Chapter 8
Regenerative Rehabilitation: Synergizing Regenerative Medicine Therapies with Rehabilitation for Improved Muscle Regeneration in Muscle Pathologies

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8.1 Introduction

With recent advances in the understanding of the molecular basis for tissue regeneration, regenerative medicine therapies for a host of musculoskeletal disorders are becoming available at an ever increasing pace. One promising area for the application of such therapies is toward the regeneration of skeletal muscle tissue. A host of disorders and pathologies contribute to the loss of skeletal muscle, including muscular dystrophies, acute trauma, tumor resection, and age-related sarcopenia. While some of these disorders have a relatively mild impact on the loss of muscle strength and function, others are so severe that they lead to the need for limb amputation or, in the worst cases, death. Therefore, regenerative medicine strategies are critical for the treatment of many musculoskeletal disorders.

While advances in muscle tissue regeneration are occurring at an unprecedented pace, the use of rehabilitation to support such procedures has traditionally received less attention. The cellular- and tissue-level response to mechanical loading has been well described in the musculoskeletal system; however, little is known regarding how this process could be leveraged to facilitate muscle regeneration. In the musculoskeletal system, there is a wealth of knowledge regarding the cellular- and tissue-level response to mechanical loading. However, there is little information as

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to how this process could be leveraged in a targeted and specific manner in order to facilitate tissue remodeling following regenerative medicine applications for muscle regeneration. In the following sections, we will (1) review the most recent advances in regenerative medicine therapies for skeletal muscle regeneration, (2) provide an overview of the principles of mechanotransduction as they apply to the musculo-skeletal system, and finally, (3) present early evidence supporting the use of physical rehabilitation as a tool to facilitate muscle regeneration following the application of regenerative medicine technologies. While there are a number of different musculoskeletal pathologies that may benefit from the use of regenerative medicine strategies, this review will focus on two primary applications that have received the majority of research focus: (1) volumetric muscle loss and (2) muscular dystrophy. Although this field is in its infancy, the available evidence supporting the importance of physical rehabilitation in facilitating muscle regeneration provides a foundation that may guide future investigations aimed at treating many severe musculoskeletal pathologies and disorders.

8.2   Regenerative Medicine Therapies for Muscle Pathology

Young, healthy skeletal muscle has a tremendous capability for regeneration following a relatively minor injury. However, this capacity is severely diminished with disease, volumetric muscle loss, and age, all of which can dramatically affect strength and functional capacity. To address these issues, many regenerative medicine therapies are being developed to regenerate muscle tissue and restore strength and functional capacity. These therapies can be generally divided into three areas of research focus: (1) stem cell transplantation, (2) biologic and engineered scaffolds, and (3) a combination approach using both stem cells and scaffolds. The basic principles, current findings, and limitations of each type of therapy are discussed below.

8.2.1   Stem Cell Transplantation

The idea of a stem cell first took form at the turn of the twentieth century when Ernst Haeckel described the presence of *stammzelles* [1]. These were described as primordial cells with the capacity to evolve into all types of cells and multicellular organisms. Since that time, numerous studies have investigated the use of stem cells to treat a plethora of disorders and diseases. Stem cells are unspecialized cells, capable of self-renewal, that have the capacity to differentiate into specialized tissue types. These cell populations can be isolated from many different tissues; however, they are most commonly isolated from embryonic or adult tissue. Stem cells derived from embryonic tissue have the potential to form all of the specialized cell types of the body, a feature known as *pluripotency*. However, their use is highly controversial and there are many concerns that their unlimited potential could lead to the

formation of unwanted tissue types (i.e., tumors). Adult stem cells are more desirable as they can be obtained from many tissues in the body, including bone marrow, fat, and skin. These cells were originally thought to have more limited potential for tissue regeneration due to their more differentiated state. However, recent studies have demonstrated that differentiated cell populations, such as fibroblasts (from skin) and adipocytes (from fat), can be reprogrammed to an embryonic-like stem cell by transient expression of four early developmental transcription factors [2]. This finding opens up numerous therapeutic applications, allowing researchers and doctors to harness the regenerative potential of more readily available cells types and to generate patient specific stem cells for autologous transplantation.

While stem cell therapies appear promising given the potential for muscle regeneration, their clinical application has, to date, been met with limited success [3]. Stem cell-mediated muscle regeneration for Duchenne muscular dystrophy (DMD) has been the most thoroughly investigated and is one of the few pathologies for which stem cell therapy has been translated to clinical trials [3]. DMD is a progressive muscle wasting disorder caused by a loss of the protein dystrophin, resulting in the loss of functional muscle by early teenage years. Early clinical trials of myoblast injections for DMD demonstrated the safety of intramuscular injections and the ability of transplanted cells to contribute to new myoblast formation and muscle regeneration [4–11]. Unfortunately, many studies found that the newly formed fibers did not provide any meaningful functional benefit, as no improvements in muscle strength were observed [5, 8, 9, 12]. In addition, poor donor cell engraftment was often noted [13–15]. Ultimately, the limited clinical success of these early interventions was attributed to rapid cell death, poor migration, and immune rejection of the implanted cells [13–15].

