Mammalian skeletal muscle is notable for both its highly ordered biophysical structure and its regenerative capacity following trauma. Critical to both of these features is the specialized muscle extracellular matrix, comprising both the multiple concentric sheaths of connective tissue surrounding structural units from single myofibers to whole muscles and the dense interstitial matrix that occupies the space between them. Extracellular matrix-dependent interactions affect all activities of the resident muscle stem cell population (the satellite cells), from maintenance of quiescence and stem cell potential to the regulation of proliferation and differentiation. This review focuses on the role of the extracellular matrix in muscle regeneration, with a particular emphasis on regulation of satellite-cell activity.

Introduction

Our ~640 individual skeletal muscles, comprising roughly 40% of an adult human’s total body mass, are collectively responsible for maintaining posture and balance, for respiration, and for nearly all movements, from the most delicate microsurgceries and brush-strokes to marathon running and power-lifting. Possibly due to the potential for damage implicit in such diverse and critical functions, skeletal muscle is one of the most highly regenerative tissues in the body; such regeneration requires the activity of a population of tissue-specific adult stem cells referred to as satellite cells.

Briefly, muscle satellite cells are the obligate tissue-specific stem cells of skeletal muscle: if the satellite-cell population (defined by expression of the satellite-cell marker Pax7 [1]) is genetically ablated after maturity in the mouse, muscle regeneration fails [2–4]. Satellite cells are derived from a somitic lineage during development, and are thought to disperse throughout the developing musculature concomitantly with the myoblasts that contribute to embryonic and fetal myogenesis [5–7]. They are maintained in a quiescent state in the absence of physiological signals of damage, over-use or disease in a minimal niche consisting of the cell membrane of the multi-nucleate, differentiated myofiber they are associated with and its overlying basal lamina (described further below). When activated from quiescence by stress or damage, satellite cells enter the cell cycle and proliferate extensively to form a population of replacement myocytes, which fuse with each other or existing myofibers to reconstitute the muscle [8,9]. Due in large part to their potential as either vectors or targets for cell therapy of human myopathies, particular Duchenne muscular dystrophy, satellite cells have been the focus of intensive research to try to unravel the molecular mechanisms governing their maintenance and activity in vivo, in both healthy and pathological tissue. We describe briefly the role

Abbreviations
ECM, extracellular matrix; HGF, hepatocyte growth factor.
that the extracellular matrix (ECM) plays in the structure and function of healthy skeletal muscle, then address key interactions with the ECM during satellite cell-mediated muscle regeneration, with particular emphasis on potential new and emerging areas of research.

**Structure of the skeletal muscle extracellular matrix**

The architecture of each individual skeletal muscle is maintained in part by concentric arrangements of connective tissue layers, which give the muscles both structure and strength [10,11]; a recent review [12] describes the muscle ECM in detail with respect to its composition, ultrastructure and inter-connectivity. The myofiber plasma membrane is referred to as the sarcolemma; it encases each myofiber and forms the most proximal layer of the myofiber basal lamina. The sarcolemma and its associated basal lamina are a key feature of muscle fibers: first described in Bowman’s classic monograph ‘On the Minute Structure and Movements of Voluntary Muscle’ in 1840 [13] as a thin, tough, transparent membrane that remained after hyper-contracture of the contents of a myofiber, Bowman’s tubes ‘seem not improbably to consist of a very close and intricate interweaving of threads, far too minute for separate recognition’. The basal lamina and its associated interstitial matrix are of particular interest for the purposes of this review due to their physical proximity and intimate interactions with quiescent and activated satellite cells and the sarcolemma of myofibers. In fact, the ‘gold standard’ definition of a quiescent satellite cell is as originally described by Mauro in 1961 [14], and elaborated on in further electron microscopy-based studies [15]: small bipolar cells with a heterochromatic nucleus, scanty cytoplasm and tron microscopy-based studies [15]: small bipolar cells of myofibers and their associated endomysium highlight the intimate associations of each muscle fiber with the three-dimensional network of the endomysium; the endomysium also harbors capillaries and axons serving each individual muscle fiber [23].

