

REVIEW

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# Insight into muscle stem cell regeneration and mechanobiology

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## Abstract

Stem cells possess the unique ability to differentiate into specialized cell types. These specialized cell types can be used for regenerative medicine purposes such as cell therapy. Myosatellite cells, also known as skeletal muscle stem cells (MuSCs), play important roles in the growth, repair, and regeneration of skeletal muscle tissues. However, despite its therapeutic potential, the successful differentiation, proliferation, and expansion processes of MuSCs remain a significant challenge due to a variety of factors. For example, the growth and differentiation of MuSCs can be greatly influenced by actively replicating the MuSCs microenvironment (known as the niche) using mechanical forces. However, the molecular role of mechanobiology in MuSC growth, proliferation, and differentiation for regenerative medicine is still poorly understood. In this present review, we comprehensively summarize, compare, and critically analyze how different mechanical cues shape stem cell growth, proliferation, differentiation, and their potential role in disease development (Fig. 1). The insights developed from the mechanobiology of stem cells will also contribute to how these applications can be used for regenerative purposes using MuSCs.

**Keywords** Skeletal muscle stem cells, Mechanobiology, Stiffness, Viscoelasticity, Strain, Topography

## Introduction of muscle stem cells

### Functional role of muscle stem cells

Functional skeletal muscles are integral in daily activities, and they undergo consistent loading stress. When they are unable to support the excessive tensile strength, muscle injury inevitably occurs on post-mitotic cells that do not possess the capability to undergo cell division. As such, the main role of muscle tissue regeneration falls solely on MuSCs which play important roles in the growth, repair, and regeneration of skeletal muscle homeostasis [1]. They exist within quiescent cells in healthy adult mammals, accounting for around 2.5–6.5% of all muscle fiber-associated nuclei. In the resting state, they are mitotically quiescent in the G0 phase, suggesting the cells do not undergo active proliferation. These quiescent MuSCs are characterized by the expression of paired box transcription factor paired box 7 (Pax7) that plays an important role in the maintenance and self-renewal of MuSCs [2, 3] (Fig. 1).

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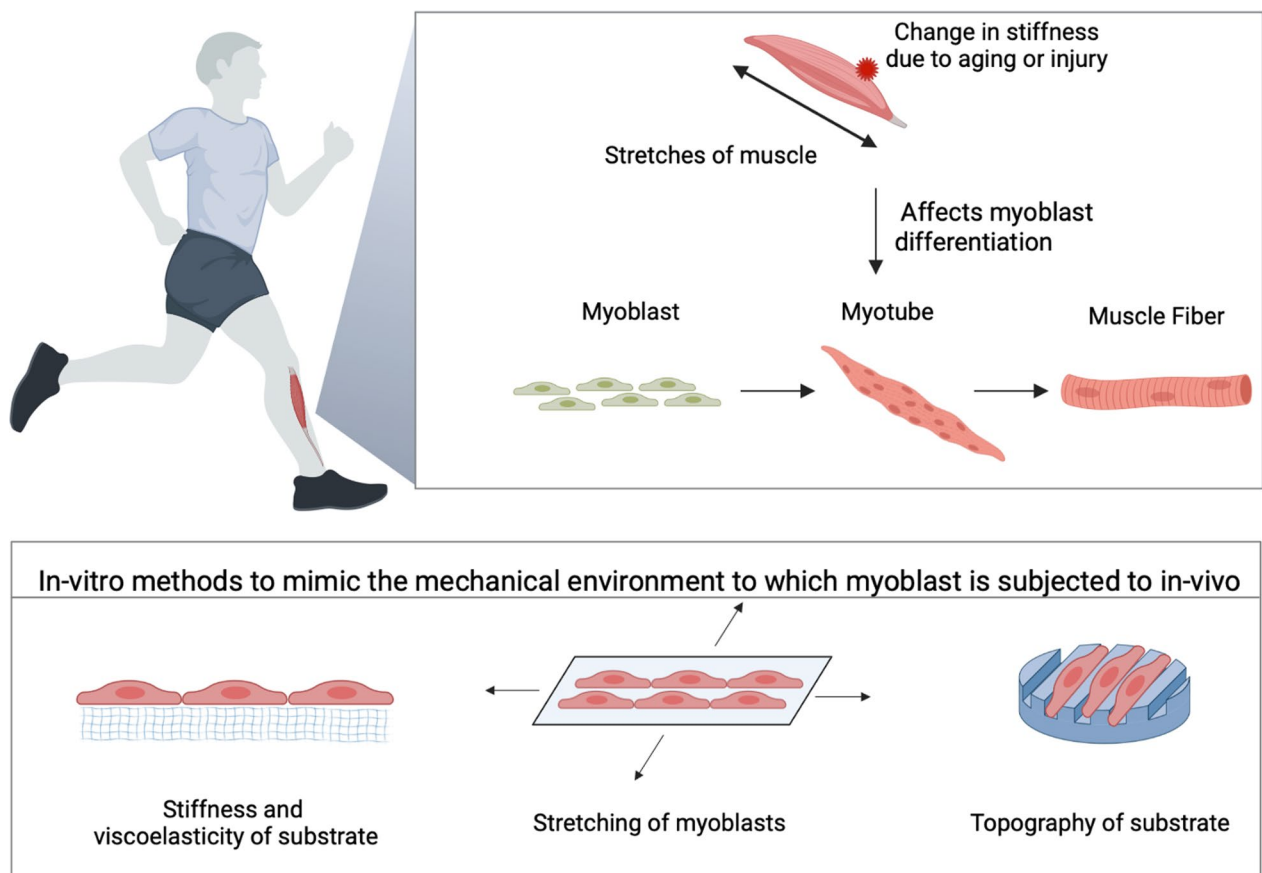
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**Fig. 1** A schematic diagram that summarizes the role of mechanical forces in myoblast differentiation

The proliferative capability of MuSCs serves two purposes; (1) replenishment of the MuSCs pool in the stem cell niche and (2) generation of committed myoblasts that are key to muscle tissue regeneration. PAX7 is an important transcription factor that plays a role in skeletal precursor cells proliferation [3, 4], while myoblast determination protein 1 (MyoD) is one of the earliest key markers in muscle cell commitment and differentiation and increase in MyoD expression is critical for further differentiation of myoblasts into myotubes [5, 6]. To replenish the MuSCs niche, an individual MuSC undergoes symmetric cellular division to give rise to identical  $\text{MyoD}^{\text{low}}/\text{Pax7}^{\text{high}}$  daughter cells that are capable of self-renewal to meet future demands of muscle tissue regeneration [7, 8]. When muscle tissue injury occurs, a small population of MuSCs undergoes asymmetric cellular division, with a self-renewing daughter clone ( $\text{MyoD}^{\text{low}}/\text{Pax7}^{\text{high}}$ ) and a committed daughter clone ( $\text{MyoD}^{\text{high}}/\text{Pax7}^{\text{low}}$ ) that exits the cell cycle. These  $\text{MyoD}^{\text{high}}/\text{Pax7}^{\text{low}}$  committed cells eventually differentiate into myoblasts expressing MyoD, myogenic regulatory factor 4 (Mrf4) and myogenic regulatory factor 5

(Myf5) [9]. Subsequent differentiation of these myoblasts into myofibers is accompanied by the decrease of Pax3/7 and Myf5 expression and an increase of myogenin and myosin heavy chain (MHC) [10, 11]. As such, muscle tissue homeostasis is balanced by the constant self-renewal process of ( $\text{MyoD}^{\text{low}}/\text{Pax7}^{\text{high}}$ ) MuSCs that are capable of self-renewal and  $\text{MyoD}^{\text{high}}/\text{Pax7}^{\text{low}}$  MuSCs for further commitment toward repairing muscle tissue damage. In fact, when Pax7 + MuSCs are eliminated in mice through transgenic targeting of Pax7<sup>Cre</sup>, the mice experienced > 90% failure in muscle regeneration [12–15].

Inside the skeletal muscle, MuSCs reside in a specialized niche that is located between the muscle fiber plasma membrane and the basal lamina that surrounds the muscle fiber [16–18]. Inside this specialized niche, the muscle fiber basal lamina consists of extracellular matrix (ECM) components such as entactin, fibronectin, laminin and type IV collagen [19] and MuSCs are able to interact with them through the expression of  $\alpha$ 7- and B1-integrins [20]. As a result, various growth factors such as basic fibroblast growth factors (bFGF), epidermal growth factor (EGF) and hepatocyte growth factor

(HGF), and insulin-like growth factor-1 (IGF-1) promote the survival and proliferation of MuSCs [21–24].