The limited success of stem cell therapies has highlighted a number of barriers to translation. The method of delivery, commonly via direct injection into the target tissue of interest, often leads to formation of a bolus of cells at the injection site [16]. Cells in the center of the injection site are therefore not able to get the nutrients or signals they need to thrive and differentiate, often leading to massive cell death. In addition, cells must be able to migrate away from the injection site in order to effectively integrate into the area of interest, which is not possible with a bolus of cells. On the other hand, there is also concern as to methods to maintain the cells in the location of interest. Without some form of "anchoring" within the target tissue, there is potential that the injected cells may migrate away from the area of interest, negating any regenerative benefit of the implanted cells.

If the cells do remain viable following transplantation, another concern is the ability of stem cells to differentiate into the target tissue. As growth factors are partially responsible for guiding cells to differentiate into one of many tissue types, there is some concern regarding the growth factors to which donor stem cells are exposed in an injured/diseased environment. If the cells are implanted into an inhospitable microenvironment, such as is the case in either "diseased" or acutely injured tissue, cells may be exposed to growth factors that promote further pathogenesis. If cells are exposed to such deleterious factors, there may be a risk for terminal differentiation toward an unwanted phenotype, such as fibrosis [17]. Studies are

needed to investigate the optimal time at which stem cells should be introduced into an environment, especially if the introduction of cells occurs following an acute injury.

In an attempt to address many of the known limitations of stem cell therapies, novel techniques to improve donor cell incorporation into muscle tissue are being investigated. Improvements in the isolation and manipulation of stem cells have the potential to improve engraftment potential and encourage functional muscle regeneration. For example, applications utilizing stem cell populations typically involve isolation and expansion of the cell population on tissue culture plates prior to implantation. However, it was later shown that even short-term culture of muscle stem or satellite cells results in a myoblast population with a greatly diminished regenerative potential [18–20]. To avoid the deleterious effects of cell culture, advances in cell sorting using flow cytometry have enabled the improved identification and isolation of fresh muscle satellite cells [19]. Recent studies have demonstrated the robust engraftment efficiency of such freshly isolated satellite cells, as demonstrated by a significant increase in muscle force production, as compared to cultured populations [21]. These studies represent an important step toward clinical translation of these therapies through improved methodology for cell isolation.

8.2.2 Biologic and Engineered Scaffolds

The use of scaffold materials for the replacement of injured or diseased tissue has gained considerable interest in recent years. This process involves the use of either naturally occurring or engineered/synthetic materials, sometimes combined with bioactive molecules, to reconstruct or restore living tissues. These scaffolds not only provide structural support for infiltrating progenitor cells, but, additionally, they facilitate tissue formation by enabling cell attachment, migration, proliferation, and differentiation [22]. To be effective, the scaffold must be able to bridge any tissue defect, interact with the surrounding tissue, and encourage new, functional tissue formation. Arguably, one of the most important properties of any scaffold is that it must not elicit an immune response. Both biologic and engineered scaffolds offer many of these advantages; however, there are specific benefits to each.

8.2.2.1 Biologic Scaffolds

Biologic scaffolds are derived through the decellularization of various source tissues and organs using detergents and/or enzymes, leaving behind only the extracellular matrix (ECM). The ECM is the secreted product of the resident cells of every tissue and organ in the body and is composed of various structural and cell adhesion proteins and glycans. Structural proteins within the ECM, such as collagen and elastin, provide structure and resilience to the tissue, while cell adhesion proteins such as fibronectin and laminin provide integrin-binding sites. These integrin-binding

sites activate intercellular signaling pathways that are important for regulating expression of ECM proteins. Glycans are an especially important component of the ECM as they provide a reservoir for signaling molecules and growth factors, which help to direct cell differentiation upon surgical implantation.

