MUSC AND THEIR NICHE
The most abundant tissue in the human body is skeletal muscle, representing about 40% of total body weight. This tissue allows body posture, breathing, and voluntary movements, organism metabolism, and maintenance of body temperature. Skeletal muscle is a highly organized tissue composed of blood vessels, nerves, connective tissue, and long multinucleated muscle cells named myofibers. Myofibers harbor multiple proteins and enzymes, allowing the production of contractile forces for movements such as locomotion and posture. Muscle stem cells (MuSC), also known as satellite cells, participate in postnatal myofiber growth. However, when the tissue reaches homeostasis, their numbers decline as they enter and remain in a quiescent state of adult stages. In 1961, Alexander Mauro identified satellite cells in a specialized niche between the myofiber sarcolemma and the basal lamina. He defined satellite cells as "merely dormant myoblasts... ready to recapitulate the embryonic development of skeletal muscle fiber when the main multinucleate cell is damaged."2

The niche is a local anatomic milieu instructing MuSC to participate in tissue formation, maintenance, and repair. The conditional mouse model Pax7creERT2; R26RDTA allows the specific depletion of MuSC through the CRE-mediated activation (in PAX7-expressing cells) of diphtheria toxin fragment A (DTA), an inhibitor of protein translation that kills specifically DTA-positive cells. The use of this recombinase-based lineage tracing mouse model showed that elimination of MuSC abrogates tissue repair upon acute injury6–10, thus suggesting that MuSC are the major source of muscle regeneration. Several intrinsic and extrinsic factors regulate MuSC function in the stem cell niche. Extrinsic signals sent by the niche are essential to maintain cell stemness, as described 40 years ago by Schofield for hematopoietic stem cells.11 The stem cell niche plays a critical role in transmitting mechanical signals to MuSC to sense the structure, stiffness, and strength of the muscle.12 The niche enables MuSC to maintain their quiescent state and provides them the ability to proliferate, migrate, self-renew, progress through the myogenic lineage, and differentiate to repair the damaged tissue (Fig. 1).

The ECM is a complex network within the stem cell niche (Fig. 2) that plays a critical role during tissue remodeling. Besides maintaining muscle structure and stiffness, the ECM also regulates the local concentration of soluble factors in the tissue.13 Multiple muscle resident cell populations have been identified as major producers of these factors or contribute to ECM remodeling during tissue repair. For example, fibro-adipogenic progenitors (FAPs) are interstitial mesenchymal progenitors producing ECM components and soluble factors such as wnt1 inducible signaling pathway protein 1 (WISP1), bone morphogenic protein 1 (BMP1), and follistatin14–18, that support differentiation of myogenic progenitors, further aiding myogenesis.14,19,20 Endothelial cells are another example of muscle-resident cells in the juxtavascular stem cell niche and are known to regulate MuSC quiescence and self-renewal through the Dll4-Notch pathway (Fig. 3).

In healthy adult tissue, the specific location and orientation of MuSC set a constant interaction with the basal lamina on the basal side and the myofibers on their apical side. In addition, myofibers play an essential role in regulating MuSC fate.22 However, how MuSC perceive signals from their surrounding environment has been an emerging topic in the last years. For example, M-cadherin and N-cadherin, which are components of adhesive junctions, are expressed at sites of direct contact between MuSC and myofibers, and regulate MuSC quiescence through the canonical Wnt/β-Catenin signaling.23 Likewise, the non-canonical Wnt pathway regulates the homeostatic maintenance of the MuSC pool.24,25.
Brack group showed that Wnt4, secreted by the myofibers, positively regulates MuSC quiescence by activating Rho-GTPase and repressing yes-associated protein (YAP) \(^{24}\). Wnt7a, another secreted factor from the WNT family, has been shown to mediate symmetric MuSC expansion through its binding to frizzled-7 (FZD7) and Syndecan-4 (SDC4) \(^{25,26}\), two cell-surface receptors expressed in quiescent cells \(^{25,27,28}\).

Activation of the Notch signaling pathway requires direct cell-cell binding between MuSC and myofibers \(^{29}\), which includes the Delta-Like ligands (DIL) expressed by myofibers and the Notch receptors 1, 2, and 3 (NOTCH1, NOTCH2, NOTCH3) expressed by MuSC \(^{30-32}\). This signaling pathway participates in the maintenance of MuSC in a quiescent state \(^{33,34}\) and prevents their progression into the cell cycle \(^{30,32,35}\). Other studies have also identified Notch as the mediator of MuSC self-renewal \(^{31}\) through direct regulation of Pax7 \(^{36}\).

All these studies have contributed to our understanding of the complexity of the MuSC niche and how signals coming from different sources can regulate MuSC stemness and myogenic properties. The use of lineage tracing and single-cell RNA sequencing has allowed the construction of a cell atlas of adult skeletal muscle in healthy and regenerative conditions and has uncovered significant heterogeneity of the different cell types in this tissue \(^{37-42}\). However, the relative contribution of each cell population to the ECM composition is still actively under study. Thus, understanding the MuSC niche composition and defining the role of ECM components is relevant for developing therapeutic interventions to improve muscle repair and ameliorate muscle-associated diseases.

In this review, we discuss the ECM composition of the MuSC microenvironment and summarize the relevance of multiple ECM proteins on MuSC function during muscle repair. Finally, we describe the impact of the ECM proteins in the niche on regulating MuSC function in muscle-associated diseases and during aging and discuss the developing approaches currently under study to better characterize the ECM and prospectively improve muscle healing.

**THE ECM IN THE MUSC MICROENVIRONMENT**

The basal lamina is a thin layer of the basement membrane, composed mainly of the ECM proteins Laminin and Collagen. It can be described as a biophysical barrier that provides mechanical support to the tissue and protects the MuSC from the external signals coming from their microenvironment, thus preventing their activation and entry into the myogenic program \(^{43}\).

In adults, cells are constantly rebuilding and remodeling the ECM to maintain tissue homeostasis \(^{44-46}\). The ECM mechanical
properties change as a muscle grows and influence its length and tension. Muscle stiffness increases throughout life, and that can be explained by the biochemical composition of the ECM and its rearrangement\(^49,50\). As age stiffness increases and MuSC function is progressively impaired, it is important to understand how ECM components are regulated since some have been known as contributors to muscle tissue stiffness. Previous studies have explored whether substrate elasticity regulates MuSC properties and muscle regeneration. Indeed, culturing MuSC on pliant hydrogel mimicking the elasticity of the in vivo native muscle enhanced MuSC survival and self-renewal, prevented their differentiation, and maintained their ability to repair muscle upon transplantation, compared to traditional culture on rigid plastic\(^51\). This is an example showing that soft substrates enhanced MuSC function and stemness. However, there is an optimal elasticity of the tissue since the proliferation of primary myoblasts is influenced by a specific stiffness of the gel substrate, not being too soft or rigid\(^52\). Likewise, reducing the collagen content in muscle (in the collagen VI null mouse model) increased the elasticity of the tissue, leading to a loss of MuSC self-renewal\(^53\).

