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Stem Cell Differentiation Toward the Myogenic Lineage for Muscle Tissue Regeneration: A Focus on Muscular Dystrophy

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Stem cell differentiation toward the myogenic lineage for muscle tissue regeneration: A focus on muscular dystrophy

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The authors declare no conflict of interest

Abstract

Skeletal muscle tissue engineering is one of the important ways for regenerating functionally defective muscles. Among the myopathies, the Duchenne muscular dystrophy (DMD) is a progressive disease due to mutations of the dystrophin gene leading to progressive myofibers degeneration with severe symptoms. Although current therapies in muscular dystrophy are still very challenging, important progress has been made in materials science and in cellular technologies with the use of stem cells. It is therefore useful to review these advances and the results obtained in a clinical point of view. This article focuses on the differentiation of stem cells into myoblasts, and their application in muscular dystrophy. After an overview of the different stem cells that can be induced to differentiate into the myogenic lineage, we introduce scaffolding materials used for muscular tissue engineering. We then described some widely used methods to differentiate different types of stem cell into myoblasts. We highlight recent insights obtained in therapies for muscular dystrophy. Finally, we conclude with a discussion on stem cell technology. We discussed in parallel the benefits brought by the evolution of the materials and by the expansion of cell sources which can differentiate into myoblasts. We also discussed on future challenges for clinical applications and how to accelerate the translation from the research to the clinic in the frame of DMD.

Contents

1. Introduction

Duchenne muscular dystrophy (DMD) is a progressive disease caused by mutations in the X-linked dystrophin gene that is characterized by a lack of dystrophin protein and chronic cycles of myofiber degeneration/regeneration, leading to the replacement of muscular tissue with adipose and fibrotic tissue in the advanced stages of the disease [1, 2]. This muscular degeneration progressively leads to severe symptoms such as disability and eventually death. Dystrophin is an important 427-kDa cytoskeletal protein that links the actin cytoskeleton of muscle cells to extracellular matrix (ECM) proteins, such as laminin, via the transmembrane dystrophin-glycoprotein complex (DGC) composed of dystroglycan, sarcoglycan, sarcospan, dystrophin, syntrophin and α-dystrobrevin [3, 4]. This mechanical anchoring stabilizes the sarcolemma of muscle cells under force transmission and favors the structural organization of proteins involved in cellular signaling [5]. The lack of dystrophin compromises the sarcolemma integrity, disturbs the Ca^{2+} and NO homeostasis, induces the over-activation of calcium proteases due to calcium entry into the intracellular compartment, destroys the mitochondria and generates oxidative stress, resulting in cellular death and muscular fiber necrosis [6-8]. A popular animal model for studying DMD is the mdx mouse, which has an identical mutation in the dystrophin gene similar to human but displays less severe clinical features due to the upregulation of utrophin, which partially rescues the functionality assumed normally by dytrophin, in addition to the presence of revertant fibers [9, 10]. Other animal models include the dystrophin/utrophin double knockout (mdx/utrn$^{-/-}$) mouse, which exhibits similarly severe clinical features as human patients, the dystrophin-deficient golden retriever dog (GRMD), and some others models like a rat model generated more recently by genetic modifications, the pig, the zebrafish, or the *Drosophila melanogaster*, [11-17].

To restore the muscle functionalities three main therapeutic approaches (the gene therapy, the pharmacological therapy and the cell therapy) using several strategies are usually developed (Figure 1) [18]. The gene therapy focuses on the restoration of the dystrophin complex by the delivery of a corrected gene or a change in the reading frame such as by exon-skipping [19-21]. The pharmacological therapy focuses on the development of drugs against the recurrent tissue inflammation and the consecutive health problems due to DMD [22, 23]. The cell therapy focuses on the rescue of the tissue functionalities by cell delivery [24-26]. In the following, we further emphasis on the use of stem cells and their important potential benefit in the treatment of such myopathy.
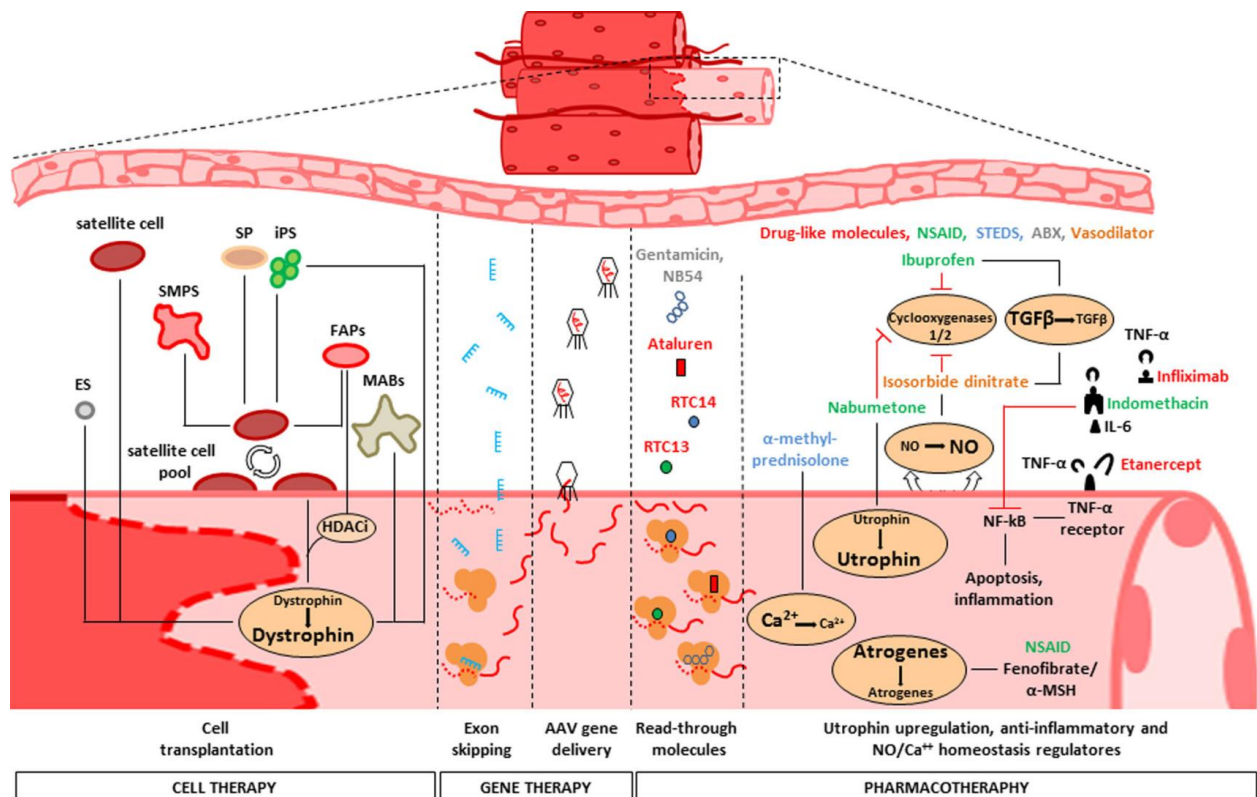
Figure 1: Schematic of main therapeutic approaches for muscle regeneration in dystrophinopathies. Reprinted with permission from Berardi et al. 2014 [22]©Berardi, Annibali, Cassano, Crippa and Sampaolesi.

The aim of regenerative medicine is to replace or restore biological tissues or organs to reestablish normal function. Accomplishing this goal requires ready access to an unlimited number of specific cell types to fabricate biological tissue and a lack of rejection upon tissue transplantation. To avoid an immunological response, autologous cells from the patient are usually used. However, due to the balance between cell differentiation and cell proliferation, the proliferative capacity of biologically functional but terminally differentiated cells is limited. To obtain the high number of cells required for transplantation, less differentiated cells with greater proliferative capacity are desired. Stem cells can self-regenerate, undergo unlimited proliferation, and differentiate into several different cell types; they are located in the body in pools of a few cells and directly enable tissue regeneration *in vivo*. Consequently, stem cells represent a remarkable cell source for tissue reconstruction. Satellite cells (SCs) are stem cells that are directly used by the body for muscle regeneration [27]. Many studies have used SCs for muscular tissue reconstruction. However, the discovery of other stem cell types that can differentiate into the myogenic lineage has increased the possibilities for muscular tissue engineering, with specific advantages and disadvantages associated with each stem cell type.

