Review Article

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Decellularized extracellular matrix as a promising biomaterial for musculoskeletal tissue regeneration

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Abstract: The emergence of tissue engineering provides an alternative therapeutic strategy for various regeneration. It is the crucial step for choosing an ideal scaffold to support the cellular behaviors of various functional cells. Various biomaterials have been found or synthesized and applied to tissue repair. Among these biomaterials, as a natural-derived material, decellularized extracellular matrix (dECM) derived from cells, tissues, and organs is attracting more and more interest due to its good biocompatibility, biodegradability, and the ability to mimic a microenvironment similar to extracellular matrix. More and more researchers utilized dECM derived from cells, tissues, and organs to fabricate tissue-engineered scaffolds to repair musculoskeletal tissues, since the bioactive molecules of dECM, such as fibrous proteins, proteoglycans, and adhesive glycoproteins, could provide various bioactive cues for tissue regeneration and remodeling. The physiochemical properties of dECM can be enhanced by changing decellularization and modification techniques. In addition, dECM can act as carriers of drugs, factors, or exosomes, delivering agents to injured tissues and promoting tissue repair and regeneration. Therefore, we conduct this review to discuss the current status and challenges of dECM in repairing the musculoskeletal system. Furthermore, the fabrication and modification of dECM were also discussed in our study.

Keywords: decellularized extracellular matrix, tissue regeneration, tissue repair, bone, cartilage

1 Introduction

Tissue engineering, as defined by Langer and Vacanti in 1993, is a biomedical engineering discipline that uses living cells, biocompatible materials, and suitable biological factors, as well as combinations, to create tissue-like structures for tissue or organ replacement [1–4]. Tissue or organ failure caused by trauma, tumor, genetic diseases, or surgical removal is considered a worldwide healthcare challenge. The emergence of organ transplantation brings hope to patients suffering from tissue or organ failure [5–7]. Nevertheless, the shortage of organ donors, the increasing number of people on transplant waiting lists, and an aging population necessitate the development of novel methods to restore the function of damaged organs and tissues [8–10]. Tissue engineering provides an alternative and promising therapeutic strategy for tissue or organ regeneration by integrating various scientific disciplines, such as cell biology, material science, engineering, and developmental biology [11–14]. The first step in the construction of tissue or organ substitutes is to fabricate a scaffold to support the survival of various seed cells [15].

An ideal scaffold can provide a native cellular microenvironment that allows various cells to function as they do in the native tissue [16]. Various biomaterials, including

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synthetic and natural materials, have been synthesized and developed as scaffolds for tissue engineering applications. Synthetic biomaterials, such as polylactic, polyglycolic, and polycaprolactone family of polymers, have been widely used in the medical community [17]. In addition, some synthetic biomaterials have already been commercialized and fabricated into the plastic intraocular lens or bioresorbable sutures [18]. However, the structure and composition of synthetic biomaterials are different from native tissue or organ. In addition, synthetic biomaterials are unable to induce tissue remodeling after implantation [19]. Natural biomaterials are found in nature and have not been made by humans [20]. Compared with synthetic biomaterials, nature biomaterials have the advantages of biocompatibility, biodegradability, and remodeling and have been widely applied in the repair or replacement of damaged human tissues and organs [21]. In addition, natural biomaterials are more effective in capturing the native tissue properties and inducing repair [22].

Among these natural materials, decellularized extracellular matrix (dECM) is a promising biomaterial for various tissue regeneration. dECM refers to the biological materials that is treated to remove the cell components in the tissue. It contains many proteins, fibrous proteins, proteoglycans (PGs), adhesive glycoproteins, and other matrix components, which can provide bioactive cues for tissue repair and remodeling [23,24]. Many studies have shown that dECM can be applied to multiple areas, including skin, liver, heart, and brain, and exhibits a synergistic effect to support and control cell behaviors, including cell survival, growth, migration, and differentiation during the process of tissue repair [25,26]. In addition, the biological and structural arrangements of various dECM could provide a network for tissue regeneration.

Allogeneic or xenogeneic donor tissue requires decellularization before implantation to avoid disease transmission and reduce inflammatory responses [27]. Various decellularized technology has been applied to remove cell residues and surface antigens and preserve the inherent inner structure and components of target tissue [28,29]. The process of decellularization in the fabrication of dECM-based biomaterials can be divided into physical, chemical, biological/enzymatic, or a combination of these methods [30]. As a natural polymer, dECM plays a crucial role in tissue homeostasis and maturation because it contains various factors and proteins required for cell growth and differentiation [31]. dECM is often prepared directly in the form of hydrogels or as carriers of drugs, factors, or exosomes, delivering bioactive agents to injured tissues and promoting tissue repair and regeneration. In addition, various modification methods can be used to improve the mechanical as well as biological properties of dECM-derived hydrogels. Recently, the importance of musculoskeletal tissue engineering has been magnified, which has triggered the active development of musculoskeletal tissue-derived dECM [32–34]. Therefore, we conduct this review to discuss the current status and role of dECM in the musculoskeletal system. Furthermore, the fabrication and modification of dECM were also discussed in our study.

2 Bioactive molecules in the dECM

The dynamic remodeling of the extracellular matrix (ECM) of musculoskeletal tissue exerts a mechanical force on cells. It modifies biochemical mediators near the cell membrane, thereby initiating cell-signaling cascades that produce changes in gene expression and cell behavior [35]. In addition, the composition and inner structure of ECM can also be affected by cellular changes [35]. The ECM plays an essential role in directing musculoskeletal tissue-specific development [36]. More and more researchers fabricated dECM for musculoskeletal tissue engineering by mimicking native musculoskeletal tissues [37]. At the same time, the dECM can serve as a repository of bioactive molecules to support various cellular behaviors, including adhesion, proliferation, migration, and differentiation [38–40]. The uniqueness and diversity of structural and functional components in the spatial distribution of dECM make it well distinguished from other biomaterials [41,42]. Thoroughly clarifying the biological components of dECM will enhance its further applications in musculoskeletal tissue regeneration.

The highly flexible and dynamic properties of the ECM in regulating cellular behavior are essential to perform biological functions, which allows remodeling throughout life. Orderly organization of ECM provides not only physical scaffolds to endow musculoskeletal tissue with specific mechanical properties but also a necessary living place for various cells, such as osteoblasts, skeletal muscle cells, tendon cells, and vascular endothelial cells, thanks to the musculoskeletal tissue-specific reservoir of structural and functional proteins within it. On the contrary, the disorganization of ECM may result in an extensive range of musculoskeletal diseases, such as osteogenesis imperfecta, and sarcopenia [37,43]. The ratios of ECM composition and structure vary among different tissue, and the common biomacromolecules of ECM in musculoskeletal tissues have been extensively studied [44]. The common biomacromolecules of ECM in musculoskeletal tissues include structural proteins (collagen and elastin), PGs (hyaluronic acid; HA), adhesive polysaccharides (fibronectin and laminin), and integrin adhesome. The typical composition and tissue

Table 1: Typical composition and tissue sources of ECM

ECM components	Tissue sources
Structural proteins	
Collagen	Bone, tendon, and cartilage [46,47]
Elastin	Blood vessels, ligaments, and cartilage [48]
PGs	
HA	Cartilage [49]
Aggrecan	Invertebrate cartilage and subchondral bone [50]
Versican	Bone marrow, ligament, lung [51]
Adhesive polysaccharides	
Fibronectin	Plasma, surfaces of cells [52]
Laminin	Placenta, basal lamina [46]
Integrin adhesome	
Integrins	bone, cartilage [53,54]

sources of ECM in musculoskeletal tissues are shown in Table 1. The ECM can not only provide simple physical assistance but also participate in the separation, establishment, and maintenance of differentiated musculoskeletal tissues, the transmission of mechanical forces, releasing of growth factors and signaling, and tissue polarization [39,45].

2.1 Structural proteins

Fibrous proteins, also known as structural proteins, are one of the significant classes of secreted macromolecules that make up the dECM derived from musculoskeletal tissues [55,56]. Structural proteins include collagen and elastin, which are present in the ECM in the form of fibrils and exhibit mechanical strength of musculoskeletal tissues, such as bone and cartilage [57]. Collagen is the main component of ECM in musculoskeletal tissues, accounting for 25–35%, also known as a single subgroup of ECM proteins, and the most abundant protein in musculoskeletal tissues [58,59]. For example, collagen in bone tissue maintains the mechanical properties of the ECM and supports the network structure of tissue. Its basic structure is a triple helix structure formed by three polypeptide chains entangled with each other [60]. The molecular formula of collagen is shown in Figure 1. The synthesis and deposition of collagen are regulated by autocrine and paracrine hormones, and the degradation is also regulated by enzymes (such as collagenase and serine protease) that are synthesized and secreted by cells [61]. This is because collagen molecules contain a variety of cell signals that contain polypeptide sequences of the binding site [62]. Collagen has good biocompatibility and low immunogenicity and is the earliest and most widely used scaffold material in musculoskeletal tissue regeneration. Collagen is mainly distributed in the musculoskeletal tissues. It can provide tensile strength for the musculoskeletal tissues, connect the frameworks of tissues, and affect the behavior of cells, such as osteoblasts and chondrocytes [63,64]. Another crucial structural protein is elastin, which is rich in glycine, proline, alanine, and valine. Elastin is the main component of elastic fibers, which together with collagen fibers impart musculoskeletal tissues' elasticity and tensile strength, respectively. Elastin possesses a highly resilient ability, which can adjust the mechanical properties of musculoskeletal tissues, such as skeletal muscle and tendon, and enable tissue with elasticity to recover its shape under repetitive tensile forces [63–65].

2.2 PGs

PGs play an essential role in the preparation of dECM hydrogel-based biomaterials by regulating the self-assembly of collagen fibers or forming nucleation sites of collagen fibers, thereby accelerating the cross-linking of collagen and contributing to the gel properties of the ECM [66]. Different biological functions of PGs depend on different glycosaminoglycans (GAGs) (*e.g.*, HA, heparan sulfate PGs (HSPGs), chondroitin sulfate PGs (CSPGs), dermatan sulfates PGs, keratan sulfates PGs (KSPGs)) [56,67], therefore, in the process of decellularization, in addition to effectively removing cellular components, the retention of bioactive factors such as GAGs as much as possible are also regarded as essential criteria for evaluating decellularization methods [68]. GAGs expressing extensively in ECM of musculoskeletal tissues are linear anionic polysaccharides, which can be bound with a large number of water and combined with active proteins such as growth factors and cytokines in the covalently bound region, thereby slowing and controlling its release in the body to perform biological functions [69]. In addition, GAGs are involved in various stages of bone tissue metabolism, such as calcification, bone regeneration, and bone remodeling. HA is a unique GAG not linked to a core protein. HA is a non-sulfated GAG composed of multiple repeating disaccharides (D-glucuronic acid and *N*-acetyl-D-glucosamine), which exists in the body as a salt, and is mainly distributed in cartilage [70].

HA is a natural biomaterial that can be extracted and modified and used as a tissue engineering scaffold for cartilage regeneration. The molecular formula of HA is shown in Figure 2. It is currently the mainstream product in the market, but expensive, has short clinical effects, and requires repeated injections. HSPGs are distributed on the cell surface and in the ECM and can not only bind to a variety of ECMs but

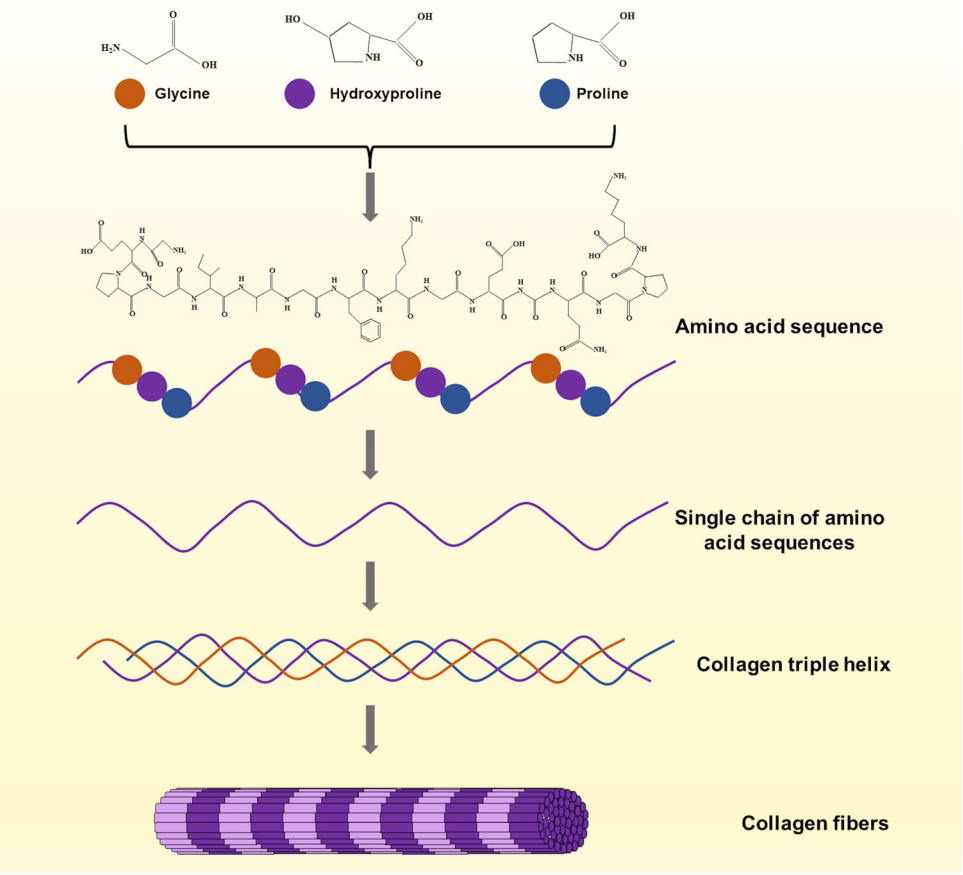

Figure 1: The molecular formula of collagen.

also effectively bind to a variety of growth factors, promoting efficient binding of growth factors to receptors and signal transduction. It has been reported in the literature that HSPGs have high affinity with basic fibroblast growth factor (bFGF) and can act as a co-receptor of bFGF to promote the efficient binding of bFGF to the receptor and the activation of downstream signals, which is beneficial to tissue damage repair [71].

