparticles were able to mitigate gene expression and protein secretion related to inflammation when the cells were

cultured in both regular and inflammatory environments. Experiments validating the efficacy of this cell reprogramming strategy in vivo after a TBI are necessary to determine if controlling monocyte-derived macrophage phenotype could have therapeutic efficacy. Moreover, modulating inflammation to a constructive magnitude and duration is likely the first step toward implementing these cells as a therapy. Controlling the extent of homing, phagocytosis, and cytokine secretion will also be imperative if they are to become a clinical treatment. This strategy along with other macrophage-directed TBI therapies offers promising avenues to provide a pro-regenerative environment that can improve the efficacy of rehabilitation and maximize recovery.

13.5 Current and Future Approaches to Mitigate Neuronal and Axonal Loss

Despite the heterogeneity of brain injury, all forms of TBI—whether mild or severe, focal or diffuse—are thought to result in some form of neuronal and/or axonal loss (Meaney et al. 2014; Dixon 2017; Kaur and Sharma 2018). While the location and extent of neuronal or axonal loss depend on the specific injury mechanisms, the presence of such degeneration appears to be ubiquitous. The initial event resulting in TBI causes mechanical tissue deformation that may lead to relatively rapid necrotic cell death (Kaur and Sharma 2018). In focal brain injuries such as hematomas, hemorrhages, and contusions, a large proportion of the initial neuronal loss is concentrated around the injury site (Kaur and Sharma 2018). In diffuse brain injury, inertial impulse loading leads to widespread axonal damage throughout the brain (DAI), as well as acute plasmalemma permeabilization affecting soma in the gray matter (Singleton and Povlishock 2004; Cullen et al. 2011; Johnson et al. 2013b; Meaney et al. 2014; Kaur and Sharma 2018; Keating et al. 2020). The disruption of axonal integrity caused by DAI leads to structural, metabolic, and neurochemical impairments that in turn initiate Wallerian degeneration of the axons, a series of hallmark pathologies that begins with disrupted axonal transport and ultimately leads to complete degeneration and self-destruction (Johnson et al. 2013b; Koliatsos and Alexandris 2019). This pathology generally develops quickly but can persist chronically following even a single injury event in the human brain (Povlishock and Christman 1995; Johnson et al. 2013b). Additionally, across both focal and diffuse TBI, the primary insult that results in initial neuronal death is followed by secondary (or indirect) injury that is driven by events including neuroinflammation, excitotoxicity, oxidative stress, and mitochondrial dysfunction (Wang and Jin 2015; Russo and McGavern 2016; Wofford et al. 2019b; Ladak et al. 2019). This secondary injury further exacerbates the initial neuronal loss resulting from the primary injury, causing a continual neuronal loss for weeks, months, and even years after the initial injury. Thus, acute pathology can lead to widespread axonal degeneration and programed neuronal loss that may greatly exceed any necrotic cell

death that occurs at the time of the initial injury. In addition, the adult CNS has an extremely limited capacity to regenerate following injury (Fry 2001; Illis 2012), owing to an extremely limited capacity for neuronal replacement, an inability of axons to regenerate on their own absent directed guidance, coupled with an inflammation-induced inhibitory environment that further limits regenerative potential (Fry 2001; Kyritsis et al. 2014). The meager regenerative capacity of CNS neurons limits the potential for recovery from TBI. This deficiency has inspired research into the development of a variety of therapies specifically designed to promote neuronal replacement and/or axon regeneration following brain injury.

As described in the previous section on current approaches to rehabilitation, diet and exercise can enhance plasticity—and to some degree neurogenesis—in the brain, facilitating compensatory rewiring to achieve functional recovery. In addition to these contemporary approaches for enhancing plasticity, there are currently three advanced regenerative strategies being explored to replace lost neuronal populations following TBI (Grade and Götz 2017): (1) transplantation of exogenously-sourced stem cells, or differentiated neurons derived from stem cells (Kassi et al. 2018; Clervius et al. 2019; Liao et al. 2019), (2) direct reprogramming of existing cell populations in the brain (Torper and Götz 2017; An et al. 2018; Wang and Zhang 2018), and (3) redirection of endogenous neural stem and/or progenitor cells (NSPCs) into an injured brain region (Bellenchi et al. 2013; Rolfe and Sun 2015; Hayashi et al. 2018; Purvis et al. 2020). The majority of neuronal replacement techniques have been designed to repopulate areas afflicted by focal injury such as cerebral ischemia or focal TBI. Indeed, the first thing that comes to mind for most people when they hear "regenerative therapy" is stem cell transplantation. This technique involves transplanting a bolus of stem cells either directly into or nearby a region of brain injury. Transplanted stem cells have been shown to reduce cognitive and motor deficits caused by experimental TBI (Haus et al. 2016; Spurlock et al. 2017) and to offer neuroprotection in the penumbra by reducing inflammation, mitigating chronic glial activation, and augmenting endogenous neurogenesis in preclinical models of TBI (Kassi et al. 2018; Clervius et al. 2019). While exogenous stem cells have the ability to survive, integrate, and fire action potentials following transplantation into the brain (Tennstaedt et al. 2015; Falkner et al. 2016), differentiation and functional integration to restore lost neural circuitry remains a challenge, and benefits observed in preclinical studies are primarily attributed to release of neurotrophic factors from the transplanted cells (Rolfe and Sun 2015; Yamashita et al. 2017; Xiong et al. 2018). Furthermore, the efficacy of mesenchymal stem cell-derived exosomes for improving functional recovery in a preclinical model of TBI coupled with a lack of differentiation of transplanted cells shows that the benefits of exogenous stem cell transplantation are independent of differentiation or neuronal replacement (Zhang et al. 2020). In fact, one of the most difficult challenges in stem cell transplantation is promoting and ensuring survival and functional integration of the transplanted cells (Liu and Huang 2007; Uemura et al. 2010). Limited cell survival indicates that the beneficial effects of transplanted cells (i.e., growth/neurotrophic factors released from stem cells into the injury site) are likely short-lasting, suggesting that multiple transplants would be required to promote enduring

neuroprotection and regeneration over time. In addition to a lack of cell differentiation and low neuronal survival rates, exogenous stem cell transplantation often comes with the risk of immune rejection (Barker and Widner 2004) and retention of epigenetic memory of the transplanted cells (Kim et al. 2010).

Current regenerative treatments are not limited to exogenous stem cells. Another widely investigated neuronal replacement approach is direct in vivo reprogramming of one somatic cell type (e.g., fibroblasts, astrocytes, NG2 glia, reactive glial cells, early post-mitotic neurons) into another (i.e., a specific neuronal phenotype lost due to injury) without intermediately generating induced pluripotent stem cells (Xu et al. 2015a; Torper and Götz 2017; An et al. 2018; Wang and Zhang 2018). A variety of techniques have been used to induce reprogramming including lineage-specific regulatory transcription factors (Guo et al. 2014; Heinrich et al. 2014) and microRNAs (Ambasudhan et al. 2011; Yoo et al. 2011). Small molecules have also been used for direct reprogramming, bypassing the need for invasive genetic manipulation techniques (Hu et al. 2015; Li et al. 2015). While direct cell reprogramming circumvents issues of immune rejection and tumor formation caused by exogenous transplants, this approach has the potential to introduce dangerous genetic mutations causing deleterious side effects and inherently relies on reducing the quantity of other cell types presumably necessary to brain function. Additionally, although conversion efficiencies have improved over time and have fairly high efficacy (Guo et al. 2014; Gascón et al. 2016), current reprogramming techniques still struggle to reliably generate sufficient numbers of functional, subtype-specific neurons (Torper and Götz 2017; Wang and Zhang 2018).

A third class of neuronal replacement techniques has focused on endogenous NSPCs as a source to replace neurons lost due to brain injury. There is a substantial body of research detailing a variety of different experimental technologies that are designed to redirect endogenous NSPCs in the brain from their site of origin into a site of brain injury (recently reviewed by Purvis et al. 2020) (Purvis et al. 2020). Endogenous neuroblasts are a particularly attractive cell source to replace lost neuronal populations because there is no risk of immune rejection as occurs with exogenous stem cell transplants and there is no requirement for invasive reprogramming techniques for cells to acquire a neuronal phenotype (Bellenchi et al. 2013). Most endogenous neuronal replacement techniques target NSPCs that arise from the subventricular zone as these cells already possess the inherent ability to depart from their site of origin and migrate toward regions of neuronal injury (Ramaswamy et al. 2005; Thored et al. 2007; Lindvall and Kokaia 2015; Kaneko et al. 2018), an observation that has also been reported in the human brain (Jin et al. 2006; Minger et al. 2007). However, the quantity of endogenous neuroblasts that mature into functional neurons in injured regions is insufficient to improve functional recovery without experimental intervention (Kojima et al. 2010; Kernie and Parent 2010; Hayashi et al. 2018). Various pharmacological strategies, biomaterial scaffolds, and emerging tissue-engineering techniques have been created to enhance the migration of subventricular zone-derived NSPCs and promote survival and integration following their arrival into regions of neuronal injury.

Pharmacological strategies include utilization of neurotrophic factors such as epidermal growth factor (Teramoto et al. 2003), stromal-derived factor 1 (Ohab et al. 2006), or BDNF (Schäbitz et al. 2007) to augment NSPC production within the subventricular zone and migration of these cells into injured regions. The majority of pharmacological techniques have administered neurotrophic factors directly into the lateral ventricles (Teramoto et al. 2003; Kolb et al. 2007; Schäbitz et al. 2007), but subcutaneous (Ohab et al. 2006; Popa-Wagner et al. 2010) and intranasal (Ma et al. 2008) administration methods have also shown efficacy at augmenting NSPC migration into regions of injury. The efficacy of neurotrophic factor administration for promoting endogenous NSPC infiltration can be further augmented when the factors are administered in biomaterial hydrogels (Wang et al. 2012). However, such pharmacological interventions have overall shown limited efficacy for altering endogenous NSPC arrival into regions of injury, and the transient effectiveness indicates that repeated administration over time is likely needed to produce clinical efficacy. There is also limited research demonstrating whether these pharmacological techniques can lead to actual functional recovery following experimental TBI. Additionally, while compounds such as growth and neurotrophic factors do provide chemoattractive cues that guide NSPCs toward regions of injury, cells must migrate through harsh, unfamiliar territory to reach distant locations in the brain. For instance, NSPCs often migrate along blood vessels, with branching blood vessels often leading NSPCs astray and preventing a majority of cells from reaching their destination (Kojima et al. 2010; Grade et al. 2013; Hayashi et al. 2018).

This challenge has led to the development of a variety of acellular biomaterial scaffolds designed to directly intercept the subventricular zone neurogenic niche and span directly into an injured brain region, providing a pathway to guide endogenous NSPC migration and circumventing the need for NSCPs to travel along inefficient, indirect routes to reach injured destinations (Oliveira et al. 2018; Purvis et al. 2020). One concern with using endogenous NSCPs to replace lost neurons is that these precursors are multipotent, meaning that they have the potential to differentiate into neurons or glial cells once they arrive at a site of injury (Lim and Alvarez-Buylla 2016). To circumvent this problem, biomaterial hydrogels are often engineered to contain neurotrophic factors to encourage neuronal differentiation upon arrival at an injury site (Fon et al. 2014a, b; Zhou et al. 2016; Clark et al. 2016). Some of the most efficacious of these acellular scaffolds are constructed from laminin, mimicking the material properties of blood vessels that traditionally support endogenous NSPC migration toward regions of injury (Ajioka et al. 2015; Fujioka et al. 2017; Gundelach and Koch 2018). These biomaterial scaffolds have effectively augmented the delivery of new, mature neurons into neuron-deficient brain regions (Wang et al. 2012; Gundelach and Koch 2018; Jinnou et al. 2018; Motamed et al. 2019) and have contributed to behavioral recovery following preclinical brain injury (Clark et al. 2016; Jinnou et al. 2018). These scaffolds are an attractive strategy for endogenous NSPC redirection as they offer an enhanced level of spatial control for cell migration and they can be engineered to mimic signaling mechanisms that typically support endogenous NSPC migration.