### **Muscle stem cells in aging and their influence on cell microenvironment**

MuSCs possess a high regenerative potential which can be used to replenish lost MuSCs. However, the regenerative capacity of the MuSCs declines during the natural aging process; the properties of muscle tissues start to change as the muscle stem niche starts to undergo remodeling [25]. Damage to skeletal muscle tissue eventually results in fibrosis and stiffening of the extracellular matrix (ECM) [26, 27]. Consequently, the microenvironment of MuSCs is remodeled, suggesting that a greater collagen deposition increases muscle stiffness [28]. This stiffness of the microenvironment may be due to alterations to collagen and advanced glycation end-products (AGE), which impair MuSCs proliferation [29]. Many studies have shown that the aging MuSC niche also plays a key role in the impairment of self-renewal ability in MuSCs [30–33].

There is still controversy over the aging effects that result in the loss of MuSCs. While some studies have revealed that there were no differences in MuSC counts in young and aged muscles [34–36], others have shown that estimated MuSC counts increase or decrease with age [37]. Aged MuSCs exhibit impaired proliferative potential due to a delayed response to activation [34, 38]. These aged MuSCs also exhibit susceptibility to tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-induced apoptosis [39, 40]. Rejuvenation of activation ability in aging muscle stem cells is dependent on physical exercise that restores Cyclin D1 expression.

### **Current challenges in the differentiation and proliferation processes of muscle stem cells**

MuSCs are a rare population of cells, accounting for around 2.5–0.5% of all muscle fiber-associated nuclei. Due to the limited amount found in the human body, several groups have started to look at other types of stem cells such as mesenchymal stem cells (MSCs). There are several pieces of evidence showing the potential of MSCs giving rise to MuSCs after transplantation into mdx mouse model [41, 42]. Stem cells are undifferentiated cells that possess the ability to undergo self-renewal and differentiate into different lineages such as cardiac [43], hepatic [44], neuronal [45], and pancreatic [46, 47]. In general, stem cells can be differentiated in a stepwise guided process using growth factors, chemicals, and small molecules of which developmental potency decreases at each specific stage. The differentiated endpoint cells can be used for cell and drug therapies.

The development and growth of skeletal muscle cells from the embryo stage to the adult stage are generally well understood. For the past few decades, researchers have also been searching for ways to generate skeletal muscle cells from pluripotent stem cells in vitro. These efforts are made possible with our understanding of the biochemical and molecular aspects of directed differentiation and directed reprogramming of stem cells at different stages to skeletal muscle cells [48]. Traditionally, the activation of signaling pathways was often carried out by using specific growth factors, small molecules, and chemicals. However, usage of these growth factors is very costly due to the short half-life of the proteins. Moreover, these growth factors may inevitably induce certain unintended signaling pathways that may be detrimental to the survival, proliferation, and differentiation of MuSCs.

While MuSCs have been shown to promote muscle regeneration, they often exhibit loss of potency when they are expanded ex vivo. MuSCs also exhibit limited in vivo migration, suggesting limited regenerative potential in injury sites. It has also been shown that MuSCs rapidly lose their stemness with the removal of their stem cell niche [49, 50]. There is an urgent need to recapitulate the MuSC niche in vitro so that they are able to remain in a quiescent state. A cocktail combination of 4 different factors, interleukin 1 $\alpha$  (IL-1 $\alpha$ ), interleukin 13 (IL-13), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon  $\gamma$  (INF- $\gamma$ ), promoted the proliferation and differentiation of myosatellite cells for more than 20 passages. The myosatellite cells that are expanded ex vivo under such conditions expressed high *Pax7* and low *MyoD* expression levels and were able to repopulate the stem cell niche [51].

Mechanical forces are being investigated to understand how they can complement the use of growth factors in promoting MuSC regeneration and maintaining the stemness of muscle stem cells. In addition, in consideration of the fact that skeletal muscle tissues are always under mechanical loading and stretching, it makes sense to investigate the role of mechanical forces in promoting MuSCs proliferation and differentiation, which in turn results in improved muscle regeneration. In the following section, we review such influences.

### **Mechanobiology of skeletal muscle stem cells**

In the body's natural microenvironment, stem cell fate and proliferation are constantly regulated by biophysical cues. Biological cells are known to sense mechanical forces and their mechanical environment and translate these mechanical cues into biochemical signals. Examples include the effect of fluidic shear stress, strain, and ECM stiffness on endothelial cells [52, 53], smooth muscle cells [54], and cancer cells [55], respectively. Similarly, cells of the musculoskeletal system are also capable

of sensing mechanical forces. Additionally, it has been observed that skeletal muscle cells change their size and structural properties in response to mechanical stimulation [56].

Stem cells are also capable of sensing mechanical cues and mediating their differentiation accordingly. They sense mechanical forces and the environment through various mechanosensory mechanisms. Cell density, shape, and the adhesion area of human mesenchymal stem cells (hMSC) have been shown to drive their commitment switch between osteogenic or adipogenic fates—larger adhesion area drives hMSC commitment toward osteogenesis and activates ras homology family member A (RhoA) expression. RhoA then signals through its effector—Rho-associated protein kinase (ROCK) to activate myosin II and cytoskeletal tension activity [57]. Another signaling pathway that is involved in the mechanosensing of hMSC is the Yes-associated protein/Taffazin (YAP/TAZ) pathway. The spreading of hMSC on a larger adhesion area increases the localization of YAP/TAZ in the nucleus and this regulation is independent of the Hippo pathway. In addition, ECM stiffness, which is known to regulate hMSC, also affects YAP/TAZ activation. Regulation of stiffness-mediated differentiation of hMSC is abrogated when YAP/TAZ pathway is inhibited, suggesting the important role of YAP/TAZ pathway in the mechanoregulation of hMSC differentiation. YAP/TAZ activity also requires the tension of the actin cytoskeleton and Rho [58]. Cell spread is measured by cell aspect ratio—a ratio between width and length; the ratio was found to influence stem cell lineage commitment in a microfilament and ROCK-dependent manner [59]. The aforementioned evidence shows that stem cells sense their mechanical environment partially through adhesion and cytoskeletal activity. These will be discussed in detail in the next section.

## **Influence of mechanical factors on muscle stem cell properties**

### **Substrate stiffness**

Stiffness, commonly known as rigidity, is a mechanical property that measures the resistance of the material to deformation. It is measured by Young's modulus—a ratio between stress and strain ( $E = \sigma/\epsilon$ ) in the unit of Pascals. Stiffness characterization of biological cells and ECM have been shown to be important for understanding cellular physiology, development, and pathological progression of diseases [60–65]. In particular, ECM stiffness has been shown to regulate cell migration [66, 67], apoptosis [68], and proliferation [69]. ECM stiffness is also known to govern stem cell behavior. For example, adipose-derived stem cells cultured on hydrogels with a linear stiffness gradient revealed that cell aspect ratio

and expression of mechanosensitive markers lamin A, nucleus YAP translocation, and myocardin-related transcription factor A (MRTFa) were positively correlated to substrate stiffness via myosin and Rho/ROCK signaling [70].

Out of all mechanical cues, ECM stiffness plays a major role in stem cell differentiation. hMSC commits to lineages toward the cell type where in vivo stiffness matches the stiffness of the matrix. For instance, while soft polyacrylamide gels that mimic brain and fat tissue environment drive neurogenic and adipogenic differentiation respectively, stiff polyacrylamide gels that mimic the bone drive osteogenic differentiation [58, 71]. Cytoskeleton tension is involved in stiffness-regulated differentiation. A similar observation was also made when MSCs were cultured within 3-dimensional arginylglycylaspartic acid (RGD)-alginate microspheres [72]. Hence, stem cells would differentiate into skeletal muscle cells if they were grown on ECM with stiffness that emulates those of skeletal muscles.

To maintain MuSCs stemness, a 3D micro scaffold (~1–2 kPa) composed of collagen, recombinant laminin and  $\alpha 4\beta 1$  integrin was used in muscle stem cell culture and they were able to maintain muscle cell stemness [73]. The stiffness of mouse skeletal muscle and myoblast cell lines (C2C12) was found to be around 12 kPa through atomic force microscopy (AFM) indentation [74, 75]. Myoblasts were also found to exhibit a higher level of differentiation, striation, and fusion to myotubes when they were cultured on collagen-coated polyacrylamide gel substrates that mimic the stiffness of skeletal muscles (~12 kPa). Interestingly, there was little striation when myoblasts were cultured on stiff glass substrates [75].