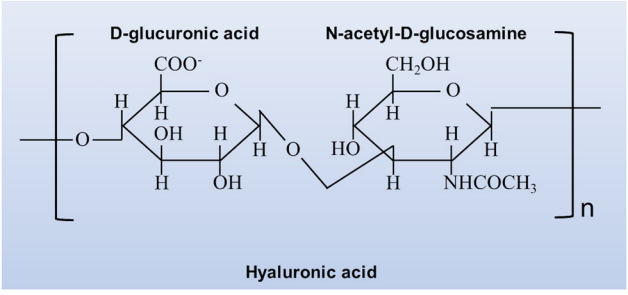

Figure 2: The molecular formula of HA.

Versican and aggrecan are key ECMs belonging to CSPGs, which are present in blood vessel walls and cartilaginous tissues [72]. In studies of neural tissue regeneration, astrocytes respond to soluble factors and influence their environment by secreting CSPGs (a kind of ECM component). It varies with mature/resting *vs* reactive astrocytes [73,74]. Continued high CSPG release prevented reactive astrogliosis in 3D *in vitro* models, which is critical for maintaining neuronal health and functional synapses in musculoskeletal tissues. Therefore, the secretion of CSPGs was regarded as a marker of astrocyte maturation and reactive astrogliosis [75].

CS and DS are composed of repeating disaccharide units [76,77]. CS disaccharides include β-D-glucuronic acid and *N*-acetyl-D-galactosamine, while DS disaccharides contain α-L-iduronic acid, which is often transformed from β-D-glucuronic acid, and thus CS and DS usually exist in a single chain in a hybrid form, which is why many research have studied them as a whole (CS-DS) [78]. The wide variety of structures exhibited by CS-DS polysaccharides depends on different sulfation patterns [79]. Furthermore, increasing

studies have shown that different sulfidation patterns endow CS-DS with different functions in biological processes [77,80]. Besides DS being a kind of GAGs, KS is also one of the main types of this. KS is a polymer of disaccharide composed of *N*-acetylglucosamine and galactose [81,82]. KSPGs (*e.g.*, lumican and fibromodulin) contain 1–3 chains of KS N-linked to the core proteins *via* a mannose-containing linkage oligosaccharide [81,83]. In general, GAGs (except HA) are covalently combined with the core protein to form PG monomers and then combine with HA through connexins to form PG multimers, also known as mucopolysaccharides.

2.3 Adhesive polysaccharides

The ECM contains many secreted macromolecules, which interweave to form a unique network structure known as a microenvironmental niche of musculoskeletal tissues such as bone and cartilage. However, adhesive polysaccharides (*e.g.*, fibronectin, laminin) play a crucial role in stabilizing the network structure of musculoskeletal tissues. As a vital component of the ECM, fibronectin can effectively mediate the differentiation and development of bone tissue. During the process of skeletal development, fibronectin can provide a unique stem cell niche to regulate cellular behaviors [84]. In addition, mutations in fibronectin have been confirmed to lead to skeletal dysplasia [85]. Laminin-1 and -5 can also enhance the osteogenic differentiation of various stem cells [86]. In addition, chondrocytes and stem cells can express laminins, which can provide a suitable matrix microenvironment and enhance chondrogenesis [87]. Fibronectin is closely associated with osteoarthritis progression [88]. The previous study demonstrated that the fibronectin content in osteoarthritis is higher than that of normal cartilage [89]. Laminin-111 can regulate the cellular behaviors of myoblast, including proliferation, migration, and myofiber formation. In addition, laminin-111 can enhance muscle regeneration by promoting fiber formation and satellite cell expansion [90]. Alheib *et al.* demonstrated that a hydrogel functionalized with laminin can effectively enhance skeletal muscle regeneration [91]. Additionally, fibronectin and laminin are involved in neural development, which can provide adhesion support to donor cells, mediate subsequent cell signaling events, and participate in peripheral nerve repair and regeneration by promoting cell survival, cell migration, and neurite outgrowth. Studies have shown that neural stem cells complexed with fibronectin- or laminin-based scaffolds were transplanted into the brains of traumatized mice. The cells delivered in the scaffolds were more widely distributed in the injured brain and had higher survival rate compared to controls. Furthermore, behavioral analyses showed that mice engrafted with neural stem cells within a laminin-based scaffold performed significantly better than untreated mice on a spatial learning task, supporting the opinion of a positive correlation between functional recovery and donor cell survival [92].

2.4 Integrin adhesome

Cell behaviors are influenced by itself integrating signals from ECM. In turn, the formation of ECM depends on macromolecules synthesized and secreted by cells under the control of various signals, so there is an important dynamic communication between cells and ECM or between cells and other cells. In this process, “integrin adhesome” plays a critical role. Integrin adhesome is a group of proteins with elaborate network structures that mediate the interaction between integrin adhesions and the actin cytoskeleton [93]. Integrin adhesome plays an important role in supporting the physical integration of cytoskeleton and ECM-bound cells, which maintain the particular structure of tissues or organs [93]. Moreover, the integrin adhesome enables cells to sense and respond to the chemical and mechanical stimulus from the external microenvironment [93,94]. In addition, integrin adhesome can affect the morphogenesis, migration, proliferation, differentiation, and survival of cells during the reconstruction of musculoskeletal tissue [95,96]. For example, the regulation of stem cell proliferation, adhesion, and regeneration is achieved through integrin-modulated activation of focal adhesion kinase and phosphoinositide 3-kinase signaling pathways [97,98]. In turn, signaling pathways can also regulate integrin transmission [99]. Integrin adhesome includes associated cytoskeleton, various actin regulators, and adaptor proteins that connect cytoskeletal structures to the cytoplasmic tail of integrins, and integrins that link the ECM to the intracellular cytoskeleton [100]. Integrins are composed of α and β subunit heterodimers through non-covalent linking. In mammals, 18 α subunits and 8 β subunits can combine to form 24 integrin dimers with different tissue and matrix-binding specificity. [101]. Integrins, as transmembrane ECM receptors or adhesion receptors, belong to a family of glycoproteins involved in the scaffolding function of adhesion bodies and in adhesion-mediated signaling that affect adhesion itself with multiple cellular downstream targets [102]. Integrins help intracellular and extracellular signals to communicate with each other. It can transmit information from ECM to the interior of cells by coordinating with intracellular linkage molecules such as FAK, α6β1 integrin, α9 integrin, β1 integrin, and αvβ3 integrin. In turn, its β subunit can also bind

to intracellular activators to influence the affinity of integrins for ECM ligands and ultimately transmit intracellular signals to the ECM, affecting ECM assembly, cell adhesion, proliferation, and homing of stem cells [103–105]. Integrins and other cell surface receptors mediate cellular behavior, and musculoskeletal tissue development in response to ligands present within the ECM. Even with subtle changes in the structural and mechanical properties of the ECM, cellular transcriptional events and associated cellular phenotypes and functions are affected by them [106,107]. For example, αvβ3, αvβ5, α5β1, α6β4, α4β1, and αvβ6 integrins in tumor cells have tumor-promoting functions, and their high expression and signal transduction are associated with disease progression in various tumor types such as breast tumors [108,109].

2.5 Specific molecules in the ECM of musculoskeletal tissue

Articular cartilage is a kind of hyaline cartilage, which is composed of the cartilage ECM, fibers, and chondrocytes. The cartilage ECM has a composition similar to that of the connective tissue ECM, but it contains more CS and HA [110]. The fibers in articular cartilage mainly consist of collagen fibrils (type II collagen). The bone ECM can be divided into organic and inorganic ECM. Organic ECM mainly includes bone collagen fibers (type I collagen), glycosaminopolysaccharides, and a variety of glycoproteins (osteocalcin, osteonectin, osteopontin). The inorganic ECM is mainly composed of hydroxyapatite crystals, which are needle-like and arranged along the long axis of collagen fibers. Skeletal muscle plays an important role in the production of movement and maintenance of upright posture. The ECM of skeletal muscle consists of elastin, PGs, glycoproteins, and collagens [111]. In addition, collagen acts as the major structural protein and accounts for 1–10% of muscle mass dry weight [112]. As ECM-rich tissue, tendons connect muscles and bones. In addition, tendons play an important role in transmitting various forces between bones and muscles [113]. The tendon ECM predominantly consists of collagen (type I collagen), which provides structural support and accounts for more than half of the dry weight of tissue [114]. The ECM of the peripheral nerve consists of collagens, laminin-2, PGs, glycoproteins, and non-PG polysaccharides [115].

3 Classification of dECM materials

With the rapid development of tissue engineering and regenerative medicine, dECM has attracted extensive attention because of its good biophysical and biochemical properties, which can directly or indirectly regulate cell proliferation, adhesion, migration, and differentiation. According to the source of ECM, dECM scaffolds can be divided into organ/tissue-derived dECM scaffolds [116,117] and cell-derived dECM scaffolds [30,118].

3.1 Organ/tissue-derived dECM

In recent years, organ/tissue-derived dECM has become a research hotspot in the field of tissue engineering due to its similar structure and composition to the target tissue [119]. Organ/tissue-derived dECM reproduces the physiological environment with high fidelity to *in vivo* conditions and promotes tissue-specific cell development and maturation [120]. The organ/tissue-derived dECM can be prepared and used in different forms, such as whole organ/tissue shapes [121], patch-like shapes [122], coating materials for two-dimensional (2D) cell culture substrates [123], and injectable gels [124]. In addition, organ/tissue-derived dECM scaffolds serve as reservoirs for site-specific bioactive molecules and cell-matrix interactions. The memory factors and cues of original ECM can retain disparate tissue-specific memories that can drive tissue-specific differentiation [120]. In addition, the mechanical properties of organ/tissue-derived dECM were similar to that of host [125,126]. Furthermore, the complex internal microstructures of organ/tissue-derived dECM, such as pore morphology and collagen fiber arrangement, can direct cell adhesion, proliferation, and differentiation [127]. However, organ/tissue-derived dECM has disadvantages of difficulty for large-scale *in vitro* analysis.

3.2 Cell-derived dECM

In recent years, dECM derived from cells cultured *in vitro* (cell-derived dECM) has been widely used for tissue repair and regeneration [30]. Currently, the decellularization methods of cell-derived dECM can be classified into physical, chemical, and enzymatic treatments [22]. In addition, cell-derived ECM substrates can also be fabricated into 3D cell-derived ECM pellets or 3D cell-derived ECM scaffolds. The schematic illustration of fabricating cell-derived ECM, ECM Pellet, and cell-derived ECM scaffolds is shown in Figure 3. Compared with organ/tissue-derived dECM, cell-derived dECM can more completely duplicate the native ECM microenvironment for cells to survive and be more accessible to be isolated and extracted

[128]. In addition, it is very easy to obtain the ECM model from small tissue regions [27]. The large amount of ECM produced by cells is rich in secreted macromolecules and some growth factors [129]. Various cell types can be cultured *in vitro* to produce a variety of cell-derived ECM for the subsequent decellularization process [130]. Bone marrow mesenchymal stem cells (BMSCs)-derived dECM can act as a scaffold for chondrocyte proliferation, chondrocyte phenotype maintenance, and promotion of chondrocyte expansion and redifferentiation [128]. In bone regeneration, dECM derived from human BMSCs exhibit a good osteogenic ability to repair mouse calvarial defects [131]. However, for cell-derived dECM, it is challenging to obtain decellularized 3D constructs whose composition, mechanical properties, and microstructure are identical to native ECM [27].

3.3 Other classifications methods of dECM materials

According to the method of reconstitution or application, dECM are scaffolds that exist in both solid and liquid forms. Solid dECM can be used directly as a biomaterial without disrupting the ECM microstructure. Solid scaffolds can be categorized by application, including tissue-engineered dECM patches/sheets [132–134] and whole tissues [116,135–137]. The dECM can be solubilized into bioinks for 3D bioprinting because of converting into hydrogel by a pre-gel fluid [138,139]. Thereby, soluble dECM has become an essential form of dECM-based biomaterials, classified as injectable hydrogels, 2D and 3D hydrogels, and combinatorial patches composed of dECM and other biomaterials. Depending on the origin of the ECM, dECM scaffolds include autologous dECM and allogeneic dECM. Since autologous dECM scaffolds face source limitations and surgical complications, most dECM scaffolds are derived from allogeneic or xenogeneic donor tissue; however, there may be structural and mass composition differences in allogeneic or xenogeneic dECM as well as immunogenicity problems caused by incomplete decellularization [140].

4 Construction of dECM

Autologous transplantation is currently the gold standard for tissue reconstruction. The use of allogeneic or xenogeneic decellularized tissue scaffolds brings hope to patients suffering from tissue defects [141]. Allogeneic or xenogeneic donor tissue requires decellularization before implantation to avoid disease transmission and reduce inflammatory responses [27]. The ideal decellularized technology can remove cell residues and surface antigens and preserve the inherent inner structure and components of target tissue [28,29]. The inflammatory response *in vivo* is triggered by two common antigens within dECM, DNA, and cell surface oligosaccharide molecule α-Gal (Galα1,3-Galβ1-4GlcNAc-R) [142]. Fully removing the DNA of dECM is beneficial to decrease its immunogenicity [143]. The previous studies demonstrated that dECM with a content of less than 50 ng/mg DNA was less likely to induce a severe immune response after

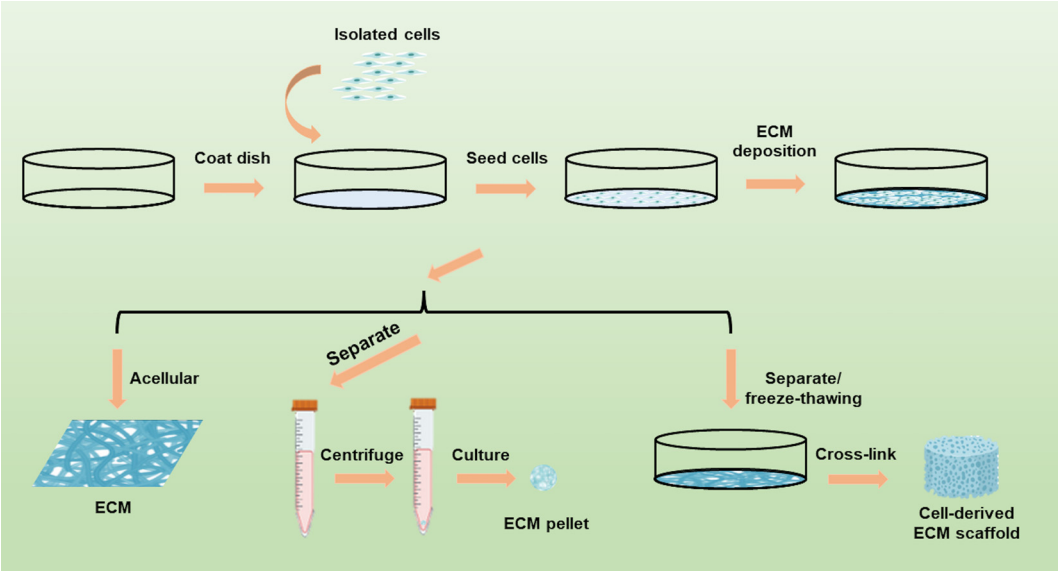

Figure 3: The schematic illustration of fabricating cell-derived ECM, ECM Pellet, and cell-derived ECM scaffolds.

implantation [144,145]. To avoid an immune response triggered by residual α-Gal in the dECM, the α-galactosidase was used to treat allografts or xenografts before implantation [146]. The previous studies showed that dECM treated with α-galactosidase decreased the immune response *in vivo* [147].

