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# The Extracellular Matrix Complexome from Skeletal Muscle

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<http://dx.doi.org/10.5772/62253>

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## Abstract

The various layers of the extracellular matrix, forming the endomysium, perimysium and epimysium of skeletal muscles, provide essential structural and mechanical support to contractile fibres. Crucial aspects of muscle elasticity and fibre contractility are dependent on proper cell–matrix interactions. A complex network of collagen fibres, non-fibrillar collagens, proteoglycans, matricellular proteins, matrix metalloproteinases, adhesion receptors and signalling molecules maintain the physical structure for force transmission within motor units, embed critical cellular structures such as capillaries and motor neurons, and enable essential sarcolemma-matrix adhesion processes and signalling cascades. The systems biological concept of protein complexomes, which assumes the existence of interconnectivities between large protein assemblies, can be readily applied to the proteins within the extracellular space of muscles. Recent proteomic studies confirm that the extracellular matrix complexome has considerable influence on the integrity and cellular functions of skeletal muscle fibres. Adaptations or changes in the organization of the extracellular matrix play a crucial role during fibre regeneration following injury, extensive neuromuscular activity or pathophysiological insults. This chapter outlines the molecular components of the matrixome from skeletal muscles and discusses the extracellular matrix in relation to myogenesis, maturation of motor units, adaptation to changed functional demands and myofibrosis in muscular disorders.

**Keywords:** collagen, fibrosis, matricellular, matrixome, proteoglycan

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## 1. Introduction

In addition to the universal physiological, metabolic and regulatory challenges of almost all cellular entities in the body, muscle tissues have to constantly adapt to a variety of biological

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issues related to high energy demand, elevated levels of cellular stress and enormous physical strains during excitation–contraction–relaxation cycles [1]. The survival of skeletal muscle fibres therefore depends heavily on (i) a high degree of physiological adaptability, (ii) a unique level of tissue plasticity, (iii) efficient molecular chaperoning to prevent proteotoxic insults and (iv) a sophisticated repair machinery that can counter-act frequent cellular injuries [2]. A crucial stabilizing element that is intrinsically involved in this continuous maintenance of contractile tissues is the extracellular matrix (ECM) [3]. On the one hand, the complex layers of the muscle ECM provide the physical structure for force transmission between contracting fibres and their surrounding tissue environment [4], and on the other hand the ECM functions as an embedding medium for essential supportive components of muscles such as capillaries and motor neurons [5].

The composition and organization of the ECM adapts considerably in response to changed functional or structural demands during myogenesis, fibre maturation and exercise-induced changes [6]. During the natural aging process and in association with a variety of muscular disorders, a hyperactive connective tissue may trigger myofibrosis with a detrimental impact on muscle elasticity and fibre contractility [7]. Physiological or pathological changes in the muscle ECM frequently mirror the different phases of altered muscle structure and function [8]. The main component of the ECM is represented by collagen, which exists in a large number of isoforms that connect with proteoglycans, matricellular proteins and adhesion receptors to form an elaborate extracellular network and tight cell–matrix interactions [9].

This chapter provides an overview of the molecular components of the ECM from skeletal muscle and describes the proteomic concept of the ECM complexome. The formation, maturation and flexibility within the various layers of the ECM in developing, maturing and adapting skeletal muscles is outlined, as well as the crucial role of myofibrosis in neuromuscular pathology.

## 2. Molecular and cellular structure of the extracellular matrix

In skeletal muscles, the ECM is involved in a variety of processes during development, contractile maturation, fibre regeneration following injury, physiological adaptations to changed functional demands and the natural aging process [8]. As outlined in **Figure 1**, the ECM is a highly dynamic non-cellular system that undergoes frequent cycles of modifications, degradation and reassembly. The muscle ECM functions on the one hand as an embedding and stabilizing structural support and on the other hand as a cellular interaction and signalling medium. The molecular lattice of collagens and proteoglycans with its associated matricellular proteins, enzyme systems and adhesion receptors mediates various physiological and biochemical mechanisms, including (i) the overall maintenance of muscle tissue stability and elasticity, (ii) the mechanical transduction of force from the contractile fibres to their anchoring tissues, (iii) cytoskeletal coupling to enable the efficient execution of frequent excitation–contraction–relaxation cycles, (iv) the provision of signalling pathways at the fibre periphery, (v) the preservation of neuromuscular homeostasis, and (vi) the physical scaffold and embed-

ding medium of cellular constituents, such as contractile fibres, capillaries, motor neurons and satellite cell pools. Cell–matrix interactions are of central importance during cell adhesion and cell migration and thus essential during both embryonic and adult myogenesis.