In this review, we first provide an overview of the different stem cells that can differentiate into the myogenic lineage. We then introduce some fabrication techniques and various scaffolding

materials that are widely used with stem cells in muscular tissue engineering. Next, we described various techniques for differentiating stem cells into myoblasts and myotubes. We then highlight recent insights into cell and genetic therapies for muscular dystrophy. Finally, we conclude with potential challenges and future research directions for the use of stem cells in the frame of DMD and their translation into the clinic.

2. Stem cells for muscular tissue engineering

In addition to their ability to differentiate into several cell types and thereby generate different tissues, stem cells exhibit vast diversity and heterogeneity [28, 29]. Therefore, a variety of stem cell types, which we describe in the following, may be used for muscle regeneration.

Adult stem cells can be isolated from postnatal tissues. These cells are multipotent but have less proliferative potential than embryonic stem cells (ESCs). The major advantage of adult stem cells over ESCs is their ability to be isolated from individual patients. Adult stem cells have been isolated from many different tissues and are usually designated based on their tissue of origin, such as mesenchymal stem cells (MSCs), adipose-derived stem cells (ASCs), or adult neural stem cells (NSCs). In the body, these cells serve as a reservoir for cell and tissue regeneration. For example, muscle fiber regeneration in the body is enabled by **SCs**, which are quiescent stem cells named based on their location wedged between the plasma membrane of the muscle fiber and the basal lamina [30]. These cells are characterized as $CD56^+$/$Pax7^+$/M-cadherin/$\alpha7$ integrin/ CD34/Syndecan-4 [31, 32]. During muscle fiber regeneration, SCs are activated and proliferate, giving rise to two different pools of SCs via a specific cell division mechanism [33, 34]. One

pool of cells is Pax7$^+$/Myf5$^-$ and maintains the pool of SCs, whereas the other pool is Pax7$^+$/Myf5$^+$ and will further differentiate and fuse together to form new myofibers [34].

ESCs are derived from the inner cell mass of blastocysts at an early stage, 4-5 days post-fertilization. ESCs, which in human have a size around 14 μm, are pluripotent and can be propagated indefinitely in culture in their undifferentiated state [35]. Due to these characteristics, ESCs are an attractive cell source for tissue and organ regeneration. However, the use of ESCs raises ethical concerns because the process of isolating the inner cell mass from the trophectoderm destroys the embryo. Moreover, ESCs are necessarily allogeneic and therefore may induce an immune response upon transplantation [36]. Furthermore, ESCs propagate indefinitely, raising concerns about potential tumorigenicity [37]. To avoid these problems, ESCs have been differentiated into myoblasts before transplantation in mice, resulting in stable engraftment and myofiber regeneration without the formation of teratomas [38, 39].

Located in the interstitium near the blood vessel, **muscle side population cells (SPs)** are characterized as Sca-1$^+$/ABCG2$^+$/CD45$^-$/CD43$^-$/c-kit$^-$/Pax7$^-$. Alone or in the presence of myogenic cells, SPs can differentiate into SCs [40-42]. Interestingly, intravenously injected SPs can migrate to sites of injured muscle and restore the muscle [43]. Furthermore, muscle regeneration by a sub-group of Sca-1$^+$/ABCG2$^+$/CD45$^-$/Pax7$^+$/Syndecan-4$^+$ SPs has been observed in an injured mouse model [44]. Similarly, another group of PW1$^+$/Pax7$^-$ cells increased in population and differentiated into SCs when injected into injured muscle [45, 46].

Mesoangioblast cells are also derived from the interstitium, are multipotent and are characterized by the surface markers CD34$^+$/c-kit$^-$/Flk1$^+$/Sca-1$^+$/NKX2.5$^-$/Myf5$^-$/Oct4$^-$ [30]. These cells maintain their multipotency in *in vitro* culture and are efficient at regenerating

myofibers both *in vitro* and in severely diseased animal models [47, 48]. **Pericytes** closely surround the endothelial cells of small blood vessels and capillaries; human pericytes are characterized by the surface markers CD45^{-}/CD34^{-}/CD56^{-}/CD144^{-}/CD146^{+}/PDGFR-β1^{+}/NG2 proteoglycans^{+} [49]. After transplantation via systemic injection, pericytes increase the SC pool and regenerate myofibers [49]. AC133^{+} or CD133^{+} cells can differentiate into hematopoietic cells, endothelial cells, or muscle cells when co-cultured with myoblasts or Wnt^{+} fibroblasts [50].

In addition to SCs, **muscle-derived stem cells (MDSCs)** have been isolated from muscle. MDSCs are multipotent and are characterized as Sca-1^{+}/CD45^{-}/CD34^{-}/Flk1^{+}/Desmin^{+}/M-cadherin^{-} [51]. MDSCs can be used to regenerate myofibers after systemic delivery [52]. MDSCs transplanted into a dystrophic dog enabled myofiber regeneration, satellite cell pool replenishment, and long-term dystrophin expression [53].

Induced pluripotent stem cells (iPS) are somatic cells that have been reprogrammed into an ESC-like state [54]. These cells have a huge potential in regenerative medicine, disease modeling and drug discovery applications [55]. Takahashi et al. showed that pluripotency can be induced in adult human dermal fibroblasts by the nuclear transfer of four transcription factors (Oct3/4, Sox2, c-Myc and Klf4) [56]. iPS cells may overcome the ethical drawbacks of ESCs while maintaining the major advantage of pluripotency. However, as for ESCs, iPS cells may induce an immunological response after transplantation [57]. Different methods have been developed to deliver the four transcription factors with or without viral vectors; interestingly, iPS cells can be generated from genetically diseased tissues to serve as disease models for specific studies [58-60]. Myogenic progenitors can be derived from iPS cells and have been shown to engraft well after transplantation, to repopulate the stem cell niche, and to regenerate myofibers [61, 62]. Hosoyama et al. derived myogenic progenitors from human ESCs, iPS cells and diseased iPS

cells by culturing aggregated cells, named EZ spheres, in culture medium with high concentration of fibroblast growth factor-2 (FGF-2) and epidermal growth factor (EGF) [63]. By selective expansion of myogenic mesenchymal cells cultured in embryoid bodies, Awaya et al. derived ESCs and iPS cells into myogenic precursors, which showed long term and stable engraftment into damaged muscle of immunodeficient mice [64]. Filareto *et al.* obtained iPS cells from fibroblasts harvested from double knock-out dystrophin/utrophin mice, corrected the dystrophin gene, and observed good cell engraftment and improved muscle strength in dystrophic mice after cell injection [65].

3. Importance of scaffolds in muscle tissue engineering

Traditionally, regenerative strategies have been based on *ex vivo* engineered constructs that use autologous cells that can be re-implanted into the patient [66]. Among these strategies, tissue engineering (TE) is a multidisciplinary field involving engineering and bioscience techniques aimed at fabricating biological tissues for repairing or regenerating functional tissue. The concept of TE involves the use of a scaffold to support the growth and proliferation of cells, which are usually harvested from the patient, followed by the transplantation of this construct into the patient (Figure 2). Suitable integration of the construct with the surrounding tissue of the defective part is necessary as the scaffold is resorbed and new natural tissue is generated. Therefore, the scaffold plays a key role in TE and should recapitulate the attributes of the natural ECM as much as possible. Indeed, the direct *in vivo* environment of a cell comprises the ECM, the neighboring cells and a plethora of growth factors and signaling molecules [67]. The ECM provides mechanical support to the cell and is a map that spatially and temporally regulates cellular functions, such as cell proliferation, migration, differentiation and tissue organization.

