Chapter 12

Research on Skeletal Muscle Diseases Using Pluripotent Stem Cells

Lorena de Oñate, Elena Garreta, Carolina Tarantino, Elena Martínez, Encarnación Capilla, Isabel Navarro, Joaquín Gutiérrez, Josep Samitier, Josep Maria Campistol, Pura Muñoz-Cánovas and Nuria Montserrat

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60902

Abstract

The generation of induced pluripotent stem cells (iPSCs), especially the generation of patient-derived pluripotent stem cells (PSCs) suitable for disease modelling *in vitro*, opens the door for the potential translation of stem-cell related studies into the clinic. Successful replacement, or augmentation, of the function of damaged cells by patient-derived differentiated stem cells would provide a novel cell-based therapy for skeletal muscle-related diseases. Since iPSCs resemble human embryonic stem cells (hESCs) in their ability to generate cells of the three germ layers, patient-specific iPSCs offer definitive solutions for the ethical and histo-incompatibility issues related to hESCs. Indeed human iPSC (hiPSC)-based autologous transplantation is heralded as the future of regenerative medicine. Interestingly, during the last years intense research has been published on disease-specific hiPSCs derivation and differentiation into relevant tissues/organs providing a unique scenario for modelling disease progression, to screen patient-specific drugs and enabling immunosupression-free cell replacement therapies. Here, we revise the most relevant findings in skeletal muscle differentiation using mouse and human PSCs. Finally and in an effort to bring iPSC technology to the daily routine of the laboratory, we provide two different protocols for the generation of patient-derived iPSCs.

Keywords: Pluripotent stem cells, Myogenic differentiation, Disease modelling, Patient-specific induced pluripotent stem cells, Muscular dystrophy

1. Introduction

Regenerative Medicine aims to restore the loss of function in tissues and organs due to any cause (trauma, stress, aging, or disease) by the replacement of dysfunctional structures with competent cells, tissues, or organs. In order to achieve this goal Regenerative Medicine takes advantage of different forefront methodologies, such the use of stem cells, gene therapy, and tissue engineering among others.

1.1. Human embryonic stem cells (hESCs)

The isolation and derivation of hESCs by Thompson and colleagues in 1998 attracted significant attention in the Regenerative Medicine field [1]. Indeed, regenerative cell transplantation therapies have been expected to treat incurable diseases, such as spinal cord injury [2], neurodegenerative disease [3], heart failure [4,5], diabetes [6], and retinal disease [7].

Nowadays, clinical application of hESCs still shows many concerns regarding the use of human embryos, tissue rejection after transplantation, and tumour formation. However, hESCs possess the dual ability to proliferate indefinitely without phenotypic alterations, and more importantly, to differentiate, theoretically, into all cell types in the human body. These qualities suggest extensive utility of hESCs in applications varying from the definition of differentiation protocols, to the generation of drug screening platforms for disease treatment. Thus, hESCs represent an ideal source for understanding skeletal muscle development and disease, such skeletal muscle.

1.2. Induced pluripotent stem cells (iPSCs)

In 2006 Professor Shinya Yamanaka and colleagues [8] showed for the very first time, that by introducing different transcription factors the epigenetic status of somatic cells could be reverted to pluripotency. In particular, the Japanese team ectopically induced the expression of specific transcription factors related with embryonic stem cells (ESCs) biology, generating in a period of only 30 days, cells that were identical to mouse ESCs (mESCs) in terms of self-renewal capacity, expression of endogenous pluripotency-related factors, and *in vivo* and *in vitro* differentiation potential to give rise to cells belonging to the three germ layers of the embryo (ectoderm, mesoderm, endoderm). This discovery was awarded with the Nobel Price of Medicine in 2012 to Professor Shinya Yamanaka.

While, at first, somatic reprogramming was described using mouse embryonic fibroblasts, the Japanese team could show that also a reduced formula of the original "Yamanaka cocktail" could be used to reprogram human somatic cells towards human iPSCs (hiPSCs) [9]. Since 2007 different research groups, including us, have shown that iPSC technology can be applied to reprogram a huge variety of human somatic cells, independently of their embryonic origin [10–13]. Interestingly, during the last years the generation of protocols avoiding the use of lentiviral or retroviral vectors for the expression of Yamanaka factors has involved the definition of novel strategies for hiPSCs generation, including the use of recombinant proteins [14,15], episomal vectors [16], or mRNAs [17,18], among others [13]. Thus, the generation of

http://dx.doi.org/10.5772/60902

hiPSCs, especially the generation of patient-derived iPSCs suitable for disease modelling *in vitro*, opens the door for the potential translation of patient-derived iPSCs into the clinic. Successful replacement or augmentation of the function of damaged cells by patient-derived differentiated stem cells would provide a novel cell-based therapy for skeletal muscle-related diseases.

Satellite cells (SCs), the adult stem cell pool in skeletal muscle, are often compromised in patients with muscle dystrophies (MDs). Over the last decades the understanding of the transcription factors and intrinsic and extrinsic signals that govern SCs or terminally differentiated myogenic cells have represented a good starting point for the definition of protocols for the generation of myogenic cells from PSCs (both from mouse and human ESCs and iPSCs). In the same manner, the generation of patient-derived cell platforms can help us to develop experimental strategies toward generating muscle stem cells, either by differentiating patient-specific iPSCs or by converting patient's somatic cells towards myogenic cells (transdifferentiation). Overall, the possibility to generate disease-free patient iPSCs can help us to identify which are the mechanisms driving muscle disease, and more importantly, to develop new compounds for treating MDs (Figure 1).

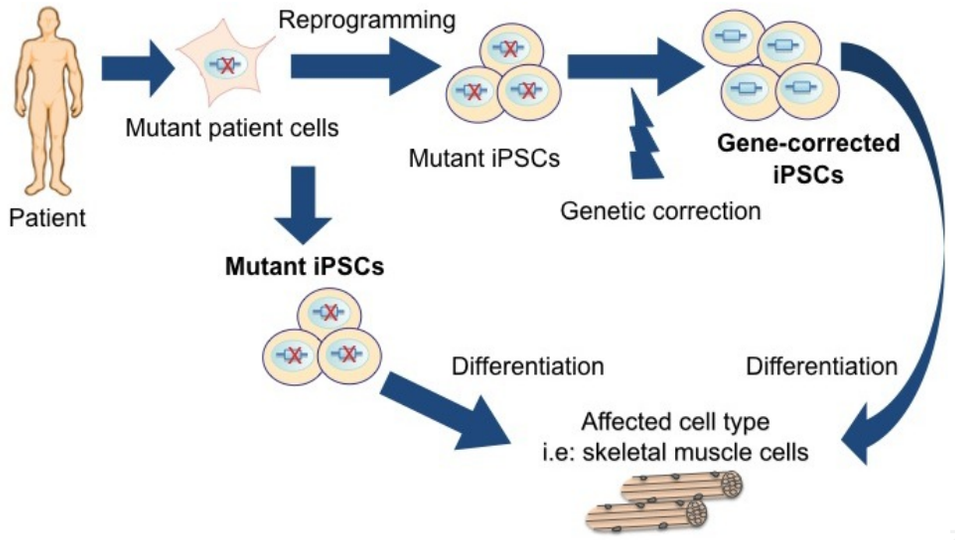

Figure 1. Patient iPSCs represent an unprecedented tool for the generation of i*n vitro* platforms for disease modelling and the definition of protocols for PSCs differentiation. The correction of the genetic defect(s) leading to disease may help to understand the molecular and cellular mechanisms driving disease gestation and progression, and more importantly, to identify novel mechanisms leading to muscle regeneration.

2. General approaches to induce *in vitro* differentiation of pluripotent stem cells (PSCs)

Both mouse and human PSCs are routinely cultivated in the presence of feeder layers. PSCs grow on the feeder layers as colonies (Figure 2). Generally, human and mouse PSCs are

enzymatically dissociated with trypsin, acutase, or dispase to obtain a suspension of single cells, which is then transferred for subculture and expansion or differentiation purposes. For mouse PSCs, LIF can substitute for feeder layers. However, since LIF is not effective for human PSCs, in the last years different chemically defined media have been generated in order to sustain human PSCs culture and expansion in feeder-free substrates.