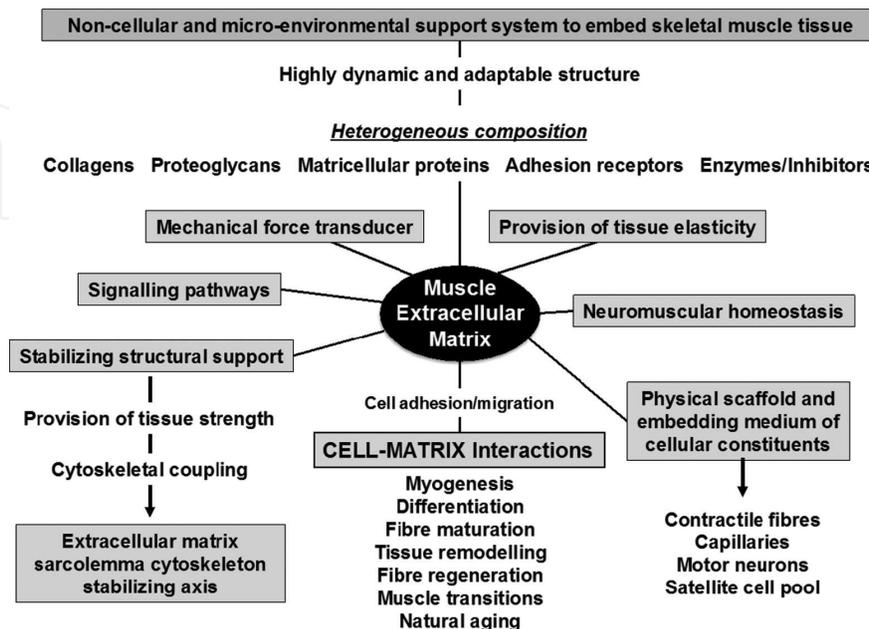
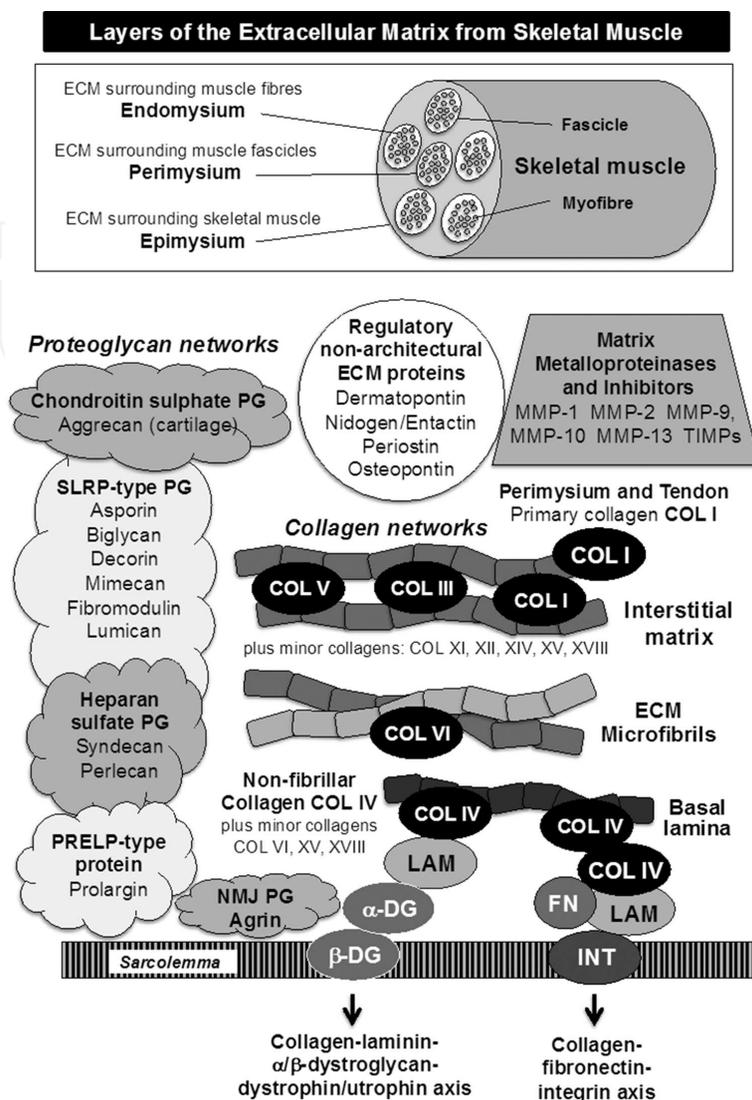


Figure 1. Overview of the complex functions and interactions of the extracellular matrix from skeletal muscles.

The distinct layers of the ECM that surround muscle fibres, muscle fascicles and the entire skeletal muscle are formed by the endomysium, perimysium and epimysium, respectively [3]. Figure 2 shows diagrammatically the arrangements of the ECM from skeletal muscles and lists the main molecular constituents, including various isoforms of collagen, proteoglycans, matricellular proteins, crosslinking proteins and matrix metalloproteinases. Crucial adhesion systems that maintain sarcolemma–matrix interactions are marked. They include the collagen–laminin- $\alpha/\beta$ -dystroglycan-dystrophin/utrophin axis and the collagen–fibronectin–integrin axis that link the basal lamina via the plasmalemma to the underlying membrane cytoskeleton [10].

The stabilizing linkage between the outside and inside of muscle cells, provided by the ECM–sarcolemma–cytoskeleton axis, is of critical importance for maintaining normal contractile functions [8]. Primary or secondary abnormalities in individual binding partners of these surface complexes may result in severe neuromuscular disorders, as discussed in below section on the role of the ECM in skeletal muscle pathology and myofibrosis.