The ECM is a 3D, highly hydrated viscoelastic network formed by various proteoglycans, glycosaminoglycans and fibrillar proteins exhibiting nanoscale structures, which determine the cell-matrix interactions. For example, collagen fibrils, which are one of the most important components of the ECM, are dozens of micrometers long and between 260 and 410 nm wide [68]. This nano/micro-fibrillar environment can be recapitulated *in vitro* with nanofiber scaffolds. These scaffolds must be biodegradable to enable gradual replacement with regenerated tissue and secreted ECM. To improve the cell-material interactions, the surface of the scaffolds may be functionalized with a protein coating or by chemical conjugation [69].
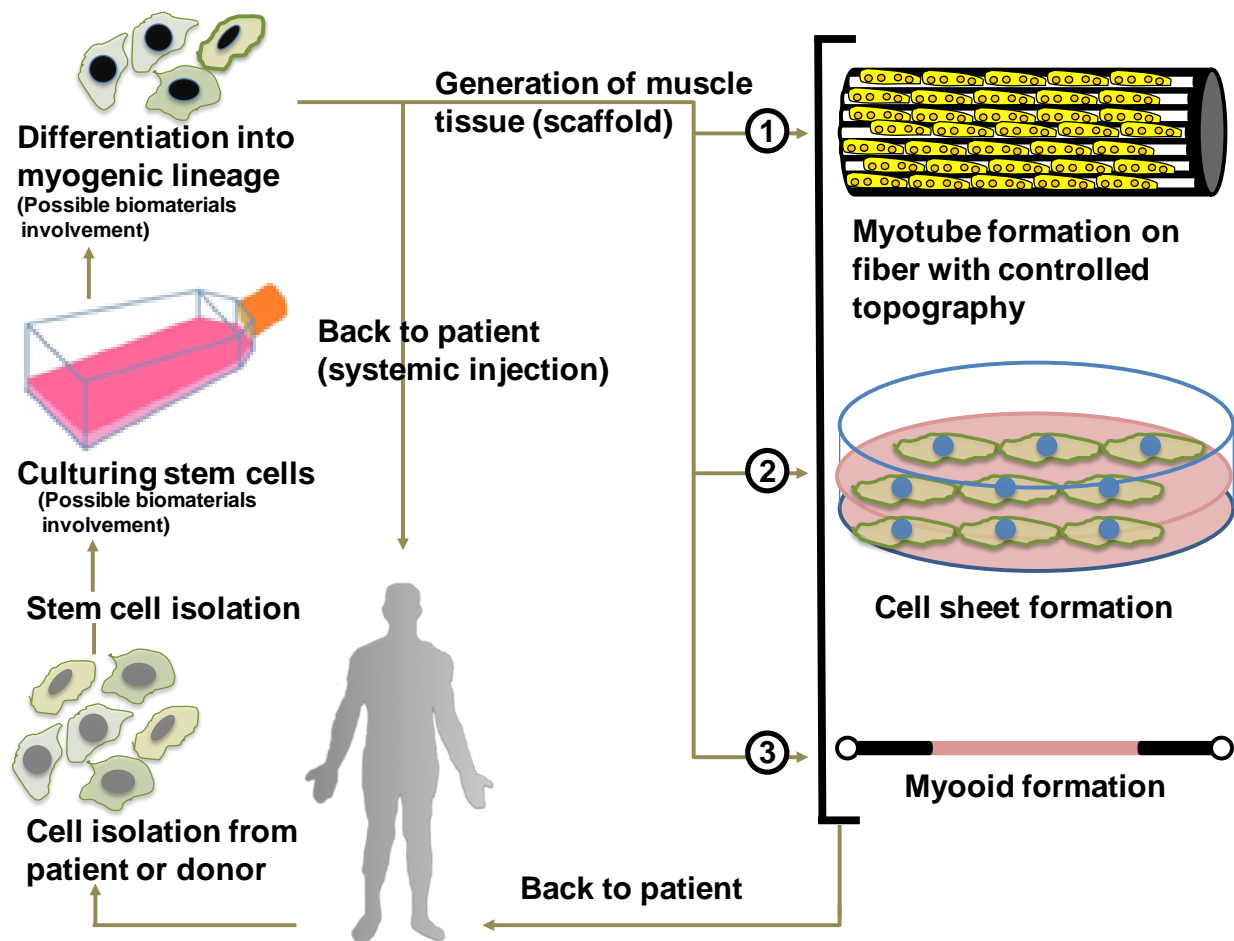
Figure 2: Schematic of the concept of muscle tissue engineering using stem cells. Additional steps with potential biomaterials involvement are indicated. Cells differentiated into the myogenic lineage may be delivered to the patient directly via systemic injection or after further differentiation into muscular tissue on a biomaterial scaffold, as indicated by the three examples of culture techniques.

4. Scaffold fabrication techniques and materials used for tissue constructs with stem cells

Engineering materials with improved cell-material interactions requires control of physical properties (e.g., shape and stiffness) and chemical properties (functional groups and surface modifications). Numerous techniques have been developed for the fabrication of scaffolds and readers can refer to different reviews or book chapters [70-72]. In the following, we introduced some techniques, such as electrospinning, phase separation, and self-assembly, which permit the production of nanofiber scaffolds in a regulated manner.

4.1. Electrospinning

Electrospinning is a versatile and cost-effective technique for producing polymeric fibers with diameters ranging from a few nanometers to a few micrometers [70]. Polymeric jets are ejected from a spinneret under high electrical tension and collected onto a counter electrode [73]. Nanofibers can be produced in different shapes, such as solid fiber, porous, core shell, yarn, hollow yarn or bundle, depending on the specific application [74]. The orientation (aligned or random) of the produced nanofibers can be controlled to match the native muscle tissue organization [75]. The use of a sacrificial material blended with a polymer enables increased cell

penetration into the scaffold, and photopatterning is possible with acrylated electrospun polymers [76, 77]. Moreover, electrospun fibers may be subjected to surface modification post-electrospinning to enhance specific cellular interactions [70, 78]. Currently, numerous natural (e.g., gelatin and chitosan) or synthetic (e.g., polycaprolactone (PCL) and polyethylene glycol (PEG)) polymers have been electrospun and used as scaffolds for myogenic differentiation [79]. For example, Huang *et al.* compared the differentiation of C2C12 cells cultured on poly(L-lactide) (PLLA) nanofibrous scaffolds and on micropatterned polydimethylsiloxane (PDMS) membranes [80]. Also, Riboldi *et al.* used an electrospun of polyesterurethane to study the myogenesis with C2C12 cells [81]. In another study, Li et al. used polyaniline/gelatine composite nanofibers as substrate for myoblasts and showed comparable cell attachment and proliferation than the control in Petri dishes [82]. Hybrid materials have also been used with stem cell. For example, McKeon-Fischer and Freeman used electrospun of PLLA-gold nanoparticles composite nanofibers to improve myoblasts differentiation [83]. Also, Ostrovidov *et al.* showed that the inclusion of multi-walled carbon nanotubes (MWNTs) in gelatin nanofiber scaffolds favored myotube formation by enhancing the mechanotransduction [84]. Finally, Liao *et al.* studied the differentiation of C2C12 cells on electrospun polyurethane (PU) scaffolds stimulated with electrical pulses and mechanical stretches [85].

4.2 Phase separation

In phase separation, an homogeneous polymer-solvent solution is separated into two phases (a polymer-rich phase and a polymer-lean phase) by the addition of another immiscible solvent or by decreasing the temperature below a binodal solubility curve [70, 86]. The solvent is then removed by freeze-drying, resulting in the formation of a microporous polymer structure. This technique enables the fabrication of nanofibers with diameters of 50 to 500 nm, similar to the

size of native collagen [87]. Phase separation can also be combined with others techniques for additional control on the features of the fabricated scaffold. Thus, porogens such as paraffin, sugar or salt can be added to the polymer solution as sacrificial component for opening predesigned micropores by porogen leaching to improve cell penetration into the scaffold [88]. Ma and colleagues have fabricated molds with anatomical shapes, by using CT-scanning and solid free-form technology, for their use in phase separation [89]. Also, Nieponice et al. fabricated a tubular poly(ester urethane)urea (PEUU) by phase separation that they loaded homogeneously with MDSCs under rotation and cultured in spinner flask during 7 days [90]. They observed high cell viability and proliferation without stemness loss. Gradients in porosity and pore size have also been obtained in porous gelatin hydrogels by phase separation [91].

4.3 Self-assembly

 Self-assembly is a process based on the spontaneous arrangement of building blocks into a precise structure [68]. The building blocks assemble due to non-covalent interactions (e.g., hydrogen bonding and ionic bonding), and the resulting structures are stabilized by numerous interactions. Peptide amphiphiles (PAs) or cell-laden hydrogels have been used in the self-assembly process, and self-assembled nanofibers have been produced by carefully designing the building blocks [92-94]. Importantly, due to their size, the self-assembled fibers create a 3D environment that mimics the *in vivo* environment, which is favorable for cell development and differentiation [95]. Self-assembly can also be combined with other technical processes. For example, to improve cell attachment and spreading, Tambralli *et al.* used peptide self-assembly to coat an electrospun PCL [96].

Table 1: Examples of scaffolding materials for muscle tissue engineering.

