Received: 14 August 2023 | Revised: 16 November 2023 | Accepted: 9 December 2023

DOI: 10.1111/cts.13703

REVIEW

Considerations for enhanced mesenchymal stromal/stem cell myogenic commitment in vitro

Simone Grobbelaar[1,2] | **Anne E. Mercier**[1] | **Iman van den Bout**[1,3] | **Chrisna Durandt**[2] | **Michael S. Pepper**[2]

[1]Department of Physiology, School of Medicine, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

[2]Institute for Cellular and Molecular Medicine, Department of Immunology, and South African Medical Research Council Extramural Unit for Stem Cell Research and Therapy, School of Medicine, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

[3]Centre for Neuroendocrinology, Department of Immunology, School of Medicine, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

Correspondence
Michael S. Pepper, Institute for Cellular and Molecular Medicine, Department of Immunology, and South African Medical Research Council Extramural Unit for Stem Cell Research and Therapy, School of Medicine, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa.
Email: michael.pepper@up.ac.za

Funding information
South African Medical Research Council, Grant/Award Number: SAMRC-RFA-UFSP-01-2013/STEM CELLS

Abstract

The generation of tissue from stem cells is an alluring concept as it holds a number of potential applications in clinical therapeutics and regenerative medicine. Mesenchymal stromal/stem cells (MSCs) can be isolated from a number of different somatic sources, and have the capacity to differentiate into adipogenic, osteogenic, chondrogenic, and myogenic lineages. Although the first three have been extensively investigated, there remains a paucity of literature on the latter. This review looks at the various strategies available in vitro to enhance harvested MSC commitment and differentiation into the myogenic pathway. These include chemical inducers, myogenic-enhancing cell culture substrates, and mechanical and dynamic culturing conditions. Drawing on information from embryonic and postnatal myogenesis from somites, satellite, and myogenic progenitor cells, the mechanisms behind the chemical and mechanical induction strategies can be studied, and the sequential gene and signaling cascades can be used to monitor the progression of myogenic differentiation in the laboratory. Increased understanding of the stimuli and signaling mechanisms in the initial stages of MSC myogenic commitment will provide tools with which we can enhance their differentiation efficacy and advance the process to clinical translation.

INTRODUCTION

"Mesenchymal stromal/stem cell" (MSC) is an umbrella term for a heterogeneous cell population with multipotent differentiation potential and immunomodulatory properties.[1] A key characteristic of these cells is the ability to differentiate into multiple mesodermal cell types in vitro, including adipogenic, osteogenic, myogenic, and

chondrogenic lineages.[2] These cells are cell culture plastic adherent in vitro and express characterizing surface proteins such as cluster of differentiation (CD)73, CD105, and CD90, amongst others.[2] MSCs can be isolated from multiple sources including bone marrow, adipose tissue, umbilical cord blood, Wharton's jelly, synovial fluid, dental pulp, and peripheral blood.[3] These cells are classified by their source of origin, for example, bone marrow-derived MSCs (BM-MSCs) or adipose-derived stromal/stem cells (ASCs) which are obtained from the stromal vascular fraction (SVF).[4]

These cells hold promise for cell-based therapies and regenerative medicine, and are the focus of intense research and development programs. Globally, there are hundreds of clinical trials investigating the use of MSCs to treat various medical conditions.[5] MSCs have been utilized in the treatment of graft-versus-host disease; osteoarthritis; liver, kidney, cardiac, and lung diseases; autoimmune disorders; neurological diseases; and spinal cord injuries.[5] Although a limited number of adverse effects have been reported, several MSC-based therapies have been approved for clinical use by international regulatory bodies.[6] These include Prochymal® (Remestemcel-L; Osiris Therapeutics Inc.), an intravenous formulation of allogeneic BM-derived MSCs used for the treatment of pediatric acute graft-versus-host disease, and Alofisel® (Darvadstrocel; Cx601; TriGenix and Takeda), an ASC-derived product indicated for the treatment of perianal fistulas in Crohn's disease.[7,8]

Protocols for MSC tri-lineage differentiation (osteogenesis, chondrogenesis, and adipogenesis) in vitro have been extensively published. However, MSCs are also able to differentiate into the myogenic lineage. Despite the development of more advanced protocols, the efficiency of MSCs differentiation into the myogenic lineage has rarely been more than 15%.[9,10] In contrast, at least 40% of primary human myoblasts (satellite cells; PHM) differentiate into myotubes in vitro.[11] This suggests that only PHMs should be considered for therapeutic purposes. However, current protocols used for MSC myogenesis can be improved upon to achieve comparable levels of differentiation. Having access to more than one source of MSCs is likely to enhance the therapeutic potential for muscle regeneration applications.

An important obstacle in the translation of MSCs into cell-based regenerative therapies is the cellular heterogeneity encountered within these isolates. This is due in part to inadequate standardization and reproducibility of isolation, propagation, and differentiation protocols. MSC heterogeneity has been observed between tissue sources, clonal subpopulations, and human donors. This heterogeneity may encompass different stages of cellular senescence and attenuated cell renewal, as well as variable cell viability and differentiation capabilities which correlate to donor age. Advanced donor age may result in telomere shortening in MSCs.[12] Similarly, differences in donor comorbidities have been linked to variation in differentiation capability, with decreased differentiation capacity possibly caused by the dysregulation of pathways associated with stem cell maintenance such as Notch, Hedgehog, and Wnt signaling.[13] Selich et al.[14] demonstrated that clonal diversity of MSCs significantly decreases from as early as the third passage in vitro, while variation in differentiating potential could be observed between clonal populations.[14] Together, these data highlight that both intra- and inter-donor heterogeneity of MSCs can affect their differentiation potential. Research to identify markers or parameters with which MSCs can be stratified would be useful to identify those that have the best characteristics for regenerative therapies.

Along with the heterogeneity already described, tissue sources of MSCs can also affect their differentiation potential in vitro. For instance, bone marrow-derived MSCs have higher osteogenic proclivity, with a decreased adipogenic potential when compared with adipose-derived MSCs.[15] Similarly, MSCs derived from skeletal muscle have an inherent myogenic predilection, while they are unable to differentiate into osteogenic precursors. Lastly, umbilical cord blood-derived MSCs retain a predisposition to differentiate into chondrogenic pathways.[16] Bana et al.[17] reported an upregulation of myogenic markers such as desmin and α-myosin heavy chain (α-MHC) in chorion-derived MSCs (C-MSCs) when compared with umbilical cord blood-derived (UCB)-MSCs. However, umbilical cord tissue-derived MSCs showed enhanced myogenic differentiation when compared with UCB-derived MSCs.[18] These studies highlight the value of careful deliberation when selecting the appropriate cell sources when attempting MSC differentiation into specific lineages for translational applications. Further research exploring the value of tissue source, clonal-subpopulation selection during in vitro culture, and suitability of individual cell donors may greatly improve the efficacy of MSC differentiation for clinical purposes.