In the past 20 years, the FDA has approved many biologic scaffolds for use in soft tissue repair, including the reinforcement of tendon repairs and soft tissue grafts [23]. Commercially available biologic scaffolds have been derived from a variety of tissues, including the small intestine, dermis, urinary bladder, pericardium, and heart values. More recently, these scaffold materials have been repurposed for the treatment of severe muscle injuries. Studies have suggested that surgical implantation of biologic scaffolds encourages the site-appropriate, functional remodeling of muscle tissue in individuals with volumetric muscle loss [24–26]. Upon implantation into a muscle, the ECM is infiltrated by mononuclear cells and is gradually degraded (Fig. 8.1). The resulting degradation products, including bioactive peptides, growth factors, and cytokines, are released from the ECM to influence and direct multipotent stem/progenitor cell recruitment, proliferation, and differentiation,
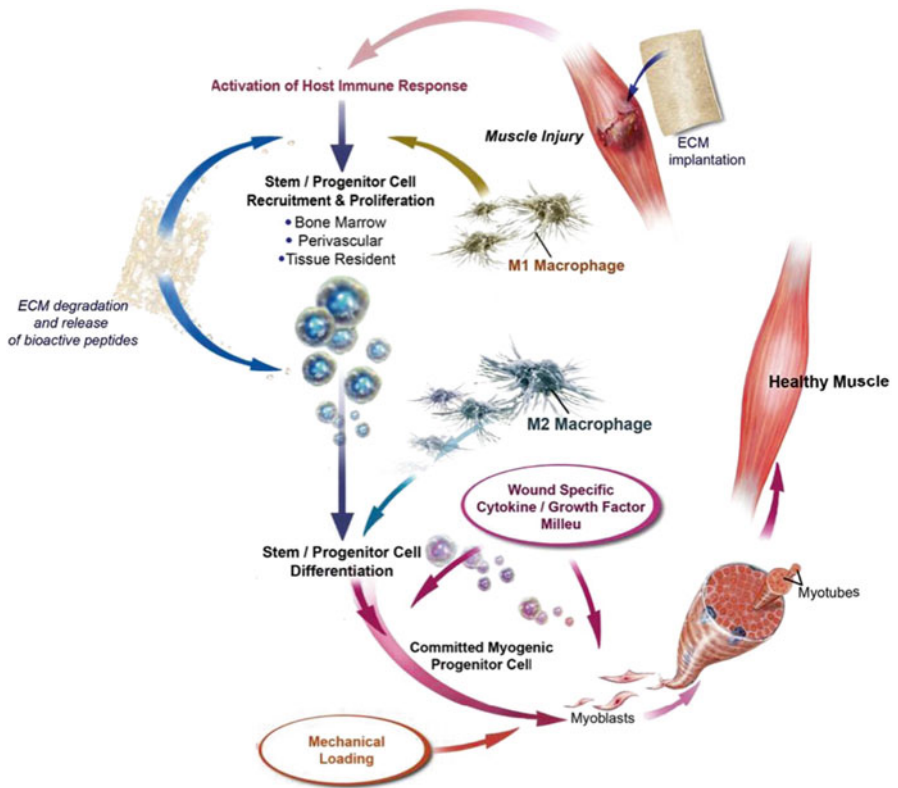

Fig. 8.1 Schematic displaying the cascade of events leading to the formation of healthy skeletal muscle following ECM implantation (adapted from Wolf et al. [22])

all of which contribute to the formation of site-appropriate tissue. In addition, degradation products have been suggested to modulate the innate immune response and encourage tissue remodeling. Preclinical studies have demonstrated that ECM implantation into areas of large volumetric muscle loss promotes an anti-inflammatory, remodeling macrophage phenotype (M2), rather than the default pro-inflammatory macrophage phenotype (M1) [27–29]. Previous studies have demonstrated that the macrophage phenotype (M1 vs. M2) is a major determining factor in the host tissue response, with increased scar tissue formation and poorer functional outcomes observed in individuals presenting with an M1 macrophage phenotype [28–30].

Scaffold degradation is a critical component of constructive tissue remodeling. If scaffold degradation is prohibited, muscle tissue formation will not occur. Badylak et al. demonstrated that chemical cross-linking of scaffold materials inhibits degradation and the release of biologic factors, leading to impaired tissue remodeling [27, 31]. Previously, biologic scaffolds were chemically cross-linked to strengthen the scaffold, allowing it to withstand the large tensile forces generated in vivo. However, as degradation of the scaffold appears to be a critical step in the process of muscle remodeling following ECM implantation, this practice would appear detrimental to muscle regeneration therapies.

8.2.2.2 Biologic Scaffold Implantation for Skeletal Muscle Repair

The successful formation of functional skeletal muscle following ECM implantation has been demonstrated in preclinical models of volumetric muscle loss in the abdominal wall, quadriceps, and gastrocnemius/Achilles tendon complex [26, 31–33]. These studies have utilized many scaffold source materials (e.g., small intestinal submucosa and urinary bladder matrix), different animal model species (e.g., mouse, rat, rabbit, canine), and various defect sizes (approximately 15–75 % of the affected muscle).

This regenerative medicine approach has been investigated in small case studies, with encouraging results reported. Mase et al. evaluated the implantation of ECM into the quadriceps muscle of a former military service member who had sustained a traumatic skeletal muscle injury to the quadriceps muscle 3 years previously [25]. In this proof-of-principle case report, dramatic improvements in knee extensor torque, power, and work were observed 16 weeks following ECM implantation. In addition, the participant reported that his cycling and walking endurance had improved and he was able to walk up and down stairs more easily and with greater stability.

