Journal of Muscle Research and Cell Motility (2020) 41:297–311
https://doi.org/10.1007/s10974-019-09550-w

Skeletal muscle cell transplantation: models and methods

Amber L. Mueller[1] · **Robert J. Bloch**[1]

Received: 4 April 2019 / Accepted: 1 August 2019 / Published online: 7 August 2019

Abstract
Xenografts of skeletal muscle are used to study muscle repair and regeneration, mechanisms of muscular dystrophies, and potential cell therapies for musculoskeletal disorders. Typically, xenografting involves using an immunodeficient host that is pre-injured to create a niche for human cell engraftment. Cell type and method of delivery to muscle depend on the specific application, but can include myoblasts, satellite cells, induced pluripotent stem cells, mesangioblasts, immortalized muscle precursor cells, and other multipotent cell lines delivered locally or systemically. Some studies follow cell engraftment with interventions to enhance cell proliferation, migration, and differentiation into mature muscle fibers. Recently, several advances in xenografting human-derived muscle cells have been applied to study and treat Duchenne muscular dystrophy and Facioscapulohumeral muscular dystrophy. Here, we review the vast array of techniques available to aid researchers in designing future experiments aimed at creating robust muscle xenografts in rodent hosts.

Keywords Xenograft · Transplantation · Muscular dystrophy · FSHD · Satellite cell · Myoblast transfer therapy

Introduction

Engraftment of skeletal muscle cells is commonly used to study muscle wasting, regeneration, and differentiation in the context of muscle injury and muscular dystrophies. Muscle cell transplantation has also been proposed as a therapeutic treatment for volumetric muscle loss, traumatic muscle contusion, muscular dystrophies and other myopathies (Partridge et al. 1989; Vilquin et al. 2005; Quenneville and Tremblay 2006; Halum et al. 2008; Ambrosio et al. 2010; Morosetti et al. 2011; Lavasani et al. 2014; Negroni et al. 2015; Kim et al. 2016; Barthelemy and Wein 2018). The term, " xenograft", is used to describe the transplantation of tissue or cells from a donor of one species into a host of a different species. Most commonly, and throughout this review, "xenograft" describes human muscle tissue or mononuclear muscle precursor cells engrafted into a rodent host.

Myoblast transfer therapy was pioneered in the 1990s with the goal of treating Duchenne muscular dystrophy (DMD) by delivering healthy, dystrophin-expressing myoblasts to dystrophic muscle groups. Though these initial trials showed little clinical benefit (reviewed in Smythe et al. 2000; Mouly et al. 2005; Skuk and Tremblay 2015), these studies provided much insight into the conditions necessary for muscle cell engraftment, as well as the factors that limit its success. Pivotal studies by Riederer et al. identified three major hurdles to successful xenotransplantation, namely early progenitor cell death, limited cell proliferation, and poor migration within engrafted muscle (Riederer et al. 2012). They showed that muscle cell differentiation within the first 3 days after engraftment prevents proliferation and migration of muscle progenitor cells and that methods to increase proliferation and inhibit early differentiation resulted in a greater number of muscle fibers formed by the engrafted cells (Riederer et al. 2012). Other groups have investigated the use of other cell types, including satellite cells, induced pluripotent stem (iPS) cells, and mesangioblasts in engraftment.

Recent advances in methods for xenografting have focused on identifying new immunodeficient mice and myogenic cell types, adapting methods to injure the host muscle prior to engraftment, and intervening to encourage proliferation of the engrafted cells and reduce proliferation of host

✉ Robert J. Bloch
rbloch@umaryland.edu

Amber L. Mueller
amberl.mueller@gmail.com

[1] Department of Physiology, University of Maryland School of Medicine, 655 W. Baltimore St., Baltimore, MD 21201, USA

cells after engraftment. Currently, xenografts continue to be used to study muscle differentiation and regeneration, stem cell and satellite cell biology, revascularization and reinnervation of muscle, and the control of fibrosis and liposis. For clinical purposes, xenografts are also being used to study corrected-cell and gene therapies for muscular dystrophies, including DMD, as models for Facioscapulohumeral muscular dystrophy (FSHD), and to examine potential small molecule and virally-based therapies for these and other muscle diseases (Vilquin et al. 2005; Morosetti et al. 2011; Sakellariou et al. 2016; Chen et al. 2016; Barthelemy and Wein 2018; Mueller et al. 2019). Together, these studies employ a vast array of methods. We present this comprehensive review of these methods to help researchers design and combine approaches to improve the quality of their xenografts to study muscle regeneration, muscle diseases, and potential cell therapies.

Skeletal muscle cell transplantation methods

General schema

The techniques used to xenograft muscle cells and tissue vary widely and are largely dependent on their application and the goals of the specific study. Some variables include cell type, mechanism under investigation, and limitations in time and resources that affect throughput. Generally, xenografts are generated in immunodeficient rodent hosts that have undergone some form of muscle preinjury to create a niche for muscle-derived cells or tissue to engraft (Fig. 1). The graft is typically initiated by direct intramuscular injection or by surgical implantation of myogenic cells or tissue, respectively, but systemic cell delivery is also being explored. Engraftment can be followed for several days, weeks, or months, often with interventions to enhance muscle fiber development (Fig. 1).

The success of engraftment is determined by the proportion of the engrafted muscle that is of donor origin. Brimah et al. suggested that the best way to identify human regions of mosaic fibers is with human-specific antibodies to spectrin (h-spectrin) and to the nuclear envelope protein, lamin A/C (h-lamin A/C) (Brimah et al. 2004). Unlike immunolabeling for human-β-galactosidase (produced by cells transformed to express it) and, perhaps, human dystrophin, h-spectrin does not spread in host muscle and is likely to be a better indicator of myonuclear domain size (Brimah et al. 2004). Recently, however, it was reported that the h-spectrin antibody can detect false-positives in regenerating muscles of several immunodeficient mouse strains, possibly due to cross-reaction with utrophin (Rozkalne et al. 2014). Several human specific antibodies should therefore be used to ensure accurate quantification. Antibodies to human lamin A/C, spectrin, and dystrophin are most commonly used for this purpose.

Additional considerations in evaluating the success of engraftment are the ability of the engrafted human cells or tissue to repopulate the satellite cell niche, to be innervated by murine motor neurons, and to acquire an adequate blood

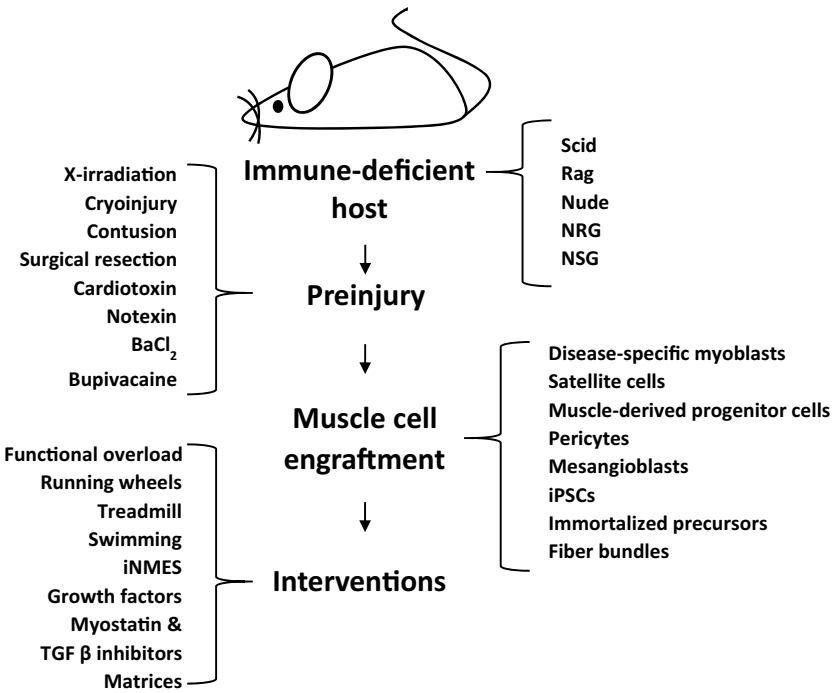

Fig. 1 General schematic of xenograft transplantation method including common protocol variations

supply. These factors will affect survival of the graft, and, most importantly for many studies, its ability to improve function. The most significant factor, however, is the requirement that the engrafted cells or tissue not be rejected by the host immune system.

Immunodeficient rodent hosts

In order for cross-species transplantation (xenografting) to be successful, the host's immune system must not reject tissue or cells from the donor. Several immunodeficient rodent strains are available that permit human cell engraftment, including nude mice and rats, *scid* mice, *Rag,* and NOD mouse variations. The Jackson Laboratory provides a comprehensive list of immunodeficient mouse strains and their properties (https://www.jax.org/jax-mice-and-services/find-and-order-jax-mice/most-popular-jax-mice-strains/immunodeficient-mouse-and-xenograft-host-comparisons).

Some of the earliest muscle xenografting studies utilized severe combined immunodeficient (*scid*) mice which largely lack T and B lymphocytes (Huard et al. 1994; Skuk et al. 1999) yet still possess a partially functional innate immune system (Lovik 1995). *Scid* mice have been used for studies of nerve regeneration by multipotent muscle progenitor cells (Lavasani et al. 2014) and for studies of a potential cell therapy for FSHD using mesangioblasts (Morosetti et al. 2011). Recent studies of DMD have utilized the dystrophin-deficient *mdx* mouse crossed into the *scid*, nude, and other immunodeficient genetic backgrounds (Morrison et al. 2000; Farini et al. 2007; Walsh et al. 2011; Chirieleison et al. 2012; Martinez-Sarra et al. 2017; Benedetti et al. 2018; Meng et al. 2015) (reviewed by McGreevy et al. 2015). Nude mice and rats, while lacking mature T cells, are otherwise immune-competent and have limited applications for xenografting. *Scid* mice are preferred in some instances, although their typically short lifespan and "leaky" lymphocyte phenotype limit their usefulness for long-term studies, which require the complete absence of adaptive immunity (The Jackson Laboratory) (Cooper et al. 2001).