SATELLITE CELLS AND MYOGENESIS: SUMMARIZING THE KEY ELEMENTS INVOLVED IN MYOGENIC DIFFERENTIATION IN VIVO

During embryogenesis, the limbs and skeletal muscle are derived from the paraxial mesoderm somites. As these somites mature, they become confined to the dermomyotome.[19] Subsequently, paired box protein (Pax) 3- and 7-expressing muscle progenitor cells arise from

the dermomyotome driven by the sonic hedgehog (Shh), neurogenic locus notch homolog (Notch), and Wingless/Integrated (Wnt) signaling pathways, and are maintained throughout embryogenesis (Figure 1). During development, $Pax3^+$ cells act as founder cells for initial muscle fiber formation. $Pax7^+$ cells potentially stimulate the formation of secondary fibers and are the source of the satellite stem cell pool, which is responsible for adult skeletal muscle repair and maintenance.[20]

Skeletal muscle is composed of cylindrical, contractile, multinucleated myofibrils.[21] Myogenesis is a systematic and coordinated process involving the regulation of gene expression by multiple transcription factors (TFs), including members of the basic helix–loop–helix family.[22] Both satellite and myogenic precursor cells can be induced to form myoblasts, which proliferate prior to exiting the cell cycle, expressing myogenic regulatory factors and undergoing morphological changes.[23] These cells ultimately fuse to create multinucleated myotubes, which then fuse to form characteristic fully differentiated myofibers. Although there are similarities between embryonic myogenesis and postnatal muscle regeneration, distinct differences have been described.[24]

Differentiation of myogenic precursor cells into committed myoblasts is under the primary control of the TFs sineoculis homeobox homolog (Six) 1 and 4 and differs from the early lineage signaling in satellite cells. Six1/4 contain a DNA-binding domain, as well as binding sites for myogenic cofactors.[25,26] During myogenesis Six1/4 associate with eyes absent homolog (Eya) 1 and 2, resulting in their translocation to the nucleus. Here they sequentially induce the expression of Pax3, myogenic differentiation (MyoD), myogenin (MyoG), and myogenic regulatory factor 4 (Mrf4). In turn, Pax 3 along with Six 1/4 enhance the expression of myogenic factor 5 (Myf5) and MyoD (Figure 2).[27] Myf5 and MyoD direct both myogenic precursor and satellite cells towards myogenesis, while MyoG and beta-myosin heavy chain (MyHC) are responsible for their terminal myogenic differentiation.[25]

Myf5 binds to myogenic promoter regions together with MyoD and MyoG. MyoD induces MyoG expression which in turn initiates terminal differentiation and cell fusion (Figure 3).[27,28] The concurrent action of MyoD and MyoG also upregulates late-stage myogenic TFs such as Mrf4.[29] Myf5 is downregulated by the increased expression of MyoG, while MyoD and MyoG expression are abrogated in response to the expression of later-stage myogenic TFs.[28,29] In addition to Pax3/7, the structural intermediate filament, desmin, is also detectable during early myogenesis. Myoblastic desmin is initially expressed at low levels, but increases once Myf5 and MyoD are upregulated in activated and differentiating myoblasts (Figure 4).[30]

The regulation of MyoD is quite complex, with the initial characterization of two enhancer regions located 24 kb upstream from the transcription initiation locus.[31] Further research has revealed that various TFs could interact with these enhancers at various stages of differentiation, which may differ depending on the type of

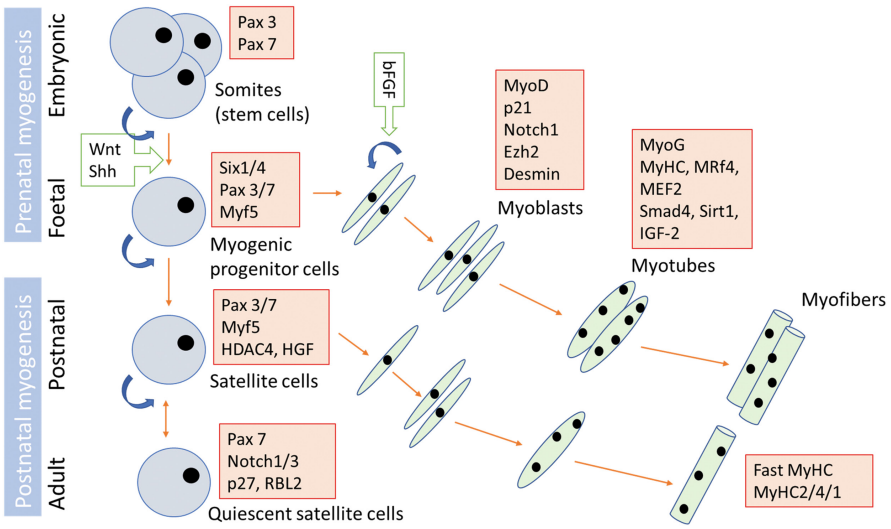

FIGURE 1 Selected signaling pathways, coding genes, and growth factors regulating embryonic and postnatal myogenic differentiation. Not shown are the numerous non-coding RNAs that are integral to this process. Paired box protein (Pax) 3 and 7, sonic hedgehog (Shh), neurogenic locus notch homolog (Notch), Wingless/Integrated (Wnt), sineoculis homeobox homolog (Six) 1 and 4, myogenic differentiation (MyoD), myogenin (MyoG) and myogenic regulatory factor 4 (Mrf4), myogenic factor 5 (Myf5), beta-myosin heavy chain (MyHC), histone deacetylase 4 (HDAC4), hepatocyte growth factor (HGF), retinoblastoma-like protein 2 (RBL2), myocyte enhancer factor 2 (MEF2), insulin-like growth factor 2 (IGF-2), mothers against decapentaplegic homolog 4 (Smad4), NAD-dependent deacetylase sirtuin-1 (Sirt1), enhancer of zeste homolog 2 (EZH2), beta-fibroblast growth factor (bFGF), and myosin heavy chain (MyHC) 2/4/1.

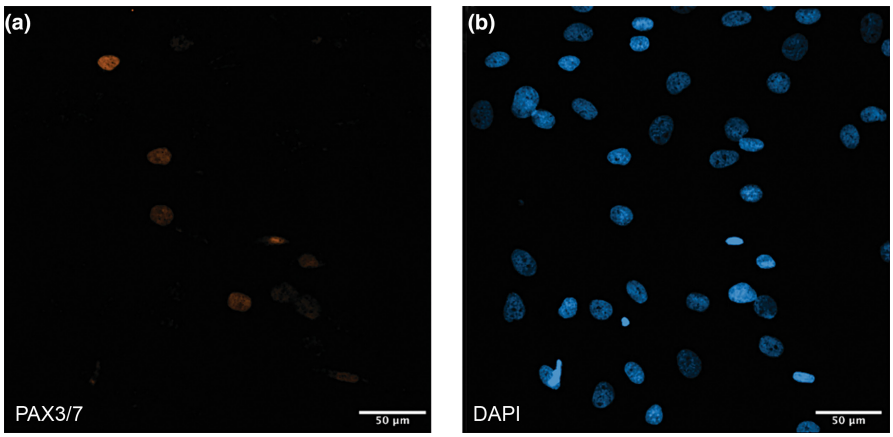

FIGURE 2 Paired box protein 3 and 7 expressed on differentiating muscle progenitors. Single-channel images of a cell culture of primary human myoblasts expressing Pax3/7 (orange) (a) with nuclei stained with DAPI (blue) (b). Scale bar = 50 µm.

