Decellularised extracellular matrix-based injectable hydrogels for tissue engineering applications

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Key Words:
decellularisation methods; decellularised extracellular matrix; injectable hydrogels; tissue engineering

From the Contents

ABSTRACT

Decellularised extracellular matrix (dECM) is a biomaterial derived from natural tissues that has attracted considerable attention from tissue engineering researchers due to its exceptional biocompatibility and malleability attributes. These advantageous properties often facilitate natural cell infiltration and tissue reconstruction for regenerative medicine. Due to their excellent fluidity, the injectable hydrogels can be administered in a liquid state and subsequently formed into a gel state in vivo, stabilising the target area and serving in a variety of ways, such as support, repair, and drug release functions. Thus, dECM-based injectable hydrogels have broad prospects for application in complex organ structures and various tissue injury models. This review focuses on exploring research advances in dECM-based injectable hydrogels, primarily focusing on the applications and prospects of dECM hydrogels in tissue engineering. Initially, the recent developments of the dECM-based injectable hydrogels are explained, summarising the different preparation methods with the evaluation of injectable hydrogel properties. Furthermore, some specific examples of the applicability of dECM-based injectable hydrogels are presented. Finally, we summarise the article with interesting prospects and challenges of dECM-based injectable hydrogels, providing insights into the development of these composites in tissue engineering and regenerative medicine.

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Introduction

Loss of tissue structure and function is usually due to tissue damage, defects, and lesions. Repair and regeneration of abnormal tissues remain significant clinical challenges.[1, 2] Considerably, achieving tissue regeneration and substantial functional recovery requires the simulation of the microenvironment of tissues.[3-5] However, the microenvironment of tissues is complex and variable and varies with tissue type and health status. Therefore, accurate simulation of the tissue microenvironment is an important area of research. In this context, the extracellular matrix (ECM), a three-dimensional (3D) reticular structure, has garnered enormous interest from researchers, providing structural support and transmitting appropriate signalling cues to the cells. In addition, various proteins (collagen, fibronectin, proteoglycans, and glycoproteins) and growth factors present in the ECM are supplied to the deposited cells to improve the impaired proliferation, differentiation, and migration capabilities.[6] Since the concept of decellularisation was first introduced by Poel[7] in 1948, there have been many studies in different directions on obtaining decellularised ECM (dECM) from cells, tissues, or organs. Typically, these methods can be broadly classified into three types: physical, chemical, and enzymatic techniques.[8] The decellularisation process is capable of eliminating the cellular components that trigger the immune response while retaining

the tissue-specific structure and a variety of ECM components. Thus, the component with improved biocompatibility is therefore considered an excellent biomaterial for *in vivo* use, especially for tissue reconstruction. Extensive research has evidenced the development of various forms of dECM (hydrogels, bioinks, and organised porous structures) for different applications in the biomedical field.[9-14] In particular, dECM-based biomaterials have been approved by the United States Food and Drug Administration for the manufacture of medical devices for clinical applications, which have been widely investigated as new alternatives for regenerative therapy.[15-23]

Hydrogel is a hydrophilic material with structural properties that absorb and retain water. Its porosity and water absorption capacity can be controlled by regulating the preparation process. Hydrogels have received extensive attention from researchers due to their specific properties of high binding affinity and excellent biodegradability.[24-26] Regarding dECM-based hydrogels, they retain important ECM proteins, making them relatively stable at certain temperatures and pH values. dECM hydrogel is rich in numerous biomolecules, including structural proteins, glycosaminoglycans, and type I collagen (Col-I), which are of particular importance for tissue maintenance and repair. Derived from different sources, dECM preserves specific biomacromolecules and biological signals, endowing dECM hydrogels with excellent biocompatibility and the ability to stimulate cell proliferation. They can serve as effective scaffolding materials to promote tissue regeneration.[27] However, the dECM hydrogel suffers from several shortcomings, such as lower mechanical strength than that of natural tissues, requiring the adjustment of the structure towards better mimicking the tissue regeneration microenvironment.[28] With different preparation approaches, several advancements have been evidenced over the past decade toward providing excellent injectability and achieving tuneable mechanical properties.[29-31] Considering their superior properties, dECM-based hydrogel materials offer a promising application in the direction of regenerative medicine.[32, 33]

This review initially focuses on the latest developments of dECM-based injectable hydrogels for tissue engineering, summarising the different preparation methods with the evaluation of injectable hydrogel properties. Then, the current research on dECM-based injectable hydrogel is presented with some specific examples (**Figure 1**). Finally, we summarise the article with the prospects and challenges of dECM-based injectable hydrogel, providing insights into the development of these composites for tissue engineering and regenerative medicine. To ensure the timeliness of this comprehensive review, a detailed methodology was used to conduct a literature search using the most renowned scientific databases, such as Web of Science and PubMed. We have used the most relevant keywords, such as "decellularised extracellular matrix", "injectable hydrogel", and "decellularisation methods" for the literature search in the notified search engines. It should be noted that the literature published in the last 5 years was given prior importance, focusing on recent advances in the field.

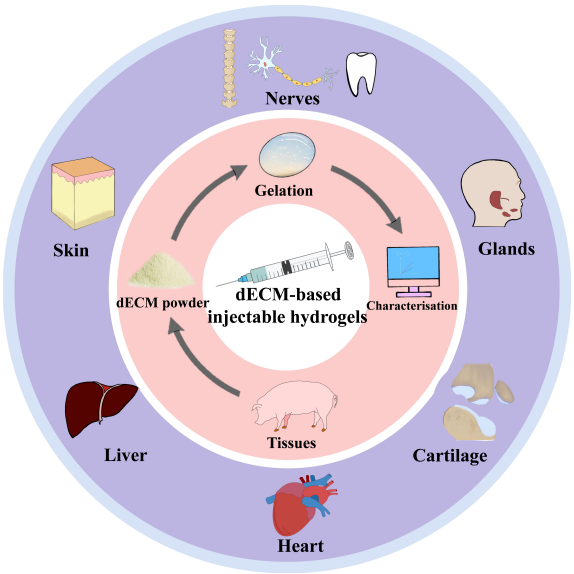

Figure 1. Schematic illustrating the preparation and application of dECM-based injectable hydrogels. Created using Microsoft PowerPoint 2016 and Procreate® 5.3.9. dECM: decellularised extracellular matrix.

