Review Article

Narrative review of the choices of stem cell sources and hydrogels for cartilage tissue engineering

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Abstract: Stem cell-based therapy is a promising treatment for cartilage defects due to the pluripotency, abundant sources and low immunogenicity of stem cells. Hydrogels are a promising class of biomaterials for cartilage engineering and are characterized by bioactivity, degradability and elasticity as well as provide water content and mechanical support. The combination of stem cells and hydrogels opens new possibilities for cartilage tissue engineering. However, the selection of suitable types of stem cells and hydrogels is difficult. Currently, various types of stem cells, such as embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), and peripheral blood mononuclear cells (PBMSCs), and various types of hydrogels, including natural polymers, chemically modified natural polymers and synthetic polymers, have been explored based on their potential for cartilage tissue engineering. These materials are used independently or in combination; however, there is no clear understanding of their merits and disadvantages with regard to their suitability for cartilage repair. In this article, we aim to review recent progress in the use of stem cell-hydrogel hybrid constructs for cartilage tissue engineering. We focus on the effects of stem cell types and hydrogel types on efficient chondrogenesis from cellular, preclinical and clinical perspectives. We compare and analyze the advantages and disadvantages of these cells and hydrogels with the hope of increasing discussion of their suitability for cartilage repair and present our perspective on their use for the improvement of physical and biological properties for cartilage tissue engineering.

Keywords: Stem cells; hydrogels; cartilage tissue engineering

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Introduction

Cartilage-related tissue defects are a common cause of disability and account for 6% of disabled people over 30 years of age (1). Cartilage defects are a major problem in orthopedic surgery and are commonly caused by traumatic injury, disease and aging. Persistent cartilage injury may induce irrevocable deterioration of joints, leading to osteoarthritis and disabilities (2). Due to the absence of vascularization, nutrient supply and proper endogenous

progenitor cells, the self-repair capability of cartilage is quite limited.

Traditional treatments for cartilage-related tissue defects include microfracture surgery or autologous chondrocyte implantation. Despite the common use of traditional treatments in clinical practice, they have notable shortcomings that cannot be neglected. Microfracture may induce cartilage regeneration by drilling tiny holes that penetrate the cartilage and subchondral bone to bring stem cells and biomolecules to the defects. However, microfracture frequently leads to the formation of fibrocartilage, which has inferior biofunctions compared to articular cartilage (3,4). Autologous chondrocyte implantation has a satisfactory surgical outcome and has been used clinically for two decades. Nevertheless, due to the long harvest time of autologous chondrocytes, the shortage of chondrocyte sources and their low effectiveness in aged patients, additional surgery may be required, and the possibility of donor morbidity has to be considered (5).

Cartilage tissue engineering has been proposed as a more effective treatment, which can be achieved by two main approaches. One approach is to mimic the architectural features and biological functions of native cartilage by regenerating articular cartilage, osteochondral interface tissues and zonal structures into a highly complex composition using advanced manufacturing technology. Another approach is to deliver appropriate biomaterials as an artificial extracellular matrix (ECM) to facilitate cell growth, proliferation, and differentiation at the sites of the defects, thus leaving the regeneration of the articular cartilage and subchondral bone to native biological processes (6).

Hydrogels are versatile biomaterials used in cartilage tissue engineering due to their unique properties, which are similar to the characteristics of natural ECMs. The structure, composition, and biochemical and mechanical properties of hydrogels are conveniently tunable to suit various desired applications (7). Hydrogels can serve as ECMs to control cell morphology, proliferation, and differentiation at a defect site (8,9). Moreover, cell-laden hydrogels can be manufactured by advanced techniques with patient-customized compositions (10,11). Hence, it is widely accepted that hydrogels loaded with cells and growth factors have great potential to address the challenge of regenerating full-thickness cartilage (9). In the literature, chondrocytes embedded in various types of hydrogels have demonstrated a well-maintained cell phenotype (12). However, the application of chondrocytes has certain limitations. First, chondrocytes are harvested from healthy cartilage tissue at a non-weight-bearing site, which may lead to morbidity of the donor site. Second, the number of chondrocytes is limited and it takes as long as 3 to 5 weeks for the *in vitro* expansion of cells. Third, the proliferation capacity of chondrocytes is low, especially in the case of chondrocytes derived from aged patients.

Thus, stem cell-based therapy is considered to be a more promising approach to treat cartilage defects because of its strong chondrogenic potential and proliferative capacity. Different types of stem cells are suitable for cartilage engineering with easy access, such as mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), and peripheral blood mononuclear cells (PBMSCs). MSCs are the most widely used stem cells; they are derived from a variety of sources (such as bone marrow, adipose tissue and muscle) and can proliferate without differentiation for up to 40 generations. MSCs can interact with local biochemical stimuli and generate a number of growth factors for tissue restoration. The use of ESCs and iPSCs is emerging in cartilage engineering due to their pluripotency and potential to differentiate into almost all cell lineages, including chondrogenic lineages.

In this review, we will briefly revisit the hydrogels explored in cartilage repair, focus on the recent advances in the utilization of stem cells combined with hydrogels for cartilage repair and discuss the advantages and disadvantages of various options in the hope that we can shed light on these topics for future studies and for the clinical development of stem cells and biomaterial-based cartilage regeneration. We present the following article in accordance with the Narrative Review reporting checklist (available at http://dx.doi.org/10.21037/atm-20-2342).

Methods used for the literature search

This systematic research was performed using the Web of Science and PubMed databases with the following search string: “stem cell” OR “mesenchymal stem cells” OR “embryonic stem cells” OR “induced pluripotent stem cells” OR “peripheral blood mononuclear cells” AND “hydrogel” OR “hyaluronic acid” OR “chitosan” OR “alginate” OR “agarose” AND “cartilage repair” OR “cartilage rehabilitation”. Papers published in English up to April 2019 were selected.