Borrowing from other xenografting fields, Cooper and colleagues demonstrated that the recombinase-activating gene 2 and the common cytokine receptor γ chain gene (*Rag2*$^{-}$/γc^{-}) double mutant mouse is superior to the *scid* mouse for studies of myoblast engraftment because it is completely devoid of B and T lymphocytes and produces defective natural killer cells, allowing muscle xenografts to persist long enough to reach maturity (Cooper et al. 2001). This mouse strain, as well as an alymphoid triple mutant variation lacking the C5 complement gene (C5^{-}/Rag2^{-}/γc^{-}), were used by several groups to perform many of the seminal studies that led to the development of myoblast transfer therapy for DMD (Goldman et al. 1998; Cooper et al. 2001; Guigal et al. 2002; Morgan et al. 2002; Cooper et al. 2003; Brimah et al. 2004; Chicha et al. 2005; Silva-Barbosa et al. 2005; Mamchaoui et al. 2011; Riederer et al. 2012; Bencze et al. 2012; Vallese et al. 2013; Meng et al. 2015). Additional variant strains that are immunodeficient but also lack specific proteins linked to muscular dystrophies, such as α- (Sgca-null/scid/beige) or β-sarcoglycan (sgcb^{-}/Rag2^{-}/γc^{-}), have also been developed and used for xenografting (Tedesco et al. 2012; Martinez-Sarra et al. 2017; Lorant et al. 2018; Torrente et al. 2004; Meng et al. 2011).

More recently the NOD *scid* gamma (NSG) mouse has become the leading standard in xenografting cancer and stem cells because it is able to engraft the widest range of cell types and has a longer lifespan than *scid* mice (The Jackson Laboratory). These mice lack T and B lymphocytes and natural killer cells and have defective dendritic cells and macrophages (The Jackson Laboratory). The NSG mice and the dystrophic NSG-mdx have been widely used for studies of satellite cell regeneration and corrected-cell therapies for DMD and other myopathies (Xu et al. 2015; Benedetti et al. 2018; Arpke et al. 2013; Young et al. 2016).

Finally, a variation of the NOD mouse, the NOD *Rag* gamma (NRG) mouse, was first examined in xenografting studies by Silva-Barbosa et al. (2005). This strain better tolerates the use of DNA-damaging agents and irradiation, making it the best option for studies requiring the elimination of host muscle fibers and stem cells by these methods. We have confirmed that the NOD-Rag mouse can withstand local X-irradiation of the hindlimb up to 25 Gy, which completely inhibits regeneration of murine muscle, with little or no consequences to health or life span (Sakellariou et al. 2016; Mueller et al. 2019).

A recent study of cell therapy for volumetric muscle defect repair used nude rats to engraft human myogenic precursor cells and showed functional improvements in the injured rat muscle (Kim et al. 2016). Immunocompromised Rag2-knockout rats are commercially available but to our knowledge have not yet been used in muscle grafting experiments. Rats may be particularly useful hosts when larger samples of engrafted tissue are required, but the greater availability and lower costs for purchase, breeding and housing immunodeficient mice provide substantial advantages.

Preinjury

Generating robust xenografts of muscle cells generally requires one or more forms of preinjury in order to create a niche for muscle cells to engraft (Fig. 1). Preinjury methods include ionizing radiation, mechanical disruption, and the use of myotoxic pharmacological agents. Typically, X-irradiation is used to disrupt the host satellite cell niche for studies which aim to avoid host-derived muscle regeneration, while myotoxins or mechanical injury is used to destroy host muscle fibers.

Irradiation

High dose X-irradiation is used to limit the formation of host-donor mosaic fibers during regeneration and to enhance the formation of xenografts that are highly enriched in fibers of donor origin. Host satellite cell proliferation is inhibited by 16-18 Gy X-irradiation (Morgan et al. 1990). Host satellite cells are more effectively eliminated by 18 Gy X-irradiation than by myotoxins such as $BaCl_2$, notexin or cardiotoxin, and as a result, the irradiated muscles regenerate significantly less than the intoxicated ones (Boldrin et al. 2012). A subset of mouse host satellite cells are irradiation-resistant, however, and become activated after large scale injury (Brimah et al. 2004; Heslop et al. 2000). Higher doses are therefore needed to suppress the contributions of myogenesis of host cells to the xenografts. Slower rates of irradiation of the mouse hindlimbs significantly increased the number of fibers that formed from the donor cells (Gross et al. 1999). More recently, utilizing the irradiation-resistant NRG mouse strain (Pearson et al. 2008), our group delivered 25 Gy X-irradiation to mouse hindlimbs, shielding the body with lead to prevent damage to other tissues, and showed that the human fibers (labeled for h-spectrin) with centrally located nuclei of murine origin (not labeled for human lamin A/C) constituted less than 1.5% of all centrally nucleated myofibers (CNFs) in the xenografts (Sakellariou et al. 2016). Irradiation is also reported to increase laminin deposition in areas of damage, aiding myoblast engraftment (Silva-Barbosa et al. 2008).

Mechanical disruption

Cryoinjury has been widely used to induce muscle damage to create a niche for engrafting muscle progenitor cells. Typically, cryolesions are created by surgically exposing the muscle of interest and inducing two or more cycles of freeze-thawing with a metal rod first cooled in liquid nitrogen and then applied to the muscle for several seconds (Cooper et al. 2001; Brimah et al. 2004; Silva-Barbosa et al. 2005, 2008; Mamchaoui et al. 2011; Riederer et al. 2012; Hardy et al. 2016; Lorant et al. 2018). In a comparative study, more myogenic cells were found to be engrafted in cryoinjured TAs than in cardiotoxin-injured TAs after 4 weeks (Silva-Barbosa et al. 2005). A more physiologically relevant form of mechanical injury is performed by dropping a small steel ball directly onto the muscle of interest. This produces a high-energy blunt injury, or contusion, and has been used to study muscle allografting and the role of inflammation in muscle regeneration in mice (Ambrosio et al. 2010; Liu et al. 2017, 2018, 2019; Xiao et al. 2016). Contusion and cryoinjury induce local muscle fiber damage, while largely preserving the original basal lamina surrounding each muscle fiber. They are therefore most effective for studies of xenografting muscle precursor cells into discreet areas to study regeneration and their incorporation into regenerating muscle.

Surgical removal of large sections of muscle has also been used to create a niche for engraftment, especially prior to xenografting bundles of muscle tissue, to model repair by progenitor cells after volumetric muscle loss, and to test potential cell therapies for muscle wasting (Zhang et al. 2014; Kim et al. 2016; Kuhn et al. 2017; Baker et al. 2017). Conversely, surgical excision of the entire *tibialis anterior* (TA) has been used to functionally overload a synergistic muscle, the *extensor digitorum longus* (EDL), which induces degeneration and regeneration in the EDL (Ambrosio et al. 2009). Likewise, muscle atrophy and denervation have also been used to induce muscle wasting prior to xenografting (Lavasani et al. 2014). Each of these methods of mechanical disruption induce different forms of injury, to varying degrees of physiological relevance and, more importantly, usefulness in addressing particular questions.

Although not a mechanical disruption per se, exercise (e.g., intensive swimming) has been used prior to initiating a graft in dystrophic mice, to promote greater muscle turnover and create a niche more receptive to engraftment (Torrente et al. 2001, 2004; Benchaouir et al. 2007).

Myotoxins and chemical injuries

Several commercial myotoxins and chemicals have been developed and compared to injure muscle, leading to the death of myofibers and subsequent regeneration. Boldrin et al. (2012) showed that many more satellite cells (initially introduced exogenously) were present in muscles 3 days and 4 weeks after intoxication with $BaCl_2$, notexin, or cardiotoxin than after X-irradiation. Notably, the three toxins were indistinguishable in this respect. A more recent study compared cardiotoxin, notexin, and $BaCl_2$ to cryoinjury in immune-competent mice (Hardy et al. 2016). They assayed necrosis, markers of inflammation, satellite cell proliferation, fibrosis and vascularization, and showed that each method had distinct regeneration profiles. Interestingly, cardiotoxin was the only method in which cytokines were eventually restored to normal levels, whereas $BaCl_2$, notexin and freeze injury caused inflammatory cytokines to remain elevated over the duration of the study (Hardy et al. 2016). In another report, however, levels of inflammatory cells in cardiotoxin-treated TAs in immune-compromised murine hosts were higher than in cryoinjured TAs (Silva-Barbosa et al. 2005). For cardiotoxin and notexin, there was batch to batch variation in some of the measured parameters (Hardy et al. 2016) which we have also seen in our unpublished studies (Llach, Mueller et al., in preparation). Several questions still remain, including the relative effects of these injuries in immunodeficient mice, where inflammation

may be suppressed. The majority of the studies on muscle injury methods have been performed on immune-competent muscle, which may respond differently. The effects of the immune system during muscle injury have a major impact on regeneration (Tidball 2005, 2011; Yang and Hu 2018), which means immune-competent muscle may respond differently to injury than immunodeficient muscle. Therefore, a comprehensive analysis of these preinjury methods in immunodeficient mice should be investigated.

There are also several other sources of myotoxins that have not been tested in comparative studies, including cardiotoxins from different snake species and different methods for their partial or complete purification (Hodges et al. 1987; Chien et al. 1994; Mir et al. 2008). One of the most widely used forms of cardiotoxin, previously supplied by Sigma-Aldrich, is no longer produced, which has led us and other investigators in search of the closest replacement. In our experience, none of the other commercially available toxins are comparable to the Sigma-Aldrich cardiotoxin in their ability to kill murine muscle fibers in immunodeficient mice and subsequently to support engraftment. Indeed, in our studies, $BaCl_2$ seems to be superior to commercial toxins in damaging mouse muscle fibers and for subsequent engraftment of donor cells (Llach, Mueller et al., in preparation). As dosing is limited by the fact that high concentrations of $BaCl_2$ is cardiotoxic and can kill the treated mice, we hope that other investigators will continue to test potential myotoxins with the goal of identifying the optimal reagent for xenografting.

Cell types

The consideration of which cells are best suited for engineering of skeletal muscle tissue has been reviewed (Fishman et al. 2013). Protocols for xenografting skeletal muscle have utilized a number of different cell types for studies of regeneration after injury or atrophy, disease modeling, and cell therapy strategies. Early xenografting studies identified muscle precursor cells based on desmin immunolabeling, following primary cell isolation from muscle tissue (Cooper et al. 2001; Brimah et al. 2004; Riederer et al. 2012). Engraftment of primary cells into adult host muscle is generally difficult, however, unless the regenerative capacity of the endogenous satellite cell population has been reduced (e.g., by X-irradiation). Recently, however, methods to isolate muscle precursor cells from adult biopsy, autopsy, and fetal tissue have expanded and several discreet lineages with varying ability to form fibers and to differentiate into satellite cells in muscle xenografts have been identified (Lorant et al. 2018; Negroni et al. 2015).