Collagens form tight helical structures and function as the main structural protein species in the extracellular space. Muscle-associated collagens are highly abundant in the interstitial matrix, ECM microfibrils and the basal lamina [11]. Collagen isoform COL I, the most abundant protein in the mammalian body [12], is the primary collagen in the perimysium and tendon. The interstitial matrix contains mostly collagen COL I, COL III and COL V. Minor types of



**Figure 2** Diagrammatic presentation of the various layers of the extracellular matrix (ECM) from skeletal muscle and an overview of the molecular components that form the interstitial matrix, microfibrils and the basal lamina. Abbreviations: COL, collagen; DG, dystroglycan; FN, fibronectin; LAM, laminin; INT, integrin; MMP, matrix metalloproteinase; NMJ, neuromuscular junction; PG, proteoglycan; PRELP, proline-arginine-rich end leucine-rich repeat protein; SLRP, small leucine-rich repeat proteoglycans; TIMP, tissue inhibitors of matrix metalloproteinases.

collagens in this extracellular region are COL XI, XII, XIV, XV and XVIII and are mostly expressed during muscle development [3]. The main structural constituent in ECM microfibrils is collagen isoform COL VI. Microfibrils provide structural support during the physical strains of continuing contraction–relaxation cycles. Directly overlaying the sarcolemma membrane is the basal lamina consisting of the main non-fibrillar collagen isoform COL IV and a few minor constituents, including COL VI, XV and XVIII. The collagen network of the basement membrane interacts with two crucial plasmalemma adhesion complexes, the integrin complex and the dystrophin–glycoprotein complex [10]. These sarcolemma-bridging protein assemblies form the laminin–dystroglycan axis [13] and the fibronectin–integrin ( $\alpha7\beta1$ ) axis [14], whereby

the ECM glycoprotein fibronectin mediates the connection between laminin-211 ( $\alpha 2\beta 1\gamma 1$  merosin) and collagen COL IV [15].

A variety of regulatory ECM proteins are involved in matrix assembly and the modulation of cell–matrix interactions, such as dermatopontin, nidogen/entactin, periostin (osteoblast-specific factor OSF-2) and osteopontin [16–18]. Matricellular proteins represent non-architectural ECM components and are crucial factors during muscle development and fibre repair. Matrix metalloproteinases are an important class of ECM-associated enzymes that regulate the degradation of ECM proteins and support tissue integrity during phases of collagen deposition and muscle regeneration [19]. The main isoforms present in skeletal muscles are matrix metalloproteinases MMP-1, MMP-2, MMP-9, MMP-10 and MMP-13 [20,21]. The tissue inhibitors of matrix metalloproteinases, named TIMP, are endogenous regulatory factors involved in the formation, adaptation and controlled degradation of the ECM [22]. TIMP molecules play a crucial role in the migration and differentiation of muscle stem cells during regeneration following cellular injury [19].

A large variety of proteoglycans fill the gaps between collagen molecules and thereby form an integral part of the highly complex ECM structure. Proteoglycan molecules are highly glycosylated with glycosaminoglycans at multiple sites along the peptide backbone [23]. Skeletal muscles contain small leucine-rich repeat proteoglycans (SLRP), heparan sulfate proteoglycans and chondroitin sulphate proteoglycans. Muscle-associated proteoglycans of the type SLRP are asporin, biglycan, decorin, mimecan (osteoglycin), fibromodulin and lumican. Asporin is mostly found in the cartilage matrix. Biglycan is a small SLRP-type proteoglycan that interacts with  $\alpha$ -sarcoglycan and  $\gamma$ -sarcoglycan of the dystrophin-glycoprotein complex [24]. Decorin is the primary proteoglycan molecule of the perimysium and tendon structures [25]. Fibromodulin is involved in collagen fibril formation, which is illustrated by the biomedical fact that fibromodulin-deficient tendons exhibit abnormal collagen fibrils [26]. The heparan sulfate proteoglycan syndecan is transiently up-regulated during tissue differentiation and is involved in stem cell maintenance and muscle regeneration [27]. Perlecan is located to the basement membrane and its expression is also transiently increased during muscle differentiation [28]. The chondroitin sulphate proteoglycan named aggrecan forms large aggregates in cartilage [29]. A proline-arginine-rich end leucine-rich repeat protein (PRELP) is presented by prolargin of the basal lamina. At the highly differentiated neuromuscular junction region, the large proteoglycan molecule agrin is present and forms via  $\alpha$ -dystroglycan a tight linkage to the utrophin-glycoprotein complex. Agrin is essential for the normal development of the neuromuscular junction and agrin-induced clustering processes are crucial for the anchoring of the acetylcholine receptor complex in the junctional folds [30].

### **3. Skeletal muscle development and the extracellular matrix**

Skeletal muscle fibres derive from the mesoderm [31] and represent one of the most abundant cell types in the body. Myofibres play a key physiological role in the provision and regulation of locomotion, breathing, postural control, heat homeostasis and metabolic integration [32–