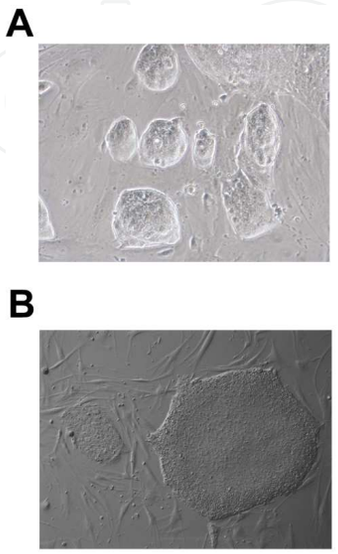

Figure 2. PSCs are typically maintained in mitotically inactivated supportive cells. A) Mouse iPSCs cultured on top of irradiated mouse embryonic fibroblasts grow in tight colonies that are further trypsinized for subculture or differentiation purposes. B) Human iPSCs cultured on top of human irradiated dermal fibroblasts grow as colonies with defined borders.

As an option for culturing human PSCs without feeder cells, Matrigel™ has proven to be a useful alternative enabling the stable culture of human PSCs. Moreover, we have also shown that Matrigel™ allows the generation of hiPSCs without animal-derived feeder cells [19]. Since Matrigel™ was derived from Engelbreth-Holm-Swarm mouse sarcoma cells [20], other types of matrices which do not contain animal-derived agents have been tested and used as feeder-cell substitutes for the successful maintenance and generation of human PSCs; such as CellStart [21,22], recombinant proteins [23–25], and synthetic polymers [26,27].

The culture media used in the early generation of hESCs contained fetal bovine serum [1]. In order to remove unspecific agents that might cause the differentiation of hESCs, knockout serum replacement (KSR) has now been established as a defined material for maintaining hESCs [28] and is also traditionally used for hiPSC generation [9,12,29,30]. In this regard, mTeSR1 medium was developed as a chemically defined medium for maintaining human PSCs [31]. Importantly, in the last years several authors have reported the generation of commercially developed *xeno*-free media for maintaining hiPSCs, and such media have already been used successfully for iPSCs generation. These media include: TeSR2 [32], NutriStem [33], Essential E8 [24], and StemFit [34].

When factors that sustain PSCs stemness are deprived from the media, PSCs spontaneously differentiate into derivatives of the three embryonic germ layers. This capacity has been

http://dx.doi.org/10.5772/60902

profited for more than 30 years in order to direct PSCs to the desired cell product. In this regard, up to day, an infinite number of protocols have been established to promote the development of the cell type of interest.

The following are basic strategies to induce *in vitro* differentiation of PSCs cells:

a. **Embryoid Bodies' (EBs) formation**: In contrast to monolayer cultures, EBs are spherical structures that allow PSCs culture in suspension (Figure 3). The three-dimensional structure, including the establishment of complex cell-adhesions and paracrine signaling within the EB microenvironment, enables differentiation and morphogenesis. For that reason, the first protocols for muscle differentiation took advantage of EB induction from mESCs, followed by different periods of exposure to specific cell culture media in which serum, mitogenic factors, and essential substrates (such as amino-acids or glutamine) were formulated. In that manner, those first assays proved the feasibility of mESCs to give rise to myogenic cells, setting the bases for the definition of robust protocols for the differentiation of muscle cells from human PSCs. Up to day, most of the protocols for the generation of myogenic cells from PSCs make use of the differentiation of EBs derived from either wild type or transgenic PSCs.

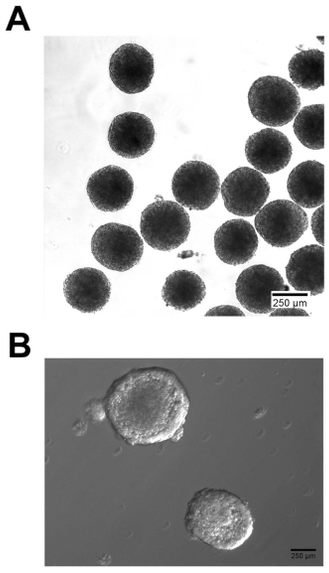

Figure 3. PSCs are capable to differentiate into cells belonging to the three somatic germ layers of the embryo. The generation of EBs from PSCs is a common method for producing different cell lineages for further purposes. A) EBs from mouse iPSCs grown in suspension. B) EBs derived from human iPSCs grown in suspension.

b. **Modification of medium composition**: Monolayers of PSCs and also EBs have been traditionally subjected to changes in nutrient composition, (i.e, reduction/increase of serum concentration, addition/removal of a growth factor or addition/removal of cytokines, among others) in order to induce their differentiation towards the desired cell type. These changes are conducted in order to promote changes on gene expression profiles and cell proliferation rates. In this manner, by means of relatively simple methods, PSCs are artificially guided towards the desired cell type. Although these methodologies have proven low efficiency yields for specific cell types (i.e., motorneurons, hepatic cells; among

others), they are extremely valuable when combined together with PSCs in which the expression of master factors critical for differentiation are under the control of hormones (i.e., tamoxifen inducible reporters) or antibiotics (i.e., puromycin, or hygromycin, among others). The control of expression of the specific transcription factor of choice (i.e., MyoD1) together with the addition of specific molecules mimicking tissue development [i.e., insulin like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF)] has demonstrated good results when differentiating mouse or human PSCs towards myogenic cells.

c. **Genetic manipulation of PSCs**: Forced expression of transcription factors can direct differentiation of PSCs toward specific lineages. In the last years, the generation of platforms for transgene expression in PSCs has emerged as one of the most potent tools for PSCs differentiation. Whereas the first studies took advantage of exogenous gene expression systems (i.e., lentivirus or retrovirus), nowadays the use of integrative vectors are limited, since they incur uncertain risks for potential cell-based therapeutic applications [35]. In this regard, the use of excisable vectors (i.e., transposons; [36,37], or mRNAs [17]) offer an unprecedented opportunity for the derivation of differentiated PSCs suitable for regenerative medicine.

d. **Use of extracellular matrix (ECM) and signaling molecules**: Unlike *de novo* embryonic muscle formation, muscle regeneration in higher vertebrates depends on the injured tissue retaining of an ECM scaffolding that serves as a template for the formation of muscle fibers [38]. In this regard, the interaction between cells and ECM via integrins determines the expression of signaling molecules that affect PSCs differentiation [39]. Of note, when mouse iPSCs have been cultured in the presence of matrigel, myogenesis (this is, proliferation of myoblasts and further fusion into myotubes) has been positively induced [40]. Similar results have been observed when using collagen-based matrix for the differentiation of human iPSCs expressing a Dox-inducible expression cassette of MyoD1 [41]. For the organization and alignment of muscle fibers not only the composition of the ECM but also its anisotropic architecture are essential. To address this, a number of strategies have been developed to organize myotubes: topography-based approaches based on the use of nanofibers, [42], microabrated surfaces [43], and microcontact printing of ECM proteins such as fibronectin [44] and vitronectin [45]. In a complementary approach, biochemical cues have also been introduced to promote cell alignment and differentiation. By using inkjet bioprinting, spatially defined patterns of myogenic and osteogenic cells were derived from primary muscle-derived stem cells (MDSCs) as a response to BMP-2 patterning [46]. The combination of topographical and biochemical signaling has also been explored by coating sub-micron polystyrene fibers with either FGF-2 or BMP-2 to provide spatial control of cell fate and alignment in order to mimic native tissue organization [47]. The vast majority of these works present cells to static microenvironments. Latest trends point out the relevance of presenting cells to spatially and temporally dynamic microenvironments [48]. Surfaces with gradient concentrations of growth factors (BMP-2 and BMP-7) have shown to successfully drive cell differentiation [49,50]. Although not yet a reality, these strategies appear a promising way to direct the differentiation of PSCs [51].

e. **Use of biomatrices mimicking skeletal muscle niche:** Tissue engineering approaches have been used to design synthetic and natural 3D scaffold materials to mimic the structural, biochemical, and mechanical properties of the stem cell niche [52,53]. Synthetic

http://dx.doi.org/10.5772/60902

and natural scaffolds have been designed to provide support for muscle growth and allow myofibroblasts to rebuild their native ECM. Natural scaffolds based on ECM proteins such as fibrin and collagens have been used to form hydrogels for musculoskeletal tissue engineering [54–56]. Commercially available ECM substitutes such as Matrigel™ hydrogels are also showing promising results in the differentiation of PSCs towards cardiomyocytes [57]. However, current ECM protein-based scaffolds are limited by their immune rejection and scaling up technologies. Synthetic scaffolds, which can be fabricated with ideal architectures at the nanoscale, pore sizes and mechanical properties, represent an advantageous solution to mimic the 3D ECM microenvironment (Figure 4). Technologies such as electrospinning, which allows organizing the polymers into thin sheets of fibrous meshes, are promising in this field [58,59]. Recently, it has been proved the reprogramming of mouse fibroblasts onto cardiomyocyte-like cells on polyethylene glycol (PEG) hydrogels functionalized with laminin and RGD peptides [60]. This opens new perspectives toward the use of custom-engineered synthetic scaffolds in the differentiation of PSCs to muscle cells. Finally, the use of acellularized tissue scaffolds is also being explored in muscle regeneration. They offer a native ECM with the optimal biochemical and mechanical properties for cell culture and preserve the architectural features of the tissue. Their use as a matrix supporting the commitment of cardiac muscle cells has been recently reported, thus showing the potential of this approach [61].