Several decellularization techniques have been used to fabricate dECM-based biomaterials [148]. The process of decellularization in the fabrication of dECM-based biomaterials can be divided into physical, chemical, and biological/enzymatic or a combination of these methods [30]. Physical decellularization techniques include sonication, solution agitation, snap-freezing, scraping, pressure gradients, non-thermal irreversible electroporation, and supercritical fluids [64]. The chemical decellularization techniques remove cellular components by applying acids and bases, hypotonic and hypertonic solutions, detergents, alcohols, and other solvents [149]. Sodium dodecyl sulfate (SDS), sodium deoxycholate, Triton-X100, and peracetic acid (PAA) are commonly used as decellularized regents in musculoskeletal tissue regeneration [150,151]. The biological decellularization techniques can be divided into enzymatic and non-enzymatic. Many enzymes, including collagenases, trypsin, dispases, nucleases, thermolysin, and lipases, have been utilized in biological decellularization techniques [152]. In non-enzymatic decellularization techniques, chelating agents, such as ethylene diamine tetra-acetic acid and ethylene glycol tetra-acetic acid, can enhance the cell dissociation from ECM proteins by interrupting cell attachment to collagen and fibronectin [153]. The classifications and mechanism of physical, chemical, and biological decellularization techniques in fabricating dECM-based scaffolds is shown in Figure 4. Apart from a single decellularization strategy, more and more combinatorial decellularization methods have been used for the fabrication of decellularized tissues or organs. However, the differences between single decellularization methods and combinatorial decellularization methods still need further studies.

5 Modification and processing of dECM

dECM is a natural polymer, which can play a crucial role in tissue homeostasis and maturation because it contains various factors and proteins required for cell growth and differentiation [31], regulates biological balance, and has lower toxicity and immunogenicity [148]. Compared with other natural polymers such as sodium alginate [154], collagen [155], and HA [156], dECM is more biomimetic and can also be fabricated as the dECM-derived hydrogels with a three-dimensional network structure through specific

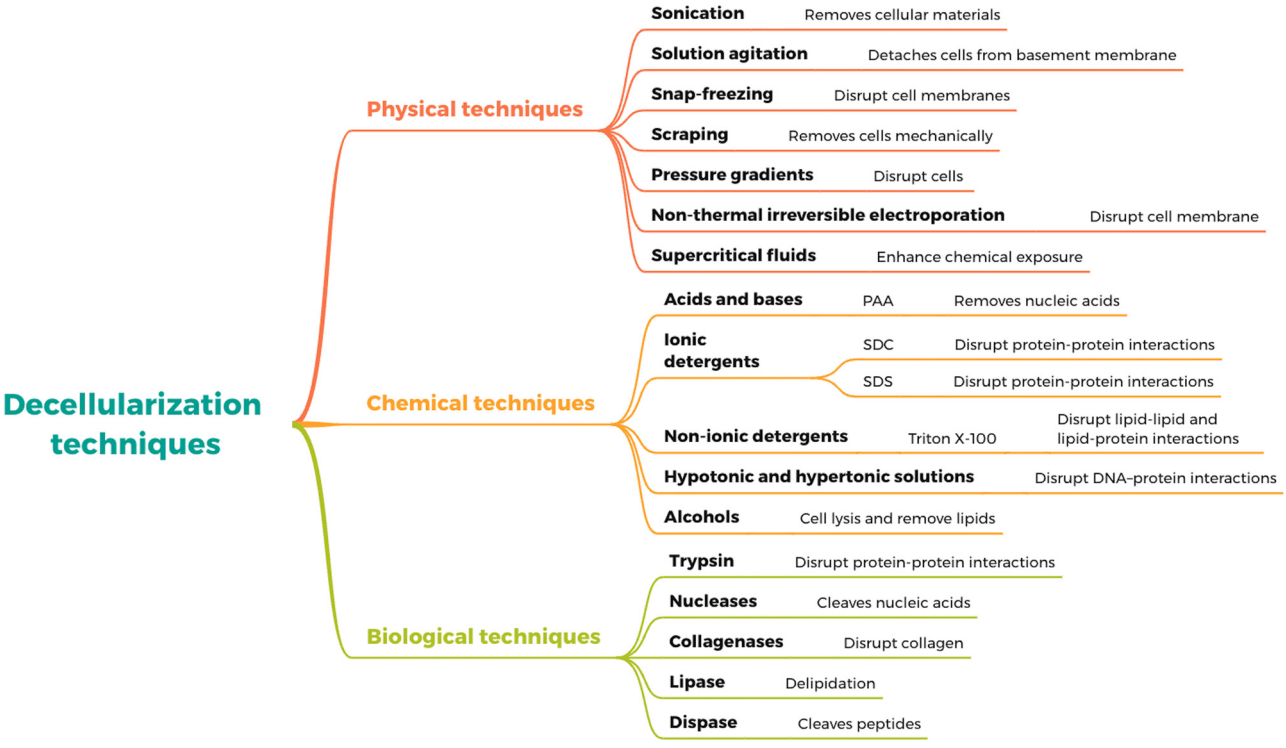

Figure 4: The classifications and mechanism of physical, chemical, and biological decellularization techniques in fabricating dECM-based scaffolds.

methods, which provide a cell-friendly environment that can hold a large amount of water. Therefore, it can simulate the native microenvironment for cells growing *in vitro* [41,157]. The dECM-derived hydrogel is not only similar in composition and structure to the natural ECM and can promote tissue regeneration but also induces the proliferation and differentiation of stem cells in the host and regulates cell signaling pathways and gene expression, which is in line with the printability that bioinks need to meet, good mechanical properties, and biocompatibility [38]. In conclusion, dECM-derived hydrogels are one of the most promising biomaterials for tissue engineering applications due to their advantages, such as good biocompatibility, biodegradability, and the ability to mimic a microenvironment similar to ECM. It can reach beyond the limitations of traditional manufacturing techniques through new biofabrication strategies to reproduce the complex structures of natural tissue, such as textile-based technology (form stronger supporting structures) and 3D bioprinting (have more complex and controlled architectures) to construct natural hydrogels [158–160].

dECM is often prepared directly in the form of hydrogels or as carriers of drugs, factors, or exosomes, delivering bioactive agents to injured tissues and promoting tissue repair and regeneration. Due to the short half-life of these agents or their easy loss of blood circulation in the body, the repair effect of systemic or local administration on tissue damage is limited. Xu *et al.* mixed a homogenized solution of the rat-derived acellular spinal cord with a solution of bFGF, which was mixed together utilizing electrostatic or receptor-mediated interactions. Subsequently, the composite scaffold was encapsulated into a heparin modified poloxamer solution to prepare a temperature-sensitive hydrogel, which reconstructed a three-dimensional matrix structure for dECM and controlled the release of the neuroprotective factor bFGF loaded in the material. The experimental results show that this type of composite scaffold in the form of combined therapy protects the nerves of spinal cord injury by stably delivering bFGF to the rat spinal cord hemisection model, thereby increasing the survival rate of neurons and improving the functional recovery of spinal cord tissue [161].

6 dECM in tissue regeneration

6.1 Bone regeneration

Bone is a multifunctional hard tissue that supports the mechanical movements of the body and preserves important minerals [162]. Bone tissue can heal itself with little to no formation of scar tissue [163]. However, the bone tissue cannot repair the defects by itself whenever the severity of the bone damage is excessive [164,165]. The gold treatment standard for significant bone defects is based on autografts [166]. However, the amount of autologous bone grafts is limited, and complications at the harvesting site, such as pain, infection, or bleeding, could result in additional donor-site morbidity [162,167]. Therefore, additional implantation of bone repair materials is required for bone defect repair. Various bone repair biomaterials have been applied in bone regeneration [168,169]. However, the regenerative capacity of most bone repair biomaterials is limited due to insufficient similarity in composition and tissue structure. Among these bone repair biomaterials, the bone dECM is considered one of the most promising bone substitutes due to its unique bioactivity [170].

As an essential noncellular constituent of bone tissue, the bone ECM, consisting of organic and inorganic phases, possesses a supportive network to regulate the behaviors of various cells [171–173]. Nature ECM used in bone tissue engineering mainly includes three primary forms, demineralized bone matrix (DBM), deproteinized bone ECM, and bone dECM [149]. DBM includes bone ECM without mineral contents and increases the expression of osteogenic markers *in vitro* and *in vivo* [149,174]. In addition, the processing (sterilization, demineralization, and processing time) and patients' basic condition (age, gender, and comorbidities) can affect the osteogenic ability of DBM [175]. Deproteinized bone ECM includes inorganic components of bone tissue, while the organic components were removed [176]. However, the cell debris in DBM and deproteinized bone ECM can lead to the host immune rejection. The bone dECM can effectively decrease the immune response by removing cellular and nuclear contents [177]. In addition, the bone dECM is mainly composed of collagen, glycoproteins, and various bone matrix proteins (osteonectin, osteopontin, bone sialoprotein, and osteocalcin), which play a crucial role in bone regeneration and remodeling [178]. The biomacromolecules of bone dECM can modulate the fate of osteoblast-lineage cells, mainly including MSCs, osteoblasts, osteocytes, and osteoclasts [179]. Table 2 shows the functions of the biomacromolecules of bone dECM in the osteoblast-lineage cells.

Various bone dECM have been used for bone regeneration due to their similarity to the native bone matrix and excellent osteoinductive and biomechanical properties. The matrix architecture and mineral content have an influence on the bioactivity of bone dECM. When the density of bone dECM scaffolds is about $0.434 \pm 0.015\ mg/mm^3$, dECM scaffolds exhibit better bioactivity in balancing nutrient transport, cell attachment, cell proliferation, matrix production,

Table 2: Functions of the biomacromolecules of bone dECM in the osteoblast-lineage cells

Biomacromolecules	MSCs	Osteoblasts	Osteocytes	Osteoclasts
Collagen (Type I)	—	Osteogenesis (+) [180]	Osteocyte mineralization (+) [181]	Osteoclast formation (−) [182]
Collagen (Type III)	—	Osteogenesis (+), mineralization (+) [183]	—	—
Biglycan	Proliferation (+) [184]	—	—	Osteoclast precursors differentiation (−) [185]
Thrombospondins-1	Proliferation (+) [186]	Mineralization (−) [187]	—	Osteoclast differentiation and activity (+) [188]
Thrombospondins-2	Osteogenesis (+) [189]	Mineralization (+) [190]	—	Osteoclastogenesis (+) [191]
Bone sialoprotein	—	Osteoblast differentiation (+), mineralization (+) [192]	—	Osteoclast migration and bone resorption (+) [193]
Matrix extracellular phosphoglycoprotein	—	—	Osteocyte mineralization (+) [194]	—
Osteopontin	Proliferation (+), migration (+) [195]	Mineralization (−) [196]	—	Osteoclast activity and sealing zone formation (+) [196]
Osteocalcin	Osteogenesis (+) [195]	Mineralization (−) [197]	—	—
Matrix Gla Protein	—	Osteoblast differentiation (+), mineralization (+) [198]	—	Osteoclast differentiation and bone resorption (−) [199]
Dentin matrix protein-1	Pluripotency (+) [200]	—	Osteocyte apoptosis (−) [201]	—
R-spondin2	—	Osteoblast differentiation (+) [202]	—	—
Periostin	—	Osteoblast differentiation (+) [203]	—	—

and scaffold mechanical strength [204]. In addition, the bone mineral in bone dECM can enhance bone markers expression and robust accumulation of new bone matrix [204]. The bone dECM can be constructed into hydrogels for bone regeneration. Gothard *et al.* fabricated a versatile bone repair composite hydrogel by combining decellularized, demineralized bone ECM and alginate [205]. The *in vivo* results showed that the composite hydrogel exhibited a good heterotopic osteogenic capacity (Figure 5). In another study, Parthiban *et al.* demineralized and decellularized human bone fragments combined with methacrylate groups to form photocrosslinkable bone dECM hydrogels for bone regeneration [206]. Although the bone dECM hydrogel exhibits good osteoinductivity, the architecture and topology of bone dECM are not preserved. The cell-derived dECM can be used as a coating on the surface of osteogenic scaffolds. Kang *et al.* modified umbilical vein-derived endothelial cells (HUVECs)-derived ECM onto the surface of β-TCP scaffolds. The results demonstrated that the cell-derived ECM enhanced the osteogenic ability of β-TCP scaffolds by activating MAPK/ERK signaling pathway *in vivo* [207]. In another study, the MSCs-derived ECM coated on the surface of bioceramic-polymer hybrid scaffolds could enhance the survival and osteogenic differentiation capacity of MSCs seeded onto the scaffolds [208]. The strategy of dECM coating onto the scaffolds can exert its biological function and avoid the weak mechanical properties of dECM alone.

Combining scaffolds with seed cells is a classic therapeutic strategy for tissue repair using tissue engineering principles. Various bone dECM possess good biocompatibility and bioactivity and can be used as carriers of osteogenic cells for bone repair [209]. Fröhlich *et al.* demonstrated that the combination of human adipose-derived stem cells and bone dECM scaffolds enhanced the formation of dense and stable bone tissue structures *in vivo* [210]. The bone dECM can provide an excellent internal microenvironment for biological behaviors such as adhesion and proliferation of seed cells. However, further research is needed on the fate of stem cells loaded on bone dECM after implantation. In addition, although the paracrine function of stem cells has been demonstrated, the molecular biological mechanism involved in paracrine still needs to be further studied [118].

