The Potential of Decellularized Extracellular Matrix Scaffolds in Tendons and Ligament Repair and Regeneration

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Abstract. The repair and regeneration of tendons and ligaments represent a continues clinical challenge. Ligaments and tendons are lack of blood supply and are subjected to constant stress, the regeneration process would take a long time. The conventional methods have limitations such as infection risks, high reintervention rates, and stress deprivation. Regenerative medicine and tissue engineering provide the potential for regenerating torn or ruptured tissues. Scaffolds are an essential component of tissue engineering, which provide physical support for cell adhesion and proliferation and provide tissue structure, similar to the extracellular matrix (ECM) in original tissue. ECM is the three-dimensional macromolecular network that provides support and connects cells. The decellularized ECM (dECM), which is derived from natural or engineered tissues, exhibits superior biocompatibility, mechanical properties closer to native tissues, and biodegradability, making it a suitable scaffold for tendon and ligament regeneration. This review summarizes the advanced research on dECM tissue engineering scaffold in ligament regeneration, compares several commonly used decellularization methods and the mechanical properties of the resulting regenerated ligament tissue. Moreover, discussing the methods used to enhance the mechanical properties and structural stability of dECM scaffold, the research trends in dECM as a scaffold for ligament regeneration are proposed.

Keywords: Regenerative medicine, extracellular matrix, decellularization, tissue engineering scaffold.

1. Introduction

In the worldwide, there are always challenges in the clinical repair and regeneration of ligaments and tendons. The injuries affect hundreds of thousands of athletes each year and seriously impact their future sports careers [1]. The damage caused by sports injuries, trauma, or infections lead to loss of function in muscles, joints, and bones [1, 2]. These functions loss thereby negatively affecting the patient's normal life and sports performance, with a serious economic burden. Compared with muscles, the ligaments and tendons have poor blood supply and are usually under constant mechanical load [3]. Therefore, the repair and regeneration process would take a long time. The conventional repair therapy uses implants to strengthen the connection between ligaments and bones [4]. However, these implants will not promote ligament regeneration at the cellular level, and will also increase the risk of infection [5]. Moreover, this therapy has a high reintervention rate, which cause multiple injuries to patients [4, 5]. And the regenerative therapy is also used to treat ligament tears and ruptures. The artificial ligament implants are used to replace the injured ligament tissue. But the artificial ligaments have the limitations of low biocompatibility, low cell viability, mismatched mechanical properties and high immunogenicity.

Over the previous decades development in tissue engineering and regenerative medicine, the importance and indispensability of tissue engineering scaffolds have been demonstrated by numerous studies [6, 7]. Tissue engineering scaffolds improve the biological and mechanical properties of engineered tissues and enhance the cell adhesion, which provides support for cell proliferation and differentiation [7]. The tissue engineering scaffolds are typically fabricated using additive manufacturing methods [8]. There are various additive manufacturing methods, such as 3D printing and electrospinning, can be used to fabricate scaffolds with better strength, biocompatibility, and

cellular activity [6, 9]. These scaffolds are able to support cell proliferation and differentiation to form target tissues, which mimic the structure and functions of the extracellular matrix (ECM) [10].

In human-derived tissue, ECM is the three-dimensional macromolecular network that provide support and connect cells [11]. The main components of ECM include various collagens, glycoproteins, and proteoglycans [12]. These matrix components interact with cell adhesion receptors (e.g. integrin) to form a complex network [12]. As showed in Fig. 1, this network provides a physical scaffold for cell proliferation and activities and regulates cell function and physiological processes through signal transduction [4, 12-14]. In tissue engineering research, synthetic polymers and natural materials (e.g. silk fibroin, alginate, etc.) are synthesized as composites, and various fabrication methods have been used to fabricate tissue engineering scaffolds to mimic the unique 3D structures of the ECM [15]. However, compared with the native ECM, the artificial tissue scaffolds have limitations in mechanical properties, biocompatibility, cell activity, and high immunogenicity, which proves the artificial tissue scaffolds still cannot perfectly simulate the functions of the native ECM [16]. Therefore, people thought of using the ECM itself directly as a tissue engineering scaffold.