Human bone marrow- and dental-derived MSCs, or gingival MSCs, were able to undergo myogenic differentiation when they were encapsulated in alginate microspheres (enriched with myogenic cocktail) with well-tuned stiffness. Both MSC grown on alginate microspheres with a stiffness of 15 kPa, compared with 5 and 45 kPa, had the highest expression of MyoD protein, suggesting that a stiffness of 15 kPa (stiffness close to the stiffness of skeletal muscle) provides an optimal mechanical environment for myogenic differentiation [76].

Self-renewal of stem cells is essential because they replicate and regenerate more stem cells to help in tissue regeneration. MuSCs cultured on soft poly(ethylene glycol) (PEG) hydrogel substrates that have a stiffness of 12 kPa, compared with cells cultured on stiff plastic dishes, exhibited self-renewal phenotype in vitro. These stem cells were successfully transplanted into mice and contributed to muscle regeneration [77]. It has also been shown that hematopoietic and progenitor cells cultured on elastic tropoelastin had a higher expansion of



undifferentiated cells compared with culture multiwell plates [78]. Human MuSCs were also shown to remain quiescent and maintain their 'stemness' when they were cultured on soft poly-L-lysine/hyaluronic acid films, while their counterparts cultured on stiff films were activated and began to differentiate and proliferate [79].

Changes in stiffness can occur in muscle injury. Muscle stiffness was found to increase from around 13–20 kPa after mice muscles were injured using barium chloride ( $\text{BaCl}_2$ ), and this stiffness remained elevated after completion of muscle regeneration 28 days after injury. Further, strain-promoted azide–alkyne cycloaddition (SPAAC) hydrogels with a PEG precursor were used to mimic the stiffness of injured muscles and myoblast cell line (C2C12) cells cultured on them exhibited increased proliferation and migration, and localization of YAP/TAZ to the cell nucleus [80]. A similar observation was also made by increasing muscle stiffness from 12 kPa to approximately 25 kPa in the first three days post-injury [81]. Importantly, PEG-based hydrogel stiffness was found to synergize with NOTCH signaling to regulate muscle stem cell fate—Jagged-1 tethered on stiff hydrogel (42 kPa) as compared with soft hydrogel (12 kPa), reduced myoblast myogenin expression and that is dependent on ROCK [81]. Isolated shrunken and collapsed myofibers cultured *ex vivo* were also found to have significantly higher stiffness (2 kPa) compared with their intact counterparts (0.5 kPa) and the provision of a stiffer microenvironment for satellite cells in turn promoted muscle progenitor cells (MPCs) proliferation. MPCs that were grown on a 2 kPa polyacrylamide-based substrate that mimics collapsed myofibers stiffness exhibited higher proliferation and reduced spontaneous differentiation compared to MPCs grown on a 0.5 kPa [82]. At the cellular level, MuSCs stiffness increased by 2.9-fold during skeletal muscle regeneration after  $\text{BaCl}_2$ -induced injury. High 3-dimensional niche stiffness (21.7 kPa) increased the amount of planar orientation of self-renewal division via the Wnt family member 7a (Wnt7a) and non-canonical Wnt pathway, as compared with low 3D niche stiffness (5.9 kPa) [62].

ECM stiffness has also been studied in the context of skeletal muscle aging, a condition that is characterized by declining muscle strength, which leads to reduced mobility and muscle function. This condition has been partly attributed to the defects in MuSC regeneration [25]. Aged muscles that were injured were found to have high ECM deposition, rather than undergoing myofiber repair (Grounds, 1998), which in turn increases muscle stiffness [83, 84]. MuSCs that were grown on decellularized aged ECM (with high stiffness) exhibited lower myogenic and higher fibrogenic markers than those grown on decellularized young ECM. Fibroblasts grown on stiff silicone

gels (32 kPa) that mimic the stiffness of aged muscle expressed higher YAP/TAZ nuclear translocation and secreted soluble mediators that inhibit myogenesis [84]. Furthermore, another study found that only one-third of MuSCs from aged mice retain their renewal ability and capacity to repair myofibers. This defect was rescued by culturing aged muscle stem cells on soft PEG-based hydrogel (12 kPa) in conjunction with p38 $\alpha$  and p38 $\beta$  inhibition. Interestingly, tuning substrate stiffness alone was not sufficient to enhance cell renewal suggesting that mechanical and biochemical cues are required to synergistically modulate stem cell renewal in aged muscle [85].

### Substrate viscoelasticity

Most studies characterized the effect of ECM stiffness on stem cell fate. However, it is important to note that ECM-like fibrin and collagen are viscoelastic materials [86, 87]. Hence, investigating the effect of elasticity alone does not uncover the true mechanobiological behavior of stem cells. In contrast to pure elastic material, viscoelastic materials exhibit a time-dependent response to an applied load due to the flow and remodeling of the ECM. In particular, ECM exhibits time-dependent stress relaxation: cells cultured on a viscoelastic material first experience the stiffness of the material, and then the traction force of cells reduces as ECM is relaxed over time. Cell spreading and proliferation on viscoelastic materials that exhibit stress relaxation were larger than those cultured on purely elastic ECM with the same stiffness. Fibroblasts cultured on viscoelastic material were also found to have high nuclear translocation of YAP than those cultured on pure elastic materials. These effects are mediated through actomyosin-based contractility and integrin adhesions [88].

Consistent with the above observation [88], hMSCs cultured on polyacrylamide gel with higher loss modulus, a measure of energy dissipation under dynamic mechanical testing, exhibited higher spread area, greater proliferation, and differentiation potential. Interestingly, hMSCs were more sensitive to changes in loss modulus, as compared with the change in stiffness. Changing the loss modulus by two orders of magnitude resulted in the same fold-change in cell spread area as differences in stiffness by three orders of magnitude. In the presence of the inductive myogenic, adipogenic, and osteogenic medium, hMSCs cultured on the substrate with higher loss modulus expressed significantly higher levels of differentiation markers, suggesting that higher loss modulus of the substrate is more favorable for MSC differentiation. This observation was attributed to higher cell spread area and loss of cytoskeletal tension through the loss of energy resulting from material creep within the substrates with high loss modulus [89]. The same group went

on to prove that substrate loss modulus-mediated Rac1 activation, increased N-Cadherin expression, and higher hMSCs motility and lamellipodial protrusion rates are the underlying mechanisms for enhanced differentiation toward smooth muscle cell lineage [90]. Another study by Chaudhuri et al. (2016) also supported this observation—MSCs spreading, proliferation, YAP nuclear translocation, and osteogenic differentiation were enhanced when they were cultured on polyacrylamide hydrogels with faster relaxation time [91].

Although ECM viscoelasticity has an important role in stem cell differentiation, its role in skeletal stem cell differentiation has not been investigated. This remains a research gap that should be filled to have a comprehensive understanding of the role of ECM mechanics on skeletal stem cell differentiation.

### Stretching

Skeletal muscle is a mechanically dynamic tissue, and they contract and relax up to 17% of their length to produce voluntary motion [92]. Mechanical strain has been shown to result in skeletal muscle strengthening and hypertrophy [93] and maintenance of muscle progenitors [94]. Hence it is important to understand the role of mechanical strain in the regeneration of skeletal muscle cells—particularly self-renewal and myogenesis of muscle stem cells and their precursor cells. In most studies, mechanical strain is characterized as a ratio of change in length (transverse or longitudinal length) to the original length; it is often expressed as a percentage.

Biaxial cyclic strain (10%, 0.17 Hz) was found to inhibit hESC differentiation, as evidenced by the upregulated expression of Oct4 and SSEA-4. Mechanical strain also promoted hESC self-renewal while maintaining their pluripotency. Medium conditioned by strained-hESCs did not inhibit unstrained-hESC differentiation, suggesting that only mechanical force, but not other soluble mediators, was responsible for the inhibition of differentiation [95]. The same group went on to demonstrate that the same mechanical strain-induced TGF $\beta$ 1, activin A, Nodal expression and Smad 2/3 phosphorylation are responsible for strain-induced inhibition of hESC differentiation and stimulation of self-renewal [96]. However, mechanical strain does not inhibit commitment in all types of lineages. Cyclic strain (10%, 1 Hz) inhibited osteogenic and neurogenic differentiation of bone marrow-derived MSCs but promoted smooth muscle differentiation of these cells. Round cells, among other cell shapes, were most sensitive to mechanical strain, while spindle cells and amebocytes were only responsive to mechanical strain for vascular smooth muscle and neurogenic differentiation [97].