Results of the literature search

The results of the literature search are shown in *Figure 1*. The initial search resulted in 1,497 articles. After exclusion of duplicates, 644 articles remained, which were then subjected to a manual review based on their titles and abstracts. A total of 274 articles were reviews, editorial or conference presentations, and 138 papers were not related to stem cells. Out of the remaining articles, 89 papers were irrelevant to stem cells and 81 papers did not involve hydrogels. Thus, 62 studies remained after screening. We further excluded 12 articles without an available full-text, 24 studies without *in vivo* observations, and a clinical study that did not specify the clinical protocol. Finally, 22 preclinical studies and 3 clinical studies were included in the present review.

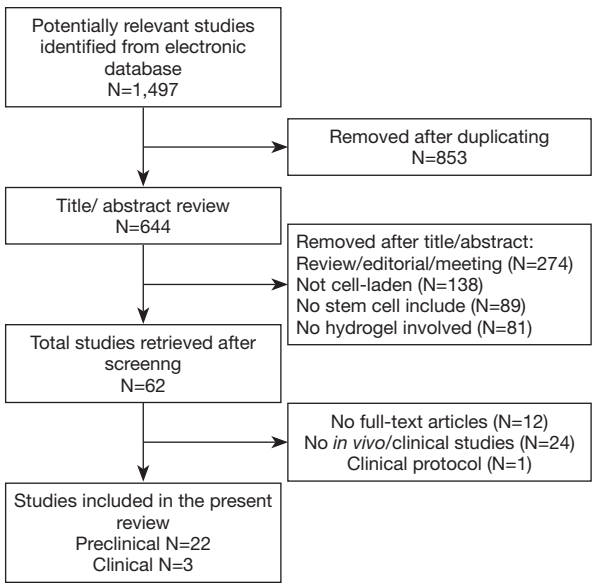

Figure 1 Flowchart of literature search.

Hydrogels for cartilage reconstruction

Hydrogels are a series of ECM-mimicking polymeric biomaterials that have a high water content, porosity, biocompatibility and biodegradability. As shown in *Figure 2*, hydrogels are injectable, which is a minimally invasive method unlike implantation surgery, and can be formed into any desired shape to match irregular defects (13,14). During the past decade, a rich variety of hydrogels have been developed from natural polymers, chemically modified natural polymers, or synthetic polymers or have been used in combination for regeneration of cartilage tissues. Hydrogels based on natural polymers can be divided into two groups: (I) polysaccharides, such as hyaluronic acid (HA), chitosan, alginate and agarose; (II) proteins, such as collagen, gelatin, and fibroin (15-20). A variety of hydrogels based on synthetic polymers, such as polyethylene glycol (PEG), poly N,N-dimethylacrylamide (PDMAAm) and polyvinyl alcohol (PVA), have been reported (21-23).

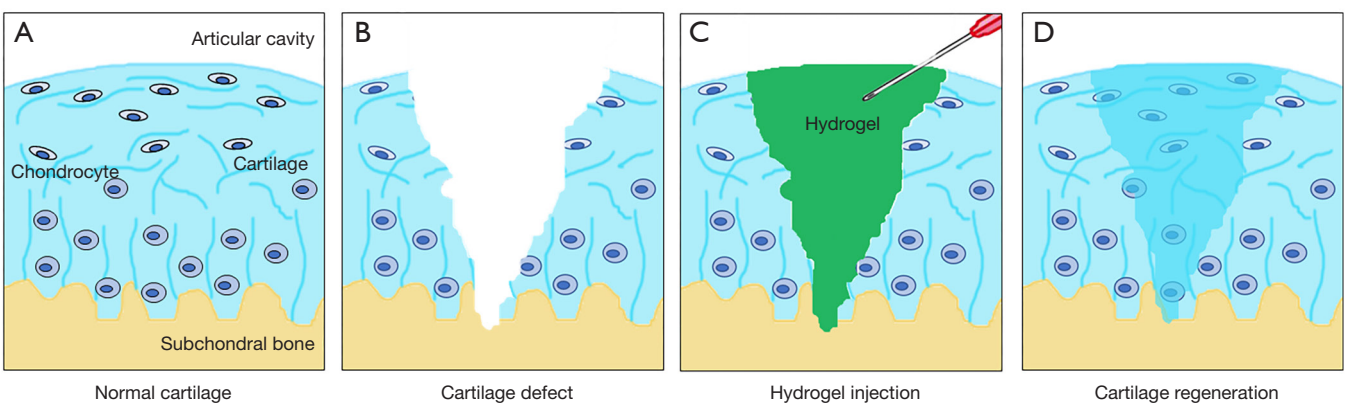

Figure 2 Ideograph of cartilage repair by hydrogels. (A) The composition of normal cartilage, including well organized cartilage, chondrocytes in cartilage and the supporting subchondral bone; (B) in various diseases, cartilage is impaired, including the layer of cartilage and the subchondral bone, which is often irregular; (C) the injection of a hydrogel by an injection syringe is a minimally invasive method that can form any desired shape to match irregular defects; (D) regeneration of the cartilage by reconstruction of the cartilage layer and the subchondral bone.

Furthermore, natural and synthetic polymers can be fabricated into combinations, which provide additional possibilities for selection.