An alternative technology that seeks to further enhance NSPC migration from the subventricular zone into injured brain regions is the tissue-engineered rostral migratory stream (TE-RMS) (O'Donnell et al. 2018, 2021; Purvis et al. 2020). This "living scaffold" contains living astrocytes encapsulated within a preformed three-dimensional hydrogel column and is designed to emulate the structure and function of the endogenous rostral migratory stream, thus providing an "implantable highway" that recapitulates the mechanisms with which subventricular zone-derived NSPCs naturally migrate in the adult brain (Winter et al. 2016; O'Donnell et al. 2018, 2021; Katiyar et al. 2018). Similar to the acellular scaffolds discussed above, the TE-RMS is intended for implantation to span from the subventricular zone neurogenic niche toward a region of brain injury. It is predicted that this scaffold will integrate into the brain following implantation, becoming enwrapped by blood vessels that surround and support the scaffold. While further research is needed to demonstrate the ability of this emerging technology to promote functional recovery following injury, the TE-RMS is predicted to be particularly effective at NSPC redirection and functional integration due to the ability of NSPCs to actively communicate with cells contained within the scaffold, thus recapitulating both the physical (e.g., glial tube structure (Gengatharan et al. 2016)) and chemical (e.g., Slit/Robo signaling (Kaneko et al. 2017)) cues that traditionally guide subventricular-zone derived NSPC migration (O'Donnell et al. 2018, 2021). Additionally, by replicating the mechanisms with which NSPCs migrate endogenously, the TE-RMS is designed to *slowly* introduce new neurons into regions of injury over time (rather than introducing a large quantity of new cells all at once as occurs with exogenous stem cell transplantation). It is hypothesized that providing slow, sustained delivery of NSPCs over time will increase cell survival and augment the ability of redirected cells to functionally integrate into existing circuitry as compared to traditional cell transplantation or reprogramming techniques.

In addition to repopulating neuron-deficient brain regions with single neurons, strategies are being explored to replace long-distance axonal tracts that have been lost or damaged as a result of brain injury. Axons are generated early during embryonic development when neuronal targets are close to one another (Tau and Peterson 2010). As the brain develops, the length of axonal pathways increases simultaneous with the growth of bone and connective tissue, leading to the establishment of long-distance "stretch-grown" axonal tracts throughout the brain. Following injury, the adult CNS is generally unable to re-grow long-projecting axons due to a combination of an inhibitory environment, limited intrinsic neuronal growth capacity, and insufficient directed axon guidance to appropriate distant targets (Fawcett 2002; Curinga and Smith 2008; Fitch and Silver 2008; Huebner and Strittmatter 2009). Due to this lack of axon regeneration, the effects of widespread axonal loss in white matter following TBI can be devastating and permanent. Cell transplantation and replacement strategies cannot sufficiently restore the anatomical features of long, damaged axonal pathways. Strategies to encourage targeted and long-distance axon re-growth encompass two broad techniques: reducing the inhibitory environment that prevents axon regrowth (Stichel et al. 1999; Bradbury et al. 2002; Mingorance et al. 2006) and increasing the intrinsic regeneration ability of

axons (Jain et al. 2004; Yip et al. 2010; Liu et al. 2010). Various biomaterial-based tubular conduits have been developed that promote axon regeneration following spinal cord injury in vivo (Tsai et al. 2004; Moore et al. 2006; Silva et al. 2010). While these strategies have demonstrated some success at eliciting axon regeneration, achieving sufficient axonal growth rates and proper targeting in vivo remain major challenges. Therefore, these strategies have had minimal success at functionally restoring lost axonal connections.

In addition to repopulating neuron-deficient brain regions with single neurons, another emerging tissue engineering approach seeks to replace entire circuits that have been lost due to brain injury. For example, micro-tissue engineered neural networks (micro-TENNs) are implantable living scaffolds consisting of neurons and preformed axonal tracts contained within a hydrogel structure that are designed to re-establish long-distance neuronal connections in the brain (Struzyna et al. 2015a, b, 2017). This is the first technology created to simultaneously replace multiple discrete neuronal populations *and* their long-distance axonal connections, introducing the possibility of targeted neurosurgical reconstruction designed to facilitate functional axonal replacement and/or regeneration following brain injury (Struzyna et al. 2015a, b, 2017; Harris et al. 2016). Micro-TENNs have been created with both unidirectional and bidirectional architectures (Struzyna et al. 2015a) to recapitulate specific neuronal tracts including the nigrostriatal (Struzyna et al. 2018) and corticothalamic (Struzyna et al. 2015b) pathways. Notably, micro-TENNs have been shown to exhibit functional connectivity (Dhobale et al. 2018) and have been successfully engineered from human embryonic stem cells (Struzyna et al. 2018). Further advancements in this technology may introduce significant potential to restore functional neuronal connectivity following TBI.

Patients that suffer a severe TBI may experience damage to connections between the deep brain areas that constitute the ARAS—such as those between pons and thalamus or pons and basal forebrain—resulting in prolonged Disorders of Consciousness (DoC) (Edlow et al. 2012, 2013; Snider et al. 2019, 2020). The resultant disruption of activation of higher-order brain circuitry and lack of awareness of self or environment can render traditional cognitive and exercise rehabilitation approaches unworkable. DoC patients require specialized rehabilitative approaches focused on restoring awareness (Schnakers and Monti 2017; Provencio et al. 2020; Edlow et al. 2021). In the future, it may be possible to replace lost connections between select areas of the ARAS, and micro-TENNs for replacing ponto-thalamic afferents offer a focused, promising target for initial testing, with thalamocortical reconstruction as a secondary goal. While these new approaches are exciting, it should be stressed that these technologies are many years away from clinical application. Investigating tissue engineering approaches to regenerative rehabilitation from traumatic DoC will require preclinical studies, and while a preclinical model of traumatic DoC does not currently exist, efforts are underway to make these studies possible (O'Donnell et al. 2019).

In general, there are several questions and challenges that remain to be addressed regarding the ability of the abovementioned technologies to restore lost neuronal populations to regions of brain injury (Aboody et al. 2011; Purvis et al. 2020). One

of the biggest challenges for all neuronal replacement techniques is the ability to generate a sufficient number mature, phenotype-specific neurons to effectively restore function to an injured brain region. Across all neuronal replacement technologies, more research is needed to demonstrate that new neurons appropriately mature, differentiate (i.e., express relevant synaptic structures and synaptic markers), and functionally integrate with preexisting circuitry. There are also numerous manufacturing, safety, and regulatory considerations as these technologies move toward clinical utilization. While significant hurdles remain, these evolving technologies demonstrate considerable potential for neuronal and/or axon tract replacement following TBI.

13.6 Conclusions and Future Directions

Current efforts to promote plasticity and regeneration during rehabilitation from TBI are limited in their potency, specificity, and efficacy. Diet and exercise have been found to provide some improvement to regenerative potential during rehabilitation, but the ceiling is unfortunately low compared to other regenerative therapies under preclinical investigation. Emerging strategies for regenerative rehabilitation, from more traditional therapeutic approaches intended to mitigate anti-regenerative TBI endophenotypes, to more innovative approaches like tissue engineering and microglial depletion/replacement, offer significant potential for removing limits to rehabilitation to maximize functional recovery. Of course, spurring neurogenesis is only part of the challenge—new neurons need to end up where they are needed, integrate within appropriate 3D architecture, mature into correct phenotypes, and form functionally meaningful connections. Just as these nascent regenerative therapies are intended to enhance traditional rehabilitative therapies, those traditional rehabilitative therapies may help to improve the microenvironment of new neurons and therefore indirectly aid in addressing these challenges. For example, exercise and diet can reduce harmful inflammation, promote neurotrophic factor release, and improve plasticity, creating a more favorable environment for the survival and functional integration of implanted neural networks and/or re-routed endogenous neurons. As such, next-generation pro-regenerative therapies should be integrated with the existing—and effective—framework for cognitive rehabilitation after TBI.

Despite the lack of any approved treatment for mitigating neurodegenerative cascades and/or improving recovery from TBI, there are many potential therapeutics and even more potential therapeutic targets under preclinical investigation. For example, device-based plasticity (e.g., transcranial direct current stimulation, transcranial magnetic stimulation) may have a role as an adjunct to traditional rehab but is beyond the scope of the current article. We only discussed a fraction of these potential therapeutics and targets in this chapter, while illustrating the chronic challenges limiting recovery from TBI and the various ways in which regenerative rehabilitation strategies could be employed to maximize recovery. Unfortunately, neurotrauma therapeutics have a history of translational failure, due

in part to the heterogeneity of TBI, but also largely due to an overreliance on rodent models that do not sufficiently recreate the mechanisms and manifestations of the injury to provide reliable efficacy testing. Small animal models of TBI are essential to study specific endophenotypes, identify potential therapeutic targets, and test mechanisms of action for novel therapeutics. However, large animal models that better represent the mechanisms of biomechanical injury, neurophysiological sequelae, and neuropathological distribution of human TBI must be utilized to bridge the gap between small animal models and clinical trials to establish a viable translational pipeline for neurotrauma. By engaging in research addressing the challenges of both plasticity and ongoing injury, targeting specific endophenotypes based on the heterogeneity of TBI, and progressing through a carefully considered translational pipeline from small animals to large gyrencephalic animals, emergent strategies for regenerative rehabilitation can be brought to bear to maximize recovery after TBI.

Financial support was provided by the Department of Veterans Affairs [BLR&D I01-BX005017 (Cullen); RR&D IK2-RX003376 (O'Donnell); RR&D IK2-RX003651 (Swanson)] and the National Institutes of Health [NINDS R01-NS117757 (Cullen); NINDS R03-NS116301 (Cullen); NINDS F32-NS116205 (Wofford)]. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Veterans Affairs or the National Institutes of Health.

References

Aboody K, Capela A, Niazi N et al (2011) Translating stem cell studies to the clinic for CNS repair: current state of the art and the need for a Rosetta stone. Neuron 70:597–613. https://doi.org/10.1016/j.neuron.2011.05.007

Ajioka I, Jinnou H, Okada K et al (2015) Enhancement of neuroblast migration into the injured cerebral cortex using laminin-containing porous sponge. Tissue Eng A 21:193–201. https://doi.org/10.1089/ten.TEA.2014.0080

Alahmari KA, Marchetti GF, Sparto PJ et al (2014) Estimating postural control with the balance rehabilitation unit: measurement consistency, accuracy, validity, and comparison with dynamic posturography. Arch Phys Med Rehabil 95:65–73. https://doi.org/10.1016/j.apmr.2013.09.011

Alam A, Thelin EP, Tajsic T et al (2020) Cellular infiltration in traumatic brain injury. J Neuroinflammation 17:1–17. https://doi.org/10.1186/s12974-020-02005-x

Alawieh A, Langley EF, Weber S et al (2018) Identifying the role of complement in triggering neuroinflammation after traumatic brain injury. J Neurosci 38:2519–2532. https://doi.org/10.1523/JNEUROSCI.2197-17.2018

Alawieh A, Chalhoub R, Mallah K et al (2021) Complement drives synaptic degeneration and progressive cognitive decline in the chronic phase after traumatic brain injury. J Neurosci. https://doi.org/10.1523/JNEUROSCI.1734-20.2020

Alyenbaawi H, Kanyo R, Locskai LF et al (2021) Seizures are a druggable mechanistic link between TBI and subsequent tauopathy. eLife 10:e58744. https://doi.org/10.7554/eLife.58744

Ambasudhan R, Talantova M, Coleman R et al (2011) Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. Cell Stem Cell 9:113–118. https://doi.org/10.1016/j.stem.2011.07.002

An N, Xu H, Gao W-Q, Yang H (2018) Direct conversion of somatic cells into induced neurons. Mol Neurobiol 55:642–651. https://doi.org/10.1007/s12035-016-0350-0

Anselmo AC, Mitragotri S (2014) Cell-mediated delivery of nanoparticles: taking advantage of circulatory cells to target nanoparticles. J Control Release 0:531–541. https://doi.org/10.1016/j.micinf.2011.07.011.Innate

Anselmo AC, Gilbert JB, Kumar S et al (2015) Monocyte-mediated delivery of polymeric backpacks to inflamed tissues: a generalized strategy to deliver drugs to treat inflammation. J Control Release 199:29–36. https://doi.org/10.1016/j.jconrel.2014.11.027

Asehnoune K, Seguin P, Allary J et al (2014) Hydrocortisone and fludrocortisone for prevention of hospital-acquired pneumonia in patients with severe traumatic brain injury (Corti-TC): a double-blind, multicentre phase 3, randomised placebo-controlled trial. Lancet Respir Med 2:706–716. https://doi.org/10.1016/S2213-2600(14)70144-4

Bambakidis T, Dekker SE, Sillesen M et al (2016) Resuscitation with valproic acid alters inflammatory genes in a porcine model of combined traumatic brain injury and hemorrhagic shock. J Neurotrauma 33:1514–1521. https://doi.org/10.1089/neu.2015.4163

Banz Y, Rieben R (2012) Role of complement and perspectives for intervention in ischemia-reperfusion damage. Ann Med 44:205–217. https://doi.org/10.3109/07853890.2010.535556