These studies revealed the sensitivity of MuSC to the biophysical properties of skeletal muscle. Consequently, it will be relevant to identify more components of the MuSC microenvironment that regulate tissue stiffness to develop approaches mimicking their native niche.

In the following sections, we describe how ECM proteins regulate MuSC and which approaches have been developed to bioengineer substrates to study biomechanical signals in vitro and in vivo. Three-dimensional (3D) scaffolds mimicking the properties and mechanical integrity of the native tissue are essential to understand how biomechanical signals modulate and/or stimulate MuSC function and fate and to develop tissue engineering approaches to improve tissue repair.

THE COMPLEXITY OF THE ECM IN SKELETAL MUSCLE

Previous studies on mammary gland development have proposed a model in which there is a “dynamic reciprocity” between the ECM and mammary cells\(^54\). In fact, there is a bidirectional interaction between cells and their microenvironment\(^55\): through cell surface receptors, ECM components directly interact with cells and regulate their fate, proliferation, differentiation, migration, and adhesion (Fig. 3).

In skeletal muscle, the remodeling of the ECM represents an essential step during tissue repair since it impacts MuSC behavior and function. Myofibers are surrounded by ECM composed of glycoproteins, proteoglycans, collagens, matrix metalloproteinases (MMP), and matricellular proteins\(^56\). These factors have been shown to contribute to the regulation of MuSC function, muscle growth, and repair\(^57\). Furthermore, there is constant cooperation between ECM proteins and secreted molecules. For example, growth factors contribute to the production of the ECM proteins, and ECM proteins can either present or sequester growth factors to the cells\(^58\).

Below, we detail three categories of ECM proteins known to modulate MuSC function during muscle repair—collagens, fibrillins, and laminins, which have been extensively studied in the muscle field. Other ECM proteins, including fibrillins, tenascin-c (TnC), versican, periostin, and thrombospondins (TSP), are summarized in Table 1 and discussed in the next section, as they have been mostly studied in the context of muscle-associated diseases. Other components of the stem cell niche, including...
soluble factors, proteoglycans, and MMPs, have been previously reviewed in depth.\textsuperscript{12,44,59–61}

**Collagens**

Collagen is one of the most abundant ECM glycoproteins in the body and has a widespread distribution among tissues.\textsuperscript{60} In vertebrates, there are 40 collagen genes identified that form 29 collagen-type homo- and/or heterotrimeric members.\textsuperscript{62} Collagen is composed of three chains that wind together to form a triple-helical region responsible for the structural conformation of the protein.\textsuperscript{53} The distribution of specific collagen heterotrimers is heterogeneous and can be tissue and region-specific. For instance, collagen IV (\(\alpha_3, \alpha_4, \alpha_5\)) and (\(\alpha_5\)\(_2\)(\(\alpha_6\)), is exclusive to neuromuscular junctions, whereas the same type of Collagen, but with different chains, is more abundant near the basement membrane of the myofibers.\textsuperscript{64,65}

Collagen undergoes two major post-translational modifications in the endoplasmic reticulum: hydroxylation, which participates in its thermal and mechanical stability in the triple helix, and glycosylation, which regulates the collagen-assembled form.\textsuperscript{66,67} Fibroblasts are one of the major producers of collagens. Other cell types such as chondrocytes, MuSC, and committed myoblasts can also secrete several types of collagens, including type I, V, or VI\textsuperscript{53,62,67–69}.

**Type I Collagen.** Type I collagen has been studied in vitro using the myoblast cell line C2C12 (immortalized mouse myoblast cell line) and MuSC associated with single isolated myofibers.\textsuperscript{67} The Bou-Gharios group showed that Collagen-I is highly expressed in undifferentiated C2C12 myoblasts, and this expression is down-regulated at the beginning of differentiation. Consistently, differentiation of C2C12 cells is inhibited when these cells are treated with soluble collagen.\textsuperscript{67} However, more recently, it has been shown that when collagen type-I is used as a biomaterial in a 3D culture, it supports the myogenic progression of C2C12 myoblasts allowing their elongation, proliferation, attachment, and their alignment for myogenic differentiation and fusion into multinucleated myotubes.\textsuperscript{70} The myogenic potential of immortalized human C25Cl48 myoblasts was also tested in 3D collagen type-I culture, but it was reduced, and these cells were unable to contract spontaneously.\textsuperscript{70} Furthermore, isolated MuSC from mouse extensor digitorum longus muscle, cultured on Matrigel and embedded in collagen type-I for several days, could not elongate or form myotubes.\textsuperscript{70} These findings reveal some of the challenges of studying ECM components in vitro and highlight the need to understand further their impact on MuSC in 2D versus 3D cultures, to better recapitulate the tissue microenvironment. Additional studies will be essential to determine how Collagen-I regulates MuSC differentiation and explore how this ECM protein contributes to skeletal muscle regeneration in vivo. Crystal structure analysis and in vitro studies using recombinant domains have detected collagen-I binding sites on integrins ITG, Calcitonin (CALCR), syndecans (including syndecan-4 (SDC4)), α-dystroglycan, and EGF receptors. These stem cells are able to interact with endothelial cells via Vegf and Notch signaling. MuSC fate is also regulated by signals emitted by FAPs (WISP1, IL6, and follistatin).

**Collagen V.** Collagen-V (COLV) is a glycoprotein produced by MuSC in murine models.\textsuperscript{58} In vitro, treatment with COLV delayed the entry of freshly isolated MuSC into the cell cycle, proliferation, and differentiation, while treatment with other collagens (COL-I and COL-VI) did not have this effect. Accordingly, specific deletion of collagen-V encoding genes in MuSC resulted in a spontaneous exit from quiescence and promoted differentiation, leading to the exhaustion of the MuSC pool.\textsuperscript{68} Collagen-V is a ligand for the calcitonin receptor, a marker of quiescent MuSC, and this binding
Collagen-I

It is highly expressed in undifferentiated C2C12 myoblasts. Differentiation of C2C12 is inhibited when these cells are treated with collagen-type I.
It supports the myogenic progression of C2C12 cells when used as a biomaterial in 3D culture. Collagen-type I treatment impairs murine MuSC elongation of differentiation into myotubes.

Collagen-V

It participates in the maintenance of MuSC in its quiescent niche.

Collagen-VI (Col6)

Essential for structural support, maintenance, and differentiation of muscle. Mutations in COL6 lead to Bethlem myopathies and Ullrich congenital muscular dystrophies.
It is highly expressed by freshly isolated MuSC and regulates their self-renewal.

Col6α1+/− mouse model recapitulates Bethlem myopathy phenotype.

Fibronectin

Knockout murine adult models not viable — embryonic death at day 8.5. In skeletal muscle, it is transiently expressed during tissue remodeling. It promotes symmetric expansion and self-renewal of MuSC.
It is a preferential adhesion substrate for MuSC. Laminin-111 (Merosin)

Laminin-211-deficient mice (dy/*, mouse model for MDC1A)—a decrease of PAX7+ cells and increase of asymmetric cell divisions leading to failure of MuSC expansion; myofibers are smaller and muscle size is not recovered postnatally.

