MIT Open Access Articles

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

The MIT Faculty has made this article openly available. ***Please share*** how this access benefits you. Your story matters.

Citation	Ostrovidov, Serge, Xuetao Shi, Ramin Banan Sadeghian, Sahar Salehi, Toshinori Fujie, Hojae Bae, Murugan Ramalingam, and Ali Khademhosseini. "Stem Cell Differentiation Toward the Myogenic Lineage for Muscle Tissue Regeneration: A Focus on Muscular Dystrophy." Stem Cell Reviews and Reports (September 2, 2015).
As Published	http://dx.doi.org/10.1007/s12015-015-9618-4
Publisher	Springer US
Version	Author's final manuscript
Citable link	http://hdl.handle.net/1721.1/103523
Terms of Use	Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.

Massachusetts Institute of Technology

DSpace@MIT

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

Serge Ostrovidov[1], Xuetao Shi[2], Ramin Banan Sadeghian[1], Sahar Salehi[1], Toshinori Fujie[3], Hojae Bae[4], Murugan Ramalingam[1, 5] and Ali Khademhosseini[1, 4, 6, 7, 8, 9 *]

[1]WPI-Advanced Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan.

[2]Guangzhou Higher Education Mega Center, South China University of Technology, Panyu district, Guangzhou 510006, China.

[3]Department of Life Science and Medical Bioscience, School of Advanced Science and Engineering, Waseda University, Tokyo 162-8480, Japan.

[4]College of Animal Bioscience and Technology, Department of Bioindustrial Technologies, Konkuk University, Hwayang-dong, Kwangjin-gu, Seoul 143-701, Republic of Korea.

[5]Centre for Stem Cell Research, Christian Medical College Bagayam Campus, Vellore 632002, India.

[6]Biomaterials Innovation Research Center, Division of Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston 02139, MA, United States of America.

[7]Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge 02139, MA, United States of America.

[8]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston 02115, MA, United States of America.

[9]Department of Physics, King Abdulaziz University, Jeddah 21569, Saudi Arabia.

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.

Keywords: Muscle, DMD, Muscular dystrophy, Stem cells, Tissue engineering, Scaffold

Contents

1. Introduction……………………………………………………………….………................3

2. Stem cells for muscular tissue engineering……..………………………………………..........7

3. Importance of scaffolds in muscle tissue engineering……………………………………… 10

4. Scaffold fabrication techniques and materials used for tissue constructs with stem cells ...….12

4.1 Electrospinning…...…………………………………………………………….…12

4.2 Phase separation…………...……………………………………………………………13

4.3 Self-assembly…………...……………………………………………………………14

5. Scaffolds used with stem cells……..……………………………………………………….15

6. Methods for obtaining myogenic progenitor cells from stem cells...…………………….......22

6.1 Myoblast derivation from ESCs……………..…………………...…………………23

6.2 Myoblast derivation from iPS cells……………………...…………………...........26

6.3 Myoblast derivation from mesangioblasts……………………………………………27

7. Applications of stem cell therapy in Duchenne muscular dystrophy………………………...29

Conclusion……………………………………………………………………..…...…...33

Acknowledgements…….…………………………………………………………………….35

References……………………………………………………………..……………….36

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.

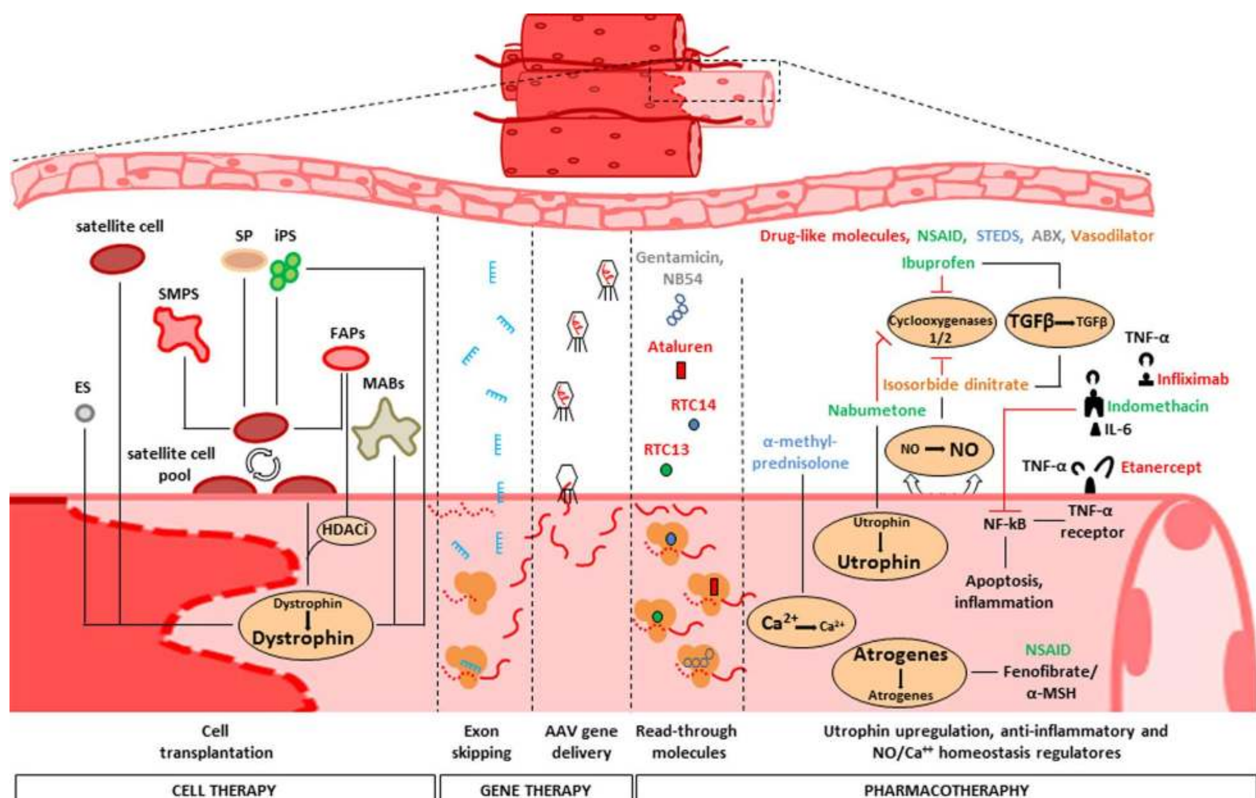


Figure 1: Schematic of main therapeutic approaches for muscle regeneration in dystrophinopathies. Reprinted with permission from Berardi et al. 2014 [22]©Berardi, Annibali, Cassano, Crippa and Sampaolesi.

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/α7 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 $Sca\text{-}1^+/ABCG2^+/CD45^-/CD43^-/c\text{-}kit^-/Pax7^-$. 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 $Sca\text{-}1^+/ABCG2^+/CD45^-/Pax7^+/Syndecan\text{-}4^+$ 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 $CD34^+/c\text{-}kit^-/Flk1^+/Sca\text{-}1^+/NKX2.5^-/Myf5^-/Oct4^-$ [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 $CD45^-/CD34^-/CD56^-/CD144^-/CD146^+/PDGFR\text{-}\beta1^+/NG2$ 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 $Sca\text{-}1^+/CD45^-/CD34^-/Flk1^+/Desmin^+/M\text{-}cadherin^-$ [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].