In the context of skeletal muscle regeneration, MSCs, myoblast, and skeletal MuSCs were stretched to investigate the effects of strain on stem cell proliferation and differentiation. Mouse myoblast (C2C12) subjected to uniaxial strain (12.2–14.2%, 0.5 Hz) aligned perpendicular to the direction of strain [98]. This has been shown to be dependent on strain amplitude as other studies that applied lower strain magnitude (7%, 0.5 Hz, and 2%, 1 Hz) reported lower orientation angle (7% strain) or no alignment (2% strain) [99, 100]. Alignment was not observed when C2C12 were subjected to equibiaxial strain (approximately 10%, 0.5 Hz). Moreover, equibiaxial strain resulted in cell membrane damage. Uniaxial strain, but not equibiaxial strain, enhanced myogenic differentiation, evidenced by myotube/myoblast ratio and myosin-positive myotubes [98]. In contrast to that, a study has shown that equibiaxial strain (3%, 0.05 Hz) also enhanced myotube formation [101]; the cause for the discrepancy observed between this study and Pennisi et al. (2011)'s is not clear.

Not all studies supported the effect of strain in the inhibition of differentiation: In a study by Boonen et al. (2010), C2C12 and MPCs subjected to uniaxial ramp strain (0–2%) for two days, followed by intermittent dynamic strain (2–6%, 3 h on, 3 h off, 1 Hz) showed suppressed myotube formation and maturation [100]. High uniaxial cyclic strain (17%, 1 Hz) was also found to promote the proliferation of C2C12 and inhibited myotube formation in C2C12 culture via the activation of NF- $\kappa$ B, Ras-related C3 botulinum toxin substrate 1 (Rac-1), and focal adhesion kinase (FAK) [102]. The inconsistency of the effect of uniaxial strain is likely due to the lower or higher strain magnitude (less than 10% or above 15%) and different strain application strategies adopted by them.

Skeletal muscle inflammation can result in muscle protein degradation and inhibit myogenesis [103]. Myotube formation was inhibited in C2C12 treated with pro-inflammatory cytokine TNF- $\alpha$  through the activation of NF- $\kappa$ B pathway and induction of nitric oxide synthase 2A (NOS2A) and nitric oxide (NO) production [104, 105]. Equibiaxial cyclic strain (3, 6, 9, 12, and 18%, 0.05 Hz) was found to inhibit TNF- $\alpha$ -induced expression of NOS2 in a magnitude-dependent manner, and it (3%, 0.05 Hz) also rescued impairment of myogenesis caused by TNF- $\alpha$  [101].

The effect of mechanical strain on muscle stem cells was also investigated. Primary isolated satellite cells, as opposed to transformed myoblastic cell lines such as C2C12, provide better insight into how mechanical strain affects myogenesis as these cells are stem-like cells that differentiate into myoblasts in vivo. Mechanical strain (10%, 0.25 Hz) promoted bovine satellite cell proliferation

and reduced myogenic differentiation via the activation of extracellular signal-regulated kinase (ERK) [106].

Although skeletal muscle stem cells are the most studied cell type in tissue regeneration, its lack of differentiation capacity poses a challenge for use in therapeutics. Other sources of stem cells such as adipocyte-derived stem cells (ASCs) and bone marrow-derived mesenchymal stromal cells (BMSCs) are sought after as an alternative to skeletal muscle stem cells. ASCs can participate in muscle regeneration by fusion or co-culturing with myoblasts to form myotubes [107–109]. Uniaxial strain (15%, 0.5 Hz) has been shown to enhance ASCs fusion with skeletal myocytes. More myotubes were formed when ASCs are strained while cocultured with mouse skeletal muscle myoblasts (C2C12) [110]. As for BMSCs, uniaxial strain (10%, 0.17 Hz) enhanced myogenic differentiation and reduced proliferation and motility. Strain also aligned BMSCs parallel to the strain direction, and this is different from what was reported in myoblasts [98, 110, 111].

### Topography

Topographical cues can significantly affect the cellular morphology and phenotype of various cell types such as epithelial cells [112, 113], fibroblasts [114], and endothelial cells [115]. Stem cells were also found to be influenced by topography. ESCs grown on Polydimethylsiloxane (PDMS) gratings with 600 nm features and spacing showed stronger alignment and elongation. The same study also showed that nanotopographic cues altered the organization of various cytoskeletal components, including F-actin, vimentin,  $\gamma$ -tubulin, and  $\alpha$ -tubulin, and the observed changes in proliferation and morphology were abolished by the effect of actin-disrupting agents like cytochalasin D and latrunculin B [116].

Skeletal muscles consist of highly aligned multinucleated myotubes and their alignment is required for myotube fusion [117]. During in vivo myogenesis, grooves formed between muscle fibers have a depth and width of between 1–4.5  $\mu\text{m}$  and 2–3  $\mu\text{m}$ , respectively. Myoblasts grew and aligned themselves along the grooves and differentiated into well-aligned and fused skeletal muscle tissue [118]. Therefore, it is not surprising that topography can direct stem cell alignment and differentiation toward myogenesis. Primary myoblasts and C2C12 myoblasts were found to align well along grooves with a range of subcellular widths between 2 and 75  $\mu\text{m}$  [119–124]. In addition to width, the depth of grooves was found to affect myoblast alignment too. C2C12 cells seeded in 2  $\mu\text{m}$  wide and 7  $\mu\text{m}$  deep PDMS grooves aligned better than those seeded in 2  $\mu\text{m}$  wide and 2  $\mu\text{m}$  deep grooves—this is likely because the shallow grooves did not provide sufficient contact guidance for cell alignment. C2C12

cells seeded in 2  $\mu\text{m}$  wide and 7  $\mu\text{m}$  deep grooves also had better formation and fusion of multinucleated myotubes [124]. Grooves are also found naturally in cellulose. Decellularized green onion has natural grooves measuring 20  $\mu\text{m}$  wide by 10  $\mu\text{m}$  deep, and C2C12 myoblasts grown on them were aligned with more myotube formation [120]. Similar observations were also made when C2C12 myoblasts were grown on submicron grooves made of polystyrene, polycaprolactone, and PEG-based hydrogel [125–127].

With advances in biomaterials, the effect of nanoscale topography on muscle stem cells can be investigated. For example, cellulose nanowhiskers (CNWs) extracted from *Asciidiella aspersa* and *Halocynthia roretzi* have widths of 6–7 nm and 10–15 nm, respectively. C2C12 myoblasts seeded on this nanoscale topography showed a higher degree of multinucleated myotube fusion with fibrillar fibronectin deposition on the surface of highly oriented CNWs [128, 129]. Similarly, C2C12 cells grown on alginate nanofibers made using a modified electrospinning process had higher adhesion, alignment and myotube formation [127].

MSCs derived from bone marrow, fetal tissue, and adipose grown on microcontact printed-20  $\mu\text{m}$  wide fibronectin lanes had aligned actin filaments and vinculin, and elongated nuclei. The alignment caused by the lanes was found to drive myogenic differentiation in these cells too [130]. Similarly, ASCs seeded in 100  $\mu\text{m}$  wide and 27  $\mu\text{m}$  deep grooves made of polyacrylamide hydrogel exhibited a higher level of alignment and myotube fusion compared to unpatterned control [131]. Some studies have also attempted to grow cells on a complex 3D contour. For instance, a study developed a novel molding technique to replicate human skeletal muscle myoblast features and contour by creating an inverted pattern on poly(urethane acrylate)(PUA). hMSCs grown on 3D ‘pockets’ with myoblasts features took the shape and alignment of the topography and are committed to myogenesis, compared with hMSCs grown on a flat control [132].

### Conclusion

In summary, MuSCs possess high regenerative capabilities for muscle tissue repair and regeneration. However, current understanding and knowledge of MuSCs biology were always based on 2D monolayer cultures on a tissue culture dish. This outdated approach does not recapitulate the in vivo 3D microenvironment where external mechanical cues like substrate stiffness, strain, and topography play important roles in MuSC survival and proliferation. Moreover, the biological effects whereby MuSCs are governed by mechanobiological forces are not well-studied. Hence, there is a need to carry out in vitro

3D studies to understand how mechanobiological forces govern MuSCs self-renewal, proliferation, and survival. Further work may involve high-throughput screening of MuSC response to mechanobiological cues to elucidate novel mechano-signaling pathways. Such approaches may support the use of mechanobiology forces to prime MuSCs for enhanced survival and proliferation. This will also lead to the discovery of certain drugs that will augment MuSCs growth. One possible suggestion would be to incorporate both aspects of biomechanical cues (novel biomaterials and external mechanical forces) and exogenous biological signals (growth factors such as EGF, HGF, and IGF) to promote muscle tissue regeneration. A thorough understanding of the mechanical forces regulating MuSCs allows for the development of bio-inspired extracellular matrix which can serve to increase mature muscle fiber differentiation.