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Preparation of Decellularised Extracellular Matrix-Based Injectable Hydrogels

Decellularisation methods

The decellularisation process refers to the removal of the cellular components of a tissue or organ, which can be utilised as a cellular scaffold and drug delivery vehicle, among others. Along this line, several decellularisation methods can be employed, including physical methods,[34-42] chemical methods,[43-52] enzymatic methods,[53, 54] and combinatorial methods (**Table 1**).

Table 1. Several commonly applied physical, chemical, and enzymatic methods for dECM preparation

Method	Technology	Specific method and effect	Reference
Physical methods	Cyclic freeze-thawing	• By subjecting the cells to a repeated freeze-thawing process, the cell membranes rupture, leading to cell lysis and achieving the removal of cells; • Range of temperature −196–37°C.	34-36
	Supercritical carbon dioxide fluid	• Cause rupture or denaturation of the cell membrane, which releasing intracellular material.	37-39
	Tissue electroporation	• By applying an electric field, the potential maintained by the cell membrane is disrupted, disrupting the lipid bilayer and affecting cellular homeostasis, ultimately leading to decellularisation; • Often used in decellularising small-sized soft tissues.	40
	Ultrasonication	• Use ultrasonic cavitation to disrupt cells and perform decellularisation. • May result in DNA breaks.	41, 42
Chemical methods	Ionic (e.g. SDS, SDC)	• Disrupt lipid-protein interactions without disrupting protein-protein interactions; • Disrupt cell membranes so that intracellular material can flow out.	43-46
	Non-ionic (e.g., Triton X-100, Tween-20)	• As a surfactant; • Disrupt cell membranes and inducing cell lysis.	47-49
	Zwitterionic detergents (CHAPS)	• Disrupt the cell membrane; • Often used in the decellularisation process of neuronal cells.	50
	Hypotonic/hypertonic solutions	• Use osmotic pressure to rupture cell membranes.	51, 52
Enzymatic methods	Nucleases	• Cut DNA strands to deactivate DNA.	53
	Trypsin	• Used in combination with chelating agents such as ethylenediaminetetraacetic acid; • Degrade intercellular adhesion molecules.	54

Note: CHAPS: 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propane sulfonate; DNA: deoxyribonucleic acid; SDS: sodium dodecyl sulfate; SDC: sodium deoxycholate.

The physical-based techniques involve the application of mechanical forces to disrupt and remove cells. Several physical techniques include cyclic freeze-thawing and supercritical carbon dioxide fluid, among others. These methods can break cells apart and separate cellular debris from the ECM. Cyclic freeze-thawing is one of the earliest methods of physical decellularisation, involving the repeated freeze-thaw-freeze process by controlling the cooling and warming of the tissue. During the freeze-thaw process, the microstructure gradually changes with the formation and disappearance of ice and the decrease of elastic modulus. Thus, multiple cycles of the freeze-thawing process may destroy the dECM properties. In an instance, Luo et al.[35] utilised dECM using cyclic freeze-thawing to alter mechanotransduction signals and promote M2 polarisation of macrophages, ultimately achieving wound healing. The process included freezing the porcine peritoneum in liquid nitrogen for 5 minutes and then thawing, which was repeated for freeze-thaw cycles, involving temperatures ranging from −196°C to 25°C. Finally, the successful decellularisation was confirmed by histological staining of dECM, with no visible nuclei. In addition, the DNA content was determined to be less than 50 ng/mg dry weight, which met the routine requirements.[55] These findings suggested that decellularisation could be achieved by cyclic freezing and thawing. Giang et al.[37] utilised the supercritical fluid technology to remove cellular immunogenic components and fabricate an innovative acellular dermal matrix for allografts. Further, the successful decellularisation was demonstrated by histological staining.

In chemical-based methods, several chemicals can be used to break down cellular components, such as detergents, acids, and bases. These chemicals disrupt cell membranes and dissolve cellular components. The decellularisation process is successful after washing off the agents. However, it should be noted that increasing the length of exposure to detergents could provide better removal of cellular material outside, which, however, might also damage dECM. Therefore, to achieve the desired decellularisation, it is necessary to optimise the process to

reduce the damage to the ECM caused by detergents and complete the decellularisation process at a rapid rate. Alshaikh et al.[43] designed three different experimental protocols to investigate the effect of sodium deoxycholate (SDC) *vs.* sodium dodecyl sulfate (SDS) on porcine ovarian decidualisation. Although SDS, as a decellularisation reagent, was excellent for removing various cellular components, such as DNA, it might also damage the ECM significantly more than other reagents. In both experimental protocols containing SDS, the damage to ovarian tissue appeared to be larger than SDC, which retained more collagen. Both SDS and SDC significantly reduced the DNA of the treated tissues by disrupting cell membranes, indicating their favourability for decellularisation. Bae et al.50 showed that amphoteric detergents with nuclease were optimal for neural decellularisation. It was experimentally verified that the use of amphoteric detergents and nuclease decellularised cells resulted in better removal of cellular components and minimised damage to the ECM during decellularisation compared to non-ionic and anionic detergents.

To this end, enzymatic-based methods employ various enzymes to digest cellular components selectively, such as nucleases (e.g., DNase and RNase) to degrade nucleic acids, proteases (e.g., trypsin, pepsin) to disrupt cell membranes, and lipases to destroy lipid-lipid interactions. These enzymatic methods can be gentle and specific but may require longer processing times. As the enzyme-based method is better at protein-protein linkages and DNA removal, it is slightly less effective at disrupting cell membranes compared to other methods. Therefore, it is often used in combination with chemical and physical methods to achieve improved cell removal. Hong et al.[56] used a combination of chemical and enzymatic methods to fabricate dECM from the porcine brain to repair the injured spinal cord. The dECM hydrogel promoted macrophage polarisation towards the M2 phase. The dECM hydrogel applied to a rat model of spinal cord injury presented significant changes in the ratio of M1 and M2 macrophages in the damaged spinal cord. They were stirred in 0.02% trypsin for 1 hour at 37°C and decellularised in 3.0% Triton X-100 and 1.0 M sucrose for 60 minutes. After washing, 4.0% SDC was added and treated for 60 minutes. Finally, it was finally sterilised for testing, achieving excellent decellularisation.