Bao W, He F, Yu L et al (2018) Complement cascade on severe traumatic brain injury patients at the chronic unconscious stage: implication for pathogenesis. Expert Rev Mol Diagn 18:761–766. https://doi.org/10.1080/14737159.2018.1471985

Barker RA, Widner H (2004) Immune problems in central nervous system cell therapy. NeuroRx 1:472–481. https://doi.org/10.1602/neurorx.1.4.472

Barnes DE, Byers AL, Gardner RC et al (2018) Association of mild traumatic brain injury with and without loss of consciousness with dementia in US military veterans. JAMA Neurol 75:1055–1061. https://doi.org/10.1001/jamaneurol.2018.0815

Barreto GE, Sun X, Xu L, Giffard RG (2011) Astrocyte proliferation following stroke in the mouse depends on distance from the infarct. PLoS One 6:e27881. https://doi.org/10.1371/journal.pone.0027881

Bell DR, Guskiewicz KM, Clark MA, Padua DA (2011) Systematic review of the balance error scoring system. Sports Health 3:287–295. https://doi.org/10.1177/1941738111403122

Bellenchi GC, Volpicelli F, Piscopo V et al (2013) Adult neural stem cells: an endogenous tool to repair brain injury? J Neurochem 124:159–167. https://doi.org/10.1111/jnc.12084

Blomstrand E (2001) Amino acids and central fatigue. Amino Acids 20:25–34. https://doi.org/10.1007/s007260170063

Bradbury EJ, Moon LDF, Popat RJ et al (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416:636–640. https://doi.org/10.1038/416636a

Brambilla L, Martorana F, Rossi D (2013) Astrocyte signaling and neurodegeneration: new insights into CNS disorders. Prion 7:28–36. https://doi.org/10.4161/pri.22512

Brasure M, Lamberty GJ, Sayer NA et al (2013) Participation after multidisciplinary rehabilitation for moderate to severe traumatic brain injury in adults: a systematic review. Arch Phys Med Rehabil 94:1398–1420. https://doi.org/10.1016/j.apmr.2012.12.019

Brown BN, Sicari BM, Badylak SF (2014) Rethinking regenerative medicine: a macrophage-centered approach. Front Immunol 5:1–11. https://doi.org/10.3389/fimmu.2014.00510

Bulger EM, May S, Brasel KJ et al (2010) Out-of-hospital hypertonic resuscitation following severe traumatic brain injury: a randomized controlled trial. JAMA 304:1455–1464. https://doi.org/10.1001/jama.2010.1405

Cash A, Theus MH (2020) Mechanisms of blood–brain barrier dysfunction in traumatic brain injury. Int J Mol Sci 21:3344. https://doi.org/10.3390/ijms21093344

CDC (2019) Surveillance report of traumatic brain injury-related emergency department visits, hospitalizations, and deaths—United States, 2014. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services

Chan MWY, Viswanathan S (2019) Recent progress on developing exogenous monocyte/macrophage-based therapies for inflammatory and degenerative diseases. Cytotherapy 21:393–415. https://doi.org/10.1016/j.jcyt.2019.02.002

Chang C-Y, Liang M-Z, Chen L (2019) Current progress of mitochondrial transplantation that promotes neuronal regeneration. Transl Neurodegener 8:17. https://doi.org/10.1186/s40035-019-0158-8

Chen Y, Swanson RA (2003) Astrocytes and brain injury. J Cereb Blood Flow Metab 23:137–149

Chen W, Huang J, Hu Y et al (2020) Mitochondrial transfer as a therapeutic strategy against ischemic stroke. Transl Stroke Res 11:1214–1228. https://doi.org/10.1007/s12975-020-00828-7

Ciechanowska A, Ciapała K, Pawlik K et al (2020) Initiators of classical and lectin complement pathways are differently engaged after traumatic brain injury-time-dependent changes in the cortex, striatum, thalamus and hippocampus in a mouse model. Int J Mol Sci 22:45. https://doi.org/10.3390/ijms22010045

Clark AR, Carter AB, Hager LE, Price EM (2016) In vivo neural tissue engineering: cylindrical biocompatible hydrogels that create new neural tracts in the adult mammalian brain. Stem Cells Dev 25:1109–1118. https://doi.org/10.1089/scd.2016.0069

Clervius H, Baig M, Mahavadi A, Gajavelli S (2019) Human neural stem cell transplants to address multiple pathologies associated with traumatic brain injury. Neural Regen Res 14:1699. https://doi.org/10.4103/1673-5374.255620

Cohen AS, Pfister BJ, Schwarzbach E et al (2007) Injury-induced alterations in CNS electrophysiology. Prog Brain Res 161:143–169. https://doi.org/10.1016/S0079-6123(06)61010-8

Cole JT, Mitala CM, Kundu S et al (2010) Dietary branched chain amino acids ameliorate injury-induced cognitive impairment. Proc Natl Acad Sci U S A 107:366–371. https://doi.org/10.1073/pnas.0910280107

Corps KN, Roth TL, McGavern DB (2015) Inflammation and neuroprotection in traumatic brain injury. JAMA Neurology 72:355. https://doi.org/10.1001/jamaneurol.2014.3558

Cotman CW, Berchtold NC, Christie L-A (2007) Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends Neurosci 30:464–472. https://doi.org/10.1016/j.tins.2007.06.011

Cullen DK, Vernekar VN, LaPlaca MC (2011) Trauma-induced plasmalemma disruptions in three-dimensional neural cultures are dependent on strain modality and rate. J Neurotrauma 28:2219–2233. https://doi.org/10.1089/neu.2011.1841

Cullen DK, Harris JP, Browne KD et al (2016) A porcine model of traumatic brain injury via head rotational acceleration. Methods Mol Biol 1462:289–324. https://doi.org/10.1007/978-1-4939-3816-2_17

Curinga G, Smith GM (2008) Molecular/genetic manipulation of extrinsic axon guidance factors for CNS repair and regeneration. Exp Neurol 209:333–342. https://doi.org/10.1016/j.expneurol.2007.06.026

Dalakas MC, Alexopoulos H, Spaeth PJ (2020) Complement in neurological disorders and emerging complement-targeted therapeutics. Nat Rev Neurol 16:601–617. https://doi.org/10.1038/s41582-020-0400-0

De Blasio D, Fumagalli S, Orsini F et al (2019) Human brain trauma severity is associated with lectin complement pathway activation. J Cereb Blood Flow Metab 39:794–807. https://doi.org/10.1177/0271678X18758881

De Paoli F, Staels B, Chinetti-Gbaguidi G (2014) Macrophage phenotypes and their modulation in atherosclerosis. Circ J 78:1775–1781. https://doi.org/10.1253/circj.CJ-14-0621

Dekker SE, Bambakidis T, Sillesen M et al (2014) Effect of pharmacologic resuscitation on the brain gene expression profiles in a swine model of traumatic brain injury and hemorrhage. J Trauma Acute Care Surg 77:906–912.; discussion 912. https://doi.org/10.1097/TA.0000000000000345

Denny-Brown DE, Russell WR (1941) Experimental concussion: (section of neurology). Proc R Soc Med 34:691–692

Desestret V, Riou A, Chauveau F et al (2013) In vitro and in vivo models of cerebral ischemia show discrepancy in therapeutic effects of M2 macrophages. PLoS One 8:e67063. https://doi.org/10.1371/journal.pone.0067063

Devine JM, Zafonte RD (2009) Physical exercise and cognitive recovery in acquired brain injury: a review of the literature. PM & R 1:560–575. https://doi.org/10.1016/j.pmrj.2009.03.015

Dhobale AV, Adewole DO, Chan AHW et al (2018) Assessing functional connectivity across 3D tissue engineered axonal tracts using calcium fluorescence imaging. J Neural Eng 15:056008. https://doi.org/10.1088/1741-2552/aac96d

Diaz-Arrastia R, Agostini MA, Madden CJ, Van Ness PC (2009) Posttraumatic epilepsy: the endophenotypes of a human model of epileptogenesis. Epilepsia 50(Suppl 2):14–20. https://doi.org/10.1111/j.1528-1167.2008.02006.x

Dixon KJ (2017) Pathophysiology of traumatic brain injury. Phys Med Rehabil Clin N Am 28:215–225. https://doi.org/10.1016/j.pmr.2016.12.001

Ducruet AF, Hassid BG, Mack WJ et al (2008) C3a receptor modulation of granulocyte infiltration after murine focal cerebral ischemia is reperfusion dependent. J Cereb Blood Flow Metab 28:1048–1058. https://doi.org/10.1038/sj.jcbfm.9600608

Ducruet AF, Zacharia BE, Hickman ZL et al (2009) The complement cascade as a therapeutic target in intracerebral hemorrhage. Exp Neurol 219:398–403. https://doi.org/10.1016/j.expneurol.2009.07.018

Early AN, Gorman AA, Van Eldik LJ et al (2020) Effects of advanced age upon astrocyte-specific responses to acute traumatic brain injury in mice. J Neuroinflammation 17:115. https://doi.org/10.1186/s12974-020-01800-w

Edlow BL, Takahashi E, Wu O et al (2012) Neuroanatomic connectivity of the human ascending arousal system critical to consciousness and its disorders. J Neuropathol Exp Neurol 71:531–546. https://doi.org/10.1097/NEN.0b013e3182588293

Edlow BL, Haynes RL, Takahashi E et al (2013) Disconnection of the ascending arousal system in traumatic coma. J Neuropathol Exp Neurol 72:505–523. https://doi.org/10.1097/NEN.0b013e3182945bf6

Edlow BL, Keene CD, Perl D et al (2018) Multimodal characterization of the Late Effects of TBI (LETBI): a methodological overview of the LETBI project. J Neurotrauma. https://doi.org/10.1089/neu.2017.5457

Edlow BL, Claassen J, Schiff ND, Greer DM (2021) Recovery from disorders of consciousness: mechanisms, prognosis and emerging therapies. Nat Rev Neurol 17:135–156. https://doi.org/10.1038/s41582-020-00428-x

Elliott JE, De Luche SE, Churchill MJ et al (2018) Dietary therapy restores glutamatergic input to orexin/hypocretin neurons after traumatic brain injury in mice. Sleep 41:zsx212. https://doi.org/10.1093/sleep/zsx212

Elmore MRP, Najafi AR, Koike MA et al (2014) Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neuron 82:380–397. https://doi.org/10.1016/j.neuron.2014.02.040

Elvington A, Atkinson C, Zhu H et al (2012) The alternative complement pathway propagates inflammation and injury in murine ischemic stroke. J Immunol 189:4640–4647. https://doi.org/10.4049/jimmunol.1201904

Emani SM, McCully JD (2018) Mitochondrial transplantation: applications for pediatric patients with congenital heart disease. Transl Pediatr 7:16975–16175

English K, Shepherd A, Uzor N-E et al (2020) Astrocytes rescue neuronal health after cisplatin treatment through mitochondrial transfer. Acta Neuropathol Commun 8:36. https://doi.org/10.1186/s40478-020-00897-7

Erblich B, Zhu L, Etgen AM et al (2011) Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. PLoS One 6:e26317. https://doi.org/10.1371/journal.pone.0026317

Escartin C, Galea E, Lakatos A et al (2021) Reactive astrocyte nomenclature, definitions, and future directions. Nat Neurosci 24:312–325. https://doi.org/10.1038/s41593-020-00783-4

Esslinger P, Zappala G, Chakara F, Barrett A (2007) Cognitive impairments. In: Brain injury medicine: principles and practice, 2nd edn. Demos Medical Publishing, New York, NY, US, pp 990–1001

Eucker SA, Smith C, Ralston J et al (2011) Physiological and histopathological responses following closed rotational head injury depend on direction of head motion. Exp Neurol 227:79–88. https://doi.org/10.1016/j.expneurol.2010.09.015

Evans MA, Shields CW IV, Krishnan V et al (2019) Macrophage-mediated delivery of hypoxia-activated prodrug nanoparticles. Adv Ther 3(2):1900162. https://doi.org/10.1002/adtp.201900162

Falkner S, Grade S, Dimou L et al (2016) Transplanted embryonic neurons integrate into adult neocortical circuits. Nature 539:248–253. https://doi.org/10.1038/nature20113

Fattouch K, Bianco G, Speziale G et al (2007) Beneficial effects of C1 esterase inhibitor in ST-elevation myocardial infarction in patients who underwent surgical reperfusion: a randomised double-blind study. Eur J Cardiothorac Surg 32:326–332. https://doi.org/10.1016/j.ejcts.2007.04.038

Fawcett J (2002) Repair of spinal cord injuries: where are we, where are we going? Spinal Cord 40:615–623. https://doi.org/10.1038/sj.sc.3101328