Material	Cells	Reference
Natural materials		
Dynamic alginate-collagen hydrogel	Human pluripotent stem cells	[75]
Alginate microbeads	Urine-derived stem cells	[97]
Shape memory alginate scaffold	Autologous myoblasts	[98]
Acellular scaffold	Recruited host progenitor stem cells	[99]
Hydroxybutyl chitosan fibers	Human mesenchymal stem cells	[100]
Chitosan/β-glycerophosphate/collagen	Satellite cells	[101]
Elastin	Human mesenchymal stem cells	[102]
Fibrin hydrogel	Human umbilical cord stem cells	[103]
Gelatin-MWCTs nanofibers	C2C12 cells	[84]
Hyaluronic acid	Adipose stem cells	[104]
Silk-alginate hydrogel	Mouse embryonic stem cells	[105]
Gelatin-silk hydrogel	Pluripotent cells	[106]
Synthetic materials		
Polyesterurethane	C2C12 cells	[81]
Polyhydroxybutyrate	C2C12 cells	[107]
Poly(lactic-co-glycolic acid) (PLGA)	C2C12 cells	[108]
Poly(L-lactic acid) (PLLA)	Recruited host progenitor stem cells	[109]
Polystyrene nanomembrane	C2C12 cells	[110]
Poly(ε-caprolactone) (PCL)/collagen	Human mesenchymal stem cells	[111]
Poly(glycolic acid) (PGA)	Myoblasts	[112]
Graphene oxide (GO)/poly(ε-caprolactone) (PCL) composite fibrous scaffolds	Human cord blood mesenchymal stem cells	[113]
Graphene oxide (GO)	C2C12 cells	[114]
PCL/PANI nanofibers	C2C12 cells	[115]

5. Scaffolds used with stem cells

Cell-supportive scaffolds can be used in combination with stem cell therapy to direct stem cell fate and to interact with the host tissue (Figure 3). The cell-material interactions are rich and complex cell signaling pathways are induced by these interactions. The material stiffness, roughness, topography, degradation by-products, and properties for sequestering or presenting growth factors are important factors that influence the stem cell fate [116, 117]. Ideal scaffolds

must recapitulate as many features of the stem cell niche as possible because these niches house and regulate the stem cells in the body [118]. Therefore, the scaffolds should have appropriate micro/nano features, adequate mechanical properties and present fixation sites for cells and growth factors [119]. The effects of nanofeatures on cell attachment, migration, proliferation, and differentiation have been described previously [68], and we present here some of the more recent findings using stem cells. Using a precise arrangement of RGD (Arg-Gly-Asp) ligands, Schvartzman *et al.* showed that the minimal matrix adhesive unit to promote cell spreading was a cluster of 4 RGD ligands with gaps less than 60 nm between the RGD clusters [120]. By increasing the symmetry of a lattice of pits, McMurray *et al.* fabricated a nanostructured surface in PCL that promoted the cell proliferation and maintained the stemness of MSCs in culture for up to eight weeks [121]. Stem cell differentiation has also been regulated by nanotopography or by the presence of functional chemical groups. Both, the order of symmetry in a lattice of nanopits and the matrix rigidity affected the stem cell differentiation [122-124]. In an example with neural stem cells, a study showed that these cells preferentially differentiate into neurons when they are encapsulated in a nanofiber scaffold presenting the epitope isoleucine-lysine-valine-alanine-valine (IKVAV) [125]. This signaling to cells via nanofeatures is a key component of scaffold development for cell-based therapies, and understanding the stem cell niches and the cell-materials interactions will enable a better translation from research to the clinic. Currently, the scaffold research field is expanding, and many platforms with increasing complexities in their signaling to cells have been fabricated to interact with stem cells and to guide their behavior (Table 1). These biomaterial platforms can have different forms (fibrous meshes, hydrogels, polymeric substrates, porous scaffolds, acellular scaffolds) and have multi-functionalities, ranging from mechanically supporting stem cells, modulating the cell behavior

and organization, delivering spatiotemporally growth factors or drugs, to protecting cells or protein functionalities from immune response when transplanted, recruiting host cell, eliciting specific gene expression, and being biodegradable [126]. These protection and promotion of the cellular functions by the biomaterials render the delivery of cells to the site of damaged muscle via scaffolding strategy interesting compared to direct cell delivery by injection [127].

Nanofiber scaffolds

As skeletal muscles are naturally anisotropic structures, scaffolds with aligned nanofibers will be preferred over randomly oriented nanofibers because the alignment of the stem cells by contact guidance will favor myoblast fusion, gene expression, and differentiation [128-130]. Ricotti et al. showed that aligned nanofibers of poly(hydroxybutyrate) promotes the differentiation of C2C12 and H9c2 compared to random nanofiber scaffolds [107]. Dang *et al.* cultured human MSCs on aligned nanofibers of the thermoresponsive polymer hydroxybutyl chitosan and produced a cell sheet of aligned human MSCs by putting the fibers below the lower critical solution temperature (LSCT) [100]. The use of polymer blend allows obtaining composite nanofibers with improved properties inherited from both polymers. Choi et al. showed that electrospun scaffolds of a poly(ε-caprolactone)/collagen blend with aligned fibers enhanced the alignment of human skeletal muscle cells and myotube formation [111]. Due to their shape and the nature of the polymer used, nanofibers induce topographical constraint on cells, have a certain stiffness which influences the cell behavior, and have natural or fabricated adhesive sites for cell attachment. The use of a conductive polymer such as polyaniline (PANI), polythiofene (PEDOT), or polypyrrole (PPY) introduced an additional signaling way to cells through the electrical

conductivity of the polymer. Jun et al. fabricated poly(L-lactide-co-ε-caprolactone) (PCL)/ polyaniline (PANI) composite nanofibers by electrospinning and showed enhanced C2C12 differentiation on the electrically conductive composite fibers, without the use of electrical stimulation pulses, compared to PCL fibers used as a control [131]. Ku et al. also used an electroconductive scaffold of PCL-PANI nanofibers and observed a synergy between the cell alignment by contact guidance and the electrical conductivity on myoblast differentiation [115]. Different nanomaterials such as gold particles, carbon nanotubes, graphene can be included into the polymeric solution to obtain hybrid nanofibers with improved mechanical, electrical and cell adhesive properties [83, 84, 132]. Chaudhuri et al. observed excellent differentiation into myoblasts of human cord blood derived MSCs cultured on electrospun fibrous meshes of graphene oxide-polycaprolactone (GO-PCL) composite [133]. Chemical signaling can also be added directly on the scaffold or in the culture medium. Thus, high myogenic differentiation was observed by Leung et al. when human ECs were cultured on aligned chitosan-polycaprolactone nanofibers in culture medium containing Wnt3a protein [134, 135].

Hydrogels

Hydrogels are 3D polymeric network with high content of water (> 90%) and with viscoelastic properties mimicking those of biological tissues [136]. The polymeric network is stabilized by chemical or physical crosslinks of the polymeric chains and hydrogels have the property of swelling in presence of water. Natural hydrogels (e.g. gelatin, collagen, fibrin) have adhesive sites for cell attachment and strong interactions with cells, while synthetic hydrogels (e.g. PEG, poly(vinyl alcohol) (PVA), poly(2-hydroxyethyl methacrylate) (PHEMA)) lack of adhesive sites for cell attachment and have weak interactions with cells [137]. Due to their tunable properties,

hydrogels are appealing for biological and biomedical applications. Thus, Neal et al. used a sacrificial outer molding method to fabricate muscle strips in cylindrical fibrin hydrogels suspended between two anchoring ends [138]. The fabricated 3D fascicle-like muscle constructs with tunable diameter have high volumetric cell density with aligned C2C12 cells along the cylindrical axis, due to the compaction of the hydrogels. Furthermore, by transfecting C2C12 with a light-activated cation channel, channelrhodopsin 2 (ChR2), and using the same sacrificial outer molding method, muscle strips that can be optically stimulated were fabricated [139, 140]. Researchers observed an optimal strip diameter of 500 μm generating a peak twich stress of 1.28 kPa under stimulation and showed that optical stimulation can be as good as electrical stimulation. Chan et al made a further development by fabricating bundles of muscle strips containing a co-culture of muscle cells and human embryonic kidney cells (HEK293T) transfected with ChR2 [141]. In another study, Shi et al. fabricated a cell-responsive grooved microfiber of gelatin methacryloyl (GelMA), which showed enhanced cell supportive properties and allowed to co-culture different cell types in the fiber core and on the microstructured surface of the fiber that induced cell alignment [142]. Also, Gilbert et al. cultured *in vitro* muscle stem cells on soft PEG hydrogel mimicking the elasticity of muscle (12 kPa) and showed that cells kept their stemness properties contrary to those cultured on Petri dishes (~3 GPa) [143]. Transplanted in mice these stem cells showed also good engraftment and dynamic proliferation. Desiderio *et al.* used a hyaluronic acid (HA) scaffold crosslinked with L-lysine methyl ester dihydrochloride after activation with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)/1-hydroxybenzotriazole hydrate (HOBt). After the scaffolds were seeded with a subpopulation of adipose stem cells (ACs), they were engrafted subcutaneously in mice for 30 days. The results demonstrated that CD34$^+$/Ng2$^+$ ACs differentiated into myogenic cells