Lorant et al. composed an excellent list of features of adult stem cells, distinct from satellite cells, that have been identified for potential use in xenografting, and specifically for DMD cell therapies (See Table S1 of Lorant et al. 2018). Briefly, these include myoblasts, $CD133^+$ cells, hematopoietic stem cells, pericytes, mesangioblasts, muscle-derived stem cells, mesenchymal stem cells, myo-endothelial cells, and "side population" cells derived from interstitial tissue (Lorant et al. 2018). The properties of each cell type, including their stability, proliferation and differentiation ability, ability to integrate into host muscle and to home to muscle through the bloodstream, are also listed in Table S1 of Lorant et al. (2018), which readers should consult for details and relevant references.

In some cases, the same cell types vary in their ability to generate muscle, perhaps due to factors associated with the murine strain used and the mode of delivery (Dellavalle et al. 2007; Meng et al. 2011). Other human sources include fetal and neonatal muscle-derived stem cells and myogenic precursor cells (Brimah et al. 2004; Riederer et al. 2012; Silva-Barbosa et al. 2005, 2008), immortalized cells (Mamchaoui et al. 2011; Sakellariou et al. 2016; Mueller et al. 2019), iPS cells (reviewed in Danisovic et al. 2018), dental pulp pluripotent-like stem cells (Martinez-Sarra et al. 2017), and satellite cells (Hall et al. 2017). Muscle-derived stem/progenitor cells isolated from adult skeletal muscle have even been used to study nerve regeneration and dysphagia (Lavasani et al. 2014; Walz et al. 2008; Lavasani et al. 2013; Kuhn et al. 2017).

Immortalized cells

As noted above, successful xenografting of myogenic cells is a function of the ability of these cells to survive, proliferate, migrate throughout the muscle compartment, and eventually differentiate into myofibers in the host environment (Riederer et al. 2012). A key determinant of engraftment ability is the maintenance of myogenic precursor cells in a proliferative state. Reversible cell immortalization overcomes the issues of limited proliferation and cell cycle senescence in culture. The advantages of studying immortalized cells include the relative ease of preparation (transfection of primary cultures, followed by derivation of individual clones, if desired), the cell's retention of the properties of the parent primary cells from which they were derived (e.g., Mueller et al. 2019), the ease of culture, and the available of essentially unlimited numbers of cells for engraftment.

Several methods of immortalization have been developed, which usually include transduction of human telomerase reverse transcriptase (hTERT) and another anti-senescence gene. hTERT maintains telomere ends so that otherwise post-mitotic cells can undergo continuous population doublings in culture. As other inhibitors of proliferation remain, however, complimentary transduction with another anti-senescence gene is necessary. Currently the two primary choices as partners of hTERT are Bmi-1

and CDK4 (Cudre-Mauroux et al. 2003; Zhu et al. 2007). Bmi-1 negatively regulates cell cycle-repressing genes p16 and p19Arf, allowing cells to avoid senescence (Jacobs et al. 1999). CDK4 directly regulates the cell cycle, allowing cells to progress through G1 phase. Both methods overcome problems with previous immortalization methods that resulted in major chromosomal abnormalities and phenotypic changes (Cudre-Mauroux et al. 2003; Zhu et al. 2007). DMD cells immortalized with Bmi-1 and hTERT are currently being used to develop corrected-cell therapies for DMD, while cells immortalized with CDK4 and hTERT are being used to model FSHD and to study other complex muscular dystrophies (Benedetti et al. 2018; Stadler et al. 2011; Sakellariou et al. 2016; Mamchaoui et al. 2011; Arandel et al. 2017; Mueller et al. 2019).

Satellite cells

Recent studies have made significant advances in the methods for transplanting satellite cells and activated muscle stem cells (Sacco et al. 2008; Boldrin et al. 2012; Boldrin and Morgan 2012; Charville et al. 2015; Hall et al. 2017). Several have also begun to clarify the similarities and differences in *Pax7* expression, cell-surface marker expression, and the regenerative potential of human compared to murine satellite cells (Reimann et al. 2004; Boldrin and Morgan 2012; Xu et al. 2015; O'Connor et al. 2009; Decary et al. 1997). To assess their characteristics in vivo, Xu et al. isolated satellite cells from biopsies of several human muscles and engrafted them into the mouse TA (Xu et al. 2015). They showed that xenografted $CD56^{+}/CD29^{+}$ human myoblasts reconstituted the satellite cell niche and formed several clusters of human fibers within the graft. Upon reinjury, they saw robust expansion of the engrafted human muscle fibers, indicating that the regenerative capacity of these human satellite cells is maintained. Importantly, they saw no major differences in the relative quantity or ability of the human satellite cells to engraft between the biopsied muscles groups, suggesting that large numbers of satellite cells can be isolated from multiple muscle groups from a single human donor (Xu et al. 2015). Ishii et al. (2018) found similar results with satellite cells that were isolated and maintained in the presence of laminin E8 to promote their "stemness". Upon engraftment, these cells repopulated the satellite cell niche and promoted muscle regeneration (Ishii et al. 2018).

The ability of myogenic cells to repopulate the satellite cell niche is necessary for studies of the possible role of satellite cells in disease and regeneration between healthy and diseased human muscle. Certain cell types are able to generate both muscle fibers and to become Pax7+ satellite cells upon transplantation. Among the cell types capable or repopulating the satellite cell niche are blood- and muscle-derived $CD133^{+}$ cells (Torrente et al. 2004; Benchaouir et al. 2007); human mesoangioblast/pericyte-derived cells (Dellavalle et al. 2011), side-populations, interstitial cells (Asakura et al. 2002) and normal or immortalized human muscle precursor cells (Ehrhardt et al. 2007; Mueller et al. 2019).

iPS cells

Several laboratories have recently begun to explore the use of iPS cells in skeletal muscle grafts. Pluripotent stem cells can be selected for their myogenic capacity, or programmed to become myogenic by transformation to express Pax3 and/or Pax7, or MyoD, or by the addition of factors to the culture medium (Mizuno et al. 2010; Darabi et al. 2011; Swartz et al. 2016; Torihashi et al. 2015). These cells have several advantages, including the ease of preparing them from individuals with a wide range of muscle disorders without requiring biopsies, and, like immortalized human muscle precursor cells, their availability in unlimited numbers in culture. They are already being examined in the context of different diseases of muscle (Jiwlawat et al. 2019; Nakajima et al. 2019; Steele-Stallard et al. 2018; Salani et al. 2012; Kim et al. 2019); (reviewed in Piga et al. 2019; Xia et al. 2018; Danisovic et al. 2018; Pourquie et al. 2018). In xenografting studies, iPS cells transfected to express Pax3/Pax7 engraft better than fetal myoblasts and reseed the satellite cell niche in the engrafted muscle (Incitti et al. 2019). Similarly, myogenic cells created by expressing Pax3 alone in human iPS cells developed into small bundles in tissue culture that remained functional upon engraftment into immunocompromised mice (Rao et al. 2018). Zhao et al. reprogrammed fibroblasts from dystrophic mdx mice with site-specific recombinases to create dystrophin-positive myogenic precursors that successfully engrafted into mdx muscles, albeit in relatively low numbers (Zhao et al. 2014). Similar studies were reported by Cai et al. (2016), Goudenege et al. (2012) and Beck et al. (2011), although with different cell populations and transformation methods. Tedesco et al. (2012) derived mesangioblast-like cells from iPS cells isolated from α-sarcoglycan-null mice, corrected the genetic deficit, and introduced them into sarcoglycan-null immunodeficient mice. They found limited regions of the muscles that were successfully engrafted, as indicated by the presence of α-sarcoglycan.

Although iPS cells require considerable effort to prepare and characterize prior to use in transplantation studies, they may well define the future of xenografting to study human muscle development, examine the pathogenic mechanisms of different myopathies and muscular dystrophies, and test a range of therapeutics in preparation for clinical trials. Their use in treating diseases of muscle is also under consideration (Maffioletti et al. 2014; Hagan et al. 2018; Pareja-Galeano et al. 2016; Loperfido et al. 2015).

Cellular properties and culture conditions affecting engraftment

Importantly, the age of donor cells, measured by age of the donor, mean population doublings prior to engraftment, and telomere length, have all been reported to affect regenerative capacity (Decary et al. 1997; Cooper et al. 2003; Brimah et al. 2004; Schafer et al. 2006). The greater the number of population doublings and the shorter the telomere length, the lower the ability to engraft, as measured by the number of donor fibers formed in xenografts of muscle precursor cells (Decary et al. 1997; Cooper et al. 2003; Brimah et al. 2004). The severity of the donor's myopathy is also inversely correlated with xenografting capacity, perhaps due to progenitor cell exhaustion in affected muscles (Schafer et al. 2006; Morosetti et al. 2007). Culture conditions can also impact the myogenicity of donor cells. Our group and others have observed that culturing muscle precursor cells under conditions that increase proliferation, while suppressing differentiation, increases the fiber number within xenografts formed by those cells. Some of these conditions include the use of high serum concentrations, culture on laminin, heat shock treatment, and co-culture with IGF-1, bFGF and others (Silva-Barbosa et al. 2008; Sakellariou et al. 2016; Brimah et al. 2004; Danisovic et al. 2018; Riederer et al. 2008).

Cell delivery

The method of cell delivery is also an important consideration in xenograft studies. While most studies have relied on single intra-muscular injection, progressive cell delivery (multiple injections) and systemic delivery have been explored. Kim et al. (2016) showed that progressive intramuscular injections of cells over 4 weeks restored greater muscle force, increased muscle mass and differentiation, reduced fibrosis and increased vascularization in a volumetric muscle defect repair model, as compared to a single injection. Several studies suggest $CD133^+$ muscle progenitor cells isolated from muscle or blood could be introduced systemically to deliver gene therapies for DMD, although engraftment of patient-derived $CD133^+$ cells, in particular, has only been variably successful (Torrente et al. 2004, 2007; Gavina et al. 2006; Benchaouir et al. 2007; Negroni et al. 2009; Meng et al. 2014, 2018). Mesangioblasts and other pericytes may also be systemically delivered, and have been characterized in xenografts as a potential cell therapy approach for FSHD (Morosetti et al. 2007, 2011). Pericytes delivered intra-arterially in three bouts were able to colonize and generate new muscle fibers in hindlimb muscles of *scid/mdx* mice (Dellavalle et al. 2007), but, as noted above, another study using intra-arterial injection reported that muscle-derived cells did not contribute to regeneration in all hosts (Meng et al. 2011). The variability in the success of xenografting following systemic introduction of donor cells suggests that critical factors remain to be discovered and refined before this method can be more widely adopted.

Interventions to enhance engraftment

Once myogenic precursor cells are delivered to muscle, several methods of intervention can be used to enhance engraftment (Fig. 1). Due to the inverse relationship between the proliferation of myogenic cells and their differentiation into myotubes and myofibers, interventions aimed at increasing differentiation should start at least 3 days after engraftment (Riederer et al. 2012). Interventions can largely be divided into two main categories: exercise-mimicking and molecular.