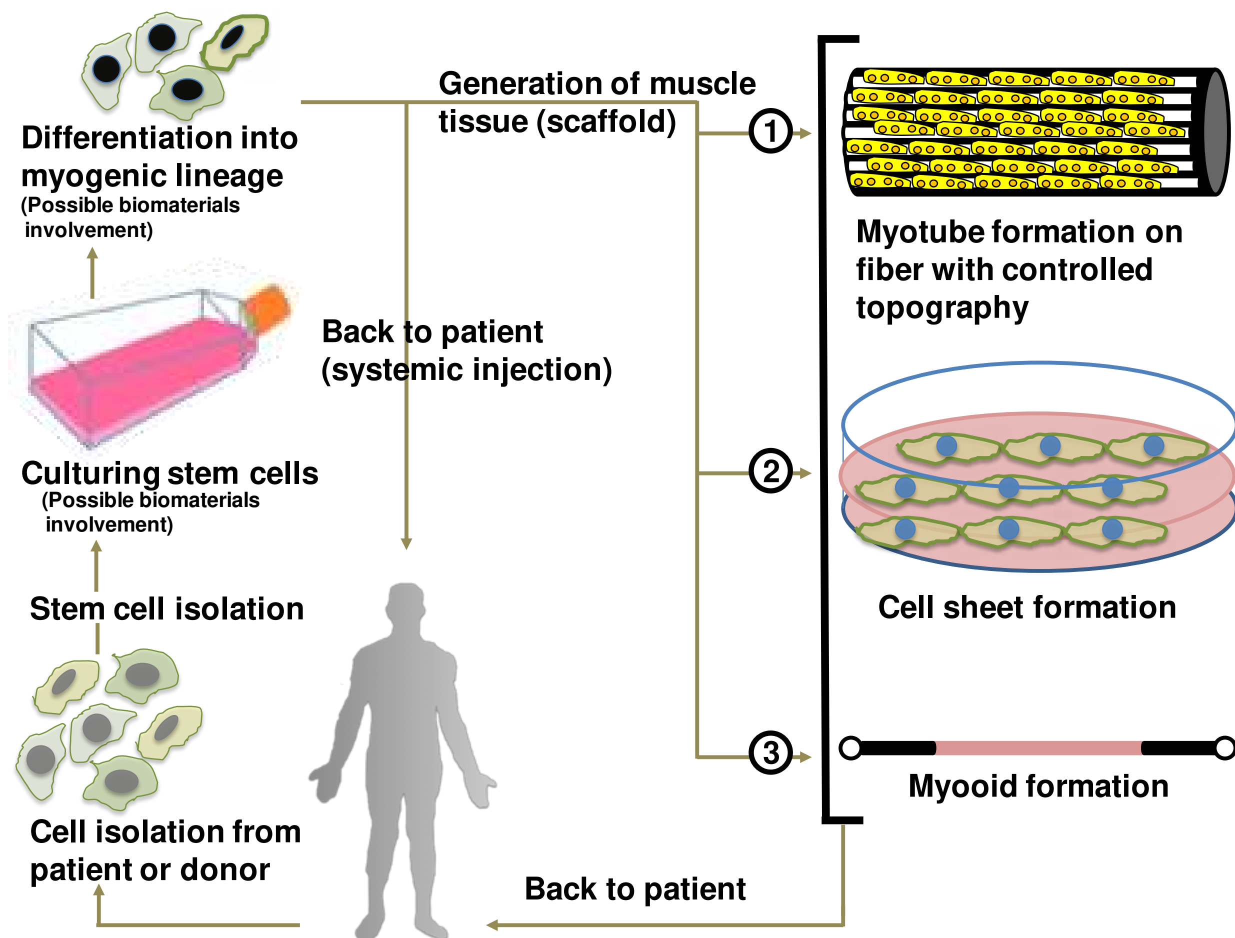


Figure 2: Schematic of the concept of muscle tissue engineering using stem cells. Additional steps with potential biomaterials involvement are indicated. Cells differentiated into the myogenic lineage may be delivered to the patient directly via systemic injection or after further differentiation into 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.

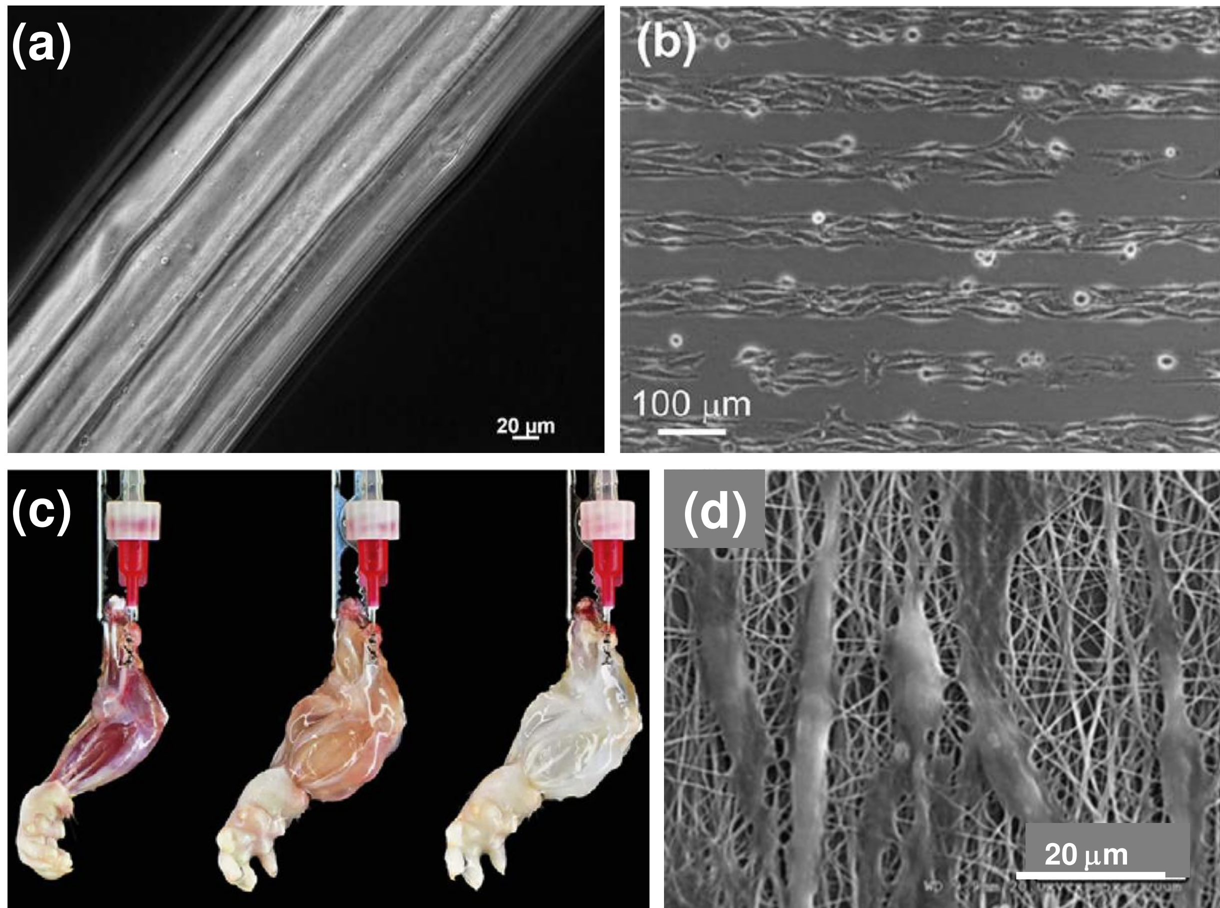


Figure 3: Pictures of different scaffolds used for muscle tissue engineering. (a) Grooved GelMA fiber, (b) Polystyrene nanomembrane with patterned lines of C2C12 cells, (c) Decellularized mouse limb, (d) Aligned PCL/collagen nanofibers with human skeletal muscle cells. Reprinted with permission from 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.

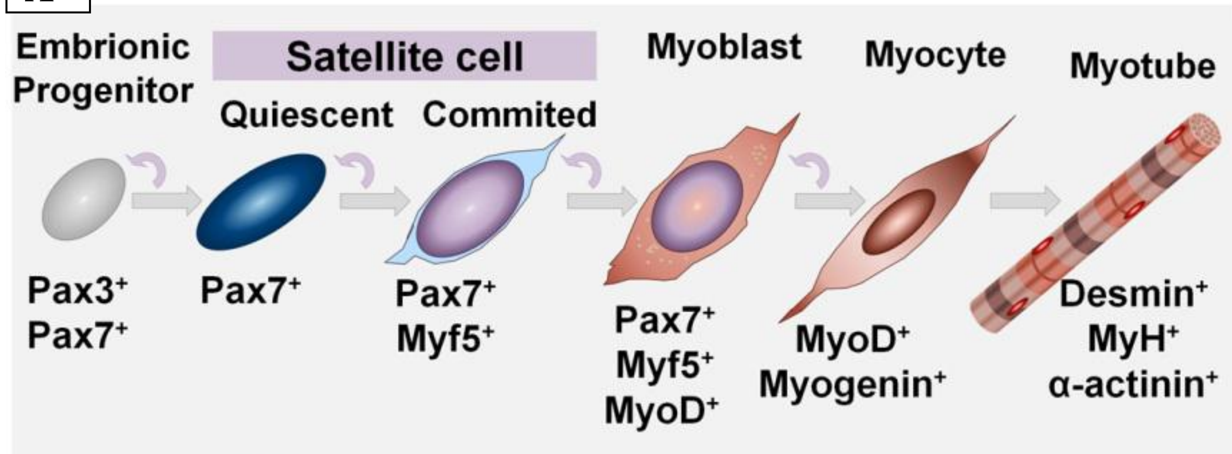
Reference	**Darabi *et al.*, 2011** [177]	**Mizuno *et al.*, 2010 [176]**
Starting cells	iPAX7-miPSCs	miPSCs
EB acquisition	Hanging drop	Hanging drop
Duration (days)	5	4-6
Myogenic cell differentiation		
Media	DMEM, 2% HS	DMEM, 10% FCS, 5% HS
Factors		NEAA, β-ME
Duration (days)	7	27

6.3 Myoblast derivation from mesoangioblasts

Mesoangioblasts are characterized as $CD34^{+}$/c-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].