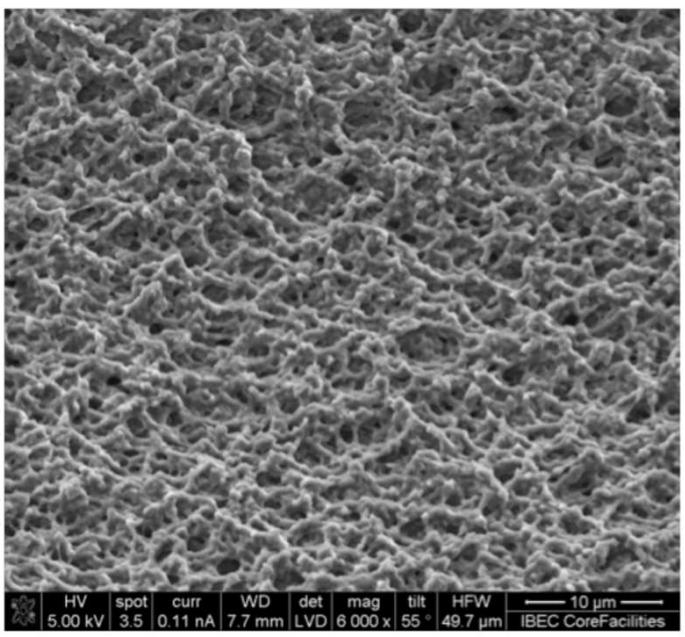

Figure 4. Bio-inert polyethylene glycol (PEG)-based hydrogels have been designed as the scaffold substrate for biomimetic matrices supporting muscle migration in three dimensions. The picture shows PEGDA hydrogel (MW 550 KDa) cross-linked by UV light.

f. Co-culture with supportive cells (feeder cells): The co-culture of mouse and human PSCs (either as monolayers or EBs) with feeder cells has been traditionally used in order to induce PSCs differentiation [39]. The stromal cell line OP9 [62,63], which is derived from

newborn mouse calvaria, supports hematogenesis [64,65]. The preadipose cell line PA6 [66] promotes neural differentiation of mouse and human PSCs [10,11,67]. In this regard, Baghavati [68] showed that the co-culture of mESCs together with primary muscle cells suffice for myogenic differentiation, since donor-derived myofibers generated by co-culturing mouse EBs on top of primary muscle fibers could be occasionally found on the surface of the host muscle.

3. Strategies to induce *in vitro* differentiation of mouse and human PSCs towards myogenic cells

3.1. Generation of myogenic precursors and/or terminally differentiated multinucleated myogenic cells by exogenous expression of transcription factors in PSCs

During the last 30 years successful generation of myogenic precursors from mouse and human PSCs has been achieved by exogenous expression of transcription factors crucial for myogenic differentiation. PSCs are especially amenable for genome editing because they can undergo extensive tissue culture manipulations, such as drug selection and clonal expansion, while still maintaining their pluripotency signature and genome stability. In his regard, different authors have explored the possibility to generate PSCs stable cell lines that express the myogenic transcription factor of interest under the control of specific drugs (i.e., antibiotics). The myogenic transcription factor of interest is normally subcloned into a viral vector, which possesses high infection efficiency when transducing PSCs. Other methods involve the use of non-integrative vectors such as transposons or excisable lentiviral vectors. Following these strategies different authors have shown that PSCs monolayers or PSCs-derived EBs can be converted into myogenic cells (see below).

3.1.1. Early studies of myogenic differentiation from mESCs by exogenous expression of transcription factors

Already in 1992 Dekel and colleagues showed that, when mESCs were electroporated with MyoD1 cDNA driven by the Beta-actin promoter, some cells could be converted to skeletal muscle cells [69]. Moreover, authors showed that contracting skeletal muscle fibers could be generated when the transfected cells were allowed to differentiate *in vitro*, via EBs, in low-mitogen-containing medium. Although those studies failed to develop efficient protocols for the generation of high yields of myogenic cells, they helped to understand that environmental factors should control MyoD expression and its myogenic differentiation function, and more importantly, that MyoD was required for the establishment of the myogenic program but not for its maintenance.

Thus, those first observations served as a starting point for the definition of enriched cell culture media for mESCs differentiation towards myogenic cells, and more importantly, for the generation of fine-tuned systems in order to control the expression of the desired myogenic factor at a precise moment during the onset of differentiation. Alongside this line, Ozasa and

colleagues [70] established a mESC line by introducing a MyoD transgene controlled by a Tet-Off system (ZHTc6-MyoD). The possibility to induce MyoD expression during the time course of differentiation, allowed mESCs to differentiate almost exclusively into the myogenic lineage in the absence of doxycycline, and without pre-differentiation into EBs. To start the differentiation process, Ozasa and colleagues removed doxycycline and used a differentiation medium containing 4% fetal bovine serum (FBS). Under those conditions and only after 7 days, primed cells started to fuse into myotubes, and occasionally light muscle contractions were observed. In that study the potential of the generated cells to differentiate into myofibers *in vivo* was also investigated by intramuscular injections into *mdx* mice and clusters of dystrophin-positive myofibers were detected in the injected area.

3.1.2. Myogenic differentiation from human PSCs by exogenous expression of transcription factors

In the same manner, within the last years several studies have demonstrated the possibility to generate myocytes, and even multinuclear myotubes from both hESCs and patient hiPSCs by means of different systems in which the expression of MyoD is driven under the control of soluble factors during the time course of differentiation. In this regard, early in 2012 two different reports indicated that mesodermal [71] or mesenchymal cells [72] could be generated from iPSCs, demonstrating a high potential for myogenic differentiation in response to *MyoD* over-expression.

Also Rao and colleagues (2012) generated a transgenic Tet-inducible MyoD cassette in which all the transgenic elements were inserted in hESCs making use of lentiviral vectors. In that particular study, authors were able to generate multinucleated myotubes with 90% of efficiency in a period that lasted only 10 days. Later on, Yasuno and colleagues [37] improved a previous protocol [36] for the generation of terminal multinucleated cells from iPSCs derived from patients affected with Carnitine palmitoyltransferase II (CPT II). Their protocol consisted in the transduction of a self-contained Tet-inducible MyoD1 expressing *piggyBac* vector (Tet-MyoD1 vector) and transposase into hiPSCs by lipofection. This system allowed the indirect monitoring of induced *MyoD* expression in response to doxycycline by co-expression of a red fluorescent protein (mCherry). Moreover, in that particular setting authors increased the purity of the generated myocytes by culturing the cells in low glucose conditions, a condition that was also reported to increase differentiated cardiomyocytes out of undifferentiated iPSCs based on the substantial biochemical differences in glucose and lactate metabolism between differentiated cells and undifferentiated iPSCs [73].

Very recently, Abujarour and colleagues [41] found that it is possible to derive myotubes from control iPSC and iPSC lines from patients with either Duchenne or Becker muscular dystrophies. In particular, by using a lentiviral system expressing MyoD under the control of a Tet-inducible promoter, and under-optimized culture conditions, the authors achieved an efficient myogenic differentiation setting the bases for the production of scalable sources of normal and dystrophic myoblasts for further use in disease modelling and drug discovery.

MyoD1 has not been the sole transcription factor of choice when differentiating human PSCs towards myogenic cells. Iacovino and colleagues [74] generated an unprecedented system in which it was possible to integrate the gene of interest into the desired cells (mESCs, kidney

murine cells and hESCs) by means of a system that authors called inducible cassette exchange (ICE). In that particular setting, authors were able to integrate one single copy of Myf5 into mESCs and hESCs. Overall, Iacovino and colleagues showed that Myf5 expression is sufficient to promote the myogenic commitment of nascent mesoderm thereby establishing a novel and rapid method of differentiating mESCs and hESCs into skeletal muscle tissue.