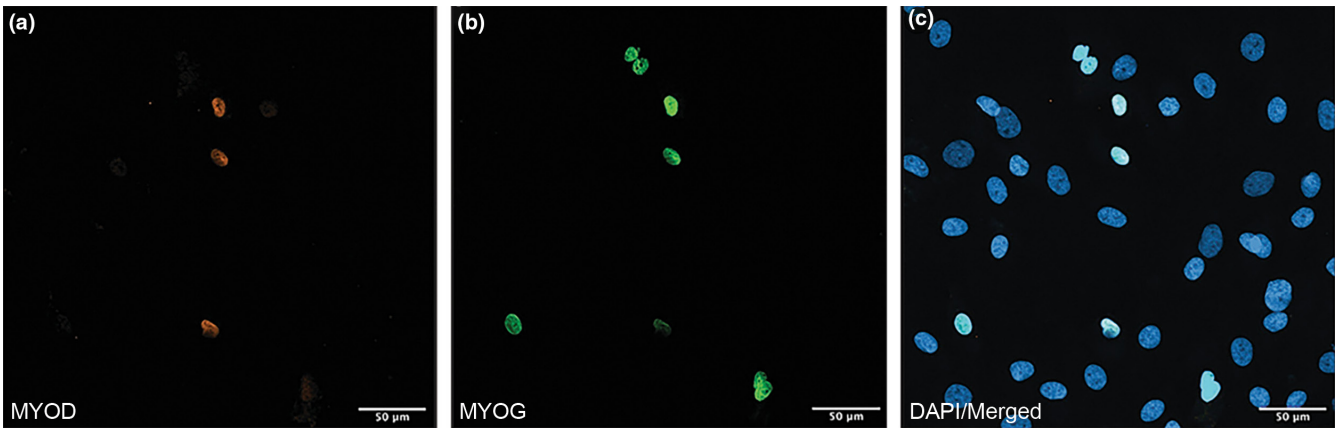

FIGURE 3 Myogenic differentiation and myogenin expressed on differentiating muscle progenitors. Primary human myoblasts were stained for the myogenic proteins myogenic differentiation (MyoD) in orange (a), myogenin (MyoG) in green (b), and nuclei in blue (c; merged image). Merged channel indicates that MyoD is only expressed in MyoG-positive cells while MyoG is not correlated to MyoD expression. Scale bar = 50 µm.

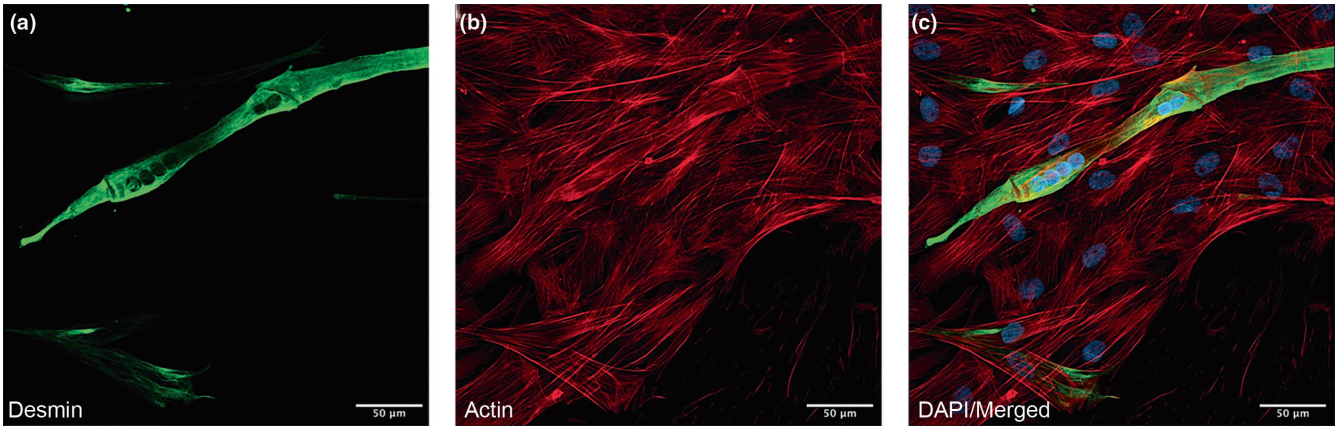

FIGURE 4 Desmin is expressed in differentiating muscle progenitors. Primary human myoblasts were induced for differentiation before being processed for desmin expression (a; green). Actin filaments were visualized with phalloidin (b; red) and nuclei with DAPI (c; merged image; nuclei stained blue). A multinucleated myotube positive for desmin expression is clearly visible. Scale bar = 50 µm.

progenitor cell.[32] Epigenetic changes such as DNA methylation, histone modification, and non-coding RNA interactions also play an integral part in MyoD expression.[23] There is a close relationship between regulation of the cell cycle and MyoD expression, suggesting that MyoD may be a master regulator for myogenesis. Cyclin-dependent

kinase (CDK) 9/cylinT2 is recruited to the transcription complex by MyoD where it phosphorylates the carboxyl terminal of RNA polymerase (pol) II to promote transcription.[33] MyoD also arrests the cell cycle in the G_1/S phase by enhancing the expression of p21. p21 inhibits CDKs and proliferating-cell nuclear antigen (PCNA) that are both necessary for cell cycle progression.[34] It is proposed that this crosstalk between MyoD and cell cycle signaling allows myoblasts to shift from proliferation to differentiation.[35] Moreover, MyoD has been implicated in the regulation of a number of other factors. MyoD regulates the expression of cell division cycle (cdc) 6 to unwind chromatin for replication, allowing cells to enter into the cell cycle.[36] Li et al.[37] demonstrated that MyoD upregulates miRNA-223, which inhibits the expression of both insulin-like growth factor 2 (IGF-2) and zinc finger E-box binding homeobox 1 (ZEB1). IGF-2 inhibition results in repression of myoblastic proliferation, while ZEB1 downregulation promotes differentiation.[37] Understanding the role of MyoD as a master regulator is important in the context of the in vitro application of chemical compounds used to direct progenitor cells into the myogenic lineage, which is discussed later in this review.

The three-dimensional (3D) skeletal muscle extracellular matrix (ECM) scaffold plays an important role in the proliferation, migration, alignment, and differentiation of myocytes. The main constituents of this ECM are collagens (of which types I and III are most abundant), proteoglycans, glycoproteins, and elastin.[38] The transfer of stimuli from the external environment into myofibrils and the triggering of intracellular signaling cascades is mediated by bidirectionally acting integrins.[39] Satellite cell activation entails the recruitment of these cells into the cell cycle for proliferation prior to being committed to differentiation by dedicated TFs. Damage to the muscle fibers or the basal lamina promotes satellite cell activation, with fibroblast growth factor 2 and alternatively spliced isoforms of IGF (mechano growth factor and IGF-1Ea) released from the injured myofibrils being significant triggers for the activation and mobilization of quiescent satellite cells from their niche.[40]

The satellite cell niche is more than just an anatomical location. It is a complex arrangement that allows for the control, activation, migration, proliferation, and differentiation of satellite cells, orchestrated by microenvironmental cues.[41] These cues are regulated by a complex interaction between endothelial cells, macrophages, fibro/adipogenic progenitors, the ECM, and the satellite cells. Within adult skeletal muscle tissue, satellite cells remain quiescent in their niche between the sarcolemma and the surrounding basal lamina.[41] Ras homolog family member A (RhoA) has been implicated in the control of actin cytoskeleton organization and polymerization, satellite recruitment to sites of disruption, and muscle growth and regeneration.[42] Mechanical transduction converts physical stimuli into biochemical messages which are transmitted to the nucleus to induce transcription of essential genes.[42] While quiescent, satellite cells express Pax3/7 in the absence of MyoD.[43] When the integrity of the niche is disrupted in response to stimuli (damage to the muscle, mechanical or chemical disruption), satellite cells downregulate Pax3/7 expression with the concurrent upregulation of MyoD, leading to myogenic differentiation.[41]

With the disruption or injury of the muscle through mechanical sheer forces, autocrine and paracrine factors are released. Such factors include hepatocyte growth factor (HGF), nitric oxide (NO), IGFs, and basic fibroblast growth factor (bFGF), which regulate satellite cell proliferation and differentiation.[44] Increased NO also stimulates HGF expression in stromal and satellite cells, and is present as a soluble factor during muscle regeneration.[45] In turn, HGF binds to c-MET leading to the activation of the MAPK/ERK pathway which directs satellite cell migration.[45] IGF signaling directs satellite cell differentiation through a calcium-mediated response via the action of calcineurin.[46] Moreover, it increases the expression of nuclear factor of activated T-cells (NFAT), a TF implicated in the upregulation of Myf5.[46] bFGF stimulates myoblast proliferation while inhibiting their differentiation.[47] Additionally, monocytes migrate to the niche where they enhance the recruitment and survival of satellite cells through the release of soluble factors and via direct interactions.[48] Other physiological cues implicated in the activation of satellite cells include Notch signaling, phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) signaling, matrix metalloproteinases, and ECM components such as versican, glypicans, fibrillin-2, and deposition of laminin-α1 and -α5.[40,49] A more detailed description of satellite cell activation can be found in the review by Fu et al.[49]