A



B

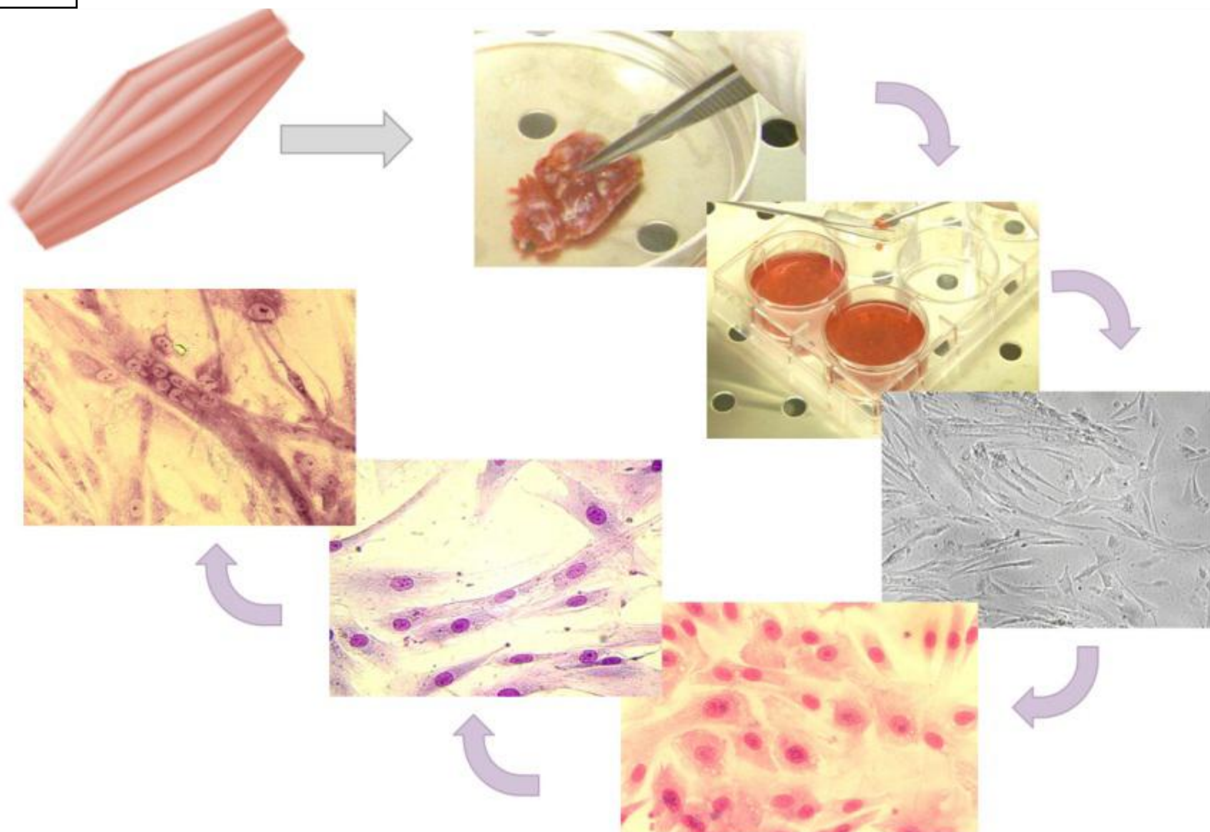


Figure 4. Myogenic cell characterization and culture. The myogenic cell lineage can be identified in each differentiation state and follows tightly regulated proliferation and differentiation cycles. From the embryonic state until terminal differentiation into muscle fibers, an intricate network of transcription 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].

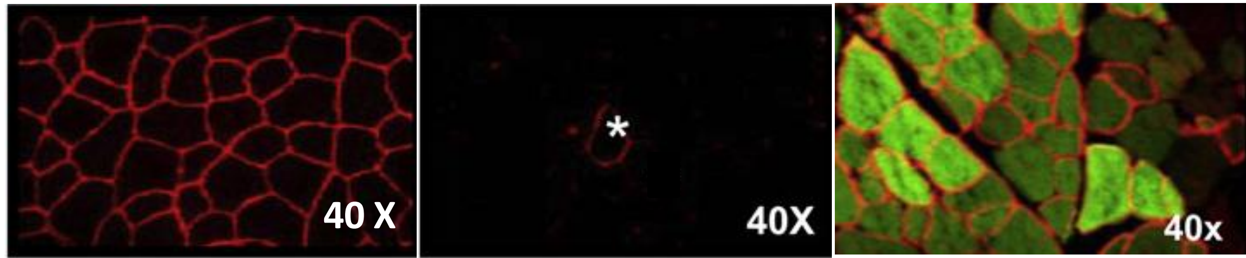


Figure 5: Immunostainning (in red) for dystrophyn protein in transverse frozen sections of Gastrocnemius muscle obtained from (a) wild type C57HBL/ka mouse, (b) mdx mouse, (c) mdx mouse transplanted 4 weeks previously with 11 000 green fluorescence positive (GFP^+) skeletal muscle progenitors showing 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

This work was supported by the World Premier International Research Center Initiative (WPI), MEXT, Japan.

References

1 Miyagoe-Suzuki Y., Fukada S., Takeda S. (2012). Muscle satellite cells and Duchenne muscular dystrophy. In Muscular Dystrophy, Dr. Madhuri Hegde (Ed.), ISBN: 978-953-51-0603-6, InTech, DOI: 10.5772/30769. Available from: http://www.intechopen.com/books/muscular-dystrophy/muscle-satellite-cells-and-muscular-dystrophy.

2 Goncalves, M. A. F. V., de Vries, A. A. F., Holkers, M., et al. (2006). Human mesenchymal stem cells ectopically expressing full-length dystrophin can complement Duchenne muscular dystrophy myotubes by cell fusion. *Human Molecular Genetics*, 15(2), 213-221.

3 Gawlik, K. I., Holmberg, J., & Durbeej, M. (2014). Loss of dystrophin and β-sarcoglycan significantly exacerbates the phenotype of laminin α2 chain-deficient animals. *The American Journal of Pathology*, 184(3), 740-752.

4 Ervasti, J. M., & Sonnemann, K. J. (2008). Biology of the striated muscle dystrophin–glycoprotein complex. In: Kwang, W. J. (Ed.), International Review of Cytology (pp. 191-225). Academic Press.

5 Constantin, B. (2014). Dystrophin complex functions as a scaffold for signalling proteins. *Biochimica et Biophysica Acta*, 1838(2), 635-642.

6 Ervasti, J. M., & Campbell, K. P. (1991). Membrane organization of the dystrophin-glycoprotein complex. *Cell*, 66(6), 1121-1131.

7 Mosqueira, M., Zeiger, U., Förderer, M., Brinkmeier, H., & Fink, R. H. A. (2013). Cardiac and respiratory dysfunction in Duchenne muscular dystrophy and the role of second messengers. *Medicinal Research Reviews*, 33(5), 1174-1213.

8 Onopiuk, M., Brutkowski, W., Young, C., et al. (2015). Store-operated calcium entry contributes to abnormal Ca2+ signalling in dystrophic mdx mouse myoblasts. *Archives of Biochemistry and Biophysics*, 569, 1-9.

9 Arechavala-Gomeza, V., Kinali, M., Feng, L., et al. (2010). Revertant fibres and dystrophin traces in Duchenne muscular dystrophy: Implication for clinical trials. *Neuromuscular Disorders*, 20(5), 295-301.

10 Manning, J., & O'Malley, D. (2015). What has the mdx mouse model of duchenne muscular dystrophy contributed to our understanding of this disease? *Journal of Muscle Research and Cell Motility*, 36(2), 155-167.

11 Berry, S. E., Liu, J., Chaney, E. J., & Kaufman, S. J. (2007). Multipotential mesoangioblast stem cell therapy in the mdx/utrn-/- mouse model for Duchenne muscular dystrophy. *Regenerative Medicine*, 2(3), 275-288.

12 Zatz, M., Vieira, N. M., Zucconi, E., et al. (2015). A normal life without muscle dystrophin. *Neuromuscular Disorders*, 25(5), 371-374.

13 Vieira, N. M., Guo, L. T., Estrela, E., et al. (2015). Muscular dystrophy in a family of Labrador Retrievers with no muscle dystrophin and a mild phenotype. *Neuromuscular Disorders*, 25(5), 363-370.

14 Larcher, T., Lafoux, A., Tesson, L., et al. (2014). Characterization of dystrophin deficient rats: A new model for Duchenne muscular dystrophy. *PLoS ONE*, 9(10), e110371.

15 Klymiuk, N., Thirion, C., Burkhardt, K., et al. (2011). 238 tailored pig model of Duchenne muscular dystrophy *Reproduction, Fertility and Development*, 24(1), 231-231.

16 Neuman, S., Kaban, A., Volk, T., Yaffe, D., & Nudel, U. (2001). The dystrophin / utrophin homologues in Drosophila and in sea urchin. *Gene*, 263(1–2), 17-29.