Interventions that mimic exercise can include voluntary exercise, such as housing mice in cages with running wheels or performing bilateral hindlimb engraftment which encourages the mouse to use both legs in order to ambulate (Llach, Mueller et al., in preparation). Functional overloading of the EDL by surgical removal of the TA, treadmill exercise, and swimming protocols have also been used to improve engraftment of muscle derived stem cells (Ambrosio et al. 2009, 2010; Fakhfakh et al. 2012a, b). Intermittent neuromuscular electrical stimulation, performed while the mouse is under anesthesia, also increases engraftment by increasing fiber number, size, and maturity (Sakellariou et al. 2016; Mueller et al. 2019).

Molecular interventions to improve tissue and cell engraftment include local or systemic injection of small molecules, synthetic matrices, growth factors, and myostatin and TGF-β inhibitors (Brimah et al. 2004; Fakhfakh et al. 2012a, b; Gerard et al. 2012). Of these, the best studied are interventions which modulate the TGF-beta signaling pathway, known to play an important role in muscle regeneration. In myopathies that involve dysregulated muscle repair, myostatin, a TGF-β superfamily myokine, and TGF-β overexpression at the site of muscle injury inhibit cell proliferation, delay differentiation, and promote the formation of fibrosis in place of degenerating myofibers (reviewed in Burks and Cohn 2011; Kim and Lee 2017). Blocking myostatin signaling by either overexpressing follistatin, an endogenous myostatin antagonist, or a dominant-negative form of the TGF-β superfamily receptor, ActRIIB, improves the transplantation of human myoblasts in *Rag/mdx* TAs (Benabdallah et al. 2008; Fakhfakh et al. 2011). These experiments were followed by methods that could be applied to humans such as systemic delivery of ActRIIB/Fc and oral treatment with Losartan, a small molecule that decreases TGF-β expression. Combined with forced swimming to encourage muscle turnover, ActRIIB/Fc treatment led to increased body weight and increased hypertrophy of dystrophin-positive fibers in the TAs of Rag-null/mdx mice engrafted with human myoblasts (Fakhfakh et al. 2012a, b). Losartan

treatment increases myoblast proliferation and fusion, while decreasing negative regulators of muscle repair (Fakhfakh et al. 2012a, b). Combined, these studies show that modulating the activity of the TGF-β superfamily can be used to increase myogenicity in xenografting protocols.

Co-injection of fibrin gel with human myoblasts significantly increases cell survival after implantation and the number of dystrophin-positive fibers formed at 3 weeks post-engraftment in *Rag/mdx* mice (Gerard et al. 2012). A pro-survival cocktail, containing Matrigel, IGF-I and several other factors, provides the same benefit (Laflamme et al. 2007; Gerard et al. 2012). Keratin hydrogels, biodegradable polymers, decellularized tissue scaffolds, and growth-factor releasing matrices have also been suggested as potential tools for muscle repair (Saxena et al. 1999; Lee et al. 2000; Richardson et al. 2001; Levenberg et al. 2005; Baker et al. 2017; Urciuolo and De Coppi 2018), although comprehensive studies of their utility in xenografting have not yet been performed.

Recent muscle xenograft applications

FSHD xenograft models and cell therapy

FSHD is a debilitating muscle wasting disease caused by the pathogenic misexpression of the transcription factor, DUX4. DUX4 is a primate-lineage gene located within 3.3 kb repeated units in the distal end of chromosome 4 (reviewed in Tassin et al. 2013). The downstream gene targets of the mouse *Dux* paralog are unlike the targets of DUX4 (Sharma et al. 2013; Eidahl et al. 2016), and the epigenetic regulation of DUX4 expression is likely dependent on other human genes (reviewed in Daxinger et al. 2015; DeSimone et al. 2017; Hamel and Tawil 2018; Himeda et al. 2018). Thus, models of FSHD that reproduce the genetic and epigenetic features of mature human muscle cannot be readily generated by standard transgenic or knockout methods. Recently, two novel models were developed by engrafting immortalized human muscle cells or muscle fibers into the mouse hindlimb to allow for studies of endogenous human gene expression in a living tissue (Chen et al. 2016; Sakellariou et al. 2016; Mueller et al. 2019).

Chen et al. used a xenograft model of FSHD to test the efficacy of an antisense phosphorodiamidate morpholino oligonucleotide (PMO) to suppress DUX4 expression (Chen et al. 2016). They surgically removed the TA and EDL muscles from NRG mice and engrafted 8 × 3 × 1 mm bundles of biopsy or autopsy human donor muscle collected from FSHD patients and control relatives into the anterior compartment of the mouse hindlimb (Zhang et al. 2014; Chen et al. 2016). After 4–6 months they electroporated the engrafted muscle with the PMO and collected the tissue 2 weeks later. They saw a reduction in *DUX4* and *DUX4*-target expression in FSHD patient-derived muscle xenografts treated with the PMO, providing evidence that antisense PMOs may be effective in treating FSHD (Chen et al. 2016). This method, while effective for testing proof-of-principle FSHD therapies, is limited by the difficulty of obtaining muscle biopsies, the time it takes to achieve sufficient engraftment, the variability in the quality of the grafts it produces, and the relatively small number of xenografts that can be generated by each biopsy.

Our laboratory has developed a xenograft model of FSHD which avoids most of these issues. Most importantly, it is scalable, to allow for larger studies of FSHD pathophysiology and in vivo testing of potential FSHD therapeutics. Our method uses immortalized human muscle precursor cells (hMPCs) from FSHD patients and control relatives engrafted into the TA of NRG mice, which has been irradiated and intoxicated by myotoxins to create a niche for new human-derived muscle to develop (Sakellariou et al. 2016). We see robust engraftment by 4 weeks and the genetic and epigenetic profiles of our xenografts replicate those of FSHD patients (Sakellariou et al. 2016; Mueller et al. 2019). An advantage of our method is that it occasionally produces a TA muscle in the mouse that is completely human in origin. The disadvantage is that this is infrequent, as the xenografts typically contain only 400–900 human fibers. However, because we are using immortalized FSHD cell lines, rather than primary cells or bundles of muscle fibers, we are able to generate an almost infinite number of genetically identical grafts for studies of both FSHD pathophysiology and drug screening. We are currently testing several potential therapeutic compounds and gene therapies to treat FSHD.

The potential for cell therapy to treat FSHD has also been studied using xenografts. FSHD myoblasts (Vilquin et al. 2005) or mesangioblasts (Morosetti et al. 2011) isolated from unaffected muscles of patients contribute to the regeneration of murine muscle fibers after myotoxin or cryoinjury, respectively. One report described a defective homing mechanism in FSHD mesangioblasts derived from severely affected muscles, which should be considered in designing autologous cell therapies (Morosetti et al. 2011). Combined, the FSHD xenograft models and FSHD cell transfer studies suggest that xenografting will be a pivotal technique going forward to elucidate the mechanism of muscle wasting in FSHD and to design and test therapeutic strategies to treat this debilitating disease.

Xenografts in studies of Duchenne muscular dystrophy

DMD is an X-linked disease caused by mutations in the dystrophin gene, which is required for sarcomeric and sarcolemmal integrity (Mokri and Engel 1975; Hoffman et al. 1987;

Porter et al. 1992). Several xenograft strategies to study and treat DMD have been reported, including those that involve gene therapy, cell therapy, or a combination of the two, which aim to replenish the muscle with dystrophin. These have been extensively reviewed (Shimizu-Motohashi et al. 2016; Barthelemy and Wein 2018; Shimizu-Motohashi et al. 2019; Danisovic et al. 2018; Lorant et al. 2018) and suggest that novel methods of engraftment have the potential to lead to new approaches to treating DMD.

Pericytes, but not satellite cells, isolated from DMD biopsies and corrected with human mini-dystrophin, were delivered to muscle tissue by intra-arterial injection. These cells were able to traffic to and engraft the TA of *scid/mdx* mice to form dystrophin-positive muscle fibers (Dellavalle et al. 2007). Similarly, in another study using intra-arterial delivery, exon-skipped DMD-CD 133^+ cells colonized the TA and were able to improve muscle morphology, function, and dystrophin expression in *scid/mdx* mice (Benchaouir et al. 2007). However, a more recent study concluded that while DMD-CD133^+ cells may regenerate muscle fibers in muscle xenografts, they do not repopulate the satellite cell niche like healthy donor-derived CD133^+ cells (Meng et al. 2018). This lack of myogenic potential may be due to exhaustion of the muscle stem cells (Heslop et al. 2000), leading to an increase in CD133^+ expression in non-myogenic cells (Meng et al. 2018). In another study, CRISPR/Cas9 reframed human iPS-derived skeletal muscle cells engrafted into the TAs of NSG-*mdx* mice and led to the expression of dystrophin and β-dystroglycan, restoring the dystrophin-glycoprotein complex (Young et al. 2016). Human adipose-derived mesenchymal stem cells delivered either locally or systemically have also shown great promise for xenotransplantation, expressing dystrophin in muscles of *mdx* mice and golden retriever muscular dystrophy dogs (Rodriguez et al. 2005; Vieira et al. 2012).

In one of the most promising series of studies to date, Tedesco et al. corrected *mdx*-derived mesangioblasts using a human artificial chromosome (HAC) encoding human dystrophin and engrafted those cells into *mdx* mice. This yielded dystrophin-positive fibers and satellite cells that persisted in the host for up to 8 months (Tedesco et al. 2011). This study led to further studies, which showed that HACs encoding dystrophin can be introduced into immortalized DMD-patient-derived myoblasts or mesangioblasts and then engrafted into the TAs of *scid/mdx* mice to form dystrophin-corrected muscle fibers (Benedetti et al. 2018). These authors also designed a synthetic HAC which expresses all of the necessary components for this strategy to be used as a novel gene therapy: genes for controlled levels of dystrophin protein expression, reversible immortalization, inducible differentiation, and regulated cell death (Benedetti et al. 2018). These and other xenograft studies of potential cell therapies including TALEN and CRISPR-corrected DMD-patient-derived iPS cells are ongoing (Li et al. 2015). Future publications will reveal if these strategies are successful.

Discussion

Xenografts of human muscles in mice provide an important tool for studies of human muscle physiology and pathophysiology, as well as unique tissues with which to test the specificity and efficacy of drugs targeted to human diseases in general and to diseases of muscle in particular. Ideally, the xenografts should have a large number of myofibers of human origin in a defined portion of the muscle, that can be isolated and studied without significant contamination by murine host tissue. Most studies have not had this as an aim, however. Rather, they have aimed to learn if engraftment methods can be used to restore normal gene expression and suppress pathophysiology in murine models of diseases such as DMD. These studies have produced promising results, but their applicability to human health is so far limited by the difficulties in introducing human myogenic precursor cells into patients by systemic injection, such that they access all the skeletal muscles of the human body affected by dystrophinopathy, while avoiding attack by the immune system.