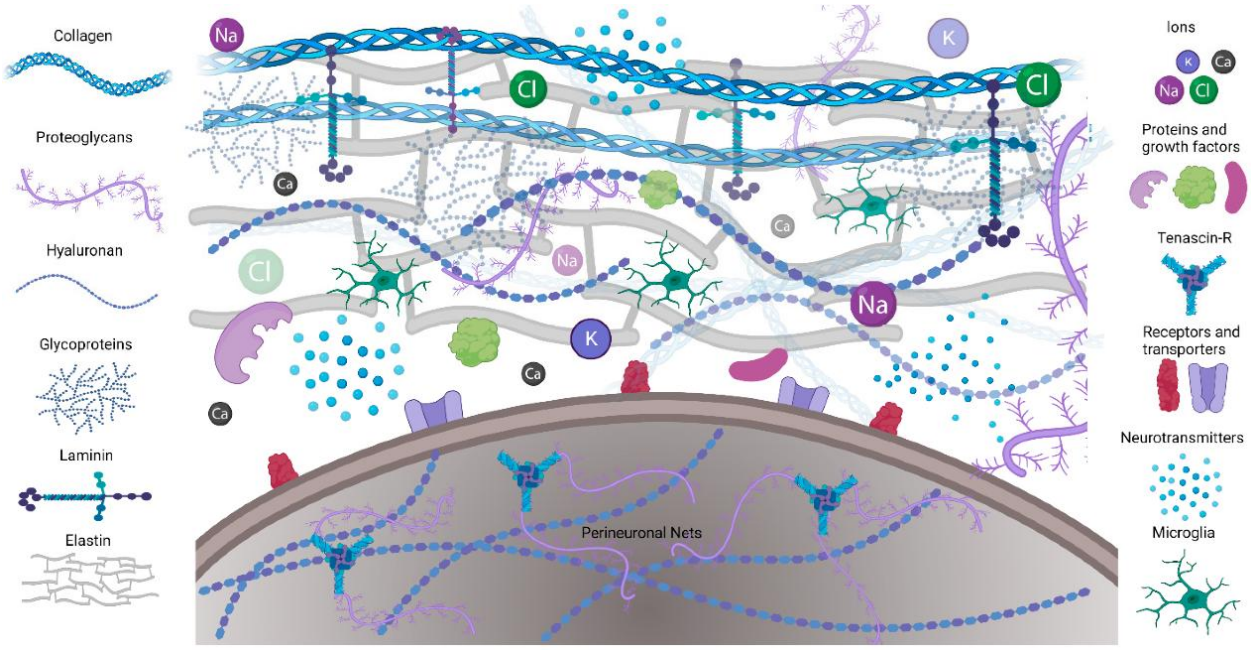

Figure 1. Schematic illustrating the ECM composition [14]

Decellularization is a treatment method that removes cellular components from tissues, leaving only the extracellular matrix (ECM). Numerous past and ongoing studies are exploring appropriate decellularization methods for different tissues. Decellularized tissues retain the ECM, which, after washing, becomes a dECM scaffold, suitable for further tissue engineering studies. The effectiveness of dECM scaffolds in regenerative medicine is influenced by a combination of tissue source, decellularization methods, evaluation of decellularization, and recellularization strategies.

2. Decellularized ECM tissue engineering scaffold

In recent research, the most commonly researched tissue engineering scaffolds are fabricated from synthetic materials. Synthetic materials have controllable physical properties, and they are easy to process. However, they cannot fully reproduce the complex signaling networks of the extracellular matrix. The dECM scaffolds are more similar to the native tissue environment. They not only exhibit better biocompatibility and offer stronger support for cell adhesion and organization, but also have higher biological activity, which promotes cell proliferation, differentiation, and the synthesis of new ECM. In addition, dECM scaffolds are biodegradable, the dECM scaffold would be resorbed and replaced by newly produced ECM during cell proliferation. The mechanical properties of dECM scaffolds also offer guidance and stimulation during cell proliferation, particularly promoting the

alignment of fibroblasts on the scaffold. Therefore, dECM scaffolds show great potential for ligament repair and regeneration in tissue engineering.

2.1. Tissue Sourcing

The properties of dECM scaffolds are highly related to the source tissue [17]. The commonly researched tissue sources include autologous, allogeneic, xenogeneic tissues, and tissues produced through in vitro culture. In clinical applications, autologous and allogeneic tissues are the most direct options, such as tendons or ligaments obtained from patients or donors [18]. These tissues have highly structural and compositional similarity with the target ligament, which provide enough mechanical and biological features. However, this method faces limitations including the shortage of donor tissues, ethical problems, and the risks of immune rejection.

