Advanced hydrogels for the repair of cartilage defects and regeneration

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ABSTRACT

Cartilage defects are one of the most common symptoms of osteoarthritis (OA), a degenerative disease that affects millions of people worldwide and places a significant socio-economic burden on society. Hydrogels, which are a class of biomaterials that are elastic, and display smooth surfaces while exhibiting high water content, are used for the deployment of hydrogels for medical applications. We also highlight the development of advanced hydrogels for cartilage defects. Nevertheless, the success of autologous chondrocyte implantation (ACI), and matrix-induced autologous chondrocyte implantation (MACI) are the most common strategies for the repair of small cartilage defects. In recent years, various kinds of hydrogels have been developed and applied for the repair of cartilage defects in vitro or in vivo, some of which are hopeful to enter clinical trials. In this review, recent research findings and developments of hydrogels for cartilage defects repair are summarized. We discuss the principle of cartilage regeneration, and outline the requirements that have to be fulfilled for the deployment of hydrogels for medical applications. We also highlight the development of advanced hydrogels with tailored properties for different kinds of cartilage defects to meet the requirements of cartilage tissue engineering and precision medicine.

1. Introduction

Osteoarthritis (OA) is a widely occurring degenerative disease and a leading cause of disability that affects more than 303 million people globally [1]. Despite not being fatal, OA places a substantial burden on societies around the world, especially those with ageing populations. The pain and further mobility limitations impact both physical and mental wellbeing of patients. Although OA mostly occurs in elderly people, young people can be affected, especially after joint injuries. Due to the increase in the age of many societies, the predicted number of people with OA will increase by 50% over the next 20 years [2]. To ameliorate this situation, the development of new treatment strategies for OA are urgently needed, as current therapies are often not satisfactory for most patients. Development of new therapeutic strategies is difficult, as the pathogenesis of OA is heterogeneous and complex.

Based on studies over the last decade, one of the major pathological characteristics of OA are defects of articular cartilage [3–5]. The development of OA and cartilage defects is a vicious circle: catabolic and proinflammatory mediators in the OA joints lead to the excess production of proteolytic enzymes and break down the cartilage; the defect in the cartilage in turn amplifies the inflammation in the joint [6]. As cartilage is an important tissue that serves by reducing joint friction between bones, its breakdown may contribute to the deterioration of joint function [7–9]. Once cartilage defects occur, the limited self-repair capability of the tissue is insufficient and no significant regeneration will occur due to the complex structure of cartilage, where no blood vessels, nerves or lymphatic tissue exists [10–12].

In the clinic, osteochondral autografts, microfracture surgery, autologous chondrocyte implantation (ACI), and matrix-induced autologous chondrocyte implantation (MACI) are the most common strategies for the repair of small cartilage defects. Nevertheless, the success of these strategies is often complicated by the formation of fibroccartilage which impairs joint function [13]. Some other limitations such as shortage of chondrocyte source and low effectiveness, often observed in older patients, were summarized by Yang et al. in a recent review article [14]. To address these issues, improved and new strategies are needed to
### Overview of hydrogels with different polymer types for cartilage tissue engineering: chemical structure and features for cartilage engineering.

| Name (Abbreviation) | Chemical structure | Features for cartilage engineering | Limitations | Refs |
|---------------------|--------------------|-----------------------------------|-------------|------|
| **Synthetic polymers** | | | | |
| Polyethylene glycol (PEG) | ![PEG structure](image) | 1. Biocompatible 2. Easy to be functionalized | Biologically inert | [47–51] |
| Poly (N-vinylcaprolactam) (PVCL) | ![PVCL structure](image) | 1. Thermosensitive 2. Cyto compatible to chondrocytes and MSCs | Moderate wettability, weak mechanical properties, low anti- biofouling | [42,157] |
| Polyvinyl alcohol (PVA) | ![PVA structure](image) | 1. Mechanical strong 2. MSCs chondrogenic differentiation | Biologically inert | [19,37,38,158] |
| Poly (2-methacryloyloxyethyl phosphorylcholine) (PMPC) | ![PMPC structure](image) | 1. Monomer and polymer biocompatible 2. Lubricant 3. Mechanical strong | Do not support cell attachment | [39–41,159] |
| **Polysaccharides** | | | | |
| Chondroitin sulfate (CS) | ![CS structure](image) | 1. Component of natural cartilage 2. Regulate hypertrophy during MSC chondrogenesis 3. Promote cartilage ECM production | Rapid degradation | [11,34,54–56] |
| Hyaluronic Acid (HA) | ![HA structure](image) | 1. Component of natural cartilage and tissue 2. Promote cartilage marker gene expression of chondrocytes 3. Easy to be functionalized | Do not support cell attachment | [62,64,68,160,161] |
| Chitosan | ![Chitosan structure](image) | 1. Drug delivery capacity | Low solubility and high viscosity | [81–83,162] |
| **Alginate** | | | | |
| 1. Fast cross-linking and mechanical strong 2. Able to 3D printing/bioprinting 3. Can be used as gene carriers | Non-biodegradable and elicit immunological responses | [86–89,91] |
| **Cellulose** | | | | |
| 1. Can be sulfated 2. Nanofibrils similar to collagen fibrils found in tissue ECM | Lack of mechanical properties | [92,93,95] |
| **Proteins** | | | | |
| Collagen | ![Collagen structure](image) | 1. Natural extracellular matrix (ECM) protein 2. Immunomodulation | Limited number of functional groups for crosslinking | [30,99,100,103,163] |
| Gelatin | ![Gelatin structure](image) | 1. Promote cell adhesion 2. Easy to be modified for UV crosslinking 3. Able to 3D printing/bioprinting | Poor mechanical properties and low thermal stability | [35,104,107,130] |
| Silk Fibroin (SF) | ![Silk Fibroin structure](image) | 1. Mimicking the collagen structure of native cartilage 2. Immunocompatible | Limited options for anchoring growth factor | [111,113,114,164] |

(continued on next page)
alleviate the problem. The chondrogenic capabilities of mesenchymal stem cells (MSC) have made them promising in cell therapy for cartilage defects. MSC, combined with materials such as hydrogels, can be delivered to the defect site of cartilage and promote cartilage regeneration. Also, hydrogels can be designed to enhance the chondrogenesis of MSC.