Taking advantage of Iacovino´s system [74], Darabi and colleagues generated an improved version of ICE system in order to generate mESCs in which Pax7 expression was controlled under the control of doxycycline, and they succeeded in inducing the myogenic program in mouse cultures [75,76]. Later on, the same authors generated inducible Pax7 hESCs and hiPSCs with a doxycycline-inducible lentiviral vector encoding Pax7 (iPax7 and the expression of the Pax 7 transgene was detected by incorporating an IRES-GFP reporter downstream of the Pax7 gene. Next, iPax7 hESCs and hiPSCs were induced to generate EBs and after three days doxycycline was added into the media in order to induce Pax7 expression. Following 4 days of induction, Pax7+GFP+ cells were purified by FACS and expanded in a secondary monolayer culture in a medium containing doxycycline and bFGF. Under those conditions iPax7 hESCs and hiPSCs expressed markers of early muscle differentiation (Pax7 and Pax3), and terminally differentiated when iPax7 hESCs and iPSCs were subjected to differentiation-inducing conditions (culture media with 5% horse serum and withdrawal of doxycycline and bFGF). Finally, Darabi and colleagues demonstrated that transplantation of Pax7-derived myogenic progenitors into dystrophin-deficient mice (mdx) promotes extensive and long-term muscle regeneration accompanied by functional improvement [77].

3.2. Generation of myogenic precursors and/or terminally differentiated multinucleated myogenic cells by soluble factors

Although in the last years different authors have shown the possibility to generate myogenic cells from human PSCs by means of the ectopic expression of specific transcription factors, these methods do not reflect normal development, and most importantly, are not suitable for therapeutic purposes or *in vitro* disease modelling. For this reason, in the last years different groups have investigated the possibility to expose EBs or monolayers of mouse and human PSCs to different culture media mimicking muscle development. In order to monitor and control the myogenic signature of the produced cells, authors have isolated the different potential populations based on the acquisition of surface markers related to myogenic fate (i.e., paraxial mesoderm) by means of FACS. In the same manner, the majority of these studies have relayed in the analysis of the expression of myogenic-related markers by common techniques such as the expression of myogenic-related factors by polymerase chain reaction or immunohistochemistry. In that way, the different protocols evaluate the efficiency of their method quantifying the percentage of cells that are differentiated towards myogenic cells.

3.2.1. Early studies in myogenic differentiation from mESCs by soluble factors

Although EBs exposed to undefined differentiation cell culture media spontaneously develop skeletal muscle cells and other cells *in vitro*, transplantation of EBs without any induction to direct development along a specific pathway leads to a failure of integration into recipient

tissues and often forms teratomas in transplanted tissues. Thus, the definition of the specific conditions able to instruct PSCs towards myogenic cells requires establishment of robust conditions able to guide cells through the different stages of muscle differentiation.

In the first moment authors thought that the co-culture of EBs on top of freshly isolated muscle cells could serve as a novel method for myogenic differentiation. Although authors showed that differentiated cells generated by this method developed vascularized and muscle tissue when transplanted in dystrophic mice (mdx mice), still the number of engrafted cells was too low for potential applications in a clinical setting [68]. Later, Zheng and colleagues [78] showed that human EBs (hEBs) from two different hESCs lines cultured in the presence of differentiation media with different percentages of animal serum with or without Epidermal growth factor and 5-azacytidine could give rise to myogenic precursors. Interestingly, in that same work authors demonstrated that when those hESC-derived myogenic precursors were transplanted in NOD-SCID mice they could incorporate into the host muscle efficiently and become part of regenerating muscle fibers; giving rise to myocytes, myotubes, and myofibers, as well as satellite cells.

3.2.2. Generation of myogenic cells from human PSCs by soluble factors

In the quest for protocols suitable for regenerative purposes, Barberi and colleagues [79,80] developed simple feeder-free-monolayer culture systems in order to generate mesenchymal precursors that could be further differentiated towards myogenic cells from hESCs. In those studies multipotent mesenchymal precursors (MMPs) were purified for the acquisition of CD73 surface marker using FACS technology. First, MMPs were maintained in inactivated foetal serum and in the presence of the mouse skeletal myoblast line C2C12 [79]. Later, Barberi and colleagues could avoid the use of C2C12 cells by using serum-free N2 medium. Moreover, in that work authors further purified skeletal muscle myoblasts by means of a second FACS analysis for the neural cell adhesion molecule (NCAM), a marker of embryonic skeletal muscle. Those changes allowed for the expansion of hESC-derived myoblasts in a serum-free N2 medium in the presence of insulin [80].

Following a similar strategy Sakurai and colleagues [81] differentiated a murine ESC line towards paraxial mesodermal progenitors. Specifically, authors selected paraxial mesodermal progenitors based on the expression of platelet-derived growth factor receptor α (PDGFR-α) and the absence of Flk-1–a lateral mesodermal marker. Later on, the same authors demonstrated that mESCs could be directed toward the paraxial mesodermal lineage by a combination of bone morphogenetic protein (BMP) and Wnt signaling under chemically-defined conditions [82]. Interestingly, the same group developed a protocol for the generation of paraxial mesoderm progenitors from both miPSCs and hiPSCs. Although some differences in growth factor requirement between mESCs and miPSCs cells were observed, the PDGFR-α+ population derived from miPSCs was almost identical to that of mESCs. Importantly, the work of Sakurai and colleagues showed that, under their specific conditions, two different lines of hiPSCs could be differentiated towards PDGFR-α+/KDR- cells. Those progenitors could be further differentiated into osteocytes, chondrocytes, and skeletal muscle cells, demonstrating the suitability of their procedures for the generation of myogenic cells for regenerative purposes.

Notably, other authors have shown the possibility to generate PDGFR-α+ from hESCs [83]. However, those same authors showed few engraftments of transplanted hESCs-derived myogenic cells into injured skeletal muscle. Interestingly, the same authors have recently demonstrated that, by incorporating Wnt3a in culture medium, myogenic commitment is rapidly achieved from hESCs, and more significantly, that those cells can contribute to finally regenerate cardiotoxin-injured skeletal muscle of NOD/SCID mice [84]. In the same line, other authors have demonstrated that the inhibition of GSK3B and treatment with FGF2 could specifically promote skeletal muscle differentiation. In particular, Xu and colleagues [85] have demonstrated that simultaneous inhibition of GSK3B, activation of adenyl cyclase and stimulation with FGF2 during EBs formation could promote the generation of myogenic precursors that terminally differentiate *in vitro* and act as satellite cells upon transplantation. Also, Borchin and colleagues [86] have shown that human PSCs can be differentiated towards Pax3/Pax7 double positive cells after GSK3B and FGF2 exposure.

4. Induced pluripotent stem cells from patients: how to model muscle disease in the Petri dish

The possibility to direct cell differentiation from human PSCs opens the door for the development of massive platforms for the study of muscle differentiation and disease progression. Moreover, the possibility to combine gene-editing strategies allowing for the correction of the genetic disorder leading to muscle disease, together with the generation of myogenic cells from patients' cells, represents an unprecedented opportunity for the establishment of *in vitro* systems for the study of MDs.

So far, different groups have demonstrated the suitability of patient iPSCs approaches in order to model MDs. Abujarour and colleagues [41] have derived myotubes from Duchenne Muscular Distrophy (DMD) and Becker Muscular Distrophy (BMD) hiPSCs. In particular, authors showed that myotubes derived from MDM and BMD iPSCs could respond to insulin-like growth factor 1(IGF-1) and wingless-type MMTV integration site family member 7A (Wnt7a) in a similar manner to primary myotubes. These results point out that iPSC derived from MDM and BMD patients have no intrinsic barriers preventing from myogenesis, and thus represent a scalable source of normal and dystrophic myoblast for further use in disease modelling and drug discovery.

Recently, Tedesco and colleagues [71] generated iPSCs from fibroblasts and myoblasts from limb-girdle muscular dystrophy 2D (LGMD2D) patients, developing the first protocol for the derivation of mesoangioblast-like cells from these iPSCs. Moreover, authors expanded and genetically corrected patient iPSC-derived mesoangioblasts *in vitro* by means of a lentiviral vector for the expression of human α-sarcoglycan in striated muscle cells. When LGMD2D disease free iPSC-derived mesoangioblasts were transplanted into α-sarcoglycan-null immunodeficient mice authors showed that they were capable to generate muscle fibers expressing α-sarcoglycan. Interestingly, when the same experiments were conducted using mouse-derived mesoangioblasts authors showed a functional amelioration of the dystrophic pheno-

type and restoration of the depleted progenitors in α-sarcoglycan-null immunodeficient mice. Overall, Tedesco and colleagues showed that transplantation of genetically corrected mesoangioblast-like cells derived from iPSCs from LGMD2D patients could represent a novel therapeutic approach for these patients.