Many studies have proved that xenogeneic tissues, such as porcine or bovine tendons and pericardium, are also potential tissue sources of tendon and ligament dECM scaffold. These tissues are readily available at a lower cost. And their immunogenicity is significantly reduced by the decellularization process. Therefore, xenogeneic tissues are an important alternative to human-derived sources. In recent studies, engineered tissues grown on artificial scaffolds or ECM scaffolds that were constructed in vitro have also been proven to be promising options. Fibroblasts, tendon cells, or stem cells can be cultured in vitro to secrete and deposit ECM. This type of dECM solves the limitation of donor shortage and can also be customized by changing cell types, culture conditions, and growth.

2.2. Decellularization Methods

The aim of decellularization is to remove cellular components and immunogenic substances while preserving the structure, components and functions of the ECM [18, 19]. According to recent studies, the commonly used strategies could be classified into three main categories: physical, chemical, and enzymatic methods [20].

The physical methods remove cells primarily by disrupting the cell membrane through external forces. The repeated freeze–thaw cycles can cause membrane rupture and the release of intracellular contents, which is suitable for some soft tissues [21]. The high hydrostatic pressure (HHP) is a recently announced physical technique [22]. The tissue will be subjected to hundreds of megapascals of pressure, the pressure rapidly disrupts cell membranes while causing minimal damage to the ECM structure.

The chemical decellularization methods mainly involve the use of surfactants to remove cells. The commonly used ionic surfactants, like sodium dodecyl sulfate (SDS), is able to disrupt cell and nuclear membranes and eliminate DNA effectively [23]. But it may also damage the collagen and other structural proteins. And the non-ionic surfactants, such as Triton X-100, are milder and are applied in combination with other methods. Other chemical agents, including hydrogen peroxide, ammonium hydroxide, and deoxycholic acid, could also be used in specific tissues. However, they react more aggressively with tissues, leading to the degradation of ECM components and structure. Therefore, a balance must be maintained between cell removal efficiency and ECM preservation [19].

The enzymatic methods mainly rely on the enzymes to degrade cellular components [16]. DNase and RNase are commonly employed to degrade residual nucleic acids, while trypsin can be used to remove the extracellular proteins. The enzymatic treatment is usually combined with physical or chemical methods to enhance decellularization efficiency while minimizing ECM damage.

2.3. Evaluation of the decellularization

The quality of decellularization directly determines the safety and functionality of dECM scaffold in tissue engineering. An ideal decellularization method should be able to remove cells and immunogenic components completely, while preserving the structure, composition, and bioactivity of the ECM [19]. It should also have non-cytotoxicity and good biocompatibility [19]. Therefore,

researchers evaluate the decellularization by the content of cellular residue, the integrity of ECM structure and composition, mechanical properties, immunogenicity, and cell viability [24].

2.3.1. Cell residue detection

The residual DNA content and nuclear integrity are the key criteria for evaluating the effectiveness of decellularization. The fluorescence-based quantitative assays are accurately measure DNA content in tissues. Histological staining techniques, such as DAPI and H&E staining, provide direct visualization of nuclear residues. For scaffolds intended for use in tissue engineering, the accepted standards include a residual DNA content of less than 20 ng per mg of dry tissue and a DNA fragment length of less than 200 bp.

2.3.2. ECM structure and composition integrity

The main components in ECM are collagen and glycosaminoglycans, which determine the mechanical properties of the ECM scaffold and play important roles in regulating cell behavior. Scanning electron microscopy (SEM) can be used to check the fiber orientation, porosity, and three-dimensional structure of the ECM. Furthermore, immunohistochemistry and immunoblotting are used to assess the retention of key molecules, including collagen types I, III, and IV, as well as laminin.

2.3.3. Immunogenicity and cytotoxicity

Common methods for evaluating the immunogenicity of scaffolds include immunohistochemistry, Western blotting, and ELISA, which are used to detect residual levels of major immune-related molecules such as MHC antigens and α-Gal epitopes.

3. Recent research

3.1. Porcine Serous Pericardium dECM Ligament Tissue Scaffold

In the study by Suzuki et al. porcine serous pericardium was used to fabricate a dECM scaffold for ligament in vitro reconstruction [25]. The combination of decellularization methods was applied and showed promising results. Specifically, the tissue was treated with high hydrostatic pressure (HHP), followed by washing with DNase and $MgCl_2$ solution to enhance decellularization efficiency. For comparison, a control group was decellularized by perfusion with surfactants, enabling evaluation of dECM scaffolds generated by different methods.