Using physical-based methods, there is no risk of denaturing substances, such as proteins, in the tissues. Accordingly, several physical methods, such as supercritical carbon dioxide-based and cyclic freeze-thawing-based approaches, can be operated in large quantities, facilitating high-volume industrial production. However, the physical method suffers from a major shortcoming, i.e., failing to achieve the comprehensive removal of cells. To this end, the chemical method is more effective than the physical-based approach in removing cells that cause immune rejection. Although better than the physical method, the chemical method suffers from a major limitation of induced toxicity by the applied reagents, such as SDS and SDC, among others. However, extensive washes in the subsequent processing can eliminate the toxic concentration of the chemicals. The enzymatic methods require the cooperation of chemical or physical methods, needing the release of the intracellular components to the outside of the cell membrane. Moreover, biological enzymes are used to achieve the effect of decellularisation. However, the use of large quantities of enzymes may increase production expenses due to their high cost.[57] Although a variety of different methods contribute to decellularisation, using a combined method is the best option for excellent decellularisation. A combinatorial strategy must be used to achieve optimal decellularisation while minimising damage to the ECM structure.[58-60]

In addition to decellularisation, the characterisation of dECM plays an important role in demonstrating its quality. Several techniques include infrared absorption spectroscopy, histological observations, DNA quantification, biocompatibility, and scanning electron microscopy, among others. Through several experimental comparisons and constant updating of protocols, a combination of notified decellularisation methods could achieve better results than individual approaches. Although each decellularisation method has its advantages and challenges, the choice of method depends on factors such as the type of tissue or organ being decellularised, desired preservation of ECM structure and composition, and intended application in tissue engineering or transplantation. In addition, the optimisation of decellularisation protocols is an ongoing area of research that is being done to improve efficiency and efficacy while minimising damage to the ECM.

Gelation of dECM hydrogel

The basic principle of dECM hydrogel formation is the physical reaction of collagen self-assembly, which is often influenced by various proteins (e.g. glycosaminoglycans) contained in the dECM.[33, 61] Firstly, the prepared dECM powder is digested with enzymes to prepare a pre-gel. Subsequently, cross-linking is induced by adjusting pH, controlling temperature (typically at 37°C), and salt ion concentration.[62-65] Typically, a pH value of around 7 is optimal for gel formation in most tissues with dECM. Further, a phosphate-buffered saline at a concentration of 0.1 M in a volume ratio of 1/10 or 1/9 is usually added to adjust the ionic concentration.[62, 66] Several reports demonstrated the successful preparation of dECM hydrogel of different tissues for tissue engineering applications.[30, 67-72]

Notably, the differences in tissue source and the chosen decellularisation protocols can have an impact on dECM gelation. Fernández-Pérez et al.[73] analysed the gelation kinetics of ECM hydrogel by turbidimetric analysis. The technique was based on the physical reaction of collagen self-assembly, and the turbidity of the samples increased with the self-assembly process. The probing results showed that the curves of all the samples showed an inverted S-shape, and with time, the samples were stabilised for a while before gelation. Among the samples, those treated with freeze-thaw exhibited the shortest gel time, while hydrogel treated with SDS decellularisation presented the longest gel time.

In addition, the use of different digestion methods can cause differences in dECM gel properties.[66, 74, 75] The most common method to achieve solubilisation is digestion with pepsin for 24–72 hours. The speed and progress of digestion are influenced

by factors such as stirring rate, enzyme type, and substrate concentration. However, the dECM hydrogel prepared by enzymatic digestion suffers from poorer mechanical stability compared to those of their source. Thus, the resulting hydrogel can be treated with chemical cross-linking agents or by adding synthetic functional groups or polymers to the dissolved dECM.

Characterisation of Decellularised Extracellular Matrix-Based Injectable Hydrogel

The dECM-based injectable hydrogel typically requires the following tests: (i) Rheological testing: Evaluate the viscosity, elasticity, and rheological properties of the gel to ensure its adaptability and stability during the injection process. In an instance, Sawkins et al.[76] determined the rheological properties of bovine demineralised bone matrix and bovine exfoliated cell matrix hydrogel using a parallel-plate rheometer. They compared them with Col-I hydrogel of the same concentration. Both bovine exfoliated cell matrix and bovine demineralised bone matrix hydrogel showed slower gelation rates than Col-I at the same total protein concentration. Wang and coworkers[77] tested the gelation kinetics of dECM hydrogel at 37°C using a rheometer. The highest values of G' [storage modulus] and G'' [loss modulus] were found for dECM hydrogel with a dECM concentration of 12 mg/mL. The gelation time at this suitable concentration was reasonable. (ii) Biocompatibility testing: Including cytotoxicity testing, skin irritation testing, and subcutaneous injection experiments to ensure the safety and biocompatibility of the gel to human tissues.[78] Wang et al.[77] observed normal cell growth and value addition by inoculating and culturing the cells at different time points. The results indicated that the dECM hydrogel was biocompatible. (iii) Injection performance testing: Assessing the fluidity, homogeneity, and controllability of the gel in the injection device to ensure a smooth injection process can be confirmed by observing its passage through the needle.[63] The method used in the preparation of dECM-based injectable hydrogels influences their injectability in varying degrees. For instance, powdered dECM is injected directly by re-swelling in an aqueous suspension. However, the injectability of powdered dECM often depends on the appropriate selection of the needle with an inner diameter correlated to the powder particle size.[79] (iv) Stability testing: Examine the stability of the gel under different temperatures, humidity, and storage conditions to ensure its quality stability during long-term storage and transportation. (v) Biodegradability testing: The degradation rates can be tested both *in vivo* and *in vitro*. On the one hand, the *in vivo* degradation assessment generally uses bacterial collagenase to simulate degradation conditions. On the other hand, the *in vitro* degradation can be manifested by adding equal volumes of collagenase and 0.25 M $CaCl_2$ to 0.1 M Tris-base, incubating for a certain period and then testing the weight change before and after the incubation.[80] *In vivo* degradation testing can be done with the help of fluorescently labelling the substrate with Alexa Fluor 568 succinimidyl ester, removing the tissue immediately after euthanasia, and then performing frozen sections. Finally, the presence of hydrogel can be identified from the histological sections.[81] (vi) Swelling properties testing: The hydrogel swelling characteristics test is used to characterise the properties of water gel. The solvent molecules can enter the gel network structure during swelling. This property makes the hydrogel a good carrier for the drug delivery application.

Applications of Decellularised Extracellular Matrix-Based Injectable Hydrogels in Tissue Engineering

The development of biocompatible dECM-based injectable hydrogels is quite rapid and promising for tissue engineering. Along this line, various dECM-based materials have been synthesised in the fields of tissue engineering and regenerative medicine. In this section, several applications of engineering tissues are discussed.