Laminin-111

Laminin 111 can compensate for the loss of laminin-211 when used as a treatment in muscle tissue of dy/* mice.
Laminin-111 treatment decreases muscle pathology in murine and canine models of Duchenne muscular dystrophy.
Maintains MuSC polarity and mediates asymmetric cell division.

Fibrillin-2 (Fbn2)

Mutations in the FBN2 gene cause congenital contractual arachnodactyly (CCA) or Beals syndrome.
Stimulates cell attachment of human dermal fibroblast.
Enhances lung epithelial regeneration by modulating basal epithelial stem cells proliferation, migration, and reducing cellular senescence.
Absence of FBN2—reduced muscle mass, delay in forelimb muscle differentiation, contractions, infiltration of fat into the limb connective tissue space, and abnormal activation of BMP signaling.

Fibrillin-1 (Fbn1)

Mutations in FBN1 cause Marfan syndrome.
Sequesters latent TGF-β in the extracellular space.
Fbn1-deficient mice—delayed muscle regeneration, inhibited MuSC proliferation and differentiation, and increased TGF-β activity.

Periostin (Postn)

It is upregulated in dystrophic muscle and its deletion improves muscular dystrophy pathology.

Tenascin-C (TnC)

Promotes MuSC expansion and enhances muscle repair.
It is secreted by necroptotic myofibers and promotes the proliferation of MuSC through EGRF interaction.
Modulates cell proliferation, survival, adhesion, migration, and differentiation of different cell types, including neural crest cells, fibroblasts, and tumor cells.

Thrombospondin-1 (TSP-1)

Accumulated in the skeletal muscle of patients with Dysferlinopathies (myopathy caused by a mutation in the dysferlin gene). Upregulation of TSP-1 correlates with higher chemotactic capacity and macrophage infiltration and activity into the muscle.
Causes an increase in mitochondrial density in skeletal muscle, leading to a performance benefit and increased metabolic efficiency.
TSP-1 is an antiangiogenic regulator in skeletal muscle. Through the CD36 receptor, TSP-1 regulates basal skeletal muscle capillarity.
TSP-1, expressed by visceral adipose tissues, induces muscle fibrosis, insulin resistance, and adipose tissue dysfunction during obesity progression. Besides, in cultured C2C12 myoblast cells, secreted TSP-1 inhibits insulin signaling associated with the activation of stress signaling (JNK, p38, and IKK).

Thrombospondin-2(TSP-2)

TSP-2 is transiently expressed after injury (hindlimb ischemia), including in muscle fibers.

Table 1. The functional regulation of ECM proteins in skeletal muscle and muscle-associated diseases.

| Gene          | Major roles                                                                 | References                                                                 |
|--------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Collagen-I   | It is highly expressed in undifferentiated C2C12 myoblasts. Differentiation of C2C12 is inhibited when these cells are treated with collagen-type I. It supports the myogenic progression of C2C12 cells when used as a biomaterial in 3D culture. Collagen-type I treatment impairs murine MuSC elongation of differentiation into myotubes. | Alexakis et al.67, Prüller et al.70 |
| Collagen-V   |                                                                             |                                                                           |
| Collagen-VI  | Essential for structural support, maintenance, and differentiation of muscle. Mutations in COL6 lead to Bethlem myopathies and Ullrich congenital muscular dystrophies. It is highly expressed by freshly isolated MuSC and regulates their self-renewal. | Cescon et al.76, Lampe and Bushby129 |
| Laminin-111  | Laminin 111 can compensate for the loss of laminin-211 when used as a treatment in muscle tissue of dy/* mice. Laminin-111 treatment decreases muscle pathology in murine and canine models of Duchenne muscular dystrophy. Maintains MuSC polarity and mediates asymmetric cell division. | Rooney et al.113,114, Barraza-Flores et al.; Goudenege et al.; Rooney et al.142–144, Rayagiri et al.111 |
| Laminin-211  | Laminin-211-deficient mice (dy/*, mouse model for MDC1A)—a decrease of PAX7+ cells and increase of asymmetric cell divisions leading to failure of MuSC expansion; myofibers are smaller and muscle size is not recovered postnatally. | Nunes et al.116 |
| Fibrillin-2   | Mutations in the FBN2 gene cause congenital contractual arachnodactylyy (CCA) or Beals syndrome. Stimulates cell attachment of human dermal fibroblast. Enhances lung epithelial regeneration by modulating basal epithelial stem cells proliferation, migration, and reducing cellular senescence. Absence of FBN2—reduced muscle mass, delay in forelimb muscle differentiation, contractions, infiltration of fat into the limb connective tissue space, and abnormal activation of BMP signaling. | Belleh et al.; Lee et al.; Tsipouras et al.204–206, Brinckmann et al.207, Gilpin et al.208, Sengle et al.172 |
| Fibrillin-1   | Mutations in FBN1 cause Marfan syndrome. Sequesters latent TGF-β in the extracellular space. Fbn1-deficient mice—delayed muscle regeneration, inhibited MuSC proliferation and differentiation, and increased TGF-β activity. | Dietz et al.129, Neptune et al.180, Cohn et al.181 |
| Periostin (Postn) | It is upregulated in dystrophic muscle and its deletion improves muscular dystrophy pathology. | Lorts et al.145 |
| Tenascin-C | Promotes MuSC expansion and enhances muscle repair. It is secreted by necroptotic myofibers and promotes the proliferation of MuSC through EGRF interaction. Modulates cell proliferation, survival, adhesion, migration, and differentiation of different cell types, including neural crest cells, fibroblasts, and tumor cells. | Tierney et al.69, Zhou et al.155, Akbareian et al.; Midwood et al.; Orend et al.; Sauge et al.; Tucker205–213 |
| Thrombospondin-1 (TSP-1) | Accumulated in the skeletal muscle of patients with Dysferlinopathies (myopathy caused by a mutation in the dysferlin gene). Upregulation of TSP-1 correlates with higher chemotactic capacity and macrophage infiltration and activity into the muscle. Causes an increase in mitochondrial density in skeletal muscle, leading to a performance benefit and increased metabolic efficiency. TSP-1 is an antiangiogenic regulator in skeletal muscle. Through the CD36 receptor, TSP-1 regulates basal skeletal muscle capillarity. TSP-1, expressed by visceral adipose tissues, induces muscle fibrosis, insulin resistance, and adipose tissue dysfunction during obesity progression. Besides, in cultured C2C12 myoblast cells, secreted TSP-1 inhibits insulin signaling associated with the activation of stress signaling (JNK, p38, and IKK). | De Luna et al.; Urao et al.165,166, Frazier et al.214, Audet et al.215, Inoue et al.; Matsugi et al.216,217, Krady et al.218 |
| Thrombospondin-2(TSP-2) | TSP-2 is transiently expressed after injury (hindlimb ischemia), including in muscle fibers. | |
mediates the interaction with MuSC. In this study, the authors further showed that MuSC are retained in their niche, in a quiescent state, through Notch-Collagen-V-Calcitonin receptor signaling\textsuperscript{68}. Further studies have shown that Notch signaling induces the transcription of the quiescence-specific microRNA-708 (miR708), which consequently inhibits tensin3, a component of the focal adhesion complex\textsuperscript{75}. Through the Notch-miR708-Tensin3 axis, MuSC migration is inhibited, and quiescence is stabilized, thus maintaining the MuSC pool\textsuperscript{76}. As Notch signaling is required to maintain MuSC quiescence and allows cell–cell communication, it will be relevant to broaden our understanding of ECM-interaction and Notch receptor/ligands on controlling MuSC function during muscle regeneration.