Yet, our current understanding of how biomechanical cues and biochemical signals orchestrate is far from comprehensive. Extensive characterization of mechanobiological aspect of MuSCs renewal, survival, and differentiation needs to be carried out. The combination of the therapeutic effects of growth factors into novel biomaterials with well-tuned mechanics would help push the frontiers of skeletal muscle regeneration.

#### Abbreviations

MuSCs	Muscle stem cells
Pax7	Paired box 7
MyoD	Myoblast determination protein 1
Mrf4	Myogenic regulatory factor 4
Mrf5	Myogenic regulatory factor 5
Pax3	Paired box 3
MHC	Myosin heavy chain
bFGF	Basic fibroblast growth factors
EGF	Epidermal growth factor
HGF	Hepatocyte growth factor
IGF-1	Insulin-like growth factor 1
ECM	Extracellular matrix
AGE	Advanced glycation end-product
TGFβ	Transforming growth factor β
NF-κB	Nuclear factor kappa B
IL-6	Interleukin 6
DMD	Duchenne muscular dystrophy
DGC	Dystrophin glycoprotein complex
IL-1α	Interleukin 1α
IL-13	Interleukin 13
TNF-α	Tumor necrosis factor-α
INF-γ	Interferon γ
hMSC	Human mesenchymal stem cells
RhoA	Ras homology family member A
ROCK	Rho-associated protein kinase
YAP	Yes-associated protein
TAZ	Tafazzin
MRTFa	Myocardin-related transcription factor A
RGD	Arginylglycylaspartic acid
PEG	Poly(ethylene glycol)
SPAAC	Strain-promoted azide-alkyne cycloaddition
MPC	Muscle progenitor cells
Wnt7a	Wnt family member 7a
Rac-1	Ras-related C3 botulinum toxin substrate 1

FAK	Focal adhesion kinase
NOS2A	Nitric oxide synthase 2A
NO	Nitric oxide
ERK	Extracellular signal-regulated kinase
ASCs	Adipocyte-derived stem cells
BMSCs	Bone marrow-derived mesenchymal stromal cells
PDMS	Polydimethylsiloxane
CNWs	Cellulose nanowhiskers
PUA	Poly(urethane acrylate)

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#### Author contributions

KTP and LLSW performed conceptualization; KTP, LLSW, and IW provided resources; KTP, LLSW, and SC performed writing—original draft preparation; all authors performed writing—review and editing; KTP performed visualization and project administration; IW and KTP carried out supervision. All authors have read and agreed to the published version of the manuscript.

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#### Declarations

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#### References

- Rudnicki MA, Le Grand F, McKinnell I, Kuang S. The molecular regulation of muscle stem cell function. *Cold Spring Harb Symp Quant Biol.* 2008;73:323–31. <https://doi.org/10.1101/sqb.2008.73.064>.
- Kuang S, Chargé SB, Seale P, Huh M, Rudnicki MA. Distinct roles for Pax7 and Pax3 in adult regenerative myogenesis. *J Cell Biol.* 2006;172(1):103–13.
- Seale P, Sabourin LA, Giris-Gabardo A, Mansouri A, Gruss P, Rudnicki MA. Pax7 is required for the specification of myogenic satellite cells. *Cell.* 2000;102(6):777–86.
- Relaix F, Montarras D, Zaffran S, Gayraud-Morel B, Rocancourt D, Tajbakhsh S, et al. Pax3 and Pax7 have distinct and overlapping functions in adult muscle progenitor cells. *J Cell Biol.* 2006;172(1):91–102.
- Cornelison DDW, Olwin BB, Rudnicki MA, Wold BJ. MyoD<sup>−/−</sup> satellite cells in single-fiber culture are differentiation defective and MRF4 deficient. *Dev Biol.* 2000;224(2):122–37.
- Sabourin LA, Giris-Gabardo A, Seale P, Asakura A, Rudnicki MA. Reduced differentiation potential of primary MyoD<sup>−/−</sup> myogenic cells derived from adult skeletal muscle. *J Cell Biol.* 1999;144(4):631–43.
- Collins C. Satellite cell self-renewal. *Curr Opin Pharmacol.* 2006;6(3):301–6.