HA hydrogels

HA is the most abundant native component in cartilage and an important component in organizing the cartilage ECM into resilient structures. HA is able to interact with chondrocytes via surface receptors (such as RHAMM and CD44) (14), which contributes to the morphogenesis, proliferation, and inflammation activity of chondrocytes and has stimulatory effects on the chondrocyte metabolism (24). HA is also able to stimulate the synthesis of chondroitin-6-sulphate, collagen type II, glycosaminoglycan, hydroxyproline, and DNA in chondrocytes. Hence, an effort has been made to develop chondrocyte-laden HA hydrogels for the regeneration of cartilage tissues (25,26). HA hydrogels have been demonstrated to support the early differentiation of MSCs into the chondrogenic linage and enhance cartilage tissue formation *in vitro* and *in vivo* (27,28). MSC-laden HA hydrogels can promote neocartilage formation with increased collagen type II and aggrecan production (29). However, HA hydrogels have certain disadvantages. The mechanical properties of HA hydrogels are poor. HA is easily hydrolyzed and rapidly degraded at body temperature, which limits its applications. To overcome these drawbacks, a series of modifications have been developed. Chemical modifications of HA can be achieved via reacting its carboxylic groups with various hydroxyl- or amine-bearing motifs to form derivatives with improved biocompatibility and controlled biodegradability (30). Conjugation with tunable amounts of sulfate groups produces HA hydrogels with significantly slower degradation by hyaluronidase and improved protein sequestration compared to unmodified HA hydrogels, thus promoting chondrogenesis and suppressing the hypertrophy of encapsulated MSCs both *in vitro* and *in vivo* (16). Reactions with the hydrazine-modified elastin-like protein can produce elastin-like protein-HA hydrogels, which can increase the expression of chondrogenic marker genes and enhance soluble sulfated glycosaminoglycan (sGAG) deposition while minimizing the undesirable fibrocartilage phenotype (31).

Alginate hydrogels

Alginate is a polysaccharide extracted from brown algae and can be physically crosslinked by divalent cations at room temperature, which makes it useful in molding, spraying, and 3D bioprinting (32). Alginate hydrogels are characterized by favorable scaffold formation, high biocompatibility, low toxicity, lack of immunogenicity and relatively low cost (33,34). Cartilage engineering using bone marrow-derived MSCs (BMSCs) suspended in alginate hydrogels can enhance the regeneration of chondral defects and promote mechanically functional repair tissue (35) while exhibiting negligible inflammatory and oxidative stress responses. Furthermore, a recent study revealed that the development of MSC-laden alginate hydrogels that mimic the effects of hypoxia on encapsulated stem cells results in a more stable, cartilage-like tissue (36). However, alginate hydrogels have certain limitations for tissue engineering. First, alginate hydrogels lack long-term stability and have low mechanical strength in the physiological environment within a relatively short timeframe. Second, alginate hydrogels have low cellular adhesiveness and interaction and provide limited support for cellular functions (37). Therefore, alginate is often used in a modified form, such as with a sulfate modification, or is combined with other materials, which can facilitate cell spreading, proliferation, and collagen II synthesis and render the material more suitable for 3D printing (38).

Agarose hydrogels

Agarose is a type of polysaccharide that forms thermally reversible hydrogels at 17–40 ℃ and is soluble at temperatures over 65 ℃ (37). The strengths of agarose hydrogels include their stability at body temperature and lack of native ligands for cell-material interactions. Additionally, agarose hydrogels have excellent biocompatibility, stiffness and viscoelasticity. A number of studies have demonstrated that agarose hydrogels promote chondrocyte phenotype maintenance in cartilage regeneration (39,40). Agarose hydrogels can support chondrogenic differentiation and cartilaginous tissue formation by the encapsulated MSCs (41). The temperature-responsive gelation ability of agarose hydrogels enables the design of injectable cell-laden hydrogels for minimally invasive treatment of cartilage defects (42). However, compared with other hydrogels (such as collagen and alginate), agarose hydrogels are inadequate in supporting ECM generation, chondrocyte proliferation and cell phenotype maintenance (43,44).

Chitosan hydrogels

Chitosan is derived from chitin, the second most abundant natural biopolymer from renewable sources, such as crustacean shells and mushrooms envelopes (45). Chitosan and cartilage glycosaminoglycan have similar structures that are biodegradable and biocompatible. Chitosan hydrogels prepared by enzymatic crosslinking can support proliferation, maintain the chondrogenic phenotype and morphology, and boost the deposition of the cartilaginous ECM of chondrocytes and MSCs *in vitro* (46,47). Long-term *in vitro* culture and *in vivo* subcutaneous implantation of MSC-laden chitosan hydrogels demonstrates their ability to support chondrogenesis and hypertrophy of MSCs (41). However, the preparation of chitosan is complex since it is insoluble in water and has to be dissolved in an acetic acid solution, which requires tedious washing steps. Recently, water soluble chitosan has been introduced and characterized as having limited water uptake, shorter gelation times and tighter hydrogel structures (48).

Collagen/Gelatin hydrogels

Collagen is the most abundant native structural protein component of the ECM. Collagen II is the major type of collagen in cartilage. Collagen has been widely used in tissue engineering because of its weak antigenicity and high compatibility with other biomaterials. Collagen is able to maintain the natural morphology and secretion of the cartilage-specific ECM of chondrocytes (49). Collagen II hydrogels have been shown to have the best ability of all collagen types to induce and maintain MSC chondrogenic differentiation in the absence of transforming growth factor (TGF)-β1 (50). Collagen can support the formation of microspheres by MSC due to its fibrous meshwork, which provides a good environment for MSC survival, growth and differentiation (51).