and formed skeletal myofibers, whereas $CD34^+/Ng2^-$ ACs differentiated into adipose tissue [104]. To improve their functionalities, hydrogels can be conjugated to molecules, peptides, proteins or other hydrogels. Thus, Dang et al. conjugated the peptide QHREDGS, derived from angiopoeitin-1, to PEG hydrogels to improve the adhesion, morphology and viability of human iPS cells in cultures [144]. The same group also modified chitosan and chitosan-collagen hydrogels with QHREDGS to improve the morphology and functionalities of cardiomyocytes in cultures and after hydrogel injection in a rat model of myocardial infarction [145-147]. Synthetic hydrogels such as PEG are often bioconjugated with RGD peptide, which is a motif found in ECM and recognized by integrins for cellular attachment. For example, Salinas and Anseth studied the effects of the RGD presentation in PEG hydrogels on human MSCs attachment and observed improved encapsulated cell survival when RGD peptides were covalently linked to the hydrogels via a single arm spacer [148]. Also, Fuoco et al. compared young (piglet) and adult (boar) pericytes in muscle and blood vessel regeneration and showed that composite PEG-fibrinogen hydrogel rejuvenate aged pericytes and re-established their myogenic and angiogenic capabilities [149, 150]. The same group showed that a PEG-fibrinogen hydrogel loaded with mesoangioblasts can induce the formation of an artificial muscle *in vivo*, replacing the void created by ablation of a large defect in mice *tibialis anterior* [151]. Using a combination of collagen and alginate hydrogels, Dixon et al. fabricated a platform with a changing environment that switched human pluripotent stem cells from proliferation to differentiation [152].

Numerous nanomaterials such as metallic nanocluster, polymeric nanofibers, carbon nanotubes, graphene, synthetic clays, silicate nanoplatelets, polymeric nanoparticles, and nanocrystals can be integrated into hydrogels to form nanocomposite hydrogels for tissue engineering applications [153, 154]. For example, Ramon-Azcon et al. aligned carbon nanotubes into GelMA hydrogels

by dielectrophoresis to improve the electrical conductivity of the material for fabricating contractile myotubes [155]. Also, Pek et al. used thixotropic PEG-silica hydrogels to study the differentiation of human MSCs induced by mechanotransduction due to the cell tractions on the thixotropic material [156, 157]. Hydrogels are also excellent carriers for drug loading and allow long time and spatio-temporal drug releases [158, 159]. In one study, Liu *et al.* differentiated human urine-derived stem cells (USCs) into a myogenic lineage by culturing them on alginate microbeads encapsulating a cocktail of growth factors and then subcutaneously engrafted these cells into nude mice [97, 160]. Hydrogels can also be designed to be injectable. The gel formation in situ can be triggered by pH, temperature, ionic strength environment, and several chemical reactions of conjugation [161]. Due to the viscoelastic properties of hydrogels, such gel formation in situ is minimally invasive and allows effective molding and space filling of any voids let by defects. Liu et al. developed an injectable fibrin hydrogel loaded with human umbilical cord MSCs that exhibited good cell viability and successful myogenic differentiation [103]. In addition, Ding *et al.* cultured SCs encapsulated in an injectable thermoresponsive chitosan/β-glycerophosphate/collagen (C/GP/Co) hydrogel. Four weeks after subcutaneous injection into the dorsum of nude mice, good cell viability and maintenance of stemness were observed [101].

Along with advances in scaffolding materials, progress has also been made in the generation of muscular tissue from stem cells. Different methods have been established to differentiate stem cells into myoblasts, and we detail specific techniques in the following section.
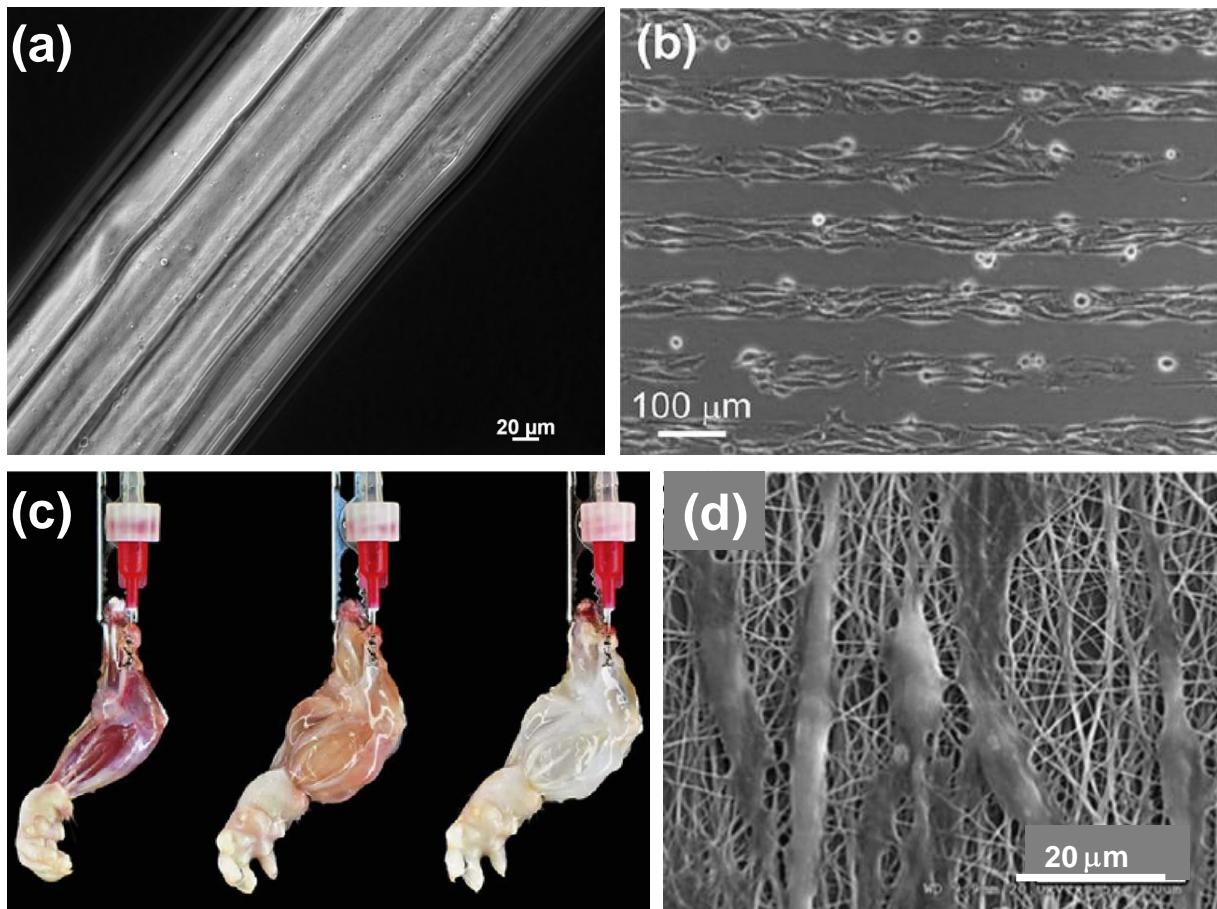
Figure 3: Pictures of different scaffolds used for muscle tissue engineering. (a) Grooved GelMA fiber, (b) Polystyrene nanomembrane with patterned lines of C2C12 cells, (c) Decellularized mouse limb, (d) Aligned PCL/collagen nanofibers with human skeletal muscle cells. Reprinted with permission from Fujie et al. 2013 [110] ©2013, American Chemical Society; Bernhard J Jank et al. 2015 [162] ©2015, Elsevier Ltd; and Jin San Choi et al. 2008 et al. [111] ©2008, Elsevier Ltd, (photos b, c, d, respectively).

6. Methods for obtaining myogenic progenitor cells from stem cells

Because many different stem cells can differentiate into the myogenic lineage, several methods based on the stem cell types have been developed to induce cell differentiation until myoblasts capable of fusing together and forming new myofibers.