**Collagen-VI.** Collagen-VI (COL6) is one of the major components of the ECM and is ubiquitously expressed in multiple tissues, including skeletal muscle, nervous system, adipose tissue, skin, and cartilage\textsuperscript{76}. It exerts several functions such as regulation of cell differentiation, inhibition of apoptosis, and maintenance of cell stemness. Until a few years ago, three chains, α1, α2, and α3, were known to be encoded by COL6A1, COL6A2, and COL6A3 genes. In 2008, three other chains were identified in mice and humans: α4, α5, and α6\textsuperscript{77}. COL6 leads to the formation of the characteristic beaded microfilaments network in the basement membrane of muscle. This cell-adherent ECM protein is required for structural support, maintenance, and differentiation of muscle\textsuperscript{77}. Collagen-VI has been described as being a vital component of the MuSC niche\textsuperscript{53}. This ECM protein is adjacent to the surface of MuSC and highly expressed by freshly isolated MuSC while following MuSC activation, the expression of collagen-VI decreases. When Col6a1−/− murine muscles are subjected to injury, muscle regeneration is delayed and there is a significant reduction in MuSC numbers, suggesting that collagen-VI regulates MuSC self-renewal and is needed for the maintenance of the MuSC pool. In vitro experiments showed that collagen-VI promotes the maintenance and survival of PAX7-expressing MuSC. The authors demonstrated that collagen-VI is involved in regulating muscle stiffness and MuSC function depends on this mechanical property of the tissue. Transplantation of fibroblasts from wild-type mice into Col6a1−/− tibialis anterior muscles induced the production and deposition of collagen-VI and led to an increase in MuSC in collagen-VI deficient mice\textsuperscript{53}. These studies define a relevant role of collagen-VI in the maintenance of the MuSC pool in their own niche. In vitro studies have shown that collagen-VI can bind multiple integrins\textsuperscript{74,76,78,79} including α1β1 and α2β1 in melanoma cells\textsuperscript{78}. However, how collagen-VI communicates with MuSC to regulate their pool is still incompletely understood.

Overall, collagens are among the most abundant proteins in the body and regulate MuSC during muscle repair. Although the precise contribution of type-I collagen on regulating MuSC differentiation is still unclear, types-V and VI are needed to modulate self-renewal and maintain the stem cell pool. Additional studies are required to identify the cell surface receptors allowing the interaction between collagens and MuSC and other muscle-resident cell types to understand how they regulate MuSC function. Collagens can either directly bind to a MuSC-surface receptor or regulate the expression of soluble factors that will further modulate MuSC function. Similarly, these studies might contribute to a better understanding of how MuSC respond to the microenvironment stiffness. These discoveries are necessary to develop innovative tissue modeling approaches, including the engineering functional skeletal muscle in vitro.

**Fibronectins** are one of the major components of the ECM, mainly expressed during embryonic development and transiently upregulated during tissue remodeling\textsuperscript{86,80–82}. Fibronectin is a multidomain glycoprotein with a high molecular weight, existing as a dimer composed of two identical subunits (monomers) (~250 kDa per subunit) linked covalently through C-terminal pair of disulfide bonds\textsuperscript{83}. Each monomer is composed of 12 FN type I (FNI), 2 FN type II (FNII), and 15-17 FN type III (FNIII) repeats, which enable high-affinity binding to growth factors, including insulin-like growth factor binding-protein-3, fibroblast growth factor (FGF)-2, and vascular endothelial growth factor (VEGF)-A\textsuperscript{84}. In humans, the gene encoding for fibronectin protein can generate up to 20 variants due to alternative mRNA splicing. This process is dependent on age, stage of development, and cell type, and each variant plays specific roles, including fibronectin secretion, adhesion, liver fibrosis, and skin wound healing\textsuperscript{85}. In addition to alternative splicing, fibronectin can also undergo multiple post-translational modifications such as phosphorylation and glycosylation\textsuperscript{85}. In mouse fibroblasts, phosphorylation of fibronectin participates in cell spreading, proliferation, and cell traction forces\textsuperscript{86}. In human U2OS (human bone osteosarcoma) and HeLa (human cervical adenocarcinoma epithelial) cell lines, five N-glycosylation sites (N430, N528, N542, N1007, and N1244) were identified in the fibronectin protein. When N-linked glycans were cleaved using N-glycosidase F (PNGase F), adhesion and migration functions of fibronectin were significantly reduced, indicating that N-glycans of fibronectin are involved in cell adhesion and migration\textsuperscript{87}. However, the post-translational modifications of fibronectin have not been extensively explored, either during muscle development or adult muscle tissue repair. In the future, it will be relevant to explore and compare whether fibronectin post-translational modifications are conserved and/or differ in these two contexts and whether they have a role in regulating MuSC function. Fibronectin is present in both the ECM and in the plasma, thus existing as two distinct forms—insoluble and soluble, respectively. Fibronectin in the plasma is synthesized by hepatocytes in the liver, while in muscle, Fibronectin is produced by multiple cell types, including fibroblasts, chondrocytes, endothelial cells, myoblasts, myotubes, and MuSC\textsuperscript{76,80,85}.

**Table 1 continued**

| Gene                        | Major roles                                                                 | References                |
|-----------------------------|------------------------------------------------------------------------------|---------------------------|
| Thrombospondin-4 (TSP-4)    | Maintains structure and function of myotendinous junctions and regulates    | Senina-Adogoravi and Plow; Vanhoutte et al; Frolova et al\textsuperscript{169,197,205} |
|                            | muscle integrity. Ameliorates vesicular trafficking of dystrophin protein. Mice lacking TSP-4 showed a decreased muscle mass and poorer performance of limbs. Lack of TSP-4 caused spontaneous dystrophic changes in muscle with aging and accelerated muscular dystrophy in murine models. |                                                                                      |
| Versican (Vcan)             | Regulates proliferation and inhibits differentiation of satellite cells isolated from pectoralis major muscle of F and RBC2 turkey lines. Clearance of VCAN by ADAMTS proteins enhances the fusion of C2C12 myoblasts. | Stupka et al; Velleman et al\textsuperscript{221,222}              |

In previous publications, several ECM proteins have been identified in the microenvironment of MuSC\textsuperscript{53,98,223}. This table summarizes the major roles of some of those ECM proteins, with a particular focus on skeletal muscle and muscle-associated diseases.
In multiple animal models, the deletion of fibronectin affects tissue development. For example, in zebrafish, the knockdown of fibronectin impairs somite formation and disrupts muscle morphogenesis\textsuperscript{90}. In *Xenopus laevis*, fibronectin is essential for the attachment and migration of mesodermal cells. In addition, knockdown of fibronectin delays gastrulation and impairs convergence and extension, thus affecting body axis formation\textsuperscript{92,97}. Furthermore, fibronectin is upregulated after appendage amputation in amphibians and is involved in multiple cellular mechanisms required for wound healing, such as cell growth and migration\textsuperscript{92,93}. Finally, in murine models, embryos lacking this protein die before birth at day 8.5 and show deformed hearts, cardiovascular defects, and impaired formation of notochord and somites\textsuperscript{94}.