Nerves

Peripheral nervous system

The peripheral nervous system transmits messages between the central nervous system and the body. These peripheral nerve injuries are a common type of traumatic lesions to the nervous system.[82] In this context, the dental pulp belongs to the peripheral nervous system. Its primary function is to provide sensation to the teeth. Typically, the most common approach for treating pulpal injuries is root canal therapy, which begins with the complete removal and debridement of the damaged area of the pulp and is filled with an inert biomaterial. The entire process results in the regeneration of the diseased area, leading to a complete healing of the tooth structure with an exceptional recovery. Depending on the treatment process, inert biomaterials have become a hot research topic, and dECM, a new material with good biocompatibility, has emerged as an excellent choice for regenerative medicine.

Yuan et al.[78] successfully developed porcine pulp-derived dECM hydrogels. Considering their excellent rheological properties, biocompatibility, and experimental validations, porcine pulp-derived dECM hydrogels could promote cell migration and angiogenesis *in vitro*, making them excellent scaffolding materials to treat dental pulp and promote pulp regeneration. Regarding the materials required for pulp tissue engineering, it is important to establish a tissue-specific microenvironment conducive to pulp regeneration (**Figure 2**). Firstly, porcine pulp-derived dECM hydrogel could provide many different filling structures with variable morphology. Secondly, it should be able to offer critical biological signals to effectively induce the differentiation of dental pulp stem cells. Therefore, the porcine pulp-derived dECM hydrogel was considered one of the best options. In *in vitro* experiments, dECM hydrogel offered excellent injectability and could support the survival, odontogenic differentiation, and neurogenic differentiation of dental pulp stem cells in a 3D culture environment. Compared to treatment with gelatin methacrylate hydrogel, porcine pulp-derived dECM hydrogel offered significantly more pro-regenerative capacity.

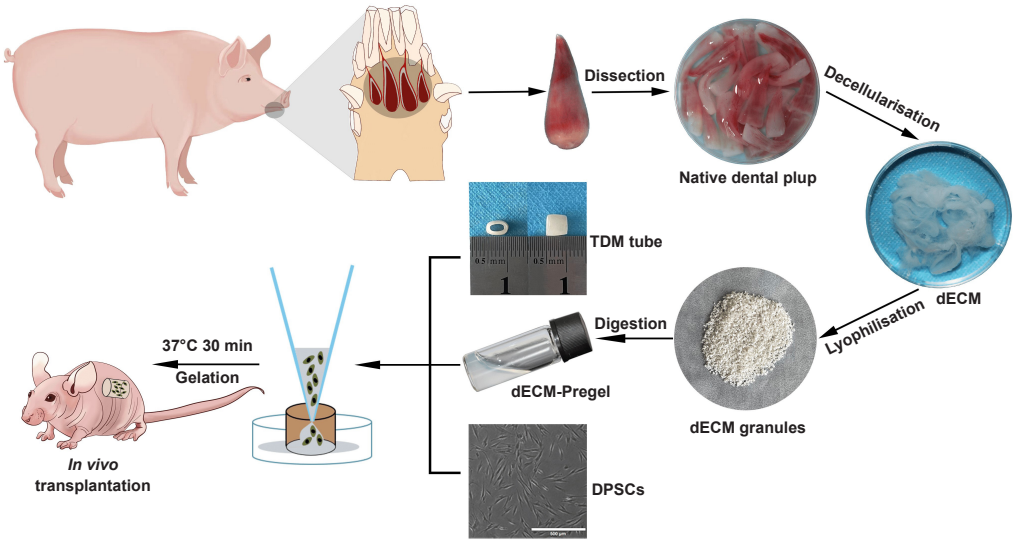

Figure 2. The process of injectable xenogeneic dental pulp decellularised extracellular matrix hydrogel promoting functional dental pulp regeneration. Reprinted from Yuan et al.[78] dECM: decellularised extracellular matrix; DPSCs: dental pulp stem cells; TDM: treated dentin matrix.

Central nervous system

Damage to the central nervous system can lead to loss of sensory and motor function, paralysis, and even death. The repair of the nervous system is a major challenge due to the lack of ability to regenerate neurons after injury. The tissue-derived dECM hydrogels are a class of natural injectable materials that can be used for neural tissue repair. However, these may suffer from a limitation of rapid biodegradability that may disrupt the reconstruction of nerve tissue *in vivo*. Výborný et al.[83] investigated the cross-linking properties of genipin and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride with dECM at concentrations ranging from 0.5 to 10 mM to improve the stability of dECM hydrogels derived from human umbilical cord. It was found that cross-linking improved the strength of the material without affecting the functionality of the dECM hydrogel at both genipin and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride concentrations of 1 mM. In addition, *in vivo* investigations showed that dECM hydrogels cross-linked with genipin prolonged retention time in the ischaemic foci of the cerebral cortex compared to uncross-linked dECM. In comparison, genipin was found to be more suitable for in situ cross-linking of dECM than N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride at a concentration of 1 mM. In another study, Liu et al.[84] developed a host-guest (HG) composite hydrogel containing brain dECM and matrix metallopeptidase (MMP)-responsive HG to promote the survival and differentiation of human induced pluripotent stem cell-derived neural stem cells *in vivo*, demonstrating to be a promising platform for regulating stem cell survival and differentiation.

The spinal cord is part of the central nervous system, and spinal nerves are distributed to the extremities, body walls, and viscera. Traumatic spinal cord injury is one of the clinical challenges facing orthopaedic surgeons, where patients suffer a disruption of the structural stability of the spine, resulting in permanent motor deficits, sensory disturbances, and autonomic dysfunction.[85] Hong et al.[56] used a porcine brain for decellularisation to obtain an injectable porcine brain dECM hydrogel for the treatment of the injured spinal cord. The *in vitro* experimental results conducted with different concentrations of dECM hydrogel demonstrated that the dECM hydrogel could promote neuronal cell proliferation and differentiation, as well as macrophage polarisation from the M1 phenotype to the regenerative M2 phenotype. Further, the *in vivo* experiments validated that the hydrogel reduced the size of the injury cavity and successfully promoted motor repair. These findings provided new therapeutic options and ideas for the future treatment of spinal cord injury.