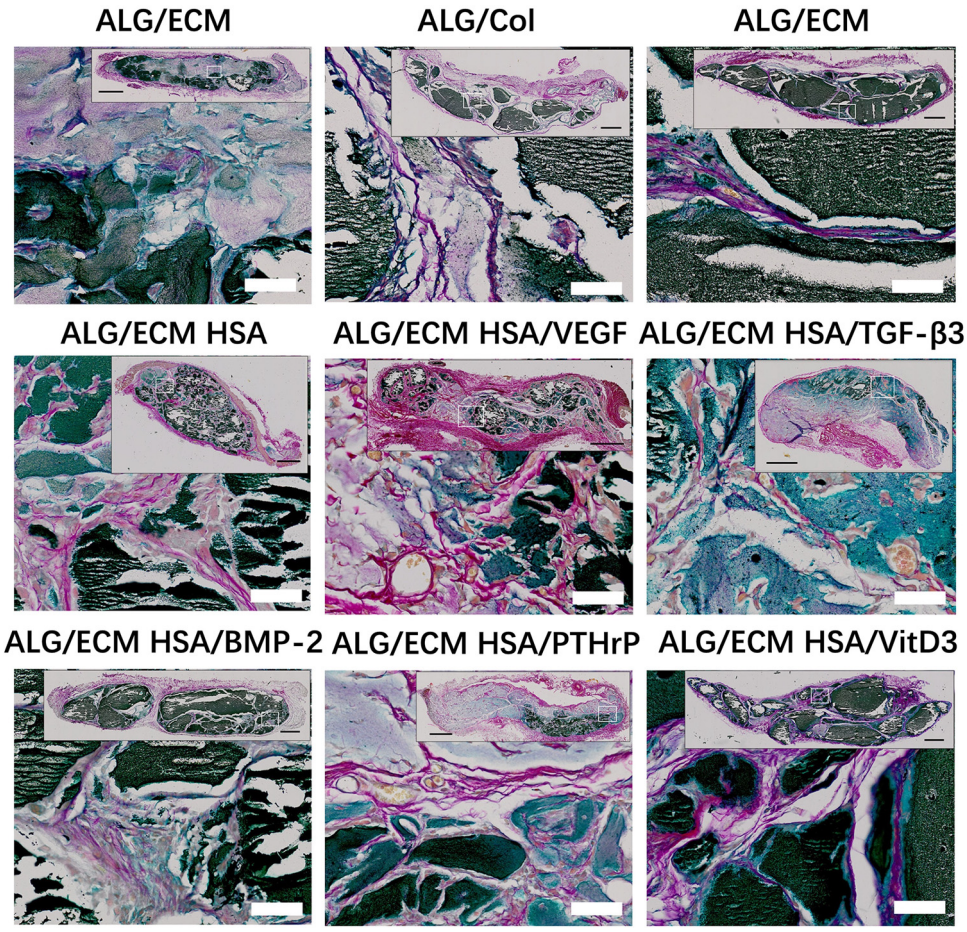

Figure 5: The Von Kossa staining after implantation. ALG, alginate; HSA, human serum albumin [205].

The loading of bioactive substances, including osteogenic and angiogenic factors, can further improve the osteogenic ability of the bone dECM. As a concentrate of platelet-rich plasma (PRP) protein derived from whole blood, PRP contains various blood-derived growth factors and cytokines, which could decrease local inflammation and promote the healing process of different kinds of damaged tissues [211]. Leng *et al.* mixed PRP with bone dECM for critical-size radial defect regeneration in rabbit model [212]. The *in vivo* results demonstrated that the addition of PRP could reduce immune response and enhance bone formation *in vivo*. Stromal cell-derived factor-1 (SDF-1) is a chemokine involved in the homing and recruitment of stem cells [213]. Chen and Lv coated collagen/hydroxyapatite mixture onto the bone dECM and loaded it with SDF-1α [214]. The *in vivo* results showed that the release of SDF-1α from the scaffold enhanced endogenous MSCs recruitment and bone regeneration (Figure 6). Various inorganic components, such as hydroxyapatite, can be incorporated into bone dECM scaffolds to enhance the osteoinductive properties of scaffolds [215,216].

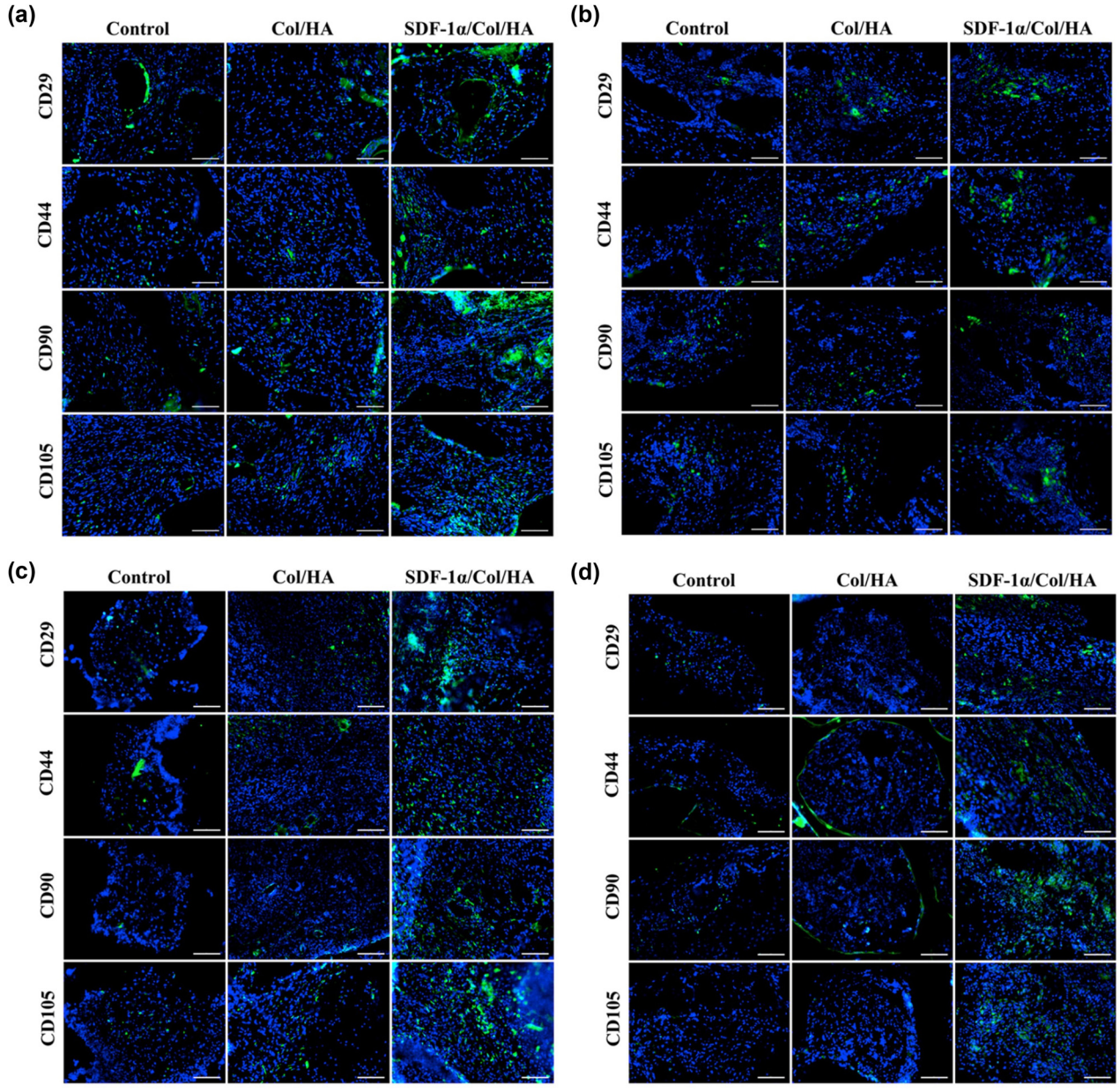

Figure 6: CD105, CD90, CD44, and CD29 immunofluorescent staining of dECM scaffolds after implantation on day 5 (a), day 10 (b), week 4 (c), and week 8 (d). The CD105, CD90, CD44, and CD29 (green) are surface markers of MSCs, which confirmed *in vivo* recruitment of stem cells by dECM scaffolds The blue represents the nucleus [214].

6.2 Articular cartilage regeneration

Articular cartilage defects or injuries from trauma or osteoarthritis present a substantial challenge to repair [217]. Articular cartilage is the primary connective tissue that sits at the edge of bone tissue and distributes concentrated loads and facilitates low-friction movement. In addition, the regenerative capacity of articular cartilage is poor owing to its nonvascular property [218]. To avoid further deterioration caused by articular cartilage defects, autologous cartilage transplantations, Autologous periosteal transplantation, and microfracture are used in clinical treatment. However, all these treatments have limitations [219]. The autologous cartilage or periosteal transplantation need second surgery, while microfracture can lead to regenerated tissue that is fibrous in nature [219]. Given these limitations, tissue engineering has become a potential therapeutic strategy for cartilage repair [220]. Articular cartilage is composed of dispersed chondrocytes and a dense ECM and is rich in water, up to 80% [221]. The ECM in cartilage could affect the cell behaviors of chondrocytes, including cell attachment, migration, and proliferation [222]. Cartilage dECM scaffolds can preserve natural tissue ECM and possess good biological and biomechanical properties. In addition, the cartilage dECM scaffolds possess low immunogenic ability by eliminating specific homogenous/xenogeneic cells [223]. Therefore, cartilage dECM has been regarded as a promising therapy for articular cartilage repair.

The dense cartilage matrices increase the difficulty for reagents to penetrate during the decellularization procedures. Breaking down the cartilage matrix into fragments can enhance the surface area, and then reagents can easily penetrate into the cartilage matrix [219]. After decellularization procedures, the cartilage fragments are rebuilt into a porous scaffold by freeze-drying. Improving the porosity of cartilage dECM is beneficial for cellular infiltration [224]. In addition, the relatively dense ECM could prevent chondrocytes from migrating into the cartilage dECM scaffolds *in vitro* and *in vivo*, leading to the failure of cartilage regeneration. Li *et al.* utilized laser modification to create micropores within cartilage dECM scaffolds to enhance the migration of seeded chondrocytes into the scaffold [225]. The results showed that laser-modified cartilage dECM scaffolds could enhance the degree of decellularization and were conducive to cell adhesion compared with intact cartilage dECM scaffolds. Yang *et al.* fabricated a cartilage dECM scaffold with 3D interconnected micropores, which could support the adherence, proliferation, and differentiation of BMSCs to chondrocytes [226]. Articular cartilage exhibits both stress-strain and tension-compression nonlinearity under compressive forces. In addition, the articular cartilage provides resistance to compressive joint loading.

Currently, many researchers utilize various cartilage dECM scaffolds in cartilage regeneration. Chen *et al.* successfully fabricated a cartilage dECM scaffold by utilizing rabbit-derived fibrocartilage. The *in vitro* studies showed that the scaffolds exhibited a good ability for cell-loading and chondrogenic induction [227]. Nie *et al.* fabricated a decellularized, tissue-engineered hyaline cartilage graft for articular cartilage repair *via* the decellularization process [228]. The *in vivo* results demonstrated hyaline-like cartilaginous neo-tissue formed after implantation in porcine knee joints. In another study, Chen *et al.* utilized dECM-chitosan compound to treat knee osteoarthritis [229] (Figure 7a). The *in vivo* study demonstrated that the cartilage dECM could alleviate knee joint pain in rats and significantly delay the progression of knee osteoarthritis in rats (Figure 7b). Kim *et al.* constructed polymeric polycaprolactone nanofibers decorated with cartilage-derived dECM as a chondroinductive scaffold material to mimic cartilage-specific microenvironment for cartilage repair [230]. The results showed that adipose-derived stem cells (ADSCs) in the nanofibril composites significantly increased the expression of chondrogenic gene markers compared to those in pellet culture. The antler cartilage is a unique regenerative cartilage that has the potential for cartilage repair.

Although knee articular cartilage is the most used cartilage source for cartilage dECM scaffolds, more and more studies are now trying to use cartilage from other parts for articular cartilage repair. Chu *et al.* utilized deer antler cartilage dECM scaffold with high collagen content and GAGs for cartilage regeneration [231] (Figure 8a). The *in vivo* results demonstrated that the deer antler cartilage dECM scaffold exhibited a better cartilage regeneration ability than the porcine cartilage dECM scaffold (Figure 8b). Das *et al.* fabricated a goat conchal cartilaginous dECM scaffold for osteochondral defects in rabbits [232]. The *in vitro* results demonstrated that cartilaginous dECM scaffolds could enhance cellular infiltration and proliferation. The *in vivo* results confirmed that cartilaginous dECM scaffolds possessed good biocompatibility without immune response or tissue rejection. In another study, Ortiz-Arrabal *et al.* fabricated a novel biomaterial obtained by decellularizing sturgeon chondral endoskeleton tissue for use in cartilage tissue engineering [233]. The *in vivo* results supported the biocompatibility of decellularized sturgeon cartilage, as well as its ability to sustain cell adhesion, proliferation, and differentiation (Figure 8c). Changchen *et al.* compared auricular cartilage dECM and costal cartilage dECM [234]. The results found that the auricular cartilage dECM had a larger pore size, more pores, and a higher degradation rate than

the costal cartilage dECM. In addition, the auricular cartilage dECM had a higher cell proliferation rate and more prominent immunomodulatory effect than the costal cartilage dECM *in vitro*. Although enormous *in vitro* and *in vivo* studies have confirmed the chondrogenic ability of cartilage dECM scaffolds, the weak mechanical ability of cartilage dECM scaffolds still needs further research.