In murine regenerating skeletal muscle, fibronectin is highly expressed in the microenvironment surrounding MuSC\textsuperscript{26}. The Rudnicki group showed that fibronectin is expressed by MuSC during muscle repair and is strongly upregulated when MuSC are in an activated state and primed for myogenic commitment. This study demonstrated that committed muscle myogenic cells release high amounts of fibronectin into their niche. This work also showed that this ECM protein stimulates WNT7a activity through the frizzled-7 and syndecan-4 coreceptor complex to induce MuSC symmetric expansion and self-renewal\textsuperscript{26}(Fig. 3). This ability of fibronectin in promoting MuSC self-renewal seems to be specific to adulthood since acute downregulation of fibronectin in fetal MuSC does not affect their expansion\textsuperscript{99}. Furthermore, knockdown of fibronectin in adult MuSC leads to a decrease in the number of committed myogenic cells and a reduction in symmetric divisions numbers, therefore impairing muscle repair\textsuperscript{26}. It has been previously shown that fibronectin delays myoblasts fusion in vitro, suggesting that this glycoprotein inhibits myogenic differentiation\textsuperscript{95}. These findings, combined with Bentzinger’s results showing that once MuSC enter the differentiation program, fibronectin expression is downregulated\textsuperscript{99}, suggest that low levels of Fibronectin are required to allow MuSC differentiation and promote tissue repair.

Besides syndecan-4, MuSC can also adhere to fibronectin through integrins (including alpha-5 (\(\alpha 5\))-integrin)\textsuperscript{96–98}. A recent study explored the interaction between fibronectin and integrins during nuclear positioning\textsuperscript{99}. Multinucleated myoblasts secrete fibronectin and deposit it at the surface of muscle cells to attract myonuclei to the cell periphery. Fibronectin secreted by myofibroblasts mediates peripheral nuclear positioning and directly modulates myofiber differentiation through activation of \(\alpha 5\)-integrin via focal adhesion kinases (FAK), SRC, and small Rho guanosine triphosphatase (GTPases) Cdc42\textsuperscript{99}. Besides its function of regulating the migration of mesodermal cells during embryonic development and controlling the symmetric expansion and self-renewal of MuSC in adulthood, this work has shown that fibronectin is also required for nuclear movements within the developing myofibers.

In addition, a 3D time-lapse analysis exploring MuSC motility on single living fibers has demonstrated that primary cells plated on fibronectin showed a round shape and minimally adhered to this substrate. Also, the specific blockade of integrin-\(\alpha 5\), a fibronectin-receptor, increased the velocity of MuSC, suggesting that fibronectin regulates MuSC motility\textsuperscript{97}. Thus, it will be relevant to determine how fibronectin contributes to MuSC movements, for instance, to define the intracellular pathways that are activated once fibronectin binds to this integrin receptor. Also, it is currently unknown whether the deletion of fibronectin affects MuSC adhesion to the myofibers or to their stem cell niche. Nonetheless, it is already known that fibronectin promotes cell adhesion and ECM assembly\textsuperscript{100}. However, this process is deregulated in aged MuSC, thus affecting the aged stem cell niche and the regenerative response of skeletal muscle\textsuperscript{98}.

\(\beta 1\)-integrin is a relevant MuSC receptor that contributes to the maintenance of MuSC homeostasis and supports MuSC expansion and self-renewal during muscle regeneration\textsuperscript{100}. This group has shown that \(\beta 1\)-integrin acts in cooperation with FGF2, a growth factor for MuSC\textsuperscript{101}. Furthermore, it has been shown that fibronectin treatment on muscle cells affects FGF-ERK MAP kinases cascades\textsuperscript{98}. Rozo et al. have tested FGF2 responsiveness in the presence of fibronectin in myoblasts lacking \(\beta 1\)-integrin and have observed that this response was compromized, suggesting that there is a crosstalk between \(\beta 1\)-integrin, fibronectin, and FGF2. This effect seems to be exerted through the binding of the arginine-glycine-aspartic acid motif\textsuperscript{102}; a common integrin-binding domain present in multiple ECM proteins, including fibronectin and laminin\textsuperscript{102}. Additional studies are necessary to characterize the specificity of this fibronectin-FGF2-\(\beta 1\)-integrin interaction on regulating MuSC homeostasis.

**Laminins**

Laminins are ubiquitously distributed throughout multiple tissues and laminin isoforms are tissue-specific. These ECM proteins play several biological roles, including tissue structure and maintenance, cell migration, adhesion, proliferation, differentiation, and survival\textsuperscript{103}. Laminins are the major cell adhesive components of the basement membrane. They are heterotrimeric glycoproteins with three polypeptide chains \(\alpha, \beta, \gamma\) encoded by different genes\textsuperscript{56,104}. These chains bind together at the coiled-coil region of laminin, forming heterotrimeric isoforms with about ~400–900 kDa of molecular weight\textsuperscript{89}. Mice lacking a \(\alpha\) or \(\gamma\) chains of laminin showed developmental defects leading to embryonic lethality or severe disorders after birth\textsuperscript{105–107}, suggesting that these ECM proteins are essential for early development.

About 13–30% of the total molecular weight of these heavy glycoproteins is attributed to N-linked glycosylation\textsuperscript{108,109}. In addition, N-glycosylation affects cell spreading, adhesion, scattering, and migration in human cells, as well as tumor growth and metastasis in animal models\textsuperscript{109,110}, making this post-translational modification a potential target for the development of therapeutic approaches. However, these post-translational modifications have not been defined in skeletal muscle yet.

The function of laminins is dependent on ECM-binding proteins (e.g., collagen-IV), as well as cell-surface binding receptors, including \(\alpha 7\)-integrin\textsuperscript{103} and \(\alpha 6\)-integrin\textsuperscript{111}. Additionally, laminins bind to the \(\alpha\)-dystroglycan receptor, a component of the dystrophin–glycoprotein complex known to lead to muscular dystrophies when mutated\textsuperscript{103}. These binding events are responsible for basement membrane assembly and mechanical linkage of the cell to this membrane\textsuperscript{112}.

**Laminin-111.** The laminin-111 (chains \(\alpha 1\)B1\(\gamma 1\)) isoform is mainly expressed in embryonic skeletal muscle. In adults, activated MuSC secrete laminin-111 and this ECM protein is deposited into their basal lamina. It next binds its receptor \(\alpha 6\beta 1\)-integrin, leading to the maintenance of MuSC polarity and mediating asymmetric cell division\textsuperscript{111}. This ECM protein has been studied in the context of muscular dystrophies, where it has been shown its potential treatment to alleviate these diseases\textsuperscript{113,114}.