Glands

Glands play a major role in regulating food digestion, growth, and metabolism, and include two main categories: exocrine and endocrine glands. The physiological effects and pathological changes of various glands are closely related to life. Several factors result in various diseases related to glands, often having a significant impact on the metabolism, in severe cases, even leading to death. Several malignant diseases of the glands, such as pancreatic cancer and breast cancer, are major clinical concerns. Kojima et al.[86] investigated the efficacy of dECM hydrogels and MSCs in a rodent pancreatitis model. MSCs were embedded in dECM hydrogel and transplanted into the rat pancreas. The results demonstrated the anti-inflammatory and antifibrotic effects of this combined treatment. Wang and coworkers[65] prepared an injectable porcine submandibular gland decellularised gel for functional reconstruction of salivary gland defective tissues. The results showed that this dECM hydrogel not only improved cell viability and proliferation and significantly promoted the migration and recruitment of submandibular gland mesenchymal stem cells to form functional vesicles or duct-like structures, but also effectively inhibited the fibrosis of the glandular tissues.

Cartilage

The ECM of cartilage is normally gelatinous and similar to that of connective tissue, in which the components that determine its mechanical properties include the collagen network and proteoglycans. The collagen network mainly determines the tensile strength of cartilage, while the proteoglycans determine the osmotic swelling and elastic properties of the cartilage.[87]

Zeng et al.[88] developed an injectable, porcine cartilage dECM-based hydrogel for repairing cartilage defects (**Figure 3**). The dECM-based hydrogel showed excellent biocompatibility and immunomodulation *in vitro* and *in vivo*. The dECM hydrogels loaded with urine-derived stem cells were characterised, indicating excellent material properties to achieve cartilage repair. Yu and coworkers[89] proposed an interesting treatment option to address cartilage damage, suggesting that repairing cartilage might require restoring both the cartilage joint and the subchondral bone simultaneously. In addition to cartilage repair, the subchondral bone could provide mechanical support for cartilage joints, achieving a substantially improved cartilage repair treatment in the long run if treated simultaneously. The solution was designed as a two-layer injectable gel structure as a scaffold, carrying chondrocytes and bone marrow mesenchymal stem cells (BMSCs) for cartilage repair. For different repair sites, a multi-layered dECM construct was fabricated for subchondral and cartilage constructs. Firstly, agarose was used as the continuous phase to generate a Ca^{2+} cross-linked sodium alginate/agarose layer for repairing the subchondral bone. In addition, the cartilage dECM/agarose layer was prepared to enhance the mechanical strength of dECM by adding agarose to repair the cartilaginous joints. Next, chondrocytes were co-cultured with BMSCs, which complemented each other to reduce the number of chondrocytes and accomplish improved cartilage repair. Chondrocytes could alleviate the problems of cartilage hypertrophy and endochondral osteogenesis brought about by BMSCs, inducing their differentiation to chondrocytes. BMSCs could secrete transforming growth factor-β and bone morphogenic protein-2, reducing the differentiation to chondrocytes so that they could continuously provide cartilage matrix to execute repair comprehensively. Finally, the plastic morphology of the gel could perfectly fit the repair site, and the establishment of the injectable gel system could avoid secondary injury to the joint while opening the joint cavity for treatment. Moreover, the porous structure of the gel could provide a suitable environment for cell growth. Through animal experiments, it could be clearly concluded that the double-layer structure was significantly better than the single-layer structure in repairing both cartilage joints and subchondral bone (**Figure 4**). These hydrogels are expected to be biomaterials that could substantially promote cartilage regeneration.

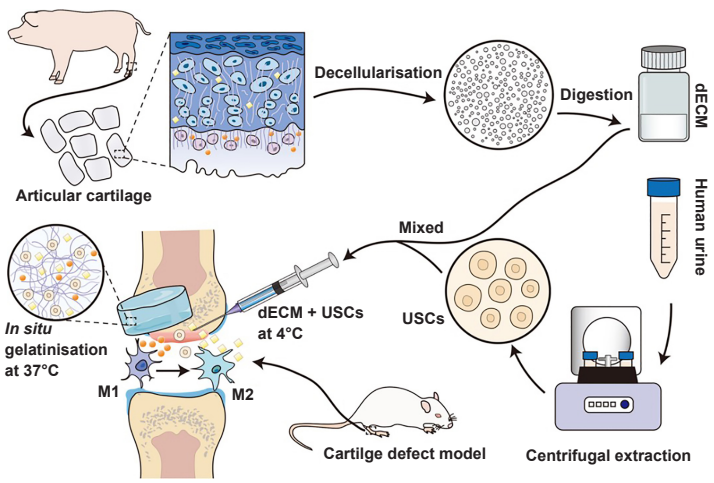

Figure 3. Schematic illustration of the injectable decellularised cartilage matrix hydrogel encapsulating human USCs for immunomodulatory and cartilage defect regeneration. Reprinted from Zeng et al.[88] dECM: decellularised extracellular matrix; M1: classical activated macrophages; M2: alternatively activated macrophages; USCs: urine-derived stem cells.

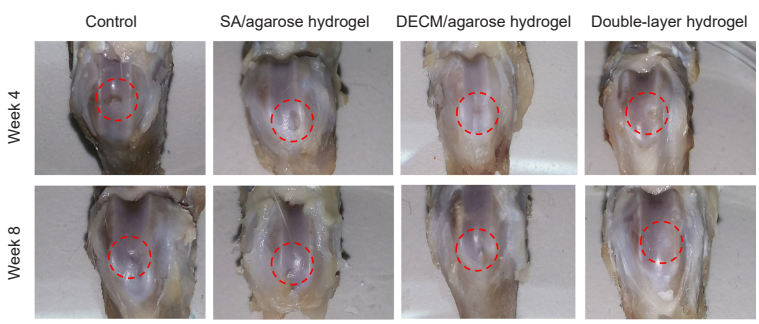

Figure 4. The appearance of articular cartilage injury samples with different hydrogels at weeks 4 and 8. The red circle shows the new articular cartilage. Reprinted from Yu et al.[89] DECM: decellularised extracellular matrix; SA: sodium alginate.