17 McGreevy, J. W., Hakim, C. H., McIntosh, M. A., & Duan, D. (2015). Animal models of Duchenne muscular dystrophy: From basic mechanisms to gene therapy. *Disease Models & Mechanisms*, 8(3), 195-213.

18 Pozzobon, M., Franzin, C., Piccoli, M., & De Coppi, P. (2014). Fetal stem cells and skeletal muscle regeneration: A therapeutic approach. *Frontiers in Aging Neuroscience*, 6(article 222), 1-6.

19 Braun, R., Wang, Z., Mack, D. L., & Childers, M. K. (2014). Gene therapy for inherited muscle diseases: Where genetics meets rehabilitation medicine. *American Journal of Physical Medicine & Rehabilitation*, 93(11), S97-S107.

20 Jarmin, S., Kymalainen, H., Popplewell, L., & Dickson, G. (2014). New developments in the use of gene therapy to treat Duchenne muscular dystrophy. *Expert Opinion on Biological Therapy*, 14(2), 209-230.

21 Al-Zaidy, S., Rodino-Klapac, L., & Mendell, J. R. (2014). Gene therapy for muscular dystrophy: Moving the field forward. *Pediatric Neurology*, 51(5), 607-618.

22 Berardi, E., Annibali, D., Cassano, M., Crippa, S., & Sampaolesi, M. (2014). Molecular and cell-based therapies for muscle degenerations: a road under construction. *Frontiers in Physiology*, 5(article 119), 1-13.

23 Abdel-Hamid H, & Clemens PR (2012). Pharmacological therapies for muscular dystrophies. *Current Opinion in Neurology*, 25(5), 604-608.

24 Filareto, A., Darabi, R., & Perlingeiro, R. C. R. (2012). Engraftment of ES-derived myogenic progenitors in a severe mouse model of muscular dystrophy. *Journal of Stem Cell Research & Therapy*, 10(1), S10-001.

25 Sakai, H., Sato, T., Sakurai, H., et al. (2013). Fetal skeletal muscle progenitors have regenerative capacity after intramuscular engraftment in dystrophin deficient mice. *PLoS ONE*, 8(5), e63016.

26 Roca I, Requena J, Edel MJ, & AB, A.-P. (2015). Myogenic precursors from iPS cells for skeletal muscle cell replacement therapy. *Journal of Clinical Medicine*, 4(2), 243-259.

27 Mauro, A. (1961). Satellite cells of skeletal muscle fibers. *Journal of Biophysical and Biochemical Cytology*, 9(2), 493-495.

28 Ostrovidov, S., Hosseini, V., Ahadian, S., et al. (2014). Skeletal muscle tissue engineering: Methods to form skeletal myotubes and their applications. *Tissue Engineering Part B: Reviews*, 20(5), 403-436.

29 Fishman, J. M., Tyraskis, A., Maghsoudlou, P., et al. (2013). Skeletal muscle tissue engineering: Which cell to use? *Tissue Engineering Part B: Reviews*, 19(6), 503-515.

30 Yin, H., Price, F., & Rudnicki, M. A. (2013). Satellite cells and the muscle stem cell niche. *Physiological Reviews*, 93(1), 23-67.

31 Montarras, D., Morgan, J., Collins, C., et al. (2005). Direct isolation of satellite cells for skeletal muscle regeneration. *Science*, 309(5743), 2064-2067.

32 Fu, X., Wang, H., & Hu, P. (2015). Stem cell activation in skeletal muscle regeneration. *Cellular and Molecular Life Sciences*, 72(9), 1663-1677.

33 Zammit, P. S., Golding, J. P., Nagata, Y., et al. (2004). Muscle satellite cells adopt divergent fates: A mechanism for self-renewal? *The Journal of Cell Biology*, 166(3), 347-357.

34 Kuang, S., Kuroda, K., Le Grand, F., & Rudnicki, M. A. (2007). Asymmetric self-renewal and commitment of satellite stem cells in muscle. *Cell*, 129(5), 999-1010.

35 Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., et al. (1998). Embryonic stem cell lines derived from human blastocysts. *Science*, 282(5391), 1145-1147.

36 Preynat-Seauve, O., Krause, K.-H., & Villard, J. (2011). The immune barriers of cell therapy with allogenic stem cells of embryonic origin. In: Artmann, G. M., Minger, S.&Hescheler, J. (Ed.), Stem Cell Engineering (pp. 181-197). Springer Berlin Heidelberg.

37 Ben-David, U., & Benvenisty, N. (2011). The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nature Reviews Cancer*, 11(4), 268-277.

38 Barberi, T., Bradbury, M., Dincer, Z., et al. (2007). Derivation of engraftable skeletal myoblasts from human embryonic stem cells. *Nature Medicine*, 13(5), 642-648.

39 Zheng, J. K., Wang, Y., Karandikar, A., et al. (2006). Skeletal myogenesis by human embryonic stem cells. *Cell Research*, 16(8), 713-722.

40 Penton, C. M., Thomas-Ahner, J. M., Johnson, E. K., McAllister, C., & Montanaro, F. (2013). Muscle side population cells from dystrophic or injured muscle adopt a fibro-adipogenic fate. *PLoS ONE*, 8(1), e54553.

41 Asakura, A., Seale, P., Girgis-Gabardo, A., & Rudnicki, M. A. (2002). Myogenic specification of side population cells in skeletal muscle. *The journal of Cell Biology*, 159(1), 123-134.

42 Meeson, A. P., Hawke, T. J., Graham, S., et al. (2004). Cellular and molecular regulation of skeletal muscle side population cells. *Stem Cells*, 22(7), 1305-1320.

43 Bachrach, E., Li, S., Perez, A. L., et al. (2004). Systemic delivery of human microdystrophin to regenerating mouse dystrophic muscle by muscle progenitor cells. *Proceedings of the National Academy of Sciences -U. S. A.*, 101(10), 3581-3586.

44 Tanaka, K. K., Hall, J. K., Troy, A. A., et al. (2009). Syndecan-4-expressing muscle progenitor cells in the SP engraft as satellite cells during muscle regeneration. *Cell Stem Cell*, 4(3), 217-225.

45 Mitchell, K. J., Pannerec, A., Cadot, B., et al. (2010). Identification and characterization of a non-satellite cell muscle resident progenitor during postnatal development. *Nature Cell Biology*, 12(3), 257-266.

46 Montarras, D., L'Honoré, A., & Buckingham, M. (2013). Lying low but ready for action: the quiescent muscle satellite cell. *FEBS Journal*, 280(17), 4036-4050.

47 Sampaolesi, M., Blot, S., D'Antona, G., et al. (2006). Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature*, 444(7119), 574-579.

48 Meregalli, M., Farini, A., Belicchi, M., et al. (2013). Perspectives of stem cell therapy in Duchenne muscular dystrophy. *FEBS Journal*, 280(17), 4251-4262.

49 Dellavalle, A., Sampaolesi, M., Tonlorenzi, R., et al. (2007). Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. *Nature Cell Biology*, 9(3), 255-267.

50 Torrente, Y., Belicchi, M., Sampaolesi, M., et al. (2004). Human circulating AC133+ stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *The Journal of Clinical Investigation* 114(2), 182-195.

51 Usas, A., & Huard, J. (2007). Muscle-derived stem cells for tissue engineering and regenerative therapy. *Biomaterials*, 28(36), 5401-5406.

52 Torrente, Y., Tremblay, J.-P., Pisati, F., et al. (2001). Intraarterial injection of muscle-derived Cd34+Sca-1+ stem cells restores dystrophin in mdx mice. *The Journal of Cell Biology*, 152(2), 335-348.

53 Rouger, K., Larcher, T., Dubreil, L., et al. (2011). Systemic delivery of allogenic muscle stem cells induces long-term muscle repair and clinical efficacy in Duchenne muscular dystrophy dogs. *The American Journal of Pathology*, 179(5), 2501-2518.

54 Yamanaka, S. (2007). Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell*, 1(1), 39-49.

55 Singh, V. K., Kalsan, M., Kumar, N., Saini, A., & Chandra, R. (2015). Induced Pluripotent Stem Cells: Applications in regenerative medicine, disease modelling and drug discovery. *Frontiers in Cell and Developmental Biology*, 3(2), 1-18.

56 Takahashi, K., Tanabe, K., Ohnuki, M., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, 131(5), 861-872.

57 Zhao, T., Zhang, Z.-N., Rong, Z., & Xu, Y. (2011). Immunogenicity of induced pluripotent stem cells. *Nature*, 474(7350), 212-215.

58 Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T., & Yamanaka, S. (2008). Generation of mouse induced pluripotent stem cells without viral vectors. *Science*, 322, 949-953.

59 Park, I.-H., Arora, N., Huo, H., et al. (2008). Disease-specific induced pluripotent stem cells. *Cell*, 134(5), 877-886.

60 Tanaka, A., Woltjen, K., Miyake, K., et al. (2013). Efficient and reproducible myogenic differentiation from human iPS cells: Prospects for modeling Miyoshi myopathy *in vitro*. *PLoS ONE*, 8(4), e61540.