More recently, Sicari et al. evaluated muscle regeneration and patient function following ECM implantation in five individuals with volumetric muscle loss [26]. All patients had a reported 58–90 % loss of muscle volume, as compared to their unaffected extremity. Prior to surgery, subjects underwent a personalized preoperative physical therapy program targeting specific strength and functional deficits until they reached a plateau in performance, defined as a period of 2 weeks with no appreciable improvements (<2 %) in strength or function. The purpose of this

preoperative physical therapy program was to maximize strength and function so that any improvements observed following surgery could be attributed to the surgical intervention and not to rehabilitation alone. Following plateau, subjects underwent surgery, which included excision of local scar tissue and ECM implantation into the defect area. After surgery, patients underwent 6 months of physical therapy and then returned for muscle biopsies, imaging, and assessment of muscle strength and function. Histological evaluation revealed perivascular stem cell mobilization, angiogenesis, and de novo formation of skeletal muscle. Imaging results indicated the formation of dense tissue, consistent with the appearance of skeletal muscle, in the region of ECM implantation. In addition, increased force production and/or improvements in activities of daily living were observed in four of the five patients 6 months after ECM implantation.

While the current clinical studies utilizing biologic scaffolds provide promising findings, there are a number of limitations that must be considered when interpreting the results. First, these studies include a heterogeneous sample of patients and do not include control subjects for comparison. In addition, investigators were not blinded as to the surgical limb/location, which could influence the results. It is also important to note that although participants in clinical trials demonstrated improvements in strength and function, there was not a total recovery as compared to control limbs. Subjects in these trials were only followed up to 6 months, and it's possible that muscle regeneration may continue well beyond that. Future studies should include later time points to determine if additional gains in strength and function occur. Finally, there is a possibility that the scar tissue debridement performed during the surgery may play a role in the improvements observed following surgery. However, this is unlikely due to the fact that the majority of these patients have previously undergone such surgeries without any appreciable improvement.

Although biologic scaffolds are advantageous due to their native complex structure and the availability of bioactive molecules within the matrix [34, 35], there are additional factors that need to be considered with their use. Given that the ECM is the secreted product of resident cells, each scaffold will have variations in architecture and biochemical composition. This may be problematic for studying muscle regeneration in patients as different scaffolds may affect the remodeling of muscle tissue in different patients. In addition, while it has been observed that ECM transplantation promotes site-specific tissue remodeling, the underlying mechanism by which this occurs has yet to be fully elucidated. Therefore, it is difficult to determine which factor has the most influence on the process of muscle remodeling.

8.2.2.3 Engineered Scaffolds

Engineered scaffolds have been used for over 50 years for skeletal muscle repair and reconstruction [22]. Their use is desirable for a number of reasons. First, engineered scaffolds are readily available and can be manufactured as needed, unlike biologic scaffolds that may have more limited availability. In addition, engineered scaffolds can be manufactured in a highly reproducible manner, which may allow for more

controlled delivery of the product to the patient. Engineered scaffolds are typically made of polypropylene, poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), and polyurethanes and can be made in many different configurations (e.g., meshes, foams, hydrogels, and electrospun scaffolds) [22]. Polypropylene was one of the earliest materials used for muscle repair and was desirable due to its high mechanical strength, durability, and low cost to manufacture. However, as a nonbiodegradable material, it elicits a cascade of immunological events resulting in fibrotic tissue deposition and thus has limited application for muscle regeneration [36, 37]. The use of PLGA has been investigated more extensively for tissue engineering and is most commonly used in biodegradable sutures [38–40]. As a scaffold for muscle tissue regeneration, PLGA is desirable as it is biodegradable and its degradation products are nontoxic [39, 40]. PLGA scaffolds have been shown to promote cell adherence, proliferation, and formation of new three-dimensional tissues, and porous PLGA scaffolds have been shown to promote vascularization and cell infiltration upon implantation [41–43]. Both PCL and polyurethanes are biodegradable. However, their degradation rates are typically slower than biological scaffolds and, thus, they are often used in combination with other components, such as bioactive molecules or growth factors, or are chemically modified [22, 44–46].

While engineered scaffolds have commonly been used for reinforcement and repair of muscle tissue, their application for muscle regeneration continues to be challenging. Engineered scaffolds lack the bioactive molecules found in biologic tissue that facilitate progenitor cell recruitment upon scaffold remodeling. The addition of specific growth factors, such as hepatocyte growth factor, insulin-like growth factor-1, and fibroblast growth factor, may overcome some of the limitations of engineered scaffolds and recreate some of the "niche" properties vital for site-specific tissue remodeling. In addition, synthetic scaffolds tend to elicit a pro-inflammatory foreign body reaction upon implantation, leading to scar tissue formation both within and around the implanted scaffold [37, 47]. Given this response, considerable research has been focused on the development of hybrid scaffolds, including the addition of bioactive coatings and biologically derived materials. Scaffolds are now being developed that are capable of providing the timed release of specific factors necessary for different stages of tissue repair, providing both the spatial and temporal cues necessary to support the normal regenerative process in skeletal muscle.

8.2.3   *Biologic and Engineered Scaffolds Combined with Cells*

While the implantation of biologic or engineered scaffolds is appealing for use in individuals with volumetric muscle loss, it is unknown if the same procedure can be effective for muscle remodeling in the presence of muscle pathology, such as is observed in muscular dystrophy or age-related sarcopenia.