Heart

The heart, as one of the vital organs of the human body, is composed of myocardium, valves, and blood vessels. Cardiovascular-based diseases have become the most dreadful clinical concern over the past few decades. Myocardial infarction is one among the several cardiovascular-based diseases that leads to rapid cardiomyocyte death. The predominant reason for cardiovascular-based diseases is the minimal intrinsic regenerative capacity of the human heart, as cardiomyocytes are terminally differentiated cells lacking division and expansion capabilities.[90-93] The dECM-based injectable hydrogel in the field of cardiac tissue engineering is aimed at stimulating the differentiation of stem cells and haemodialysis reconstruction of ischaemic cardiac tissue. Currently, dECM-based injectable hydrogels prepared from the decellularisation of small intestinal submucosa have been shown to promote the repair of ischaemic cardiac tissues and are ready for clinical settings.[93, 94]

Wang and colleagues[95] co-cultured human umbilical vein endothelial cells (HUVECs) and fibroblasts to observe cell differentiation on small intestinal submucosa hydrogel and Col-I hydrogel. The authors demonstrated that capillary formation could only be observed on small intestinal submucosa hydrogel after 1 week. In addition, several studies evaluated the cardiogenic capacity of dECM hydrogel derived from cardiac tissue to compare dECM hydrogel obtained from different tissues. Seo and coworkers[96] conducted experiments on rats, indicating that, after 3 days of subcutaneous injection, the initial vessel formation of cardiac dECM hydrogel increased 3-fold compared with Col-I. Due to a major limitation of poor mechanical tunability, the cardiac dECM hydrogels could be modified to achieve replicated cardiac mechanics. Diaz and colleagues[97] prepared an injectable myocardium-derived dECM hydrogel to study the efficacy and mechanism of preventing deterioration of negative left ventricular remodelling in a small animal model of chronic myocardial infarction (**Figure 5**). The authors found preserved left ventricular volume, ameliorated apical wall thickening and reduced fibrosis in 4 weeks after injection of a myocardial matrix hydrogel. Moreover, they found that the dECM hydrogel was sufficient to prevent further negative left ventricular remodelling by modulating the immune response, down-regulating pathways related to the progression of heart failure and fibrosis, and up-regulating genes associated with myocardial contraction to prevent further negative left ventricular remodelling. Kong and colleagues[98] developed a hybrid hydrogel, i.e., dECM/glycopeptide hybrid hydrogel, which was co-assembled from dECM derived from porcine heart and immunomodulatory glycopeptides for endogenous tissue regeneration after myocardial infarction (**Figure 6**). It was demonstrated that this dECM hydrogel, without drugs or stem cells, could attract host cell infiltration, controlling the process of macrophage differentiation and promoting endothelial cell proliferation, thus effectively improving cardiac repair.

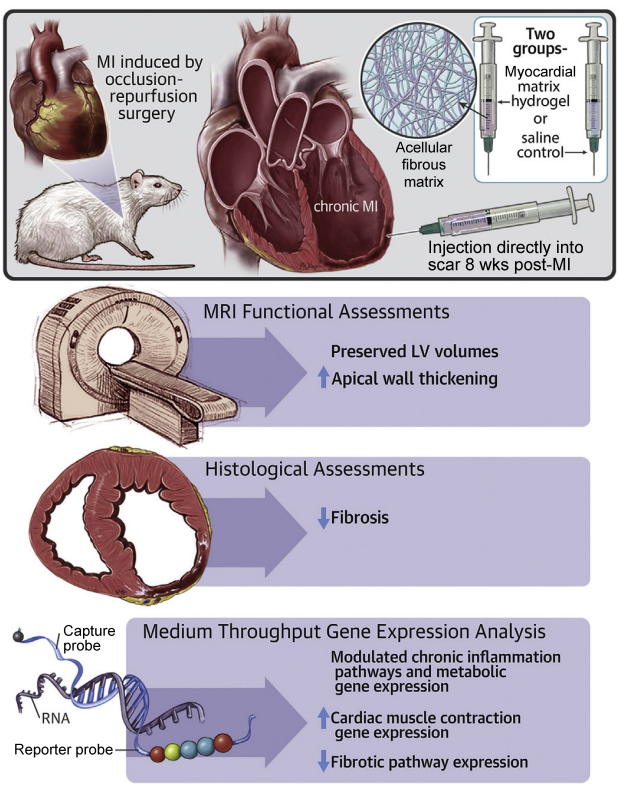

Figure 5. Visual summary of injectable myocardial matrix hydrogel attenuating negative left ventricular remodelling in a chronic myocardial infarction model. Reprinted from Diaz et al.[97] LV: left ventricular; MI: myocardial infarction; MRI: magnetic resonance imaging.