Xenografting human myogenic cells into murine muscle to achieve muscles that are exclusively human in origin to model muscular dystrophies is also technically challenging, although noteworthy strides in this direction have recently been made. The major difficulty so far is eliminating all of the host muscle fibers within a particular compartment, and then ensuring that the hMPCs that are introduced to replace them distribute widely enough and are robust enough to repopulate the muscle compartment with human fibers. The fact that intoxicated muscles tend to degenerate only partially seems to be the major stumbling block here, likely because the myotoxins typically used in these experiments do not access all of the sub-compartments of the muscle. The reason for this is unclear, although it may be due to the persistence of diffusion barriers created by connective tissue. Multiple injections into widely spaced sites might solve this problem, but the toxicity to mice of some of the reagents, such as $BaCl_2$, limits the usefulness of this approach. We were only able to generate intact TA muscles with almost exclusively human myofibers using the cardiotoxin preparation from Sigma, which was never fully defined chemically and which is no longer commercially available. In its continued absence, other approaches, such as freezing and thawing the host muscle, may be the better alternative.

Limiting the ability of the endogenous murine tissue to regenerate is also a significant issue. X-irradiation is so far the most widely used method to do so, but most immunodeficient murine strains are damaged by the doses of X-rays

needed to entirely eliminate the contribution of murine satellite cells to the xenograft, and even NRG mice, which are more radiation-resistant, show occasional skin lesions following irradiation of their hindlimbs. Other side effects of X-irradiation, such as inflammation, may also occur. Alternative radiation sources, such as gamma radiation, may prove to be both gentler and more efficient (Caiozzo et al. 2010). To date, gamma irradiation has not yet been tested in muscle for its usefulness in xenografting experiments.

The literature is clear about the best sources of donor cells for engraftment, however, at least when numerous grafts containing large numbers of fibers of human origin are needed. Immortalized myogenic precursor cells, cultured with a minimal number of population doublings, have now been widely used and have in most cases given excellent results. They are easy to grow in large numbers and provide an almost unlimited source of identical donor material. This provides a significant advantage over transplantation of primary cells or tissues, including intact human muscle fibers or fiber bundles, which are by definition limited to clinical samples obtained through biopsies, which is neither always possible nor without risk. Furthermore, immortalized hMPCs can also be transfected to express particular proteins or other macromolecules, allowing very specific tests of the roles of these molecules in disease or disease prevention. The major disadvantage of the immortalized cells is that immortalization by hTERT and either Bmi-1 or CDK4 makes the cells potentially tumorigenic and thus unsuitable for any clinical studies unless they are remortalized first. Remortalization is possible if the transgenes are introduced between sites susceptible to Cre or other excision sequences, but this would have to be very efficient, indeed, to eliminate the possibility of tumor formation following clinical use. Tumorigenicity of hMPCs in mice has not been a common problem in xenografting studies, however, making them nearly ideal for studies of pathogenic mechanisms and of therapies for muscle diseases. As the field progresses, hMPCs may ultimately be replaced by iPS cells, which have the advantage that they can be more easily derived from patients with different genetic variations, but methods for xenografting of iPS cells are still not well developed.

Although the discussion above summarizes the many advantages of xenografting, the approach has several disadvantages. First, success in generating large, robust xenografts requires the use of immune-compromised mice, which means that the contribution of the immune system to diseases and disease progression cannot be addressed with these methods (Tidball 2011; Yang and Hu 2018). As a cautionary tale, allografts of bone marrow mesenchymal stem cells in immune-competent mice resulted in increased inflammation, oxidative stress, fibrosis, and impaired regeneration (Liu et al. 2019). This is also a serious drawback for studies of the inflammatory diseases of muscle, such as polymyositis and dermatomyositis, as well as of autoimmune diseases of muscle, such as myasthenia gravis. Another drawback is that, unless introduction of the donor cells is systemic, xenografting is typically limited to a particular muscle or muscle group, and thus cannot recapitulate the rostro-caudal or dorso-ventral position of the muscles affected by disease in the human body. Although they can reach maturity, the muscles in the grafts may not fully report on the responses of muscles in aging individuals or in individuals with diseases that have progressed significantly. Finally, the methods are quite labor intensive and are thus much more difficult to generate and maintain than spontaneous, transgenic or knockout murine models of human muscle diseases.

Human-to-mouse muscle xenografts have one obvious advantage over other murine models, however: they are comprised of human tissue and thus provide the best source of mature human muscle, outside of the clinic, to study the specificity and efficacy of drugs designed to treat human diseases. One example of particular interest to us is the observation that overexpression of DUX4, the pathogenic agent in FSHD, causes many genetic changes in murine muscle that are not seen in human FSHD muscle. Because of their ability to report specifically on the biochemical and molecular responses of human muscle, even if they may not yet reliably report on physiological changes, we believe that xenografts should be used by academic laboratories and pharmaceutical companies routinely as part of preclinical drug trials.

The range of options open to researchers interested in using xenografts in their studies is large but it should not be daunting. Some methods are clearly adapted to study particular questions related to muscle (e.g., satellite cell biology, the satellite cell niche, muscle regeneration, etc.) whereas others are more suited to examine disease mechanisms or to test potential therapeutics. There are nevertheless several key questions in muscle biology that may be addressable with xenografting methods that have not yet been tried, such as those related to the formation and extent of myonuclear domains, and the dosage of particular genes required to keep fibers healthy or to cause disease. Our aim has been to summarize the field and to encourage investigators not only to use established methods, but also to test new approaches that may be applicable to these and other compelling questions in muscle biology.

References

Ambrosio F, Ferrari RJ, Fitzgerald GK, Carvell G, Boninger ML, Huard J (2009) Functional overloading of dystrophic mice enhances muscle-derived stem cell contribution to muscle contractile capacity. Arch Phys Med Rehabil 90:66–73

Ambrosio F, Ferrari RJ, Distefano G, Plassmeyer JM, Carvell GE, Deasy BM, Boninger ML, Fitzgerald GK, Huard J (2010) The synergistic effect of treadmill running on stem-cell

transplantation to heal injured skeletal muscle. Tissue Eng A 16:839–849

Arandel L, Polay Espinoza M, Matloka M, Bazinet A, De Dea Diniz D, Naouar N, Rau F, Jollet A, Edom-Vovard F, Mamchaoui K, Tarnopolsky M, Puymirat J, Battail C, Boland A, Deleuze JF, Mouly V, Klein AF, Furling D (2017) Immortalized human myotonic dystrophy muscle cell lines to assess therapeutic compounds. Dis Model Mech 10:487–497

Arpke RW, Darabi R, Mader TL, Zhang Y, Toyama A, Lonetree CL, Nash N, Lowe DA, Perlingeiro RC, Kyba M (2013) A new immuno-, dystrophin-deficient model, the NSG-mdx(4Cv) mouse, provides evidence for functional improvement following allogeneic satellite cell transplantation. Stem Cells 31:1611–1620

Asakura A, Seale P, Girgis-Gabardo A, Rudnicki MA (2002) Myogenic specification of side population cells in skeletal muscle. J Cell Biol 159:123–134

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 A 23:572–584

Barthelemy F, Wein N (2018) Personalized gene and cell therapy for duchenne muscular dystrophy. Neuromuscul Disord 28:803–824

Beck AJ, Vitale JM, Zhao Q, Schneider JS, Chang C, Altaf A, Michaels J, Bhaumik M, Grange R, Fraidenraich D (2011) Differential requirement for utrophin in the induced pluripotent stem cell correction of muscle versus fat in muscular dystrophy mice. PLoS ONE 6:e20065

Benabdallah BF, Bouchentouf M, Rousseau J, Bigey P, Michaud A, Chapdelaine P, Scherman D, Tremblay JP (2008) Inhibiting myostatin with follistatin improves the success of myoblast transplantation in dystrophic mice. Cell Transpl 17:337–350

Benchaouir R, Meregalli M, Farini A, D'Antona G, Belicchi M, Goyenvalle A, Battistelli M, Bresolin N, Bottinelli R, Garcia L, Torrente Y (2007) Restoration of human dystrophin following transplantation of exon-skipping-engineered DMD patient stem cells into dystrophic mice. Cell Stem Cell 1:646–657

Bencze M, Negroni E, Vallese D, Yacoub-Youssef H, Chaouch S, Wolff A, Aamiri A, Di Santo JP, Chazaud B, Butler-Browne G, Savino W, Mouly V, Riederer I (2012) Proinflammatory macrophages enhance the regenerative capacity of human myoblasts by modifying their kinetics of proliferation and differentiation. Mol Ther 20:2168–2179

Benedetti S, Uno N, Hoshiya H, Ragazzi M, Ferrari G, Kazuki Y, Moyle LA, Tonlorenzi R, Lombardo A, Chaouch S, Mouly V, Moore M, Popplewell L, Kazuki K, Katoh M, Naldini L, Dickson G, Messina G, Oshimura M, Cossu G, Tedesco FS (2018) Reversible immortalisation enables genetic correction of human muscle progenitors and engineering of next-generation human artificial chromosomes for Duchenne muscular dystrophy. EMBO Mol Med 10:254–275

Boldrin L, Morgan JE (2012) Human satellite cells: identification on human muscle fibres. PLoS Curr 3:Rrn1294

Boldrin L, Neal A, Zammit PS, Muntoni F, Morgan JE (2012) Donor satellite cell engraftment is significantly augmented when the host niche is preserved and endogenous satellite cells are incapacitated. Stem Cells 30:1971–1984

Brimah K, Ehrhardt J, Mouly V, Butler-Browne GS, Partridge TA, Morgan JE (2004) Human muscle precursor cell regeneration in the mouse host is enhanced by growth factors. Hum Gene Ther 15:1109–1124

Burks TN, Cohn RD (2011) Role of TGF-beta signaling in inherited and acquired myopathies. Skelet Muscle 1:19

Cai WF, Huang W, Wang L, Wang JP, Zhang L, Ashraf M, Wu S, Wang Y (2016) Induced pluripotent stem cells derived muscle progenitors effectively mitigate muscular dystrophy through restoring the dystrophin distribution. J Stem Cell Res Ther 6:1000361

Caiozzo VJ, Giedzinski E, Baker M, Suarez T, Izadi A, Lan M, Cho-Lim J, Tseng BP, Limoli CL (2010) The radiosensitivity of satellite cells: cell cycle regulation, apoptosis and oxidative stress. Radiat Res 174:582–589