The cytoskeleton of each myofiber is physically attached to the basal lamina, mainly through integrins and dystrophin–glycoprotein complexes [24,25]; these connections are continued via additional cross-linking molecules such as entactin, nidogen and agrin to the interstitial matrix between myofibers (Fig. 1) [26]. It is disruptions in this linkage that are most often responsible for human myopathies, including Duchenne’s muscular dystrophy [27]. The interstitial matrix that surrounds myofibers accounts for 1-10% of muscle tissue, and fills all of the space between muscle fibers while maintaining mechanical continuity with tendons. The major roles of the interstitial ECM are to transduce mechanical force from the muscles and to serve as a structural support for the muscle and its associated blood vessels and nerves. It is more porous than the basal lamina, and is composed of fibrous components providing tensile strength (primarily collagens) and proteoglycans (including chondroitin, heparan and dermatan sulfates). Proteoglycans comprise 10% of the weight but 90% of the volume of the matrix and provide both a labile environment for other cells and components to move within and a sink for other matrix-associated molecules (proteases, chemokines, cytokines, mitogens, and growth factors) [16]. The most significant fibrous components of the interstitial matrix are the collagens: collagen comprises roughly 90% of the protein mass of the ECM. Local fibroblasts secrete collagen VI into the interstitium [28], where the triple-helical subunits form double-beaded collagen VI microfibrils by end-to-end association. Collagen VI in the interstitial matrix binds the muscle ECM into functional units and provides structure and stability.

Bundles of muscle fibers bounded by epimysia are then formed into fascicles, which are surrounded by the perimysium; this layer contains and organizes larger blood vessels and nerves as well as connecting to the interstitial matrix. The whole muscle is then surrounded by the epimysium, which broadens and flattens at the extremities of each muscle to form the myotendinous junction. The perimysium and epimysium differ from the endomysium in their composition (primarily collagens I and III rather than collagens IV and VI, and various complements of proteoglycans) and function; their specific role in preservation of...
muscle architecture and function is also somewhat less well defined [12]. The epimysium, perimysium and endomysium are linked by inter-connecting collagen fibers to evenly and effectively distribute contractile forces without damaging the muscle structure during movement [29].

As expected, loss or mutation of basal lamina or interstitial matrix components frequently leads to muscle pathologies [30]: ~50% of congenital muscular dystrophies are the result of deficiencies in laminin-2 (congenital muscular dystrophy type 1A; MDC1A), mutations in collagen IV have pleiotropic effects that include some forms of myopathy, and defects in collagen VI lead to Ullrich myopathy (the second most frequent form of congenital muscular dystrophy) and Bethlem myopathy [31]. While myopathies caused by defects in the structure of the endomysium itself are the most common in terms of patient numbers, disruptions in the connectivity between the myofiber cytoskeleton and the endomysium, including Duchenne’s muscular dystrophy, collectively represent a larger class of mutations. In all of these cases, a direct molecular connection to satellite-cell biology is unclear: structural proteins of mature muscle such as these are not expressed by undifferentiated satellite cells, but, as the cells responsible for muscle repair and homeostasis, they are necessarily affected by the chronic over-activation, inflammation and alteration of the muscle environment itself that accompany muscle disease. As described below, significant gaps remain in our understanding of what signals satellite cells receive from the muscle ECM and how they respond, even in healthy tissue; it is important to bear in mind that parallel studies on the signals prevalent in pathological muscle are also being performed, but are not discussed further here.

**Signaling to satellite cells by the extracellular matrix**

It has been argued that ECM signals are at least as important as soluble signals in regulating cellular determination, differentiation, proliferation, survival, polarity and migration [32]. In the case of satellite cell/ECM signaling interactions, there are published examples of regulation and presentation of ‘soluble’ factors by the ECM, specific adhesion signaling, and biophysical stress- or stiffness-induced signals that are each critical for satellite-cell activity and function. It is likely that not only do all three signaling modalities directly affect satellite-cell activity, but that they are cooperative and interactive.

The niche of the quiescent satellite cell comprises the sarcolemma of the host muscle fiber and the interior side of the basal lamina, thus any niche factors involved in maintenance of quiescence must derive from these two matrix sources: during quiescence, satellite cells are isolated electrically and chemically...
from both the myofiber cytoplasm and the extracellular environment [33]. Because of the difficulties in studying quiescent satellite cells in situ, comparatively little is known regarding the input of the niche to satellite-cell activity; however, it has been established that disruption of the matrix components of the quiescent niche negatively affect satellite-cell activity [34], while preservation of the niche in cell transplants dramatically enhances engraftment and function [35].