The physiological process directing satellite cells to differentiate into fully functioning muscle fibers is complex. However, as they have been primed for this specific function in vivo, they retain this ability in vitro. In contrast, the molecular processes behind the dedication of MSCs into the myogenic lineage remain unclear. There is a paucity of information on the precise cellular mechanisms and signaling pathways involved in the early stages of myogenic commitment of MSCs, from indication to MyoD expression. The preceding discussion on satellite cell myogenesis has thus been presented to provide a background on how chemical and mechanical induction may be used to manipulate MSCs into a myogenic fate. Research to determine more effective strategies of in vitro MSC differentiation into the myogenic lineage, particularly using

human-equivalent reagents, would aid in understanding the signaling involved and might promote the development of therapeutic strategies using the muscle generated.

CURRENT IN VITRO METHODS TO COMMIT MESENCHYMAL STROMAL/STEM CELLS TO MYOGENESIS

In vitro satellite cell reactivation and differentiation can be stimulated by a carefully curated culture medium via growth medium supplementation. In mouse myoblast (C2C12) or satellite cell/PHM cultures, this is often accomplished through starvation, or may occur spontaneously upon cell confluency.[50] Conditions differ when the same is attempted with MSCs. Factors which influence the efficacy of MSC differentiation into the myogenic pathway include chemical induction constituents, cell culture substrates, and mechanical strain.

Chemical inducers for mesenchymal stromal/stem cell myogenesis

Zuk et al.[9] were the first to demonstrate that ASCs isolated from the SVF, the adipocyte-free cellular fraction of adipose tissue, can be differentiated into a myogenic lineage over a period of 42 days by supplementing the induction medium with dexamethasone and hydrocortisone. This induction protocol was modified in 2002 by Mizuno et al.,[51] who used only hydrocortisone. The efficiency of myogenic differentiation has been further improved by exposing ASCs to the global demethylating agent 5-azacytidine (5-Aza) for 21 days.[52]

Glucocorticoids (GCs)

The use of dexamethasone for the induction of MSC myogenesis is well established, particularly when used in combination with hydrocortisone.[18] GCs are used clinically as anti-inflammatory and immunosuppressive agents. Long-term therapeutic use causes muscle atrophy due to GC-mediated proteolysis and increased levels of myostatin (a negative regulator of myogenesis).[53,54] However, commitment of MSCs to myogenesis in vitro can be achieved with a combination of GCs. The molecular mechanism through which the GCs, particularly dexamethasone, achieve in vitro myogenic commitment of MCSs has not yet been clearly defined. Dexamethasone appears to augment the formation of force-producing sarcomeric structures in skeletal muscle cells in a rat model.[54] Moreover, it increases cellular proliferation and augments the expression of MyoD, MyoG, and dysferlin in vitro.[54,55]

The dose and timing of MSC exposure to GCs may influence the efficiency of differentiation, proliferation, and expression of myogenic markers.[54] Dexamethasone may either promote or inhibit myogenesis in vitro, depending on the timing of the treatment. Han et al.[56] reported that C2C12 cells treated with dexamethasone at the myotube stage atrophied, while those exposed during the myoblast stage increased MyHC expression and resultant myotube growth. McRae et al.[57] demonstrated that dexamethasone promotes myotube fusion in vitro by attenuating the synthesis of versican, an ECM proteoglycan that forms part of the transitional matrix during muscle regeneration. The transitional matrix, composed of ECM factors such as hyaluronic acid, fibronectin, and tenascin, promotes myoblast proliferation, alignment, and fusion.[58] This matrix diminishes as myocytes fuse and myotubes regenerate. Tenascin and hyaluronic acid appear to form a boundary, allowing muscle to regenerate and form attachments to tendons, ligaments, or bone.[58]

Differentiating myoblasts use focal adhesions to align themselves before fusing into myotubes.[59] Preceding this, actin filaments anchor to focal adhesions in the membrane to begin the formation of myofibrils.[60] Focal adhesions and their associated actin filaments are essential for myoblast fusion and contractile force production.[60] β1 integrin forms the core of the focal adhesions, creating an extracellular receptor for ECM ligands, while connecting to the actin cytoskeleton via adapter molecules such as vinculin intracellularly. Lin et al.[61] proposed that dexamethasone may promote muscle regeneration by directly modulating kinesin-1 to accelerate myotube fusion and enhance mitochondrial movement during differentiation. Kinesin-1 recruits β1 integrin to focal adhesions during differentiation. Inhibition of kinesin-1 in differentiating C2C12 cells causes a significant decrease in expression of MyHC 1 and 2, causing a delay in cell fusion.[61] This suggests that dexamethasone promotes myogenic differentiation through kinesin-1.

5-Azacytidine

The demethylating agent 5-Aza is incorporated into DNA and forms irreversible covalent bonds with DNA-cytosine methyltransferase at cytosine-phosphate-guanine (CpG) sites, inhibiting its methylation activity.[62] The role of methylation in regulating gene expression has been well described. Hypermethylation of promoter regions is associated with decreased gene expression, while hypomethylation is correlated with increased expression.[63]

Pretreatment with 5-Aza reprograms the epigenetic landscape to enhance cell stemness and differentiation potential by restricting methylation of key regulatory elements.[64] 5-Aza is incorporated into DNA during the S-phase of the cell cycle after which the enzymatic activity of methyltransferase is permanently silenced, resulting in the expression of previously inhibited GpC regions in the daughter cells.[65]

Multiple studies have demonstrated that 5-Aza incorporation leads to the upregulation of factors essential for myogenic differentiation including Myf5, MyoD, MyoG, and desmin.[52,66–68] 5-Aza-mediated demethylation also enhances myotube formation in the later stages of myogenesis by upregulating the IGF-1 pathway, which is involved in the hypertrophic process.[69] Montesano et al. (2013)[62] demonstrated that 5-Aza significantly upregulates p21, promoting myoblastic differentiation. Burlacu et al.[52] reported that 5-Aza-exposure results in a sequential decrease in osteocalcin and alkaline phosphatase expression in rat BM-MSCs, while connexin 43 is upregulated. By 14 days, the cells take on a myocyte-like phenotype and express multiple cardiomyogenic proteins and genes (MyoD, desmin, α-actinin, and troponin T). 5-Aza appears to stimulate myogenesis through the demethylation of myogenic promoters, upregulation of essential myogenic proteins (MyoD, Myf5, MyoG, and desmin), and by regulating the cell cycle (through p21). Table 1 summarizes the main chemical components (GCs and 5-Aza) used for myogenic differentiation in vitro.

Myogenesis-enhancing cell culture substrates

Cell culture substrates have important effects on cell behavior in vitro. Cells exist in 3D microenvironments in vivo, with physical niches for stem cells which contribute to their stemness. The efficiency of myogenesis in vitro can be influenced by ECM substrates, including collagen type I (Col I), fibronectin, and ECM mimetics such as Matrigel®. Engler et al.[73] demonstrated that contractile cells sense their immediate microenvironment in terms of its mechanical and molecular characteristics, and that optimal myocyte fusion occurs on substrates that mimic tissue-like environments.