Gelatin is a hydrolyzed product derived from collagen and is the major ECM component in cartilage tissue. Gelatin has excellent cell adhesion, high biocompatibility and biodegradability. However, it has a drawback: the low stability of its physical crosslinks at body temperature. Therefore, chemical modification of gelatin hydrogels is required before they can be used in tissue engineering (52). Methacryloyl-modified gelatin (GelMA) retains most of the functional amino acid motifs, thus inheriting the excellent cell adhesive properties of gelatin. MSC-laden GelMA hydrogels have been demonstrated to be able to stimulate abundant production of aggrecan and type II collagen *in vitro* (53).

Silk fibroin hydrogels

Silk fibroin is a novel type of biomaterial used in tissue engineering and has excellent biocompatibility, robust mechanical properties, slow degradability and an abundant supply. A cellular study revealed that chondrocyte/MSC-laden fibroin hydrogels have the abundant native cartilage-like ECM of aggrecan and collagen type II (54); therefore, silk fibroin is a suitable candidate type for hydrogel generation for cartilage tissue engineering. However, studies have suggested that MSCs produce lower levels of cartilage ECM when loaded on fibroin hydrogels compared with the properties achieved in the case of alginate and chitosan hydrogels (41), indicating the lower potential of fibroin hydrogels for cartilage repair. Additional studies on silk fibroin are required before a conclusion can be drawn about its potential for cartilage repair.

Synthetic polymers-based hydrogels

Hydrogels based on synthetic polymers exhibit highly tunable biocompatibility, biodegradability, biochemical characteristics and mechanical properties. PEG-based hydrogels in combination with organic growth factors support the adhesion and proliferation of MSCs, ESCs and chondrocytes, which can differentiate into chondrogenic linages (55). PEG diacrylate (PEGDA)-based hydrogels have been widely studied for their ability to induce cartilage regeneration. PEGDA hydrogels loaded with MSCs can facilitate improved cartilage ECM deposition, adjacent cartilage tissue growth, and cartilage tissue formation compared to the standard microfracture treatment (56). The PEGDA/fibrinogen composite hydrogel has been shown to enhance chondrogenesis of MSCs while minimizing hypertrophic differentiation (57), which suggests that composite hydrogels can be designed to improve cartilage tissue regeneration. Thermosensitive chitosan-pluronic (CP) hydrogels have been synthesized by grafting pluronic onto chitosan. CP hydrogels are soluble at room temperature but turn semisolid at body temperature; thus, CP hydrogels can be delivered by injection while providing mechanical support for chondrocyte growth (46). Fibrin/PLGA hydrogels loaded with MSCs have been designed for full-thickness cartilage defect repair (58). However,

Table 1 Merits and demerits of different types of hydrogels

Types of hydrogels	Merits	Demerits
Hyaluronic acid	Interact with chondrocytes	Poor mechanical properties and hydrolytic reactions
	Promote cellular morphogenesis and proliferation	Fast degradation
	Stimulate cellular metabolism	
Alginate	Favorable scaffold forming and good biocompatibility	Lacks long-term stability
	Low toxicity, non-immunogenicity and relatively low cost	Low cellular adhesiveness and interaction ability
		Limited support for cell function
Agarose	Stable at body temperature and lacks native ligands	Poor in supporting ECM generation, chondrocyte growth and maintain cell phenotype
	Excellent biocompatibility and good stiffness and viscoelasticity	
Chitosan	Similar structure with cartilage glycosaminoglycan	Insoluble in water
	Biodegradable and biocompatible	Requires tedious washing steps
Collagen/ Gelatin	Weak antigenicity Good integration with other biomaterials	Unstable physically crosslink at the physiological temperatures
	Excellent cell adhesion capacity	
	High biocompatibility and biodegradability	
Silk fibroin	Excellent biocompatibility and robust mechanical properties	Novel type of biomaterial and need more studies *in vivo*
	Slow degradability and abundant supply	
Synthetic polymers	Highly tunable biocompatibility, biodegradability, biochemical characteristics and mechanical properties	Low cellular adhesiveness and high cost

hydrogels based on synthetic polymers have the common disadvantages of relatively low cellular adhesiveness and high cost compared to natural polymer hydrogels.

Choice of hydrogels for cartilage engineering?

We compared and summarized the merits and disadvantages of the discussed hydrogels in *Table 1*. In the analyzed list of the hydrogels, alginate is not suitable due to its short-term stability and low cellular adhesiveness; additional work needs to be performed before synthetic polymers-based hydrogels can be applied in the clinic. HA, agarose and collagen may be more suitable than alginate and can be improved via chemical modifications to enhance their biocompatibility and controlled biodegradability. Silk fibroin is a new player in the field and is very promising. However, there are only a few studies on silk fibroin, and additional work is required to establish its potential *in vivo*. Compared to the limited number of types of natural hydrogels, synthesized polymers have substantially higher variability, and this category is continuously expanding. In general, natural hydrogels have higher biocompatibility and cell viability than chemically modified and synthetic hydrogels, while chemically modified and synthetic hydrogels have widely tunable mechanical properties and suitable biodegradability for effective cartilage regeneration and clinical translation.

A few studies have performed comparative analyses of the cartilage regeneration effects of various hydrogels. A 2019 study compared the effects of various polysaccharides-gellan gum (GG), alginate, agarose and HA hydrogels on cartilage regeneration *in vitro* and *in vivo* and found that all the hydrogels, except alginate, resulted in tissue regeneration, while the effects of GG and agarose were better (59).

Chondrogenesis of stem cells encapsulated in hydrogels

Stem cells can differentiate into various tissue-forming cells, including chondrogenic and osteogenic lineages for cartilage

and bone regeneration. The most commonly studied stem cells include MSCs, iPSCs, ESCs, and PBMSCs. Here, we review recent advances in the application of various stem cells for the repair of the cartilage defects.