In contrast to the limited understanding of ECM signaling during quiescence, the critical role of the matrix in the earliest events of the satellite-cell response is well established. Initial activation of the satellite cell occurs downstream of physical stretch [36,37] or rupture of the myofiber membrane and basal lamina [38]. Nitric oxide synthase-1 is anchored to the myofiber sarcolemma by association with the dystrophin–glycoprotein complex, and has mechanosensory as well as enzymatic functions: following myofiber stretch, a bolus of nitric oxide (NO) is released locally [39]. This leads to release of the active form of hepatocyte growth factor/scatter factor (HGF) (which is itself sequestered within the ECM [40]), potentially through activity of the matrix metalloprotease MMP-2 [41,42]. HGF remains the only protein factor definitively shown to directly activate satellite cells from quiescence [43,44], thus these matrix-mediated events are critical to satellite-cell activity.

Once activated, satellite-cell proliferation and differentiation are modulated by multiple extracellular signaling pathways, the majority of which include a matrix component; however, this aspect of ‘soluble’ factor signaling is rarely taken into account in in vitro studies. For example, binding of fibroblast growth factors (FGFs) to their receptors requires specific sequences of heparan sulfates as components of the ternary complex [45], transforming growth factor-β ligands must bind to proteoglycans in the matrix to be ‘presented’ to their cellular receptors [46], and complexes of HGF with its receptor c-Met may include fibronectin or vitronectin as well as integrins [47]. In particular, a requirement for ligand binding and presentation by proteoglycans, in cis or in trans, introduces an additional level of specificity to the organism, and analytical difficulty for the researcher: carbohydrate chains are added and modified posttranslationally via multiple steps at various locations within the cell. The length, sequence, epimerization and sulfation pattern of the sugars comprising the carbohydrate chains of matrix proteoglycans are extremely dynamic [48], and are also highly specific in their capacity for interaction with growth factors such as FGF [49]. Thus, the co-localization of growth factor, receptor and proteoglycan does not necessarily, or even usually, imply either ligand–receptor binding or downstream signaling activity [50]. Proteoglycan side chains are by far the most complex biopolymer; however, current technology for analyzing even the sequence of carbohydrates lags dramatically behind that for DNA, RNA or protein [51]. Unfortunately, realistic integration of this aspect of satellite cell/ECM signaling in vivo is beyond the current capacity of the field. However, progress made primarily in vitro has identified roles for ECM molecules in directing the satellite-cell response to soluble growth factors. Work in the Brandan laboratory has identified roles for the ECM proteoglycans decorin, biglycan and dermanan sulfate in modulating the bioavailability and signaling potential of key growth factors, including FGF2 and HGF [52] and transforming growth factor-β [53]; other groups have identified similar effects on myostatin [54]. It is important to note that these interactions are with extracellular proteoglycans that presumably act in trans, unlike the interactions in cis that characterize cell-surface proteoglycans expressed by satellite cells themselves, such as syndecans and glypican [55–58].

In addition to ECM molecules produced by either muscle fibroblasts or differentiated myofibers, quiescent and activated satellite cells have themselves been shown to be a source of ECM components that affect multiple aspects of satellite-cell activity. Most recently, an increase in fibronectin expression by satellite cells immediately following activation has been implicated in active remodeling of the local ECM to promote self-renewal by facilitating Wnt7a binding to a complex of its receptor and the transmembrane heparan sulfate proteoglycan syndecan-4 [59].

Another modality of signaling from the ECM is via integrins, with or without coordinated signaling of a growth factor receptor. Adhesion signaling to myoblasts/myocytes has been shown to be necessary for myogenesis, particularly in the case of differentiation. Hauschka and Konigsberg were the first to demonstrate the requirement for a specific ECM factor (collagen I) for differentiation of chick myoblasts [60], and many studies since have highlighted the importance of both complete ECM and its components for differentiation of myogenic cell lines and primary myoblasts from developing chick and mouse [61–65]. Integrins are heterodimeric transmembrane adhesion receptors with specificity for various ECM components based on the identity of the α and β chains that they comprise [66]. While satellite cells appear transcriptionally competent to express nearly all known integrin chains [67], only a limited number of functional dimers have...
shown a biological activity or phenotype. Of these, the laminin receptor integrin α7β1 is the most prominent [11]. Integrin α7β1 is localized throughout the sarcolemma, myotendinous junctions and neuromuscular junctions of myofibers [68], where it mediates adhesion to laminin as well as interacting with the dystroglycan–glycoprotein complex, syndecans and sulfatides [11,30,69]. It is also considered a molecular marker for the satellite-cell population even in quiescence [70], where its primary role appears to be in migration [67,71,72] (discussed further below). Of the other integrins expressed by satellite cells and their progeny, most have not yet been correlated with a molecular function, although activity in proliferation, differentiation and fusion has been suggested. Making analysis more complicated is the key role that many of these same integrins play in the maintenance and function of differentiated muscle: not only do integrin α7β1 deficiencies result in myopathy [69], but changes in integrin α7β1 expression are associated with muscle pathologies caused by many other different mutations as well [73]. Unraveling the roles of specific integrins in vivo within a tissue in which myofibers and satellite cells co-exist and co-express the same integrins will most likely require cell type-specific genetic deletions and careful downstream analysis, as affected satellite cells will give rise to affected and/or chimeric myofibers.