61 Darabi, R., Arpke, R. W., Irion, S., et al. (2012). Human ES- and iPS-derived myogenic progenitors restore dystrophin and improve contractility upon transplantation in dystrophic mice. *Cell Stem Cell*, 10(5), 610-619.

62 Meregalli, M., Farini, A., Sitzia, C., & Torrente, Y. (2014). Advancements in stem cells treatment of skeletal muscle wasting. *Frontiers in Physiology*, 5(article 48), 1-12.

63 Hosoyama, T., McGivern, J. V., Van Dyke, J. M., Ebert, A. D., & Suzuki, M. (2014). Derivation of myogenic progenitors directly from human pluripotent stem cells using a sphere-based culture. *Stem Cells Translational Medicine*, 3, 564-574.

64 Awaya, T., Kato, T., Mizuno, Y., et al. (2012). Selective development of myogenic mesenchymal cells from human embryonic and induced pluripotent stem cells. *PLoS ONE*, 7(12), e51638.

65 Filareto, A., Parker, S., Darabi, R., et al. (2013). An ex vivo gene therapy approach to treat muscular dystrophy using inducible pluripotent stem cells. *Nature Communications*, 4, 1549.

66 Schuurman, W., Khristov, V., Pot, M. W., et al. (2011). Bioprinting of hybrid tissue constructs with tailorable mechanical properties. *Biofabrication*, 3(2), 1-7.

67 Sant, S., Hancock, M. J., Donnelly, J. P., Iyer, D., & Khademhosseini, A. (2010). Biomimetic gradient hydrogels for tissue engineering. *The Canadian Journal of Chemical Engineering*, 88(6), 899-911.

68 Ostrovidov Serge, Seidi Azadeh, Kaarunya Sampathkumar, et al. (2014). Introduction to nanobioscience: A tissue engineering perspective. In: Valeri Kharkin, Chunli Bai, Osama O. Awadelkarim & Kapitza, S. (Ed.), *Nanoscience and Nanotechnologies*. In *Encyclopedia of Life Support Systyem (EOLSS)*, developped under the auspices of the UNESCO. Paris, France: Eolss Publishers.

69 Ramalingam, M., & Khademhosseini, A. (2012). Micropatterned Biomaterials for Cell and Tissue Engineering. In: John P . Fisher , A. G. M., Joseph D . Bronzino , and Donald R . Peterson (Ed.), Tissue Engineering Principles and Practices (pp. 1-18). CRC Press.

70 Ostrovidov, S., Seidi, A., Ahadian, S., Ramalingam, M., & Khademhosseini, A. (2013). Micro- and nanoengineering approaches to developing gradient biomaterials suitable for interface tissue engineering. In: Murugan Ramalingam, E. J., Seeram Ramakrishna, Ali Khademhosseini (Ed.), Micro and nanotechnologies in engineering stem cells and tissues (pp. 52-79). Hoboken, New Jersey: John Wiley & Sons, Inc.

71 Bajaj, P., Schweller, R. M., Khademhosseini, A., West, J. L., & Bashir, R. (2014). 3D Biofabrication strategies for tissue engineering and regenerative medicine. *Annual Review of Biomedical Engineering*, 16, 247-276.

72 Dolatshahi-Pirouz, A., Nikkhah, M., Kolind, K., Dokmeci, M. R., & Khademhosseini, A. (2011). Micro- and nanoengineering approaches to control stem cell-biomaterial interactions. *Journal of Functional Biomaterials*, 2(3), 88.

73 Murugan, R., & Ramakrishna, S. (2006). Nano-featured scaffolds for tissue engineering: A review of spinning methodologies. *Tissue Engineering*, 12(3), 435-447.

74 Mukherjee, S., Venugopal, J. R., Ravichandran, R., et al. (2013). Nanofiber technology for controlling stem cell functions and tissue engineering. In: Murugan Ramalingam, E. J., Seeram Ramakrishna, Ali Khademhosseini (Ed.), Micro and nanotechnologies in engineering stem cells and tissues (pp. 27-51). John Wiley & Sons, Inc.

75 Murugan, R., & Ramakrishna, S. (2007). Design strategies of tissue engineering scaffolds with controlled fiber orientation. *Tissue Engineering*, 13(8), 1845-1866.

76 Baker, B. M., Gee, A. O., Metter, R. B., et al. (2008). The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers. *Biomaterials*, 29(15), 2348-2358.

77 Sundararaghavan, H. G., Metter, R. B., & Burdick, J. A. (2009). Electrospun fibrous scaffolds with multiscale and photopatterned porosity. *Macromolecular Bioscience*, 10(3), 265-270.

78 Seidi, A., Ramalingam, M., Elloumi-Hannachi, I., Ostrovidov, S., & Khademhosseini, A. (2011). Gradient biomaterials for soft-to-hard interface tissue engineering. *Acta Biomaterialia*, 7(4), 1441-1451.

79 Lim, S. H., & Mao, H.-Q. (2009). Electrospun scaffolds for stem cell engineering. *Advanced Drug Delivery Reviews*, 61(12), 1084-1096.

80 Huang, N. F., Patel, S., Thakar, R. G., et al. (2006). Myotube assembly on nanofibrous and micropatterned polymers. *Nano Letters*, 6(3), 537-542.

81 Riboldi, S. A., Sadr, N., Pigini, L., et al. (2008). Skeletal myogenesis on highly orientated microfibrous polyesterurethane scaffolds. *Journal of Biomedical Materials Research Part A*, 84A(4), 1094-1101.

82 Li, M., Guo, Y., Wei, Y., MacDiarmid, A. G., & Lelkes, P. I. (2006). Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications. *Biomaterials*, 27(13), 2705-2715.

83 McKeon-Fischer, K. D., & Freeman, J. W. (2011). Characterization of electrospun poly(L-lactide) and gold nanoparticle composite scaffolds for skeletal muscle tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine*, 5(7), 560-568.

84 Ostrovidov, S., Shi, X., Zhang, L., et al. (2014). Myotube formation on gelatin nanofibers-multi-walled carbon nanotubes hybrid scaffolds. *Biomaterials*, 35(24), 6268-6277.

85 Liao, I. C., Liu, J., Bursac, N., & Leong, K. (2008). Effect of electromechanical stimulation on the maturation of myotubes on aligned electrospun fibers. *Cellular and Molecular Bioengineering*, 1(2), 133-145.

86 Nam, Y. S., & Park, T. G. (1999). Porous biodegradable polymeric scaffolds prepared by thermally induced phase separation. *Journal of Biomedical Materials Research Part A*, 47(1), 8-17.

87 Liu, X., & Ma, P. X. (2009). Phase separation, pore structure, and properties of nanofibrous gelatin scaffolds. *Biomaterials*, 30(25), 4094-4103.

88 Zhang R, & Ma PX (2000). Synthetic nano-fibrillar extracellular matrices with predesigned macroporous architectures. *Journal of Biomedical Materials Research* 52(2), 430-438.

89 Holzwarth, J. M., & Ma, P. X. (2011). 3D nanofibrous scaffolds for tissue engineering. *Journal of Materials Chemistry*, 21(28), 10243-10251.

90 Nieponice, A., Soletti, L., Guan, J., et al. (2008). Development of a tissue-engineered vascular graft combining a biodegradable scaffold, muscle-derived stem cells and a rotational vacuum seeding technique. *Biomaterials*, 29(7), 825-833.

91 Van Vlierberghe, S., Cnudde, V., Dubruel, P., et al. (2007). Porous gelatin hydrogels: 1. Cryogenic formation and structure analysis. *Biomacromolecules*, 8(2), 331-337.

92 Yu, Y.-C., Roontga, V., Daragan, V. A., et al. (1999). Structure and dynamics of peptide-amphiphiles incorporating triple-helical proteinlike molecular architecture. *Biochemistry*, 38(5), 1659-1668.

93 Du, Y., Lo, E., Ali, S., & Khademhosseini, A. (2008). Directed assembly of cell-laden microgels for fabrication of 3D tissue constructs. *Proceedings of the National Academy of Sciences -USA*, 105(28), 9522-9527.

94 Hartgerink, J. D., Beniash, E., & Stupp, S. I. (2002). Peptide-amphiphile nanofibers: A versatile scaffold for the preparation of self-assembling materials. *Proceedings of the National Academy of Sciences -USA*, 99(8), 5133-5138.

95 Zhang, S., Gelainb, F., & Zhaoc, X. (2005). Designer self-assembling peptide nanofiber scaffolds for 3D tissue cell cultures. *Seminars in Cancer Biology*, 15(5), 413-420.

96 Tambralli , A., Blakeney , B., Anderson , J., et al. (2009). A hybrid biomimetic scaffold composed of electrospun polycaprolactone nanofibers and self-assembled peptide amphiphile nanofibers. *Biofabrication*, 1(2), 025001.