**Laminin-211.** Laminin-211 (chains \(\alpha 2\)B1\(\gamma 1\)), also named merosin, is the most abundant isoform expressed in the basement membrane and surrounds muscle fibers in adult skeletal muscle\textsuperscript{103,115}. Laminin-211 controls muscle function through its binding to cell-surface receptors \(\alpha 7\)-integrin, \(\alpha\)-dystroglycan, and syndecans\textsuperscript{103}. The consequences of the truncated deficiency form of the laminin \(\alpha\)-2 chain (lacking the N-terminal LN domain) in skeletal muscle have been previously explored in murine...
tissues. In this mouse model, myofibers are smaller and muscle size is not recovered postnataly. This phenotype is accompanied by a decrease in PAX7-positive MuSC and myogenin-positive cells, suggesting that these mice fail to generate sufficient numbers of MuSC to support fetal myofiber growth. Moreover, Nunes et al. observed an abnormal overactivation of JAK-STAT (Janus kinase–signal transducer and activator of transcription) signaling. We and others have previously shown that STAT3 promotes MuSC progression into committed myogenic progenitors. Also, stat3-knockdown stimulated symmetric MuSC divisions on cultured myofibers. Thus, Nunes et al. suggested that in mice lacking laminin-211, activation of this signaling leads to an increase of the frequency of asymmetric cell divisions at the expense of symmetric cell divisions, impairing the expansion of the MuSC pool.

The potential use of laminins as therapeutic tools has been previously explored by culturing isolated MuSC on the laminin-E8 fragment (C-terminal half of the coiled-coil region of laminin protein, which maintains the integrin-binding activity). The authors have demonstrated that the use of these fragments allowed the establishment of an artificial niche favorable for MuSC expansion and maintained MuSC in an undifferentiated state through modulation of JNK and p38 signaling, two pathways essential for MuSC function. ECM components are important not only to provide oostasis and regenerative response but also to modulate MuSC adhesion and migration during muscle repair.

Collagen. Type VI collagen is one of the most studied ECM proteins in muscle since mutations in collagen-VI are responsible for several forms of human muscle diseases, including Bethlem myopathies and Ullrich congenital muscular dystrophies. A genetic mouse model lacking one of the collagen-VI chains, the α1 (Col6a1−/−), recapitulated the Bethlem myopathy phenotype. These mice showed myofiber necrosis and phagocytosis, changes in fiber diameter, mitochondrial dysfunction, and spontaneous apoptosis leading to myofibers degeneration and defective autophagy. Mtaneous muscular dystrophies (group of diseases affecting skeletal muscles) are associated with several human diseases, including Junctional epidermolysis bullosa (group of disorders characterized by abnormal fragility of the skin) or Congenital muscular dystrophies (group of diseases affecting skeletal muscles). A mouse model for human merosin-deficient congenital muscular dystrophy type 1A (MDC1A), named dyW, has been used to study the consequences of truncated deficiency of laminin-α2 chain in skeletal muscle. This work has shown that the onset of this dystrophy occurs prenatally. In MCD1A, laminin-111 delivery has been studied as a substitute for the loss of laminin-211 in adults. Indeed, in the dyW mouse model, the laminin-111 treatment prevented muscle disease progression and promoted muscle repair. This treatment also induced an increase in myofiber size and number of regenerating myofibers, and expression levels of PAX7 and myogenin, suggesting a restoration of muscle regeneration. Therefore, the authors proposed that laminin-111 could counteract the loss of laminin-211 and prevent muscle disease progression of patients with this muscular dystrophy. Duchenne muscular dystrophy (DMD) is the most common childhood form of muscular dystrophy, affecting 1 in 3500 male births and inducing progressive muscle weakness. Several invertebrate and mammalian models have been utilized to study the consequences of this genetic degenerative neuromuscular disorder, including Caenorhabditis elegans, Drosophila melanogaster, zebrafish, rats, pigs, cats, dogs, and several mouse models, being the mdx mouse model the most widely used. This lethal disease is caused by mutations in the dystrophin gene, leading to the lack of a dystrophin protein. In myofibers, dystrophin connects the cytoskeleton to the ECM through the sarcosome. Dystrophin is expressed not only in differentiated myofibers but also in activated MuSC, in which this protein regulates MuSC polarity and asymmetric division. Dystroglycan is a membrane component of the dystrophin-glycoprotein complex and establishes the transmembrane link between laminin-2 and dystrophin, indicating that ECM proteins can interact and regulate dystrophin. In mdx mice and Golden retriever muscular dystrophy dog models, laminin-111 treatment decreased muscle pathology by reducing muscle fiber damage and fibrosis. These findings indicate that ECM components, particularly laminin-111, can be used as therapeutic tools for muscle diseases.

Dysregulation of ECM proteins compromises tissue repair in muscle-associated pathological contexts

Several studies have demonstrated the relevance of the stem cell niche in coordinating cell-cell communication and controlling MuSC function. ECM components are important not only to provide structural integrity to the niche and regulate tissue stiffness but also to modulate MuSC adhesion and migration during muscle repair. However, ECM components significantly change their expression in muscle diseases and during aging, compromising tissue homeostasis and regenerative response.

ECM proteins in muscle-associated diseases

Multiple mutations or dysfunctions of ECM components are related to muscle-associated diseases. The most common is primary myopathies and, in particular, muscular dystrophies. Muscular dystrophies are a group of inherited diseases characterized by progressive weakness and muscle loss.
However, whether these interactions modulate MuSC function has not been extensively studied.

**Tenascin-C.** Tenascin-c (TNC) is expressed during embryonic development and increases rapidly after birth. In adults, it is transiently expressed during tissue injury and regeneration, including skeletal muscle. In dystrophic muscles from mice, dogs, and humans, the glycoprotein TNC has been detected in degenerating and regenerating areas, whereas, in healthy adult skeletal muscle, this protein was not detectable. However, little is known about the function of tenascin-c in this pathology. MuSC secrete TNC, and this ECM protein promotes MuSC expansion and enhances muscle repair. Another study exploring necroptosis, a type of programmed cell death, proposed that necrotic myofibers promote MuSC proliferation by activating the TNC–epidermal growth factor receptor (EGFR) kinase cascade. Indeed, the authors demonstrated that TNC is released by EGFR to promote MuSC proliferation. Other studies have shown that activation of Egfr signaling promoted the production of myogenic progenitors through stimulation of asymmetric divisions. These studies are required to address whether EGF influences the proliferation of isolated MuSC and how the microenvironment regulates this factor. A recent study has further shown that TNC is also expressed by muscle glial cells activated upon nerve injury, in accordance with the role of TNC on neuro-muscular junction formation and stabilization. These studies suggest that MuSC, glial cells, and myofibers are sources of TNC in skeletal muscle. However, little is known about the potential of other muscle-resident cells to express this protein in other physiological and disease conditions. Furthermore, the molecular mechanism through which TNC promotes MuSC expansion and whether it regulates their function (including migration, differentiation, self-renewal, or quiescence) in a regenerative environment is still unclear. Additionally, initial evidence of activated necroptosis in dystrophin-deficient mouse and human muscles shows how preventing this type of programmed cell death could be a potential therapeutic tool to improve muscular dystrophies. Thus, further studies exploring the function of TNC in dystrophic models would improve the development of therapeutic approaches for this muscle disease.