Charville GW, Cheung TH, Yoo B, Santos PJ, Lee GK, Shrager JB, Rando TA (2015) Ex vivo expansion and in vivo self-renewal of human muscle stem cells. Stem Cell Rep 5:621–632

Chen JC, King OD, Zhang Y, Clayton NP, Spencer C, Wentworth BM, Emerson CP Jr, Wagner KR (2016) Morpholino-mediated knockdown of DUX4 toward facioscapulohumeral muscular dystrophy therapeutics. Mol Ther 24:1405–1411

Chicha L, Tussiwand R, Traggiai E, Mazzucchelli L, Bronz L, Piffaretti JC, Lanzavecchia A, Manz MG (2005) Human adaptive immune system Rag2-/-gamma(c)-/- mice. Ann N Y Acad Sci 1044:236–243

Chien KY, Chiang CM, Hseu YC, Vyas AA, Rule GS, Wu W (1994) Two distinct types of cardiotoxin as revealed by the structure and activity relationship of their interaction with zwitterionic phospholipid dispersions. J Biol Chem 269:14473–14483

Chirieleison SM, Feduska JM, Schugar RC, Askew Y, Deasy BM (2012) Human muscle-derived cell populations isolated by differential adhesion rates: phenotype and contribution to skeletal muscle regeneration in Mdx/SCID mice. Tissue Eng A 18:232–241

Cooper RN, Irintchev A, Di Santo JP, Zweyer M, Morgan JE, Partridge TA, Butler-Browne GS, Mouly V, Wernig A (2001) A new immunodeficient mouse model for human myoblast transplantation. Hum Gene Ther 12:823–831

Cooper RN, Thiesson D, Furling D, Di Santo JP, Butler-Browne GS, Mouly V (2003) Extended amplification in vitro and replicative senescence: key factors implicated in the success of human myoblast transplantation. Hum Gene Ther 14:1169–1179

Cudre-Mauroux C, Occhiodoro T, Konig S, Salmon P, Bernheim L, Trono D (2003) Lentivector-mediated transfer of Bmi-1 and telomerase in muscle satellite cells yields a duchenne myoblast cell line with long-term genotypic and phenotypic stability. Hum Gene Ther 14:1525–1533

Danisovic L, Culenova M, Csobonyeiova M (2018) Induced pluripotent stem cells for duchenne muscular dystrophy modeling and therapy. Cells 7:253

Darabi R, Pan W, Bosnakovski D, Baik J, Kyba M, Perlingeiro RC (2011) Functional myogenic engraftment from mouse iPS cells. Stem Cell Rev 7:948–957

Daxinger L, Tapscott SJ, van der Maarel SM (2015) Genetic and epigenetic contributors to FSHD. Curr Opin Genet Dev 33:56–61

Decary S, Mouly V, Hamida CB, Sautet A, Barbet JP, Butler-Browne GS (1997) Replicative potential and telomere length in human skeletal muscle: implications for satellite cell-mediated gene therapy. Hum Gene Ther 8:1429–1438

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 9:255–267

Dellavalle A, Maroli G, Covarello D, Azzoni E, Innocenzi A, Perani L, Antonini S, Sambasivan R, Brunelli S, Tajbakhsh S, Cossu G (2011) Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. Nat Commun 2:499

DeSimone AM, Pakula A, Lek A, Emerson CP Jr (2017) Facioscapulohumeral muscular dystrophy. Compr Physiol 7:1229–1279

Ehrhardt J, Brimah K, Adkin C, Partridge T, Morgan J (2007) Human muscle precursor cells give rise to functional satellite cells in vivo. Neuromuscul Disord 17:631–638

Eidahl JO, Giesige CR, Domire JS, Wallace LM, Fowler AM, Guckes SM, Garwick-Coppens SE, Labhart P, Harper SQ (2016) Mouse Dux is myotoxic and shares partial functional homology with its human paralog DUX4. Hum Mol Genet 25:4577–4589

Fakhfakh R, Michaud A, Tremblay JP (2011) Blocking the myostatin signal with a dominant negative receptor improves the success of human myoblast transplantation in dystrophic mice. Mol Ther 19:204–210

Fakhfakh R, Lamarre Y, Skuk D, Tremblay JP (2012a) Losartan enhances the success of myoblast transplantation. Cell Transplant 21:139–152

Fakhfakh R, Lee SJ, Tremblay JP (2012b) Administration of a soluble activin type IIB receptor promotes the transplantation of human myoblasts in dystrophic mice. Cell Transplant 21:1419–1430

Farini A, Meregalli M, Belicchi M, Battistelli M, Parolini D, D'Antona G, Gavina M, Ottoboni L, Constantin G, Bottinelli R, Torrente Y (2007) T and B lymphocyte depletion has a marked effect on the fibrosis of dystrophic skeletal muscles in the scid/mdx mouse. J Pathol 213:229–238

Fishman JM, Tyraskis A, Maghsoudlou P, Urbani L, Totonelli G, Birchall MA, De Coppi P (2013) Skeletal muscle tissue engineering: which cell to use? Tissue Eng B Rev 19:503–515

Gavina M, Belicchi M, Rossi B, Ottoboni L, Colombo F, Meregalli M, Battistelli M, Forzenigo L, Biondetti P, Pisati F, Parolini D, Farini A, Issekutz AC, Bresolin N, Rustichelli F, Constantin G, Torrente Y (2006) VCAM-1 expression on dystrophic muscle vessels has a critical role in the recruitment of human blood-derived CD133 + stem cells after intra-arterial transplantation. Blood 108:2857–2866

Gerard C, Forest MA, Beauregard G, Skuk D, Tremblay JP (2012) Fibrin gel improves the survival of transplanted myoblasts. Cell Transpl 21:127–137

Goldman JP, Blundell MP, Lopes L, Kinnon C, Di Santo JP, Thrasher AJ (1998) Enhanced human cell engraftment in mice deficient in RAG2 and the common cytokine receptor gamma chain. Br J Haematol 103:335–342

Goudenege S, Lebel C, Huot NB, Dufour C, Fujii I, Gekas J, Rousseau J, Tremblay JP (2012) Myoblasts derived from normal hESCs and dystrophic hiPSCs efficiently fuse with existing muscle fibers following transplantation. Mol Ther 20:2153–2167

Gross JG, Bou-Gharios G, Morgan JE (1999) Potentiation of myoblast transplantation by host muscle irradiation is dependent on the rate of radiation delivery. Cell Tissue Res 298:371–375

Guigal N, Rodriguez M, Cooper RN, Dromaint S, Di Santo JP, Mouly V, Boutin JA, Galizzi JP (2002) Uncoupling protein-3 (UCP3) mRNA expression in reconstituted human muscle after myoblast transplantation in RAG2-/-/gamma c/C5(-) immunodeficient mice. J Biol Chem 277:47407–47411

Hagan M, Ashraf M, Kim IM, Weintraub NL, Tang Y (2018) Effective regeneration of dystrophic muscle using autologous iPSC-derived progenitors with CRISPR-Cas9 mediated precise correction. Med Hypotheses 110:97–100

Hall MN, Hall JK, Cadwallader AB, Pawlikowski BT, Doles JD, Elston TL, Olwin BB (2017) Transplantation of Skeletal Muscle Stem Cells. Methods Mol Biol 1556:237–244

Halum SL, Hiatt KK, Naidu M, Sufyan AS, Clapp DW (2008) Optimization of autologous muscle stem cell survival in the denervated hemilarynx. Laryngoscope 118:1308–1312

Hamel J, Tawil R (2018) Facioscapulohumeral muscular dystrophy: update on pathogenesis and future treatments. Neurotherapeutics 15:863–871

Hardy D, Besnard A, Latil M, Jouvion G, Briand D, Thepenier C, Pascal Q, Guguin A, Gayraud-Morel B, Cavaillon JM, Tajbakhsh S, Rocheteau P, Chretien F (2016) Comparative study of injury models for studying muscle regeneration in mice. PLoS ONE 11:e0147198

Heslop L, Morgan JE, Partridge TA (2000) Evidence for a myogenic stem cell that is exhausted in dystrophic muscle. J Cell Sci 113(Pt 12):2299–2308

Himeda CL, Jones TI, Virbasius CM, Zhu LJ, Green MR, Jones PL (2018) Identification of epigenetic regulators of DUX4-fl for targeted therapy of facioscapulohumeral muscular dystrophy. Mol Ther 26:1797–1807

Hodges SJ, Agbaji AS, Harvey AL, Hider RC (1987) Cobra cardiotoxins. Purification, effects on skeletal muscle and structure/activity relationships [published errtum appears in Eur J Biochem 1988 Feb 1;171(3):727]. Eur J Biochem 165:373–383

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

Huard J, Verreault S, Roy R, Tremblay M, Tremblay JP (1994) High efficiency of muscle regeneration after human myoblast clone transplantation in SCID mice. J Clin Invest 93:586–599

Incitti T, Magli A, Darabi R, Yuan C, Lin K, Arpke RW, Azzag K, Yamamoto A, Stewart R, Thomson JA, Kyba M, Perlingeiro RCR (2019) Pluripotent stem cell-derived myogenic progenitors remodel their molecular signature upon in vivo engraftment. Proc Natl Acad Sci USA 116(10):4346–4351

Ishii K, Sakurai H, Suzuki N, Mabuchi Y, Sekiya I, Sekiguchi K, Akazawa C (2018) Recapitulation of extracellular LAMININ environment maintains stemness of satellite cells in vitro. Stem Cell Rep 10:568–582

Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M (1999) The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. Nature 397:164–168

Jiwlawat N, Lynch EM, Napiwocki BN, Stempien A, Ashton RS, Kamp TJ, Crone WC, Suzuki M (2019) Micropatterned substrates with physiological stiffness promote cell maturation and Pompe disease phenotype in human induced pluripotent stem cell-derived skeletal myocytes. Biotechnol Bioeng. https://doi.org/10.1002/bit.27075

Kim J, Lee J (2017) Role of transforming growth factor-beta in muscle damage and regeneration: focused on eccentric muscle contraction. J Exerc Rehabil 13:621–626

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

Kim EY, Barefield DY, Vo AH, Gacita AM, Schuster EJ, Wyatt EJ, Davis JL, Dong B, Sun C, Page P, Dellefave-Castillo L, Demonbreun A, Zhang HF, McNally EM (2019) Distinct pathological signatures in human cellular models of myotonic dystrophy subtypes. JCI Insight 4:e122686

Kuhn MA, Black AB, Siddiqui MT, Nolta JA, Belafsky PC (2017) Novel murine xenograft model for the evaluation of stem cell therapy for profound dysphagia. Laryngoscope 127:E359-e63

Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE (2007) Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol 25:1015–1024

Lavasani M, Lu A, Thompson SD, Robbins PD, Huard J, Niedernhofer LJ (2013) Isolation of muscle-derived stem/progenitor cells based on adhesion characteristics to collagen-coated surfaces. Methods Mol Biol 976:53–65

Lavasani M, Thompson SD, Pollett JB, Usas A, Lu A, Stolz DB, Clark KA, Sun B, Peault B, Huard J (2014) Human

muscle-derived stem/progenitor cells promote functional murine peripheral nerve regeneration. J Clin Invest 124:1745–1756

Lee KY, Peters MC, Anderson KW, Mooney DJ (2000) Controlled growth factor release from synthetic extracellular matrices. Nature 408:998–1000

Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC, Marini R, van Blitterswijk CA, Mulligan RC, D'Amore PA, Langer R (2005) Engineering vascularized skeletal muscle tissue. Nat Biotechnol 23:879–884

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 4:143–154

Liu X, Liu Y, Zhao L, Zeng Z, Xiao W, Chen P (2017) Macrophage depletion impairs skeletal muscle regeneration: the roles of regulatory factors for muscle regeneration. Cell Biol Int 41:228–238

Liu X, Zeng Z, Zhao L, Xiao W, Chen P (2018) Changes in inflammatory and oxidative stress factors and the protein synthesis pathway in injured skeletal muscle after contusion. Exp Ther Med 15:2196–2202

Liu X, Zhen L, Zhou Y, Chen Y, Chen P, Xiao W (2019) BMSC transplantation aggravates inflammation, oxidative stress, and fibrosis and impairs skeletal muscle regeneration. Front Physiol 10:87

Loperfido M, Steele-Stallard HB, Tedesco FS, VandenDriessche T (2015) Pluripotent stem cells for gene therapy of degenerative muscle diseases. Curr Gene Ther 15:364–380

Lorant J, Saury C, Schleder C, Robriquet F, Lieubeau B, Negroni E, Leroux I, Chabrand L, Viau S, Babarit C, Ledevin M, Dubreil L, Hamel A, Magot A, Thorin C, Guevel L, Delorme B, Pereon Y, Butler-Browne G, Mouly V, Rouger K (2018) Skeletal muscle regenerative potential of human mustem cells following transplantation into injured mice muscle. Mol Ther 26:618–633

Lovik M (1995) The SCID (severe combined immunodeficiency) mouse–its biology and use in immunotoxicological research. Arch Toxicol Suppl 17:455–467

Maffioletti SM, Noviello M, English K, Tedesco FS (2014) Stem cell transplantation for muscular dystrophy: the challenge of immune response. Biomed Res Int 2014:964010

Mamchaoui K, Trollet C, Bigot A, Negroni E, Chaouch S, Wolff A, Kandalla PK, Marie S, Di Santo J, St Guily JL, Muntoni F, Kim J, Philippi S, Spuler S, Levy N, Blumen SC, Voit T, Wright WE, Aamiri A, Butler-Browne G, Mouly V (2011) Immortalized pathological human myoblasts: towards a universal tool for the study of neuromuscular disorders. Skelet Muscle 1:34

Martinez-Sarra E, Montori S, Gil-Recio C, Nunez-Toldra R, Costamagna D, Rotini A, Atari M, Luttun A, Sampaolesi M (2017) Human dental pulp pluripotent-like stem cells promote wound healing and muscle regeneration. Stem Cell Res Ther 8:175

McGreevy JW, Hakim CH, McIntosh MA, Duan D (2015) Animal models of Duchenne muscular dystrophy: from basic mechanisms to gene therapy. Dis Model Mech 8:195–213

Meng J, Adkin CF, Xu SW, Muntoni F, Morgan JE (2011) Contribution of human muscle-derived cells to skeletal muscle regeneration in dystrophic host mice. PLoS ONE 6:e17454

Meng J, Chun S, Asfahani R, Lochmuller H, Muntoni F, Morgan J (2014) Human skeletal muscle-derived CD133(+) cells form functional satellite cells after intramuscular transplantation in immunodeficient host mice. Mol Ther 22:1008–1017

Meng J, Bencze M, Asfahani R, Muntoni F, Morgan JE (2015) The effect of the muscle environment on the regenerative capacity of human skeletal muscle stem cells. Skelet Muscle 5:11

Meng J, Muntoni F, Morgan J (2018) CD133 + cells derived from skeletal muscles of Duchenne muscular dystrophy patients have a compromised myogenic and muscle regenerative capability. Stem Cell Res 30:43–52

Mir R, Sinha M, Sharma S, Singh N, Kaur P, Srinivasan A, Singh TP (2008) Isolation, purification, crystallization and preliminary crystallographic studies of sagitoxin, an oligomeric cardiotoxin from the venom of Naja naja saggitifera. Acta Crystallogr F 64:545–547

Mizuno Y, Chang H, Umeda K, Niwa A, Iwasa T, Awaya T, Fukada S, Yamamoto H, Yamanaka S, Nakahata T, Heike T (2010) Generation of skeletal muscle stem/progenitor cells from murine induced pluripotent stem cells. Faseb J 24:2245–2253

Mokri B, Engel AG (1975) Duchenne dystrophy: electron microscopic findings pointing to a basic or early abnormality in the plasma membrane of the muscle fiber. Neurology 25:1111–1120

Morgan JE, Hoffman EP, Partridge TA (1990) Normal myogenic cells from newborn mice restore normal histology to degenerating muscles of the mdx mouse. J Cell Biol 111:2437–2449

Morgan JE, Gross JG, Pagel CN, Beauchamp JR, Fassati A, Thrasher AJ, Di Santo JP, Fisher IB, Shiwen X, Abraham DJ, Partridge TA (2002) Myogenic cell proliferation and generation of a reversible tumorigenic phenotype are triggered by preirradiation of the recipient site. J Cell Biol 157:693–702

Morosetti R, Mirabella M, Gliubizzi C, Broccolini A, Sancricca C, Pescatori M, Gidaro T, Tasca G, Frusciante R, Tonali PA, Cossu G, Ricci E (2007) Isolation and characterization of mesoangioblasts from facioscapulohumeral muscular dystrophy muscle biopsies. Stem Cells 25:3173–3182

Morosetti R, Gidaro T, Broccolini A, Gliubizzi C, Sancricca C, Tonali PA, Ricci E, Mirabella M (2011) Mesoangioblasts from facioscapulohumeral muscular dystrophy display in vivo a variable myogenic ability predictable by their in vitro behavior. Cell Transpl 20:1299–1313

Morrison J, Lu QL, Pastoret C, Partridge T, Bou-Gharios G (2000) T-cell-dependent fibrosis in the mdx dystrophic mouse. Lab Invest 80:881–891

Mouly V, Aamiri A, Perie S, Mamchaoui K, Barani A, Bigot A, Bouazza B, Francois V, Furling D, Jacquemin V, Negroni E, Riederer I, Vignaud A, St Guily JL, Butler-Browne GS (2005) Myoblast transfer therapy: is there any light at the end of the tunnel? Acta Myol 24:128–133

Mueller AL, O'Neill A, Jones TI, Llach A, Rojas LA, Sakellariou P, Stadler G, Wright WE, Eyerman D, Jones PL, Bloch RJ (2019) Muscle xenografts reproduce key molecular features of facioscapulohumeral muscular dystrophy. Exp Neurol 320:113011

Nakajima T, Sakurai H, Ikeya M (2019) In vitro generation of somite derivatives from human induced pluripotent stem cells. J Vis Exp 146:e59359

Negroni E, Riederer I, Chaouch S, Belicchi M, Razini P, Di Santo J, Torrente Y, Butler-Browne GS, Mouly V (2009) In vivo myogenic potential of human CD133 + muscle-derived stem cells: a quantitative study. Mol Ther 17:1771–1778

Negroni E, Gidaro T, Bigot A, Butler-Browne GS, Mouly V, Trollet C (2015) Invited review: stem cells and muscle diseases: advances in cell therapy strategies. Neuropathol Appl Neurobiol 41:270–287

O'Connor MS, Carlson ME, Conboy IM (2009) Differentiation rather than aging of muscle stem cells abolishes their telomerase activity. Biotechnol Prog 25:1130–1137

Pareja-Galeano H, Sanchis-Gomar F, Emanuele E, Gallardo ME, Lucia A (2016) IPSCs, a promising tool to restore muscle atrophy. J Cell Physiol 231:259–260

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 337:176–179

Pearson T, Shultz LD, Miller D, King M, Laning J, Fodor W, Cuthbert A, Burzenski L, Gott B, Lyons B, Foreman O, Rossini AA,

Greiner DL (2008) Non-obese diabetic-recombination activating gene-1 (NOD-Rag1 null) interleukin (IL)-2 receptor common gamma chain (IL2r gamma null) null mice: a radioresistant model for human lymphohaematopoietic engraftment. Clin Exp Immunol 154:270–284

Piga D, Salani S, Magri F, Brusa R, Mauri E, Comi GP, Bresolin N, Corti S (2019) Human induced pluripotent stem cell models for the study and treatment of Duchenne and Becker muscular dystrophies. Ther Adv Neurol Disord 12:1756286419833478

Porter GA, Dmytrenko GM, Winkelmann JC, Bloch RJ (1992) Dystrophin colocalizes with beta-spectrin in distinct subsarcolemmal domains in mammalian skeletal muscle. J Cell Biol 117:997–1005

Pourquie O, Al Tanoury Z, Chal J (2018) The long road to making muscle in vitro. Curr Top Dev Biol 129:123–142

Quenneville SP, Tremblay JP (2006) Ex vivo modification of cells to induce a muscle-based expression. Curr Gene Ther 6:625–632

Rao L, Qian Y, Khodabukus A, Ribar T, Bursac N (2018) Engineering human pluripotent stem cells into a functional skeletal muscle tissue. Nat Commun 9:126

Reimann J, Brimah K, Schroder R, Wernig A, Beauchamp JR, Partridge TA (2004) Pax7 distribution in human skeletal muscle biopsies and myogenic tissue cultures. Cell Tissue Res 315:233–242

Richardson TP, Peters MC, Ennett AB, Mooney DJ (2001) Polymeric system for dual growth factor delivery. Nat Biotechnol 19:1029–1034

Riederer I, Negroni E, Bigot A, Bencze M, Di Santo J, Aamiri A, Butler-Browne G, Mouly V (2008) Heat shock treatment increases engraftment of transplanted human myoblasts into immunodeficient mice. Transpl Proc 40:624–630