The composition of the substrate has a significant effect on MSC myogenic differentiation. Liu et al.[74] reported that Col I significantly enhanced myocyte migration and differentiation, but not proliferation, through increased focal adhesion kinase (FAK) phosphorylation via interleukin 6 and nuclear factor kappa B (NFκB). Vaz et al.[75] demonstrated that fibronectin promotes the migration, alignment, and fusion of C2C12 myoblasts, while Grefte et al.[76] showed that seeding and

TABLE 1 Chemical components used to induce myogenesis and their reported effects.

Chemical inducer	Cell type/source	Effect	Reference(s)
Glucocorticoids	Skeletal muscle units (rat soleus muscle)	Increases the expression of MyoD; stimulates myoblast proliferation; improves myotube fusion	70
	Skeletal muscle units (rat soleus muscle)	Increases expression of MyoD and MyoG	54
	Hindlimb tibialis anterior muscles from dystrophic mdx mice	Promotes in vitro myotube fusion by attenuating versican expression	57
	C2C12 myoblasts	Induces myogenesis through a kinesin-1-mediated mechanism	61
	Human stromal vascular fraction	Increases the expression of MyoD and MyHC	9,51
5-Azacytidine	C2C12 myoblasts	Regulates the cell cycle to enhance myogenic differentiation	62
	Mouse atrial tissue	Transdifferentiation of cardiac muscle cells into skeletal muscle by upregulating the expression of MyoD and MyoG	71
	Rat BM-MSCs	Increases calcium channel sensitivity; promotes expression of MyoD, desmin, α-actinin, and troponin T	52
	Human ASCs	Increases expression of MyoD, MyoG, desmin, MyHC	67,68,72

Abbreviations: ASCs, adipose-derived stromal/stem cells; BM-MSCs, bone marrow-derived mesenchymal stromal/stem cells; MyHC, beta-myosin heavy chain; MyoD, myogenic differentiation; MyoG, myogenin.

differentiating satellite cells on Matrigel resulted in significantly enhanced proliferation, migration, expression of myogenic markers, and fusion capability when compared with cells cultured and differentiated on Col I. Matrigel is composed of multiple ECM factors including laminin, collagen IV, heparan sulfate proteoglycans, and entactin/nidogen.[77]

Conventional two-dimensional (2D) cell culture forces cells to propagate in monolayers, which means external conditions are uniform, nutrients are equally distributed, and access to nutrients and other factors is unimpeded. However, there are several critical limitations in such a culture system with respect to muscle tissue engineering: it does not represent the architecture of the niche and its ECM microenvironment; cellular cross-talk is only bidirectional as cells do not form 3D structures; it does not allow 3D muscle structural organization; and formation of terminally differentiated contractile cells is inefficient.[78,79] To mitigate these limitations, researchers have explored the establishment of 3D cell culture environments which aim to support multidirectional interactions using scaffolds. If the niche architecture and composition is accurately reproduced in vitro, it will support improved myogenesis.[80]

3D scaffolds can be constructed from hydrogels synthesized from proteins such as collagen, alginate, gelatin, fibrin, or Matrigel.[81–84] Compared with 2D cell cultures, 3D cultures of myocytes efficiently generate terminally differentiated and contractile muscle fibers.[83] Using satellite cells, Pollot et al.[84] found that hydrogel scaffolds consisting of either fibrin or collagen demonstrated superior myogenic ability to agarose or alginate scaffolds. Scaffolds may also be designed to direct and maintain specific alignments (by adding microgrooves), which significantly improve the formation of functional muscle fibers.[85,86] Nakayama et al.[87] reported that when compared with randomly orientated scaffolds, myoblasts co-cultured with endothelial cells formed highly organized functional muscle fibers with synchronized contractability and vascularization when seeded and induced on linear scaffolds. Witt et al.[88] reported that a 3D environment enhanced the myogenic ability of rat BM-MSCs co-cultured with myoblasts, further demonstrating 3D culture potential for the enhancement of myogenesis in co-culture conditions.

In summary, pertinent considerations when selecting a culture substrate for myogenesis include substrate stiffness, its effect on myocyte migration, alignment and fusion, 2D or 3D culture environments, and the inclusion of other cell types in a co-culture condition. Additionally, it appears that substrates consisting of multiple ECM components such as Matrigel provide an advantage to myogenic differentiation over single component substrates.

Mechanical induction and dynamic culture conditions

Mechanical stimulation significantly enhances in vitro myogenesis in MSCs, primary myoblasts, and immortalized myoblast cell lines. Various strain regimens have been employed to enhance myogenesis in 2D and 3D environments (comprehensively reviewed by Somers et al., 2017).[89] In human MSCs, Huri et al.[67,68] demonstrated that applying uniaxial strain enhances myogenic induction by significantly increasing the expression of myogenic factors such as MyoD, Pax3/7, desmin, and ultimately MyHC when compared with cells cultured under static conditions. In addition, dynamic cultures demonstrate superior myoblast alignment and fusion capabilities. However, mechanical induction alone does not commit MSCs to myogenesis and is dependent on a combined effect together with biochemical stimulation.[68]

Similar results were obtained by Ergene et al.[90] in which MSCs isolated from adipose tissue seeded onto fibrin hydrogel scaffolds were induced using 5-Aza and subjected to uniaxial cyclic strain. MSCs induced under these conditions showed enhanced expression of MyoD, MyoG, desmin, and MyHC when compared with MSCs induced without uniaxial strain. These results further indicate that biochemical and biomechanical stimulation act synergistically to increase myogenic differentiation efficiency.

The mechanisms through which tensile strain enhances myogenesis in vitro have not been clarified. Torsoni et al.[91] reported that cyclic strain enhances FAK phosphorylation in cardiac myocytes through RhoA/ROCK signaling pathways, possibly by coordinating upstream factors associated with the actin cytoskeleton. In addition, Anderson et al.[92] found that C2C12 cells failed to respond to cyclic strain when cultured in the presence of a FAK inhibitor. This suggests that FAK activation is an essential component for cellular alignment and enhanced differentiation in strain-induced myogenesis. Strain-induced signaling may also be involved in the upregulation of key myogenic factors.[67,68,72]

The type of strain (whether uni- or biaxial) can influence cellular alignment and differentiation capability. Uniaxial strain appears to be superior, as it facilitates cellular alignment by imitating the linear application of force as is observed in muscle contraction. C2C12 myoblasts exposed to uniaxial strain demonstrated improved alignment, fusion, and expression of myogenic markers when compared with cells exposed to biaxial strain.[93] Additionally, cells with a myogenic origin tend to align perpendicular to strain, while cells with a non-myogenic origin (such as MSCs) tend to align parallel to the strain, suggesting that the response to mechanical cues may vary between cell populations.[68,89,93] Like the induction of focal adhesion signaling by the substrate component and stiffness, strain may also facilitate the formation and correct

signaling of focal adhesions which seem to be essential for efficient differentiation, cell alignment, and fusion.