Fitch MT, Silver J (2008) CNS injury, glial scars, and inflammation: inhibitory extracellular matrices and regeneration failure. Exp Neurol 209:294–301. https://doi.org/10.1016/j.expneurol.2007.05.014

Fleminger S, Oliver DL, Lovestone S et al (2003) Head injury as a risk factor for Alzheimer's disease: the evidence 10 years on; a partial replication. J Neurol Neurosurg Psychiatry 74:857–862. https://doi.org/10.1136/jnnp.74.7.857

Fletcher-Sandersjöö A, Thelin EP, Maegele M et al (2020) Time course of hemostatic disruptions after traumatic brain injury: a systematic review of the literature. Neurocrit Care. https://doi.org/10.1007/s12028-020-01037-8

Fluiter K, Opperhuizen AL, Morgan BP et al (2014) Inhibition of the membrane attack complex of the complement system reduces secondary neuroaxonal loss and promotes neurologic recovery after traumatic brain injury in mice. J Immunol 192:2339–2348. https://doi.org/10.4049/jimmunol.1302793

Fon D, Al-Abboodi A, Chan PPY et al (2014a) Effects of GDNF-loaded injectable gelatin-based hydrogels on endogenous neural progenitor cell migration. Adv Healthc Mater 3:761–774. https://doi.org/10.1002/adhm.201300287

Fon D, Zhou K, Ercole F et al (2014b) Nanofibrous scaffolds releasing a small molecule BDNF-mimetic for the re-direction of endogenous neuroblast migration in the brain. Biomaterials 35:2692–2712. https://doi.org/10.1016/j.biomaterials.2013.12.016

Franke H, Verkhratsky A, Burnstock G, Illes P (2012) Pathophysiology of astroglial purinergic signalling. Purinergic Signal 8:629–657. https://doi.org/10.1007/s11302-012-9300-0

Fry EJ (2001) Central nervous system regeneration: mission impossible? Clin Exp Pharmacol Physiol 28:253–258. https://doi.org/10.1046/j.1440-1681.2001.03417.x

Fujioka T, Kaneko N, Ajioka I et al (2017) β1 integrin signaling promotes neuronal migration along vascular scaffolds in the post-stroke brain. EBioMedicine 16:195–203. https://doi.org/10.1016/j.ebiom.2017.01.005

Gallaway M, Scheiman M, Mitchell GL (2017) Vision therapy for post-concussion vision disorders. Optom Vis Sci 94:68–73. https://doi.org/10.1097/OPX.0000000000000935

Gardner RC, Yaffe K (2014) Traumatic brain injury may increase risk of young onset dementia. Ann Neurol 75:339–341. https://doi.org/10.1002/ana.24121

Gardner RC, Yaffe K (2015) Epidemiology of mild traumatic brain injury and neurodegenerative disease. Mol Cell Neurosci 66:75–80. https://doi.org/10.1016/j.mcn.2015.03.001

Gardner RC, Burke JF, Nettiksimmons J et al (2014) Dementia risk after traumatic brain injury vs nonbrain trauma: the role of age and severity. JAMA Neurol 71:1490–1497. https://doi.org/10.1001/jamaneurol.2014.2668

Garrett MC, Otten ML, Starke RM et al (2009) Synergistic neuroprotective effects of C3a and C5a receptor blockade following intracerebral hemorrhage. Brain Res 1298:171–177. https://doi.org/10.1016/j.brainres.2009.04.047

Gascón S, Murenu E, Masserdotti G et al (2016) Identification and successful negotiation of a metabolic checkpoint in direct neuronal reprogramming. Cell Stem Cell 18:396–409. https://doi.org/10.1016/j.stem.2015.12.003

Genda EN, Jackson JG, Sheldon AL et al (2011) Co-compartmentalization of the astroglial glutamate transporter, GLT-1, with glycolytic enzymes and mitochondria. J Neurosci 31: 18275–18288. https://doi.org/10.1523/JNEUROSCI.3305-11.2011

Gengatharan A, Bammann RR, Saghatelyan A (2016) The role of astrocytes in the generation, migration, and integration of new neurons in the adult olfactory bulb. Front Neurosci 10:149. https://doi.org/10.3389/fnins.2016.00149

Gentleman SM, Leclercq PD, Moyes L et al (2004) Long-term intracerebral inflammatory response after traumatic brain injury. Forensic Sci Int 146:97–104. https://doi.org/10.1016/j.forsciint.2004.06.027

Getts DR, Terry RL, Teague Getts M et al (2014) Therapeutic inflammatory monocyte modulation using immune-modifying microparticles. Sci Transl Med 6:1–14

Gomez-Pinilla F, Gomez AG (2011) The influence of dietary factors in central nervous system plasticity and injury recovery. PM R 3:S111–S116. https://doi.org/10.1016/j.pmrj.2011.03.001

Grade S, Götz M (2017) Neuronal replacement therapy: previous achievements and challenges ahead. NPJ Regen Med 2:29. https://doi.org/10.1038/s41536-017-0033-0

Grade S, Weng YC, Snapyan M et al (2013) Brain-derived neurotrophic factor promotes vasculature-associated migration of neuronal precursors toward the ischemic striatum. PLoS One 8:e55039. https://doi.org/10.1371/journal.pone.0055039

Graney PL, Ben-Shaul S, Landau S et al (2020) Macrophages of diverse phenotypes drive vascularization of engineered tissues. Sci Adv 6:eaay6391. https://doi.org/10.1126/sciadv.aay6391

Griffin AD, Turtzo LC, Parikh GY et al (2019) Traumatic microbleeds suggest vascular injury and predict disability in traumatic brain injury. Brain 142:3550–3564. https://doi.org/10.1093/brain/awz290

Grovola MR, Paleologos N, Wofford KL et al (2020) Mossy cell hypertrophy and synaptic changes in the hilus following mild diffuse traumatic brain injury in pigs. J Neuroinflammation 17:44. https://doi.org/10.1186/s12974-020-1720-0

Grovola MR, Paleologos N, Brown DP et al (2021) Diverse changes in microglia morphology and axonal pathology during the course of 1 year after mild traumatic brain injury in pigs. Brain Pathol 31:e12953. https://doi.org/10.1111/bpa.12953

Gundelach J, Koch M (2018) Redirection of neuroblast migration from the rostral migratory stream into a lesion in the prefrontal cortex of adult rats. Exp Brain Res 236:1181–1191. https://doi.org/10.1007/s00221-018-5209-3

Guo Z, Zhang L, Wu Z et al (2014) In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell 14: 188–202. https://doi.org/10.1016/j.stem.2013.12.001

Gyoneva S, Kim D, Katsumoto A et al (2015) Ccr2 deletion dissociates cavity size and tau pathology after mild traumatic brain injury. J Neuroinflammation 12:1–12. https://doi.org/10.1186/s12974-015-0443-0

Harris JP, Struzyna LA, Murphy PL et al (2016) Advanced biomaterial strategies to transplant preformed micro-tissue engineered neural networks into the brain. J Neural Eng 13:016019. https://doi.org/10.1088/1741-2560/13/1/016019

Haus DL, López-Velázquez L, Gold EM et al (2016) Transplantation of human neural stem cells restores cognition in an immunodeficient rodent model of traumatic brain injury. Exp Neurol 281:1–16. https://doi.org/10.1016/j.expneurol.2016.04.008

Hay JR, Johnson VE, Young AMH et al (2015) Blood-brain barrier disruption is an early event that may persist for many years after traumatic brain injury in humans. J Neuropathol Exp Neurol 74: 1147–1157. https://doi.org/10.1097/NEN.0000000000000261

Hayakawa K, Esposito E, Wang X et al (2016) Transfer of mitochondria from astrocytes to neurons after stroke. Nature 535:551–555. https://doi.org/10.1038/nature18928

Hayashi Y, Jinnou H, Sawamoto K, Hitoshi S (2018) Adult neurogenesis and its role in brain injury and psychiatric diseases. J Neurochem 147:584–594. https://doi.org/10.1111/jnc.14557

Hazeldine J, Lord JM, Belli A (2015) Traumatic brain injury and peripheral immune suppression: primer and prospectus. Front Neurol 6:235. https://doi.org/10.3389/fneur.2015.00235

Hazy A, Kowalski E, Groot N, Theus M (2020) Peripheral immune response following traumatic brain injury. In: Advancement and new understanding in brain injury, pp 1–25. https://doi.org/10.5772/intechopen.93597

Heinrich C, Bergami M, Gascón S et al (2014) Sox2-mediated conversion of NG2 glia into induced neurons in the injured adult cerebral cortex. Stem Cell Reports 3:1000–1014. https://doi.org/10.1016/j.stemcr.2014.10.007

Henry RJ, Ritzel RM, Barrett JP et al (2020) Microglial depletion with CSF1R inhibitor during chronic phase of experimental traumatic brain injury reduces neurodegeneration and neurological deficits. J Neurosci 40:2960–2974. https://doi.org/10.1523/JNEUROSCI.2402-19.2020

Hikawa N, Takenaka T (1996) Myelin-stimulated macrophages release neurotrophic factors for adult dorsal root ganglion neurons in culture. Cell Mol Neurobiol 16:517–528. https://doi.org/10.1007/BF02150231

Hitti FL, Piazza M, Sinha S et al (2020) Surgical outcomes in post-traumatic epilepsy: a single institutional experience. Oper Neurosurg (Hagerstown) 18:12–18. https://doi.org/10.1093/ons/opz043

Horn LB, Rice T, Stoskus JL et al (2015) Measurement characteristics and clinical utility of the Clinical Test of Sensory Interaction on Balance (CTSIB) and modified CTSIB in individuals with vestibular dysfunction. Arch Phys Med Rehabil 96:1747–1748. https://doi.org/10.1016/j.apmr.2015.04.003

Hsieh CL, Niemi EC, Wang SH et al (2014) CCR2 deficiency impairs macrophage infiltration and improves cognitive function after traumatic brain injury. J Neurotrauma 1688:1–13. https://doi.org/10.1089/neu.2013.3252

Hu W, Qiu B, Guan W et al (2015) Direct conversion of normal and alzheimer's disease human fibroblasts into neuronal cells by small molecules. Cell Stem Cell 17:204–212. https://doi.org/10.1016/j.stem.2015.07.006

Hubbard WB, Dong J-F, Cruz MA, Rumbaut RE (2021) Links between thrombosis and inflammation in traumatic brain injury. Thromb Res 198:62–71. https://doi.org/10.1016/j.thromres.2020.10.041

Huebner EA, Strittmatter SM (2009) Axon regeneration in the peripheral and central nervous systems. Results Probl Cell Differ 48:339–351

Hutchison JS, Ward RE, Lacroix J et al (2008) Hypothermia therapy after traumatic brain injury in children. N Engl J Med 358:2447–2456. https://doi.org/10.1056/NEJMoa0706930

Illis LS (2012) Central nervous system regeneration does not occur. Spinal Cord 50:259–263. https://doi.org/10.1038/sc.2011.132

Injury C on CRT for TB, Populations B on the H of S, Medicine I of (2012) Cognitive rehabilitation therapy for traumatic brain injury: evaluating the evidence. National Academies Press, Washington, DC

Itoh T, Imano M, Nishida S et al (2011a) Exercise increases neural stem cell proliferation surrounding the area of damage following rat traumatic brain injury. J Neural Transm 118: 193–202. https://doi.org/10.1007/s00702-010-0495-3

Itoh T, Imano M, Nishida S et al (2011b) Exercise inhibits neuronal apoptosis and improves cerebral function following rat traumatic brain injury. J Neural Transm 118:1263–1272. https://doi.org/10.1007/s00702-011-0629-2

Iverson GL, Koehle MS (2013) Normative data for the balance error scoring system in adults. Rehabil Res Pract 2013:846418. https://doi.org/10.1155/2013/846418

Izzy S, Mazwi NL, Martinez S et al (2017) Revisiting grade 3 diffuse axonal injury: not all brainstem microbleeds are prognostically equal. Neurocrit Care 27:199–207. https://doi.org/10.1007/s12028-017-0399-2

Jackson JG, O'Donnell JC, Takano H et al (2014) Neuronal activity and glutamate uptake decrease mitochondrial mobility in astrocytes and position mitochondria near glutamate transporters. J Neurosci 34:1613–1624. https://doi.org/10.1523/JNEUROSCI.3510-13.2014

Jain A, Brady-Kalnay SM, Bellamkonda RV (2004) Modulation of Rho GTPase activity alleviates chondroitin sulfate proteoglycan-dependent inhibition of neurite extension. J Neurosci Res 77: 299–307. https://doi.org/10.1002/jnr.20161

Jin K, Wang X, Xie L et al (2006) Evidence for stroke-induced neurogenesis in the human brain. Proc Natl Acad Sci U S A 103:13198–13202. https://doi.org/10.1073/pnas.0603512103