MSCs: extensively used and targeted reconstruction

MSCs have become the most extensively used stem cells in regenerative medicine because their sources are abundant (such as bone morrow, adipose tissue, muscle, periodontal ligament and synovial fluid); MSCs have low immunogenicity, no ethical concerns, and a minimal teratoma risk (60). MSCs are able to proliferate without differentiation for up to 40 generations (61) and interact with local biochemical stimuli to generate a number of growth factors that provide multiple biofunctions for tissue restoration (60). MSCs can be derived from a variety of sources, including bone morrow, adipose tissue, muscle, periodontal ligament, synovial fluid, etc.; the various MSCs differ in their surface marker expression, growth factor secretion and differentiation potential. MSCs derived from birth-associated neonatal tissues, including the umbilical cord, placenta, amnion, and cord blood, have a better proliferative capacity, higher availability, longer life span, and higher differentiation potential than MSCs obtained from distinct mature adult tissues of adipose, muscle, and bone origin (62). MSCs encapsulated in various hydrogels have been tested to target the reconstruction of cartilage tissues in preclinical and clinical settings (35,63-87) (*Tables 2*,*3*). Various hydrogels exhibit variable abilities to support chondrogenesis and osteogenesis of MSCs. MSCs encapsulated in collagen hydrogels show enhanced *in vitro* formation of osteochondral interface tissues with a zonal structure consisting of a pure cartilage layer, a calcified cartilage layer, and a subchondral bone layer (88). The results of this study suggested that MSC-laden hydrogels can be promising biomaterials for osteochondral interface regeneration.

Bone marrow-derived MSCs (BMSCs)

BMSCs are the most commonly used stem cells in cartilage tissue engineering (*Table 2*). BMSCs have been used for injection with hydrogels in focal defects to promote cartilage repair in rats (75), rabbits (76), goats (87), pigs (80), and donkeys (83). Thirteen published preclinical studies compared the use of BMSCs in combination with hydrogels, and 12 studies demonstrated the benefits of using BMSC-laden hydrogels to decrease inflammation and apoptosis (77), increase their integration with healthy cartilage in the superficial and inner parts (83) and improve cartilage healing (86). Only a single study described better results in counteracting progression of osteoarthritis using MSCs and HA separately compared with the effects of their combination (79). Furthermore, a comparative clinical study (N=70) explored the efficacy of BMSC-laden HA hydrogels compared with traditional microfracture treatment. There was a significant improvement in the mean IKDC, Lysholm, and SF-36 physical component score and the visual analogue pain scores in both treatment groups without significant differences between groups (81).

A series of modification by scaffolds have been developed to improve the biofunctions of BMSCs in tissue restoration. Pre-encapsulation of bone morphogenetic protein-2 (BMP-2) in PLGA microspheres resulted in controlled release of BMP-2; the formulation was safe, easily injectable, and provided better support for cells in the BMSC-laden hydrogels (64). The incorporation of HA and PDLLA-PEG hydrogels allowed for the slow release of one-time preloaded TGF-β3, which resulted in constructs with a significantly higher mechanical strength than the conventional TGF-β3-supplemented medium loaded with BMSCs and cultured *in vitro* (89). Hypoxia can control the fate of stem cells after their implantation into the body. The delivery of DMOG, hypoxia-inducible factor (HIF) and prolyl hydroxylase inhibitors that mimic hypoxia in the microenvironment significantly reduced the mineralization of cartilaginous tissue generated by BMSCs within alginate hydrogels loaded with BMP-2 and TGF-β3 (36). Platelet lysate is an autologous source of growth factors that can be incorporated into a MSC-laden HA-TA hydrogels to induce a cartilage-like ECM deposition simultaneously with gel degradation, ultimately resulting in the formation of a tough and dense matrix (11). The addition of peptides or heterocyclic compound, such as transmembrane glycoprotein N-cadherin-derived peptides (90), icariin (91) and kartogenin (92), increases the chondrogenesis of encapsulated BMSCs in the early stages and enhances cartilage matrix production. Furthermore, new hydrogel materials, such as magnetic nanocomposites, have good mechanical properties and can provide a surface for the uniform growth of BMSCs, resulting in high rates of proliferation. Additionally, supplementation with Fe_2O_3 can enhance BMSC growth and significantly stimulate chondrocyte-related gene expression in BMSCs (93).