It is possible to adjust the elastic modulus (E) or elasticity of certain substrates (collagen and polyacrylamide gels) to mimic that of the in vivo environment.[94] This is important because the elasticity of the muscle environment is less pliant than other tissues such as neurons in the brain, but more pliant than epithelial tissues, stroma, and skin. The adjustability of matrix proteins such as collagen can thus be exploited to enhance myogenesis in vitro.[94] Rowlands et al.[95] highlighted two important factors to consider when inducing myogenesis. First, altering the elastic modulus influences the success of myogenesis, and second, the elasticity may vary depending on the coating. MyoD expression was shown to be highest when fibronectin gels with a modulus of 25 kPa were used. In contrast, cells differentiating on Col I gels showed a peak in MyoD expression when the modulus was 80 kPa. Substrate stiffness also affects integrin adhesion, with more focal adhesions being present on stiffer substrates than on more flexible counterparts, resulting in increased FAK phosphorylation and proliferation.[95]

IN VIVO APPLICATIONS OF MSCs IN MUSCLE PATHOLOGY

Muscle atrophy is a well-known adverse effect of long-term therapeutic use of GCs. Directly injecting adipose-derived MSCs into GC-induced atrophied muscle displayed promising results in murine models by reversing the atrophic phenotype via the extracellular signal regulated kinase (ERK)-1/2 signaling pathway.[96] Similarly, when MSCs obtained from adult human synovial membranes were implanted into dystrophic muscles of immunosuppressed mouse models of Duchenne muscular dystrophy, expression of dystrophin in the sarcolemma was restored.[97] Recently, Wang et al.[98] published that human umbilical-derived MSCs given to aging mice restored the strength of sarcopenic muscles. Although stem cells do display a promising avenue for muscle regeneration, there is a paucity of literature on ex vivo MSC myogenic differentiation prior to transplantation. This may possibly be attributed to the challenges associated with in vitro myogenic differentiation protocols. However, considering the potential of MSCs, more research and refinement of these protocols may yield rewarding results.

CONCLUDING REMARKS

MSCs are an attractive cellular resource for regenerative medicine, and have demonstrated the capacity to differentiate into a myogenic lineage. However, the efficacy with which it has been achieved has varied across different published studies. To enhance the process, it is important to consider appropriate culture substrates in 2D or 3D scaffolds to create an optimal environment for myogenesis, along with both biochemical and biomechanical stimulation. It appears that co-culture with endothelial cells on a stiff 3D culture substrate consisting of a mixture of constituents including at least collagen I, under unidirectional strain, may result in the most efficient differentiation. Adapting such a model for larger-scale production of functional muscle fibers, together with the utilization of human-equivalents in these culture conditions, will be the next opportunity towards tissue engineering for clinical application.

ACKNOWLEDGMENTS

The authors offer special thanks to Prof. Kathy Myburgh (Department of Physiology, University of Stellenbosch) for providing the primary human myoblasts used to generate confocal images.

FUNDING INFORMATION

This research was funded by the South African Medical Research Council in terms of the SAMRC's Flagship Award Project (SAMRC-RFA-UFSP-01-2013/STEM CELLS), the SAMRC Extramural Unit for Stem Cell Research and Therapy, and the Institute for Cellular and Molecular Medicine of the University of Pretoria.

CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

ORCID

Anne E. Mercier https://orcid.org/0000-0002-2030-9879
Iman van den Bout https://orcid.org/0000-0001-6333-1470
Michael S. Pepper https://orcid.org/0000-0001-6406-2380

REFERENCES

1. Caplan AI. Mesenchymal stem cells: time to change the name! *Stem Cells Transl Med.* 2017;6:1445-1451.
2. Dominici M, le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8:315-317.
3. Mushahary D, Spittler A, Kasper C, Weber V, Charwat V. Isolation, cultivation, and characterization of human mesenchymal stem cells. *Cytometry A.* 2018;93:19-31.
4. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose

Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy*. 2013;15:641-648.
5. Rodríguez-Fuentes DE, Fernández-Garza LE, Samia-Meza JA, Barrera-Barrera SA, Caplan AI, Barrera-Saldaña HA. Mesenchymal stem cells current clinical applications: a systematic review. *Arch Med Res*. 2021;52:93-101.
6. Baranovskii DS, Klabukov ID, Arguchinskaya NV, et al. Adverse events, side effects and complications in mesenchymal stromal cell-based therapies. *Stem Cell Investig*. 2022;9:7.
7. Elgaz S, Kuçi Z, Kuçi S, Bönig H, Bader P. Clinical use of mesenchymal stromal cells in the treatment of acute graft-versus-host disease. *Transfus Med Hemother*. 2019;46:27-34.
8. TiGenix and Takeda announce Alofisel® (darvadstrocel) receives approval to treat complex perianal fistulas in Crohn's disease in Europe. [updated: 23 March 2018; cited 20 July 2023]. https://www.takeda.com/newsroom/newsreleases/2018/tigenix-and-takeda-announce-alofisel-receives-approval-in-europe/
9. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7:211-228.
10. Barnett HH, Pursell IA, Lee L, Perez N, Caldorera-Moore M, Newman JJ. Combination of soluble factors and biomaterial scaffolds enhance human adipose-derived stem/stromal cell myogenesis. *Biochem Biophys Res Commun*. 2020;529:1180-1185.
11. Benedetti A, Cera G, de Meo D, Villani C, Bouche M, Lozanoska-Ochser B. A novel approach for the isolation and long-term expansion of pure satellite cells based on ice-cold treatment. *Skelet Muscle*. 2021;11:7.
12. Choudhery MS, Badowski M, Muise A, Pierce J, Harris DT. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. *J Transl Med*. 2014;12:8.
13. Ferrer-Lorente R, Bejar MT, Tous M, Vilahur G, Badimon L. Systems biology approach to identify alterations in the stem cell reservoir of subcutaneous adipose tissue in a rat model of diabetes: effects on differentiation potential and function. *Diabetologia*. 2014;57:246-256.
14. Selich A, Daudert J, Hass R, et al. Massive clonal selection and transiently contributing clones during expansion of mesenchymal stem cell cultures revealed by lentiviral RGB-barcode technology. *Stem Cells Transl Med*. 2016;5:591-601.
15. Xu L, Liu Y, Sun Y, et al. Tissue source determines the differentiation potentials of mesenchymal stem cells: a comparative study of human mesenchymal stem cells from bone marrow and adipose tissue. *Stem Cell Res Therapy*. 2017;8:275.
16. Sacchetti B, Funari A, Remoli C, et al. No identical "mesenchymal stem cells" at different times and sites: human committed progenitors of distinct origin and differentiation potential are incorporated as adventitial cells in microvessels. *Stem Cell Rep*. 2016;6:897-913.
17. Bana N, Sanooghi D, Soleimani M, et al. A comparative study to evaluate myogenic differentiation potential of human chorion versus umbilical cord blood-derived mesenchymal stem cells. *Tissue Cell*. 2017;49:495-502.
18. Mishra S, Sevak JK, Das A, Arimbasseri GA, Bhatnagar S, Gopinath SD. Umbilical cord tissue is a robust source for mesenchymal stem cells with enhanced myogenic differentiation potential compared to cord blood. *Sci Rep*. 2020;10:18978.
19. Buckingham M, Bajard L, Chang T, et al. The formation of skeletal muscle: from somite to limb. *J Anat*. 2003;202:59-68.
20. Maqbool T, Jagla K. Genetic control of muscle development: learning from Drosophila. *J Muscle Res Cell Motil*. 2007;28:397-407.
21. Sambasivan R, Tajbakhsh S. Skeletal muscle stem cell birth and properties. *Semin Cell Dev Biol*. 2007;18:870-882.
22. Chang CN, Kioussi C. Location, location, location: signals in muscle specification. *J Dev Biol*. 2018;6:11.
23. Luo H, Lv W, Tong Q, Jin J, Xu Z, Zuo B. Functional non-coding RNA during embryonic myogenesis and postnatal muscle development and disease. *Front Cell Dev Biol*. 2021;9:628339.
24. Tajbakhsh S. Skeletal muscle stem cells in developmental versus regenerative myogenesis. *J Intern Med*. 2009;266:372-389.
25. Bentzinger CF, Wang YX, Rudnicki MA. Building muscle: molecular regulation of myogenesis. *Cold Spring Harb Perspect Biol*. 2012;4:a008342.
26. Grifone R, Demignon J, Houbron C, et al. Six1 and Six4 homeoproteins are required for Pax3 and Mrf expression during myogenesis in the mouse embryo. *Development*. 2005;132:2235-2249.
27. Buckingham M, Rigby PW. Gene regulatory networks and transcriptional mechanisms that control myogenesis. *Dev Cell*. 2014;28:225-238.
28. Deato MDE, Marr MT, Sottero T, Inouye C, Hu P, Tjian R. MyoD targets TAF3/TRF3 to activate myogenin transcription. *Mol Cell*. 2008;32:96-105.
29. Hernández-Hernández JM, García-González EG, Brun CE, Rudnicki MA. The myogenic regulatory factors, determinants of muscle development, cell identity and regeneration. *Semin Cell Dev Biol*. 2017;72:10-18.
30. Creuzet S, Lescaudron L, Li Z, Fontaine-Pérus J. MyoD, myogenin, and desmin-nls-lacZ transgene emphasize the distinct patterns of satellite cell activation in growth and regeneration. *Exp Cell Res*. 1998;243:241-253.
31. Chen JC, Love CM, Goldhamer DJ. Two upstream enhancers collaborate to regulate the spatial patterning and timing of MyoD transcription during mouse development. *Dev Dyn*. 2001;221:274-288.
32. Wardle FC. Master control: transcriptional regulation of mammalian Myod. *J Muscle Res Cell Motil*. 2019;40:211-226.
33. Simone C, Stiegler P, Bagella L, et al. Activation of MyoD-dependent transcription by cdk9/cyclin T2. *Oncogene*. 2002;21:4137-4148.
34. Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T, Hannon GJ. Radiation-induced cell cycle arrest compromised by p21 deficiency. *Nature*. 1995;377:552-557.
35. Kitzmann M, Fernandez A. Crosstalk between cell cycle regulators and the myogenic factor MyoD in skeletal myoblasts. *Cell Mol Life Sci*. 2001;58:571-579.
36. Zhang K, Sha J, Harter ML. Activation of Cdc6 by MyoD is associated with the expansion of quiescent myogenic satellite cells. *J Cell Biol*. 2010;188:39-48.
37. Li G, Luo W, Abdalla BA, et al. miRNA-223 upregulated by MYOD inhibits myoblast proliferation by repressing IGF2 and facilitates myoblast differentiation by inhibiting ZEB1. *Cell Death Dis*. 2017;8:e3094.
38. Csapo R, Gumpenberger M, Wessner B. Skeletal muscle extracellular matrix—what do we know about its composition, regulation, and physiological roles? A narrative review. *Front Physiol*. 2020;11:253.