97 Liu, G., Pareta, R. A., Wu, R., et al. (2013). Skeletal myogenic differentiation of urine-derived stem cells and angiogenesis using microbeads loaded with growth factors. *Biomaterials*, 34(4), 1311-1326.

98 Wang, L., Cao, L., Shansky, J., et al. (2014). Minimally invasive approach to the repair of injured skeletal muscle with a shape-memory scaffold. *Molecular Therapy*, 22(8), 1441-1449.

99 Sicari, B. M., Rubin, J. P., Dearth, C. L., et al. (2014). An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss. *Science Translational Medicine*, 6(234), 234ra58.

100 Dang, J. M., & Leong, K. W. (2007). Myogenic induction of aligned mesenchymal stem cell sheets by culture on thermally responsive electrospun nanofibers. *Advanced Materials*, 19(19), 2775-2779.

101 Ding, K., Yang, Z., Zhang, Y.-L., & Xu, J.-Z. (2013). Injectable thermosensitive chitosan/β-glycerophosphate/collagen hydrogel maintains the plasticity of skeletal muscle satellite cells and supports their in vivo viability. *Cell Biology International*, 37(9), 977-987.

102 Annabi, N., Mithieux, S. M., Camci-Unal, G., et al. (2013). Elastomeric recombinant protein-based biomaterials. *Biochemical Engineering Journal*, 77, 110-118.

103 Liu, J., Xu, H. H. K., Zhou, H., et al. (2013). Human umbilical cord stem cell encapsulation in novel macroporous and injectable fibrin for muscle tissue engineering. *Acta Biomaterialia*, 9(1), 4688-4697.

104 Desiderio, V., De Francesco, F., Schiraldi, C., et al. (2013). Human Ng2+ adipose stem cells loaded in vivo on a new crosslinked hyaluronic acid-lys scaffold fabricate a skeletal muscle tissue. *Journal of Cellular Physiology*, 228(8), 1762-1773.

105 Ziv, K., Nuhn, H., Ben-Haim, Y., et al. (2014). A tunable silk-alginate hydrogel scaffold for stem cell culture and transplantation. *Biomaterials*, 35(12), 3736-3743.

106 Sun, W., Incitti, T., Migliaresi, C., et al. (2014). Genipin-crosslinked gelatin–silk fibroin hydrogels for modulating the behaviour of pluripotent cells. *Journal of Tissue Engineering and Regenerative Medicine*, doi: 10.1002/term.1868.

107 Ricotti, L., Polini, A., Genchi, G., et al. (2012). Proliferation and skeletal myotube formation capability of C2C12 and H9c2 cells on isotropic and anisotropic electrospun nanofibrous PHB scaffolds. *Biomedical Materials*, 7(3), 035010.

108 Aviss, K., Gough, J., & Downes, S. (2010). Aligned electrospun polymer fibres for skeletal muscle regeneration. *European Cells and Materials*, 19, 193-204.

109 Ju, Y. M., Atala, A., Yoo, J. J., & Lee, S. J. (2014). In situ regeneration of skeletal muscle tissue through host cell recruitment. *Acta Biomaterialia*, 10(10), 4332-4339.

110 Fujie, T., Ahadian, S., Liu, H., et al. (2013). Engineered nanomembranes for directing cellular organization toward flexible biodevices. *Nano Letters*, 13(7), 3185-3192.

111 Choi, J. S., Lee, S. J., Christ, G. J., Atala, A., & Yoo, J. J. (2008). The influence of electrospun aligned poly(ε-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes. *Biomaterials*, 29(19), 2899-2906.

112 Saxena, A. K., Marler, J., Benvenuto, M., Willital, G. H., & Vacanti, J. P. (1999). Skeletal Muscle Tissue Engineering Using Isolated Myoblasts on Synthetic Biodegradable Polymers: Preliminary Studies. *Tissue Engineering*, 5(6), 525-531.

113 Chaudhuri, B., Bhadra, D., Mondal, B., & Pramanik, K. (2014). Biocompatibility of electrospun graphene oxide-poly(ε-caprolactone) fibrous scaffolds with human cord blood mesenchymal stem cells derived skeletal myoblast. *Materials Letters*, 126, 109-112.

114 Ku, S. H., & Park, C. B. (2013). Myoblast differentiation on graphene oxide. *Biomaterials*, 34(8), 2017-2023.

115 Ku, S. H., Lee, S. H., & Park, C. B. (2012). Synergic effects of nanofiber alignment and electroactivity on myoblast differentiation. *Biomaterials*, 33(26), 6098-6104.

116 Murphy, W. L., McDevitt, T. C., & Engler, A. J. (2014). Materials as stem cell regulators. *Nature Materials*, 13(6), 547-557.

117 Lutolf, M. P., Gilbert, P. M., & Blau, H. M. (2009). Designing materials to direct stem-cell fate. *Nature*, 462(7272), 433-441.

118 Kingham, E., & Oreffo, R. O. C. (2013). Embryonic and Induced Pluripotent Stem Cells: Understanding, Creating, and Exploiting the Nano-Niche for Regenerative Medicine. *ACS Nano*, 7(3), 1867-1881.

119 Discher, D. E., Mooney, D. J., & Zandstra, P. W. (2009). Growth factors, matrices, and forces combine and control stem cells. *Science*, 324(5935), 1673-1677.

120 Schvartzman, M., Palma, M., Sable, J., et al. (2011). Nanolithographic control of the spatial organization of cellular adhesion receptors at the single-molecule level. *Nano Letters*, 11(3), 1306-1312.

121 McMurray, R. J., Gadegaard, N., Tsimbouri, P. M., et al. (2011). Nanoscale surfaces for the long-term maintenance of mesenchymal stem cell phenotype and multipotency. *Nature Materials*, 10(8), 637-644.

122 Dalby, M. J., Gadegaard, N., Tare, R., et al. (2007). The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nature Materials*, 6(12), 997-1003.

123 Huebsch, N., Arany, P. R., Mao, A. S., et al. (2010). Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nature Materials*, 9(6), 518-526.

124 Dingal, P. C. D. P., & Discher, D. E. (2014). Material control of stem cell differentiation: Challenges in nano-characterization. *Current Opinion in Biotechnology*, 28, 46-50.

125 Silva, G. A., Czeisler, C., Niece, K. L., et al. (2004). Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science*, 303(5662), 1352-1355.

126 Qazi, T. H., Mooney, D. J., Pumberger, M., Geißler, S., & Duda, G. N. (2015). Biomaterials based strategies for skeletal muscle tissue engineering: Existing technologies and future trends. *Biomaterials*, 53, 502-521.

127 Boldrin, L., Malerba, A., Vitiello, L., et al. (2008). Efficient delivery of human single fiber-derived muscle precursor cells via biocompatible scaffold. *Cell Transplantation*, 17(5), 577-584.

128 Dalby, M. J., Riehle, M. O., Johnstone, H., Affrossman, S., & Curtis, A. S. G. (2004). Investigating the limits of filopodial sensing: A brief report using SEM to image the interaction between 10 nm high nano-topography and fibroblast filopodia. *Cell Biology International*, 28(3), 229-236.

129 Hosseini, V., Ahadian, S., Ostrovidov, S., et al. (2012). Engineered contractile skeletal muscle tissue on a microgrooved methacrylated gelatin substrate. *Tissue Engineering Part A*, 18(23-24), 2453-2465.

130 Li, W.-J., Mauck, R. L., Cooper, J. A., Yuan, X., & Tuan, R. S. (2007). Engineering controllable anisotropy in electrospun biodegradable nanofibrous scaffolds for musculoskeletal tissue engineering. *Journal of Biomechanics*, 40(8), 1686-1693.

131 Jun, I., Jeong, S., & Shin, H. (2009). The stimulation of myoblast differentiation by electrically conductive sub-micron fibers. *Biomaterials*, 30(11), 2038-2047.

132 Ravichandran, R., Sridhar, R., Venugopal, J. R., et al. (2014). Gold nanoparticle loaded hybrid nanofibers for cardiogenic differentiation of stem cells for infarcted myocardium regeneration. *Macromolecular Bioscience*, 14(4), 515-525.

133 Biswadeep, C., Debabrata, B., Lorenzo, M., & Krishna, P. (2015). Myoblast differentiation of human mesenchymal stem cells on graphene oxide and electrospun graphene oxide–polymer composite fibrous meshes: importance of graphene oxide conductivity and dielectric constant on their biocompatibility. *Biofabrication*, 7(1), 015009.

134 Leung, M., Cooper, A., Jana, S., et al. (2013). Nanofiber-based in vitro system for high myogenic differentiation of human embryonic stem cells. *Biomacromolecules*, 14(12), 4207-4216.

135 Hwang, Y., Suk, S., Shih, Y.-R. V., et al. (2014). WNT3A promotes myogenesis of human embryonic stem cells and enhances in vivo engraftment. *Scientific Reports*, 4(article 5916), 1-9.