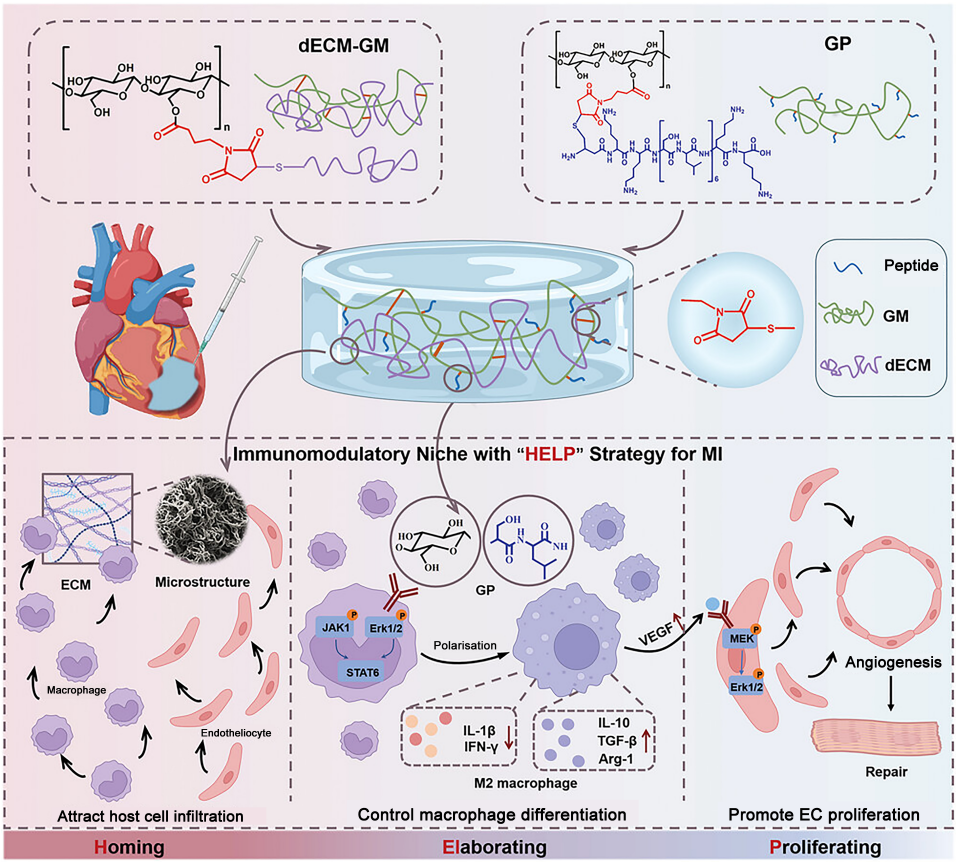

Figure 6. Schematic illustration of dECM/GP hydrogel synthesis and application for post-MI cardiac repair. Reprinted from Kong et al.[98] Arg-1: arginase-1; dECM: decellularised extracellular matrix; EC: endotheliocyte; ECM: extracellular matrix; Erk1/2: extracellular regulated protein kinases 1/2; GP: glycopeptide; GM: glucomannan; IFN-γ: interferon-γ; IL-1β: interleukin-1β; IL-10: interleukin-10; JAK1: Janus kinase type 1; M2: alternatively activated macrophages; MI: myocardial infarction; MEK: mitogen-activated protein kinase; STAT6: signal transducer and activator of transcription 6; TGF-β: transforming growth factor-β; VEGF: vascular endothelial growth factor.

Liver

As the largest internal organ in the human body, the liver offers several important functions of blood detoxification, protein synthesis, and secretion of digestive enzymes. Hepatocytes, as important functional cells of the liver parenchyma, are able to interact with non-parenchymal hepatocytes and the surrounding ECM to maintain normal liver function in a state of homeostasis.[99] Linear hepatic degeneration is the result of chronic injury or acute injury, even leading to liver failure, referred to as end-stage liver disease.[100] Liver-derived dECM hydrogel was initially used as a platform for the 3D culture of hepatocytes.[101] With ongoing research, hepatic dECM hydrogel has been further applied to regenerative applications. Jin et al.[9] initially loaded copper oxide nano-enzymes (Cu NZs) with multifunctional biomimetics onto poly(lactic-co-glycolic) acid (PLGA) nanofibres to obtain Cu NZs@PLGA nanofibres. Further, these nanofibres were hybridised with composite dECM hydrogels derived from porcine livers for the treatment of acute liver failure. The results showed that tissue necrosis and inflammation could be reduced through the scavenging of reactive oxygen species by Cu NZs and the promotion of angiogenesis by Cu NZs. The mechanical strength provided by PLGA nanofibres and functional enhancement of hepatic-like cells by dECM hydrogels synergised to reduce tissue necrotic conditions and inflammatory response, promoting liver regeneration and functional recovery (**Figure 7**). Hussein and colleagues[102] applied mouse liver decellularisation and processed it into hepatic hydrogel with the aim of developing a new treatment for hepatic fibrosis. Further, the authors experimentally demonstrated that hepatic dECM hydrogel could be used as an injectable biomaterial for hepatic tissue engineering to reduce the degree of fibrosis.

Skin

Skin is one of the largest organs of the body and the outermost protective layer, helping to shield the body from the external environment and pathogens, such as viruses. The integrity of the skin ensures maximum function. However, its lack of integrity can lead to acute physiological disorders and subsequent damage.[103] Several different cases include burns, injuries due to chronic diseases, infections, and other factors causing skin defects. These defects generally require different clinical approaches for treatment, imposing a significant and growing economic burden on both the individual patient and society.[104] Therefore, it is important to repair damaged skin tissues. Current dECM-based composites have shown many outstanding results in promoting skin regeneration. Among them, dECM hydrogels have been widely studied because of their

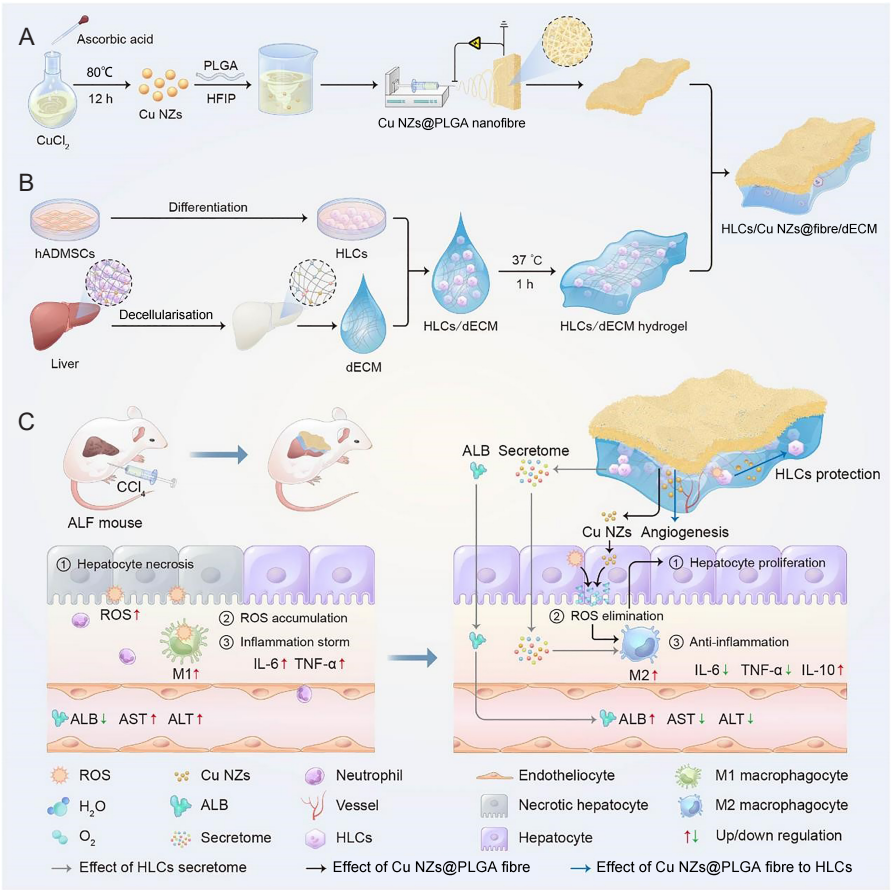

Figure 7. Schematic diagram of the composite platform consisting of Cu NZs@PLGA nanofibres and dECM hydrogels to deliver hADMSCs-derived HLCs for the treatment of CCl_4-induced acute liver failure. Reprinted from Jin et al.[9] ALB: albumin; ALF mouse: acute liver failure mouse; ALT: alanine aminotransferase; $CuCl_2$: cupric chloride; CCl_4: tetrachloromethane; Cu NZs: copper oxide nanozymes; dECM: decellularised extracellular matrix; H_2O: water; hADMSCs: human adipose-derived mesenchymal stem/stromal cells; HFIP: hexafluoroisopropanol; HLCs: hepatocyte-like cells; IL-6: interleukin-6; IL-10: interleukin-10; M1: classical activated macrophages; M2: alternatively activated macrophages; O_2: oxygen; PLGA: poly(lactic-co-glycolic) acid; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-α.