To evaluate the decellularization efficiency, DNA quantification was performed to measure residual cellular components. The residual DNA content of tissues treated with HHP and surfactants was 33.1 ± 0.8 ng/mg and 46.6 ± 0.9 ng/mg of dry weight, respectively. H&E staining and scanning electron microscopy (SEM) were also used to test the structure and morphology of the dECM scaffolds. As shown in Fig. 2, there were no marked changes in ECM shape or thickness. In HHP treated tissues, small gaps were observed between fiber bundles, whereas larger gaps were present in tissues treated with surfactants. Moreover, the SEM images revealed that fibers in HHP treated tissues have a wavy and aligned pattern along the fiber axis, while surfactant-treated tissues showed fiber disorganization and breakage (Fig. 3).

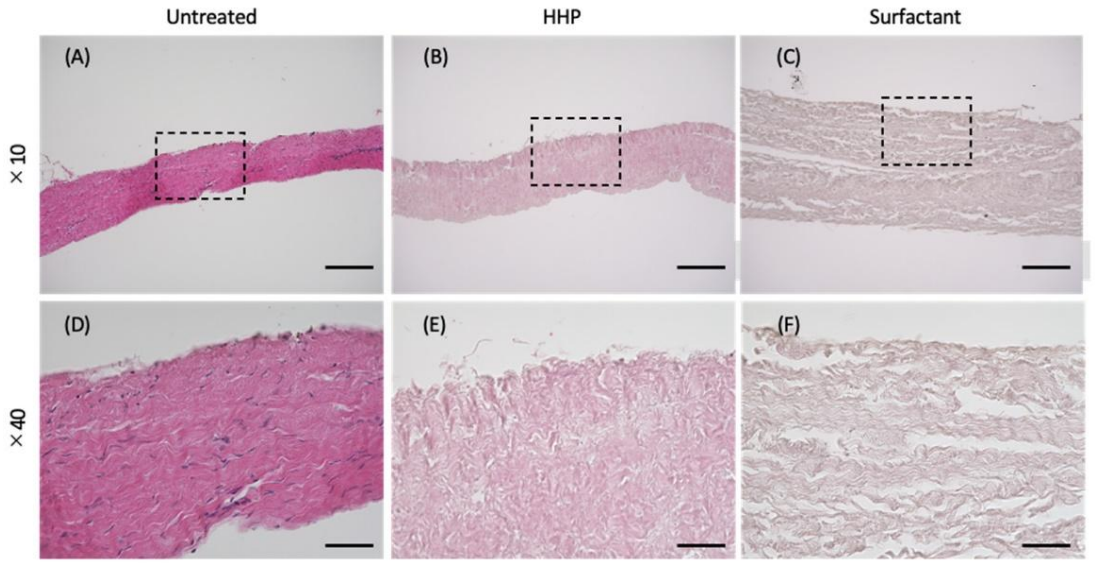

Figure 2. The results of H&E staining. Untreated pericardia tissue (A,D), HHP decellularized (B, E), surfactant decellularized (C, F) [25]