6.3 Skeletal muscle regeneration

Skeletal muscle is a highly organized and complex muscle tissue that is attached to the bones and is involved in the functioning of different parts of the body. Skeletal muscle comprises over 40% of the human body and is also highly vascularized and innervated [235]. Skeletal muscles have a robust capacity to regenerate, but under compromised conditions, including traumatic injuries, congenital defects, neuromuscular diseases, and surgical ablations, the loss of muscle functionality is inevitable [236]. The regenerative response of skeletal muscle fails when the losing muscle volume is too large [237,238]. Once muscle loss volume exceeds 20%, which is called volumetric muscle loss (VML), it overwhelms the regenerative capacity of skeletal muscle and leads to non-contractile scar tissue and muscle dysfunctionality [239]. Currently, autologous tissue transplantation is the gold standard treatment for VML. However, autologous tissue transplantation has the disadvantages of multiple surgeries and donor site morbidity. The ECM of native skeletal muscle

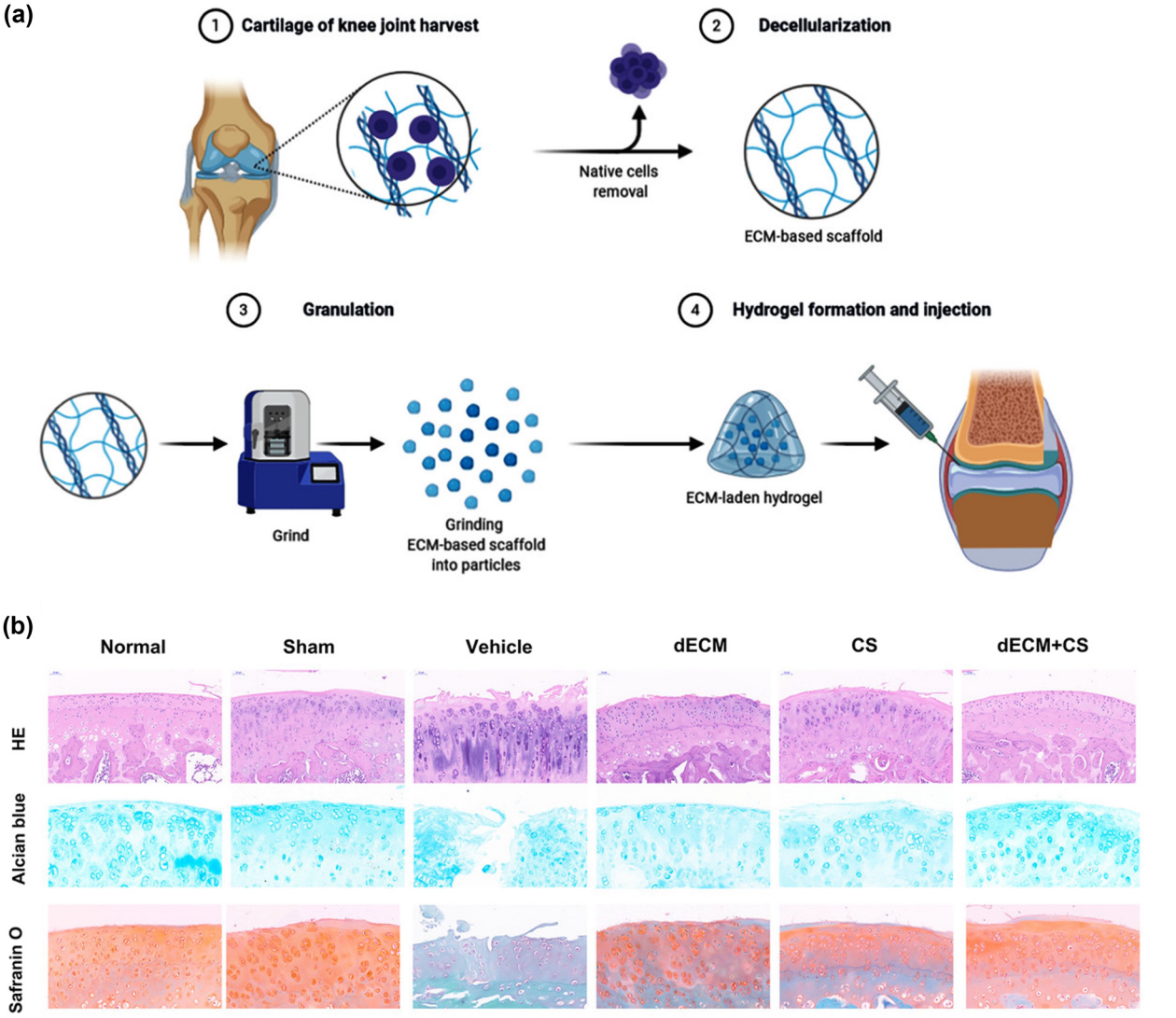

Figure 7: (a) Schematic illustration of fabricating dECM-chitosan, including cartilage of knee joint harvest, decellularization, granulation, and hydrogel formation and injection dECM-chitosan [229]. (b) The Safranin O, Alcian blue, and HE staining of knee cartilage after treatment. The histological results confirmed the dECM-chitosan could effectively regenerate articular cartilage [229].

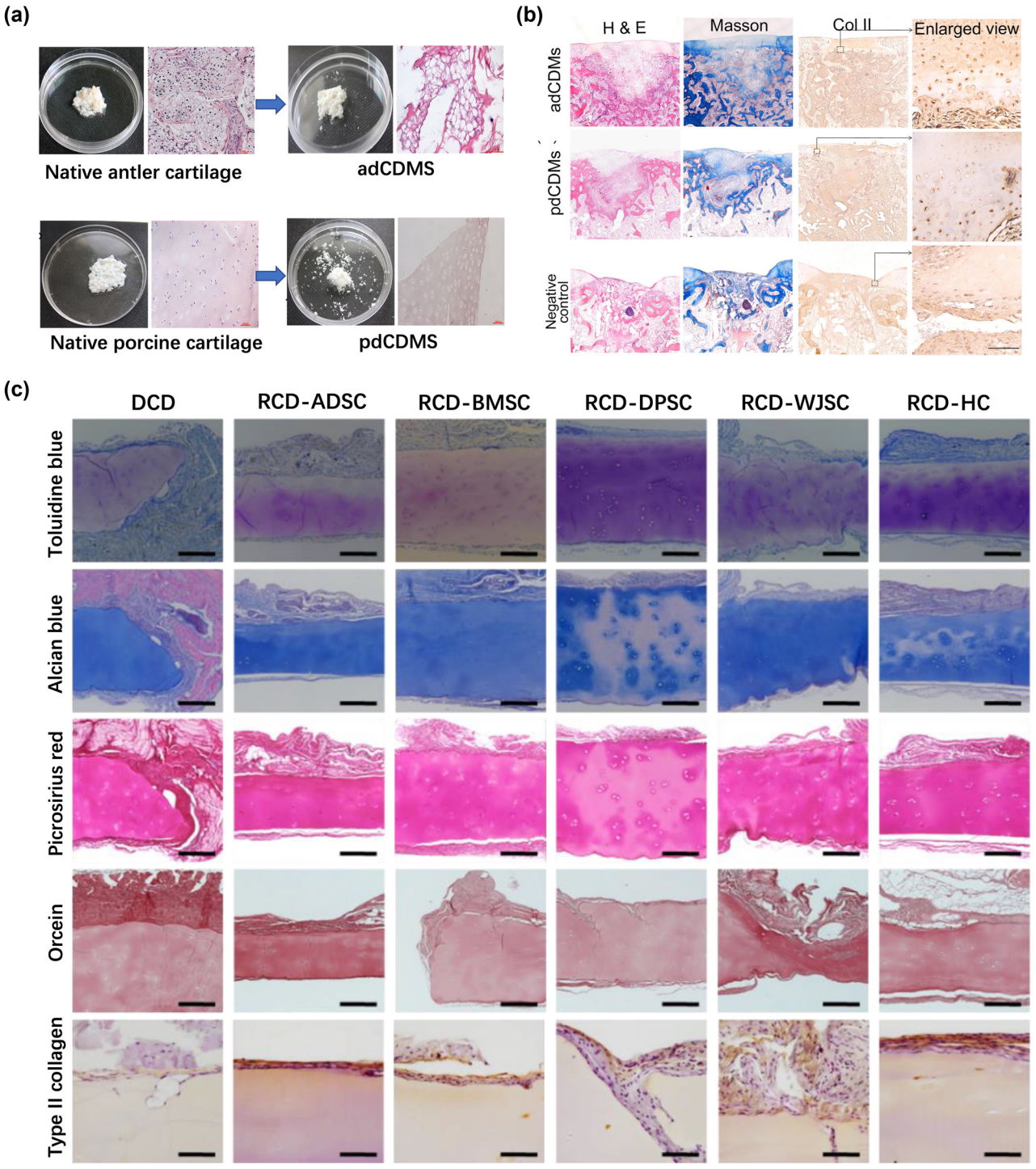

Figure 8: (a) The general view and HE staining results of deer antler and porcine joint decellularized cartilage-derived matrixs (dCDMs) [231]. (b) The histological staining [231]. (c) The histological staining of ECM components after *in vivo* implantation . The "Scale bar = 200 μm" [233].

includes various PGs and proteins, which act as gene regulators, structural materials, and modulatory binding sites [240]. dECM can preserve these biological components and is attracting more and more attention in the field of skeletal muscle regeneration.

The ideal tissue-engineered scaffolds for skeletal muscle regeneration can fill the volumetric loss and possess a good biocompatibility [241]. In addition, the chemical and physical cues inside scaffolds can provide a suitable regeneration microenvironment to modulate the biological behaviors

of various functional cells, including satellite cells (SCs) and vascular and neural cells [242,243]. Skeletal muscle dECM can promote the proliferation of vascular cells and muscle progenitor cells *in vitro*. In addition, the skeletal muscle dECM could enhance the infiltration of muscle progenitor cells and muscle proliferation [79]. The 3D organization of skeletal muscle can instruct host cells and serve as carriers for seed cells [244]. In addition, skeletal muscle dECM was found to exert anti-inflammatory and immunosuppressive effects. Many studies utilize skeletal muscle dECM as a promising scaffold for skeletal muscle regeneration. Perniconi *et al.* fabricated a skeletal muscle dECM that can replace normal tibialis anterior muscles by creating a myogenic microenvironment [245]. Hogan *et al.* fabricated a skeletal muscle dECM for skeletal muscle regeneration in a rat partial thickness tibialis anterior defect model [246]. The *in vivo* results found that skeletal muscle dECM could enhance more excellent myofiber formation in the defect site compared to the empty defect control. In another study, McClure *et al.* fabricated a muscle dECM graft by rat gastrocnemius for gastrocnemius defects [247]. The *in vivo* results showed that muscle dECM could enhance skeletal muscle regeneration with less fibrosis and more de novo neuromuscular receptors than either autograft or collagen. In addition, many researchers compare the immunogenic ability between the muscle dECM and other dECM. Lyer *et al.* demonstrated that muscle dECM could effectively reduce inflammation response in an muscle defect compared with dermal matrices [248]. Carvalho *et al.* demonstrated that xenogeneic placental ECM implanted heterotopically induced local inflammatory reactions similar to the allogeneic muscle ECM, implanted orthotopically [249]. Growth factors could enhance the muscle regenerative ability of muscle dECM. Lee *et al.* combined muscle dECM with a myogenic factor, insulin growth factor-1 (IGF-1), for the tibialis anterior muscle defect model [250]. The results showed that IGF-1/muscle dECM had a significantly greater number of myofibers when compared to both collagen and muscle dECM groups after implantation.

Currently, the *in vivo* regenerative ability of muscle dECM has been confirmed by many studies. However, most studies were conducted in mice or rat models, while only a few were conducted in large animals, such as beagles or rabbits. In addition, few studies evaluate the functional recovery of muscle dECM after implantation into skeletal muscle defects. Whether additional adjuvant therapy, such as ultrasound, would increase the *in vivo* repair capacity of skeletal muscle dECM scaffolds still need further studies.

6.4 Tendon regeneration

Tendon is a compositionally complex tissue with a predominantly mechanical function: translating muscular contractions into joint movement by transmitting forces from muscle to bone [251]. Based on the anatomical structure of the tendon, tendon tissue is strongly stressed throughout the lifespan and must sustain extreme stress up to 100 MPa [252]. The total incidence rate of tendon or ligament injuries is about 1/1,000 per year [253]. Up to 46% of musculoskeletal injuries are reported as tendon injuries, including tendinopathy [254]. The tendon injury has a significant impact on the patient's quality of life and ability to meet their health goals [255]. The tendon repair process is slow due to its limited regenerative capacity and lack of blood supply [256]. When the tendon injury occurs, the fibrotic tissue between the tendon and surrounding tissue wound leads to tendon adhesion formation [257]. In addition, the rerupture rate of certain tendons, such as Achilles tendons, after surgery can be high. Based on these issues, more and more researchers have attempted to develop various engineered tendons for clinical tendon regeneration.