Jinnou H, Sawada M, Kawase K et al (2018) Radial glial fibers promote neuronal migration and functional recovery after neonatal brain injury. Cell Stem Cell 22:128–137.e9. https://doi.org/10.1016/j.stem.2017.11.005

Johnson VE, Stewart JE, Begbie FD et al (2013a) Inflammation and white matter degeneration persist for years after a single traumatic brain injury. Brain 136:28–42. https://doi.org/10.1093/brain/aws322

Johnson VE, Stewart W, Smith DH (2013b) Axonal pathology in traumatic brain injury. Exp Neurol 246:35–43. https://doi.org/10.1016/j.expneurol.2012.01.013

Johnson VE, Weber MT, Xiao R et al (2018) Mechanical disruption of the blood–brain barrier following experimental concussion. Acta Neuropathol 135:711–726. https://doi.org/10.1007/s00401-018-1824-0

Kabadi SV, Faden AI (2014) Neuroprotective strategies for traumatic brain injury: improving clinical translation. Int J Mol Sci 15:1216–1236. https://doi.org/10.3390/ijms15011216

Kaczorowski SL, Schiding JK, Toth CA, Kochanek PM (1995) Effect of soluble complement receptor-1 on neutrophil accumulation after traumatic brain injury in rats. J Cereb Blood Flow Metab 15:860–864. https://doi.org/10.1038/jcbfm.1995.107

Kaneko N, Sawada M, Sawamoto K (2017) Mechanisms of neuronal migration in the adult brain. J Neurochem 141:835–847. https://doi.org/10.1111/jnc.14002

Kaneko N, Herranz-Pérez V, Otsuka T et al (2018) New neurons use Slit-Robo signaling to migrate through the glial meshwork and approach a lesion for functional regeneration. Sci Adv 4: eaav0618. https://doi.org/10.1126/sciadv.aav0618

Karelina K, Schneiderman K, Shah S et al (2021) Moderate intensity treadmill exercise increases survival of newborn hippocampal neurons and improves neurobehavioral outcomes after traumatic brain injury. J Neurotrauma 38(13):1858–1869. https://doi.org/10.1089/neu.2020.7389

Karlsson M, Yang Z, Chawla S et al (2020) Evaluation of diffusion tensor imaging and fluid based biomarkers in a large animal trial of cyclosporine in focal traumatic brain injury. J Neurotrauma. https://doi.org/10.1089/neu.2020.7317

Kassi AAY, Mahavadi AK, Clavijo A et al (2018) Enduring neuroprotective effect of subacute neural stem cell transplantation after penetrating TBI. Front Neurol 9:1097. https://doi.org/10.3389/fneur.2018.01097

Katiyar KS, Winter CC, Gordián-Vélez WJ et al (2018) Three-dimensional tissue engineered aligned astrocyte networks to recapitulate developmental mechanisms and facilitate nervous system regeneration. J Vis Exp: JoVE. https://doi.org/10.3791/55848

Kaur P, Sharma S (2018) Recent advances in pathophysiology of traumatic brain injury. Curr Neuropharmacol 16:1224–1238. https://doi.org/10.2174/1570159X15666170613083606

Keating CE, Cullen DK (2020) Mechanosensation in traumatic brain injury. Neurobiol Dis 148: 105210. https://doi.org/10.1016/j.nbd.2020.105210

Keating CE, Browne KD, Duda JE, Cullen DK (2020) Neurons in subcortical oculomotor regions are vulnerable to plasma membrane damage after repetitive diffuse traumatic brain injury in swine. J Neurotrauma. https://doi.org/10.1089/neu.2019.6738

Keating CE, Browne KD, Cullen DK (2021) Dietary manipulation of vulnerability to traumatic brain injury-induced neuronal plasma membrane permeability. Exp Neurol 340:113649. https://doi.org/10.1016/j.expneurol.2021.113649

Kenney K, Amyot F, Moore C et al (2018) Phosphodiesterase-5 inhibition potentiates cerebrovascular reactivity in chronic traumatic brain injury. Ann Clin Transl Neurol 5:418–428. https://doi.org/10.1002/acn3.541

Kernie SG, Parent JM (2010) Forebrain neurogenesis after focal Ischemic and traumatic brain injury. Neurobiol Dis 37:267–274. https://doi.org/10.1016/j.nbd.2009.11.002

Kerschensteiner M, Gallmeier E, Behrens L et al (1999) Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med 189:865–870. https://doi.org/10.1084/jem.189.5.865

Kim GH, Mocco J, Hahn DK et al (2008) Protective effect of C5a receptor inhibition after murine reperfused stroke. Neurosurgery 63(122–125):discussion 125-126. https://doi.org/10.1227/01.NEU.0000335079.70222.8D

Kim K, Doi A, Wen B et al (2010) Epigenetic memory in induced pluripotent stem cells. Nature 467:285–290. https://doi.org/10.1038/nature09342

Kim CC, Nakamura MC, Hsieh CL (2016) Brain trauma elicits non-canonical macrophage activation states. J Neuroinflammation 13:117. https://doi.org/10.1186/s12974-016-0581-z

Klyachko NL, Haney MJ, Zhao Y et al (2014) Macrophages offer a paradigm switch for CNS delivery of therapeutic proteins. Nanomedicine 9:1403–1422. https://doi.org/10.2217/nnm.13.115

Koch PF, Cottone C, Adam CD et al (2020) Traumatic brain injury preserves firing rates but disrupts laminar oscillatory coupling and neuronal entrainment in hippocampal CA1. eNeuro 7:ENEURO.0495-19.2020. https://doi.org/10.1523/ENEURO.0495-19.2020

Kojima T, Hirota Y, Ema M et al (2010) Subventricular zone-derived neural progenitor cells migrate along a blood vessel scaffold toward the post-stroke striatum. STEM CELLS N/A-N/A. https://doi.org/10.1002/stem.306

Kolb B, Morshead C, Gonzalez C et al (2007) Growth factor-stimulated generation of new cortical tissue and functional recovery after stroke damage to the motor cortex of rats. J Cereb Blood Flow Metab: official journal of the International Society of Cerebral Blood Flow and Metabolism 27:983–997. https://doi.org/10.1038/sj.jcbfm.9600402

Koliatsos VE, Alexandris AS (2019) Wallerian degeneration as a therapeutic target in traumatic brain injury. Curr Opin Neurol 32:786–795. https://doi.org/10.1097/WCO.0000000000000763

Kossmann T, Stahel PF, Morganti-Kossmann MC et al (1997) Elevated levels of the complement components C3 and factor B in ventricular cerebrospinal fluid of patients with traumatic brain injury. J Neuroimmunol 73:63–69. https://doi.org/10.1016/s0165-5728(96)00164-6

Kroner A, Greenhalgh AD, Zarruk JG et al (2014) TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. Neuron 83:1098–1116. https://doi.org/10.1016/j.neuron.2014.07.027

Krukowski K, Chou A, Feng X et al (2018) Traumatic brain injury in aged mice induces chronic microglia activation, synapse loss, and complement-dependent memory deficits. Int J Mol Sci 19:3753. https://doi.org/10.3390/ijms19123753

Kyritsis N, Kizil C, Brand M (2014) Neuroinflammation and central nervous system regeneration in vertebrates. Trends Cell Biol 24:128–135. https://doi.org/10.1016/j.tcb.2013.08.004

Ladak AA, Enam SA, Ibrahim MT (2019) A review of the molecular mechanisms of traumatic brain injury. World Neurosurg 131:126–132. https://doi.org/10.1016/j.wneu.2019.07.039

Lafrenaye AD, Todani M, Walker SA, Povlishock JT (2015) Microglia processes associate with diffusely injured axons following mild traumatic brain injury in the micro pig. J Neuroinflammation 12:186. https://doi.org/10.1186/s12974-015-0405-6

Lange SC, Bak LK, Waagepetersen HS et al (2012) Primary cultures of astrocytes: their value in understanding astrocytes in health and disease. Neurochem Res 37:2569–2588. https://doi.org/10.1007/s11064-012-0868-0

Langlois JA, Rutland-Brown W, Wald MM (2006) The epidemiology and impact of traumatic brain injury: a brief overview. J Head Trauma Rehabil 21:375–378

LaPlaca MC, Simon CM, Prado GR, Cullen DK (2007) CNS injury biomechanics and experimental models. Prog Brain Res 161:13–26. https://doi.org/10.1016/S0079-6123(06)61002-9

Lee S, Kivimäe S, Dolor A, Szoka FC (2016) Macrophage-based cell therapies: the long and winding road. J Control Release 240:527–540. https://doi.org/10.1016/j.jconrel.2016.07.018

Lei F, Cui N, Zhou C et al (2020) CSF1R inhibition by a small-molecule inhibitor is not microglia specific; affecting hematopoiesis and the function of macrophages. Proc Natl Acad Sci U S A 117:23336–23338. https://doi.org/10.1073/pnas.1922788117

Leinhase I, Rozanski M, Harhausen D et al (2007) Inhibition of the alternative complement activation pathway in traumatic brain injury by a monoclonal anti-factor B antibody: a randomized placebo-controlled study in mice. J Neuroinflammation 4:13. https://doi.org/10.1186/1742-2094-4-13

Lezak MD, Howieson DB, Loring DW et al (2004) Neuropsychological assessment, 4th edn. Oxford University Press, New York, NY

Li X, Zuo X, Jing J et al (2015) Small-molecule-driven direct reprogramming of mouse fibroblasts into functional neurons. Cell Stem Cell 17:195–203. https://doi.org/10.1016/j.stem.2015.06.003

Li W, Watts L, Long J et al (2016) Spatiotemporal changes in blood-brain barrier permeability, cerebral blood flow, T2 and diffusion following mild traumatic brain injury. Brain Res 1646:53–61. https://doi.org/10.1016/j.brainres.2016.05.036

Li L, Volobouеva L, Griffiths BB et al (2021) MicroRNA-338 inhibition protects against focal cerebral ischemia and preserves mitochondrial function in vitro in astrocytes and neurons via COX4I1. Mitochondrion. https://doi.org/10.1016/j.mito.2021.04.013

Liao L-Y, Lau B-M, Sánchez-Vidaña D, Gao Q (2019) Exogenous neural stem cell transplantation for cerebral ischemia. Neural Regen Res 14:1129. https://doi.org/10.4103/1673-5374.251188

Lim DA, Alvarez-Buylla A (2016) The adult Ventricular-Subventricular Zone (V-SVZ) and Olfactory Bulb (OB) Neurogenesis. Cold Spring Harb Perspect Biol 8:a018820. https://doi.org/10.1101/cshperspect.a018820

Lim HB, Smith M (2007) Systemic complications after head injury: a clinical review. Anaesthesia 62:474–482. https://doi.org/10.1111/j.1365-2044.2007.04998.x

Lim MM, Elkind J, Xiong G et al (2013) Dietary therapy mitigates persistent wake deficits caused by mild traumatic brain injury. Sci Transl Med 5:215ra173. https://doi.org/10.1126/scitranslmed.3007092

Lindsberg PJ, Ohman J, Lehto T et al (1996) Complement activation in the central nervous system following blood-brain barrier damage in man. Ann Neurol 40:587–596. https://doi.org/10.1002/ana.410400408

Lindvall O, Kokaia Z (2015) Neurogenesis following stroke affecting the adult brain. Cold Spring Harb Perspect Biol 7:a019034. https://doi.org/10.1101/cshperspect.a019034

Ling C, Sandor M, Suresh M, Fabry Z (2006) Traumatic injury and the presence of antigen differentially contribute to T-cell recruitment in the CNS. J Neurosci 26:731–741. https://doi.org/10.1523/JNEUROSCI.3502-05.2006

Liu J, Huang H-Y (2007) How to improve the survival of the fetal ventral mesencephalic cell transplanted in Parkinson's disease? Neurosci Bull 23:377–382. https://doi.org/10.1007/s12264-007-0056-4

Liu K, Lu Y, Lee JK et al (2010) PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat Neurosci 13:1075–1081. https://doi.org/10.1038/nn.2603

Liu YW, Li S, Dai SS (2018) Neutrophils in traumatic brain injury (TBI): friend or foe? J Neuroinflammation 15:1–18. https://doi.org/10.1186/s12974-018-1173-x

Loane DJ, Faden AI (2010) Neuroprotection for traumatic brain injury: translational challenges and emerging therapeutic strategies. Trends Pharmacol Sci 31:596–604. https://doi.org/10.1016/j.tips.2010.09.005

Loane DJ, Kumar A, Stoica BA et al (2014) Progressive neurodegeneration after experimental brain trauma. J Neuropathol Exp Neurol 73:14–29. https://doi.org/10.1097/NEN.0000000000000021