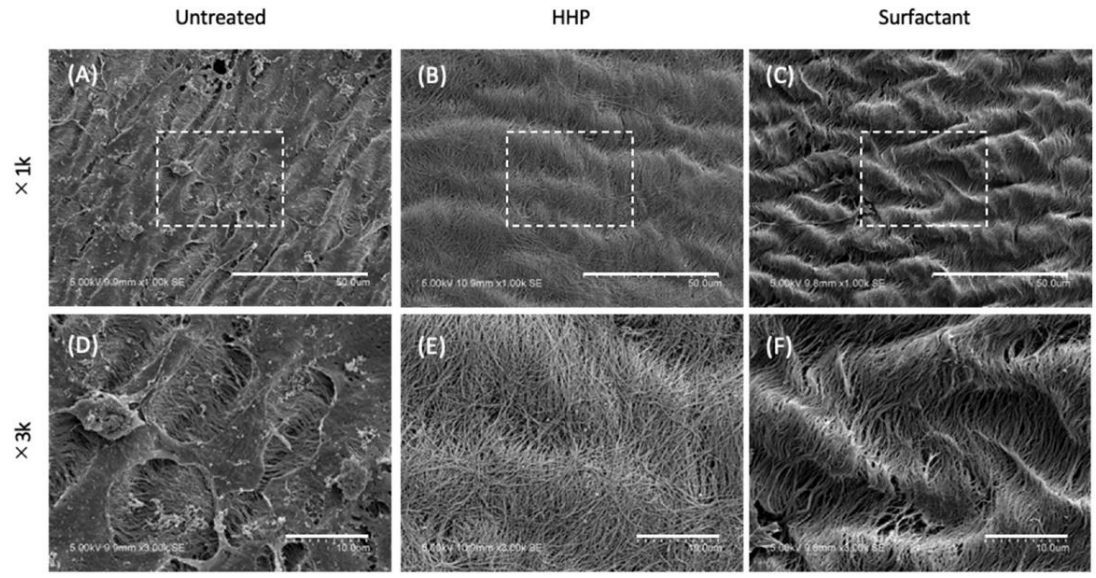

Figure 3. SEM images show the morphology of untreated pericardia tissue (A, D), HHP-decellularized tissue (B, E), surfactant decellularized tissue (C, F) [25]

In the mechanical property tests, tissues decellularized by both the HHP method and the surfactant method exhibited typical J-shaped stress-strain curves, with similar values for ultimate tensile strength, failure strain, and showed similar Young's modulus. Notably, the stress–strain profile of the HHP-treated dECM closely resembled that of the porcine anterior cruciate ligament, with no significant difference in ultimate tensile strength.

C2C12, NIH3T3 cells, and hMSCs were seeded onto the scaffolds, with culture and induction of differentiation. After incubation with Calcein-AM, cell viability and distribution were observed under fluorescence microscopy. The cells on both HHP and surfactant treated scaffolds aligned along the fiber direction and maintained oriented growth. However, NIH3T3 cell growth was inhibited on surfactant-decellularized pericardium, whereas normal proliferation was observed on HHP-derived dECM scaffolds. C2C12 cells exhibited slow growth on both types of matrices. In contrast, the growth of MSC was suppressed on both HHP and surfactant decellularized scaffolds.

3.2. Tilapia Skin Xenogeneic Acellular Tendon Scaffold

To explore more possible tissue sources and reduce the potential risk of zoonotic diseases from xenogeneic donors, recent studies have investigated the possibility of using non-mammalian tissues as decellularization sources. Liu et al. prepared decellularized extracellular matrix (dECM) scaffolds from tilapia skin and combined them with stem cells for tendon repair experiments [26]. The tissues were decellularized using SDS in combination with DNase I and RNase A to remove cellular components. The DNA quantification showed that more than 95.7% of DNA was removed after decellularization. H&E and DAPI staining confirmed the absence of nuclear residues, while SEM images demonstrated that the fiber alignment and porous architecture of the dECM remained intact.

To enhance the mechanical properties of the scaffold, they further applied EDC/NHS crosslinking to produce C-DTFS. As shown in Fig. 4, mechanical testing revealed that the ultimate tensile strength of C-DTFS in the wet state reached approximately 18 MPa, significantly higher than that of non-crosslinked DTFS (around 10 MPa). In addition, the elastic modulus of C-DTFS was also markedly increased, displaying a more stable J-shaped stress–strain curve.

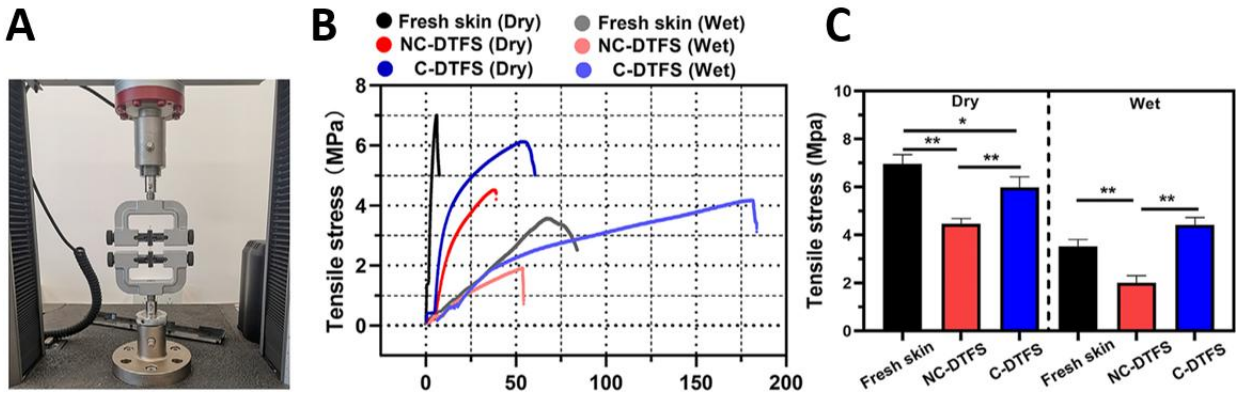

Figure 4. (A) Tensile stress measurement (B) The stress-strain curves of wet and dry skin samples (C) Maximum tensile stress of wet and dry skin samples [26]