Because hydrogels possess cartilage tissue-like features, developing hydrogels is now the frontline of research to treat cartilage defects [15, 16]. Hydrogels are three-dimensional hydrophilic polymer networks that can absorb large amounts of water while retaining their structure [17]. There is a wide range of different sources to choose from to create hydrogels (Table 1). Synthetic polymers such as polyethylene glycol (PEG), poly (vinyl alcohol) (PVA), and polyacrylamide (PAM) have been extensively used for making hydrogels in tissue engineering [18–20].

Hydrogels composed of synthetic polymers are able to exhibit high mechanical strength and good reproducibility but may be problematic due to biocompatibility issues, which have often been insufficiently investigated. Therefore, natural macromolecules including polysaccharides, proteins, and peptides that show excellent biocompatibility have continued attention as hydrogels for tissue engineering applications. In order to develop optimized protocols for cartilage regeneration, the sources of material as well as crosslinking methods have to be investigated in more detail. Several currently used methods for the preparation of natural hydrogels were summarized by Li et al. in a recent review [21].

After decades of development, findings in basic research were translated into commercial hydrogel products used for cartilage defects repair. Several products have the potential to improve or even fully heal cartilage defects: NeoCART® is a type-I collagen matrix scaffold seeded with autogenous chondrocytes and was implanted 6 weeks following arthroscopic cartilage biopsy [22]; NovoCART®3D is a biphasic collagenous scaffold consists of a spongy part with pores arranged in columns of a set size and a dense and firm membrane [23]; CaReS® is a 3D type-I collagen hydrogel seeded with autologous chondrocytes [24]. These products represent hydrogel scaffolds for embedded autologous chondrocyte implantation.

| Name (Abbreviation) | Chemical structure | Features for cartilage engineering | Limitations | Refs |
|---------------------|---------------------|-------------------------------------|-------------|------|
| Sericin             | ![Sericin structure](image1) | 1. Low cost  
2. Nutrition-supplying | Low stability in aqueous solution | [117,165] |
| Fibrin              | ![Fibrin structure](image2) | 1. Easy to be functionalized | Not chondro-permissive | [118,119] |
| Peptides            | ![Peptides structure](image3) | 1. Promote MSC chondrogenesis | Need proper peptide design, synthesis, and purification | [125,128] |

Fig. 1. Schematic of relationship of hydrogel in lab and cartilage defects repair in clinical. ACI: autologous chondrocyte implantation; MACI: Matrix-induced autologous chondrocyte implantation.
cartilage defects repair. The properties and benefits of different hydrogels used for cartilage regeneration are analyzed and summarized to direct future research. Finally, we highlight the advantage of hydrogels that can be customized to fulfill different needs for cartilage defects to meet the high standards required in cartilage regeneration and precision medicine.

2. Principle for cartilage regeneration using hydrogels

Cartilage is hypocellular, avascular, aneural, and alymphatic, resulting in limited self-repair capacity after injuries. At the time of writing, hydrogels are being used for the repair of cartilage defects in two ways: One is to encapsulate autologous cells in the hydrogel (cell-laden hydrogel) which are then been implanted into the defect site [25, 26]. The other way is to assist and induce surrounding stem cells to participate in repair [27,28]. Hydrogels with one or both above properties can be suitable for cartilage regeneration.

From the perspective of precision medicine, the methods of cartilage repair need to address different types of defects. Generally, cartilage defects can be divided into three classes, based on the depth of defects: partial-thickness defects, full-thickness defects, and osteochondral defects (Fig. 2) [29]. Partial-thickness defects are defects on the cartilage surface without penetrating the tidemark, while osteochondral defects penetrate into the bone marrow. The microenvironment of the three defects types is quite different: full-thickness defects and osteochondral defects happen in the cartilage surface and do not penetrate the tidemark. No MSC from bone marrow will migrate or be recruited to the defect areas. Full-thickness defects are deeper cartilage defects to the tidemark but do not penetrate the subchondral bone. The environment of subchondral bone matrix is conducive to the migration and adhesion of MSC. Osteochondral defects penetrate the bone marrow and allow MSCs to be recruited to the defect area.

Fig. 2. Schematic diagram of three kinds of cartilage defects: partial-thickness defects, full-thickness defects, and osteochondral defects. Partial-thickness defects happen in the cartilage surface and do not penetrate the tidemark. No MSC from bone marrow will migrate or be recruited to the defect areas. Full-thickness defects are deeper cartilage defects to the tidemark but do not penetrate the subchondral bone. The environment of subchondral bone matrix is conducive to the migration and adhesion of MSC. Osteochondral defects penetrate the bone marrow and allow MSCs to be recruited to the defect area.

3. Current state-of-art of hydrogels designed for cartilage regeneration

3.1. Hydrogel forming materials for cartilage regeneration and their sources

Hydrogels for tissue engineering can be made from various sources of both, natural and synthetic origin. This includes synthetic polymers, polysaccharides, proteins, and peptides. The features of these macromolecules for cartilage repair are listed in Table 1, and the details are described as follows.