6.1 Myoblast derivation from ESCs

There are various methods for obtaining skeletal myoblasts from ESCs. One such method involves obtaining multipotent mesenchymal precursors (MMPs, CD73$^+$) from ESCs and differentiating these precursors into myoblasts (Table 2). Briefly, ESCs are seeded at low cell density on fibronectin or collagen type IV coatings with or without a feeder layer of fibroblasts. Then, MMPs are obtained by culturing ESCs in culture medium (alpha-MEM + FBS or DMEMF12 + insulin, transferrin, and selenium) with serum for 20 days [38, 163]. In another method, ESCs are cultured in serum-free medium (SF-03) containing human recombinant bone morphogenetic protein 4 (BMP4) [164]. The generated MMPs are then screened by fluorescence-activated cell sorting (FACS) for the positive expression of CD73 or the negative expression of alpha-receptor platelet-derived growth factor (PDGFR-alpha). These MMP cells are then differentiated into skeletal myoblasts by co-culturing them for 2-3 weeks with C2C12 cells in culture medium with serum to induce cell fusion [163]. Another method involves monoculture of these MMPs in serum-free medium containing insulin for 2-3 weeks [38]. MMPs can also be cultured for 18 days in serum-free medium (SF-03) without BMP4 but with lithium chloride (LiCl), insulin-like growth factor (IGF-I), hepatocyte growth factor (HGF) and bFGF [164].

Table 2: Generation of multipotent mesenchymal precursors (MMPs) from human or murine ESCs followed by their differentiation into myogenic cells. Reprinted with permission from Salani *et al.* [165] Copyright © 2012 The Authors Journal of Cellular and Molecular Medicine © 2012 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd.

Reference	Barberi *et al.*, 2005 [163]	Barberi *et al.*, 2007 [38]	Sakurai *et al.*, 2009 [164]
Initial cell type	hESCs	hESCs	mESCs
MMP acquisition	CD73$^+$ cells	CD73$^+$ cells	
Media	αMEM, 20% inactivated FBS	DMEM/F12	Serum-free SF-03
Factors		ITS	β-ME, BMP4
Duration (days)		20	
Media	αMEM, 20% inactivated FBS	Serum-free/N2	Serum-free SF-03
Factors		Insulin	LiCl, IGF-I, HGF, bFGF
Duration (days)	14-21	14-21	18
Media	αMEM, 3% HS, 1% FBS		
Factors	C2C12 co-culture		
Duration (days)	1		

Myoblasts can also be generated from ESCs via the formation of floating embryoid bodies (EBs) that are subsequently differentiated into myoblasts (Table 3) [165]. To promote the formation of homogeneous EBs, cultures of ESCs in microwells made with different materials have been used [166-170]. Usually, ESCs are cultured on a fibroblast feeder layer with leukemia inhibitory factor (LIF) [171]. EBs form in LIF-free medium after detachment from the feeder layer and are cultured for 5 days. Several methods can be used to differentiate EBs into myoblasts. Zheng *et al.* obtained myoblasts from EBs after 2-3 weeks of culture by decreasing the serum concentration in the culture medium and adding 5-AZA, which is known to favor myogenic

lineage differentiation by hypomethylating muscle genes [172, 173]. Rohwedel *et al.* obtained myoblasts in 9 days by culturing EBs in medium containing non-essential amino acids (NEAA), beta-mercaptoethanol (β-ME), and sodium selenite [174]. Darabi *et al.* obtained myoblasts in 3 days by culturing EBs expressing Pax3 in DMEM culture medium with horse serum and doxycycline [175].

Table 3: Obtaining myoblasts from human or murine EBs cultures of ESCs. Reprinted with permission from Salani *et al.* [165] Copyright © 2012 The Authors Journal of Cellular and Molecular Medicine © 2012 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd.

Reference	Rohwedel *et al.*, 1998 [174]	Zheng *et al.*, 2006 [39]	Darabi *et al.*, 2008 [175]
Initial cell type	mESCs	hESCs	iPAX3 mESCs
EB acquisition	Hanging drop	Hanging drop	Hanging drop
Duration (days)	5	4-6	5
Myogenic cell differentiation			PDGFR-αR/Flk-1
Media	DMEM, 15% DCC-FCS	DMEM, 10% FBS	DMEM low glucose, 2% HS
Factors	L-glutamine, NEAA, β-ME, sodium selenite, transferrin	ITS, L-glutamine, EGF	Doxycycline
Duration (days)	9	14-28	7
Media	IMDM differentiation medium	DMEM, 2% HS	
Factors		Glutamine	
Duration (days)	9	14-28	
Media		DMEM, 10% HS, 10% FBS	
Factors		Glutamine, 5-AZA	

Duration (days)		14-28	

6.2 Myoblast derivation from iPS cells

iPS cells can be differentiated into myoblasts (Table 4). iPS cells are first transfected to enable positive expression of Pax7 and then cultured for 5 days to form EBs in DMEM culture medium with LIF. The myogenic progenitors are subsequently sorted by FACS based on positive PDGFR-alpha expression and negative fetal liver kinase (Flk-1) expression, and then they are differentiated into myoblasts via culture in low-serum medium for 7 days [175]. In another study, the initial selection of iPS cells based on Pax7-positivity was omitted; instead, EBs were generated, then the myogenic precursors were selected by FACS based on the positive expression of SM/C-2-6, and DMEM culture medium with 10% FBS, 5% HS, NEAA, and β-ME was used to differentiate these progenitors into myoblasts over 27 days [176].

Table 4: Obtaining myoblasts from murine iPS cells. Reprinted with permission from Salani *et al.* [165] Copyright © 2012 The Authors Journal of Cellular and Molecular Medicine © 2012 Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd.

Reference	Darabi *et al.*, 2011 [177]	Mizuno *et al.*, 2010 [176]
Starting cells	iPAX7-miPSCs	miPSCs
EB acquisition	Hanging drop	Hanging drop
Duration (days)	5	4-6
Myogenic cell differentiation		
Media	DMEM, 2% HS	DMEM, 10% FCS, 5% HS
Factors		NEAA, β-ME
Duration (days)	7	27

6.3 Myoblast derivation from mesoangioblasts

Mesoangioblasts are characterized as $CD34^+/c\text{-}kit^-/Flk1^+/NKX2.5^-/Myf5^-/Oct4^-/AP^+/CD44^+$ /$CD140a^+/CD140b^+$ and are multipotent stem cells isolated from blood vessels [1, 178]. Mesoangioblasts can be differentiated directly into myoblasts after transfection with Pax3 and Pax7 [178]. Pax3 is required for the derivation of mesoangioblasts into myoblasts *in vitro* and *in vivo* because mesoangioblasts isolated from Pax3-null mice fail to regenerate muscle fibers [179]. Mesoangioblasts proliferate well *in vitro,* preserve their multipotency and thus have been used in different animal models of muscular dystrophy [47, 48]. In cell therapy, the efficient delivery of cells to the targeted tissue is a major challenge. An important advantage of mesoangioblasts is that they can be delivered systemically via intravenous injection and can migrate to the appropriate site, cross the endothelial wall, and regenerate muscle fibers [47]. To enhance this homing property, mesoangioblasts expressing α4 integrin have been treated with stromal-derived growth factor (SDF-1) or tumor necrosis factor alpha (TNF-α) before transplantation into mdx mice, resulting in massive colonization at the diseased site (>50%) and a high muscle fiber regeneration rate (>80%) [180]. Another major problem in cell therapy is the need for a large number of progenitors cells for successful transplantation. This necessitates an *in vitro* cellular expansion phase that, depending on the progenitor type, may induce the loss of stemness or viability. Moreover, patients suffering from myopathy may have a decreased number of progenitor cells. Because patients with limb-girdle muscular dystrophy 2D (LGMD2D) lack mesoangioblasts (MABs), Tedesco *et al.* reprogrammed autologous fibroblasts into h-iPS cells. They then derived mesoangioblasts from the h-iPS cells and expanded the mesoangioblasts *in vitro*. Next, the mesoangioblasts were genetically corrected using a viral vector expressing the