As the non-cellular component of tissue, ECM retains many bioactive components, such as collagen, elastin, HA, PGs, and GAGs, which initiates crucial chemical and mechanical cues for tissue regeneration [258]. In tendon tissue engineering, more and more researchers are using intact ECM as the tendon repair scaffold due to its particular biomechanical characteristics. Tao *et al.* utilized microsection technology to optimize the decellularization process, and then fabricated a bovine tendon dECM membrane [259]. The results demonstrated that the bovine tendon dECM membrane possessed good cytocompatibility and was completely degraded at 12 weeks after subcutaneous implantation. Members of the TGF-β superfamily are actively involved in tendon development and healing in a spatiotemporally specific manner [260]. Yang *et al.* demonstrated that a soluble extract of tendon dECM enhanced the proliferation and TGF-β3-induced tenogenesis of ADSCs in both plate and scaffold cultures *in vitro*, and modulated matrix deposition of ADSCs seeded in scaffolds [260]. In another study, Ning *et al.* demonstrated that bovine tendon dECM sheet possessed a similar property as that of the native tendon, including the internal ultrastructure, biochemical compositions such as collagen, GAGs, bFGF, and transforming growth factor-β1 (TGF-β1), fibronectin, and decorin, as well as substantial mechanical strength [137]. In another study, Youngstrom *et al.* successfully fabricated equine tendon dECM with native 3D architecture by a combination of freeze/thaw cycles, incubation in 2% SDS, trypsinization, treatment with DNase-I, and ethanol (EtOH)

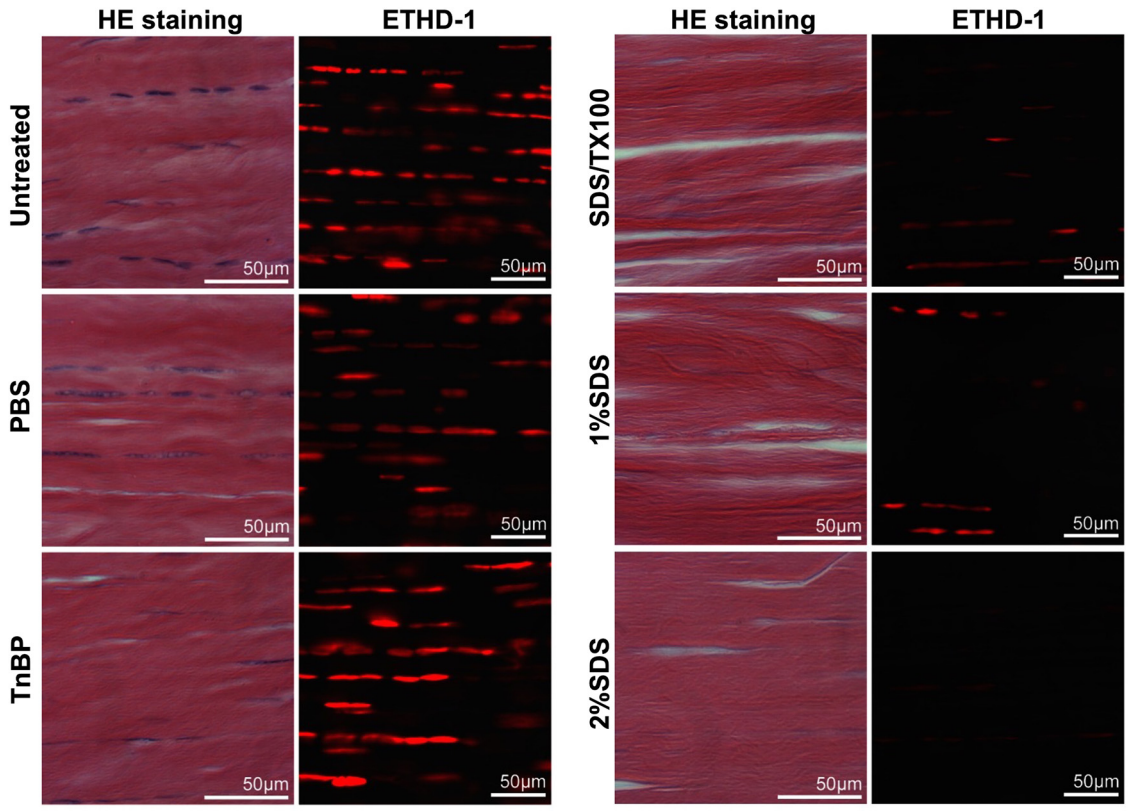

Figure 9: The HE staining and ETHD-1 staining of dECM untreated, treated with PBS, and TnBP, SDD/TX100, 1% SDS, and 2% SDS. Compared with other groups, the 2% SDS shows the best decellularization effect [261].

sterilization [261]. The results showed a marked reduction in DNA content in groups of SDS/TX100, 1% SDS, and 2% SDS (Figure 9). In addition, it has been reported that tendon dECM can promote the tendon phenotype and inhibit osteogenesis of human tendon stem/progenitor cells by regulating teno- and osteolineage-specific transcription factors Scleraxis and Runx2 [262].

Reseeding cells onto the tendon dECM has been treated as a promising approach for reconstructing damaged tendons [263]. Lohan *et al.* fabricated a tendon-like tissue by implanting porcine Achilles tendons dECM recellularized with human hamstring tendon-derived tenocytes in nude mice [264]. The *in vivo* results showed that tendons dECM recellularized tenocytes showed superior histological quality than cell-free implanted constructs [264] (Figure 10a). Song *et al.* compared tendon stem/progenitor cells with embryonic stem cell-derived mesenchymal stromal cells on the porcine dECM scaffolds [265]. The results found that compared to embryonic stem cell-mesenchymal stromal cells, tendon stem/progenitor cells combined with dECM showed more improvement in the structural and biomechanical properties of regenerated tendons *in vivo* [265]. In another study, Xie *et al.* fabricated a novel multilayered rabbit-derived tendon "book" dECM scaffold with BMSCs sheets for the repair of an Achilles tendon defect in a rabbit model [266]. The *in vivo* results showed that the book-shaped tendon dECM scaffold and BMSCs sheets could promote the regeneration of type I collagen at the wound site during healing and improve the mechanical properties of the repaired tendon [266] (Figure 10b).

6.5 Vascular regeneration

In clinical work, when musculoskeletal tissue is seriously damaged due to trauma and other reasons, it is often accompanied by severe injuries to peripheral blood vessels [267,268]. When using various tissue engineering scaffolds for musculoskeletal tissue repair, integrated repairing of the damaged blood vessels can effectively restore blood supply and shorten the repair process of musculoskeletal tissues [269–271]. Autologous vascular transplantation for peripheral vascular repair has a high failure rate due to many factors, such as the elderly donor and various comorbidities [272,273]. Currently, many synthetic materials, such as polyethylene terephthalate/Dacron, have been widely used in large-diameter vascular grafts. However, peripheral blood vessels in the musculoskeletal tissue are smaller

in diameter [274]. In addition, synthetic vascular grafts with small diameters have the disadvantages of low modulus and lack of endothelial covering, which often lead to the occurrence of intimal hyperplasia, vascular ischemia, and thrombosis [275]. Given the inherent advantages of dECM, researchers have begun to utilize cells, and 2D or tubular tissue-derived dECM for peripheral vascular repair. Dahan *et al.* fabricated vascular dECM grafts by decellularizing porcine arterial extracellular with a small diameter [276]. The results showed that vascular dECM grafts could enhance infiltration, migration, and proliferation of smooth muscle cells and HUVECs [276]. Cuenca *et al.* electrospinned polycaprolactone to the vascular dECM and loaded it with vascular endothelial growth factor (VEGF) and heparin for small-diameter vascular grafts [277]. The *in vivo* results demonstrated that the composite scaffolds could enhance the infiltration of smooth muscle cells and endothelial cells, which provides a potential vascular graft with a small diameter for vascular regeneration [277]. Kristofik *et al.* utilized a non-thrombogenic and pro-migratory ECM to modify vascular dECM grafts with a small diameter [278]. The *in vivo* results demonstrated that vascular dECM grafts after modification can enhance the reconstruction of native vessels [278]. In another study, Zhu *et al.* fabricated an anti-atherosclerotic vascular graft by incubating A20 gene-transfected endothelial progenitor cells onto vascular dECM [279]. Although many vascular dECM grafts have been constructed and exhibited a good inducing ability of endothelial cells, the repair ability of these vascular dECM grafts has not been fully investigated. In addition, most of the current vascular dECM grafts with a small diameter are used for coronary artery repair, while vascular dECM grafts with small diameter for the musculoskeletal tissue are still very rare. Microvascularization network reconstruction also plays a very important role in regeneration of musculoskeletal tissue, and further research is needed on the reconstruction mechanism of microvascular network by dECM-based scaffolds *in vivo*.

6.6 Peripheral nerve regeneration

At least 2 million people worldwide suffer annually from peripheral nerve injuries, with estimated costs of $7 billion incurred due to paralysis alone [280]. Peripheral nerve tissue damage, caused by trauma and tumor, has always been a clinical challenge for doctors due to its limited axonal regenerative capacity [281]. Peripheral nerve injury (PNI) commonly impairs movement and sensory functionalities [282]. Autologous nerve graft transplantation is the gold standard treatment for peripheral nerve tissue damage [283]. However, autologous nerve graft has the disadvantages of limited resources, multiple surgeries, high fiscal cost, and donor site morbidity [284]. Various biomaterials, such as synthetic polymers, have been applied as substitutes

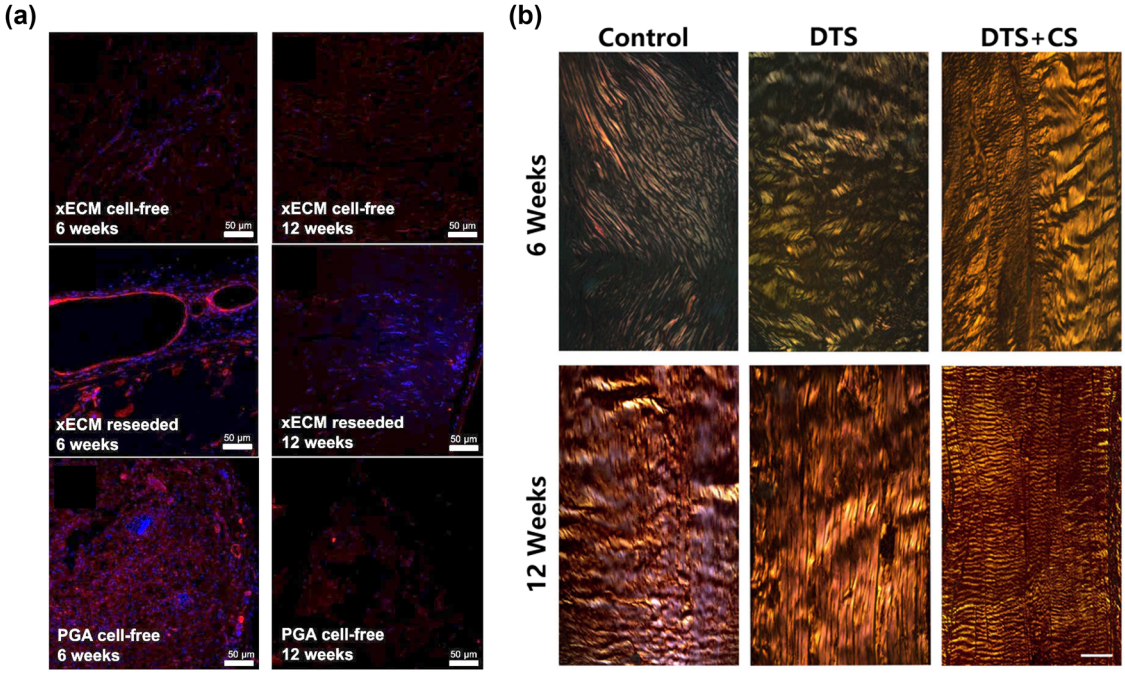

Figure 10: (a) αSMA(red)/DAPI(blue) staining of recellularized xECM after implantation [264]. (b) The collagen fiber at the healing interface. DTS, tendon dECM scaffold; DTS+CS, tendon dECM scaffold combined with cells sheet [266].

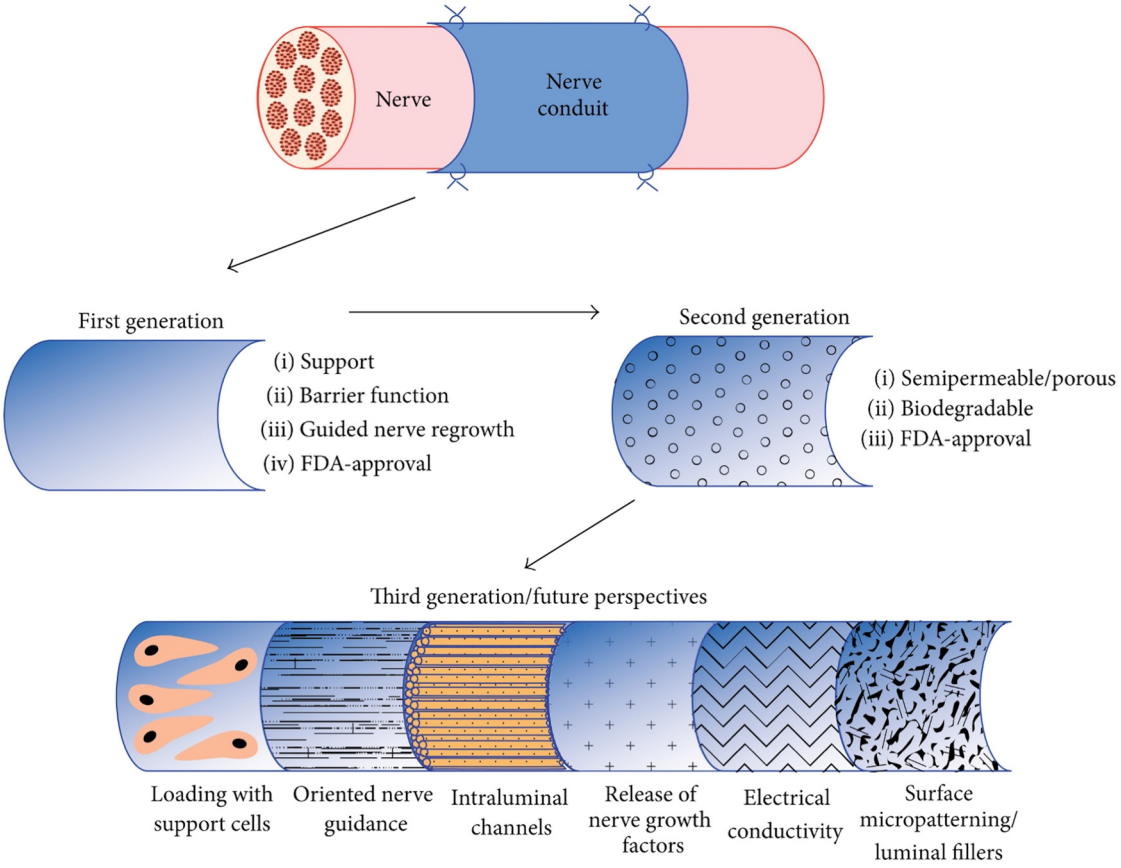

Figure 11: Schematic illustration of nerve conduit generations, including first, second, and third generations [284].

for peripheral nerve regeneration [285]. Figure 11 gives a general overview of the different nerve conduit generations [284]. However, fabricating a substitute with similar properties of the peripheral nerve by synthetic polymer is very difficult, obstructing peripheral nerve regeneration. As a promising substitute for tissue repair, dECM has been utilized in peripheral nerve regeneration. The dECM preserves various bioactive components, which can enhance the migration of Schwann cells (SCs), induce functional maturation of neural stem cells, and increase macrophage polarization [286]. Peripheral nerve repair is a complex process involving SCs proliferation and migration, formation of "Bungner bands," and new nerve extension [287]. The nerve dECM from rat sciatic nerves possesses abundant laminin and type V collagen exclusively [288]. The dECM can support the biological function of Schwann cells by providing native architecture of peripheral nerve [289].