3.1.1. Synthetic polymers

The synthetic polymers discussed in this review are industrial products. Generally, hydrogels made from synthetic polymers are mechanically strong and reproducible. In recent years, several kinds of synthetic polymers were investigated for their use in cartilage related applications.

Polyvinyl alcohol (PVA) is a reliable and high-performance carrier due to its excellent properties of film formation, emulsification, and adhesion [36]. PVA hydrogels formed by a cast-drying method were reported to have a mechanical performance similar to that of natural human cartilage, suggesting such hydrogels could be candidate for cartilage replacement [37]. Apart from the high strength, in vitro studies of PVA-chitosan hydrogels with mesenchymal stem cells (MSCs) showed chondrogenic differentiation and glycosaminoglycan (GAG) deposition, making it a promising material for cartilage repair [38].

Poly(2-methacycloxyethyl phosphorylcholine) (PMPC) is a polymer that can function as a biomimetic boundary lubricant to imitate the properties of natural cartilage [39]. Milner et al. incorporated PMPC into a double network, forming a triple network hydrogel that could promote integration of the material into the tissue during regeneration [33-35]. 4) Feasibility: Sprayable and injectable hydrogels hold great advantages in the practical applications especially in clinical settings.

Fig. 3. Schematic representation of normal cartilage (left), osteoarthritic cartilage (middle), and hydrogel-reinforced cartilage (right). GAG depletion decreases compressive stiffness and wear resistance of cartilage. To recover lost properties, a GAG-mimetic hydrogel was made by polymerizing hydrophilic zwitterionic monomers 2-methacryloyloxyethyl phosphorylcholine (MPC) using ethylene glycol dimethacrylate (EGDMA) as a crosslinker [Reprinted from Refs. [41] with permission from Wiley].

For the design of hydrogels aiming at cartilage regeneration and clinical translation, we summarized some criteria as well as suggestions for optimized outcomes. 1) Biocompatibility: Most materials for cartilage regeneration are designed to be used in the articular cavity; components that might cause inflammation and immune responses should be avoided. 2) Cell affinity: As the participation of cells is essential in the regeneration of cartilage, the material should facilitate easy cell attachment and/or embedding [25-28]. Methods for cartilage regeneration include seeding exogenous cells into engineered products and the generation of a matrix environment that induce the migration of endogenous MSC towards the injured part. 3) Tissue integration property: The adhesion between hydrogels and tissue is crucial to avoid treatment failure caused by detachment of both components, and to promote integration of the material into the tissue during regeneration [33-35]. 4) Feasibility: Sprayable and injectable hydrogels hold great advantages in the practical applications especially in clinical settings.
withstand a high amount of stress with low friction [39]. With these advantages, this hydrogel could reduce cartilage damage caused by partial implants. PMPC was considered as a biocompatible phospholipid polymer which, when polymerized, can be grafted onto a polyethylene surface to decreased friction [40]. Therefore, PMPC was used to form a double network hydrogel to restore the mechanical strength of damaged cartilage (Fig. 3) [41]. The results demonstrated that this kind of material is promising for early-stage osteoarthritis treatment.

Apart from mechanical properties, synthetic polymers can have versatile functions. Poly(N-vinylcaprolactam) (PVCL) is a cytocompatible thermosensitive polymer that can be used to fabricate injectable hydrogels for cartilage tissue engineering [42,43]. Sala et al. showed chondrocytes and MSCs exhibited high viability in PVCL hydrogels [42]. Both in vitro and in vivo tests showed that cartilage-specific extracellular matrix (ECM) was produced in a chondrocytes-laden PVCL hydrogel. Cartilage components including GAG and type II collagen increased over time with an even distribution throughout the material.

Polyethylene glycol (PEG): For a clinical application, PEG could be the most popular candidate as it was approved by Food and Drug Administration (FDA) for medical applications in pharmaceuticals and personal care products, and is considered safe even for oral consumption [44,45]. This polymer is easily modified while possessing good mechanical properties when forming hydrogels. Therefore, many research groups selected PEG as the basic material to design hydrogels for cartilage regeneration [46]. PEG hydrogels for clinical applications have been included in many “from bench to bed side” studies with promising results [28].

Recent research in this field focused on the interactions between PEG and cells, especially chondrocytes and MSCs. Basic problems here for PEG are the viability and the growth of the cells as PEG is biologically inert [47,48]. Therefore, other biological molecules are always used together with PEG to enhance the performance of the hydrogels. For example, a network of encapsulated hyaluronic acid (HA) in degradable PEG was designed to embed chondrocytes [49]. This network not only increased the number of chondrocytes and the effectiveness in deposition in tissues, but also decreased hypertrophy of cartilage. Sridhar et al.
developed a PEG norbornene hydrogel with transforming growth factor beta 1 (TGF-β1) functionalized and crosslinked with an MMP-degradable peptide (Fig. 4) [50]. During tissue regeneration, the crosslinker peptides are cleaved by enzymes present in the surrounding tissue, allowing the degradation of the artificial matrix and the creation for an optimal environment for chondrocyte development and growth. Compared to non-degradable hydrogels, this hydrogel results in the production of appropriate amounts of matrix while maintaining the chondrocytes viable. Similarly, Skaalure et al. synthesized an enzyme-sensitive PEG hydrogel crosslinked by a peptide which is derived from aggrecanase-cleavable site in aggrecan [51]. This hydrogel system was also demonstrated to promote hyaline-like cartilage regeneration while avoiding the formation of hypertrophic cartilage.

Besides, in synthetic polymers, co-polymer can be specifically designed for cartilage tissue engineering. For example, poly-D,L-lactic acid/polyethylene glycol (PLA-PEG) was used to deliver bone morphogenetic protein-2 (rhBMP-2) for articular cartilage repair. In this system, PLA-PEG permits the release of rhBMP-2 for 3 weeks. In vivo results based on New Zealand White rabbits showed that subchondral defects were completely repaired after 6 weeks [52].