**Thrombospondins.** Proteins from the TSP family are found within interstitial areas in human skeletal muscle tissues and are linked to several muscular dystrophies. Studies using the skeletal muscle of patients with dysferlinopathies (autosomal recessive muscular disorders caused by mutations in the dysferlin gene) showed an increased expression of TSP-1. This ECM protein is secreted in response to inflammation and some studies have demonstrated that TSP-1 is expressed by muscle fibers and macrophages in muscles with this specific myopathy. In dystrophin-deficient tissues, increased expression of TSP-1 in muscle fibers correlates with higher chemotactic activity promoting macrophages infiltration, suggesting that TSP-1 might contribute to inflammation. The ubiquitin-proteasome system is known to recognize and remove unfolded or misfolded proteins. Further studies have explored whether the use of ubiquitin–proteasome inhibitors can prevent the degradation of dysferlin in immortalized myoblasts with dysferlinopathies. Although the use of these inhibitors did not seem effective in restoring sarcomera repair of damaged myofibers from patients, a decrease in TSP-1 release from myotubes was observed. The authors suggested that targeting TSP-1 could be a potential therapeutic approach to reduce inflammation in patients with dysferlinopathies.

TSP-4 is transiently expressed in skeletal muscle upon injury or disease, including in dystrophic muscles (from human patients with Becker, DMD, and limb-girdle muscular dystrophies (LGMD2F)), where it is detected around macrophages infiltration and necrotic regions. Vanhoutte et al. have used two models for muscular dystrophies: the mdx mouse to study DMD and sgcd (deletion of δ-sarcoglycan) as a model for LGMD2F. Dystrophic mice lacking TSP4 showed a significant worsening of these pathologies, suggesting that this ECM component might have a protective role. Indeed, overexpression of TSP-4 in murine myofibers protected mice against muscular dystrophy. Additionally, a similar effect was observed in fruit flies, where TSP-4 treatment has rescued the muscular dystrophy phenotype, thus confirming that TSP-4 is protective and alleviates the dystrophic disease. The authors further uncovered a molecular mechanism where TSP-4 aids vesicular trafficking and chaperones dystrophin–glycoprotein and integrin attachment to the membrane, strengthening the stability and integrity of the myofiber membrane. Further studies could explore whether TSP-1 and TSP-4 interact with myofibers and if they have an impact on MuSC function.

**Fibrillins.** Other ECM components, including members of the fibrillin family, have been shown to be associated with muscle diseases. Mutations in the gene coding for fibrillin-2 (FBN2), an ECM glycoprotein, cause Congenital contractual arachnodactyly. This genetic disorder is also known as Beals syndrome and affects connective and musculoskeletal tissues. Genetic ablation of FBNI in mice leads to decreased muscle mass, increased white fat, delay in forelimb muscle development, and abnormalities in the musculature. FBN2 is an important ECM constituent of all connective tissues and maintains tissue homeostasis by sequestering TGF-β and BMP in a latent state, thus inhibiting activation of this signaling pathway. Of note, in skeletal muscle, TGF-β inhibits MuSC differentiation, thus impairing their regenerative capacity, and it has been previously reported that systemic delivery of TGF-β receptor kinase inhibitor improves muscle regeneration. Currently, the role of fibrillin-2 in muscle repair and especially in regulating MuSC function has not been comprehensively explored.

Marfan syndrome is a genetic disorder very similar to Beals syndrome and is the most common heritable disorder of connective tissues. Mutations in the fibrillin-1 gene cause this autosomal-dominant disease. Individuals affected by this disorder have a myopathy and are incapable of increasing muscle mass even with exercise. This gene codes for fibrillin-1 (FBNI), an ECM glycoprotein. Similar to fibrillin-2, this protein plays a critical role in sequestering TGF-β on a latent state. FBN1-deficient mice showed fibrosis and delayed muscle regeneration. Although this ECM protein contributes to muscle repair by modulating TGF-β, additional studies are needed to determine whether FBN1 communicates directly with MuSC through binding receptors or regulating soluble factors in the microenvironment. Altogether, these studies have shown the contribution of ECM in the regulation of MuSC function in multiple muscle-associated diseases. Therefore, understanding the molecular dynamics of the stem cell niche and identifying other relevant ECM components will represent an essential step in developing new therapies to treat these pathologies.

**The stem cell niche during aging.** Aging is associated with a decline in muscle mass, mobility, and quality of life. Aged individuals are confronted with physical limitations in part caused by age-related impairment of muscle regenerative ability. The aged population is more susceptible to injury and the muscle repair process is impaired compared to younger adults. These impairments are associated with multiple cellular and molecular defects that directly affect MuSC function. With aging, the tissue regenerative potential is reduced due to a decline of cellular quiescence and self-renewal, as well as changes in cell proliferation and activation.
Senescence, a cellular state induced by dysregulation of intrinsic and external signals leading to the functional exhaustion of the cell, affects MuSC function\textsuperscript{188-191}. In geriatric (extremely old) mice, MuSC switch from quiescent to pre-senescence, a state leading to full irreversible senescence in response to regenerative pressure caused by derepression of p16INK4a (cyclin-dependent kinase inhibitor 2A). This affects MuSC activation and expansion, resulting in a reduction of the stem cell pool and capacity to participate in muscle repair\textsuperscript{187}. The Muñoz-Cánoves team further showed that basal autophagy is crucial to maintain the MuSC quiescent state. Indeed, basal autophagy of aged MuSC is impaired, leading to an accumulation of toxic cellular waste, and as a result, MuSC enter into senescence. Thus, restoring autophagy can improve the regenerative potential of aged MuSC\textsuperscript{192}.

It has been previously demonstrated that there is an abnormal deposition of ECM components in aged tissues, leading to fibrosis and increased tissue stiffness\textsuperscript{193}. Indeed, Lukjanenko et al. have shown that some ECM components, including laminin, biglycan, and testican-1, are expressed at high levels in old muscles compared to young ones in homeostatic conditions\textsuperscript{98}. Conversely, after injury, some ECM proteins that are upregulated in young muscles, fail to do so in aged muscles. For example, lower levels of fibronectin were detected after injury in old tissues compared to young ones. This team has studied the consequences of conditional deletion of fibronectin in young mice and showed that these mice recapitulated a premature aging phenotype. Moreover, fibronectin is the preferred adhesion substrate for mouse and human muscle progenitors and modulates multiple signaling pathways\textsuperscript{98} including differentiation-inducer pathways ERK and p38 mitogen-activated protein kinases\textsuperscript{51,120,123}. Interestingly, fibronectin treatment rescued the adhesion capacity of aged MuSC, rejuvenated aged MuSC function by promoting their proliferative and myogenic potential in vivo, and improved muscle regeneration\textsuperscript{94}.