Table 2 Details of preclinical articles identified in present review

Author	Year	Animal type	Stem cell type	Hydrogel type	Study design	Results
Jia *et al.* (68)	2019	Rabbits; Chondral defect	SF-MSCs; 1×10^6/mL	Chitosan	(I) SF-MSCs + hydrogel; (II) hydrogel; (III) control. 6 animals for each group. Experimental time: 4, 8 and 12 weeks	Hydrogel/SF-MSCs was superior to using the hydrogel scaffold only and the untreated control based on gross appearance and histological grading and evaluation
Wu *et al.* (63)	2019	Minipigs; Osteochondral defect	HUC-MSCs; 5×10^6 cells	HA	(I) HUC-MSCs + HA; (II) untreated. 2 animals for each group. Experimental time: 12 weeks	Cartilage regeneration using a mixture of HUC-MSCs and HA in a large animal model may be an effective treatment for OA
Park *et al.* (67)	2019	Rats; Osteochondral defects	MSCs	HA	(I) Chondro-MSCs + HA; (II) Undiff-MSCs + HA; (III) HA; (IV) untreated. 20 animals for each group. Experimental time: 8 and 16 weeks	Transplanting undiff-MSCs resulted in overall superior cartilage repair as compared with chondro-MSCs, HA alone, or no treatment
Pascual-Garrido (66)	2019	Rabbits; Chondral defect	BMSCs	Novel photopolymerizable hydrogel	(I) hydrogel + BMSCs; (II) hydrogel; (III) control. 5 animals for each group. Experimental time: 6 months	Novel photopolymerize hydrogel is able to provide chondrogenic cues for cartilage repair in a rabbit model. *In vitro* chondrogenesis was evident when MSCs were encapsulated in the hydrogel
Critchley *et al.* (35)	2019	Rabbits; Chondral defect	BMSCs; 2×10^7 cells/mL	Alginate	(I) hydrogel + BMSCs; (II) control. 6 animals for each group. Experimental time: 3 months	Cartilage template, engineered using BMSCs, can enhance the regeneration of defects and promote the development of a more mechanically functional repair tissue
Li *et al.* (71)	2018	Rats; Chondral defect	AFF- MSCs; 1×10^6/mL	Polypegda/HA	(I) AFF-MSCs + hydrogel; (II) hydrogel; (III) control. 10 animals for each group. Experimental time: 4 and 8 weeks	AFF-MSCs/hydrogel composite significantly repair full-thickness cartilage defects generated in a rat model; smooth cartilage was formed with evidence of hyaline cartilage formation
Choi *et al.* (74)	2018	Rabbits; Osteochondral defect	BMSCs; 2×10^6 cells	HA	4 group: 3/group. (I) HA + BMSCs + Res; (II) HA + BMSCs; (III) HA; (IV) control. 3 animals for each group. Experimental time: 8 weeks	The cartilage regeneration potential of Res-treated MSCs was greater than that of untreated MSCs. The expression levels of chondrogenic markers increased and those of hypertrophic markers decreased in Rsv-treated MSCs compared with untreated MSCs
Uto *et al.* (69)	2018	Pigs; Osteochondral defect	iPSCs	Beta-TCP	(I) beta-TCP + iPSCs; (II) beta-TCP + MSCs; (III) beta-TCP. 2 animals for each group. Experimental time: 4 and 8 weeks	iPSCs transplanted into osteochondral replacement model, cartilage regeneration was observed without tumor formation

Table 2 (*continued*)

Table 2 (*continued*)

Author	Year	Animal type	Stem cell type	Hydrogel type	Study design	Results
Kim *et al.* (82)	2012	Rabbits; Osteochondral defect	BMSCs; 10^6 cells	HA	(I) MSCs + HA + 2 HA inj; (II) MSCs + HA + 1 HA inj; (III) MSCs + HA; (IV) MSCs; (V) HA; (VI) No treatment. 3 animals for each group. Experimental time: 7 weeks	Significant improvements in osteochondral defect healing at macroscopic and histological evaluation in all treatment groups compared with untreated defects; at histology, MSCs + HA + 2 HA inj showed better results than other groups
Mcilwraith *et al.* (84)	2011	Horses; Osteochondral defect	BMSCs; 2×10^6 cells	HA	(I) MFX + HA + MSCs; (II) MFX + HA. 5 animals for each group. Experimental time: 6 and 12 months	No difference in clinical and histological analysis, but significant increase in repair tissue firmness and better repair tissue quality at arthroscopic and macroscopic analysis in MSCs group with greater levels of aggrecan than in HA alone group
Saw *et al.* (85)	2009	Goats; Osteochondral defect	MSCs; 220×10^6 cells	HA	(I) MFX + 3 HA + MSCs inj; (II) MFX + 3 HA inj; (III) No treatment. 5 animals for each group. Experimental time: 24 weeks	Better cartilage repair in MSCs group at histology, with hyaline cartilage regeneration
Lee *et al.* (86)	2007	Minipigs; Osteochondral defect	BMSCs; 7×10^6 cells	HA	(I) MSCs + HA; (II) HA; (III) Saline. 9 animals for each group. Experimental time: 6 and 12 weeks	Improvement in cartilage healing at histologic and macroscopic analysis at both 6 and 12 weeks in MSCs + HA group compared with controls
Lv *et al.* (70)	2018	Sheep; OA	AD-MSCs; 1×10^7 cells & 5×10^7 cells	HA	(I) high dose AD-MSCs + HA; (II) low dose AD-MSCs + HA; (III) SVF; (IV) HA; (V) Saline. 6 animals for each group. Experimental time: 12 and 15 weeks	Better results in AD-MSCs/HA than SVF/HA in blocking OA progression and promoting cartilage regeneration
Feng *et al.* (72)	2018	Sheep; OA	AD-MSCs; 1×10^7 cells & 5×10^7 cells	HA	(I) High dosage AD-MSCs + HA; (II) low dosage AD-MSCs + HA; (III) HA; (IV) saline. 7 animals for each group. Experimental time: 14 weeks	Typical articular cartilage feature in both AD-MSCs groups and presence of AD-MSCs at synovium at 14 weeks at MRI; lower inflammatory factors from synovial fluid of AD-MSCs groups than HA alone
Desando *et al.* (73)	2018	Rabbits; OA	BMSCs	HA	(I) BMSCs + saline; (II) BMSCs + HA; (III) BMC + saline; (IV) BMC + HA; (V) HA. 12 animals for each group. Experimental time: 8 weeks	Joint repair evidence in all treatments, superior results for BMC-HA than other groups; BMSCs migrate to the meniscus while BMC in cartilage, but HA favor cells migration to cartilage

Table 2 (*continued*)