Above mentioned PVA, PMPC, PVCL, and PEG have been widely studied for cartilage tissue engineering. Hydrogels fabricated by such synthetic polymers are mechanically strong but most of them are biologically inert. The limitations of each polymer are summarized in Table 1. Therefore, native bio-macromolecules could be used to combine with synthetic polymers to simultaneously possess good mechanical strength and biological functions. For example, chondroitin sulfate (CS) is a sulfated GAG consisting of a chain of alternating N-acetylgalactosamine and glucuronic acid [53]. CS is also the main component of natural cartilage and can be used for the production of hydrogels serving as scaffolds in cartilage tissue engineering [34]. CS was reported to stimulate the secretion of glycoproteins and type II collagen by surrounding cells [54]. Although CS has good biocompatibility, it is rarely used alone due to its insufficient mechanical properties and fast degradation rates [11,55]. Recently, CS was combined with PEG to form a cartilage mimetic hydrogel, which was used to demonstrate the hypertrophy regulating capacity of CS during MSC chondrogenesis [56]. Han et al. incorporated CS into a polyacrylamide hydrogel, also demonstrating the cartilage regeneration ability of CS (Fig. 5) [57]. Besides, CS molecular chains can be modified to display physical or chemical-reactive moieties. An example is methacrylated chondroitin sulfate (CSMA) where CS chains have been modified with methacrylic anhydride. CSMA is degradable and can be UV-crosslinked to form a hydrogel scaffold that mimics the natural ECM in which chondrocytes were viable and multiplied [58]. CS is not only a macromolecule to form materials, but also is a regeneration factor for cartilage, and is worth to be studied in the future cartilage tissue engineering.

3.1.2. Polysaccharides

**Hyaluronic acid (HA)** is a linear biomacromolecule playing crucial roles in many cellular functions [59,60]. HA has fairly good biocompatibility when the molecular weight is high enough and is widely applied in cartilage tissue engineering because it is also a major component of cartilage ECM [49,61–64]. Particularly, HA possesses biological activities and therefore commonly is used to fabricate hydrogel scaffolds interacting with cells [65]. Chondrocytes encapsulated within hydrogels containing 5% HA showed more than ten-folds increasing in the expression of cartilage marker genes like Aggrecan and Sox9 compared with 1.5% HA [62].

Like chondroitin sulfate, HA can be modified in order to become more versatile for cartilage tissue engineering. Methacrylated hyaluronic acid (HAMA) is an example of a modified HA. With a photo-initiator, HAMA can be crosslinked and form hydrogels under UV irradiation. For instance, Mouser et al. added HAMA to a thermosensitive copolymer solution to form a bio-ink [66]. When cultured with chondrocytes in vitro, a cartilage-like matrix was produced, and the amounts of matrix were dose-dependent to the HAMA concentration. Shi et al. used HAMA hydrogel encapsulating kartogenin (KGN)-loaded...
nanoparticles as scaffolds to provide a matrix for cell homing and cartilage regeneration (Fig. 6) [67]. KGN is a small molecule that can induce BMSCs to develop into chondrocytes. This technology has the advantage of being a one-step procedure, with regenerated tissues being similar to natural hyaline cartilage shown by the histological tests, specific markers analysis and biomechanical tests. Another approach was the development of a photo-sensitive hydrogel by creating α-nitrobenzyl alcohol moiety modified hyaluronic acids (HA-NB); HA-NB can form hydrogels on tissue surfaces in situ under light irradiation [68,69]. The material integrates well into existing cartilage and functions as a scaffold in cartilage defect sites in order to allow stem cell-derived exosomes to be retained at defect sites, which has the potential to replace stem cell therapy in cartilage regeneration [70].

In order to increase the affinity to bind proteins, HA can be sulfated, increasing the amount of negatively charged groups [71]. The sulfated HA hydrogel demonstrated slower degradation and enhanced growth factor retention as the original scaffold consisting of unmodified HA. Chen et al. also showed that 30% thiolated hyaluronic acid (HA-SH) and 70% collagen-1 hybrid hydrogel could overcome the generally observed weak cell adhesion of HA while optimizing the gelation time to make the material suitable for injection [72]. In addition, the incorporation of HA in a chitosan-based hydrogel showed significantly increased proliferation of encapsulated chondrocytes and the deposition of cartilaginous extracellular matrix [73].

Chitosan is a polysaccharide extracted from the shells of shrimp and other crustaceans. The amino groups in chitosan make the molecule cationic. It shows antimicrobial properties and is highly bio-adhesive [26,74–78]. Liang et al. synthesized amphotytic chitosan/Carrageenan hybrid hydrogels which could induce chondrogenic differentiation of ATDC5 cells in vitro and showed good potential for medical applications for the regeneration of cartilage [79]. In order to construct a flexible hydrogel scaffold that can fill the different shapes of cartilage defects, Meng et al. blended chitosan with demineralized bone matrix particles modified with a phase display derived peptide E7 [80]. They demonstrated that the hybrid hydrogel scaffold improved MSCs survival, matrix production and chondrogenic differentiation. Methacrylated method also worked on Chitosan. Choi et al. mixed methacrylated glycol chitosan (MeGC) with type II collagen and TGF-β1, followed by exposure to visible blue light in presence of riboflavin, forming in a scaffold for mesenchymal stem cells (MSCs) in defect sites, for enhanced chondrogenic differentiation and integration into host tissue [81]. MeGC hydrogel is a good candidate to stabilize and control the release of TGF-β1 which is able to improve neocartilage formation, as well as being suitable as a drug delivery system [81,82]. Through Schiff base formation, glycol chitosan mixed with partially oxidized HA is able to form hydrogels, for the delivery of chondrocytes by non-invasive procedures [83].