Currently, various dECMs have been utilized in nerve regeneration. Kim *et al.* compared autograft nerve with isograft nerve dECM in rats 10 mm sciatic nerve model [290]. The *in vivo* results demonstrated that the nerve dECM grafts were fairly biocompatible and had comparable effectiveness to autografts for nerve regeneration. In another study, Zaminy *et al.* demonstrated that bovine-derived nerves dECM are a safe and effective approach to repair rat sciatic nerve injury [291]. In another study, Li *et al.* fabricated a novel nerve repair membrane derived from porcine nerves dECM, which could effectively prevent adhesion between the nerve anastomosis sites and the surrounding tissues and enhance nerve regeneration [282]. Changing the process of decellularization can effectively modulate the bioactivity of dECM scaffolds. In another study, García-García *et al.* demonstrated that using genipin as a crosslinker agent could be an efficient alternative to improve the biomechanical properties of Wistar rat sciatic nerve-derived acellular nerve allografts with a slight impact on the biocompatibility and histological pattern [292]. The light and fluorescent microscopy of native and acellular nerve allografts generated by Sondell and Roosens protocols are shown in Figure 12. Inducing axially aligned channels in nerve dECM grafts can effectively enhance cell penetration. Sridharan *et al.* utilized a unidirectional freeze drying to modify existing decellularization protocol of rat sciatic nerve dECM [280]. The results showed that the nerve dECM obtained from unidirectional freeze-drying possessed axially aligned channels and similar tensile properties to native nerve tissue.

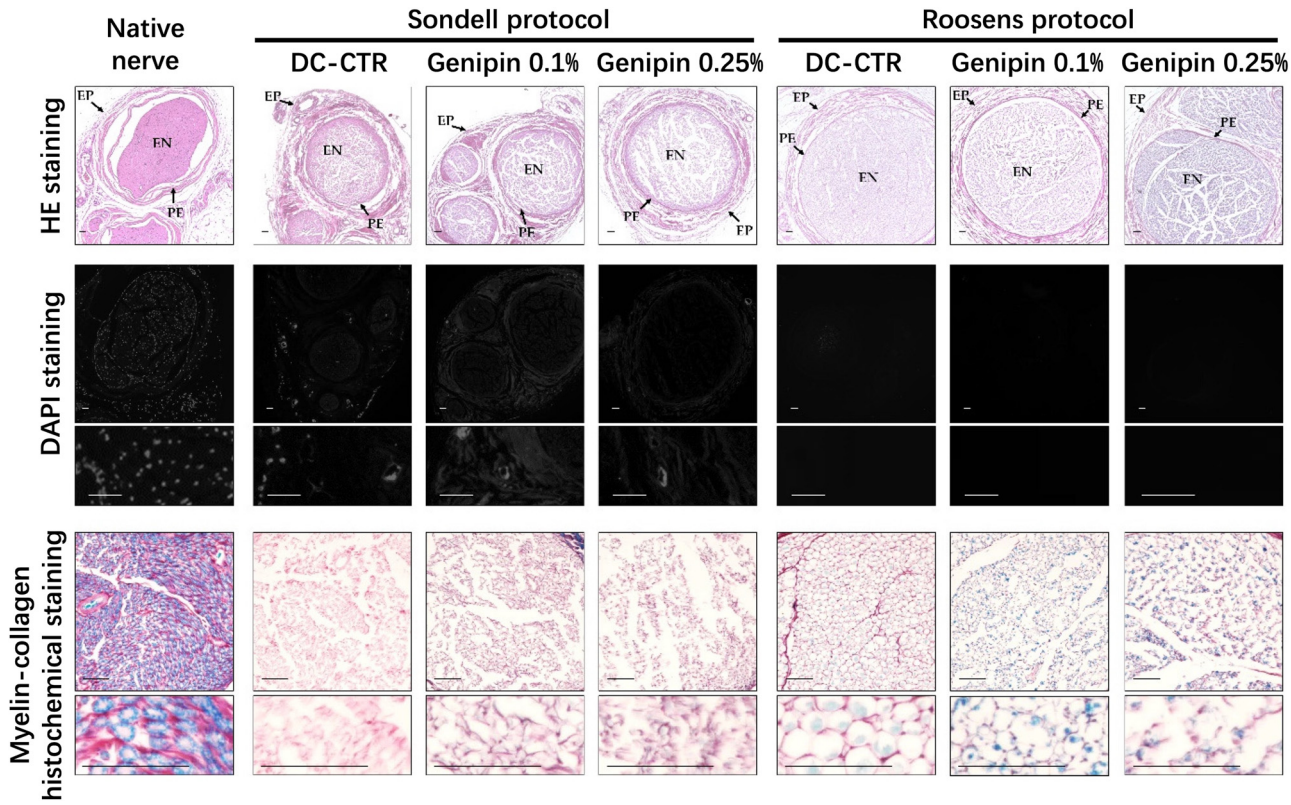

Figure 12: The HE staining, DAPI staining, and myelin-collagen histochemical staining of nerve-derived acellular nerve allografts. DC-CTR, non-crosslinked acellular nerve allografts. Genipin 0.1% and Genipin 0.25% represent acellular nerve allografts crosslinked with 0.1% genipin and 0.25%, respectively [292].

Nerve dECM derived hydrogels possess high bioactivity and can be directly injected into the injury sites of peripheral nerves. In addition, hydrogel derived from nerve dECM can also be applied to electrospinning and bioprinting for irregular nerve defects [286]. Zheng *et al.* constructed an artificial nerve guidance conduit consisting of longitudinally aligned electrospun nanofibers and porcine nerve dECM hydrogel for sciatic nerve regeneration [293]. In another study, Kong *et al.* successfully utilized pure porcine nerve dECM conduits to fabricate nerve dECM scaffolds using an electrospinning technique without other additives [294]. The *in vivo* results showed that the nerve dECM scaffolds significantly promoted the regeneration of rat sciatic nerve. In another study, the porcine nerve-derived dECM hydrogels were used as fillers in the lumen of a silicone nerve guide and placed into an 8 mm rat sciatic space model [295] (Figure 13a). The results demonstrated that porcine nerve-derived ECM hydrogels within the conduit improved electrophysiologic response and axon counts compared to empty conduit controls [295] (Figure 13b). Lin *et al.* confirmed that porcine nerve dECM hydrogel exhibited a nanofibrous structure similar to that of natural ECM and a ~280 Pa storage modulus at 10 mg/mL similar to that of native neural tissues [296]. When the lesion area is connected with a rigid tubular structure and this is filled with a hydrogel, there is a mechanical support and a suitable substrate for axonal growth [297]. In addition, nerve dECM derived hydrogels can also be applied as carriers for growth factors [297] (Figure 13c). Glial-derived neurotrophic factor (GDNF) can induce the migration of SCs and acts as a neurotrophic factor for motor axons [297]. Qiu *et al.* modified the nerve dECM scaffolds by supplementing nerve dECM hydrogel (DNMG) and GDNF and then bridged a 50 mm sciatic nerve defect in a beagle model [298]. The results demonstrated that modification of nerve dECM scaffold with DNMG and GDNF is a potential treatment of long nerve defects [298]. Li *et al.* incorporated nerve growth factor (NGF) and VEGF into porcine nerve dECM hydrogel for the treatment of PNI [299]. The porcine nerve dECM hydrogel loaded with NGF and VEGF exhibited a controlled release manner, which provides a nerve regenerative strategy based on nerve dECM hydrogel for growth factor delivery [299].

Combining stem cells with nerve dECM is another potential therapeutic strategy for repairing peripheral nerves [300]. Stem cell implantation decreases muscular atrophy while secreting various growth factors, such as NGF and brain-derived neurotrophic factor, for facilitating the sorting of

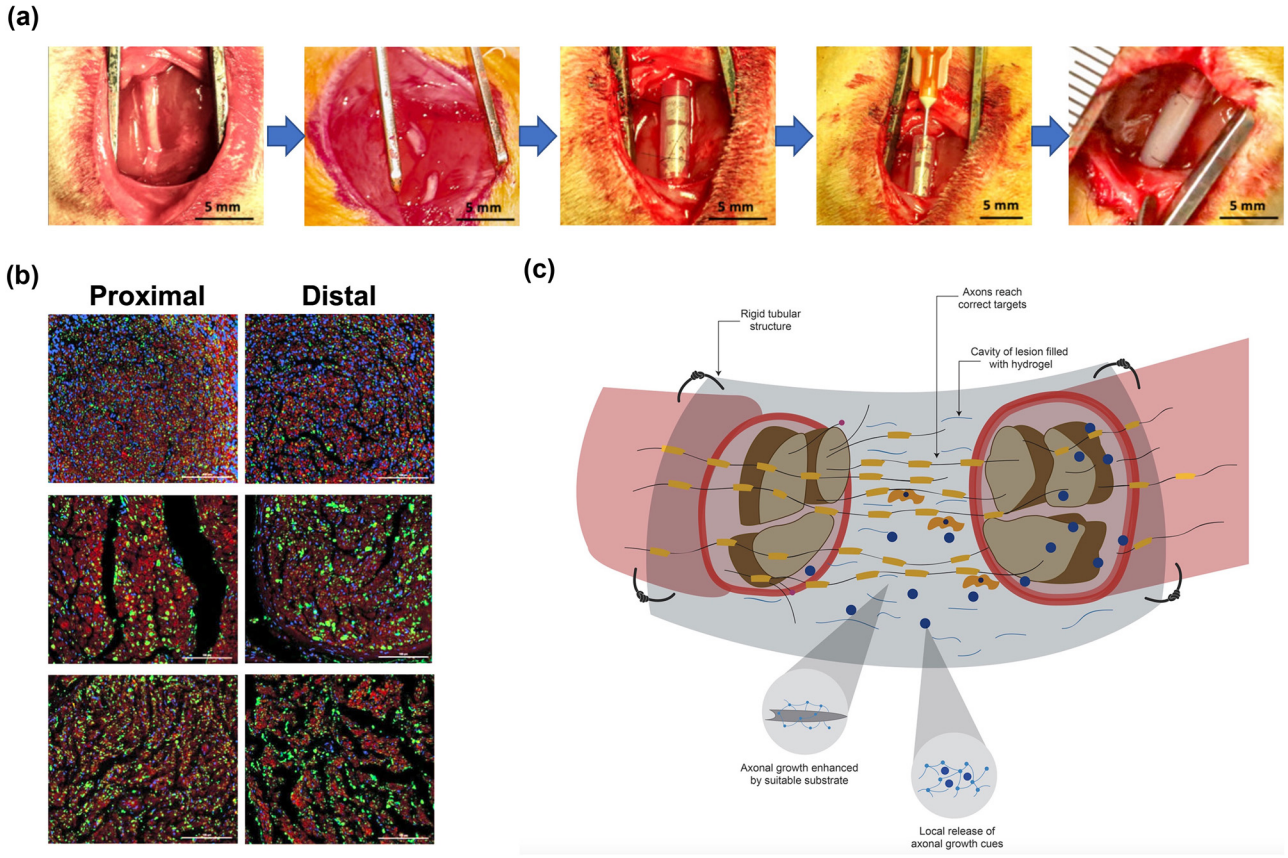

Figure 13: (a) Schematic illustration of implantation of porcine nerve-derived dECM hydrogels [295]. (b) The Axons(green)/myelin(red)/nuclei(blue) staining of porcine nerve-derived dECM hydrogels after implantation [295]. (c) The schematic illustration of repair mechanism after implantation. The dECM-based hydrogel provides mechanical support and acts as a carrier of bioactive agents [297].

axons and myelination and reducing inflammation [301]. In addition, seeding stem cells onto nerve dECM allografts permits the secretion of neurotrophic and angiogenic factors that can stimulate nerve regeneration [302]. Zhao *et al.* utilized fibrin to affix BMSCs around the nerve dECM graft for a 15 mm nerve defect in rats [303]. The results showed that the MSCs around the nerve dECM graft could effectively enhance nerve regeneration and functional recovery of peripheral nerve lesions in a 15 mm Sprague-Dawley rat sciatic nerve defects [303]. A cell sheet is a layer of cells that contains intact ECM and cell surface proteins such as growth factor receptors, ion channels, and cell-to-cell junction proteins [304]. Nakada *et al.* fabricated a sheet of stem cells derived from adipose tissue (ADSC sheet) and wrapped allogeneic nerve dECM implant to repair the sciatic nerves of Sprague-Dawley rats [305]. Compared with allogeneic nerve dECM group, the neurofilament-positive areas and axon density of the ADSC sheet-wrapped nerve group were significantly higher, which is attributable to the secretion of growth factors of ADSCs [305]. The nerve repair ability of stem cells has been confirmed by many studies [306,307]. However, the long-term fate of implanted stem cells and their definitive mechanism of action *in vivo* is not well explored [308,309]. Rbia *et al.* performed research to rack the *in vivo* distribution, and survival of MSCs seeded on a nerve dECM allograft reconstruction of a peripheral nerve defect [310]. The *in vivo* results showed that labeled MSCs seeded on the nerve dECM allograft could be detected for up to 29 days. In addition, the implanted MSCs were not detected anywhere other than the site of surgery [310].

7 dECM and bioprinting

As a promising method to construct 3D tissue substitutes, 3D bioprinting could precisely control complex 3D architecture, multiple compositions, and spatial cell distributions for various tissue regeneration, such as bone, cartilage, skin, nerve, and tendon [311,312]. Bioinks selection is the first and crucial step in the process of bioprinting [313]. Bioinks can provide a stable 3D architecture to modulate the development and maturation of tissue [314]. An ideal bioink should have the

advantages of good printability, biocompatibility, structural stability, and mechanical properties [315]. In addition, the ideal bioink possesses good suitability for chemical modifications to meet tissue-specific needs [316]. Various biomaterials have been used as bioink for the construction of tissue repair substitutes. The bioinks in the field of tissue engineering are classified as natural bioinks and synthetic bioinks [317]. Generally, synthetic bioinks, such as polyethylene glycol and pluronic acid, are easier to tailor for efficient printability [318]. On the other hand, nature-derived bioinks, such as dECM-based bioinks, have more significant potential to support cell viability and growth than synthetic bioinks [319].