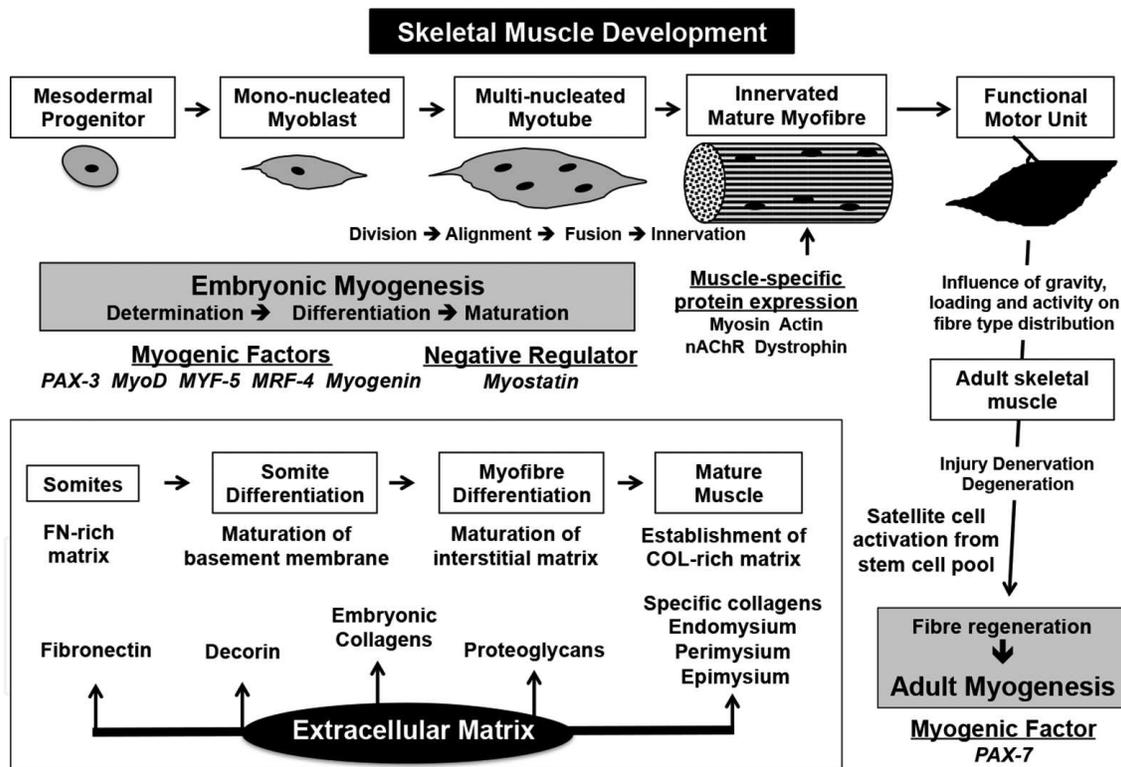
34]. The development of contractile fibres is a highly complex process and changes in the ECM play an essential role during myogenesis. Adaptations occur at the level of the basal lamina, the interstitial ECM and the collagen-rich tendon and encompass a variety of fundamental processes of development, such as the determination of cell fate, as well as proliferation, cell division, patterning and tissue transitions [35]. The most critical developmental processes occur during the initial activation of precursor cells, various differentiation steps and the final maturation of innervated myofibres. These major developmental mechanisms are regulated by a large number of genetic and signalling factors [36]. Many specialized extracellular components are involved in muscle development, which is reflected by the transient expression patterns of certain ECM molecules, such as the minor collagen isoforms COL XI, XII, XIV, XV and XVIII [3].

The molecular and cellular events that occur during the establishment of the myogenic cell lineage and resulting formation of multi-nucleated contractile fibres entail initially a fibrillar and fibronectin-rich matrix. In developmental terms, almost all myofibres of the skeletal musculature derive from mesodermal structures named somites that develop during early embryonic segmentation on both sides of the neural tube [37–39]. The developing ECM of somites is represented by a fibronectin core, a basement membrane and an outer fibronectin-containing matrix [35]. **Figure 3** outlines the initiation and control of embryonic and adult myogenesis by myogenic factors and through a complex series of spatio-temporal dependent signalling cascades. Fibronectin and its interactions with the integrin complex play a central role in polarizing and guiding somitic cells [40–42] and the ECM is crucial for somite formation and as a guiding cue during morphogenesis [43–45]. The small SLRP-type proteoglycan decorin was shown to be majorly involved in skeletal muscle development by promoting proliferation and differentiation of muscle cells through suppressing myostatin activity [46–48].

The key regulator of early myogenesis that initiates the developmental commitment into the myogenic cell lineage is the paired-type homeobox gene *PAX3* [49–51]. Interestingly, another member of the PAX transcription factor family, the *PAX3* orthologue *PAX7*, acts as a regulator of mature skeletal muscle regeneration and postnatal growth mechanisms [52]. Following the induction of mesodermal precursors, the subsequent segmentation into somites and the formation of the primary myotome involves a variety of signalling molecules and transcription factors, such as the secreted and lipid-modified family of Wnt-glycoproteins and the large group of myogenic basic helix-loop-helix muscle regulatory factors, such as MyoD, Myf5, MRF4 and myogenin [53–55]. Myogenic factors act at multiple regulatory points during embryonic myogenesis, whereby the overall genetic pathway that is responsible for the transcriptional activation of skeletal muscle-specific genes is highly complex and partially redundant [56]. A key step during muscle development is the fusion of myogenic cells that result in the formation of innervated and multi-nucleated myofibres (**Figure 3**). Although specific regulatory processes differ between embryonic, foetal, postnatal and mature regenerative myogenesis [57], the basic biological mechanisms of skeletal muscle development and injury-related adult muscle regeneration are extraordinarily similar [58,59]. A large pool of satellite cells provides a high level of regenerative capacity of the matured post-mitotic fibres

and is located between the sarcolemma and the basal lamina [60]. These mono-nucleated myogenic stem cells are activated during cycles of fibre regeneration and cellular maintenance. To provide maximum skeletal muscle performance, activated stem cells undergo a complex pattern of proliferation, differentiation and cellular fusion to form multi-nucleated and functional contractile myofibres [61]. Following maturation, the tissue mass of skeletal muscles is then regulated by catabolic and anabolic mechanisms that include signalling factors such as NF- $\kappa$ B and FoxO, as well as the mTOR pathway [62].