In the same line, other authors [36] have generated iPSCs from patients affected by Miyoshi myopathy (MM), a congenital distal myopathy caused by mutations in dysferlin. Specifically, authors demonstrated that the expression of full-length dysferlin could restore the MM associated phenotype in myotubes differentiated from MM-iPSCs. In the same line, Yasuno and colleagues [87] have shown the possibility to generate iPSCs from patients affected by Carnitine palmitoyltransferase II (CPT II) deficiency, an inherited disorder involving B-oxidation of long-chain fatty acids (FAO).

Very recently, Li and colleagues [88] have demonstrated the possibility to correct iPSCs derived from DMD patients by means of three different strategies: exon skipping, frameshifting, and exon knock-in. In their hands, exon knock-in was the most effective approach. The work of Li reveals the suitability of iPSC technology for the generation of iPSC-based approaches for MDs modelling and therapy.

Overall, these recent advances set the bases for the generation of a previously nonexisting tool for the study of MDs. The possibility to generate human models for the study of MDs by means of iPSC technology opens the door for the development of novel therapeutic compounds for MD treatment, and more importantly, to increase our understanding of MDs and muscle development.

5. Protocols for the generation of patient-specific iPSCs

Within the last years, our group has participated in the development of protocols for the derivation of patient iPSCs for disease modelling and compound screening. Taking advantage of different basic techniques that are commonly used on a daily basis in any laboratory worldwide, we have generated simple methodologies that allow the generation of patient-specific iPSCs in a period that lasts only 50 days from the moment we get the primary samples from patients (i.e., skin biopsy, lipoaspirates, etc.). In this section, we provide two concise protocols for the derivation of patient iPSCs taking advantage of retroviruses and episomal vectors.

5.1. Protocol for the generation of hiPSCs from keratinocytes derived from plucked hair samples

The development of simple methods for the generation of hiPSCs from keratinocytes from plucked hair samples offers an unprecedented scenario for the production of patient-specific iPSCs, making use of a non-invasive procedure when collecting patient samples.

Our protocol is divided into three consecutive steps, which involve: A) Isolation of keratinocytes from plucked hair samples, B) Production of retrovirus, and C) Infection of keratinocytes.

The steps are detailed below. As described elsewhere, the same protocol can be applied when reprogramming cord blood stem cells, kidney tubular epithelial cells, and dermal fibroblasts [11,12,30].

a. Isolation of keratinocytes from plucked hair

A.1 The day before hair isolation coat the required number of 35-mm culture dishes with Matrigel® (Becton Dickinson, S.A. cat. no. 356234) by adding 1 ml of Matrigel® and incubate overnight at 4 ºC.

A.2 The same day of sample recovery, prepare a non-coated 100-mm bacterial plate containing HBSS (Invitrogen cat. no. 14170-088) with 1% (vol/vol) Penicillin/Streptomycin (Invitrogen cat. no. 15140-122).

A.3 After the patient reads and signs the informed consent use tweezers to gently pull the hair out and place it on plates filled with HBSS medium. As recommended by Aasen and colleagues [89] use hair from the occipital part of the head.

A.4 Making use of forceps submerge the hair in HBSS medium. Next, cut off the external part of the hair leaving the bulb and outer root sheath.

A.5 As described by Aasen [89], at this stage two optional methodologies for growing keratinocytes from plucked hair are described: direct outgrowth and enzymatic digestion. In this section, we are going to detail how to get direct outgrowths of keratinocytes from plucked hair. For enzymatic digestion procedure follow Aasen recommendations [89].

A.6 Remove the plate from 4 ºC, aspirate the Matrigel® coating and rinse the plate with 2 ml of hESCs medium: KO-DMEM (Invitrogen, cat. no. 10829-018), 20% KOSR (Invitrogen, cat. no. 10828-028), 10 ng ml-1 bFGF (Peprotech cat. No. 100-18B), 1 mM Glutamax (Invitrogen, cat. no. 35050-038), 100 μM nonessential amino acids (Invitrogen, cat. no. 11140-050), 100 μM 2-mercaptoethanol (Sigma, cat. no. M7522), and 50 U/ml (penicillin and 50 mg/ml streptomicin).

A.7 Place gently the hair obtained from the coated culture plates.

A.8 Add few drops of hESCs medium (0.3 mL) on top of the hair sample in order to keep the hair humid. In the next 3–4 hours, add gently fresh hESCs medium (0.3 mL). The next day carefully check under the microscope that the hair sample is still attached at the bottom of the plate. Refill the plate with more hESCs medium on top of the hair, if necessary.

A.9 Add 1 ml of hESCs medium every following day. After 4 days, outgrowths of typical epithelial keratinocytes are visible.

A.10 After 10–14 days, large colonies of keratinocytes (up to 1 cm of diameter) are visible. At this stage, it is advisable to split the cells for infection or subculture to avoid cells to initiate contact-dependent differentiation.

b. Production of retrovirus

B.1 Seed out $4.3x10^6$ Phoenix Amphotropic 293 cells in 10 ml of DMEM complete medium which consists in DMEM (Invitrogen, cat. no. 11965-092), 10% FBS (Invitrogen, cat. no.

10270-106), Glutamax 2 mM, Penicillin/Streptomycin (100 U/ml, 100 μg/ml) in 100-mm culture dishes and place in a 37 ºC 5% CO2 incubator.

B.2 Next day, prepare FuGENE:DNA complex according to the manufacturer´s instructions (Roche Applied Science, cat. no. 1181509001). We recommend a ratio of 27 μl FuGENE to 9 μg plasmid DNA for every 10 cm dish. For virus production, we will make use of pMSCV-based plasmids. pMSCV-based retroviral vectors are commercially available for OCT4, SOX2, KLF4, and c-Myc in Addgene (reference numbers are: 20072, 20073, 20074, and 20075 respectively). If the infection efficiency wants to be monitored pMSCV-based retroviral vectors expressing GFP can be used (Addgene plasmid 20672).

B.3 Add the FuGENE:DNA complex solution dropwise onto media (gently). Move the plate carefully in order to distribute the transfection reaction homogenously.

B.4 Place the transfected cells at 37 ºC, with 5% CO2 overnight.

B.5 Next day, change DMEM complete medium gently (10 ml/plate) and incubate the plates overnight at 32 ºC in a 5% CO2 incubator.

B.6 Collect viral supernatants and add fresh complete DMEM medium to the different plates. Take care to avoid cells detaching from the tissue culture plates.

B.7 Every following day, for 2 days, repeat steps B.5 and B.6 in order to collect more viral supernatants.

B.8 Filter the viral supernatant through a 0.45 μm PVDF filter (Millipore® SLHV033NK) to remove any contaminant cells.

B.9 Add 1 μl polybrene (10 mg/ml; Chemicon cat. no. TR-1003-6) for each ml viral supernatant needed (final polybrene concentration of 10 μg/ml).

c. Infection of keratinocytes derived from plucked hair samples

C.1 Wash obtained keratinocyte colonies growing from a hair in hESCs medium as described in section (A) with PBS (Invitrogen, cat. no. 10010-056) and trypsinize them using 1 ml 0.25% Trypsin/EDTA (Invitrogen, cat. no. 25200-056).

C.2 After 5–8 minutes, when cells are released from the plastic surface resuspend them with 10 ml hESCs medium.

C.3 Centrifuge at 200g for 5 min.

C.4 Resuspend the pellets in 4 ml of hESCs medium.

C.5 Plate cell suspensions in the desired wells of six-well plates (Corning, cat. no. 153516) previously pre-coated with Matrigel® as explained in step A1. Seed 80.000 keratinocytes/well.

C.6 Next day add 1 ml of every single viral suspension obtained as described in steps B6-B9 for OCT4, SOX2, KLF4, and cMYC. Perform viral transduction in the same manner for GFP in order to monitor the efficiency of viral infection.

C.7 Centrifuge plates at 650g for 45 min.

C.8 Replace with 2 ml fresh hESCs medium (within 4–5 hours).

C.9 Next day, repeat steps **C.7**-**C.8** to infect cells a second time.

C.10 Change media daily with hESCs medium.

C.11 After 1–2 weeks large colonies are visible and can be picked mechanically and transferred onto irradiated human fibroblasts feeder layer (iHFF) and cultured as normal iPSCs following specifications detailed before by others [12,29,30].