A recent study has shown that aging is associated with increased muscle stiffness and pathogenic ECM architecture, resulting in aberrant myogenic progression of MuSC and affecting MuSC behavior\textsuperscript{194}. Stearns et al. demonstrated that aging directly affects the gene expression of resident fibroblasts for the secretion of ECM components through YAP/transcriptional coactivator with PDZ-binding motif (TAZ) signaling, promoting fibrogenic conversion of MuSC, thus affecting MuSC fate\textsuperscript{194}.

MuSC function is not only affected by ECM components and muscle stiffness but can also be indirectly disturbed by other cell types present in the niche. It has been recently reported that FAPs activity is impaired with aging\textsuperscript{18}. As we have previously described, FAPs secrete WISP1 (a protein regulating expansion and asymmetric commitment of MuSC), and since those cells lose their functionality with aging, WISP1 expression is affected in aged muscle. The authors demonstrated that loss of FAPs function combined with decreased WISP1 expression resulted in impaired MuSC function and muscle regeneration. Furthermore, WISP1 treatment restored MuSC myogenic potential in aged mice\textsuperscript{18}. This is another research study showing that modulation of the stem cell niche can improve age-associated failures and promote muscle repair.

Altogether, these studies demonstrate how dysregulation of ECM components in the aged microenvironment impacts MuSC function. In agreement with these studies, the exposure of old mice to the young microenvironment significantly improved their regenerative potential, suggesting that modulation of systemic factors in aged tissues could be reversed\textsuperscript{195}. Besides fibronectin, there are many other ECM components to be explored and these future studies will represent significant progress in the field to counteract muscle-aging-related pathologies, including sarcopenia, which is a chronic condition characterized by the age-related loss of skeletal muscle mass, strength, and performance\textsuperscript{196}.

Components from the MuSC microenvironment are promising targets for developing therapeutic interventions to reduce the adverse effects of sarcopenia.

**APPROACHES TO DEFINING THE ECM COMPOSITION AND USING IT AS A TOOL TO IMPROVE SKELETAL MUSCLE MAINTENANCE**

Skeletal muscle is an oriented tissue and this specific organization is required to regulate its mechanical properties and keep the spatial organization of ECM and residing cells\textsuperscript{197}. Thus, natural scaffolds retaining the mechanical integrity and the 3D ECM architecture are needed to recapitulate native tissue properties. Multiple studies showed that tissue decellularization is a powerful tool to generate naturally derived ECM and improve functional tissue regeneration upon transplantation in vivo\textsuperscript{197-200}. In fact, implanted decellularized muscles promoted host cell migration, allowed a 3D reorganization of the myofibers, and restored the MuSC pool, thus promoting functional tissue regeneration\textsuperscript{198}. However, in several of these studies, the composition of decellularized matrix is unknown, limiting our understanding of the presence and concentration of each ECM component as well as molecular mechanisms underlying their effects. Immunofluorescence experiments on tissues can be performed to enrich our knowledge about the ECM composition, however, these analyses are candidate-based approaches assessing only two or three ECM proteins at a time and rely heavily on the availability of specific antibodies. The recent development of single-cell proteomics approaches\textsuperscript{201} will overcome these limitations and enable a systematic assessment of skeletal muscle tissue composition in an unbiased manner. Finally, standard tissue digestion approaches result in loss of anatomical spatial information regarding the location of proteins in the tissue, while a central aspect of studying tissue composition is to be able to build a spatial mapping of cells and proteins, in order to evaluate their distribution and proximity to specific structures and microenvironments. Applying advanced technologies such as Nanostring Spatial Digital Profiling or STARmap\textsuperscript{202} for 3D in situ RNA sequencing or multiplex protein detection would allow single-cell analyses and protein distribution in intact issues.

As an alternative approach, in vitro cultures can be used for studying ECM proteins. Unfortunately, it is challenging to investigate ECM proteins in this context due to the lack of spatial cues as well as other tissue components that may interact with ECM proteins and modify their function. While 3D cultures are more versatile than 2D ones and enable us to recapitulate several features of native tissues, they still suffer from our limited understanding of tissue composition. The use of biomaterials able to mimic tissue properties at both the structural and functional level has significantly advanced the development of artificial matrices\textsuperscript{203}. These biomaterials have been utilized both in vitro and in vivo by injection into muscle tissues. In order to mimic the MuSC-microenvironment and improve muscle repair, multiple aspects need to be taken into account to design suitable scaffolds. The architecture of this biomaterial must mimic the stiffness of the tissue to provide the mechanical properties, and also, it has to allow cell growth, motility, adhesion, and function, including self-renewal and differentiation. Furthermore, this artificial tissue requires biocompatibility with the tissue to avoid any inflammatory response, and it has to be biodegradable to be progressively replaced by the newly formed tissue\textsuperscript{203}.

Overall, the integration of these new technologies will allow us to dissect skeletal muscle tissue composition, perform direct comparisons in different physiological and pathological contexts, and identify new approaches to modulate tissue microenvironments to promote MuSC function and tissue repair.
CONCLUSION AND PERSPECTIVES

Several studies have contributed to deepening our knowledge about the composition of the stem cell niche, including the discovery of new ECM proteins and their function in regulating MuSC and muscle repair. The construction of a cell atlas of muscle tissues is definitively an advance in the muscle field, which is contributing to the discovery of the producers of ECM components. Extending our knowledge on the cooperation between muscle-resident cell types and MuSC is essential to decipher the behavior of MuSC and the MuSC-microenvironment dynamics. This will provide a better understanding of how those muscle-resident cell types communicate with MuSC and contribute to regulating their fate and function, either with direct physical interaction or by secreting factors, including ECM proteins. These discoveries will demonstrate that not only biochemical signals but also the physical cell-cell interactions are an important factor in controlling MuSC function and behavior, as shown in previous studies.

In recent years, several studies have identified a critical role of the microenvironment in instructing MuSC behavior and temporally coordinating tissue repair and maintenance. Several ECM components have been demonstrated to modulate MuSC self-renewal and differentiation or to contribute to maintaining their quiescent state. Some of the ECM proteins we have described are transiently expressed during healing processes, suggesting that there is temporal coordination of ECM proteins to modulate MuSC. In fact, after an acute injury in healthy tissue, appropriate deposition of a transient matrix has a positive role, and it is necessary to repair the damage efficiently. However, chronic inflammation leads to a persistent accumulation of ECM components, which can be explained by the excessive accumulation of their own producers, such as fibroblast-like cells, which frequently occur in aged and dystrophic tissues. Further studies are essential to explore the original source of ECM components and identify the signaling pathways associated with these factors, to understand whether they modulate MuSC function and how MuSC respond to these signals in homeostatic versus regenerative conditions. Although several studies have contributed to deepening our knowledge about the stem cell niche, future additional studies exploring the composition of the niche and manipulating ECM components in vivo are necessary to improve the regenerative potential of MuSC and alleviate muscle-associated diseases.

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**COMPETING INTERESTS**

The authors declare no competing interests.

**ADDITIONAL INFORMATION**

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