defective SGCA gene, which encodes α-sarcoglycan. After intra-arterial injection of these corrected mesoangioblasts into a mouse with LGMD2D, good migration of the cells to the damaged site, regeneration of muscle fibers and expression of sarcoglycan were observed [181]. A clinical trial (EudraCT n∘ 2011-000176-33) has begun on the intra-arterial delivery of compatible mesoangioblasts in a boy with DMD who is less than 18 years old [181].
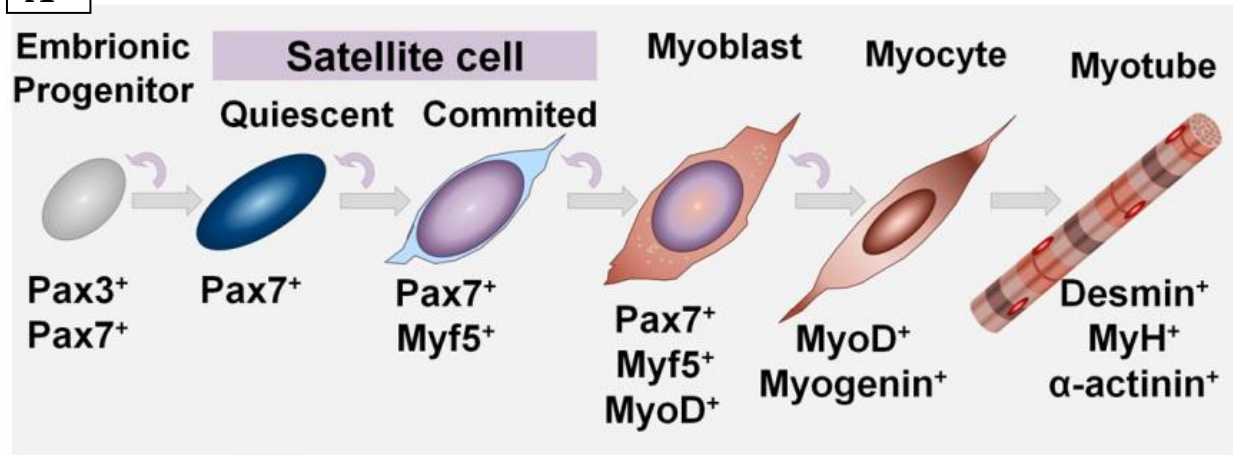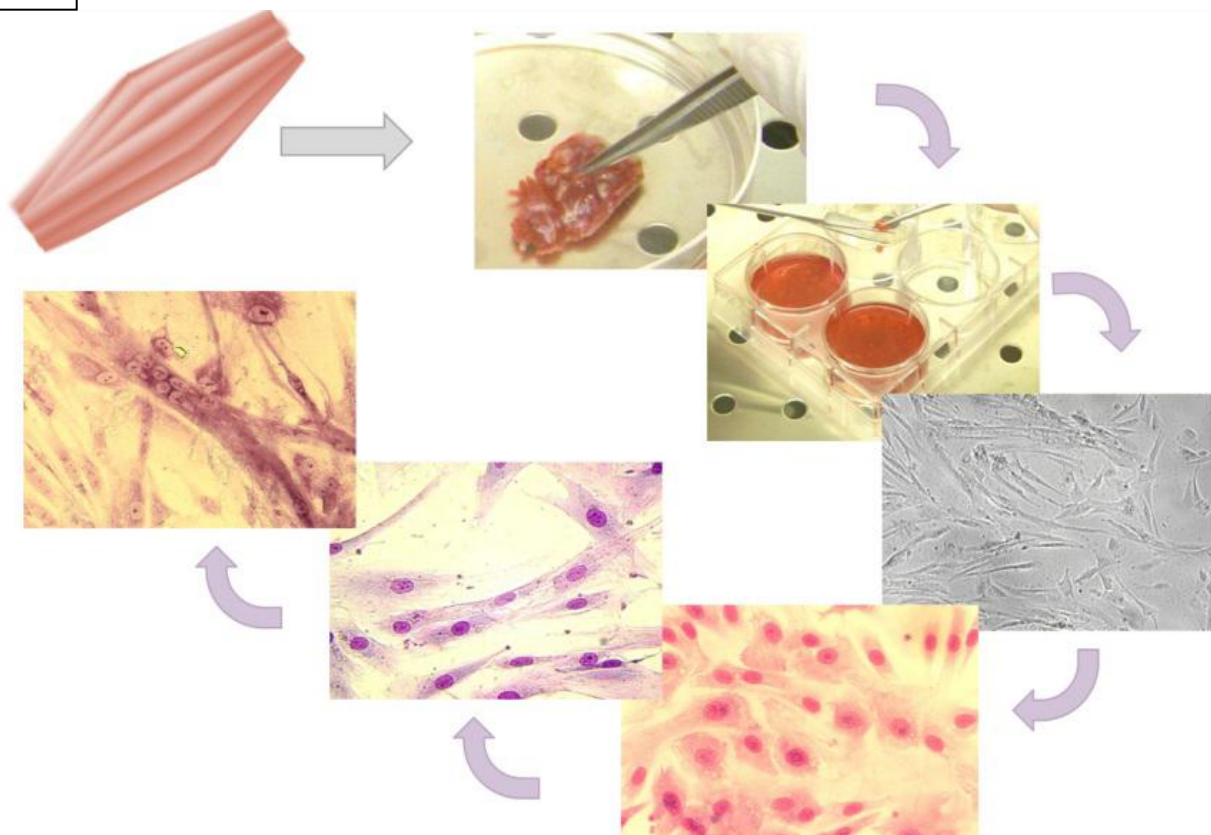
Figure 4. Myogenic cell characterization and culture. The myogenic cell lineage can be identified in each differentiation state and follows tightly regulated proliferation and differentiation cycles. From the embryonic state until terminal differentiation into muscle fibers, an intricate network of transcription factors regulates the fate of muscle progenitor cells (A). These cells can be isolated from any skeletal muscle tissue, grown in culture and re-implanted into damaged muscle to promote muscle regeneration (B). Reprinted with permission from Azzabi Zouraq et al. [182] (http://www.intechopen.com/books/regenerative-medicine-and-tissue-engineering/skeletal-muscle-regeneration-for-clinical-application).

The different techniques described previously for the derivation of stem cells into myogenic cells highlight that stem cell differentiation is dependent on the stem cell type and involves multiple steps regulated by different sets of transcription factors (Fig. 4A). Stem cells can be harvested from different tissues, cultured, and induced to differentiate into myogenic cells, which are then transplanted into a patient in an autologous manner (Fig. 4B). This cell therapy technique is particularly useful for the treatment of myopathies, such as DMD.

7. Applications of stem cell and genes therapies in Duchenne muscular   dystrophy

The first attempts to regenerate myofibers in DMD animals or patients were based on the intramuscular injection of allogenic myoblasts [183-185]. This technique permits the generation of myofibers with dystrophin, but the attempts were unsuccessful due to major technical problems, such as decreased cell viability upon transplantation and limited migration of the

transplanted cells *in situ* [186-188]. To optimize the transplantation technique and the cell engraftment, technical parameters such as the needle size, the cell number injected and the volume of injection were studied on monkeys [189]. Although, after comparing early (1991-1997) and more recent trials (2004-2007), a recent commentary by Skuk and Tremblay provides a more nuanced view on myoblast transplantation results [190], the results obtained earlier in myoblast transplantation motivated for the search of others myogenic cells. Dermal fibroblasts and dermal Sca-1$^-$ cells have also been proposed for transplantation in DMD mice because they can be converted *in vivo* into myogenic cells that are capable of fusing with myoblasts to deliver the missing gene and restore dystrophin expression [191-193]. The use of stem cells has drastically increased the number of cell sources for generating cells of the myogenic lineage. However, to obtain the high cell number required for transplantation, the stem cells must be expanded in cultures *in vitro.* If most of the stem cells proliferate well *in vitro*, apoptosis or loss of stemness may appear during this cellular expansion for some cell type [48, 194, 195]. Thus, newly harvested SCs can regenerate myofibers when transplanted into DMD mice (Figure 5), whereas transplantation of the same cells cultured *in vitro* were less effective due to the loss of proliferative capacity and stemness potential [194, 196]. By contrast, AC133 cells proliferate well in culture and maintain their stemness. Injection of a co-culture of AC133 cells and myoblasts into the muscle of dystrophic mice replenishes the satellite cell pool and regenerates myofibers [50]. An autologous transplant of AC133 cells in a dystrophic boy demonstrated the safety of the technical strategy but did not produce a substantial functional gain [197]. Systemic cell delivery through the blood stream to deficient myofiber sites has also been evaluated. Several stem cells, such as muscle SPs, MDSCs, mesoangioblasts, and pericytes, exhibit good migration to the targeted site, repopulate the stem cell niche, and regenerate myofibers with

dystrophin [43, 49, 52]. The reprogramming of somatic cells into iPS cells using only four transcription factors (*Oct4, Klf4, Sox2, C-Myc*) was a breakthrough because this technique allows a large quantity of autologous stem cells to be obtained [56]. Several methods have been developed to deliver the four transcription factors without the use of viral components [198]. Moreover, it is possible to generate diseased-iPS cells for disease models and drug development or to make genetic corrections in these cells before myogenic differentiation and transplantation [59, 61, 180, 199].