Lojovich JM (2010) The relationship between aerobic exercise and cognition. J Head Trauma Rehabil 25:184–192. https://doi.org/10.1097/HTR.0b013e3181dc78cd

Longhi L, Perego C, Ortolano F et al (2009) C1-inhibitor attenuates neurobehavioral deficits and reduces contusion volume after controlled cortical impact brain injury in mice. Crit Care Med 37:659–665. https://doi.org/10.1097/CCM.0b013e318195998a

Longhi L, Orsini F, De Blasio D et al (2014) Mannose-binding lectin is expressed after clinical and experimental traumatic brain injury and its deletion is protective. Crit Care Med 42:1910–1918. https://doi.org/10.1097/CCM.0000000000000399

Lu M, Guo J, Wu B et al (2020) Mesenchymal stem cell-mediated mitochondrial transfer: a therapeutic approach for ischemic stroke. Transl Stroke Res. https://doi.org/10.1007/s12975-020-00853-6

Ma M, Ma Y, Yi X et al (2008) Intranasal delivery of transforming growth factor-beta1 in mice after stroke reduces infarct volume and increases neurogenesis in the subventricular zone. BMC Neurosci 9:117. https://doi.org/10.1186/1471-2202-9-117

Makinde HM, Cuda CM, Just TB et al (2017) Nonclassical monocytes mediate secondary injury, neurocognitive outcome, and neutrophil infiltration after traumatic brain injury. J Immunol 199: 3583–3591. https://doi.org/10.4049/jimmunol.1700896

Manek R, Moghieb A, Yang Z et al (2018) Protein biomarkers and neuroproteomics characterization of microvesicles/exosomes from human cerebrospinal fluid following traumatic brain injury. Mol Neurobiol 55:6112–6128. https://doi.org/10.1007/s12035-017-0821-y

McCully JD, Levitsky S, del Nido PJ, Cowan DB (2016) Mitochondrial transplantation for therapeutic use. Clin Trans Med 5:16. https://doi.org/10.1186/s40169-016-0095-4

Meaney DF, Cullen DK (2016) Biomechanical basis of traumatic brain injury. In: Youmans and Winn's neurological surgery, 7th edn. Elsevier, Amsterdam

Meaney DF, Smith DH (2015) Cellular biomechanics of central nervous system injury. Handb Clin Neurol 127:105–114. https://doi.org/10.1016/B978-0-444-52892-6.00007-6

Meaney DF, Margulies SS, Smith DH (2001) Diffuse axonal injury. J Neurosurg 95:1108–1110. https://doi.org/10.3171/jns.2001.95.6.1108

Meaney DF, Morrison B, Dale Bass C (2014) The mechanics of traumatic brain injury: a review of what we know and what we need to know for reducing its societal burden. J Biomech Eng 136: 021008. https://doi.org/10.1115/1.4026364

Minger SL, Ekonomou A, Carta EM et al (2007) Endogenous neurogenesis in the human brain following cerebral infarction. Regen Med 2:69–74. https://doi.org/10.2217/17460751.2.1.69

Mingorance A, Solé M, Munetón V et al (2006) Regeneration of lesioned entorhino-hippocampal axons in vitro by combined degradation of inhibitory proteoglycans and blockade of Nogo-66/NgR signaling. FASEB J 20:491–493. https://doi.org/10.1096/fj.05-5121fje

Mocco J, Wilson DA, Komotar RJ et al (2006) Alterations in plasma complement levels after human ischemic stroke. Neurosurgery 59:28–33.; discussion 28-33. https://doi.org/10.1227/01.NEU.0000219221.14280.65

Moore MJ, Friedman JA, Lewellyn EB et al (2006) Multiple-channel scaffolds to promote spinal cord axon regeneration. Biomaterials 27:419–429. https://doi.org/10.1016/j.biomaterials.2005.07.045

Morganti JM, Jopson TD, Liu S et al (2015) CCR2 antagonism alters brain macrophage polarization and ameliorates cognitive dysfunction induced by traumatic brain injury. J Neurosci 35:748–760. https://doi.org/10.1523/JNEUROSCI.2405-14.2015

Morganti-Kossmann MC, Rancan M, Otto VI et al (2001a) Role of cerebral inflammation after traumatic brain injury: a revisited concept. Shock 16:165–177. https://doi.org/10.1097/00024382-200116030-00001

Morganti-Kossmann MC, Rancan M, Otto VI et al (2001b) Role of cerebral inflammation after traumatic brain injury: a revisited concept. Shock 16:165–177

Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8:958–969. https://doi.org/10.1038/nri2448.Exploring

Motamed S, Del Borgo MP, Zhou K et al (2019) Migration and differentiation of neural stem cells diverted from the subventricular zone by an injectable self-assembling β-peptide hydrogel. Front Bioeng Biotechnol 7:315. https://doi.org/10.3389/fbioe.2019.00315

Mucha A, Collins MW, Elbin RJ et al (2014) A Brief Vestibular/Ocular Motor Screening (VOMS) assessment to evaluate concussions: preliminary findings. Am J Sports Med 42:2479–2486. https://doi.org/10.1177/0363546514543775

Nairz M, Theurl I, Swirski FK, Weiss G (2017) "Pumping iron"—how macrophages handle iron at the systemic, microenvironmental, and cellular levels. Eur J Phys 469:397–418. https://doi.org/10.1007/s00424-017-1944-8

Nashner L (1993) Computerized dynamic Posturography. In: Newman C, Kartush J, Jacobson G (eds) Handbook of balance function testing. Mosby Year Book, St. Louis, MO, pp 280–304

Nasrallah P, Haidar EA, Stephan JS et al (2019) Branched-chain amino acids mediate resilience to chronic social defeat stress by activating BDNF/TRKB signaling. Neurobiology of Stress 11: 100170. https://doi.org/10.1016/j.ynstr.2019.100170

Nguyen H, Zerimech S, Baltan S (2021) Astrocyte mitochondria in white-matter injury. Neurochem Res. https://doi.org/10.1007/s11064-021-03239-8

O'Donnell JC, Jackson JG, Robinson MB (2016) Transient oxygen/glucose deprivation causes a delayed loss of mitochondria and increases spontaneous calcium signaling in astrocytic processes. J Neurosci 36:7109–7127. https://doi.org/10.1523/JNEUROSCI.4518-15.2016

O'Donnell J, Katiyar K, Panzer K, Dk C (2018) A tissue-engineered rostral migratory stream for directed neuronal replacement. Neural Regen Res 13:1327. https://doi.org/10.4103/1673-5374.235215

O'Donnell JC, Browne KD, Kilbaugh TJ et al (2019) Challenges and demand for modeling disorders of consciousness following traumatic brain injury. Neurosci Biobehav Rev 98:336–346. https://doi.org/10.1016/j.neubiorev.2018.12.015

O'Donnell JC, Purvis EM, Helm KVT et al (2021) An implantable human stem cell-derived tissue-engineered rostral migratory stream for directed neuronal replacement. Commun Biol 4:1–16. https://doi.org/10.1038/s42003-021-02392-8

Oberholzer M, Müri RM (2019) Neurorehabilitation of Traumatic Brain Injury (TBI): a clinical review. Medical Sciences 7:47. https://doi.org/10.3390/medsci7030047

Ohab JJ, Fleming S, Blesch A, Carmichael ST (2006) A neurovascular niche for neurogenesis after stroke. J Neurosci 26:13007–13016. https://doi.org/10.1523/JNEUROSCI.4323-06.2006

Ohno N, Kidd GJ, Mahad D et al (2011) Myelination and axonal electrical activity modulate the distribution and motility of mitochondria at CNS nodes of Ranvier. J Neurosci 31:7249–7258. https://doi.org/10.1523/JNEUROSCI.0095-11.2011

Oliveira EP, Silva-Correia J, Reis RL, Oliveira JM (2018) Biomaterials developments for brain tissue engineering. Adv Exp Med Biol 1078:323–346. https://doi.org/10.1007/978-981-13-0950-2_17

Ommaya AK, Gennarelli TA (1974) Cerebral concussion and traumatic unconsciousness. Correlation of experimental and clinical observations of blunt head injuries. Brain 97:633–654

Orsini F, De Blasio D, Zangari R et al (2014) Versatility of the complement system in neuroinflammation, neurodegeneration and brain homeostasis. Front Cell Neurosci 8:380. https://doi.org/10.3389/fncel.2014.00380

Ouyang Y-B, Xu L, Giffard RG (2005) Geldanamycin treatment reduces delayed CA1 damage in mouse hippocampal organotypic cultures subjected to oxygen glucose deprivation. Neurosci Lett 380:229–233. https://doi.org/10.1016/j.neulet.2005.01.055

Ouyang Y-B, Xu L-J, Sun Y-J, Giffard RG (2006) Overexpression of inducible heat shock protein 70 and its mutants in astrocytes is associated with maintenance of mitochondrial physiology during glucose deprivation stress. Cell Stress Chaperones 11:180–186

Ouyang Y-B, Xu L-J, Emery JF et al (2011) Overexpressing GRP78 influences Ca2+ handling and function of mitochondria in astrocytes after ischemia-like stress. Mitochondrion 11:279–286. https://doi.org/10.1016/j.mito.2010.10.007

Ouyang Y-B, Lu Y, Yue S et al (2012a) miR-181 regulates GRP78 and influences outcome from cerebral ischemia in vitro and in vivo. Neurobiol Dis 45:555–563. https://doi.org/10.1016/j.nbd.2011.09.012

Ouyang Y-B, Lu Y, Yue S, Giffard RG (2012b) miR-181 targets multiple Bcl-2 family members and influences apoptosis and mitochondrial function in astrocytes. Mitochondrion 12:213–219. https://doi.org/10.1016/j.mito.2011.09.001

Ouyang Y-B, Xu L, Lu Y et al (2013) Astrocyte-enriched miR-29a targets PUMA and reduces neuronal vulnerability to forebrain ischemia. Glia 61:1784–1794. https://doi.org/10.1002/glia.22556

Padula W, Singman E, Vicci V et al (2007) Evaluating and treating visual dysfunction. In: Brain injury medicine: principles and practice, 2nd edn. Demos Medical Publishing, New York, NY, US, pp 750–768

Patel S, Player MR (2009) Colony-stimulating factor-1 receptor inhibitors for the treatment of cancer and inflammatory disease. Curr Top Med Chem 9:599–610. https://doi.org/10.2174/156802609789007327

Paterno R, Metheny H, Cohen AS (2018) Memory deficit in an object location task after mild traumatic brain injury is associated with impaired early object exploration and both are restored by branched chain amino acid dietary therapy. J Neurotrauma 35:2117–2124. https://doi.org/10.1089/neu.2017.5170

Picone P, Porcelli G, Bavisotto CC et al (2021) Synaptosomes: new vesicles for neuronal mitochondrial transplantation. J Nanobiotechnology 19:6. https://doi.org/10.1186/s12951-020-00748-6

Popa-Wagner A, Stöcker K, Balseanu AT et al (2010) Effects of granulocyte-colony stimulating factor after stroke in aged rats. Stroke 41:1027–1031. https://doi.org/10.1161/STROKEAHA.109.575621

Povlishock JT, Christman CW (1995) The pathobiology of traumatically induced axonal injury in animals and humans: a review of current thoughts. J Neurotrauma 12:555–564. https://doi.org/10.1089/neu.1995.12.555

Powell LE, Myers AM (1995) The Activities-specific Balance Confidence (ABC) scale. J Gerontol A Biol Sci Med Sci 50A:M28–M34. https://doi.org/10.1093/gerona/50a.1.m28

Provencio JJ, Hemphill JC, Claassen J et al (2020) The curing coma campaign: framing initial scientific challenges—proceedings of the first curing coma campaign scientific advisory council meeting. Neurocrit Care. https://doi.org/10.1007/s12028-020-01028-9

Purvis EM, O'Donnell JC, Chen HI, Cullen DK (2020) Tissue engineering and biomaterial strategies to elicit endogenous neuronal replacement in the brain. Front Neurol. https://doi.org/10.3389/fneur.2020.00344

Quintana DD, Garcia JA, Sarkar SN et al (2019) Hypoxia-reoxygenation of primary astrocytes results in a redistribution of mitochondrial size and mitophagy. Mitochondrion 47:244–255. https://doi.org/10.1016/j.mito.2018.12.004

Ramaswamy S, Goings GE, Soderstrom KE et al (2005) Cellular proliferation and migration following a controlled cortical impact in the mouse. Brain Res 1053:38–53. https://doi.org/10.1016/j.brainres.2005.06.042