The tendon-derived stem cells (TDSCs) were seeded onto C-DTFS scaffolds. Results from the CCK-8 assay demonstrated continuous cell proliferation over 7 days, with a growth rate comparable to that on native tendon matrix. Furthermore, the immunofluorescence and qPCR analyses indicated that C-DTFS activated the integrin α2/β1 and TGF-β/Smad signaling pathways, upregulating the expression of tendon-related genes such as Scx, Tnmd, and Col1a1.

For the in vivo evaluation of regenerative capacity, C-DTFS combined with TDSCs was implanted into a 3mm rat Achilles tendon defect model. The collagen fibers in the C-DTFS+TDSC group were densely packed in a regular wavy pattern, which closely resembles normal tissue. And the fibers in the nano scaffold group remained less organized. Masson staining and immunohistochemistry test further proved a significant increase in type I collagen deposition and a marked reduction in inflammatory cell infiltration in the experimental group. Moreover, the ultimate tensile strength of the repaired tendon in the C-DTFS+TDSC group reached approximately 16 MPa, recovering to more than 85% of the autograft group.

This study was the first one to investigate tilapia skin as a source of dECM scaffolds. Compared with mammalian tissues, tilapia skin offers notable advantages, including abundant availability, avoidance of religious and ethical concerns, and reduced risk of zoonotic disease transmission. The combination of mild decellularization with EDC/NHS crosslinking effectively removed cellular components while preserving type I collagen fibers and the porous ECM structure. Upon recellularization with TDSCs, the scaffold demonstrated strong tenogenic induction and collagen deposition in vivo, with a repair outcome approaching that of autografts. These findings suggest tilapia skin-derived dECM as a promising alternative material for clinical tendon repair.

3.3. dECM-decorated 3D Printed Nanoscaffold

In some recent studies, researchers focused on addressing the limitation that dECM scaffolds depend on biological donors for native tissue. They aim to make tissue sources more accessible while maintaining good biocompatibility, low cytotoxicity and immunogenicity, and favorable mechanical properties. Obtaining dECM scaffolds from artificially engineered tissues has therefore been considered a feasible solution.

Sharma et al. designed a nanofiber scaffold and developed dECM decorated constructs [27]. In their research, two types of 3D nanofiber scaffolds were fabricated. The human dermal fibroblasts (HDFs) were seeded onto these scaffolds, followed by decellularization using SDS combined with freeze–thaw cycles, and getting the ECM decorated nanofiber scaffolds.

H&E and DAPI staining confirmed the nuclei had been removed completely, while SEM images showed that the 3D fibrous structure of the scaffolds was preserved. Compared with undecorated scaffolds, dECM decorated scaffolds had a richer ECM coating layer on fiber surfaces. The in vitro test demonstrated that HDFs adhered more effectively and aligned along the fiber orientation on dECM decorated scaffolds, with a significantly higher proliferation rate compared to undecorated scaffolds. Fibroblasts also displayed ordered alignment along the fiber axis.

Immunological analyses further revealed that both scaffold structure and the mode of ECM decoration influenced immunogenicity. In a subcutaneous implantation model, dECM decorated scaffolds showed greater cell infiltration and vascularization than pure nanofiber scaffolds, with markedly higher vascular density and deeper cell penetration. These findings indicate that dECM decoration not only improves cell compatibility but also promotes early-stage vascularization and tissue integration.