The porosity and pore interconnectivity of scaffolds are critical for cell infiltration and for directing tissue formation and function [320,321]. Traditional scaffold fabrication uses methods such as solvent casting/particulate leaching, gas foaming, or freeze-drying to generate and control pore structure and porosity. However, with the development of biomimetic scaffold materials, the fabrication of the inner geometry of the scaffold and the control of cell distribution cannot be achieved by traditional processes. At present, microfabrication tools, fiber-based technologies, and 3D bioprinting techniques have become emerging tools for tissue engineering. They play an essential role in constructing dECM-derived hydrogel scaffolds. Using the 3D bioprinting technique to print all the components of the tissue, and reproduce the structure similar to the tissue, to facilitate the function of the host cells and complete the regeneration of the damaged tissue, which has become the development direction of bioprinting technology. The use of dECM as a matrix material for bioprinting not only represents the complexity of native ECM but also possesses specific tissue-derived properties, a microenvironment that helps cells recreate their intrinsic morphology and function. Pati *et al.* developed a method for bioprinting cell-laden structures using porcine-derived cartilage tissue dECM as a bioink that provides an optimized microenvironment for three-dimensional tissue growth. A vital advantage of this approach is the application of tissue-specific ECM, which can provide critical clues to cell engraftment, survival, and long-term function [139].

8 Musculoskeletal tissue-specific *in vitro* models based on dECM

Currently, more and more researchers developed musculoskeletal tissue-specific *in vitro* models derived from healthy or disease tissues to study the molecular mechanism of musculoskeletal disease progression [322]. In addition, musculoskeletal tissue-specific *in vitro* models can be applied in evaluating the cellular response of various stimuli, including treatments and environmental factors [323]. While the critical step in fabricating musculoskeletal tissue-specific *in vitro* models is to fully mimic the complex biochemical and inner structural features of musculoskeletal tissues [324]. ECM cues play an important role in musculoskeletal tissue metabolism and musculoskeletal disease progression [138,325,326]. In addition, dECM meets essential criteria for optimized musculoskeletal tissue-specific *in vitro* models by mimicking the complex physical, biochemical, and architectural features of the microenvironment of healthy and diseased musculoskeletal tissues [210,327].

Although dECM possesses inherent advantages in the construction of musculoskeletal tissue-specific *in vitro* models, most studies focus on the fabrication of skeletal muscle dECM models [322]. Currently, various skeletal muscle dECM models are mainly used to study the interaction mechanism between cells and ECM. In addition, the researchers also utilize skeletal muscle dECM models to investigate the effect of various stimuli, such as drug, pH, and biological factors, on the ECM or cells reseeded onto dECM. Zhang *et al.* seeded muscle stem cells onto muscle dECM constructs derived from arsenic-exposed muscles to investigate the influence of arsenic on muscle stem cells niche [328]. Wassenaar *et al.* investigated the nature of the drug-ECM interaction by utilizing skeletal muscle dECM, which creates a new use of skeletal muscle dECM as a potential *in vitro* model to investigate the interaction mechanism between drugs and ECM [329]. In another study, Stearns-Reider *et al.* studied the influence of aged skeletal muscle ECM on muscle stem cells by using skeletal muscle dECM models [330]. The use of dECM to construct musculoskeletal tissue-specific *in vitro* models derived from healthy or diseased tissue can help to increase the understanding of pathological states of various musculoskeletal tissues, such as injury or degeneration, and help to develop dECM-based scaffolds targeting tissue microenvironment regulation for musculoskeletal tissue regeneration.

9 Challenge and future perspectives

With the rapid development of tissue engineering and regenerative medicine, more and more dECM-based scaffolds have been successfully fabricated and applied in

various tissue regeneration, especially musculoskeletal tissues. In addition, the inner native microenvironment of dECM can provide biochemical and spatial cues to support the survival of cells and modulate the process of musculoskeletal tissue remodeling. While the composition and inner structure of dECM vary from different decellularization. In addition, cell residues in dECM can induce immune rejection and decrease the therapeutic effects of dECM, so it is essential to choose the suitable decellularization method to remove cellular residues. Various physical, chemical, and biological/enzymatic or a combination of these methods have been used to decellularize tissue or organs. All these decellularization methods possess specific advantages, but few studies focus on the differences between these decellularization methods. How to change the process of decellularization to control the physicochemical characteristics of dECM, such as mechanical strength and porosity, still need further studies. In addition, the differences between different species need to be investigated for the next step of musculoskeletal tissue repair. In addition, most studies demonstrated the *in vitro* and *in vivo* tissue regenerative ability of dECM. However, it is difficult to figure out which specific components or combinations enhance the tissue repair.

Although the dECM-based scaffolds can effectively promote tissue regeneration and repair, the decellularization process would inevitably destroy the microstructure of native tissue, resulting in the decrease in the mechanical properties of dECM-based scaffolds [331–333]. In addition, dECM-based hydrogels also face the same challenge [334]. In order to expand the application of dECM-based scaffolds in the regeneration of musculoskeletal tissues, it is urgent to optimize and improve the mechanical properties of dECM-based scaffolds after decellularization [335,336]. Currently, crosslinking is a common method to optimize the properties of tissue-engineered scaffolds. In addition, the crosslinking can be used to improve the mechanical properties and biological characteristics of dECM-based scaffolds [337,338]. Generally, crosslinking can be classified into chemical crosslinking, physical crosslinking, and natural crosslinking. Chemical crosslinking refers to the crosslinking process conducted by chemical agents, such as glutaraldehyde or epoxy compounds. However, the residual chemical agents used for crosslinking are cytotoxic, which may inhibit the process of tissue repair after the implantation of dECM-based scaffolds. Physical crosslinking methods mainly include photooxidative crosslinking, thermal dehydrogenation, and ultraviolet irradiation. Although the addition of cytotoxic chemical crosslinking agents is avoided, the crosslinking efficiency of physical crosslinking is low. Natural crosslinking agents mainly include genipin, tannins, and proanthocyanidins [339]. Pinheiro *et al.* investigated the effects of three natural crosslinking agents, including genipin, proanthocyanidin, and epigallocatechin gallate, in adjusting the mechanical properties of porcine cartilage dECM [340]. The results demonstrated that all these natural crosslinking agents could increase the aggregate modulus of porcine cartilage dECM [340]. In addition, by combining other biomaterials, the mechanical properties of dECM-based scaffolds can also be improved [341,342]. In order to increase the mechanical properties of the cartilage dECM hydrogel, Beck *et al.* methacrylated porcine cartilage dECM and created methacrylated solubilized decellularized cartilage (MeSDCC) gels [343]. The results demonstrated that the methacrylated gelatin could effectively enhance the compressive modulus of MeSDCC gels [343]. In addition, the elastic compressive modulus of MeSDCC gels is similar to native porcine cartilage [343]. As a technology for preparing nanofibers, electrospinning has the advantages of high efficiency, low cost, and easy implementation, and has become one of the main methods for constructing tissue-engineering scaffolds [344]. The introduction of electrospinning technology into the construction of dECM-based scaffolds can greatly improve the mechanical properties of composite scaffolds [345,346].

Sterilization is another important step after decellularization [347,348]. Sterilization can effectively kill microorganisms colonizing the surface of the dECM-based scaffolds, fabricating a sterile microenvironment for cell adhesion and tissue repair [349]. However, sterilization inevitably impairs the biological activity and physicochemical properties of the dECM-based scaffolds. Preserving the biological activity and physicochemical properties of dECM-based scaffolds during the process of sterilization still remains a challenge [347]. Therefore, how to choose the appropriate sterilization method is essential to preserve the bioactive functions of dECM-based scaffolds. Currently, sterilization methods mainly include ethylene oxide, PAA, H_2O_2, Gamma or electron beam radiation, alcohol immersion, ultraviolet ray, and supercritical carbon dioxide [350–353]. Ethylene oxide has the advantage of strong penetrability, while ethylene oxide can react with water in scaffolds to produce toxic residuals. Gamma radiation possesses the advantages of strong penetrability and no residual toxicity. While Gamma radiation would impair the bioactivity of the dECM-based scaffold. The previous study demonstrated high Gamma radiation at 25 kGy, which impaired the architecture of dECM-based scaffolds significantly [354]. While a 5 kGy radiation dose might impair the bioactivity slightly and also be enough for sterilization [354]. PAA, EtOH, H_2O_2, and UV ray have the advantages of nontoxic products residual. Gosztyla *et al.* investigated the effect of four pathogen clearance protocols on dECM-based scaffolds, namely 0.1% PAA,

0.18% PAA + 4.8% EtOH, 0.08% PAA + 1% H_2O_2, and UV sterilization [355]. The results demonstrated that all these sterilization methods were equally effective [355]. In addition, all these sterilization methods affect the microstructure of dECM significantly [355]. In another study, Fidalgo *et al.* utilized antibiotics/antimycotic cocktail and PAA to sterilize the porcine and bovine pericardium dECM [356]. The results demonstrated that this two-step sterilization method can maintain the inner structure of dECM-based scaffolds [356]. However, the surface properties of dECM-based scaffolds were impaired during the process of the two-step sterilization method [356]. The previous study confirmed that supercritical carbon dioxide could be used for the decellularization and sterilization of dense cartilaginous biomaterials [357]. However, few studies focus on whether supercritical carbon dioxide would affect the bioactivity of dECM-based scaffolds. Although most existing sterilization methods will inevitably damage the internal structure of the dECM-based scaffolds, damage to the integrity of dECM-based scaffolds caused by sterilization can be reduced by adjusting the sterilization protocol, such as shortening sterilization time and decreasing the radiation dose. Furthermore, choosing the right sterilization method for different tissues, such as hard or soft tissues, also helps to protect the structural integrity of the dECM-based scaffolds. In addition, the differences between different sterilization methods when sterilizing various musculoskeletal tissues still need further research.

In recent years, several commercial products derived from dECM, such as ArthroFLEX®, HST-003, Allopatch HD™, GraftJacket®, and XenMatrix™, have been used to repair and regenerate musculoskeletal tissues in clinical trials [334,358]. Compared with other synthetic matrices, dECM materials are biocompatible and stable. Currently, most of these commercial dECM materials are powders or sheets, and the composition is relatively simple. More complex dECM-derived scaffolds are being developed, but are still in the early stages of translational research [358]. Compared with the commercial dECM materials, the present dECM materials reported in current research can be fabricated into various forms, such as hydrogel, sheets, pads, particles, powders, and solutions. For example, dECM hydrogels can serve as scaffolds for transplantation and injection [359]. In addition, various bioactive components, such as growth factors and drugs, can be incorporated or functionalized onto dECM-derived materials for the treatment of complex musculoskeletal tissue defects. Recently, 3D bioprinting technology has been widely used in fabricating custom tissue-engineered scaffolds with complex inner structures [360]. Combined with 3D bioprinting technology, the present dECM materials can act as bioinks to carry various cells for fabricating disease models. Compared with commercial dECM materials, the present dECM materials in the current studies are more versatile in function and have a wider range of applications. However, compared with the specific function of simple commercial dECM materials, there are still large differences in the effect of the present dECM materials in the current studies due to the addition of various components. Most present studies applied the dECM materials in animal studies, and more clinical studies are needed to confirm the regenerative and repair effect of present dECM materials on human tissues. In addition, the biosafety and indications of the present dECM materials still need to be confirmed by further studies.

Achieving complete tissue regeneration is the ultimate goal of clinical applications of dECM materials. The current clinical application of dECM materials mainly focuses on heart and skin repair. However, there are few studies focusing on the clinical application of dECM materials in musculoskeletal tissue regeneration. Dziki *et al.* reported that a 13 year-old patient with VML was treated by implantation of dECM derived from the mammalian [361]. Six months after dECM materials implantation, the muscle strength showed an improvement of 37%, and the range-of-motion and functional tasks showed an improvement of 7.1% [361]. In addition, the results of immunolabeling of ultrasound-guided biopsies confirmed that the dECM effectively enhanced skeletal muscle formation [361]. In another clinical study (ClinicalTrials.gov Identifier: NCT01292876), 17 patients suffering from injury with loss of skeletal muscle tissue were treated with dECM materials [358]. HST003 is a kind of dECM derived from human dermal fibroblasts under hypoxic conditions. In a phase 1/2 clinical trial (ClinicalTrials.gov Identifier: NCT05082831), HST003 was injected into subchondral bone following microfracture surgery for cartilage regeneration [358]. The regenerative repair ability of dECM materials on musculoskeletal tissue has been confirmed by a large number of *in vitro* studies and animal studies. However, clinical studies focusing on applying dECM in musculoskeletal tissue regeneration are still at an early stage. Compared with animal studies of dECM, clinical studies of dECM mainly focus on function recovery after implantation. In addition, the number of cases included in clinical studies is still far less than that of animal studies. Enriching the evaluation indicators of clinical studies can help to evaluate the repair effect of dECM on human musculoskeletal tissue more accurately.

10 Conclusion

As a promising biomaterial, dECM, derived from cells, tissues, and organs, has attracted more and more interest in

repairing various musculoskeletal tissue, such as bone, and cartilage, due to its good biocompatibility, biodegradability, and ability to mimic a microenvironment similar to native ECM. In addition, compared with other biomaterials, dECM contains many bioactive molecules, which can regulate the cellular behaviors of various cells, including osteoblasts, skeletal muscle cells, tendon cells, and vascular endothelial cells. However, the clinical application of dECM with tissue-specific properties to musculoskeletal tissue repair still needs to overcome some key limitations. First, various decellularization methods possess specific advantages, but few studies focus on the differences between these decellularization methods. Second, although the dECM-based scaffolds can effectively promote tissue regeneration and repair, the decellularization process would inevitably destroy the microstructure of native tissue. How to change the process of decellularization to control the physical and chemical properties of dECM, such as mechanical strength and porosity, still need further studies. Third, in order to expand the application of dECM-based scaffolds in the regeneration of musculoskeletal tissues, it is urgent to optimize and improve the mechanical properties of dECM-based scaffolds. Fourth, sterilization is an important step before dECM-based scaffolds are used for implantation. Preserving the biological activity and physicochemical properties of dECM-based scaffolds during the process of sterilization still remains a challenge. Fifth, there is still no consensus on the clinical application of dECM-based scaffolds when dealing with musculoskeletal tissue defects. The indications for dECM-based scaffolds and their long-term outcomes *in vivo* still need further research. As a whole, with the development of tissue engineering and regenerative medicine and the rise of material processing technologies, the fabrication procedure of dECM-based scaffolds will be further optimized, and more and more dECM-based scaffolds with better ability of musculoskeletal tissue repair will gradually appear.

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