136 Peppas, N. A., Hilt, J. Z., Khademhosseini, A., & Langer, R. (2006). Hydrogels in biology and medicine: From molecular principles to bionanotechnology. *Advanced Materials*, 18(11), 1345-1360.

137 Annabi, N., Tamayol, A., Uquillas, J. A., et al. (2014). 25th Anniversary article: Rational design and applications of hydrogels in regenerative medicine. *Advanced Materials*, 26(1), 85-124.

138 Neal, D., Sakar, M. S., Ong, L.-L. S., & Harry Asada, H. (2014). Formation of elongated fascicle-inspired 3D tissues consisting of high-density, aligned cells using sacrificial outer molding. *Lab on a Chip*, 14(11), 1907-1916.

139 Neal, D., Sakar, M. S., Bashir, R., Chan, V., & Asada, H. H. (2015). Mechanical characterization and shape optimization of fascicle-like 3D skeletal muscle tissues contracted with electrical and optical stimuli. *Tissue Engineering Part A*, 21(11-12), 1848-1858.

140 Sakar, M. S., Neal, D., Boudou, T., et al. (2012). Formation and optogenetic control of engineered 3D skeletal muscle bioactuators. *Lab on a Chip*, 12(23), 4976-4985.

141 Chan, V., Neal, D. M., Uzel, S. G. M., et al. (2015). Fabrication and characterization of optogenetic, multi-strip cardiac muscles. *Lab on a Chip*, 15(10), 2258-2268.

142 Shi, X., Ostrovidov, S., Zhao, Y., et al. (2015). Microfluidic spinning of cell-responsive grooved microfibers. *Advanced Functional Materials*, 25, 2250-2259.

143 Gilbert, P. M., Havenstrite, K. L., Magnusson, K. E. G., et al. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science*, 329(5995), 1078-1081.

144 Dang, L. T. H., Feric, N. T., Laschinger, C., et al. (2014). Inhibition of apoptosis in human induced pluripotent stem cells during expansion in a defined culture using angiopoietin-1 derived peptide QHREDGS. *Biomaterials*, 35(27), 7786-7799.

145 Xiao, Y., Reis, L. A., Zhao, Y., & Radisic, M. (2015). Modifications of collagen-based biomaterials with immobilized growth factors or peptides. *Methods*, dx.doi.org/10.1016/j.ymeth.2015.04.025.

146 Reis, L. A., Chiu, L. L. Y., Wu, J., et al. (2015). Hydrogels with integrin-binding angiopoietin-1–derived peptide, QHREDGS, for treatment of acute myocardial infarction. *Circulation: Heart Failure*, 8(2), 333-341.

147 Rask, F., Dallabrida, S. M., Ismail, N. S., et al. (2010). Photocrosslinkable chitosan modified with angiopoietin-1 peptide, QHREDGS, promotes survival of neonatal rat heart cells. *Journal of Biomedical Materials Research Part A*, 95A(1), 105-117.

148 Salinas, C. N., & Anseth, K. S. (2008). The influence of the RGD peptide motif and its contextual presentation in PEG gels on human mesenchymal stem cell viability. *Journal of Tissue Engineering and Regenerative Medicine*, 2(5), 296-304.

149 Fuoco, C., Sangalli, E., Vono, R., et al. (2014). 3D hydrogel environment rejuvenates aged pericytes for skeletal muscle tissue engineering. *Frontiers in Physiology*, 5(article 203), 1-8.

150 Fuoco, C., Salvatori, M., Biondo, A., et al. (2012). Injectable polyethylene glycol-fibrinogen hydrogel adjuvant improves survival and differentiation of transplanted

mesoangioblasts in acute and chronic skeletal-muscle degeneration. *Skeletal Muscle*, 2(1), 1-14.

151 Fuoco, C., Rizzi, R., Biondo, A., et al. (2015). In vivo generation of a mature and functional artificial skeletal muscle. *EMBO Molecular Medicine*, 7(4), 411-422.

152 Dixon, J. E., Shah, D. A., Rogers, C., et al. (2014). Combined hydrogels that switch human pluripotent stem cells from self-renewal to differentiation. *Proceedings of the National Academy of Sciences -USA*, 111(15), 5580-5585.

153 Gaharwar, A. K., Peppas, N. A., & Khademhosseini, A. (2014). Nanocomposite hydrogels for biomedical applications. *Biotechnology and Bioengineering*, 111(3), 441-453.

154 Carrow, J. K., & Gaharwar, A. K. (2015). Bioinspired polymeric nanocomposites for regenerative medicine. *Macromolecular Chemistry and Physics*, 216(3), 248-264.

155 Ramón-Azcón, J., Ahadian, S., Estili, M., et al. (2013). Dielectrophoretically aligned carbon nanotubes to control electrical and mechanical properties of hydrogels to fabricate contractile muscle myofibers. *Advanced Materials*, 25(29), 4028-4034.

156 Pek, Y. S., WanAndrew, C. A., Shekaran, A., Zhuo, L., & Ying, J. Y. (2008). A thixotropic nanocomposite gel for three-dimensional cell culture. *Nature Nanotechnology*, 3(11), 671-675.

157 Pek, Y. S., Wan, A. C. A., & Ying, J. Y. (2010). The effect of matrix stiffness on mesenchymal stem cell differentiation in a 3D thixotropic gel. *Biomaterials*, 31(3), 385-391.

158 Peppas, N. A., Bures, P., Leobandung, W., & Ichikawa, H. (2000). Hydrogels in pharmaceutical formulations. *European Journal of Pharmaceutics and Biopharmaceutics*, 50(1), 27-46.

159 Peppas, N. A., Keys, K. B., Torres-Lugo, M., & Lowman, A. M. (1999). Poly(ethylene glycol)-containing hydrogels in drug delivery. *Journal of Controlled Release*, 62(1-2), 81-7.

160 Chen, W., Xie, M., Yang, B., et al. (2014). Skeletal myogenic differentiation of human urine-derived cells as a potential source for skeletal muscle regeneration. *Journal of Tissue Engineering and Regenerative Medicine*, doi: 10.1002/term.1914.

161 Patenaude, M., Smeets, N. M. B., & Hoare, T. (2014). Designing injectable, covalently cross-linked hydrogels for biomedical applications. *Macromolecular Rapid Communications*, 35(6), 598-617.

162 Jank, B. J., Xiong, L., Moser, P. T., et al. (2015). Engineered composite tissue as a bioartificial limb graft. *Biomaterials*, 61, 246-256.

163 Barberi, T., Willis, L. M., Socci, N. D., & Studer, L. (2005). Derivation of multipotent mesenchymal precursors from human embryonic stem cells. *PLoS Med*, 2(6), e161.

164 Sakurai, H., Inami, Y., Tamamura, Y., et al. (2009). Bidirectional induction toward paraxial mesodermal derivatives from mouse ES cells in chemically defined medium. *Stem Cell Research*, 3(2-3), 157-169.

165 Salani, S., Donadoni, C., Rizzo, F., et al. (2012). Generation of skeletal muscle cells from embryonic and induced pluripotent stem cells as an in vitro model and for therapy of muscular dystrophies. *Journal of Cellular and Molecular Medicine*, 16(7), 1353-1364.

166 Khademhosseini, A., Ferreira, L., Blumling III, J., et al. (2006). Co-culture of human embryonic stem cells with murine embryonic fibroblasts on microwell-patterned substrates. *Biomaterials*, 27(36), 5968-5977.

167 Karp, J. M., Yeh, J., Eng, G., et al. (2007). Controlling size, shape and homogeneity of embryoid bodies using poly(ethylene glycol) microwells. *Lab on a Chip*, 7(6), 786-794.

168 Sa, S., Nguyen, D. T., Pegan, J. D., Michelle, K., & McCloskey, K. E. (2012). Round-bottomed honeycomb microwells: Embryoid body shape correlates with stem cell fate. *Journal of developmental Biology and Tissue Engineering*, 5(2), 12-22.

169 Sa, S., & McCloskey, K. E. (2012). Stage-specific cardiomyocyte differentiation method for H7 and H9 human embryonic stem cells. *Stem Cell Reviews and Reports*, 8(4), 1120-1128.

170 Nguyen, D., Sa, S., Pegan, J. D., et al. (2009). Tunable shrink-induced honeycomb microwell arrays for uniform embryoid bodies. *Lab on a Chip*, 9(23), 3338-3344.

171 Wang, X., & Yang, P. (2008). In vitro differentiation of mouse embryonic stem (mES) cells using the hanging drop method. *Journal of Visual Experiments*, 17, e825. doi: 10.3791/825.

172 Zheng, J. K., Wang, Y., Karandikar, A., et al. (2006). Skeletal myogenesis by human embryonic stem cells. *Cell Res*, 16(8), 713-722.

173 Konieczny, S. F., & Emerson Jr, C. P. (1984). 5-azacytidine induction of stable mesodermal stem cell lineages from 10T1/2 cells: Evidence for regulatory genes controlling determination. *Cell*, 38(3), 791-800.