In the case of a "diseased" muscle, it is possible there may not be an adequate supply of healthy progenitor cells to infiltrate the implanted scaffold. In addition,

ECM implantation has thus far been explored only for the replacement of an area of volumetric muscle loss, and not for entire muscle groups. For larger scale replacement of muscle tissue, there may not be enough progenitor cells available to populate the implanted scaffold. To address some of these issues, more recent studies are investigating the combined use of stem cells and biologic/engineered scaffolds as a potential technique to facilitate improved muscle regeneration. This approach provides localized delivery of various cell populations and growth factors to areas of diseased or missing skeletal muscle, providing both healthy progenitor cells and the appropriate biophysical and biochemical cues to encourage site-appropriate skeletal muscle formation.

Many different cell populations, including mesenchymal stem cells, skeletal muscle satellite cells, and myoblasts, have been used to prepare cell-seeded constructs. Following selection of the desired cell type, cells are placed on the scaffold and subsequently cultured in a bioreactor. The bioreactor is an apparatus that allows for the maintenance of a sterile environment while approximating in vivo conditions, including temperature, pH, oxygen levels, nutrients, metabolites, and regulatory molecules. In addition, physiologically relevant signals can be applied (i.e., interstitial fluid flow, shear, pressure, compression, and stretch), allowing for recreation of the in vivo physical environment. Scaffolds are maintained in the bioreactor until ready for transplantation, the duration of which may vary depending on the bioreactor conditions and cell type used.

There are many factors that need to be considered in developing the optimal cell/scaffold combination for skeletal muscle regeneration. Cell adhesion is critical for survival; therefore, any engineered construct must provide the appropriate biophysical cues to permit adhesion to the ECM. Previous studies attempting stem cell injections have failed partially due to the inability of the injected cells to attach to the host ECM. The addition of the cell adhesion ligand Arg-Gly-Asp (RGD) (cell binding domain for fibronectin) to both biologic and synthetic matrices allows stem cells to interact with the ECM and improves cell viability [48, 49]. In vitro studies have demonstrated that a minimum RGD ligand density (36 nm spacing) is required for myoblast growth on alginate gels [49, 50], and in vitro models have demonstrated that RGD-coupled alginate gels seeded with cells enhance cell viability following transplantation in mice [48]. In addition, the inclusion of ECM proteins, such as collagen, laminin, and fibronectin, along with recreation of the appropriate architecture and material stiffness, is important to recapitulate the mechanical properties of the cellular environment. Collagen VI, an important component of the ECM, has been shown to improve maintenance and survival of muscle satellite cells in vitro [51].

Along with specific ECM composition, studies have demonstrated that substrate biophysical characteristics are potent regulators of stem cell responses. Engler et al. demonstrated that mesenchymal stem cells seeded on matrices mimicking the stiffness of young, healthy skeletal muscle differentiated into myoblasts, while those seeded onto stiffer matrices differentiated toward a fibrogenic lineage [52]. Along these lines, architectural properties, such as porosity and topography, are also important considerations in the creation of synthetic environments, as these characteristics play a role in the exchange of oxygen and nutrients crucial for cell survival.

Studies have demonstrated that cells can tolerate macropore sizes ranging from 100 to 500 μm (average myofiber size ~100 μm); however, they demonstrate reduced viability when the pore size falls below 10–20 μm [53, 54]. Finally, scaffolds mimicking the collagen fibril alignment of native skeletal muscle ECM have also been found to promote regeneration of skeletal muscle in partial thickness muscle defects [55].

Although the use of cell-seeded scaffolds have not yet reached clinical trials, preclinical investigations have demonstrated promising results. Nseir et al. demonstrated that a synthetic scaffold, combined with a coculture of mouse myoblasts and either human embryonic endothelial cells or umbilical vein endothelial cells, demonstrated formation of endothelial networks both in between and around differentiating skeletal muscle fibers [56]. Shandalov et al. additionally demonstrated the fabrication of an engineered scaffold to act as a substitute for an autologous muscle flap for transplantation into a large soft tissue defect [57]. A biodegradable polymer scaffold was utilized and embedded with endothelial cells, fibroblasts, and/or myoblasts, which was then implanted into a full-thickness abdominal wall defect. After 1 week, the scaffold was shown to be highly vascularized, well integrated into the surrounding musculature, and had sufficient mechanical strength to support the abdominal viscera.

8.3 The Role of Mechanical and Electrical Stimulation in Tissue Healing and Remodeling

Skeletal muscle is a mechanosensitive tissue. That is, it responds to physical cues not only from the external environment, but also from its local microenvironment, including the ECM and surrounding cells. Both electrical and mechanical stimulation provide such physical cues to skeletal muscle and may therefore be valuable modalities to promote improved tissue healing and muscle remodeling following regenerative medicine therapies.