39. Boppart MD, Mahmassani ZS. Integrin signaling: linking mechanical stimulation to skeletal muscle hypertrophy. *Am J Physiol Cell Physiol*. 2019;317:C629-c641.
40. Rayagiri SS, Ranaldi D, Raven A, et al. Basal lamina remodeling at the skeletal muscle stem cell niche mediates stem cell self-renewal. *Nat Commun*. 2018;9:1075.
41. Yin H, Price F, Rudnicki MA. Satellite cells and the muscle stem cell niche. *Physiol Rev*. 2013;93:23-67.
42. Noviello C, Kobon K, Delivry L, et al. RhoA within myofibers controls satellite cell microenvironment to allow hypertrophic growth. *iScience*. 2022;25:103616.
43. Dumont NA, Rudnicki MA. Characterizing satellite cells and myogenic progenitors during skeletal muscle regeneration. *Methods Mol Biol*. 2017;1560:179-188.
44. Tatsumi R, Anderson JE, Nevoret CJ, Halevy O, Allen RE. HGF/SF is present in normal adult skeletal muscle and is capable of activating satellite cells. *Dev Biol*. 1998;194:114-128.
45. González MN, de Mello W, Butler-Browne GS, et al. HGF potentiates extracellular matrix-driven migration of human myoblasts: involvement of matrix metalloproteinases and MAPK/ERK pathway. *Skelet Muscle*. 2017;7:20.
46. Friday BB, Pavlath GK. A calcineurin- and NFAT-dependent pathway regulates Myf5 gene expression in skeletal muscle reserve cells. *J Cell Sci*. 2001;114:303-310.
47. Yoshida S, Fujisawa-Sehara A, Taki T, Arai K, Nabeshima Y. Lysophosphatidic acid and bFGF control different modes in proliferating myoblasts. *J Cell Biol*. 1996;132:181-193.
48. Liu X, Wu G, Shi DD, et al. Effects of nitric oxide on notexin-induced muscle inflammatory responses. *Int J Biol Sci*. 2015;11:156-167.
49. Fu X, Wang H, Hu P. Stem cell activation in skeletal muscle regeneration. *Cell Mol Life Sci*. 2015;72:1663-1677.
50. Vaughan M, Lamia KA. Isolation and differentiation of primary myoblasts from mouse skeletal muscle explants. *J Vis Exp*. 2019. doi:10.3791/60310
51. Mizuno H, Zuk PA, Zhu M, Lorenz PH, Benhaim P, Hedrick MH. Myogenic differentiation by human processed lipoaspirate cells. *Plast Reconstr Surg*. 2002;109:199-209; discussion 210–211.
52. Burlacu A, Rosca AM, Maniu H, et al. Promoting effect of 5-azacytidine on the myogenic differentiation of bone marrow stromal cells. *Eur J Cell Biol*. 2008;87:173-184.
53. Kuo T, Harris CA, Wang J-C. Metabolic functions of glucocorticoid receptor in skeletal muscle. *Mol Cell Endocrinol*. 2013;380:79-88.
54. Larson AA, Syverud BC, Florida SE, Rodriguez BL, Pantelic MN, Larkin LM. Effects of dexamethasone dose and timing on tissue-engineered skeletal muscle units. *Cell Tissue Res*. 2018;205:197-207.
55. Belanto JJ, Diaz-Perez SV, Magyar CE, et al. Dexamethasone induces dysferlin in myoblasts and enhances their myogenic differentiation. *Neuromuscul Disord*. 2010;20:111-121.
56. Han D-S, Yang W-S, Kao T-W. Dexamethasone treatment at the myoblast stage enhanced C2C12 myocyte differentiation. *Int J Med Sci*. 2017;14:434-443.
57. McRae N, Forgan L, McNeill B, et al. Glucocorticoids improve myogenic differentiation in vitro by suppressing the synthesis of versican, a transitional matrix protein overexpressed in dystrophic skeletal muscles. *Int J Mol Sci*. 2017;18:2629.
58. Calve S, Odelberg SJ, Simon H-G. A transitional extracellular matrix instructs cell behavior during muscle regeneration. *Dev Biol*. 2010;344:259-271.
59. Fulton AB, Prives J, Farmer SR, Penman S. Developmental reorganization of the skeletal framework and its surface lamina in fusing muscle cells. *J Cell Biol*. 1981;91:103-112.
60. Quach NL, Biressi S, Reichardt LF, Keller C, Rando TA. Focal adhesion kinase signaling regulates the expression of caveolin 3 and beta1 integrin, genes essential for normal myoblast fusion. *Mol Biol Cell*. 2009;20:3422-3435.
61. Lin J-W, Huang YM, Chen YQ, et al. Dexamethasone accelerates muscle regeneration by modulating kinesin-1-mediated focal adhesion signals. *Cell Death Discov*. 2021;7:35.
62. Montesano A, Luzi L, Senesi P, Terruzzi I. Modulation of cell cycle progression by 5-azacytidine is associated with early myogenesis induction in murine myoblasts. *Int J Biol Sci*. 2013;9:391-402.
63. Kribelbauer JF, Lu X-J, Rohs R, Mann RS, Bussemaker HJ. Toward a mechanistic understanding of DNA methylation readout by transcription factors. *J Mol Biol*. 2020;432:1801-1815.
64. Ong ALC, Lee SH, Aung SW, Khaing SL, Ramasamy TS. 5-azacytidine pretreatment confers transient upregulation of proliferation and stemness in human mesenchymal stem cells. *Cells Dev*. 2021;165:203659.
65. Christman JK. 5-Azacytidine and 5-aza-2′-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene*. 2002;21:5483-5495.
66. Fei W, Liu M, Zhang Y, et al. Identification of key pathways and hub genes in the myogenic differentiation of pluripotent stem cell: a bioinformatics and experimental study. *J Orthop Surg Res*. 2021;16:4.
67. Huri PY, Morrissette-McAlmon J, Grayson WL. Myogenic differentiation of ASCs using biochemical and biophysical induction. *Methods Mol Biol*. 2018;1773:123-135.
68. Yilgor Huri P, Cook CA, Hutton DL, et al. Biophysical cues enhance myogenesis of human adipose derived stem/stromal cells. *Biochem Biophys Res Commun*. 2013;438:180-185.
69. Senesi P, Luzi L, Montesano A, Terruzzi I. DNA demethylation enhances myoblasts hypertrophy during the late phase of myogenesis activating the IGF-I pathway. *Endocrine*. 2014;47:244-254.
70. Syverud BC, VanDusen KW, Larkin LM. Effects of dexamethasone on satellite cells and tissue engineered skeletal muscle units. *Tissue Eng Part A*. 2016;22:480-489.
71. Kaur K, Yang J, Eisenberg CA, Eisenberg LM. 5-azacytidine promotes the transdifferentiation of cardiac cells to skeletal myocytes. *Cell Reprogram*. 2014;16:324-330.
72. Huri PY, Wang A, Spector AA, Grayson WL. Multistage adipose-derived stem cell myogenesis: an experimental and modeling study. *Cell Mol Bioeng*. 2014;7:497-509.
73. Engler AJ, Griffin MA, Sen S, Bönnemann CG, Sweeney HL, Discher DE. Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J Cell Biol*. 2004;166:877-887.
74. Liu X, Gao Y, Long X, et al. Type I collagen promotes the migration and myogenic differentiation of C2C12 myoblasts via the release of interleukin-6 mediated by FAK/NF-κB p65 activation. *Food Funct*. 2020;11:328-338.
75. Vaz R, Martins GG, Thorsteinsdóttir S, Rodrigues G. Fibronectin promotes migration, alignment and fusion in an in vitro myoblast cell model. *Cell Tissue Res*. 2012;348:569-578.