Alginate is an anionic polysaccharide found in abundance in the cell walls of brown algae [17,84,85]. Because of its simple gelation with divalent cations like calcium ions Ca^2+; alginate is often used as an injectable hydrogel, in a non-invasive approach for cartilage repair [86,87]. Alginate was also applied in 3D bioprinting techniques because of its fast cross-linking ability [88,89]. However, alginate alone lacks bio-functionality with respect to interactions with proteins and cells. Combined HA with alginate, Park et al. showed that hybrid scaffolds could retain the ability to form gels in the presence of Ca^2+ and enhanced chondrogenic differentiation of ATDC5 [90]. Interestingly, alginate nanoparticles can also be used as gene delivery vehicles for engineering. For example, Gonzalez et al. encapsulated MSCs and nHA (nanohydroxyapatite) complexed with plasmid DNA (pDNA) encoding for pTGF-β3, pBMP2 or together into alginate hydrogels [91]. It was found that the pDNA was transported to MSCs, which did not occur in the control without alginate. This novel gene-activated alginate hydrogel successfully supported transfection of encapsulated MSCs and directed their differential orientation through different genes.

Cellulose is a linear chain polysaccharide consisting of α-glucose units and is the most abundant organic macromolecule on earth. For the construction of hydrogels, cellulose is often modified to enhance its properties. Sodium cellulose sulfate (NaCS) combined with gelatin is able to form a fibrous scaffold, in which hMSCs chondrogenesis was shown to be enhanced through increased while gene expression of type II collagen [92]. A new silated hydroxypropyl methylcellulose (Si-HPMC) hydrogel mixed with laponites, resulted in a hydrogel scaffold with improved mechanical strength that was cytocompatible and allowed oxygen diffusion [93]. When enriched with a biologically active marine exopolysaccharide (GY785), this Si-HPMC hydrogel produced an even more cartilage-like ECM [94]. Bacterial nanocellulose (BNC) is reported to possess nanofibrils similar to collagen fibrils found in tissue ECM; a bilayer BNC hydrogel has been shown to provide a suitable environment for chondrocytes to form cartilage in vitro and in vivo [95].

Polysaccharides are highly bio compatible nature macromolecules that resembles the glycan constituent of ECM. They have been widely investigated in the field of cartilage tissue engineering. However, single type of polysaccharide often has limitations to form ideal materials. For instance, CS can be biodegraded fast while chitosan has low solubility that is difficult to handle. The limitations of each polysaccharide are listed in Table 1. Combination with other category of polymers could be a solution to overcome these limitations.

### 3.1.3. Protein

**Collagen** is a type of ECM protein which naturally occurs in cartilage, supporting the growth of chondrocytes. Therefore, it is widely used in cartilage tissue engineering [96–98]. Collagen hydrogels reduce the risk of immune rejection by stimulating the ECM formation, reduce immunogenicity of exogenous cells that are seeded in engineered hydrogel constructs and promote cartilage regeneration [99]. In addition, Wong et al. found that type II collagen was capable of converting auricular chondrocytes into articular cartilage after dedifferentiation by monolayer passaging [100]. By using type I collagen as scaffold combined with stromal cell-derived factors-1 (SDF-1) as growth factor, Zhang et al. successfully induced the migration and adhesion of C-MSCs and SM-MSCs and promoted the self-repair of partial thickness defects [30]. Another study conducted by Jiang et al. indicated that collagen-based hydrogel encapsulated with MSCs could mediate the expression of Sox9 (a transcription factor regulates chondrogenesis), suggesting that appropriately designed hydrogel scaffold may work without growth factor [101]. Collagen can also be combined with other materials to form hybrid hydrogel scaffolds that have enhanced properties compared to the individual components. For instance, a native cartilage-mimic hydrogel was constructed using collagen, CS, and HA [102]. More interestingly, magnetic nanoparticles can be incorporated into type II collagen-HA-PEG hybrid hydrogels. This idea had not only beneficial effects on the engineered scaffold itself, but can also be used to transport the scaffold exactly to the cartilage defect [103], opening new avenues of cartilage tissue engineering. However, the effect of magnetic fields on the function of BMSCs remains to be investigated.