reticular 3D spatial structure and natural nutrients, providing favourable conditions for skin repair and regeneration.[105] For example, Song and colleagues[106] successfully constructed an injectable dECM hydrogel derived from porcine left ventricular myocardium loaded with adipose-derived mesenchymal stem cell-derived exosomes for skin regeneration. It offered desirable properties such as appropriate pore structure with good thermal sensitivity and biocompatibility. The dECM-based injectablehydrogels promoted vascular regeneration and firm inflammation for skin wound repair and regeneration *in vitro* and *in vivo*, respectively (**Figure 8**). Yu et al.[13] prepared covalently crosslinked dermal dECM hydrogels using dECM material derived from porcine dermis. The material subjected to ultraviolet excitation resulted in the amide bonds between methacryloyl and amino functional groups. The covalently cross-linked dermal dECM hydrogels was found to mimic the complexity of the skin's natural microenvironment with physicochemical tunability and high cellular activity, promoting the repair of skin defects throughout the skin, activating Sox9-positive hair follicle stem cells, promoting hair follicle development, facilitating scarless wound healing, and significantly enhancing angiogenesis.

Outlook and Perspectives

This review has presented the latest developments of dECM-based injectable hydrogels for tissue engineering, summarising the different preparation methods with the evaluation of injectable hydrogel properties. In addition, the current research on dECM-based injectable hydrogels is presented through many applications. Finally, we summarised the article with the prospects and challenges of dECM-based injectable hydrogel, providing insights into the development of these composites for tissue engineering and regenerative medicine.

In general, the basic preparation steps of the dECM-based injectable hydrogels are relatively mature, and their applications in regenerative medicine and tissue engineering are numerous. Although the increasing number of publications in this field reflects the growing interest in the dECM-based injectable hydrogel, several challenges remain, leading to the continuous exploration and innovation in preparation

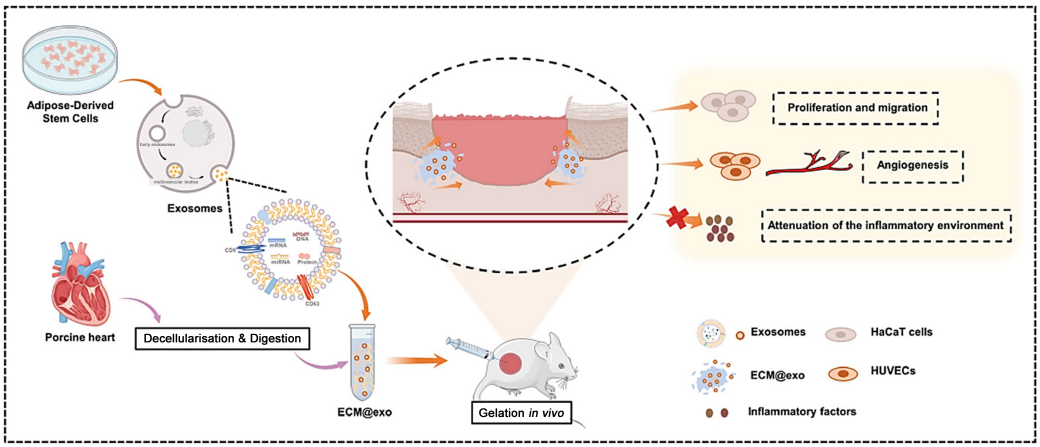

Figure 8. Preparation of ECM@exo and role of ECM@exo in the wound healing process. Reprinted from Song et al.[106] ECM@exo: extracellular matrix hydrogel@exosomes; HaCaT cells: human keratinocyte cells; HUVECs: human umbilical vein endothelial cells.

methods and applications. Firstly, the stability of the gel is the predominant concern, hampering its applicability. dECM hydrogels may lose stability after injection into the human body due to temperature and humidity changes in the surrounding environment, resulting in uneven distribution at the treatment site. To a considerable extent, incorporating additional materials (such as polylactic acid, gelatin, and chitosan-based nanoconstructs) enhances the stability of the gel's 3D network-like structure, increases the degree of cross-linking, as well as reduces the destructive effects of temperature and humidity on the gel structure. However, it is worth noting that the injectability of modified hydrogels should not be compromised while increasing the structural stability. Secondly, some dECM hydrogel may become too viscous rapidly during the injection process, affecting the injection effect due to poor fluidity. It is feasible to control the gelation time by adjusting the gel concentration and the pH value during injection, among others. In addition, the shear-thinning properties can be employed to adjust the flowability during injection. Finally, the preparation of hydrogel for injection is affected by a variety of factors, which still need to be continuously explored to arrive at a stable and reliable preparation method. Typically, the thermosensitive dECM hydrogels form a 3D network-like structure through the physical cross-linking approach. In addition to temperature, other parameters, such as pH value, protein retention in dECM, and dECM concentration, among others, affect the gelling time and quality. Moreover, it should be noted that the gelation of dECM might be dependent on the decellularisation treatment and source tissue. Therefore, continuous exploration is necessary to achieve more stable and reliable injectable hydrogels. By solving these problems, dECM-based injectable hydrogels will have great potential for medical applications due to the excellent biocompatibility of dECM. With the deepening of research and technological innovation, it is believed that the stability and controllability of the preparation of dECM-based injectable hydrogels can be improved to promote their clinical applications and contribute to human health and medical progress.

Typically, we chose dECM, a biomaterial with excellent application prospects, to compile this review article. Although dECM has been widely used recently, this perspective is strictly based on the injectable hydrogels using dECM. Considering the literature constraints, the discussion on exploring the mechanism is limited but extensively focused on the application of dECM hydrogels, highlighting the effect of various parameters on their fabrication and injectability. It should be noted that the designed dECM hydrogels offer excellent performance, which can be applied not only in the direction of injectables but also in other delivery systems based on patch[107] and 3D printing ink,[108, 109] among others. Along this line, we believe that these scaffolding materials with great research value are worth enough for more exploration and research.

Author contributions

Conceptualisation: AZC; data collection, literature reviewing, and manuscript drafting: WYG and WHW; reviewing and editing: RKK and PYX. All authors read and approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare no competing financial interest.

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