76. Grefte S, Vullinghs S, Kuijpers-Jagtman AM, Torensma R, Von den Hoff JW. Matrigel, but not collagen I, maintains the differentiation capacity of muscle derived cells in vitro. *Biomed Mater*. 2012;7:055004.
77. Aisenbrey EA, Murphy WL. Synthetic alternatives to Matrigel. *Nat Rev Mater*. 2020;5:539-551.
78. Dessauge F, Schleder C, Perruchot M-H, Rouger K. 3D in vitro models of skeletal muscle: myopshere, myobundle and bioprinted muscle construct. *Vet Res*. 2021;52:72.
79. Tan SJ, Fang JY, Wu Y, Yang Z, Liang G, Han B. Muscle tissue engineering and regeneration through epigenetic reprogramming and scaffold manipulation. *Sci Rep*. 2015;5:16333.
80. Alarcin E, Bal-Öztürk A, Avci H, et al. Current strategies for the regeneration of skeletal muscle tissue. *Int J Mol Sci*. 2021;22:5929.
81. Bettadapur A, Suh GC, Geisse NA, et al. Prolonged culture of aligned skeletal myotubes on micromolded gelatin hydrogels. *Sci Rep*. 2016;6:28855.
82. Rimington RP, Fleming JW, Capel AJ, Wheeler PC, Lewis MP. Bioengineered model of the human motor unit with physiologically functional neuromuscular junctions. *Sci Rep*. 2021;11:11695.
83. Alave Reyes-Furrer A, de Andrade S, Bachmann D, et al. Matrigel 3D bioprinting of contractile human skeletal muscle models recapitulating exercise and pharmacological responses. *Commun Biol*. 2021;4:1183.
84. Pollot BE, Rathbone CR, Wenke JC, Guda T. Natural polymeric hydrogel evaluation for skeletal muscle tissue engineering. *J Biomed Mater Res B Appl Biomater*. 2018;106:672-679.
85. Basurto IM, Mora MT, Gardner GM, Christ GJ, Caliari SR. Aligned and electrically conductive 3D collagen scaffolds for skeletal muscle tissue engineering. *Biomater Sci*. 2021;9:4040-4053.
86. Urciuolo A, Serena E, Ghua R, et al. Engineering a 3D in vitro model of human skeletal muscle at the single fiber scale. *PloS One*. 2020;15:e0232081.
87. Nakayama KH, Quarta M, Paine P, et al. Treatment of volumetric muscle loss in mice using nanofibrillar scaffolds enhances vascular organization and integration. *Commun Biol*. 2019;2:170.
88. Witt R, Weigand A, Boos AM, et al. Mesenchymal stem cells and myoblast differentiation under HGF and IGF-1 stimulation for 3D skeletal muscle tissue engineering. *BMC Cell Biol*. 2017;18:15.
89. Somers SM, Spector AA, DiGirolamo DJ, Grayson WL. Biophysical stimulation for engineering functional skeletal muscle. *Tissue Eng Part B Rev*. 2017;23:362-372.
90. Ergene E, Sezlev Bilecen D, Kaya B, Yilgor Huri P, Hasirci V. 3D cellular alignment and biomimetic mechanical stimulation enhance human adipose-derived stem cell myogenesis. *Biomed Mater*. 2020;15:055017.
91. Torsoni AS, Marin TM, Velloso LA, Franchini KG. RhoA/ROCK signaling is critical to FAK activation by cyclic stretch in cardiac myocytes. *Am J Physiol Heart Circ Physiol*. 2005;289:H1488-H1496.
92. Andersen JI, Pennisi CP, Fink T, Zachar V. Focal adhesion kinase activation is necessary for stretch-induced alignment and enhanced differentiation of myogenic precursor cells. *Tissue Eng Part A*. 2017;24:631-640.
93. Pennisi CP, Olesen CG, de Zee M, Rasmussen J, Zachar V. Uniaxial cyclic strain drives assembly and differentiation of skeletal myocytes. *Tissue Eng Part A*. 2011;17:2543-2550.
94. Chaudhuri T, Rehfeldt F, Sweeney HL, Discher DE. Preparation of collagen-coated gels that maximize in vitro myogenesis of stem cells by matching the lateral elasticity of in vivo muscle. *Methods Mol Biol*. 2010;621:185-202.
95. Rowlands AS, George PA, Cooper-White JJ. Directing osteogenic and myogenic differentiation of MSCs: interplay of stiffness and adhesive ligand presentation. *Am J Physiol Cell Physiol*. 2008;295:C1037-C1044.
96. Wang BY-H, Hsiao AWT, Shiu HT, et al. Mesenchymal stem cells alleviate dexamethasone-induced muscle atrophy in mice and the involvement of ERK1/2 signalling pathway. *Stem Cell Res Ther*. 2023;14:195.
97. De Bari C, Dell'Accio F, Vandenabeele F, Vermeesch JR, Raymackers JM, Luyten FP. Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *J Cell Biol*. 2003;160:909-918.
98. Wang C, Zhao B, Zhai J, et al. Clinical-grade human umbilical cord-derived mesenchymal stem cells improved skeletal muscle dysfunction in age-associated sarcopenia mice. *Cell Death Dis*. 2023;14:321.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Grobbelaar S, Mercier AE, van den Bout I, Durandt C, Pepper MS. Considerations for enhanced mesenchymal stromal/stem cell myogenic commitment in vitro. *Clin Transl Sci*. 2024;17:e13703. doi:10.1111/cts.13703