The systematic profiling of myogenesis using mass spectrometry-based proteomics has covered postnatal growth and development [63,64], but focused mostly on studying cell culture models [65–67] and specifically the skeletal muscle secretome during myoblast differentiation and myotube formation [68–72]. The concept that skeletal muscle cells act as secretory tissues has recently been reviewed by Pedersen [73]. Secreted myokines probably exert autocrine, paracrine or endocrine effects within the neuromuscular system and also in relation to other organ systems [74]. The skeletal muscle secretome is estimated to consist of several hundred muscle-derived peptides and proteins [75]. During muscle development,



**Figure 3.** Outline of the initiation and control of embryonic and adult myogenesis. Shown is the transition from mesodermal progenitor cells to myoblasts, followed by cellular fusion to produce multi-nucleated myotubes and finally innervated and functional myofibres. The critical role of stem cells during both embryonic development and regeneration of injured adult muscle fibres is highlighted, as well as changes in the ECM. Fibronectin (FN) and its interactions with the integrin complex are of central importance for polarizing and guiding somitic cells and the proteoglycan decorin is majorly involved in myogenesis by promoting proliferation and differentiation through suppressing myostatin activity. Changes in the ECM and the formation of a collagen (COL) matrix during muscle development are marked.

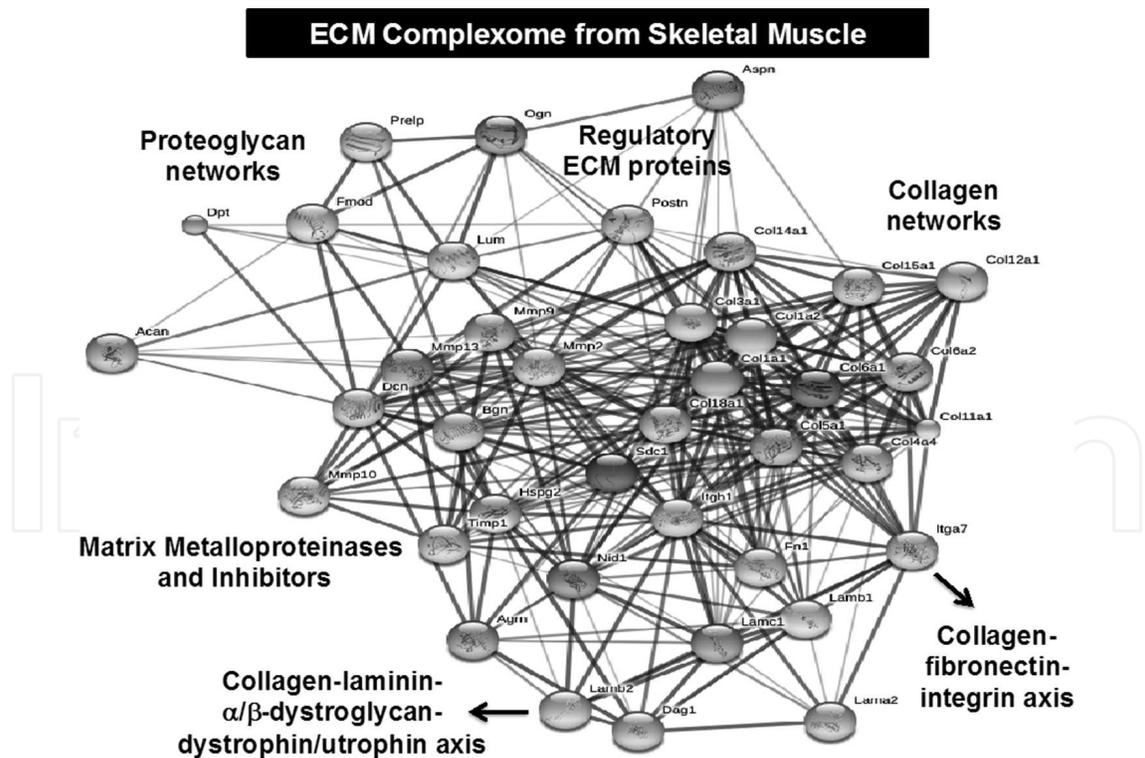
changes in ECM proteins have recently been studied in relation to human myogenesis and established that altered protein expression underlies the dramatic phenotypic conversion of primary mono-nucleated muscle cells during differentiation to form multi-nucleated myotubes [76]. The temporal profiling of the human myoblast proteome during *in vitro* differentiation highlighted the importance of ECM rearrangement during early myogenesis and showed a drastic increase in key ECM components, including several  $\alpha$ -isoforms of collagen COL VI and COL XVIII, as well as the heparan sulfate proteoglycan HSPG2 of the basement membrane, the elastin–microfibril interface–located ECM glycoprotein EMILIN2 and nidogen isoform NID2 [76]. These findings confirm the developmental concept that enhanced synthesis of ECM proteins occurs during the transition from myoblasts to syncytial myotubes [72] and that complex interactions at the cell–ECM interface facilitate the fusion of myoblasts [35].