8.3.1 Mechanical Stimulation

Mechanical stimulation of skeletal muscle influences muscle growth, morphology, and cellular differentiation. Tensile stain, compressive loads, and hydrostatic pressure all cause structural alterations in the ECM and increase force transmission both across and between the ECM and neighboring cells. Cells respond to this stimuli through the activation of intercellular signaling pathways that regulate a multitude of cellular functions that are essential for tissue development, homeostasis, and recovery from injury. The importance of mechanical stimulation on tissue healing and regeneration has been elegantly described in murine hind-limb unloading studies, which demonstrate an inhibition of the regenerative potential of skeletal muscle following injury under conditions of unloading [58, 59].

The process by which mechanical stimuli are converted into a cellular response is called "mechanotransduction." Mechanotransduction consists of three distinct phases: (1) signal transduction at the level of the receptors, (2) signal propagation, and (3) cellular response (Fig. 8.2). Briefly, during the signal transduction phase, mechanical stimuli are transmitted to mechanosensors that reside in the ECM and both within and outside the cell. A mechanosensor is a receptor that responds to changes in mechanical force. Mechanosensors include stretch activated ion channels in the plasma membrane, focal adhesion complexes (including integrins) that bridge the cytoskeleton and ECM, and basement membrane proteins in the ECM that unfold/activate in response to increased force. These sensors deform in response to the application of force, and this change triggers a cascade of biochemical signals that will ultimately influence cellular function. During the next phase, signal propagation, biochemical conversion and propagation of the transmitted mechanical signal occurs through cell signaling pathways that can either enhance or diminish the intracellular spread of the converted biochemical signal. These signals will reach a final downstream target that then modulates cell function. In the final phase, cellular response, the cell responds to the received signal. This response can occur immediately or may be delayed. In the case of an immediate response, there is as an increase or decrease in intracellular tension, changes in adhesive properties, cytoskeletal reorganization, or cellular priming for migration. Delayed responses include changes in gene expression and the synthesis of proteins that influence cell proliferation, differentiation, structural properties, and viability.
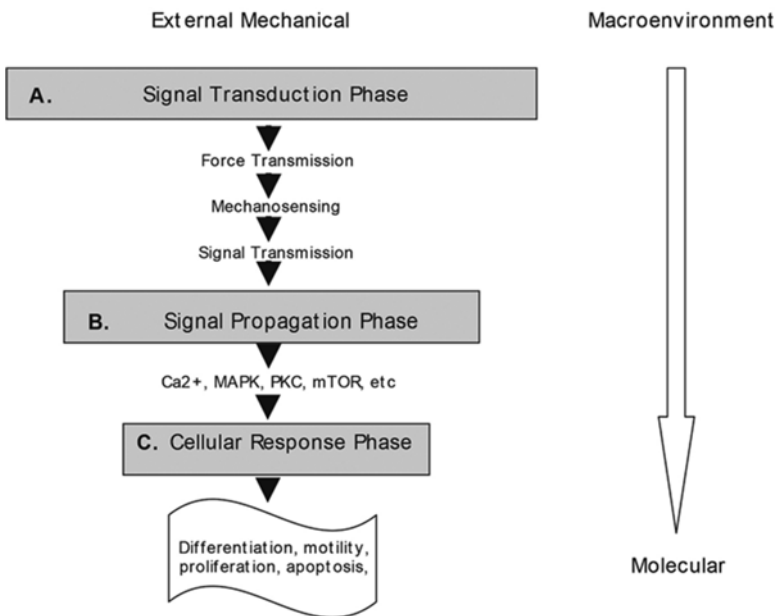

Fig. 8.2 Schematic of the phases of mechanotransduction (adapted from [60])

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While the in vivo mechanical stimulation occurs through muscle contraction or stretching, in vitro models have been developed to study of the direct influence of mechanical stimulation on cellular function [61]. There are various methods that have been implemented to apply mechanical stimulation to cells, including stretching and compressing cells [61, 62]. These methods can allow for the investigation of the underlying mechanisms by which cells directly respond to mechanical stimuli. One method to apply a mechanical stretch to cells involves culturing cells on a membrane or gel. Stretching of the membrane can then be generated in two ways, either through (1) multiaxial strain or (2) uniaxial strain (Fig. 8.3) [61]. For multiaxial strain, the membrane is stretched around a rigid frame or is deformed by applying a vacuum to the membrane, thus applying strain along multiple axes. For uniaxial strain, a stepper motor is used to increase uniaxial tension on cells seeded within either a 3D collagen gel or on a membrane. For either mode, stretching can be applied in either a cyclic or static mode to mimic different physiologic conditions, such as muscle contractions or prolonged stretching. While these methods can be applied to many different types of cells, using different stretching parameters, studies have specifically looked at the application of these methods for studying the effects on muscle cells. Specifically, studies utilizing such methods on muscle cells have demonstrated an increase in myofiber length and diameter, protein expression, and contractility when compared to static controls [61, 63–67].