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Author	Year	Animal type	Stem cell type	Hydrogel type	Study design	Results
Chiang *et al.* (76)	2016	Rabbits; OA	BMSCs (10^6 cells)	HA	(I) MSCs + HA; (II) HA; (III) Sham; (IV) Untreated. 18 animals for each group. Experimental time: 6 and 12 weeks	Less cartilage loss and surface abrasion with better histological scores and cartilage content in MSCs group compared with HA alone; engraftment of allogenic MSCs were evident in surface cartilage
Suhaeb *et al.* (79)	2012	Rat; OA	BMSCs; $3–5\times10^6$ cells	HA	(I) BMSCs + HA; (II) BMSCs; (III) HA. 7 animals for each group. Experimental time: 6 weeks	Better results with HA and BMSCs alone in counteracting OA progression with respect to their combination
Sato *et al.* (80)	2012	Pigs; OA	MSCs; 7×10^6 cells	HA	(I) MSCs + HA; (II) MSCs + saline; (III) HA; (IV) Saline. 15 animals for each group. Experimental time: 5 weeks	Histological partial defect repair only in MSCs + HA group at 5 weeks with an increase in type-II collagen content and low levels of MMP-13
Mokbel *et al.* (83)	2011	Donkeys; OA	BMSCs; $1.8–2.3\times10^6$ cells	HA	(I) BMSCs + HA; (II) HA alone. 9 animals for each group. Experimental time: 1, 2, 6 months	Reparative effect of clinical and radiological evaluation in BMSCs + HA group compared with the control; BMSCs integrated with healthy cartilage in the superficial and inner part
Murphy *et al.* (87)	2003	Goats; OA	BMSCs; 10×10^6 cells	HA	(I) HA + BMSCs; (II) HA. 6 animals for each group. Experimental time: 12 and 26 weeks	No adverse events; stimulation of the regeneration of meniscal tissue and delay of OA progression in BMSCs group
Kim *et al.* (75)	2016	Rat; OA	MSCs; 1.5×10^6 cells	SAP-SP hydrogel	(I) SAP-0.5SP; (II) SAP-SP; (III) SAP-2SP; (IV) SAP-SP-MSCs; (V) control. 7 animals for each group. Experimental time: 6 weeks	Markedly improved cartilage regeneration in the SAP-SP group showing recruitment of MSCs in the defect. SAP-SP restore articular joint function without cell transplantation
Kim *et al.* (77)	2014	Rat; OA	BMSCs (10^6 cells)	SAP hydrogel	(I) SAP-BMSCs; (II) SAP; (III) BMSCs; (IV) control. 6 animals for each group. Experimental time: 6 weeks	Evidence of chondroprotection at histological view and decrease of inflammation and apoptosis biomarkers in SAP + BMSCs group; increased BMD in SAP+ BMSCs groups relative to the controls

MSCs, mesenchymal stem cells; BMSCs, bone marrow-derived MSCs; SF-MSCs, synovial fluid-derived MSCs; HUC-MSCs, human umbilical cord-derived MSCs; OA, osteoarthritis; HA, hyaluronic acid; AFF-MSCs, arthroscopic flushing fluid MSCs; Res, resveratrol; iPSCs, induced pluripotent stem cells; beta-TCP, beta-tricalcium phosphate; inj, injection; MFX, microfracture; AD-MSCs, adipose-derived MSCs; SVF, stromal vascular fraction; BMC, bone marrow concentrate; SAP, self-assembled peptide; SP, substance P.

Table 3 Details of clinical articles identified in present review

Author	Year	Study type	Defect type	Stem cell type	Hydrogel type	Study design	Results
Pipino *et al.* (65)	2019	Comparative	Osteochondral defect	AD-MSCs	PG/GC	Lesion size: Outerbridge III–IV. Groups [2]: (I) MFX + AD-MSC (n=46); (II) MFX (n=23). Follow-up: 6, 12 and 24 months	Patient has high satisfaction rates after microfractures combined with hydrogel scaffold; histologic evaluation supported an enhanced chondrogenic environment in combined group
Saw *et al.* (78)	2013	RCT	Chondral lesion	PBMCs	HA	Number: 50. Lesions size: ICRS grade 3 and 4 lesions. Groups [2]: (I) PBPCs + HA; (II) HA. Follow-up: from 18 to 24 months	Improvement of the quality of articular cartilage repair in PBSC group at histologic and MRI evaluation
Lee *et al.* (81)	2012	Comparative	Chondral lesion	BMSCs	HA	Number: 70. Lesion size: N/A. Groups [2]: (I) BMSCs + HA; (II) MFX + BMSCs. Follow-up: 24.5 months	No significant difference between the two procedures, with less invasive and requiring only a single operation for BMSCs + HA

AD-MSCs, adipose-derived MSCs; PG/GC, polyglucosamine/glucosamine carbonate; MFX, microfracture; RCT, Randomized controlled trial; PBMCs, peripheral blood mononuclear cells; HA, hyaluronic acid; BMSCs, bone marrow-derived MSCs.

Adipose-derived MSCs (AD-MSCs)

AD-MSCs are the second most commonly used stem cells in cartilage tissue engineering, which can be isolated in high numbers with minimal manipulation even in the operation theatre and can be expanded *in vitro* without loss of their chondrogenic potential (94). In two preclinical studies, the AD-MSC-laden group had low levels of inflammatory factors from synovial fluid, blocked progression of osteoarthritis and promoted cartilage regeneration (70,72). Culturing AD-MSCs in a composite gel based on collagen/HA leads to chondrogenic differentiation of AD-MSCs stimulated by the collagen hydrogel in a dose dependent manner, with 1% HA showing the best results (94). Recently, a comparative clinical study investigated microfracture combined with a novel hydrogel (polyglucosamine/glucosamine carbonate) laden with AD-MSCs and compared the results with the effect of microfracture alone. Higher patient satisfaction rates were reported in the microfracture combined with the novel hydrogel scaffold group, while histologic evaluation supported the improvement in the chondrogenic environment in the combination group (65).