**Gelatin**, a kind of denatured collagen, is commonly used for cartilage repair as it is biocompatible, degradable, and shows good cell affinity, beside other advantageous properties [104–106]. However, major disadvantages of gelatin for cartilage repair are poor mechanical properties as well as low thermal stability [107]. To address these drawbacks, various methods have been developed. Wang et al. created a hybrid hydrogel consisting of gelatin and hydroxyphenylpropionic acid (HPA) and halloysite nanotubes (Si-HPMC) hydrogel mixed with laponites, resulted in a hydrogel scaffold with improved mechanical strength that was cytocompatible and allowed oxygen diffusion [93]. When enriched with a biologically active marine exopolysaccharide (GY785), this Si-HPMC hydrogel produced an even more cartilage-like ECM [94]. Bacterial nanocellulose (BNC) is reported to possess nanofibrils similar to collagen fibrils found in tissue ECM; a bilayer BNC hydrogel has been shown to provide a suitable environment for chondrocytes to form cartilage in vitro and in vivo [95].
hydrogel scaffolds that possessed suitable hydrophilicity for growth, embedding, and ECM secretion of cells [108], while also increasing the material strength. Methacrylic anhydride (MA) easily reacts with gelatin to form photo-crosslinkable gelatin methacrylamide (GelMA) for hydrogel scaffolds. By combining acrylamide (AM) and GelMA, Han et al. obtained a hydrogel that showed increased mechanical strength and enhanced elasticity [104]. The compression strength and storage modulus of the AM and GelMA combining hydrogel can be up to 0.38 MPa and 1000 Pa, respectively. Zhou et al. recently reported a hydrogel scaffold composed of GelMA, oxidized dextran (ODex), and gelatin used for cartilage defect repair [35]. The formed hydrogels (M-O-G hydrogels) were crosslinked by a photo-initiator lithiumphenyl-2,4,6-trimethylbenzoylphosphinate (LAP) under UV irradiation (Fig. 7). In such a system, ODex not only partly crosslinked the network through reacting with gelatin, but also provided good tissue adhesive property by Schiff base reaction with amino groups found on the surface of the cartilage. Combining these properties together, the M-O-G hydrogels were formed in situ on the cartilage defects to enhance tissue integration. An in vivo study using a rabbit osteochondral defects model, showed that cartilage was regenerated at the defect site, and the new formed cartilage possessed comparable mechanical strength to the natural one. These GelMA hydrogels were reported to be promising candidates for cartilage tissue engineering. Rothrauff et al. demonstrated GelMA hydrogel had comparable efficacy to pepsin-solubilized cartilage and tendon hydrogels in cartilage tissue engineering [109]. Similarly, Li et al. synthesized gelatin norbornene (GelNB) which can be used for encapsulating human mesenchymal stem cells [110]. This work provided researchers and clinicians with another choice of gelatin modification for articular cartilage tissue regeneration.

Silk fibroin (SF) is the main component of silk with excellent biocompatibility and biodegradability. Capable of mimicking the collagen structure of native cartilage, silk fibroin shows great potential...
in cartilage tissue engineering [111,112]. Zhou et al. synthesized methacrylated silk fibroin (MSF), which can be crosslinked by exposure to light [111]. The formed hydrogel was demonstrated to be compatible with mouse articular chondrocytes and suitable for cell adhesion. Horseradish peroxidase (HRP) mediated crosslinking was used to produce robust and interconnected porous silk fibroin scaffolds under physiological conditions [113]. Singh et al. fabricated a hydrogel composed of silk fibroin and agarose, which could be used for cartilage tissue engineering (Fig. 8) [114]. The hydrogel showed good immunocompatibility and was able to maintain of chondrogenic phenotype. To further improve mechanical properties, Yodmaung et al. generated a silk microfiber-silk hydrogel which also resulted in a favorable chondrocyte response [115]. An overview of silk fibroin-based hydrogels for cartilage defects repair and regeneration was completed by Ribeiro et al. [116].

Sericin is a protein extracted from silk fibers, and a byproduct in the silk industry, usually discarded as waste. However, like silk fibroin, sericin displays high biocompatibility. Thus, sericin can also be used to generate hydrogel for cartilage regeneration. By functionalizing the material with methacryloyl groups, the mechanical properties and degradation rates of sericin methacryloyl hydrogels can be adjusted across a wide range, making it possible to mimic native cartilage to suit different cartilage repair cases [117]. One of the advantages of using sericin is the nutrition-supplying property that allows chondrocytes proliferation even when lacking nutrition. Sericin methacryloyl hydrogels could be a promising scaffold for cartilage repair with molecular and mechanical resemblance to native cartilage and tunable properties.

Fibrin is a widely used biomaterial in the field of tissue engineering. Almeida et al. functionalized fibrin hydrogels with cartilage ECM for cartilage regeneration. Fibrin hydrogels could incorporate up to 2% (w/v) cartilage ECM particles which promoted the generation of cartilage-like tissue from freshly isolated stromal cells [118]. Snyder et al. reported a hydrogel made from chondrogenic fibrin and HA [119]. This hydrogel system could be injected, and it was possible to form a 3D network structure in situ. 3D cell culture of BMSCs in this hydrogel showed increased Sox9 mRNA expression in quantitative polymerase chain reaction (qPCR) test, suggesting early chondrogenesis and thus a high potential for articular cartilage repair.

Proteins hold great potential as biomaterials for tissue engineering because of their excellent biocompatibility and biodegradability. The limitation for most proteins is the stability: high temperature or organic solvent can lead to the denaturation of protein. This limitation makes it difficult in material processing when use proteins. Mild processing method might be developed to solve this issue and lead to the revolution of biomaterials made from proteins.

### 3.1.4. Peptides

Due to their diversity, peptides are thought as one of the most promising molecules in cartilage regeneration because they can serve as components of permeable scaffolds or bio-functional factors [120]. For instance, Link protein N-terminal peptide (LPP) in ECM of cartilage was shown to stimulate the proliferation of cartilage stem/progenitor cells (CSPCs) [121]. Peptides can mimic some functions of proteins while they have the advantage to be adjustable in size. Peptides are readily synthesized in the lab and are relatively stable. This paves the way for developing peptide hydrogel scaffolds for cartilage tissue engineering. The challenge for peptide as cartilage regeneration materials is the proper design, synthesis, and purification. Another limitation to peptides, could also exist in polysaccharides, is the potential immunogenicity and the effects of their degradation products.