8.3.2   Electrical Stimulation

Electrical stimulation is another mechanism used to modulate the tissue microenvironment. Electrical stimulation can be applied in two ways: directly to the area of interest (direct current), or indirectly, through stimulation of the nerve innervating the muscle [neuromuscular electrical stimulation (NMES)]. Direct currents have traditionally been utilized in wound healing to encourage infiltration of cells into an area of tissue damage. Following an injury, endogenous electrical currents are generated in the damaged tissue, which promotes and directs migration of cells into the area for wound healing. Efforts to enhance wound healing have therefore utilized this property to encourage cell migration through the application of external electric fields. Given that currents have a direction, the application of electrical stimulation can therefore be used to promote cell migration and alignment [68]. The use of electrical currents to influence cell alignment is of particular interest in the case of skeletal muscle where orientation of muscle fibers (aligned in parallel) is required for proper tissue functioning. While the use of electrical currents in rehabilitation is common for wound healing, there is much yet to be understood about their application in concert with regenerative medicine therapies for skeletal muscle

A Multiaxial stretch

1. Stretching frame (top view)   **Resting**   **Stretched**
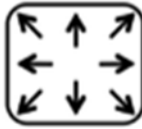
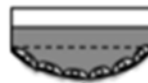Cells grown on membrane

2. Stretch by vacuum (side view)   **Resting**   **Stretched**

Cells grown on membrane

Vacuum

Uniaxial stretch

3. Stretch by stepper motor (top view)

Resting

Fixed point

Stretched
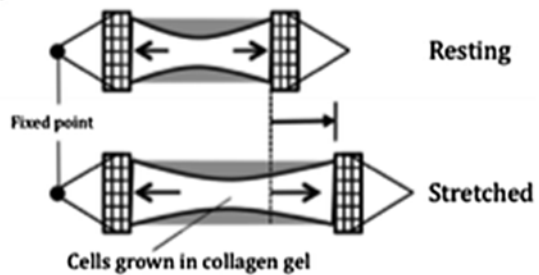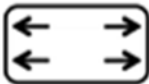
Cells grown in collagen gel

Fig. 8.3 In vitro methods to apply mechanical stimulation to cells (adapted from Passey et al. [61])

regeneration. Future studies are needed to investigate the ability of both direct and alternating currents as a method to encourage donor stem cell infiltration into an area of injury.

Electrical stimulation of the motor unit to elicit a muscle contraction may be achieved through neuromuscular electrical stimulation (NMES). NMES is a rehabilitation modality that can be used to mimic the physiologic action of neurons and recreates the mechanical environment experienced by resident muscle cells through the induced contraction of innervated muscle fibers. The transmission of electrical signals via nerve innervation is well known to play a major role in directing the process of terminal differentiation of skeletal muscle cells [69]. The application of

NMES to muscle can be used to stimulate increased cellular proliferation and survival rates, desired differentiation, and improved functionality [69]. Prior studies utilizing electrical currents to study the effects on muscle cell behavior have demonstrated increased satellite cell activation, improved differentiation, and enhanced muscle force output [70–76].

8.4 The Synergy of Rehabilitation with Regenerative Medicine Therapies to Enhance Muscle Remodeling

As described above, mechanical and electrical stimulation are powerful methods to trigger mechanotransductive responses of resident and infiltrating cells. Given the importance of mechanical and electrical stimulation on endogenous cell function and muscle healing, the prescription of targeted exercise or NMES as part of a rehabilitation protocol is a logical adjunct therapy to the application of regenerative medicine therapies. Preclinical models have provided the strongest evidence to support the use of such modalities to promote improved functional muscle regeneration. Although still in the early stages of investigation, results from recent studies are providing exciting evidence to support the use of rehabilitation approaches applied in synergy with regenerative medicine therapies to facilitate skeletal muscle regenerative potential.

8.4.1 Preclinical Models

Preclinical have demonstrated improved force production, both in vitro using cell-seeded scaffolds and in vivo, following the application of mechanical and electrical stimulation in different models of muscle regeneration. Ito et al. applied electrical stimulation (bidirectional, continuous pulses; 24 % of peak force initially, up to 50–60 % of peak force as the tissue developed) to tissue-engineered constructs seeded with C2C12 myoblasts in vitro [73]. Following stimulation, muscle constructs were fixed with two pins, one attached to a force transducer, and one to the bottom of the culture plate well. Constructs were stimulated and force production was measured. Ito et al. determined that the application of pulsed electrical stimulation resulted in increased force production in vitro, as compared to constructs that did not receive stimulation [73]. Increased force production was hypothesized to occur due to improved sarcomere organization and increased expression of myosin heavy chain, the motor protein of muscle thick filaments. Similarly, Machingal et al. demonstrated that mechanical preconditioning of muscle precursor cells seeded on biologic scaffolds prior to implantation in murine models of volumetric muscle loss demonstrated 44 % greater force production as compared to animals receiving unstimulated cell constructs [77]. Distefano et al. evaluated the ability of NMES to

improve stem cell engraftment in a murine model of muscular dystrophy (*mdx* mouse) [71]. Muscle-derived stem cells were isolated from wild-type mice and used for the experiments. The *mdx* mice were randomized into one of four treatment groups: saline injection, NMES alone, muscle-derived stem cell injection, or NMES plus muscle-derived stem cell injection. Animals in the NMES groups were stimulated 5×/week over the course of 4 weeks. The mice treated with NMES following stem cell transplantation demonstrated a twofold increase in the number of dystrophin-positive myofibers, an increased vascularity, and an accelerated recovery from a fatigue protocol, when compared to control animals that did not receive electrical stimulation [71]. In addition, several other studies have similarly demonstrated enhanced stem cell transplantation efficiency when stem cell transplantation is followed by mechanical loading, elicited via various methods such compensatory overloading [78, 79], swimming [80], or treadmill running [79, 81].