174 Rohwedel, J., Kleppisch, T., Pich, U., et al. (1998). Formation of postsynaptic-like membranes during differentiation of embryonic stem cells in vitro. *Experimental Cell Research*, 239(2), 214-225.

175 Darabi, R., Gehlbach, K., Bachoo, R. M., et al. (2008). Functional skeletal muscle regeneration from differentiating embryonic stem cells. *Nature Medicine*, 14(2), 134-143.

176 Mizuno, Y., Chang, H., Umeda, K., et al. (2010). Generation of skeletal muscle stem/progenitor cells from murine induced pluripotent stem cells. *The FASEB Journal*, 24(7), 2245-2253.

177 Darabi, R., Pan, W., Bosnakovski, D., et al. (2011). Functional myogenic engraftment from mouse iPS cells. *Stem Cell Reviews and Reports*, 7(4), 948-957.

178 Quattrocelli, M., Palazzolo, G., Floris, G., et al. (2011). Intrinsic cell memory reinforces myogenic commitment of pericyte-derived iPSCs. *The Journal of Pathology*, 223(5), 593-603.

179 Messina, G., Sirabella, D., Monteverde, S., et al. (2009). Skeletal muscle differentiation of embryonic mesoangioblasts requires Pax3 activity. *Stem Cells*, 27(1), 157-164.

180 Galvez, B. G., Sampaolesi, M., Brunelli, S., et al. (2006). Complete repair of dystrophic skeletal muscle by mesoangioblasts with enhanced migration ability. *The Journal of Cell Biology*, 174(2), 231-243.

181 Tedesco, F. S., Gerli, M. F. M., Perani, L., et al. (2012). Transplantation of Genetically Corrected Human iPSC-Derived Progenitors in Mice with Limb-Girdle Muscular Dystrophy. *Science Transational Medicine*, 4(140), 140ra89.

182 Azzabi Zouraq, F., Stölting, M., & Eberli, D. (2013). Skeletal Muscle Regeneration for Clinical Application. In: A., A. J. (Ed.), Regenerative Medicine and Tissue Engineering InTech.

183 Partridge, T. A., Morgan, J. E., Coulton, G. R., Hoffman, E. P., & Kunkel, L. M. (1989). Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts. *Nature*, 337(6203), 176-179.

184 Mendell, J. R., Kissel, J. T., Amato, A. A., et al. (1995). Myoblast transfer in the treatment of Duchenne's muscular dystrophy. *The New England Journal of Medicine*, 333(13), 832-838.

185 Sicari, B. M., Dearth, C. L., & Badylak, S. F. (2013). Tissue engineering and regenerative medicine approaches to enhance the functional response to skeletal muscle injury. *The Anatomical Record*, 297(1), 51-64.

186 Huard, J., Bouchard, J. P., Roy, R., et al. (1992). Human myoblast transplantation: Preliminary results of 4 cases. *Muscle Nerve*, 15(5), 550-560.

187 Skuk, D., Goulet, M., Roy, B., et al. (2006). Dystrophin expression in muscles of Duchenne muscular dystrophy patients after high-density injections of normal myogenic cells. *Journal of Neuropathology & Experimental Neurology*, 65(4), 371-386

188 Skuk, D., Paradis, M., Goulet, M., et al. (2010). Intramuscular transplantation of human postnatal myoblasts generates functional donor-derived satellite cells. *Molecular Therapy*, 18(9), 1689-1697.

189 Skuk, D., Goulet, M., & Tremblay, J. P. (2014). Intramuscular transplantation of myogenic cells in primates: Importance of needle size, cell number, and injection volume. *Cell Transplantation*, 23(1), 13-25.

190 Skuk, D., & Tremblay, J. P. (2014). Clarifying misconceptions about myoblast transplantation in myology. *Molecular Therapy*, 22(5), 897-898.

191 Gibson, A. J., Karasinski, J., Relvas, J., et al. (1995). Dermal fibroblasts convert to a myogenic lineage in mdx mouse muscle. *Journal of Cell Science*, 108(1), 207-214.

192 Wakabayashi, M., Ito, Y., Hamazaki, T. S., & Okochi, H. (2010). Efficient myogenic differentiation of murine dermal Sca-1 (-) cells via initial aggregation culture. *Tissue Engineering Part A*, 16(10), 3251-3259.

193 Garcia-Parra, P., Naldaiz-Gastesi, N., Maroto, M., et al. (2013). Murine muscle engineered from dermal precursors: An in vitro model for skeletal muscle generation, degeneration, and fatty infiltration. *Tissue Engineering Part C: Methods*, 20(1), 28-41.

194 Rossi, C. A., Flaibani, M., Blaauw, B., et al. (2011). In vivo tissue engineering of functional skeletal muscle by freshly isolated satellite cells embedded in a photopolymerizable hydrogel. *The FASEB Journal*, 25(7), 2296-2304.

195 Li, H., Usas, A., Poddar, M., et al. (2013). Platelet-rich plasma promotes the proliferation of human muscle derived progenitor cells and maintains their stemness. *PLoS ONE*, 8(6), e64923.

196 Cerletti, M., Jurga, S., Witczak, C. A., et al. (2008). Highly efficient, functional engraftment of skeletal muscle stem cells in dystrophic muscles. *Cell*, 134(1), 37-47.

197 Torrente, Y., Belicchi, M., Marchesi, C., et al. (2007). Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. *Cell Transplantation*, 16(6), 563-577.

198 Hou, P., Li, Y., Zhang, X., et al. (2013). Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science*, 341(6146), 651-654.

199 Darabi, R., & Perlingeiro, R. C. R. (2013). A perspective on the potential of human iPS cell-based therapies for muscular dystrophies: Advancements so far and hurdles to overcome. *Journal of Stem Cell Research & Therapy*, 3(2), 1000e113.

200 Evans, C. H., & Huard, J. (2015). Gene therapy approaches to regenerating the musculoskeletal system. *Nature Reviews Rheumatology*, 11(4), 234-242.

201 Quenneville, S. P., Chapdelaine, P., Skuk, D., et al. (2007). Autologous transplantation of muscle precursor cells modified with a Lentivirus for muscular dystrophy: Human cells and primate models. *Molecular Therapy*, 15(2), 431-438.

202 Ginn, S. L., Alexander, I. E., Edelstein, M. L., Abedi, M. R., & Wixon, J. (2013). Gene therapy clinical trials worldwide to 2012 – an update. *The Journal of Gene Medicine*, 15(2), 65-77.

203 Wang, W., Li, W., Ma, N., & Steinhoff, G. (2013). Non-viral gene delivery methods. *Current Pharmaceutical Biotechnology*, 14(1), 46-60.

204 Muntoni, F., Torelli, S., & Ferlini, A. (2003). Dystrophin and mutations: One gene, several proteins, multiple phenotypes. *The Lancet Neurology*, 2(12), 731-740.

205 Tedesco, F. (2015). Human artificial chromosomes for Duchenne muscular dystrophy and beyond: Challenges and hopes. *Chromosome Research*, 23(1), 135-141.

206 Hoshiya, H., Kazuki, Y., Abe, S., et al. (2008). A highly stable and nonintegrated human artificial chromosome (HAC) containing the 2.4 Mb entire human dystrophin gene. *Molecular Therapy*, 17(2), 309-317.

207 Zatti, S., Martewicz, S., Serena, E., et al. (2014). Complete restoration of multiple dystrophin isoforms in genetically corrected Duchenne muscular dystrophy patient-derived cardiomyocytes. *Molecular Therapy-Methods & Clinical Development*, 1(1), 1-9 doi:10.1038/mtm.2013.1.

208 Li, Hongmei L., Fujimoto, N., Sasakawa, N., et al. (2015). Precise correction of the dystrophin gene in Duchenne muscular dystrophy patient induced pluripotent stem cells by TALEN and CRISPR-Cas9. *Stem Cell Reports*, 4(1), 143-154.

209 Goyenvalle, A., Griffith, G., Babbs, A., et al. (2015). Functional correction in mouse models of muscular dystrophy using exon-skipping tricyclo-DNA oligomers. *Nature Medicine*, 21(3), 270-275.

210 Kajhoj, T. Q., Duch, M., Pedersen, F. S., Lovschall, H., & Fuchtbauer, E.M. (2015). Test of critical steps towards a combined cell and gene therapy approach for the treatment of Duchenne muscular dystrophy. *Journal of Molecular and Genetic Medicine*, 9(1), 1000160.

211 Jakob, F., Ebert, R., Rudert, M., et al. (2012). In situ guided tissue regeneration in musculoskeletal diseases and aging. *Cell & Tissue Research*, 347(3), 725-735.

212 Hutmacher, D., Duda, G., & Guldberg, R. (2012). Endogenous musculoskeletal tissue regeneration. *Cell &Tissue Research*, 347(3), 485-488.