8. Dhawan J, Rando TA. Stem cells in postnatal myogenesis: molecular mechanisms of satellite cell quiescence, activation and replenishment. *Trends Cell Biol.* 2005;15(12):666–73.
9. Tajbakhsh S, Rocancourt D, Buckingham M. Muscle progenitor cells failing to respond to positional cues adopt non-myogenic fates in myf-5 null mice. *Nature.* 1996;384(6606):266–70.
10. Knapp JR, Davie JK, Myer A, Meadows E, Olson EN, Klein WH. Loss of myogenin in postnatal life leads to normal skeletal muscle but reduced body size. *Development.* 2006;133(4):601–10.
11. Venuti JM, Morris JH, Vivian JL, Olson EN, Klein WH. Myogenin is required for late but not early aspects of myogenesis during mouse development. *J Cell Biol.* 1995;128(4):563–76.
12. Lepper C, Partridge TA, Fan C-M. An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. *Development.* 2011;138(17):3639–46.
13. McCarthy JJ, Mula J, Miyazaki M, Erfani R, Garrison K, Farooqui AB, et al. Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. *Development.* 2011;138(17):3657–66.
14. Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G. Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. *Development.* 2011;138(17):3625–37.
15. Sambasivan R, Yao R, Kissenpfennig A, Van Wittenberghe L, Paldi A, Gayraud-Morel B, et al. Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development.* 2011;138(17):3647–56.
16. Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, et al. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell.* 2005;122(2):289–301.
17. Kuang S, Gillespie MA, Rudnicki MA. Niche regulation of muscle satellite cell self-renewal and differentiation. *Cell Stem Cell.* 2008;2(1):22–31.
18. Mauro A. Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol.* 1961;9(2):493–5.
19. Sanes JR. The basement membrane/basal lamina of skeletal muscle. *J Biol Chem.* 2003;278(15):12601–4.
20. Burkin DJ, Kaufman SJ. The  $\alpha 7 \beta 1$  integrin in muscle development and disease. *Cell Tissue Res.* 1999;296(1):183–90. <https://doi.org/10.1007/s004410051279>.
21. DiMario J, Buffinger N, Yamada S, Strohman RC. Fibroblast growth factor in the extracellular matrix of dystrophic (mdx) mouse muscle. *Science.* 1989;244(4905):688–90. <https://doi.org/10.1126/science.2717945>.
22. Golding JP, Calderbank E, Partridge TA, Beauchamp JR. Skeletal muscle stem cells express anti-apoptotic ErbB receptors during activation from quiescence. *Exp Cell Res.* 2007;313(2):341–56.
23. Machida S, Booth FW. Insulin-like growth factor 1 and muscle growth: implication for satellite cell proliferation. *Proc Nutr Soc.* 2004;63(2):337–40.
24. 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(1):14–28.
25. Muñoz-Cánoves P, Neves J, Sousa-Victor P. Understanding muscle regenerative decline with aging: new approaches to bring back youthfulness to aged stem cells. *FEBS J.* 2020;287(3):406–16. <https://doi.org/10.1111/febs.15182>.
26. Blau HM, Cosgrove BD, Ho ATV. The central role of muscle stem cells in regenerative failure with aging. *Nat Med.* 2015;21(8):854–62.
27. Lukjanenko L, Karaz S, Stuelsatz P, Gurriaran-Rodriguez U, Michaud J, Damme G, et al. Aging disrupts muscle stem cell function by impairing matricellular WSP1 secretion from fibro-adipogenic progenitors. *Cell Stem Cell.* 2019;24(3):433–446.e7.
28. Alnaqeeb MA, Al Zaid NS, Goldspink G. Connective tissue changes and physical properties of developing and ageing skeletal muscle. *J Anat.* 1984;139(Pt 4):677–89.
29. Lacraz G, Rouleau A-J, Couture V, Söller T, Drouin G, Veillette N, et al. Increased stiffness in aged skeletal muscle impairs muscle progenitor cell proliferative activity. *PLoS ONE.* 2015;10(8):e0136217. <https://doi.org/10.1371/journal.pone.0136217>.
30. Chakkalakal JV, Jones KM, Basson MA, Brack AS. The aged niche disrupts muscle stem cell quiescence. *Nature.* 2012;490(7420):355–60.
31. García-Prat L, Martínez-Vicente M, Perdiguer E, Ortet L, Rodríguez-Ubrea J, Rebollo E, et al. Autophagy maintains stemness by preventing senescence. *Nature.* 2016;529(7584):37–42.
32. Price FD, von Maltzahn J, Bentzinger CF, Dumont NA, Yin H, Chang NC, et al. Inhibition of JAK-STAT signaling stimulates adult satellite cell function. *Nat Med.* 2014;20(10):1174–81.
33. Sousa-Victor P, Gutarra S, García-Prat L, Rodríguez-Ubrea J, Ortet L, Ruiz-Bonilla V, et al. Geriatric muscle stem cells switch reversible quiescence into senescence. *Nature.* 2014;506(7488):316–21.
34. Conboy IM, Conboy MJ, Smythe GM, Rando TA. Notch-mediated restoration of regenerative potential to aged muscle. *Science.* 2003;302(5650):1575–7. <https://doi.org/10.1126/science.1087573>.
35. Roth SM, Martel GF, Ivey FM, Lemmer JT, Metter EJ, Hurley BF, et al. Skeletal muscle satellite cell populations in healthy young and older men and women. *Anat Rec.* 2000;260(4):351–8. [https://doi.org/10.1002/1097-0185\(200012\)260:4%3C350::AID-AR30%3E3.0.CO;2-6](https://doi.org/10.1002/1097-0185(200012)260:4%3C350::AID-AR30%3E3.0.CO;2-6).
36. Wagers AJ, Conboy IM. Cellular and molecular signatures of muscle regeneration: current concepts and controversies in adult myogenesis. *Cell.* 2005;122(5):659–67.
37. Brack AS, Rando TA. Intrinsic changes and extrinsic influences of myogenic stem cell function during aging. *Stem Cell Rev.* 2007;3(3):226–37. <https://doi.org/10.1007/s12015-007-9000-2>.
38. Schultz E, Lipton BH. Skeletal muscle satellite cells: changes in proliferation potential as a function of age. *Mech Ageing Dev.* 1982;20(4):377–83.
39. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature.* 2007;449(7165):1003–7.
40. Jejurikar SS, Henkelman EA, Cederna PS, Marcelo CL, Urbanchek MG, Kuzon WM. Aging increases the susceptibility of skeletal muscle derived satellite cells to apoptosis. *Exp Gerontol.* 2006;41(9):828–36.
41. Liu Y, Yan X, Sun Z, Chen B, Han Q, Li J, et al. Flk-1+ adipose-derived mesenchymal stem cells differentiate into skeletal muscle satellite cells and ameliorate muscular dystrophy in MDX Mice. *Stem Cells Dev.* 2007;16(5):695–706.
42. De Bari C, Dell'Accio F, Vandenabeele F, Vermeesch JR, Raymakers JM, Luyten FP. Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *J Cell Biol.* 2003;160(6):909–18.
43. Birket MJ, Ribeiro MC, Verkerk AO, Ward D, Leitoguinho AR, den Hartogh SC, et al. Expansion and patterning of cardiovascular progenitors derived from human pluripotent stem cells. *Nat Biotechnol.* 2015;33(9):970–9.
44. Cai J, Zhao Y, Liu Y, Ye F, Song Z, Qin H, et al. Directed differentiation of human embryonic stem cells into functional hepatic cells. *Hepatology.* 2007;45(5):1229–39. <https://doi.org/10.1002/hep.21582>.
45. Shi Y, Kirwan P, Livesey FJ. Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. *Nat Protoc.* 2012;7(10):1836–46.
46. Loo LSW, Vetthe H, Soetedjo AAP, Paulo JA, Jasmen J, Jackson N, et al. Dynamic proteome profiling of human pluripotent stem cell-derived pancreatic progenitors. *Stem Cells.* 2020;38(4):542–55.
47. Loo LSW, Soetedjo AAP, Lau HH, Ng NHJ, Ghosh S, Nguyen L, et al. BCL-xL/BCL2L1 is a critical anti-apoptotic protein that promotes the survival of differentiating pancreatic cells from human pluripotent stem cells. *Cell Death Dis.* 2020;11(5):378.
48. Chal J, Pourquie O. Making muscle: skeletal myogenesis in vivo and in vitro. *Development.* 2017;144(12):2104–22.
49. Montarras D, Morgan J, Collins C, Relaix F, Zaffran S, Cumano A, et al. Direct isolation of satellite cells for skeletal muscle regeneration. *Science.* 2005;309(5743):2064–7. <https://doi.org/10.1126/science.1114758>.
50. Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM. Self-renewal and expansion of single transplanted muscle stem cells. *Nature.* 2008;456(7221):502–6.
51. Fu X, Xiao J, Wei Y, Li S, Liu Y, Yin J, et al. Combination of inflammation-related cytokines promotes long-term muscle stem cell expansion. *Cell Res.* 2015;25(6):655–73.
52. Warboys CM, Ghim M, Weinberg PD. Understanding mechanobiology in cultured endothelium: a review of the orbital shaker method. *Atherosclerosis.* 2019;285(February):170–7.
53. Ghim M, Pang KT, Arshad M, Wang X, Weinberg PD. A novel method for segmenting growth of cells in sheared endothelial culture reveals the secretion of an anti-inflammatory mediator. *J Biol Eng.* 2018;12(1):15. <https://doi.org/10.1186/s13036-018-0107-6>.