5.2. Protocol for the generation of hiPSCs from mesenchymal stem cells (MSCs) derived from adipose tissue

The possibility to generate iPSCs by means of non-integrative strategies paves the way for the development of clinical grade iPSCs from patients. Here, we detail a specific protocol for the derivation of hiPSCs from mesenchymal stem cells from adipose tissue.

Our protocol makes use of commercial episomal plasmids generated by Okita and colleagues [16]. Our method offers the possibility to generate patient-specific iPSCs in a period that last only 20 to 22 days from the moment the reprogramming experiment starts.

a. Before nucleofection

A.1 Following the Human MSC Nucleofector® Kit (DPE-1001, Amaxa) recommendations the solution for nucleofection is prepared by adding 0.5 ml of Supplement to 2.25 ml Nucleofector Solution. Human MSC Nucleofector Solution is now ready to use and is stable for 3 months at 4°C.

A.2 Under the culture hood prepare plasmid mixture by mixing 1 μg of each pCLXE episomal based plasmid (i.e., if we want to reprogram three different samples 3 μg of each pCXLE plasmid will be added to the final mixture). Plasmids are commercially available in Addgene: pCXLE-hOCT3/4-shp53-F (Plasmid #27077), pCXLE-hSK (Plasmid #27078), pCXLE-hUL (Plasmid #27080).

A.3 Pre-warm the complete Human MSC Nucleofector Solution to room temperature.

A.4 Pre-warm an aliquot mesenchymal stem cells culture medium [DMEM (Invitrogen, cat. no. 11965-092), 10% FBS (Invitrogen, cat. no. 10270-106), Glutamax 2 mM, Penicillin/Streptomycin (100 U/ml, 100 μg/ml)] at 37 °C in a 50 ml tube (500 μl per sample; 1.5 ml for 3 samples).

A.5 Prepare 6-well plates by filling the appropriate number of wells with 1 ml of culture medium containing mesenchymal stem cells culture medium and pre-incubate plates in a humidified 37 °C/5% CO2 incubator. Prepare 2 wells/sample (i.e., 6 wells for 3 samples)

b. Nucleofection and iPSCs generation

B.1 Follow Human MSC Nucleofector® Kit recommendations (http://bio.lonza.com/fileadmin/groups/marketing/Downloads/Protocols/Generated/Optimized_Protocol_90.pd).

B.2 After nucleofection transfer the nucleofected cells from the cuvettes using the plastic pipettes provided by the kit to prevent damage and loss of cells distributing the total amount

of cell suspensions into 2 wells containing the pre-warmed mesenchymal stem cells culture medium. Incubate the cells in a humidified 37 °C/5% CO2 incubator.

B.3 After 4 days transfer, wash nucleofected samples with PBS (Invitrogen, cat. no.10010-056) and trypsinize them using 1 ml 0.25% Trypsin/EDTA (Invitrogen, cat. no. 25200-056).

B.4 Transfer the nucleofected cells into six-well plates (Corning, cat. no. 153516) containing irradiated murine fibroblasts (iMEF;C57BL/6 MEF 4M IRR; Global Stem) in hESCs medium: KO-DMEM (Invitrogen, cat. no. 10829-018), 20% KOSR (Invitrogen, cat. no. 10828-028), 10 ng ml-1 bFGF (Peprotech cat. No. 100-18B), 1 mM Glutamax (Invitrogen, cat. no. 35050-038), 100 μM nonessential amino acids (Invitrogen, cat. no. 11140-050), 100 μM 2-mercaptoethanol (Sigma, cat. no. M7522), 50 U/ml (penicillin and 50 mg/ml streptomicin). Change hES media every other day.

B.5 After 20 days, large colonies are visible and can be picked mechanically and transferred onto iMEF and cultured as normal iPSCs following specifications detailed before by others [12,29,30].

Acknowledgements

L.O and E.G were partially supported by La Fundació Privada La Marató de TV3, 121430/31/32. EM and JS were partially supported by funds from the Spanish Ministry of Economy and Competitiveness (Ref PLE 2009-0164) and the Commission for Universities and Research of the Department of Innovation, Universities, and Enterprise of the Generalitat de Catalunya (2014 SGR 1442). This work was also supported by funds from the "Ministerio de Ciencia e Innovación" (grants AGL2010-17324 to E.C., AGL2011-24961 to I.N. and AGL2012-39768 to J.G.) and the Catalonian Government (grant 2009SGR-00402).N.M was partially supported by La Fundació Privada La Marató de TV3, 121430/31/32, the Spanish Ministry of Economy and Competitiveness (Ref PLE 2009-0164) and from the Catalonian Government (2014SGR 1442). CIBER-BBN is an initiative funded by the VI National R&D&i Plan 2008-2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund.

Author details

Lorena de Oñate[1], Elena Garreta[1], Carolina Tarantino[1], Elena Martínez[2], Encarnación Capilla[3], Isabel Navarro[3], Joaquín Gutiérrez[3], Josep Samitier[4], Josep Maria Campistol[5], Pura Muñoz-Cánovas[6] and Nuria Montserrat[1,7*]

*Address all correspondence to: nmontserrat@ibecbarcelona.eu

1 Pluripotent Stem Cells and Activation of Endogenous Tissue Programs for Organ Regeneration group, Institute for Bioengineering of Catalonia, Barcelona, Spain

2 Biomimetic Systems for Cell Engineering group, Institute for Bioengineering of Catalonia, Barcelona, Spain

3 Department of Animal Physiology, Faculty of Biology, University of Barcelona, Barcelona, Spain

4 Nanobioengineering group, Institute for Bioengineering of Catalonia, Barcelona, Spain; Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain

5 Renal Division, Hospital Clinic, University of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

6 Cell Biology Unit, Pompeu Fabra University, Barcelona, Spain

7 Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain

References

[1] Thomson JA. Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282(5391):1145-7.

[2] Nakamura M, Okano H. Cell transplantation therapies for spinal cord injury focusing on induced pluripotent stem cells. Cell Res [Internet]. 2013;23:70–80. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3541652&tool=pmcentrez&rendertype=abstract

[3] Ross CA, Akimov SS. Human-induced pluripotent stem cells: potential for neurodegenerative diseases. Hum Mol Genet [Internet]. 2014;23(1):1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24824217

[4] Hsiao L-C, Carr C, Chang K-C, Lin S-Z, Clarke K. Review article: stem cell-based therapy for ischemic heart disease. Cell Transplant [Internet]. 2012;22:663–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23044395

[5] Fujita J, Itabashi Y, Seki T, Tohyama S, Tamura Y, Sano M, et al. Myocardial cell sheet therapy and cardiac function. Am J Physiol Heart Circ Physiol [Internet]. 2012;303:H1169–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23001836

[6] Holditch SJ, Terzic A, Ikeda Y. Concise review: pluripotent stem cell-based regenerative applications for failing β-Cell function. Stem Cells Transl Med [Internet]. 2014;3:653–61. Available from: http://stemcellstm.alphamedpress.org/content/early/2014/03/18/sctm.2013-0184.abstract

[7] Ramsden CM, Powner MB, Carr A-JF, Smart MJK, da Cruz L, Coffey PJ. Stem cells in retinal regeneration: past, present and future. Development [Internet]. 2013;140:2576–

85. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3666384&tool=pmcentrez&rendertype=abstract

[8] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–76.

[9] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–72.

[10] Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol. 2008;26:1276–84.

[11] Giorgetti A, Montserrat N, Aasen T, Gonzalez F, Rodríguez-Pizà I, Vassena R, et al. Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. Cell Stem Cell. 2009;353–7.

[12] Montserrat N, Ramírez-Bajo MJ, Xia Y, Sancho-Martinez I, Moya-Rull D, Miquel-Serra L, et al. Generation of induced pluripotent stem cells from human renal proximal tubular cells with only two transcription factors, OCT4 and SOX2. J Biol Chem [Internet]. 2012;287:24131–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3397840&tool=pmcentrez&rendertype=abstract

[13] González F, Boué S, Izpisúa Belmonte JC. Methods for making induced pluripotent stem cells: reprogramming à la carte. Nat Rev Genet. 2011;12:231–42.

[14] Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell. 2009;381–4.

[15] Kim D, Kim C-H, Moon J-I, Chung Y-G, Chang M-Y, Han B-S, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell [Internet]. Elsevier Inc.; 2009;4(6):472–6. Available from: http://dx.doi.org/10.1016/j.stem.2009.05.005

[16] Okita K, Matsumura Y, Sato Y, Okada A, Morizane A, Okamoto S, et al. A more efficient method to generate integration-free human iPS cells. Nat Methods. 2011;8:409–12.