3.4. Structural Designed and Customized Bilayer Regeneration Promoting Scaffold

Based on the dECM scaffold, recent research also focuses on minimizing the inflammatory response and improving the efficiency of regenerative healing at the connective tissue-bone interface. Li et al. proposed an innovative, book-shaped, bilayered decellularized tendon matrix scaffold (dtECM-TAZn/Mg), aiming to address the dual challenges of difficult tendon-bone interface healing and insufficient local inflammatory regulation [28]. The research team decellularized bovine Achilles tendons using freeze-thaw cycles and nuclease treatment. H&E and DAPI staining confirmed the absence of residual cells, a significant decrease in DNA content, and collagen content comparable to native tissue. Based on this, covalent immobilization of tannic acid (TA) combined with a metal-polyphenol self-assembly method allowed for the loading of Mg^{2+} and Zn^{2+} onto the top and bottom layers of the scaffold, respectively, creating a bilayered scaffold with temporal regulation. The upper layer of dtECM-TAMg slowly releases Mg^{2+} and TA, thereby scavenging reactive oxygen species, mitigating inflammatory responses, and promoting M2 polarization of macrophages. The lower layer of dtECM-TAZn effectively promotes osteogenesis and tendon-bone interface reconstruction by releasing Zn^{2+}. As shown in Fig. 5-A, this layered, functionalized design better meets the needs of different stages of tendon-bone interface repair and regeneration than single-layer or gradient scaffolds, effectively combining early immune regulation with later osteogenesis promotion.

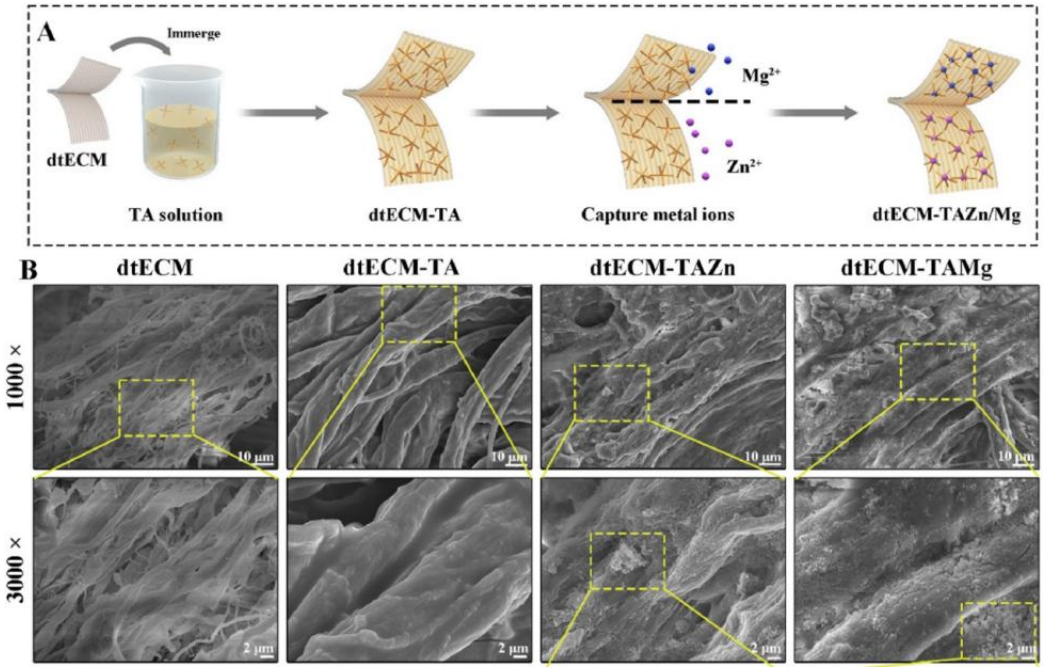

Figure 5. (A) The flow diagram of the preparation of metal phenol bioactive coatings (B) SEM images show the morphology of 4 types of bilayer scaffolds [28]

The mechanical strength test showed that the unmodified dtECM has limited strength, while the ultimate tensile strength is significantly improved after TA modification. Further loading with Zn^{2+} or Mg^{2+} achieves maximum strength due to the metal-polyphenol coordination effect. In a rat tendon repair model, the dtECM-TAZn/Mg layer achieved a maximum load of 23.5 ± 0.56 N at 8 weeks post-surgery, approaching that of healthy tendons. The stiffness of the regenerated tendons was also superior to that of the control and unmodified groups, demonstrating that the scaffold effectively restored the functional integrity of the tendon-bone interface.

As for the in vitro cell culture, the scaffold supported the adhesion and proliferation of BMSCs. The TAMg layer significantly reduced intracellular ROS levels and promoted macrophage polarization toward the M2 phenotype, demonstrating excellent anti-inflammatory and immunomodulatory properties. The TAZn layer strongly induced the upregulation of osteogenic genes (ALP and RUNX2), resulting in a 3–4-fold increase in osteogenic differentiation compared to the control group. Histological and imaging analyses further demonstrated that the dtECM-TAZn/Mg layer formed more organized collagen fibers and more mature fibrocartilage zones at both 4 and 8 weeks, accompanied by increases in bone mass and density, demonstrating superior interface healing quality compared to the control group.