An aspect of satellite cell/ECM interactions that has only recently been closely examined is the biophysical influence of substrate stiffness and organization on satellite-cell behavior. While physical stress is widely understood to influence muscle differentiation and organization, and the role of transient stretch in activation of satellite cells is well established (see discussion of nitric oxide synthase above), the effects of two-dimensional versus three-dimensional culture [74] and varying substrate rigidity on satellite-cell physiology are an emerging area of inquiry. Of particular interest, the Blau laboratory has recently described the effects of gradually altering substrate rigidity on multiple satellite-cell activities, including maintenance of stem cell potential and capacity for differentiation, using single satellite cells cultured on a wide array of poly(ethylene glycol) hydrogels [75,76]. As the great majority of in vitro studies are still performed in two dimensions on rigid surfaces that are either uncoated or coated with purified recombinant matrix factors, these new systems may serve to highlight areas where our current models of potential in vivo signaling events are incomplete or incorrect. This will be particularly important in understanding the differences in satellite-cell activity associated with either pathological or aged muscle tissue [77,78], as fibrosis and changes in matrix stiffness are characteristic of both of these conditions.

Experimental paradigms that are of increasing interest in establishing physiologically relevant biophysical influences on satellite-cell activity include simple three-dimensional culture systems using hydrogel or collagen, three-dimensional culture in reconstituted native ECM, and use of de-cellularized tissue that may be seeded with live cells. While all three of these methods are currently in use, there are significant drawbacks to each: inert or single-component hydrogels are easily created and manipulated, but necessarily lack the majority of ECM-derived signals discussed above, purified and re-aggregated muscle matrix lacks the ordered arrangement of matrix proteins as well as their carbohydrate side chains, and even de-cellularized muscle tissue implanted in vivo fails to provide an enhanced environment for myogenesis [79]. In this respect, the muscle research field lags behind studies of other tissues with regard to the ability to produce bioactive, biomimetic scaffolds for either research or clinical use, such as described for cartilage and bone [80–82]. For many of the reasons outlined here, further development of three-dimensional systems replicating both the biochemical and biomechanical properties of native muscle matrix will be a key step in developing both physiologically relevant in vitro systems and therapeutically useful clinical tools.

**Role of the extracellular matrix in satellite-cell motility**

As both the surface that motile cells adhere to and travel on and the substance that they must travel through, the various components of the ECM are critical influences on cell migration. Myogenic precursor cell motility is required for muscle development, during which migration from the somites to the presumptive muscle fields is dependent on signaling of HGF via c-met [83]. Members of the FGF and platelet-derived growth factor families have also been implicated in myoblast motility during development, and, as discussed above, all of these growth factors have requirements for matrix presentation and/or interaction to signal; however, the role of the matrix in embryonic muscle development has not been widely addressed. Thus, this area represents the increasingly rare case in which cellular and molecular mechanisms are better understood in the context of regeneration than development.

Once activated, satellite cells leave their quiescent position beneath the basal lamina, then proliferate and
migrate in the interstitial space before differentiating and fusing with a new or damaged myofiber (Fig. 2). This exit from the sub-laminar space appears to rely on physical force rather than protease activity to create a tear in the basal lamina, as it may be blocked by preventing adhesion to laminin in ex vivo fiber cultures using blocking antibodies but not by exposure to the broad-spectrum matrix metalloprotease inhibitor GM6001 (D.D.W. Cornelison, unpublished results). This results in both a change in the aspect of the basal lamina that the satellite cells are in contact with, and exposure to the interstitial matrix, with the accompanying potential for signaling. Laminin is the preferred substrate for satellite-cell motility in vitro [67,84], and engagement of laminin by α7β1 integrin is necessary for satellite-cell motility on the surface of the myofiber [67]. Once satellite cells have exited the niche and adhered to the exterior of the myofiber of the basal lamina, they are capable of extensive motility under ex vivo conditions: time-lapse observations have recorded cell velocities of up to 250 μm·h⁻¹. However, it is important to note that this does not completely or accurately mimic an in vivo situation, because the single myofiber system does not include the interstitial matrix through which cells are required to navigate in vivo, additional cells that are present in a regenerating muscle, or any matrix or soluble factors not derived from the host myofiber.