Peptide based hydrogels have already shown their superiority in biocompatibility compared to many other materials. Callahan et al. synthesized an arginine-glycine-aspartic acid (RGD) peptide and used functionalized PEGDA hydrogel scaffold to enhance cell adhesion [122]. Based on this system, they found that mechanical properties of these hydrogel scaffolds were crucial for modulating human osteoarthritic chondrocyte behavior. Peptides can also be acylated and simultaneously photopolymerized with acylated PEG to form hydrogels [123]. When bioprinting with this PEG-peptide hydrogel scaffold, hMSCs chondrogenic differentiation for cartilage formation was significantly enhanced compared with pure PEG hydrogel. Liu et al. synthesized co-polymers consist of poly (L-alanine), PEG, and poly (L-alanine-co-L-phenylalanine) for cartilage tissue engineering [124]. The formed polypeptide hydrogels displayed thermo-sensitive properties, as well as mechanical strength which can be enhanced by increasing the content of phenylalanine, leading to hyaline-like cartilage formation in vivo macroscopically and histologically. Parmar et al. developed a biodegradable peptide hydrogel that significantly enhanced chondrogenic differentiation of hMSCs [125]. They found that, in this hydrogel system, CS-binding peptides help enhance MMP7 gene expression and activity, which are thought to be relevant to chondrogenesis through accelerating collagen type II maturation and promote functioning of chondrogenic factors. In another study, a photopolymerizing hydrogel based on CS and integrin binding RGD peptides was reported to increase lubricin gene expression of the encapsulated chondrocytes [126]. Diphthynylalanine-serine (F2/S) peptide hydrogel, which is tunable in its properties, can be used to improve cartilage phenotype from differentiated pericytes. The content of type II collagen increased using this peptide hydrogel for chondrogenesis, without induction by components present in the media [127]. Lu et al. used bone marrow homing peptide (BMHP) functionalized with a self-assembling peptide (SAP) to fabricate a hydrogel scaffold for cartilage regeneration. The synthesized SAP hydrogels stimulate rabbit MSC attachment, proliferation, and chondrogenic differentiation. An in vivo test after 3 and 6 months showed that cartilage-like tissue with a smooth surface was formed on the articular cartilage defect. The study demonstrated that such hydrogel scaffolds are promising for cartilage repair without cell transplantation [128].

#### 3.2. Additive manufacturing of hydrogels for cartilage regeneration

Generally, above mentioned source materials usually form hydrogels through mold-casting strategy if not delivered in situ. A novel technique is additive manufacturing or 3D printing/bioprinting which is increasingly being deployed to fabricate biomaterials for cartilage defects repair [27,129–131]. 3D printing is able to construct scaffolds precisely to match the defective area. Combining hydrogel and cell, 3D bioprinting could allow the precise 3D space depositing of living cells that then develop into functional artificial tissue. Using this approach, Zhu et al. produced a 3D bioprinted cell-laden cartilage tissue construct [130]. GelMA and PEGDA were selected and optimized as a printable resin for cell-hydrogel bioprinting. The printed hydrogel constructs sustained release TGF-β1 and improved chondrogenic differentiation of encapsulated MSCs. Aisenbrey et al. used stereolithography-based 3D printing with photopolymerizable hydrogel to treat focal chondral defects [129]. In this study, chondrocytes were incorporated in an injectable hydrogel precursor solution which can be infiltrated into a 3D printed support structure, promoting neotissue deposition. This kind of construct minimized the damage to the surrounding tissue when placed in focal chondral defects in an osteochondral model. Costantini et al. used two coaxial-needles system, another 3D bioprinting strategy, to print a 3D biomimetic hydrogel composed of similar biopolymers present in cartilage ECM [131]. BMSCs loaded and cultured in the printed hydrogels showed enhanced viability and chondrogenic differentiation. Although 3D bioprinting is an emerging technique for tissue engineering, it has not widely been applied for the repair of cartilage defects. The current development in bioprinting is to move from form to function [132]. Novel and innovative ideas need to be developed to take full advantage of this technique for cartilage applications.

### 4. Applications and clinical trial

After extensive lab-based research and testing, some of the
developed materials mature into products with great potential for clinical applications. Currently, the use of hydrogels in the clinic is mainly to improve existing treatment strategies such as ACI, MACI, and microfracture surgery. A product consists of a 3-dimensional type I collagen scaffold and autologous chondrocytes and was developed for full-thickness cartilage defects [133]. This product proved to be effective albeit being simple and was demonstrated as a safe and effective treatment for full-thickness cartilage defects through to 5-year follow-up [134]. Another product (CaRes®) also based on collagen showed comparable results to microfracture for the treatment of patellofemoral articular cartilage lesions [135]. These results indicate collagen possesses advantages for cartilage defects repair in clinical.

Microfracture surgery is a clinical therapy to treat cartilage defects. In this method, small holes are created that penetrate subchondral bone to expose bone marrow and therein containing cells. However, about half of the procedures fail due to insufficient formation of new tissue and of fibrocartilage, but also due to unwanted bone invasion [14,28]. ChonDux hydrogel is a product that can improve this procedure. This hydrogel system consists of a PEG/HA network and a CS adhesive [29]. PEG was first modified to PEG diacrylate (PEGDA) which is able to photocrosslinked to hydrogel. Sharma et al. found HA incorporate with PEGDA could promote chondrogenesis of mesenchymal stem cells in vivo [28,136], while Wang et al. demonstrate CS adhesive helped the hydrogel retain on the cartilage [34]. With this hydrogel, the blood and bone marrow from the microfracture holes was accumulated around the defects and assists with the regeneration. A clinical trial of ChonDux for full-thickness femoral condyle defects showed 94.2% ± 16.3% of final defects filled over 24 months and the treated tissue was similar to uninjured cartilage, suggesting such a system is a safe supplementary product for microfracture therapy [137].

Stem cell therapy is a novel strategy to treat cartilage defects in OA. Cartistem® is a product composed of HA hydrogel and allogeneic human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs). Over a 7-years clinical trial, this product was demonstrated to be safe and effective for cartilage regeneration [138]. Before this, in an animal study using the minipig model, it was shown that the chondrogenic differentiation capacity of hUCB-MSCs synergistically affected the degree of cartilage regeneration potential [139]. Given that HA is one of the main ingredients in ChonDux, we think HA is an important component for cartilage tissue engineering and holds great potential for clinical translation.