Rancan M, Morganti-Kossmann MC, Barnum SR et al (2003) Central nervous system-targeted complement inhibition mediates neuroprotection after closed head injury in transgenic mice. J Cereb Blood Flow Metab 23:1070–1074. https://doi.org/10.1097/01.WCB.0000084250.20114.2C

Rich MC, Keene CN, Neher MD et al (2016) Site-targeted complement inhibition by a complement receptor 2-conjugated inhibitor (mTT30) ameliorates post-injury neuropathology in mouse brains. Neurosci Lett 617:188–194. https://doi.org/10.1016/j.neulet.2016.02.025

Roberts I, Yates D, Sandercock P et al (2004) Effect of intravenous corticosteroids on death within 14 days in 10008 adults with clinically significant head injury (MRC CRASH trial): randomised placebo-controlled trial. Lancet 364:1321–1328. https://doi.org/10.1016/S0140-6736(04)17188-2

Rolfe A, Sun D (2015) Stem cell therapy in brain trauma: implications for repair and regeneration of injured brain in experimental TBI models. In: Kobeissy FH (ed) Brain neurotrauma: molecular, neuropsychological, and rehabilitation aspects. CRC Press/Taylor & Francis, Boca Raton

Roselli F, Karasu E, Volpe C, Huber-Lang M (2018) Medusa's head: the complement system in traumatic brain and spinal cord injury. J Neurotrauma 35:226–240. https://doi.org/10.1089/neu.2017.5168

Rossi DJ, Brady JD, Mohr C (2007) Astrocyte metabolism and signaling during brain ischemia. Nat Neurosci 10:1377–1386. https://doi.org/10.1038/nn2004

Rostami E, Davidsson J, Gyorgy A et al (2013) The terminal pathway of the complement system is activated in focal penetrating but not in mild diffuse traumatic brain injury. J Neurotrauma 30:1954–1965. https://doi.org/10.1089/neu.2012.2583

Rowe RK, Harrison JL, Zhang H et al (2018) Novel TNF receptor-1 inhibitors identified as potential therapeutic candidates for traumatic brain injury. J Neuroinflammation 15:154. https://doi.org/10.1186/s12974-018-1200-y

Ruseva MM, Ramaglia V, Morgan BP, Harris CL (2015) An anticomplement agent that homes to the damaged brain and promotes recovery after traumatic brain injury in mice. Proc Natl Acad Sci U S A 112:14319–14324. https://doi.org/10.1073/pnas.1513698112

Russo MV, McGavern DB (2016) Inflammatory neuroprotection following traumatic brain injury. Science (New York, NY) 353:783–785. https://doi.org/10.1126/science.aaf6260

Rynkowski MA, Kim GH, Garrett MC et al (2009) C3a receptor antagonist attenuates brain injury after intracerebral hemorrhage. J Cereb Blood Flow Metab 29:98–107. https://doi.org/10.1038/jcbfm.2008.95

Salter MW, Stevens B (2017) Microglia emerge as central players in brain disease. Nat Med 23:1018–1027. https://doi.org/10.1038/nm.4397

Samuelsson H, Moberg M, Apró W et al (2016) Intake of branched-chain or essential amino acids attenuates the elevation in muscle levels of PGC-1α4 mRNA caused by resistance exercise. Am J Physiol-Endocrinol Metab 311:E246–E251. https://doi.org/10.1152/ajpendo.00154.2016

Sánchez MP, García-Cabrero AM, Sánchez-Elexpuru G et al (2018) Tau-induced pathology in epilepsy and dementia: notions from patients and animal models. Int J Mol Sci 19:1092. https://doi.org/10.3390/ijms19041092

Sandsmark DK, Bashir A, Wellington CL, Diaz-Arrastia R (2019) Cerebral microvascular injury: a potentially treatable endophenotype of traumatic brain injury-induced neurodegeneration. Neuron 103:367–379. https://doi.org/10.1016/j.neuron.2019.06.002

Sauerbeck AD, Fanizzi C, Kim JH et al (2018) modCHIMERA: a novel murine closed-head model of moderate traumatic brain injury. Sci Rep 8:7677. https://doi.org/10.1038/s41598-018-25737-6

Schäbitz W-R, Steigleder T, Cooper-Kuhn CM et al (2007) Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis. Stroke 38:2165–2172. https://doi.org/10.1161/STROKEAHA.106.477331

Scheiman M, Wick B (2008) Clinical management of binocular vision: heterophoric, accommodative, and eye movement disorders. Lippincott Williams & Wilkins, Philadelphia

Schnakers C, Monti MM (2017) Disorders of consciousness after severe brain injury: therapeutic options. Curr Opin Neurol 30:573–579. https://doi.org/10.1097/WCO.0000000000000495

Schwarz TL (2013) Mitochondrial trafficking in neurons. Cold Spring Harb Perspect Biol 5:a011304. https://doi.org/10.1101/cshperspect.a011304

Scott G, Zetterberg H, Jolly A et al (2018) Minocycline reduces chronic microglial activation after brain trauma but increases neurodegeneration. Brain 141:459–471. https://doi.org/10.1093/brain/awx339

Sewell DL, Nacewicz B, Liu F et al (2004) Complement C3 and C5 play critical roles in traumatic brain cryoinjury: blocking effects on neutrophil extravasation by C5a receptor antagonist. J Neuroimmunol 155:55–63. https://doi.org/10.1016/j.jneuroim.2004.06.003

Sharma R, Leung WL, Zamani A et al (2019) Neuroinflammation in post-traumatic epilepsy: pathophysiology and tractable therapeutic targets. Brain Sci 9:318. https://doi.org/10.3390/brainsci9110318

Sharma S, Ifergan I, Kurz JE et al (2020) Intravenous immunomodulatory nanoparticle treatment for traumatic brain injury sripadh. Ann Neurol 87:442–455. https://doi.org/10.1002/ana.25675

Sheldon AL, Robinson MB (2007) The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. Neurochem Int 51:333–355. https://doi.org/10.1016/j.neuint.2007.03.012

Shepard N, Handelsman J, Clendaniel R (2007) Balance and dizziness. In: Brain injury medicine: principles and practice, 2nd edn. Demos Medical Publishing, New York, NY, US, pp 779–793

Shields J, Kimbler DE, Radwan W et al (2011) Therapeutic targeting of astrocytes after traumatic brain injury. Transl Stroke Res 2:633–642. https://doi.org/10.1007/s12975-011-0129-6

Si W, He P, Wang Y et al (2019) Complement complex C5b-9 levels are associated with the clinical outcomes of acute ischemic stroke and carotid plaque stability. Transl Stroke Res 10:279–286. https://doi.org/10.1007/s12975-018-0658-3

Silva NA, Salgado AJ, Sousa RA et al (2010) Development and characterization of a novel hybrid tissue engineering–based scaffold for spinal cord injury repair. Tissue Eng A 16:45–54. https://doi.org/10.1089/ten.tea.2008.0559

Simon DW, McGeachy MJ, Bayır H et al (2017) The far-reaching scope of neuroinflammation after traumatic brain injury. Nat Rev Neurol 13:171–191. https://doi.org/10.1038/nrneurol.2017.13

Singleton RH, Povlishock JT (2004) Identification and characterization of heterogeneous neuronal injury and death in regions of diffuse brain injury: evidence for multiple independent injury phenotypes. J Neurosci 24:3543–3553. https://doi.org/10.1523/JNEUROSCI.5048-03.2004

Smith DH, Nonaka M, Miller R et al (2000) Immediate coma following inertial brain injury dependent on axonal damage in the brainstem. J Neurosurg 93:315–322. https://doi.org/10.3171/jns.2000.93.2.0315

Snider SB, Bodien YG, Bianciardi M et al (2019) Disruption of the ascending arousal network in acute traumatic disorders of consciousness. Neurology 93:e1281–e1287. https://doi.org/10.1212/WNL.0000000000008163

Snider SB, Bodien YG, Frau-Pascual A et al (2020) Ascending arousal network connectivity during recovery from traumatic coma. NeuroImage: Clinical 28:102503. https://doi.org/10.1016/j.nicl.2020.102503

Snyder RJ, Lantis J, Kirsner RS et al (2016) Macrophages: a review of their role in wound healing and their therapeutic use. Wound Repair Regen 24:613–629. https://doi.org/10.1111/wrr.12444

Spangenberg E, Severson PL, Hohsfield LA et al (2019) Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model. Nat Commun 10:1–21. https://doi.org/10.1038/s41467-019-11674-z

Spiller KL, Koh TJ (2017) Macrophage-based therapeutic strategies in regenerative medicine. Adv Drug Deliv Rev 122:74–83. https://doi.org/10.1016/j.addr.2017.05.010

Spurlock MS, Ahmed AI, Rivera KN et al (2017) Amelioration of penetrating ballistic-like brain injury induced cognitive deficits after neuronal differentiation of transplanted human neural stem cells. J Neurotrauma 34:1981–1995. https://doi.org/10.1089/neu.2016.4602

Stahel PF, Flierl MA, Morgan BP et al (2009) Absence of the complement regulatory molecule CD59a leads to exacerbated neuropathology after traumatic brain injury in mice. J Neuroinflammation 6:2. https://doi.org/10.1186/1742-2094-6-2

Stary CM, Giffard RG (2015) Advances in astrocyte-targeted approaches for stroke therapy: an emerging role for mitochondria and microRNAS. Neurochem Res 40:301–307. https://doi.org/10.1007/s11064-014-1373-4

Stichel CC, Hermanns S, Luhmann HJ et al (1999) Inhibition of collagen IV deposition promotes regeneration of injured CNS axons. Eur J Neurosci 11:632–646. https://doi.org/10.1046/j.1460-9568.1999.00466.x

Struzyna LA, Harris JP, Katiyar KS et al (2015a) Restoring nervous system structure and function using tissue engineered living scaffolds. Neural Regen Res 10:679–685. https://doi.org/10.4103/1673-5374.156943

Struzyna LA, Wolf JA, Mietus CJ et al (2015b) Rebuilding brain circuitry with living micro-tissue engineered neural networks. Tissue Eng Part A 21:2744–2756. https://doi.org/10.1089/ten.TEA.2014.0557

Struzyna LA, Adewole DO, Gordián-Vélez WJ et al (2017) Anatomically inspired three-dimensional micro-tissue engineered neural networks for nervous system reconstruction, modulation, and modeling. J Vis Exp: JoVE. https://doi.org/10.3791/55609

Struzyna LA, Browne KD, Brodnik ZD et al (2018) Tissue engineered nigrostriatal pathway for treatment of Parkinson's disease. J Tissue Eng Regen Med 12:1702–1716. https://doi.org/10.1002/term.2698

Sun Y, Ouyang Y-B, Xu L et al (2006) The carboxyl-terminal domain of inducible Hsp70 protects from ischemic injury in vivo and in vitro. J Cereb Blood Flow Metab 26:937–950. https://doi.org/10.1038/sj.jcbfm.9600246

Széplaki G, Szegedi R, Hirschberg K et al (2009) Strong complement activation after acute ischemic stroke is associated with unfavorable outcomes. Atherosclerosis 204:315–320. https://doi.org/10.1016/j.atherosclerosis.2008.07.044

Tau GZ, Peterson BS (2010) Normal development of brain circuits. Neuropsychopharmacology 35:147–168. https://doi.org/10.1038/npp.2009.115

Tennstaedt A, Aswendt M, Adamczak J et al (2015) Human neural stem cell intracerebral grafts show spontaneous early neuronal differentiation after several weeks. Biomaterials 44:143–154. https://doi.org/10.1016/j.biomaterials.2014.12.038

Teramoto T, Qiu J, Plumier J-C, Moskowitz MA (2003) EGF amplifies the replacement of parvalbumin-expressing striatal interneurons after ischemia. J Clin Invest 111:1125–1132. https://doi.org/10.1172/JCI17170

The Management of Concussion-mild Traumatic Brain Injury Working Group, The Office of Quality, Safety and Value, VA, Washington, DC, and the Office of Evidence Based Practice, U.S. Army Medical Command (2016) VA/DoD clinical practice guideline for the management of concussion-mild traumatic brain injury

Therajaran P, Hamilton JA, O'Brien TJ et al (2020) Microglial polarization in posttraumatic epilepsy: Potential mechanism and treatment opportunity. Epilepsia 61:203–215. https://doi.org/10.1111/epi.16424

Thored P, Wood J, Arvidsson A et al (2007) Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. Stroke 38:3032–3039. https://doi.org/10.1161/STROKEAHA.107.488445

Tong H, Kang W, Davy PMC et al (2016) Monocyte trafficking, engraftment, and delivery of nanoparticles and an exogenous gene into the acutely inflamed brain tissue – evaluations on monocyte-based delivery system for the central nervous system. PLoS One 11:1–21. https://doi.org/10.1371/journal.pone.0154022