The fact that satellite cells can and do move both along and between myofibers during regeneration in vivo is well established [85,86]; however, the question of whether muscle precursor cell migration is necessary for regeneration in vivo (as it is in development) remains open. Although there are theoretically adequate local populations of satellite cells available to restore lost myonuclei without recruiting cells from more distal areas [87], it is possible that the regeneration response may be both accelerated and enhanced by mobilizing additional cells. A role for long-range motility of satellite cells, including recruitment of cells from distal uninjured muscle [88], was suggested based on early in vivo analyses of muscle regeneration. Evidence for long-distance activation and motility of satellite cells includes: detection of BrdU-positive satellite cells in uninjured areas of the tibialis anterior (TA) distal to a needle injury [89], and movement of satellite cells from distal uninjured areas towards a focal crush site [90]. In addition, after free grafting of large muscles, satellite cells migrate from the central necrotic areas towards the periphery [91] then back again after revascularization [92], and have been observed to migrate from the viable half of a longitudinally split autograft into the killed half [93].

Several groups have described soluble factors that promote satellite-cell motility and migration in vitro [67]. Of these, the small chemokine SDF-1 is particularly intriguing: a key mediator of stem cell homing in several other tissues [94-96], it is expressed following injury by muscle-derived fibroblasts and is chemotactic to satellite cells [97]. The transmembrane heparan sulfate proteoglycan syndecan-4, which is expressed by satellite cells during both quiescence and activation [58], is the obligate co-receptor for SDF-1 together with CXCR4 [98], which is also a marker for satellite cells [70]; all three components of the ternary complex are implicated in satellite-cell motility [99], donor cell engraftment [100] and enhancement of muscle regeneration in vivo [101]. HGF may also play a role in addition to initial activation: it has been characterized as a potent motogen in many systems, including both developing and adult

![Fig. 2. An activated satellite cell (identified by CD34 staining, red) on an isolated mouse myofiber 18 h post-isolation; the myofiber basal lamina is stained for laminin (green). Left, schematic representation of the local matrix interactions during multiple phases of satellite-cell activity in vivo.](image)
muscle [83,102], once having been named ‘scatter factor’ on the basis of this activity [103]. It stimulates cell motility in developmental and physiologically normal contexts as well as being one of the primary signaling pathways hijacked during tumorigenesis and metastasis [104,105]. This makes the HGF/c-Met axis a frequent target for therapeutic down-regulation or inhibition [106], and many tools have been developed in the context of tumor therapy that may also be useful for evaluating the role and requirement for HGF in satellite-cell biology and motility.

The requirement for digestion and remodeling of the interstitial matrix to facilitate satellite-cell migration in vivo is suggested by experiments in which matrix-modifying enzymes such as urokinase plasminogen activator [107], matrix metalloproteases [108–113] or their inhibitors [114,115] are experimentally manipulated to achieve a positive effect on satellite-cell spread and/or muscle regeneration. This is consistent with the idea that mobilization and re-localization of satellite cells is either beneficial or necessary for successful muscle regeneration. However, while these results are promising, the overall mechanism(s) by which matrix-modifying enzymes act to enhance satellite-cell motility or activity remain elusive: indeed, the question of whether matrix metalloprotease activity should be enhanced or diminished to improve the outcome is still highly dependent on the context of the in vivo experiment and the particular enzyme being studied. Much more work focusing on both endogenously released soluble motogens and chemotactants and matrix remodeling enzymes is therefore required before a definitive role for satellite-cell migration may be determined. However, even in the absence of such a role during normal muscle regeneration, the difficulty in achieving adequate spread of therapeutically engrafted satellite cells or their progeny [116] makes a better understanding of local factors affecting satellite-cell movement through the muscle tissue an important goal.

**Signposts?**

While it was not the authors’ intent at the outset, a primary theme that emerged in writing this review is the emphasis of areas in which understanding the matrix’s place in muscle regeneration is incomplete, potentially inaccurate, or currently impossible. Although the ECM clearly plays multiple critical roles in satellite cell-mediated myogenesis, its influence is rarely taken into account in in vitro studies, and is somewhat cryptic in many in vivo situations. While mimicking the properties of the muscle ECM is difficult, it should not be impossible: advances in de-cellularized organs [81,117], purification of native muscle matrix for seeding myoblasts [61,118], and three-dimensional biomaterials-based culture systems [74,75] are promising developments that will ideally soon resolve many of our unanswered questions, and suggest new ones!

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**References**

Matrix influences on muscle regeneration


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