#### 4. Mature fibres, skeletal muscle plasticity and the extracellular matrix

Both, proteomic cataloguing studies of various skeletal muscle specimens [77–82] and the comparative expression profiling of crude skeletal muscle preparations or subcellular fractions [83–86] routinely identify ECM proteins that form the core complexes of the basal lamina, microfibrils and interstitial matrix [87]. **Figure 4** shows a bioinformatics STRING [88] map of core ECM components from skeletal muscles. In mature skeletal muscles, frequently identified ECM molecules include collagens of the interstitial matrix (COL I, COL III and COL V), the microfibrillar collagen isoform COL VI, the non-fibrillar collagen isoform COL IV of the basement membrane, components of cell–ECM adhesion complexes (laminin, fibronectin, integrins, dystrophin, utrophin), regulatory ECM proteins (dermatopontin, nidogen, periostin and osteopontin), SLRP-type proteoglycans (asporin, biglycan, decorin, mimecan, fibromodulin and lumican), heparan sulfate proteoglycans (syndecan and perlecan), the chondroitin sulphate proteoglycan aggrecan, the PRELP-type proteoglycan prolargin and the neuromuscular junction-specific proteoglycan agrin, as well as matrix metalloproteinases and their inhibitors (MMP-1, MMP-2, MMP-9, MMP-10, MMP-13 and TIMPs) [77–86]. **Figure 4** includes the protein products of the following genes: *BGN, SDC1, HSPG2, AGRN, FN1, DAG1, ACAN, DPT, POSTN, MMP1, MMP2, MMP9, MMP10, MMP13, TIMP1, COL1A1, COL1A2, COL6A1, COL6A2, COL3A1, COL5A1, COL4A4, LAMA2, LAMB1, LAMB2, LAMC1, NID1, ASPN, PRELP, PGS2, LUM, OGN, FMOD, ITGA7, ITGB1*. This encompasses essential members of the various collagen networks found in the basement membrane, the microfibrillar structures and the interstitial matrix. In addition, major proteoglycans, matricellular proteins and matrix metalloproteinases are shown, as well as the interaction sites between sarcolemmal adhesion receptor complexes and the ECM.

Individual skeletal muscles are characterized by their fibre type distribution pattern whereby the proportion of fast-twitching fibres, slow-twitching fibres and hybrid fibres is highly adaptable and changes according to specific physiological, biochemical and/or metabolic demands [89]. Alterations in physical activity affect the molecular and cellular composition of the neuromuscular system, including hypertrophy, i.e. the increase in fibre size and hyperplasia, i.e. the increase in fibre number [90]. The plasticity of the neuromuscular system is a

well-established physiological concept. Endurance training is associated with an increased aerobic capacity and elevated utilization of fatty acid oxidation [91]. In contrast, sprint training triggers higher activities of the glycolytic and phosphocreatine pathways and enhances carbohydrate metabolism [92]. Prior to systematic proteomic studies, a large number of biochemical, cell biological and physiological studies have established ECM changes in response to exercise [93–95], including collagens, adhesion receptors, growth factors, matrix proteins and matrix metalloproteinases [96–100]. Neuromuscular unloading clearly depresses collagen COL I and COL III production and reloading enhances collagen expression in fast muscles [101]. The mass spectrometric analysis of exercise indicates proteome-wide changes in the graded response of skeletal muscles to physical exercise using different training regimes [102,103]. While moderate-intensity exercise causes a shift to a more fatigue-resistant and a slower-contracting skeletal muscle phenotype, interval-exercise training is associated with changes in post-translational modifications of metabolic enzymes [104–106]. The proteomic analysis of skeletal muscle plasticity in relation to acute versus chronic exercise was recently determined using human *vastus lateralis* muscle biopsy specimens and label-free LC-MS/MS analysis [107]. While structural and mitochondrial proteins were shown to be increased after long-term exercise, components related to energy metabolism were decreased following short-term exercise. Moderate ECM changes were described for several  $\alpha$ -chains of collagen VI, fibronectin and decorin [107].