Torper O, Götz M (2017) Brain repair from intrinsic cell sources: turning reactive glia into neurons. Prog Brain Res 230:69–97. https://doi.org/10.1016/bs.pbr.2016.12.010

Tsai EC, Dalton PD, Shoichet MS, Tator CH (2004) Synthetic hydrogel guidance channels facilitate regeneration of adult rat brainstem motor axons after complete spinal cord transection. J Neurotrauma 21:789–804. https://doi.org/10.1089/0897715041269687

Uemura M, Refaat MM, Shinoyama M et al (2010) Matrigel supports survival and neuronal differentiation of grafted embryonic stem cell-derived neural precursor cells. J Neurosci Res 88:542–551. https://doi.org/10.1002/jnr.22223

Ulyanova AV, Koch PF, Cottone C et al (2018) Electrophysiological signature reveals laminar structure of the porcine hippocampus. eNeuro 5:ENEURO.0102-18.2018. https://doi.org/10.1523/ENEURO.0102-18.2018

Ulyanova AV, Cottone C, Adam CD et al (2019) Multichannel silicon probes for awake hippocampal recordings in large animals. Front Neurosci 13:397. https://doi.org/10.3389/fnins.2019.00397

VA Office of R & D VA research on TBI. https://www.research.va.gov/topics/tbi.cfm

Vaibhav K, Braun M, Alverson K et al (2020) Neutrophil extracellular traps exacerbate neurological deficits after traumatic brain injury. Sci Adv 6:1–12. https://doi.org/10.1126/sciadv.aax8847

Van Stan JH, Dijkers MP, Whyte J et al (2019) The rehabilitation treatment specification system: implications for improvements in research design, reporting, replication, and synthesis. Arch Phys Med Rehabil 100:146–155. https://doi.org/10.1016/j.apmr.2018.09.112

van Vliet EA, Ndode-Ekane XE, Lehto LJ et al (2020) Long-lasting blood-brain barrier dysfunction and neuroinflammation after traumatic brain injury. Neurobiol Dis 145:105080. https://doi.org/10.1016/j.nbd.2020.105080

Vázquez-Rosa E, Shin M-K, Dhar M et al (2020) P7C3-A20 treatment one year after TBI in mice repairs the blood-brain barrier, arrests chronic neurodegeneration, and restores cognition. Proc Natl Acad Sci U S A 117:27667–27675. https://doi.org/10.1073/pnas.2010430117

Veerhuis R, Nielsen HM, Tenner AJ (2011) Complement in the brain. Mol Immunol 48:1592–1603. https://doi.org/10.1016/j.molimm.2011.04.003

Ventura RE, Balcer LJ, Galetta SL (2014) The neuro-ophthalmology of head trauma. Lancet Neurol 13:1006–1016. https://doi.org/10.1016/S1474-4422(14)70111-5

Vink R (2018) Large animal models of traumatic brain injury. J Neurosci Res 96:527–535. https://doi.org/10.1002/jnr.24079

Wang B, Jin K (2015) Current perspectives on the link between neuroinflammation and neurogenesis. Metab Brain Dis 30:355–365. https://doi.org/10.1007/s11011-014-9523-6

Wang L-L, Zhang C-L (2018) Engineering new neurons: in vivo reprogramming in mammalian brain and spinal cord. Cell Tissue Res 371:201–212. https://doi.org/10.1007/s00441-017-2729-2

Wang Y, Cooke MJ, Morshead CM, Shoichet MS (2012) Hydrogel delivery of erythropoietin to the brain for endogenous stem cell stimulation after stroke injury. Biomaterials 33:2681–2692. https://doi.org/10.1016/j.biomaterials.2011.12.031

Wang B, Huang M, Shang D et al (2021) Mitochondrial behavior in axon degeneration and regeneration. Front Aging Neurosci 13:103. https://doi.org/10.3389/fnagi.2021.650038

Webster KM, Sun M, Crack P et al (2017) Inflammation in epileptogenesis after traumatic brain injury. J Neuroinflammation 14:10. https://doi.org/10.1186/s12974-016-0786-1

Weiss E, Dhir T, Collett A et al (2020) Effect of complement C1-esterase inhibitor on brain edema and inflammation after mild traumatic brain injury in an animal model. Clin Exp Emerg Med 7:87–94. https://doi.org/10.15441/ceem.19.050

Whyte J, Dijkers MP, Hart T et al (2014) Development of a theory-driven rehabilitation treatment taxonomy: conceptual issues. Arch Phys Med Rehabil 95:S24–32.e2. https://doi.org/10.1016/j.apmr.2013.05.034

Wilson L, Stewart W, Dams-O'Connor K et al (2017) The chronic and evolving neurological consequences of traumatic brain injury. Lancet Neurol 16:813–825. https://doi.org/10.1016/S1474-4422(17)30279-X

Winter CC, Katiyar KS, Hernandez NS et al (2016) Transplantable living scaffolds comprised of micro-tissue engineered aligned astrocyte networks to facilitate central nervous system regeneration. Acta Biomater 38:44–58. https://doi.org/10.1016/j.actbio.2016.04.021

Wofford KL, Harris JP, Browne KD et al (2017) Rapid neuroinflammatory response localized to injured neurons after diffuse traumatic brain injury in swine. Exp Neurol 290:85–94. https://doi.org/10.1016/j.expneurol.2017.01.004

Wofford KL, Cullen DK, Spiller KL (2019a) Modulation of macrophage phenotype via phagocytosis of drug-loaded microparticles. J Biomed Mater Res Part A 107:1–12. https://doi.org/10.1002/jbm.a.36617

Wofford KL, Loane DJ, Cullen DK (2019b) Acute drivers of neuroinflammation in traumatic brain injury. Neural Regen Res 14:1481–1489. https://doi.org/10.4103/1673-5374.255958
Wofford KL, Singh BS, Cullen DK, Spiller KL (2020) Biomaterial-mediated Reprogramming of Monocytes via Microparticle Phagocytosis for Sustained Modulation of Macrophage Phenotype. Acta Biomater 101:237–248. https://doi.org/10.1016/j.actbio.2019.11.021
Wofford KL, Grovola MR, Adewole DO, et al (2021) Relationships between biomechanical parameters, neurological recovery, and neuropathology following concussion in swine. bioRxiv 2021.02.09.430268. https://doi.org/10.1101/2021.02.09.430268
Wolf JA, Koch PF (2016) Disruption of Network Synchrony and Cognitive Dysfunction After Traumatic Brain Injury. Front Syst Neurosci 10:43. https://doi.org/10.3389/fnsys.2016.00043
Wolf JA, Johnson BN, Johnson VE et al (2017) Concussion Induces Hippocampal Circuitry Disruption in Swine. J Neurotrauma 34:2303–2314. https://doi.org/10.1089/neu.2016.4848
Wrisley DM, Marchetti GF, Kuharsky DK, Whitney SL (2004) Reliability, internal consistency, and validity of data obtained with the functional gait assessment. Phys Ther 84:906–918
Wu A, Ying Z, Gomez-Pinilla F (2004) Dietary Omega-3 Fatty Acids Normalize BDNF Levels, Reduce Oxidative Damage, and Counteract Learning Disability after Traumatic Brain Injury in Rats. J Neurotrauma 21:1457–1467. https://doi.org/10.1089/neu.2004.21.1457
Wu J, Holstein JD, Upadhyay G et al (2007) Purinergic Receptor-Stimulated IP3-Mediated Ca2+ Release Enhances Neuroprotection by Increasing Astrocyte Mitochondrial Metabolism during Aging. J Neurosci 27:6510–6520. https://doi.org/10.1523/JNEUROSCI.1256-07.2007
Wu A, Ying Z, Gomez-Pinilla F (2013) Exercise facilitates the action of dietary DHA on functional recovery after brain trauma. Neuroscience 248:655–663. https://doi.org/10.1016/j.neuroscience.2013.06.041
Wynn TA, Chawla A, Pollard JW (2013) Macrophage biology in development, homeostasis and disease. Nature 496:445–455. https://doi.org/10.1038/nature12034
Xiong Y, Mahmood A, Chopp M (2013) Animal models of traumatic brain injury. Nat Rev Neurosci 14:128–142. https://doi.org/10.1038/nrn3407
Xiong L-L, Hu Y, Zhang P et al (2018) Neural Stem Cell Transplantation Promotes Functional Recovery from Traumatic Brain Injury via Brain Derived Neurotrophic Factor-Mediated Neuroplasticity. Mol Neurobiol 55:2696–2711. https://doi.org/10.1007/s12035-017-0551-1
Xu L, Emery JF, Ouyang Y-B et al (2010) Astrocyte targeted overexpression of Hsp72 or SOD2 reduces neuronal vulnerability to forebrain ischemia. Glia 58:1042–1049. https://doi.org/10.1002/glia.20985
Xu J, Du Y, Deng H (2015a) Direct Lineage Reprogramming: Strategies, Mechanisms, and Applications. Cell Stem Cell 16:119–134. https://doi.org/10.1016/j.stem.2015.01.013
Xu L-J, Ouyang Y-B, Xiong X et al (2015b) Post-stroke treatment with miR-181 antagomir reduces injury and improves long-term behavioral recovery in mice after focal cerebral ischemia. Exp Neurol 264:1–7. https://doi.org/10.1016/j.expneurol.2014.11.007
Yamashita T, Liu W, Matsumura Y et al (2017) Novel Therapeutic Transplantation of Induced Neural Stem Cells for Stroke. Cell Transplant 26:461–467. https://doi.org/10.3727/096368916X692988
Yan X-X, Cai Y, Shelton J et al (2012) Chronic Temporal Lobe Epilepsy Is Associated with Enhanced Alzheimer-Like Neuropathology in 3×Tg-AD Mice. PLoS One 7:e48782. https://doi.org/10.1371/journal.pone.0048782
Yang S, Nakamura T, Hua Y et al (2006) The role of complement C3 in intracerebral hemorrhage-induced brain injury. J Cereb Blood Flow Metab 26:1490–1495. https://doi.org/10.1038/sj.jcbfm.9600305
Yip PK, Wong L-F, Sears TA et al (2010) Cortical Overexpression of Neuronal Calcium Sensor-1 Induces Functional Plasticity in Spinal Cord Following Unilateral Pyramidal Tract Injury in Rat. PLoS Biol 8:e1000399. https://doi.org/10.1371/journal.pbio.1000399
Yoo AS, Sun AX, Li L et al (2011) MicroRNA-mediated conversion of human fibroblasts to neurons. Nature 476:228–231. https://doi.org/10.1038/nature10323

You Z, Yang J, Takahashi K et al (2007) Reduced tissue damage and improved recovery of motor function after traumatic brain injury in mice deficient in complement component C4. J Cereb Blood Flow Metab 27:1954–1964. https://doi.org/10.1038/sj.jcbfm.9600497

Youle RJ, van der Bliek AM (2012) Mitochondrial Fission, Fusion, and Stress. Science 337:1062–1065. https://doi.org/10.1126/science.1219855

Zasler ND, Katz DI, Zafonte RD (eds) (2007) Brain injury medicine: Principles and practice. Demos Medical Publishing, New York, NY, US

Zhang Y, Zhang Y, Chopp M et al (2020) Mesenchymal stem cell-derived exosomes improve functional recovery in rats after traumatic brain injury: a dose response and therapeutic window study. Neurorehabil Neural Repair 34:616–626. https://doi.org/10.1177/1545968320926164

Zheng W, Watts LT, Holstein DM et al (2010) Purinergic Receptor Stimulation Reduces Cytotoxic Edema and Brain Infarcts in Mouse Induced by Photothrombosis by Energizing Glial Mitochondria. PLoS One 5:e14401. https://doi.org/10.1371/journal.pone.0014401

Zheng W, Talley Watts L, Holstein DM et al (2013) P2Y1R-initiated, IP3R-dependent stimulation of astrocyte mitochondrial metabolism reduces and partially reverses ischemic neuronal damage in mouse. J Cereb Blood Flow Metab 33:600–611. https://doi.org/10.1038/jcbfm.2012.214

Zhou K, Motamed S, Thouas GA et al (2016) Graphene Functionalized Scaffolds Reduce the Inflammatory Response and Supports Endogenous Neuroblast Migration when Implanted in the Adult Brain. PLoS One 11:e0151589. https://doi.org/10.1371/journal.pone.0151589

Zhou Y, Shao A, Yao Y et al (2020) Dual roles of astrocytes in plasticity and reconstruction after traumatic brain injury. Cell Commun Signal 18:62. https://doi.org/10.1186/s12964-020-00549-2