Gene therapy and cell therapy are complementary since the restoration or the transfer of a dystrophin gene can be done directly *in vivo* in the lesion site or *ex-vivo* in cells that will further be transplanted in the body [200]. The gene transfer requires the delivery of the gene (usually cDNA) into the host cells with its translocation to the nucleus and its high expression. This gene transfer can be done via viral vectors (e.g. retroviruses, adenovirus, vaccinia virus) or non-viral vectors (e.g. naked DNA, liposomes, polymer carriers) [201-203]. However, the human dystrophin gene is large (2.4 million base pairs) with complex transcriptional regulation [204]. The full length dystrophin mRNA has 14 000 bases and the translated protein is a rod shaped protein with four domains and a molecular weight of 427 kDa. The large size dystrophin cDNA (14 kb) cannot be integrated into conventional viral vectors and therefore different strategies were elaborated to deliver the dystrophin gene: to use a viral vector but with a shortened dystrophin cDNA, to use stem cells with a whole dystrophin gene, or to increase the vector capacity by fabricating a human artificial chromosomes containing the whole dystrophin gene [205, 206]. Zatti *et al.* showed that human cardiomyocytes from a DMD patient, which were transformed into iPS cells and then genetically corrected with an artificial human chromosome encoding the entire dystrophin genomic sequence, underwent myogenic differentiation to

generate cardiomyocytes with good sarcomeric organization, dystrophin expression and good electrical activation *in vitro* [207]. Li et al. reported on the dystrophin gene correction by three methods (exon skipping, frameshifting and exon knockin) in DMD-patient-derived iPS cells using programmable nucleases such as the transcription activator-like effector nuclease (TALEN) and the clustered regularly interspaced short palindromic repeat (CRISPR) associated 9 endonuclease [208]. After assessing the risk of off-target mutagenesis and the efficiency of the gene correction by the three methods in the different cell clones obtained, they differentiated the corrected-iPS cells into myoblasts and observed the expression of dystrophin mRNA in the different cell clones, concluding that the exon knockin method was the most efficient, since it restored the expression of the full length dystrophin mRNA similar to control cells [208]. Goyenvalle et al. 2015 used a tricyclo-DNA (tc-DNA), injected intravenously into mdx mice to induce exon 23 skipping and dystrophin protein expression in skeletal muscle, heart and also to lesser extent, the brain [209]. Others studies combined both cell and gene therapies. After transfecting mesoangioblasts with EGFP vector (encoding retroviral proteins), Khajoj et al observed that the transfected mesoangioblasts (EGFP-MA) differentiated into myotubes when co-cultured with C2C12 showing retention of their myogenic properties [210]. Furthermore, these transfected mesoangioblasts injected locally into *Gastronecmius* muscle of mice participated to the muscle regeneration. Therefore, the cell and the gene therapies research fields are promising and have substantial potential. Several recent studies have focused on a new therapeutic strategy, termed *in situ* tissue regeneration that aims to promote the *in situ* recruitment and stimulation of progenitor cells [211, 212].
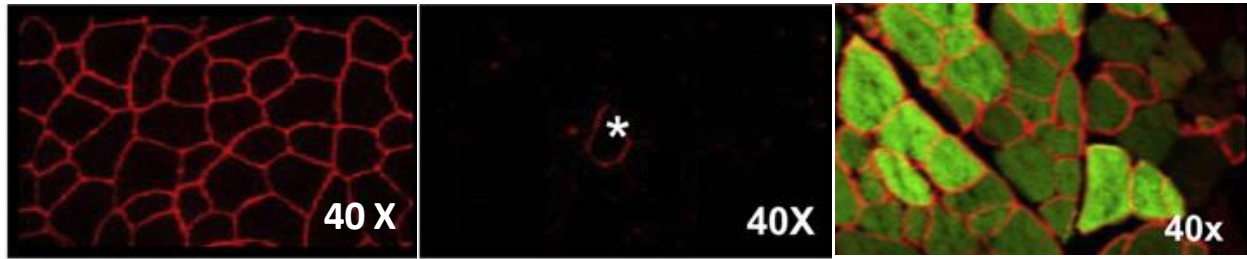
Figure 5: Immunostainning (in red) for dystrophyn protein in transverse frozen sections of Gastrocnemius muscle obtained from (a) wild type C57HBL/ka mouse, (b) mdx mouse, (c) mdx mouse transplanted 4 weeks previously with 11 000 green fluorescence positive (GFP$^+$) skeletal muscle progenitors showing restored dystrophin expression. Reprint with permission from Cerletti et al. (2008) ref [196] ©2008 Elsevier Inc.

Conclusion

A plethora of techniques, materials and fabrication tools have recently emerged to improve scaffold fabrication. These scaffolds integrate an increasing number of signals to guide the cellular behavior toward a desired goal. Thus, different features, such as geometry, stiffness, roughness, surface functionalities, growth factor incorporation via nanocarriers, nanomaterial incorporation, and external mechanical or electrical stimulation, have allowed researchers to enhance cell attachment, migration, proliferation, and differentiation. Furthermore, the shift from 2D to 3D cultures and the scaling down of the signals to cells from macro-signaling (e.g., in a Petri dish) to micro-signaling and nano-signaling (e.g., micro/nano-structured surfaces or volumes) has tremendously enhanced the cell-material interactions, resulting in increased cell functionalities. However, mimicking the complex *in vivo* cellular environment *in vitro* remains a challenge and awaits further improvements. In addition to this materials evolution, a

breakthrough in cell engineering was realized with the introduction of stem cells, which eliminate the challenges associated with organ donors. The expansion of cell sources that can give rise to myogenic progenitors has increased the possibilities for regenerating muscle, and encouraging results have already been obtained in muscular cell therapy. Moreover, techniques for cell derivation and genetic modification have further enhanced the tremendous potential of stem cell in regenerative medicine, disease modeling and drug screening.

The different approaches, tissue engineering with scaffolding materials, cell therapy, and gene therapy, used for muscular regeneration with stem cells have a complementarity, which is useful in the frame of DMD for the development of a therapeutic treatment. Since all muscles are affected in DMD, a cell delivery by systemic injection seems a preferable strategy, while scaffolding materials are usually designed to fill a muscular defect or for a localized cell delivery. However, the biomaterials can be involved at different steps of the cell therapy for DMD. Thus, biomaterials may have an important role during the expansion phase of the myogenic progenitors, by creating an environment mimicking the stem cell niche to favor the cell attachment, proliferation, and the maintain of stemness and other cell functions. Furthermore, biomaterials may also be involved in the differentiation steps to obtain myoblasts by favoring a myogenic derivation and accelerating the differentiation process. Due to their tailoring properties, versatility and injectability, hydrogels may have an important role after localized cell transplantation by protecting the cells survival and functionalities from harsh environment, recruiting host cells in the therapeutic process, and keeping low the host immune response. Importantly also, the building of muscular tissues on biomaterials help us to understand some cellular and molecular mechanisms and this understanding can be applied in the

frame of DMD. Similarly, the gene therapy is complementary to the cell therapy, as mentioned before.

Despite these advances in materials and in cell engineering, several improvements are still needed to enhance the translation from research to the clinic. One major concern is the possible host immune response after cell transplantation. This immune response can be induced by viral components if used during gene correction, or just simply because the lacked dystrophin protein in DMD diseased patients is now expressed after muscle regeneration. Therefore, most of the protocols used immunomodulation treatments during cell therapy and it should be advantageous to establish protocols, which do not requires modulation of the immune system. In addition, a totally safe way of gene correction should be demonstrated, as the stability of the corrected gene with long term monitoring studies. Similarly, the genetic stability of cells which have been reprogrammed into pluripotency and then differentiated into the myogenic lineage should be checked. There is also a need for a clinically relevant cell transplantation protocol with good manufacturing procedures at each step of the process. Another concern is that if past studies have demonstrated a local improvement at the muscle level, a real functionality and long time improvements for the patient have not yet been attained. After treatment, the muscle strength notably is still far from the one found in their natural counterparts. Therefore, more research is needed to improve both gene and cell therapies which have the potential to revolutionize the field of regenerative medicine by developing patient specific treatment. These improvements will accelerate the clinical translation and help to realize therapeutic goals.

Acknowledgements

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