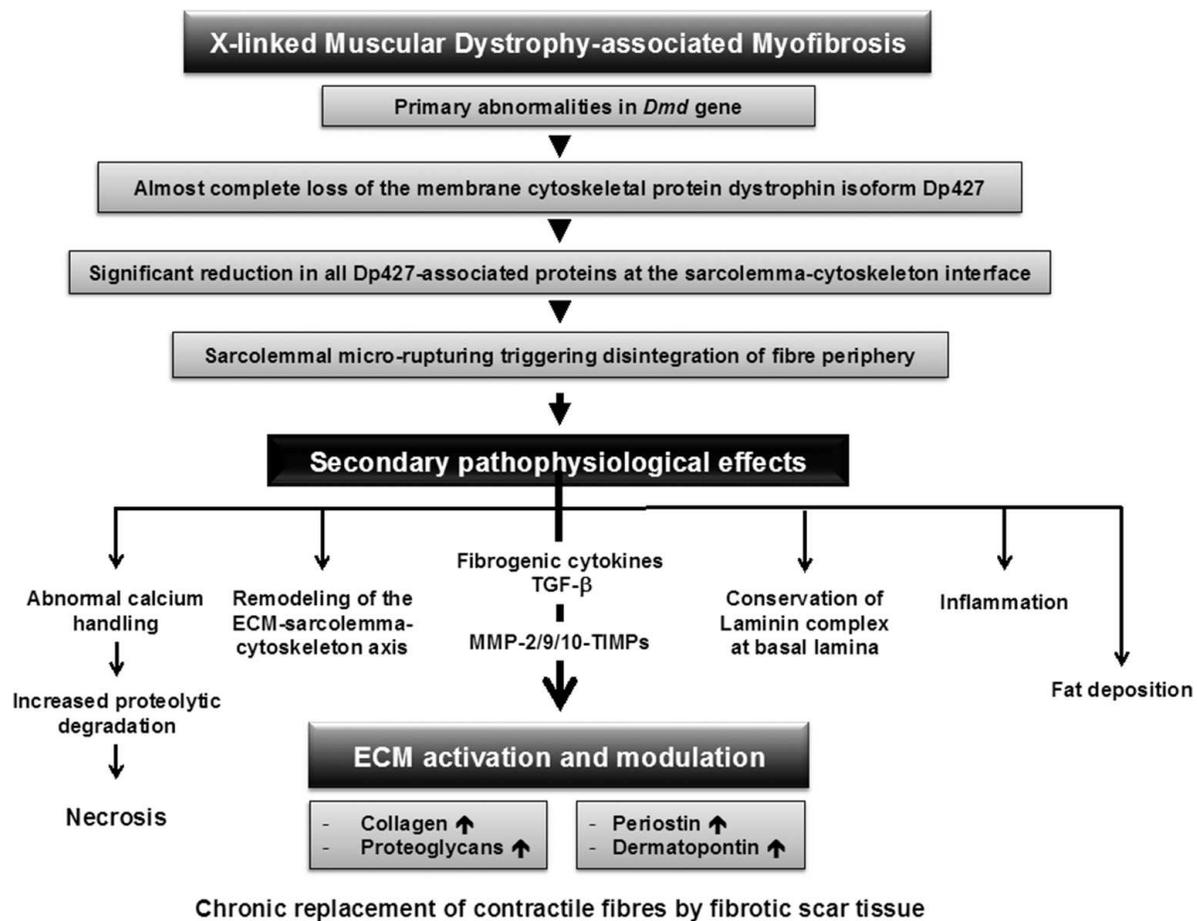
**Figure 4.** Bioinformatics STRING map of major components that form the core of the extracellular matrix (ECM) complexome. Shown are key proteins belonging to the collagen network of the basal lamina, microfibrils and the interstitial matrix, proteoglycans, matrix proteins and matrix metalloproteinases. The interaction sites of the ECM with sarcolemmal adhesion receptor complexes are shown.

An interesting non-physiological system is presented by external chronic low-frequency stimulation of fast muscles. This electro-stimulation method causes the complete activation of all affected motor units to a maximum extent [108]. During fast-to-slow transitions, skeletal muscles show a remarkable adaptation and transform physiologically and biochemically into motor units with an improved resistance to fatigue [109]. Chronic low-frequency stimulated fast muscles are characterized by decreased fibre calibres, an increase in the time-to-peak twitch tension, an increase in half-relaxation time and a significant elevation of aerobic-oxidative capacity [110]. The proteomic analysis of continuous electro-stimulation at 10 Hz has demonstrated complex biochemical changes with a significant shift from glycolytic to more aerobic-oxidative metabolism [111,112]. The ECM of transforming skeletal muscle undergoes distinct changes and exhibits increased collagen levels [113,114]. In the stimulated *latissimus dorsi* model for testing the suitability of dynamic cardiomyoplasty to treat heart failure, the collagen content was shown to be significantly elevated in the paced muscle. Although the chronically electro-stimulated muscle increased the level of fatigue resistance, distal regions of the paced *latissimus dorsi* muscle were characterized by muscular atrophy and myofibrosis [114].

## 5. Neuromuscular disorders and the extracellular matrix

A general myopathological parameter of a variety of acquired and inherited muscle diseases [115], as well as the gradual loss of contractile strength during the natural aging process [116], is the progressive accumulation of ECM components, especially collagens [7]. Inflammatory processes and tissue infiltration often accompany the loss of skeletal muscle fibres. Increased levels of non-contractile entities, such as fibrous connective and fatty tissue, within the neuromuscular system are a key pathological factor in the dysregulation of skeletal muscle function. Myofibrosis often correlates with poor motor outcome in neuromuscular disorders, such as the progressive loss of muscle strength and concomitant endomysial changes in the X-linked inherited disorder Duchenne muscular dystrophy [117]. In muscle pathology, changes in ECM components can be differentiated as being a consequence of a primary defect in the matrix of muscles, such as the ECM diseases Collagen IV myopathy [118,119] and LAMA2-related congenital myopathy [120,121], or a secondary response in the form of reactive myofibrosis, as is seen in dystrophinopathies [122,123].