Riederer I, Negroni E, Bencze M, Wolff A, Aamiri A, Di Santo JP, Silva-Barbosa SD, Butler-Browne G, Savino W, Mouly V (2012) Slowing down differentiation of engrafted human myoblasts into immunodeficient mice correlates with increased proliferation and migration. Mol Ther 20:146–154

Rodriguez AM, Pisani D, Dechesne CA, Turc-Carel C, Kurzenne JY, Wdziekonski B, Villageois A, Bagnis C, Breittmayer JP, Groux H, Ailhaud G, Dani C (2005) Transplantation of a multipotent cell population from human adipose tissue induces dystrophin expression in the immunocompetent mdx mouse. J Exp Med 201:1397–1405

Rozkalne A, Adkin C, Meng J, Lapan A, Morgan JE, Gussoni E (2014) Mouse regenerating myofibers detected as false-positive donor myofibers with anti-human spectrin. Hum Gene Ther 25:73–81

Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM (2008) Self-renewal and expansion of single transplanted muscle stem cells. Nature 456:502–506

Sakellariou P, O'Neill A, Mueller AL, Stadler G, Wright WE, Roche JA, Bloch RJ (2016) Neuromuscular electrical stimulation promotes development in mice of mature human muscle from immortalized human myoblasts. Skelet Muscle 6:4

Salani S, Donadoni C, Rizzo F, Bresolin N, Comi GP, Corti S (2012) Generation of skeletal muscle cells from embryonic and induced pluripotent stem cells as an in vitro model and for therapy of muscular dystrophies. J Cell Mol Med 16:1353–1364

Saxena AK, Marler J, Benvenuto M, Willital GH, Vacanti JP (1999) Skeletal muscle tissue engineering using isolated myoblasts on synthetic biodegradable polymers: preliminary studies. Tissue Eng 5:525–532

Schafer R, Knauf U, Zweyer M, Hogemeier O, de Guarrini F, Liu X, Eichhorn HJ, Koch FW, Mundegar RR, Erzen I, Wernig A (2006) Age dependence of the human skeletal muscle stem cell in forming muscle tissue. Artif Organs 30:130–140

Sharma V, Harafuji N, Belayew A, Chen YW (2013) DUX4 differentially regulates transcriptomes of human rhabdomyosarcoma and mouse C2C12 cells. PLoS ONE 8:e64691

Shimizu-Motohashi Y, Miyatake S, Komaki H, Takeda S, Aoki Y (2016) Recent advances in innovative therapeutic approaches for Duchenne muscular dystrophy: from discovery to clinical trials. Am J Transl Res 8:2471–2489

Shimizu-Motohashi Y, Komaki H, Motohashi N, Takeda S, Yokota T, Aoki Y (2019) Restoring dystrophin expression in duchenne muscular dystrophy: current status of therapeutic approaches. J Pers Med 9:1

Silva-Barbosa SD, Butler-Browne GS, Di Santo JP, Mouly V (2005) Comparative analysis of genetically engineered immunodeficient mouse strains as recipients for human myoblast transplantation. Cell Transpl 14:457–467

Silva-Barbosa SD, Butler-Browne GS, de Mello W, Riederer I, Di Santo JP, Savino W, Mouly V (2008) Human myoblast engraftment is improved in laminin-enriched microenvironment. Transplantation 85:566–575

Skuk D, Tremblay JP (2015) Cell therapy in muscular dystrophies: many promises in mice and dogs, few facts in patients. Expert Opin Biol Ther 15:1307–1319

Skuk D, Furling D, Bouchard JP, Goulet M, Roy B, Lacroix Y, Vilquin JT, Tremblay JP, Puymirat J (1999) Transplantation of human myoblasts in SCID mice as a potential muscular model for myotonic dystrophy. J Neuropathol Exp Neurol 58:921–931

Smythe GM, Hodgetts SI, Grounds MD (2000) Immunobiology and the future of myoblast transfer therapy. Mol Ther 1:304–313

Stadler G, Chen JC, Wagner K, Robin JD, Shay JW, Emerson CP Jr, Wright WE (2011) Establishment of clonal myogenic cell lines from severely affected dystrophic muscles—CDK4 maintains the myogenic population. Skelet Muscle 1:12

Steele-Stallard HB, Pinton L, Sarcar S, Ozdemir T, Maffioletti SM, Zammit PS, Tedesco FS (2018) Modeling skeletal muscle laminopathies using human induced pluripotent stem cells carrying pathogenic LMNA mutations. Front Physiol 9:1332

Swartz EW, Baek J, Pribadi M, Wojta KJ, Almeida S, Karydas A, Gao FB, Miller BL, Coppola G (2016) A novel protocol for directed differentiation of C9orf72-associated human induced pluripotent stem cells into contractile skeletal myotubes. Stem Cells Transl Med 5:1461–1472

Tassin A, Laoudj-Chenivesse D, Vanderplanck C, Barro M, Charron S, Ansseau E, Chen YW, Mercier J, Coppee F, Belayew A (2013) DUX4 expression in FSHD muscle cells: how could such a rare protein cause a myopathy? J Cell Mol Med 17:76–89

Tedesco FS, Hoshiya H, D'Antona G, Gerli MF, Messina G, Antonini S, Tonlorenzi R, Benedetti S, Berghella L, Torrente Y, Kazuki Y, Bottinelli R, Oshimura M, Cossu G (2011) Stem cell-mediated transfer of a human artificial chromosome ameliorates muscular dystrophy. Sci Transl Med 3:96ra78

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 4:140ra89

Tidball JG (2005) Inflammatory processes in muscle injury and repair. Am J Physiol Regul Integr Comp Physiol 288:R345–R353

Tidball JG (2011) Mechanisms of muscle injury, repair, and regeneration. Compr Physiol 1:2029–2062

Torihashi S, Ho M, Kawakubo Y, Komatsu K, Nagai M, Hirayama Y, Kawabata Y, Takenaka-Ninagawa N, Wanachewin O, Zhuo L, Kimata K (2015) Acute and temporal expression of tumor necrosis factor (TNF)-alpha-stimulated gene 6 product, TSG6, in mesenchymal stem cells creates microenvironments required for their successful transplantation into muscle tissue. J Biol Chem 290:22771–22781

Torrente Y, Tremblay JP, Pisati F, Belicchi M, Rossi B, Sironi M, Fortunato F, El Fahime M, D'Angelo MG, Caron NJ, Constantin G, Paulin D, Scarlato G, Bresolin N (2001) Intraarterial injection of muscle-derived CD34(+)Sca-1(+) stem cells restores dystrophin in mdx mice. J Cell Biol 152:335–348

Torrente Y, Belicchi M, Sampaolesi M, Pisati F, Meregalli M, D'Antona G, Tonlorenzi R, Porretti L, Gavina M, Mamchaoui K, Pellegrino MA, Furling D, Mouly V, Butler-Browne GS, Bottinelli R, Cossu G, Bresolin N (2004) Human circulating AC133(+) stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. J Clin Invest 114:182–195

Torrente Y, Belicchi M, Marchesi C, D'Antona G, Cogiamanian F, Pisati F, Gavina M, Giordano R, Tonlorenzi R, Fagiolari G, Lamperti C, Porretti L, Lopa R, Sampaolesi M, Vicentini L, Grimoldi N, Tiberio F, Songa V, Baratta P, Prelle A, Forzenigo L, Guglieri M, Pansarasa O, Rinaldi C, Mouly V, Butler-Browne GS, Comi GP, Biondetti P, Moggio M, Gaini SM, Stocchetti N, Priori A, D'Angelo MG, Turconi A, Bottinelli R, Cossu G, Rebulla P, Bresolin N (2007) Autologous transplantation of muscle-derived CD133 + stem cells in Duchenne muscle patients. Cell Transpl 16:563–577

Urciuolo A, De Coppi P (2018) Decellularized tissue for muscle regeneration. Int J Mol Sci 19:2392

Vallese D, Negroni E, Duguez S, Ferry A, Trollet C, Aamiri A, Vosshenrich CA, Fuchtbauer EM, Di Santo JP, Vitiello L, Butler-Browne G, Mouly V (2013) The Rag2(-)Il2rb(-)Dmd(-) mouse: a novel dystrophic and immunodeficient model to assess innovating therapeutic strategies for muscular dystrophies. Mol Ther 21:1950–1957

Vieira NM, Valadares M, Zucconi E, Secco M, Bueno CR Jr, Brandalise V, Assoni A, Gomes J, Landini V, Andrade T, Caetano HV, Vainzof M, Zatz M (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 Transpl 21:1407–1417

Vilquin JT, Marolleau JP, Sacconi S, Garcin I, Lacassagne MN, Robert I, Ternaux B, Bouazza B, Larghero J, Desnuelle C (2005) Normal growth and regenerating ability of myoblasts from unaffected muscles of facioscapulohumeral muscular dystrophy patients. Gene Ther 12:1651–1662

Walsh S, Nygren J, Ponten A, Jovinge S (2011) Myogenic reprogramming of bone marrow derived cells in a W(4)(1)Dmd(mdx) deficient mouse model. PLoS ONE 6:e27500

Walz PC, Hiatt KK, Naidu M, Halum SL (2008) Characterization of laryngeal muscle stem cell survival and proliferation. Laryngoscope 118:1422–1426

Xia G, Terada N, Ashizawa T (2018) Human iPSC models to study orphan diseases: muscular dystrophies. Curr Stem Cell Rep 4:299–309

Xiao W, Liu Y, Chen P (2016) Macrophage depletion impairs skeletal muscle regeneration: the roles of pro-fibrotic factors, inflammation, and oxidative stress. Inflammation 39:2016–2028

Xu X, Wilschut KJ, Kouklis G, Tian H, Hesse R, Garland C, Sbitany H, Hansen S, Seth R, Knott PD, Hoffman WY, Pomerantz JH (2015) Human satellite cell transplantation and regeneration from diverse skeletal muscles. Stem Cell Rep 5:419–434

Yang W, Hu P (2018) Skeletal muscle regeneration is modulated by inflammation. J Orthop Transl 13:25–32

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 18:533–540

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 23:3180–3188

Zhao C, Farruggio AP, Bjornson CR, Chavez CL, Geisinger JM, Neal TL, Karow M, Calos MP (2014) Recombinase-mediated reprogramming and dystrophin gene addition in mdx mouse induced pluripotent stem cells. PLoS ONE 9:e96279

Zhu CH, Mouly V, Cooper RN, Mamchaoui K, Bigot A, Shay JW, Di Santo JP, Butler-Browne GS, Wright WE (2007) Cellular senescence in human myoblasts is overcome by human telomerase reverse transcriptase and cyclin-dependent kinase 4: consequences in aging muscle and therapeutic strategies for muscular dystrophies. Aging Cell 6:515–523