8.4.2   Clinical Applications

There are very few studies investigating the use of mechanical stimulation, or rehabilitation, for muscle regeneration in clinical trials. However, recent studies provide evidence in further support of the importance of mechanical stimulation in the success of regenerative medicine therapies. As reviewed above, Mase et al. described a case study of a military service member who received biologic ECM implantation following volumetric muscle loss of his quadriceps [25]. Given preclinical findings demonstrating the importance of mechanical stimulation for effective functional remodeling following scaffold implantation [77], investigators initiated a rehabilitation protocol 4 weeks after surgery. Rehabilitation then continued for 12 weeks. As described above, 16 weeks postsurgery, marked gains in isokinetic performance were observed, and CT scans revealed the formation of new tissue at the implantation site. More recently, as additionally described above, five subjects underwent implantation of a porcine bladder-derived ECM into a region of volumetric muscle loss [26]. Once again, investigators included a postoperative rehabilitation protocol with the goal of maximizing functional incorporation of the implanted ECM. Within 24 h of the ECM implantation, subjects began a rehabilitation program that continued for the next 6 months postoperatively. At the end of the program, performance on different tests of strength and function were quantified and compared to preoperative baseline measures. As a part of the same ECM transplantation trial, Gentile et al. provided an in-depth description of the rehabilitation protocol implemented in the case of a military veteran who received surgical ECM implantation for volumetric muscle loss to his quadriceps muscle [24]. Following surgery, the subject underwent a targeted physical therapy program, including site-specific range of motion and strengthening exercises, progressing to more dynamic functional activities over 6 months. At the end of the program, the subject demonstrated gains in all of the strength and functional outcome variable quantified, with the most marked gains

observed during more dynamic tasks such as the single leg hop for distance, in which he improved 1820 % compared to presurgical values.

8.4.2.1   Limitations of Current Studies

While clinical studies demonstrate promising findings with respect to improvements in strength and/or function, along with evidence of new muscle and blood vessel formation, following ECM implantation for VML, the direct role of rehab is difficult to discern. None of these studies included control subjects receiving surgery alone without rehabilitation. Without these controls, it is difficult to directly assess the benefit of rehabilitation and the contribution to the improvements observed. In addition, each rehabilitation program included different components so it's additionally difficult to determine which aspect of the rehabilitation program may be most beneficial. Preclinical studies using both electrical and mechanical stimulation provide greater direct evidence that these modalities may play a critical role in the translation of cellular therapeutics for the treatment of muscle injuries or diseases. However, much has yet to be understood regarding the selection of the most appropriate interventions, the optimal loading frequencies and intensities, as well as the best time to initiate such interventions. For example, the application of an electrical stimulation protocol that is too aggressive has been associated with altered cellular metabolism, impaired cell viability, and even cell death [82]. Studies evaluating the utility of mechanical and electrical stimulation to enhance muscle regeneration following regenerative medicine technologies will need to take these parameters into careful consideration.

8.5   Future Directions

The synergy of physical rehabilitation and regenerative medicine therapies to optimize muscle regeneration is an exciting field of study. Although muscle regeneration is the main focus of this chapter, the use of rehabilitation to facilitate tissue remodeling following regenerative medicine therapies has been suggested for a number of different applications, including cardiac regeneration, gene therapies for muscle pathologies, and stroke [83–85]. The early evidence presented above suggests that mechanical and electrical stimulation, as applied during rehabilitation, may be powerful tools to encourage functional muscle formation following regenerative medicine therapies. Physical therapy and rehabilitation play a key role in the recovery of strength and function in individuals suffering from musculoskeletal injuries and pathologies. As such, new protocols are needed to accomplish these same goals in individuals receiving regenerative medicine therapies for muscle regeneration [86–88]. As such, there is a need for rehabilitation professionals to work closely with basic scientists toward the goal of developing clinically relevant protocols with an eye on maximized functional outcomes [87]. There is a need for

rehabilitation professionals to understand the most recent advances in regenerative medicine therapies so as to design effective programs to optimize tissue regeneration at every stage. Future studies should include well-designed clinical trials with blinded investigators and placebo control groups. The collaboration of basic scientists and clinicians promises to yield exciting advances in the treatment of a multitude of muscle injuries and pathologies in the coming years.

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