54. Humphrey JD, Schwartz MA, Tellides G, Milewicz DM. Role of mechanotransduction in vascular biology. *Circ Res*. 2015;116(8):1448–61. <https://doi.org/10.1161/CIRCRESAHA.114.304936>.
55. Chaudhuri PK, Low BC, Lim CT. Mechanobiology of tumor growth. *Chem Rev*. 2018;118(14):6499–515. <https://doi.org/10.1021/acs.chemrev.8b00042>.
56. Olsen LA, Nicoll JX, Fry AC. The skeletal muscle fiber: a mechanically sensitive cell. *Eur J Appl Physiol*. 2019;119(2):333–49. <https://doi.org/10.1007/s00421-018-04061-x>.
57. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev Cell*. 2004;6(4):483–95.
58. Dupont S, Morsut L, Aragona M, Enzo E, Giullitti S, Cord-enonsi M, et al. Role of YAP/TAZ in mechanotransduction. *Nature*. 2011;474(7350):179–83.
59. Yao X, Peng R, Ding J. Effects of aspect ratios of stem cells on lineage commitments with and without induction media. *Biomaterials*. 2013;34(4):930–9.
60. Discher DE, Janmey P, Wang Y. Tissue cells feel and respond to the stiffness of their substrate. *Science*. 2005;310(5751):1139–43. <https://doi.org/10.1126/science.1116995>.
61. Liang X, Shi X, Ostrovidov S, Wu H, Nakajima K. Probing stem cell differentiation using atomic force microscopy. *Appl Surf Sci*. 2016;366:254–9.
62. Moyle LA, Cheng RY, Liu H, Davoudi S, Ferreira SA, Nissar AA, et al. Three-dimensional niche stiffness synergizes with Wnt7a to modulate the extent of satellite cell symmetric self-renewal divisions. Discher D, editor. *Mol Biol Cell*. 2020;31(16):1703–13. <https://doi.org/10.1091/mbc.E20-01-0078>.
63. Nematbakhsh Y, Pang KT, Lim CT. Correlating the viscoelasticity of breast cancer cells with their malignancy. *Converg Sci Phys Oncol*. 2017;3(3): 034003.
64. Nakajima M, Miyajima M, Ogino I, Watanabe M, Miyata H, Karagiozov KL, et al. Leucine-rich  $\alpha$ -2-glycoprotein is a marker for idiopathic normal pressure hydrocephalus. *Acta Neurochir (Wien)*. 2011;153:1339–46.
65. Ng L, Hung H-H, Sprunt A, Chubinskaya S, Ortiz C, Grodzinsky A. Nanomechanical properties of individual chondrocytes and their developing growth factor-stimulated pericellular matrix. *J Biomech*. 2007;40(5):1011–23.
66. Guo W, Frey MT, Burnham NA, Wang Y. Substrate rigidity regulates the formation and maintenance of tissues. *Biophys J*. 2006;90(6):2213–20.
67. Pelham RJ, Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci*. 1997;94(25):13661–5. <https://doi.org/10.1073/pnas.94.25.13661>.
68. Wang H-B, Dembo M, Wang Y-L. Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am J Physiol Physiol*. 2000;279(5):C1345–50. <https://doi.org/10.1152/ajpcell.2000.279.5.C1345>.
69. Hadjipanayi E, Mudera V, Brown RA. Close dependence of fibroblast proliferation on collagen scaffold matrix stiffness. *J Tissue Eng Regen Med*. 2009;3(2):77–84. <https://doi.org/10.1002/term.136>.
70. Kim C, Young JL, Holle AW, Jeong K, Major LG, Jeong JH, et al. Stem cell mechanosensation on gelatin methacryloyl (GelMA) stiffness gradient hydrogels. *Ann Biomed Eng*. 2020;48(2):893–902. <https://doi.org/10.1007/s10439-019-02428-5>.
71. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell*. 2006;126(4):677–89.
72. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA, et al. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nat Mater*. 2010;9(6):518–26.
73. Quarta M, Brett JO, DiMarco R, De Morree A, Boutet SC, Chacon R, et al. An artificial niche preserves the quiescence of muscle stem cells and enhances their therapeutic efficacy. *Nat Biotechnol*. 2016;34(7):752–9.
74. Collinsworth AM, Zhang S, Kraus WE, Truskey GA. Apparent elastic modulus and hysteresis of skeletal muscle cells throughout differentiation. *Am J Physiol Physiol*. 2002;283(4):C1219–27. <https://doi.org/10.1152/ajpcell.00502.2001>.
75. Engler AJ, Griffin MA, Sen S, Bönnemann CG, Sweeney HL, Discher DE. Myotubes differentiate optimally on substrates with tissue-like stiffness. *J Cell Biol*. 2004;166(6):877–87.
76. Ansari S, Chen C, Xu X, Annabi N, Zadeh HH, Wu BM, et al. Muscle tissue engineering using gingival mesenchymal stem cells encapsulated in alginate hydrogels containing multiple growth factors. *Ann Biomed Eng*. 2016;44(6):1908–20. <https://doi.org/10.1007/s10439-016-1594-6>.
77. Gilbert PM, Havenstrite KL, Magnusson KEG, Sacco A, Leonardi NA, Kraft P, et al. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science*. 2010;329(5995):1078–81. <https://doi.org/10.1126/science.1191035>.
78. Holst J, Watson S, Lord MS, Eamegdool SS, Bax DV, Nivison-Smith LB, et al. Substrate elasticity provides mechanical signals for the expansion of hemopoietic stem and progenitor cells. *Nat Biotechnol*. 2010;28(10):1123–8.
79. Monge C, DiStasio N, Rossi T, Sébastien M, Sakai H, Kalman B, et al. Quiescence of human muscle stem cells is favored by culture on natural biopolymeric films. *Stem Cell Res Ther*. 2017;8(1):104. <https://doi.org/10.1186/s13287-017-0556-8>.
80. Silver JS, Günay KA, Cutler AA, Vogler TO, Brown TE, Pawlikowski BT, et al. Injury-mediated stiffening persistently activates muscle stem cells through YAP and TAZ mechanotransduction. *Sci Adv*. 2021. <https://doi.org/10.1126/sciadv.abe4501>.
81. Safaee H, Bakooshi MA, Davoudi S, Cheng RY, Martowirogo AJ, Li EW, et al. Tethered jagged-1 synergizes with culture substrate stiffness to modulate notch-induced myogenic progenitor differentiation. *Cell Mol Bioeng*. 2017;10(5):501–13. <https://doi.org/10.1007/s12195-017-0506-7>.
82. Trenz F, Lucien F, Couture V, Söller T, Drouin G, Rouleau A-J, et al. Increased microenvironment stiffness in damaged myofibers promotes myogenic progenitor cell proliferation. *Skelet Muscle*. 2016;5(1):5. <https://doi.org/10.1186/s13395-015-0030-1>.
83. Hasson CJ, Caldwell GE. Effects of age on mechanical properties of dor-siflexor and plantarflexor muscles. *Ann Biomed Eng*. 2012;40(5):1088–101. <https://doi.org/10.1007/s10439-011-0481-4>.
84. Stearns-Reider KM, D'Amore A, Beezhold K, Rothrauff B, Cavalli L, Wagner WR, et al. Aging of the skeletal muscle extracellular matrix drives a stem cell fibrogenic conversion. *Aging Cell*. 2017;16(3):518–28. <https://doi.org/10.1111/acer.12578>.
85. Cosgrove BD, Gilbert PM, Porgiglia E, Mourikoti F, Lee SP, Corbel SY, et al. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. *Nat Med*. 2014;20(3):255–64.
86. Janmey PA, Amis EJ, Ferry JD. Rheology of fibrin clots. VI. Stress relaxation, creep, and differential dynamic modulus of fine clots in large shearing deformations. *J Rheol (N Y N Y)*. 1983;27(2):135–53. <https://doi.org/10.1122/1.549722>.
87. Knapp DM, Barocas VH, Moon AG, Yoo K, Petzold LR, Tranquillo RT. Rheology of reconstituted type I collagen gel in confined compression. *J Rheol (N Y N Y)*. 1997;41(5):971–93. <https://doi.org/10.1122/1.550817>.
88. Chaudhuri O, Gu L, Darnell M, Klumpers D, Bencherif SA, Weaver JC, et al. Substrate stress relaxation regulates cell spreading. *Nat Commun*. 2015;6(1):6365.
89. Cameron AR, Frith JE, Cooper-White JJ. The influence of substrate creep on mesenchymal stem cell behaviour and phenotype. *Biomaterials*. 2011;32(26):5979–93.
90. Cameron AR, Frith JE, Gomez GA, Yap AS, Cooper-White JJ. The effect of time-dependent deformation of viscoelastic hydrogels on myogenic induction and Rac1 activity in mesenchymal stem cells. *Biomaterials*. 2014;35(6):1857–68.
91. Chaudhuri O, Gu L, Klumpers D, Darnell M, Bencherif SA, Weaver JC, et al. Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat Mater*. 2016;15(3):326–34.
92. van Donkelaar CC, Willems PJB, Muijtjens AMM, Drost MR. Skeletal muscle transverse strain during isometric contraction at different lengths. *J Biomech*. 1999;32(8):755–62.
93. Powell CA, Smiley BL, Mills J, Vandenberg HH. Mechanical stimulation improves tissue-engineered human skeletal muscle. *Am J Physiol Physiol*. 2002;283(5):C1557–65. <https://doi.org/10.1152/ajpcell.00595.2001>.
94. Esteves de Lima J, Bonnin M-A, Birchmeier C, Duprez D. Muscle contraction is required to maintain the pool of muscle progenitors via YAP and NOTCH during fetal myogenesis. *Elife*. 2016;5. Available from: <https://elifesciences.org/articles/15593>.
95. Saha S, Ji L, de Pablo JJ, Palecek SP. Inhibition of human embryonic stem cell differentiation by mechanical strain. *J Cell Physiol*. 2006;206(1):126–37. <https://doi.org/10.1002/jcp.20441>.