[17] Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell. 2010;7:618–30.

[18] Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell. 2011;8:376–88.

[19] Xia Y, Nivet E, Sancho-Martinez I, Gallegos T, Suzuki K, Okamura D, et al. Directed differentiation of human pluripotent cells to ureteric bud kidney progenitor-like cells. Nat Cell Biol [Internet]. 2013;15:1507–15. Available from: http://

eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=24240476&retmode=ref&cmd=prlinks\npapers2://publication/doi/10.1038/ncb2872

[20] Emonard H, Grimaud JA, Nusgens B, Lapière CM, Foidart JM. Reconstituted basement-membrane matrix modulates fibroblast activities in vitro. J Cell Physiol. 1987;133:95–102.

[21] Bergstrom R, Strom S, Holm F, Feki A, Hovatta O. Xeno-free culture of human pluripotent stem cells. Methods Mol Biol [Internet]. 2011;767:125–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21822871

[22] Ausubel LJ, Lopez PM, Couture LA. GMP scale-up and banking of pluripotent stem cells for cellular therapy applications. Methods Mol Biol. 2011;767:147–59.

[23] Rodin S, Domogatskaya A, Ström S, Hansson EM, Chien KR, Inzunza J, et al. Long-term self-renewal of human pluripotent stem cells on human recombinant laminin-511. Nat Biotechnol. 2010;28:611–5.

[24] Chen G, Gulbranson DR, Hou Z, Bolin JM, Ruotti V, Probasco MD, et al. Chemically defined conditions for human iPSC derivation and culture. Nat Methods. 2011;8:424–9.

[25] Miyazaki T, Futaki S, Suemori H, Taniguchi Y, Yamada M, Kawasaki M, et al. Laminin E8 fragments support efficient adhesion and expansion of dissociated human pluripotent stem cells. Nat Commun [Internet]. 2012;3:1236. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3535336&tool=pmcentrez&rendertype=abstract

[26] Mei Y, Saha K, Bogatyrev SR, Yang J, Hook AL, Kalcioglu ZI, et al. Combinatorial development of biomaterials for clonal growth of human pluripotent stem cells. Nat Mater. 2010;9:768–78.

[27] Lu HF, Narayanan K, Lim SX, Gao S, Leong MF, Wan ACA. A 3D microfibrous scaffold for long-term human pluripotent stem cell self-renewal under chemically defined conditions. Biomaterials. 2012;33:2419–30.

[28] Draper JS, Moore HD, Ruban LN, Gokhale PJ, Andrews PW. Culture and characterization of human embryonic stem cells. Stem Cells Dev. 2004;13:325–36.

[29] Montserrat N, de Oñate L, Garreta E, González F, Adamo A, Eguizábal C, et al. Generation of feeder-free pig induced pluripotent stem cells without Pou5f1. Cell Transplant. 2012;21:815–25.

[30] Montserrat N, Nivet E, Sancho-Martinez I, Hishida T, Kumar S, Miquel L, et al. Reprogramming of human fibroblasts to pluripotency with lineage specifiers. Cell Stem Cell. 2013;13:341–50.

[31] Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, et al. Derivation of human embryonic stem cells in defined conditions. Nat Biotechnol. 2006;24:185–7.

[32] Meng G, Liu S, Rancourt DE. Synergistic effect of medium, matrix, and exogenous factors on the adhesion and growth of human pluripotent stem cells under defined, Xeno-Free conditions. Stem Cells Dev. 2012; 21(11):2036-48.

[33] Sugii S, Kida Y, Kawamura T, Suzuki J, Vassena R, Yin Y-Q, et al. Human and mouse adipose-derived cells support feeder-independent induction of pluripotent stem cells. Proc Natl Acad Sci U S A. 2010;107:3558–63.

[34] Nakagawa M, Taniguchi Y, Senda S, Takizawa N, Ichisaka T, Asano K, et al. A novel efficient feeder-free culture system for the derivation of human induced pluripotent stem cells. Sci Rep [Internet]. 2014;4:3594. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3884228&tool=pmcentrez&rendertype=abstract

[35] Zhu X, Fu L, Yi F, Liu G-H, Ocampo A, Qu J, et al. Regeneration: making muscle from hPSCs. Cell Res [Internet]. 2014;24:1159–61. Available from: http://www.nature.com/doifinder/10.1038/cr.2014.91

[36] Tanaka A, Woltjen K, Miyake K, Hotta A, Ikeya M, Yamamoto T, et al. Efficient and reproducible myogenic differentiation from human iPS cells: prospects for modeling miyoshi myopathy in vitro. PLoS One. 2013; 8(4):e61540.

[37] Yasuno T, Osafune K, Sakurai H, Asaka I, Tanaka A, Yamaguchi S, et al. Functional analysis of iPSC-derived myocytes from a patient with carnitine palmitoyltransferase II deficiency. Biochem Biophys Res Commun. 2014;448:175–81.

[38] Ciciliot S, Schiaffino S. Regeneration of mammalian skeletal muscle. Basic mechanisms and clinical implications. Curr Pharm Des. 2010;16:906–14.

[39] Kurosawa H. Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells. J Biosci Bioeng. 2007;103:389–98.

[40] Mizuno Y, Chang H, Umeda K, Niwa A, Iwasa T, Awaya T, et al. Generation of skeletal muscle stem/progenitor cells from murine induced pluripotent stem cells. FASEB J. 2010;24:2245–53.

[41] Abujarour R, Bennett M, Valamehr B, Lee TT, Robinson M, Robbins D, et al. Myogenic differentiation of muscular dystrophy-specific induced pluripotent stem cells for use in drug discovery. Stem Cells Transl Med [Internet]. 2014;3:149–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24396035

[42] Huang NF, Patel S, Thakar RG, Wu J, Hsiao BS, Chu B, et al. Myotube assembly on nanofibrous and micropatterned polymers. Nano Lett. 2006;6:537–42.

[43] Shimizu K, Fujita H, Nagamori E. Alignment of skeletal muscle myoblasts and myotubes using linear micropatterned surfaces ground with abrasives. Biotechnol Bioeng. 2009;103:631–8.

[44] Altomare L, Riehle M, Gadegaard N, Tanzi M, Farè S. Microcontact printing of fibronectin on a biodegradable polymeric surface for skeletal muscle cell orientation. Int J Artif Organs. 2010;33:535–43.

[45] Molnar P, Wang W, Natarajan A, Rumsey JW, Hickman JJ. Photolithographic patterning of C2C12 myotubes using vitronectin as growth substrate in serum-free medium. Biotechnol Progr. 2007; 23(1):265-8.

[46] Phillippi JA, Miller E, Weiss L, Huard J, Waggoner A, Campbell P. Microenvironments engineered by inkjet bioprinting spatially direct adult stem cells toward muscle- and bone-like subpopulations. Stem Cells. 2008;26:127–34.

[47] Ker EDF, Nain AS, Weiss LE, Wang J, Suhan J, Amon CH, et al. Bioprinting of growth factors onto aligned sub-micron fibrous scaffolds for simultaneous control of cell differentiation and alignment. Biomaterials. 2011;32:8097–107.

[48] Wilmut I, Sullivan G, Chambers I. The evolving biology of cell reprogramming. Philos Trans R Soc Lond B Biol Sci. 2011;366:2183–97.

[49] Lagunas A, Comelles J, Oberhansl S, Hortigüela V, Martínez E, Samitier J. Continuous bone morphogenetic protein-2 gradients for concentration effect studies on C2C12 osteogenic fate. Nanomedicine Nanotechnology, Biol Med. 2013;9:694–701.

[50] Almodóvar J, Guillot R, Monge C, Vollaire J, Selimović Š, Coll JL, et al. Spatial patterning of BMP-2 and BMP-7 on biopolymeric films and the guidance of muscle cell fate. Biomaterials. 2014;35:3975–85.

[51] Sun Y, Duffy R, Lee A, Feinberg AW. Optimizing the structure and contractility of engineered skeletal muscle thin films. Acta Biomater. 2013;9:7885–94.

[52] Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. Nature. 2009;462:433–41.

[53] Vunjak-Novakovic G, Scadden DT. Biomimetic platforms for human stem cell research. Cell Stem Cell. 2011; 8(3):252-61.

[54] Liao H, Zhou G-Q. Development and progress of engineering of skeletal muscle tissue. Tissue Eng Part B Rev. 2009;15:319–31.

[55] Walters BD, Stegemann JP. Strategies for directing the structure and function of three-dimensional collagen biomaterials across length scales. Acta Biomaterialia. 2014; 10(4):1488-501.