4. Discussion

The clinical application of dECM scaffolds in tendons and ligaments regeneration mainly depends on the tissue source, decellularization methods, evaluation of decellularization, and recellularization strategies. The ultimate goal is to fabricate scaffolds with suitable biological and mechanical properties that can promote tendon and ligament regeneration.

This review compares the applications of dECM scaffolds derived from different tissue sources and decellularized methods for tendon and ligament repair. The mammalian tissue sources such as porcine pericardium and bovine tendons have high structural and mechanical similarity to target tissues. However, before further clinical research, there are still illustrates problems of immunogenicity and ethical concerns. The fish-derived tissues, such as tilapia skin, provide a low cost and low immunogenic tissue source with mechanical properties similar to native tissues. But the stability and long-term degradation still require further research. Furthermore, the engineered tissue source has been proven a promising controllable and designable abilities. The components and

structures of the dECM scaffold can be changed by different cells and growth factors. But this source needs a long cell culture time and high cost, and most studies remain at the level of subcutaneous implantation test or small-animal models. There is no much validation in large mammals under long-term loading.

For the decellularization methods, achieving complete removal of cellular components while preserving the ECM's structure and components remains the key challenge. Surfactants can effectively remove cellular contents but they may damage the ECM structure, cause the loss of ECM components with potential cytotoxicity. For the physical methods, the advanced high hydrostatic pressure and freeze–thaw cycles, better preserve ECM molecules and fiber structures, but they also showing limited efficiency in cell removal. Many recent studies have combined multiple methods to maximize the decellularization while retaining critical ECM molecules and the 3D structure. This research establishes methodological framework that promote the development of an optimal dECM scaffold for tendon and ligament repair.

The mechanical performance is a central requirement in tendon and ligament tissue engineering. The crosslinking and functionalization treatments can enhance the tensile strength and elastic modulus of dECM scaffold, nearly approaching the level of native tissues. The balancing ECM bioactivity with mechanical reinforcement is a central challenge in scaffold design.

Despite the dECM scaffolds for tendon and ligament tissue engineering have been proved potential, there is still a gap between laboratory research and clinical application. First, the most studies are based on small-animal models, lack evidence from large mammals under long-term loading. And the immunogenicity remains a risk in xenogeneic tissues, the residual antigens such as α-Gal may still trigger immune responses even when DNA content is reduced to acceptable levels. Moreover, dECM scaffold fabrication involves complex procedures, with unreliable batch-to-batch consistency. The variability of xenogeneic tissue sources may further affect the reproducibility of the scaffold. Also, the clinical translation of laboratory results also requires a standardized large-scale production guidance and reduce the cost.

Future studies are expected to continue working on advancing tissue sourcing, decellularization methods, evaluation methods, and recellularization. Tissue sources are no expanding beyond conventional porcine and bovine donors. The low-immunogenic fish tissues and cell-derived ECM, as well as ECM designs that integrate different materials to improve both biocompatibility and mechanical properties. And the dECM scaffolds is shifting from the initial usage as a passive microenvironment for cell growth to functioning as bioactive platforms with active functions. To fabricate the functionalized dECM scaffolds with immunomodulatory, pro-angiogenic, tenogenic, or Oste inductive properties would be important directions in future research. Also, the long-term in vivo studies in large animal models under load bearing conditions will be essential to evaluate the stability and biological functions of advanced dECM scaffolds, providing the foundation for clinical applications.

5. Conclusion

Overall, this review discusses the potential of decellularized ECM scaffolds for tendon and ligament repair. Numerous studies have demonstrated that these scaffolds can preserve the structure and composition of ECM while providing excellent biocompatibility and mechanical support. Future research will require comprehensive consideration of the safety and accessibility of the source, the efficiency and mildness of the process, and the targeted functional modification. Optimizing decellularization strategies, developing ECM from multiple sources, exploring customized artificial ECM scaffolds, and conducting long-term validation in large animal and clinical studies will be key steps in advancing dECM scaffolds from laboratory experiments to clinical application.

The dECM scaffolds offer more than just a substitute for synthetic materials; they provide a regenerative microenvironment more closely resembling native tissue, meeting the mechanical requirements of ligament repair while actively regulating cellular behavior and immune responses.

With the further integration of tissue engineering, biomaterials, and clinical medicine, dECM scaffolds are expected to become core tools for ligament repair and regenerative therapy, providing patients with safer, more effective, and sustainable treatment options.

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