The systematic profiling of changes in ECM components in Collagen IV myopathy and X-linked muscular dystrophy has resulted in interesting new findings in relation to primary ECM defects versus reactive fibrosis. Mutations in the genes encoding collagen isoform COL VI are the underlying cause of the severe UCMD type of Ullrich congenital muscular dystrophy and the milder BM type of Bethlem myopathy. Both disorders are characterized by skeletal muscle wasting, cycles of cellular degeneration and regeneration, and the substitution of contractile fibres with fat and connective tissue [119]. The cell biological and proteomic analysis of mouse models and biopsy material from patients afflicted with Collagen IV myopathy revealed metabolic dysregulation, enhanced cellular stress, autophagic impairment and alterations in mechano-transduction signalling pathways [124,125]. In the case of X-linked muscular dystrophy, a large number of proteomic studies have surveyed secondary changes down-



**Figure 5.** Overview of reactive myofibrosis in X-linked muscular dystrophy. The transforming growth factor TGF- $\beta$  and the activity of matrix metalloproteinases (MMP) and tissue inhibitors (TIMPs) play a crucial role in ECM activation and modulation, which is characterized by increased levels of various collagens, proteoglycans, dermatopontin and periostin. Progressive ECM accumulation triggers a chronic replacement of muscle fibres by fibrotic scar tissue leading to a loss of muscle elasticity and contractile strength.

stream of the primary abnormality in the membrane cytoskeletal protein dystrophin [126]. Muscular dystrophy-related changes affect energy metabolism, cellular signalling, the excitation–contraction–relaxation cycle, the stress response, the cytoskeletal network and the ECM [127]. The recent proteomic profiling of established genetic animal models of dystrophinopathy has revealed a drastic increase in various collagens, proteoglycans and matricellular proteins [83–86,128,129]. **Figure 5** outlines the histo- and pathobiochemical consequences of muscular changes and a hyperactive connective tissue in dystrophin-deficient skeletal muscles.

The simultaneous mass spectrometric analysis of dystrophin isoform Dp427 and collagen in moderately dystrophic *mdx-4cv* leg muscles revealed significant increases in collagens and associated ECM proteins, such as fibronectin, biglycan, asporin, decorin, prolargin, mimecan and lumican [85]. The pathoproteomic signature of the severely dystrophic *mdx-4cv* diaphragm included a significant increase in collagens and the related ECM proteins asporin, decorin,

dermatopontin, prolargin and periostin [83]. Especially interesting was the proteomic identification of dermatopontin and periostin [83,128,129]. Dermatopontin, also named tyrosine-rich acidic matrix protein TRAMP [130], is involved in matrix assembly and cell–matrix interactions [17] via interactions with decorin, TGF- $\beta$  and fibronectin [131]. High levels of dermatopontin in the dystrophic *mdx* diaphragm most likely reflect an increased demand for collagen matrix organization within Dp427-deficient fibres [128]. Periostin is a crucial matricellular protein of 93 kDa [18] that is involved in the regulation of the biomechanical properties of connective tissues and collagen fibrillogenesis [132]. Normally periostin is only temporally expressed in the muscle ECM during cellular differentiation and regeneration processes [133], making its drastic up-regulation a characteristic feature of dystrophic muscles [83,86]. Of diagnostic and therapeutic importance is the fact that muscle biopsies from Duchenne patients exhibit an elevated concentration of periostin and that the deletion of periostin clearly reduces dystrophic symptoms and myofibrosis in mice by modulating the TGF- $\beta$  pathway [134]. Interestingly, laminin-deficient muscular dystrophy also shows dysregulation of matricellular proteins as an early pathophysiological feature [120,135]. Therefore altered levels of periostin and related matricellular proteins are good biomarker candidates for the characterization of myofibrosis in relation to inherited muscular dystrophies [136]. Although the natural aging process is also associated with increased collagen levels [116], which were also shown by proteomics [137], the collagen accumulation is much less pronounced in senescent muscles as compared to muscular dystrophy.

## 6. Conclusions

In conclusion, a dynamic balance exists within the ECM system from skeletal muscles. Highly regulated cycles of protein deposition, accumulation, remodelling and degradation occur in response to development, fibre transformation, neuromuscular loading, mechanical unloading, disease processes or aging. The main components of the ECM are represented by collagen fibres, non-fibrillar collagens, matricellular proteins, proteoglycans, matrix metalloproteinases, signalling molecules and adhesion complexes. The muscle ECM forms a structural scaffold that plays a central role in the maintenance of the physical structure of motor units and provides the framework for force transmission. The ECM is also involved in signalling cascades and adhesion processes at the sarcolemma–matrix interface. The systematic mass spectrometry-based proteomic analysis of the muscle ECM has established an enormous complexity and interconnectivity of matrix proteins and confirmed the dynamic nature of collagen networks and its associated proteoglycans in health and disease. Swift adaptations or alterations in the arrangement of the ECM have been established to occur during myogenesis, fibre regeneration, increased neuromuscular activity or pathological muscle wasting. Hence, concentration changes in ECM proteins are useful indicators for studying basic cell biological or pathophysiological processes in skeletal muscles. In the future, distinct ECM molecules may be useful for designing improved diagnostic, prognostic or therapy-monitoring approaches to study neuromuscular alterations.

## Acknowledgements

Research in the author's laboratory was supported by project grants from the Deutsche Duchenne Stiftung *aktion benni & co e.V.*, Muscular Dystrophy Ireland and the Hume Scholarship programme of Maynooth University, as well as the Irish Higher Education Authority (HEA). The Programme for Research in Third Level Institutions PRTL Cycle 5 is co-funded by the Irish Government and the European Union under Ireland's EU Structural Funds Programme 2007-2013. We thank Prof. Dieter Swandulla (University of Bonn), Prof. Heinrich Brinkmeier (University of Greifswald) and Dr. Paula Meleady (Dublin City University) for their continued support of our studies into the mechanisms of muscle fibrosis.

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