[56] Tamayol A, Akbari M, Annabi N, Paul A, Khademhosseini A, Juncker D. Fiber-based tissue engineering: progress, challenges, and opportunities. Biotech Adv. 2013; 31(5): 669-87.

[57] Gu Y, Liu GH, Plongthongkum N, Benner C, Yi F, Qu J, et al. Global DNA methylation and transcriptional analyses of human ESC-derived cardiomyocytes. Protein Cell. 2014;5:59–68.

[58] Aviss KJ, Gough JE, Downes S. Aligned electrospun polymer fibres for skeletal muscle regeneration. Eur Cells Mater. 2010;19:193–204.

[59] Mertens JP, Sugg KB, Lee JD, Larkin LM. Engineering muscle constructs for the creation of functional engineered musculoskeletal tissue. Regen Med [Internet]. 2014;9:89–100. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24351009

[60] Smith AW, Hoyne JD, Nguyen PK, McCreedy DA, Aly H, Efimov IR, et al. Direct reprogramming of mouse fibroblasts to cardiomyocyte-like cells using Yamanaka factors on engineered poly(ethylene glycol) (PEG) hydrogels. Biomaterials. 2013;34:6559–71.

[61] Oberwallner B, Brodarac A, Anić P, Sarić T, Wassilew K, Neef K, et al. Human cardiac extracellular matrix supports myocardial lineage commitment of pluripotent stem cells. Eur J Cardiothorac Surg [Internet]. 2014;ezu163 –. Available from: http://ejcts.oxfordjournals.org/content/early/2014/04/25/ejcts.ezu163.long

[62] Kodama H, Nose M, Niida S, Nishikawa S, Nishikawa S. Involvement of the c-kit receptor in the adhesion of hematopoietic stem cells to stromal cells. Exp Hematol. 1994;22:979–84.

[63] Nakano T, Kodama H, Honjo T. Generation of lymphohematopoietic cells from embryonic stem cells in culture. Science. 1994;265:1098–101.

[64] Kodama H, Nose M, Yamaguchi Y, Tsunoda J, Suda T, Nishikawa S, et al. in vitro proliferation of primitive hemopoietic stem cell suported by stromal cells: evidence for the presence of a mechanism(s) other than that involving c-kit receptor and its ligand. J [Internet]. 1992;176(August). Available from: http://jem.rupress.org/content/176/2/351.abstract

[65] Liu G-H, Suzuki K, Li M, Qu J, Montserrat N, Tarantino C, et al. Modelling Fanconi anemia pathogenesis and therapeutics using integration-free patient-derived iPSCs. Nat Commun [Internet]. 2014;5:4330. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24999918

[66] Kawasaki H, Mizuseki K, Nishikawa S, Kaneko S, Kuwana Y, Nakanishi S, et al. Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. Neuron. 2000;28:31–40.

[67] Zeng X, Cai J, Chen J, Luo Y, You Z-B, Fotter E, et al. Dopaminergic differentiation of human embryonic stem cells. Stem Cells. 2004;22:925–40.

[68] Bhagavati S, Xu W. Generation of skeletal muscle from transplanted embryonic stem cells in dystrophic mice. Biochem Biophys Res Commun. 2005;333:644–9.

[69] Dekel I, Magal Y, Pearson-White S, Emerson CP, Shani M. Conditional conversion of ES cells to skeletal muscle by an exogenous MyoD1 gene. New Biol. 1992;4:217–24.

[70] Ozasa S, Kimura S, Ito K, Ueno H, Ikezawa M, Matsukura M, et al. Efficient conversion of ES cells into myogenic lineage using the gene-inducible system. Biochem Biophys Res Commun. 2007;357:957–63.

[71] Tedesco FS, Gerli MFM, Perani L, Benedetti S, Ungaro F, Cassano M, et al. Transplantation of Genetically Corrected Human iPSC-Derived Progenitors in Mice with Limb-Girdle Muscular Dystrophy. Sci Transl Med. 2012; 4(140):140ra89.

[72] Goudenege S, Lebel C, Huot NB, Dufour C, Fujii I, Gekas J, et al. Myoblasts derived from normal hESCs and dystrophic hiPSCs efficiently fuse with existing muscle fibers following transplantation. Mol Ther. 2012; 20(11):2153-67.

[73] Tohyama S, Hattori F, Sano M, Hishiki T, Nagahata Y, Matsuura T, et al. Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. Cell Stem Cell. 2013;12:127–37.

[74] Iacovino M, Bosnakovski D, Fey H, Rux D, Bajwa G, Mahen E, et al. Inducible cassette exchange: a rapid and efficient system enabling conditional gene expression in embryonic stem and primary cells. Stem Cells. 2011;29:1580–7.

[75] Darabi R, Santos FNC, Filareto A, Pan W, Koene R, Rudnicki MA, et al. Assessment of the myogenic stem cell compartment following transplantation of Pax3/Pax7-induced embryonic stem cell-derived progenitors. Stem Cells. 2011;29:777–90.

[76] Darabi R, Pan W, Bosnakovski D, Baik J, Kyba M, Perlingeiro RCR. Functional myogenic engraftment from mouse iPS cells. Stem Cell Rev Reports. 2011;7:948–57.

[77] Darabi R, Arpke RW, Irion S, Dimos JT, Grskovic M, Kyba M, et al. Human ES- and iPS-derived myogenic progenitors restore DYSTROPHIN and improve contractility upon transplantation in dystrophic mice. Cell Stem Cell. 2012;10:610–9.

[78] Zheng JK, Wang Y, Karandikar A, Wang Q, Gai H, Liu AL, et al. Skeletal myogenesis by human embryonic stem cells. Cell Res. 2006;16:713–22.

[79] Barberi T, Willis LM, Socci ND, Studer L. Derivation of multipotent mesenchymal precursors from human embryonic stem cells. PLoS Med. 2005;2:e161.

[80] Barberi T, Bradbury M, Dincer Z, Panagiotakos G, Socci ND, Studer L. Derivation of engraftable skeletal myoblasts from human embryonic stem cells. Nat Med. 2007;13:642–8.

[81] Sakurai H, Okawa Y, Inami Y, Nishio N, Isobe K. Paraxial mesodermal progenitors derived from mouse embryonic stem cells contribute to muscle regeneration via differentiation into muscle satellite cells. Stem Cells. 2008;26:1865–73.

[82] Sakurai H, Sakaguchi Y, Shoji E, Nishino T, Maki I, Sakai H, et al. In vitro modeling of paraxial mesodermal progenitors derived from induced pluripotent stem cells. PLoS One. 2012; 7(10):e47078.

[83] Hwang Y, Phadke A, Varghese S. Engineered microenvironments for self-renewal and musculoskeletal differentiation of stem cells. Regen Med. 2011;6:505–24.

[84] Hwang Y, Suk S, Shih Y-RV, Seo T, Du B, Xie Y, et al. WNT3A promotes myogenesis of human embryonic stem cells and enhances in vivo engraftment. Sci Rep [Internet]. 2014;4:5916. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25084050

[85] Xu C, Tabebordbar M, Iovino S, Ciarlo C, Liu J, Castiglioni A, et al. A zebrafish embryo culture system defines factors that promote vertebrate myogenesis across species. Cell. 2013; 155(4):909-21.

[86] Borchin B, Chen J, Barberi T. Derivation and FACS-mediated purification of PAX3+/PAX7+ skeletal muscle precursors from human pluripotent stem cells. Stem Cell Reports. 2013;1:620–31.

[87] Yasuno T, Osafune K, Sakurai H, Asaka I, Tanaka A, Yamaguchi S, et al. Functional analysis of iPSC-derived myocytes from a patient with carnitine palmitoyltransferase II deficiency. Biochem Biophys Res Commun [Internet]. Elsevier Inc.; 2014;448(2): 175–81. Available from: http://dx.doi.org/10.1016/j.bbrc.2014.04.084

[88] Li HL, Fujimoto N, Sasakawa N, Shirai S, Ohkame T, Sakuma T, et al. Precise correction of the dystrophin gene in duchenne muscular dystrophy patient induced pluripotent stem cells by TALEN and CRISPR-Cas9. Stem Cell Reports [Internet]. The Authors; 2014;4(1):143–54. Available from: http://dx.doi.org/10.1016/j.stemcr.2014.10.013

[89] Aasen T, Belmonte JCI. Isolation and cultivation of human keratinocytes from skin or plucked hair for the generation of induced pluripotent stem cells. Nat Protoc. 2010;5:371–82.

IntechOpen