96. Saha S, Ji L, de Pablo JJ, Palecek SP. TGF $\beta$ /activin/nodal pathway in inhibition of human embryonic stem cell differentiation by mechanical strain. *Biophys J*. 2008;94(10):4123–33.
97. Maeda E, Atsumi Y, Ishiguro M, Nagayama K, Matsumoto T. Shape-dependent regulation of differentiation lineages of bone marrow-derived cells under cyclic stretch. *J Biomech*. 2019;96:109371.
98. 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(19–20):2543–50. <https://doi.org/10.1089/ten.tea.2011.0089>.
99. Ahmed WW, Wolfram T, Goldyn AM, Bruellhoff K, Rioja BA, Möller M, et al. Myoblast morphology and organization on biochemically micro-patterned hydrogel coatings under cyclic mechanical strain. *Biomaterials*. 2010;31(2):250–8.
100. Boonen KJM, Langelaan MLP, Polak RB, van der Schaft DWJ, Baaijens FPT, Post MJ. Effects of a combined mechanical stimulation protocol: value for skeletal muscle tissue engineering. *J Biomech*. 2010;43(8):1514–21.
101. Chandran R, Knobloch TJ, Anghelina M, Agarwal S. Biomechanical signals upregulate myogenic gene induction in the presence or absence of inflammation. *Am J Physiol Physiol*. 2007;293(1):C267–76. <https://doi.org/10.1152/ajpcell.00594.2006>.
102. Kumar A, Murphy R, Robinson P, Wei L, Borie AM. Cyclic mechanical strain inhibits skeletal myogenesis through activation of focal adhesion kinase, Rac-1 GTPase, and NF- $\kappa$ B transcription factor. *FASEB J*. 2004;18(13):1524–35. <https://doi.org/10.1096/fj.04-2414com>.
103. Guttridge DC. Signaling pathways weigh in on decisions to make or break skeletal muscle. *Curr Opin Clin Nutr Metab Care*. 2004;7(4):443–50.
104. Di Marco S, Mazroui R, Dallaire P, Chittur S, Tenenbaum SA, Radzioch D, et al. NF- $\kappa$ B-mediated MyoD decay during muscle wasting requires nitric oxide synthase mRNA stabilization, HuR protein, and nitric oxide release. *Mol Cell Biol*. 2005;25(15):6533–45. <https://doi.org/10.1128/MCB.25.15.6533-6545.2005>.
105. Guttridge DC, Mayo MW, Madrid LV, Wang C-Y, Baldwin AS Jr. NF- $\kappa$ B-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. *Science*. 2000;289(5488):2363–6. <https://doi.org/10.1126/science.289.5488.2363>.
106. Kook S-H, Lee H-J, Chung W-T, Hwang I-H, Lee S-A, Kim B-S, et al. Cyclic mechanical stretch stimulates the proliferation of C2C12 myoblasts and inhibits their differentiation via prolonged activation of p38 MAPK. *Mol Cells*. 2008;25(4):479–86.
107. Beier JP, Bitto FF, Lange C, Klumpp D, Arkudas A, Bleiziffer O, et al. Myogenic differentiation of mesenchymal stem cells co-cultured with primary myoblasts. *Cell Biol Int*. 2011;35(4):397–406. <https://doi.org/10.1042/CBI20100417>.
108. Di Rocco G, Iachininoto MG, Tritarelli A, Straino S, Zacheo A, Germani A, et al. Myogenic potential of adipose-tissue-derived cells. *J Cell Sci*. 2008;119(14):2945–52.
109. Vieira NM, Brandalise V, Zucconi E, Jazedje T, Secco M, Nunes VA, et al. Human multipotent adipose-derived stem cells restore dystrophin expression of Duchenne skeletal-muscle cells in vitro. *Biol Cell*. 2008;100(4):231–41. <https://doi.org/10.1042/BC20070102>.
110. Andersen JL, Juhl M, Nielsen T, Emmersen J, Fink T, Zachar V, et al. Uniaxial cyclic strain enhances adipose-derived stem cell fusion with skeletal myocytes. *Biochem Biophys Res Commun*. 2014;450(2):1083–8.
111. Egusa H, Kobayashi M, Matsumoto T, Sasaki J-I, Uruguchi S, Yatani H. Application of cyclic strain for accelerated skeletal myogenic differentiation of mouse bone marrow-derived mesenchymal stromal cells with cell alignment. *Tissue Eng Part A*. 2013;19(5–6):770–82. <https://doi.org/10.1089/ten.tea.2012.0164>.
112. Brunette DM. Spreading and orientation of epithelial cells on grooved substrata. *Exp Cell Res*. 1986;167(1):203–17.
113. Teixeira AI, Abrams GA, Bertics PJ, Murphy CJ, Nealey PF. Epithelial contact guidance on well-defined micro- and nanostructured substrates. *J Cell Sci*. 2003;116(10):1881–92.
114. Walboomers XF, Monaghan W, Curtis ASG, Jansen JA. Attachment of fibroblasts on smooth and microgrooved polystyrene. *J Biomed Mater Res*. 1999;46(2):212–20. [https://doi.org/10.1002/\(SICI\)1097-4636\(199908\)46:2%3C212::AID-JBM10%3E3.0.CO;2-Y](https://doi.org/10.1002/(SICI)1097-4636(199908)46:2%3C212::AID-JBM10%3E3.0.CO;2-Y).
115. Almonacid Suarez AM, van der Ham I, Brinker MGL, van Rijn P, Harmsen MC. Topography-driven alterations in endothelial cell phenotype and contact guidance. *Heliyon*. 2020;6(6):e04329.
116. Gerecht S, Bettinger CJ, Zhang Z, Borenstein JT, Vunjak-Novakovic G, Langer R. The effect of actin disrupting agents on contact guidance of human embryonic stem cells. *Biomaterials*. 2007;28(28):4068–77.
117. Wakelam MJ. The fusion of myoblasts. *Biochem J*. 1985;228(1):1–12.
118. Wigmore PM, Maleki F, Evans DJR, McLain M. After embryonic day 17, distribution of cells on surface of primary muscle fibres in mouse is non-random. *Dev Dyn*. 1996;207(2):215–21. [https://doi.org/10.1002/\(SICI\)1097-0177\(199610\)207:2%3C215::AID-AJA9%3E3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-0177(199610)207:2%3C215::AID-AJA9%3E3.0.CO;2-K).
119. Charest JL, García AJ, King WP. Myoblast alignment and differentiation on cell culture substrates with microscale topography and model chemistries. *Biomaterials*. 2007;28(13):2202–10.
120. Cheng Y-W, Shiowski DJ, Ball RL, Whitehead KA, Feinberg AW. Engineering aligned skeletal muscle tissue using decellularized plant-derived scaffolds. *ACS Biomater Sci Eng*. 2020;6(5):3046–54. <https://doi.org/10.1021/acsbomaterials.0c00058>.
121. 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(3):537–42. <https://doi.org/10.1021/nl060060o>.
122. Lam MT, Sim S, Zhu X, Takayama S. The effect of continuous wavy micropatterns on silicone substrates on the alignment of skeletal muscle myoblasts and myotubes. *Biomaterials*. 2006;27(24):4340–7.
123. Shimizu K, Fujita H, Nagamori E. Alignment of skeletal muscle myoblasts and myotubes using linear micropatterned surfaces ground with abrasives. *Biotechnol Bioeng*. 2009;103(3):631–8. <https://doi.org/10.1002/bit.22268>.
124. Zhao Y, Zeng H, Nam J, Agarwal S. Fabrication of skeletal muscle constructs by topographic activation of cell alignment. *Biotechnol Bioeng*. 2009;102(2):624–31. <https://doi.org/10.1002/bit.22080>.
125. Gong HY, Park J, Kim W, Kim J, Lee JY, Koh W-G. A novel conductive and micropatterned PEG-based hydrogel enabling the topographical and electrical stimulation of myoblasts. *ACS Appl Mater Interfaces*. 2019;11(51):47695–706. <https://doi.org/10.1021/acsami.9b16005>.
126. Wang P-Y, Yu H-T, Tsai W-B. Modulation of alignment and differentiation of skeletal myoblasts by submicron ridges/grooves surface structure. *Biotechnol Bioeng*. 2010. <https://doi.org/10.1002/bit.22697>.
127. Yeo M, Kim G. Nano/microscale topographically designed alginate/PCL scaffolds for inducing myoblast alignment and myogenic differentiation. *Carbohydr Polym*. 2019;223:115041.
128. Dugan JM, Collins RF, Gough JE, Eichhorn SJ. Oriented surfaces of adsorbed cellulose nanowhiskers promote skeletal muscle myogenesis. *Acta Biomater*. 2013;9(1):4707–15.
129. Dugan JM, Gough JE, Eichhorn SJ. Directing the morphology and differentiation of skeletal muscle cells using oriented cellulose nanowhiskers. *Biomacromol*. 2010;11(9):2498–504. <https://doi.org/10.1021/bm100684k>.
130. Yu T, Chua CK, Tay CY, Wen F, Yu H, Chan JKY, et al. A generic micropatterning platform to direct human mesenchymal stem cells from different origins towards myogenic differentiation. *Macromol Biosci*. 2013;13(6):799–807. <https://doi.org/10.1002/mabi.201200481>.
131. Choi YS, Vincent LG, Lee AR, Kretschmer KC, Chirasatitsin S, Dobke MK, et al. The alignment and fusion assembly of adipose-derived stem cells on mechanically patterned matrices. *Biomaterials*. 2012;33(29):6943–51.
132. Lee EA, Im S-G, Hwang NS. Efficient myogenic commitment of human mesenchymal stem cells on biomimetic materials replicating myoblast topography. *Biotechnol J*. 2014;9(12):1604–12. <https://doi.org/10.1002/biot.201400020>.

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