5. Challenges

It is well known that cartilage do not self-repair due to lack of blood vessels, nerves and lymphatic tissues [140,141]. Hydrogels can act as scaffolds, elastomers, drug/nutrition carriers to help the regeneration of cartilage. The challenge here is how to select the most suitable hydrogel for a procedure to help the patients in pain due to OA. To engineer a hydrogel for cartilage repair, the first thing that should be considered is the source of the material. We know that hydrogels made from PAM and its derivative double networks are extremely strong in mechanical like natural cartilage [142,143]. However, PAM hydrogels were reported causing inflammatory reactions in breast augmentation and were banned to use by Chinese State Food and Drug Administration in 2006 [144,145]. Therefore, it might be more challenging for a clinical application to use this material as an implant for cartilage repair. Currently, materials that are able to promote MSCs chondrogenesis and maintain the phenotype of chondrocytes in vivo, have shown to be the most promising types for cartilage regeneration. A mass plethora of studies demonstrated that such materials can be used for chondrogenic differentiation of cells (an incomplete list can be found in Table 1). Apart from the type of material, parameters such as matrix elasticity, matrix stiffness, and mechanical confinement can regulate the cell fate as well [13,146,147]. Due to the complexity of the tissue, mechanical and biological considerations in terms of cell development and medical as well as physical attributes or defects, an optimal solution has yet to be found. With the increasing use of machine learning and artificial intelligence (AI), we have tools to our disposal that could help to develop better materials in the future. These techniques may be able to help us accelerated the process of trial and error, and finally obtain the ideal material for cartilage regeneration.

Another problem for cartilage tissue engineering is the way to bond the biomaterials to cartilage [34]. Without efficient bonding, even newly formed cartilage around the introduced scaffold might not integrate well with the host tissue [35]. Thus, bio-adhesive properties for the design of novel hydrogel materials have to be considered. Recent advancements in the development of hydrogels with tough adhesive properties especially to wet surfaces, may pave the way in constructing biomaterials for cartilage tissue engineering [148–150]. However, when used for cartilage tissue engineering, the biocompatibility of the glue is needed to be considered. And the mechanism of the cells traverse and modify the glue to integrate the tissue should be investigated.

Till today, we are unable to achieve engineered cartilage as it is in the human body. Although cartilage has been investigated for many decades, new technologies such as cryo electron microscopy, solid-state NMR and mass spectrometry can be used to deepen our understanding of natural cartilage to reveal the exact components and molecular structure [151,152].

6. Conclusions and outlook

In this work, we have reviewed the progress of designing hydrogels for cartilage tissue engineering during the last 5 years. We provide an overview on hydrogel structure, most of which, in fact, act as a scaffold for cell development or drug delivery. Synthetic polymers such as PVA and PEG are biologically inert and have no direct effect on cells. Factors that help to promote MSC chondrogenesis and maintain the phenotype of chondrocytes are often included as cartilage-ECM like materials such as HA, CS, and also specific peptides like arginine-glycine-aspartic acid (RGD) and Link protein N-terminal peptide (LPP). Therefore, when producing hydrogels for articular cartilage tissue engineering, some suggestions are made: 1. if a mechanically strong scaffold is needed, synthetic polymers and silk related materials might be the best choice. 2.
To enhance cell adhesion, proteins such as collagen and gelatin have been shown to be beneficial. 3. Chondrogenesis promoting factors such as HA, CS, and TGF-β1 should be added to the system to increase effectiveness of the product. 4. Hydrogels can be designed as tissue adhesive and injectable/paintable for practical applications and clinical translation. Some polymers and factors sorted by their characters are shown in Fig. 9.

In osteoarthritis, cartilage defects can be divided into three classes: partial-thickness defects, full-thickness defects, and osteochondral defects. In our opinion, it would be advantageous to consider that hydrogels should be specifically designed for their application, to treat different kinds of cartilage defects (Fig. 10). For example, scaffolds for full-thickness defects and osteochondral defects can be designed in bulk and are transplantable, with the 3D printing/bioprinting technique as an option to produce such scaffolds. Many studies focused on the treatment of full-thickness defects and osteochondral defects. But most of the cartilage defects observed in OA joints are partial-thickness, such defects need to be studied as well due to their different nature and extent of tissue damage [153, 154]. For each kind of cartilage defect, the most suitable treatment strategy should be deployed.

In our opinion, cartilage defects in OA could be treated in two ways. One is to make an artificial cartilage which might be a tough and lubricated hydrogel. The artificial cartilage can be transplanted into the defect sites or replace the whole cartilage. This kind of materials could be designed and fabricated aiming at superior properties than natural cartilage. Some super tough hydrogels such as alginate-polyacrylamide (Alg-PAM) hydrogels and polyacrylamide-alginane double network hydrogels equilibrated with aqueous solutions of calcium chloride, which have been recently reported can be considered if biocompatibility and bioadhesion are satisfactory [148, 155, 156].

The other way is to design biomaterials to facilitate self-repair in the body. Stem cells from the synovial fluids cannot perform their task to repair injured cartilage when the micro-environment of the cartilage surface is not suitable for cell adhesion [30]. Therefore, hydrogels with proper mechanical strength and excellent stem cell adhesion properties are promising materials for cartilage repair. Many of the above-reviewed development strategies to construct various kinds of hydrogel scaffolds show the promising nature of such materials for the treatment of different kinds of cartilage defects in OA. With recent developments in stem cell therapy, harvesting the synergies of both fields has the potential to provide highly satisfactory solutions for OA patients.

Declaration of competing interest
The authors declare no conflict of interest.

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