MSCs derived from other sources

Other sources of MSCs investigated for cartilage repair include synovial fluid-derived MSCs (SF-MSCs), arthroscopic flushing fluid-derived MSCs (AFF-MSCs), and umbilical cord-derived MSCs (UC-MSCs). Hydrogel laden with SF-MSCs was superior in regard to gross improvement, histological grade and evaluation compared with the effects of the hydrogel scaffold only or the untreated control in full-thickness cartilage defects in rabbits (68). Moreover, supplementation of transforming growth factor beta-3 (TGF-β3) and insulin-like growth factor 1 (IGF-1) promoted the differentiation of SF-MSCs into chondrocytes (95). Several clinical studies have reported that SF-MSCs are a viable option for the treatment of osteochondral defects, while the donor-to-donor variation should be considered and can significantly influence downstream applications (96-98). AFF-MSCs possess the typical MSC morphology and phenotype, and their encapsulation within the one-step rapid crosslinking hyper-branched polyPEGDA/HA hydrogel results in maintenance of the chondrogenic differentiation potential *in vitro* (71). AFF-MSCs lead to the repair of full-thickness cartilage defects with the formation of new hyaline cartilage

in a rat model after eight weeks of implantation (71). Similarly, a combination of human UC-MSCs and HA has been shown to be an effective treatment for osteoarthritis in a pig model (63). The composition of UC-MSCs and HA has been demonstrated to significantly improve the visual analog scale (VAS) and IKDC scores in a clinical study, which were maintained for up to 7 years at follow-up (99).

ESCs: pluripotent but it is hard to direct their differentiation

ESCs are isolated from the inner tissues of early embryos and have the potential to differentiate into almost all cell lineages in the human body (100). A study has indicated that ESCs encapsulated in PEG hydrogels can differentiate into chondrocytes and produce the neocartilage ECM (101). Recent studies have revealed that exosomes from ESCs are able to alleviate osteoarthritis by balancing the synthesis and degradation of the cartilage extracellular matrix to promote osteochondral regeneration (102,103). However, the pluripotency of ESCs is problematic because the direction of ESC differentiation is difficult to control (104). In addition, ESCs have the risk of immune rejection and ethical concerns (105). Therefore, ESCs are not a suitable choice for cartilage engineering; however, exosomes from ESCs are a potential new target of OA therapy.

iPSCs: newly developed and promising

iPSCs are obtained from somatic cells, including fibroblasts. iPSCs exhibit a similar pluripotency as ESCs and have the ability to differentiate into chondrocytes in alginate hydrogels and regenerate cartilage tissues *in vivo* (106). In a porcine model, iPSCs transplanted into osteochondral defects regenerated cartilage without tumor formation (69). iPSCs can be induced to undergo chondrogenic differentiation on a 3D micro-cavitary hydrogel interim platform. iPSCs can generate a graft, with cells exhibiting a chondrocyte phenotype with abundant and assembled type II collagen (107). Moreover, chondroinduced iPSCs show a significantly higher level of cartilage repair than chondroinduced BMSCs due to the significantly higher methylation of CpG sites in the COL10A1 promoter; the induced cells have a low hypertrophy of chondrocytes and improve cartilage repair (106). Hence, iPSCs emerge as a promising cell source for the treatment of the cartilage defects and have potential for clinical application. Additional robust *in vitro* and *in vivo* studies to optimize the chondrogenesis protocol are required (108).

PBMCs: easy to extract and develop

PBMCs can be easily extracted from peripheral blood with minimal invasiveness. Recently, PBMCs have been reported to be able to undergo chondrogenic differentiation and have a cartilage generation ability similar to that of MSCs (109). An RCT clinical trial (N=50) investigated the articular cartilage regeneration efficacy of a HA hydrogel laden with PBMCs after arthroscopic subchondral drilling; the results indicated that autologous PBMCs in combination with HA improved the quality of articular cartilage repair (evaluated by histology and MRI scanning) over the same treatment without PBMCs (78). Further studies are desirable to develop the potential of PBMCs for use in the treatment of osteochondral defects; PBMCs can be used as an autologous point-of care treatment to attract native chondrocytes from diseased tissue to aid in cartilage repair (110).

Conclusions and outlook

In this review, we summarized recent progress regarding the use of stem cell-laden hydrogel biomaterials for cartilage tissue engineering. A summary of the currently available hydrogels and sources of stem cells is illustrated in *Figure 3*. Due to their injectability, mechanical properties, outstanding biocompatibility, and proper biodegradability, stem cell-laden hydrogels have attracted increasing attention as promising tissue-engineered biomaterials for the repair of full thickness cartilage defects. Various types of stem cells encapsulated in hydrogels have been shown to be able to differentiate into chondrocytes via induction by growth factors and to promote chondrogenesis *in vitro* and *in vivo*. Currently, natural polymers and synthetic polymer-based hydrogels are widely used in cartilage engineering. The combination of natural and synthetic polymer hydrogels takes advantages of both types of hydrogel, including their higher biocompatibility and cell viability, widely tunable mechanical properties and proper biodegradability for effective cartilage regeneration, which is promising for future studies and especially clinical trials.

Articular cartilage is a complex functional structure, and several key challenges need to be overcome before full-thickness regeneration of cartilage can be achieved. First, chondrocytes proliferate slowly and easily dedifferentiate into fibroblasts (111), while MSCs laden in a hydrogel may readily become hypertrophic, resulting in the production

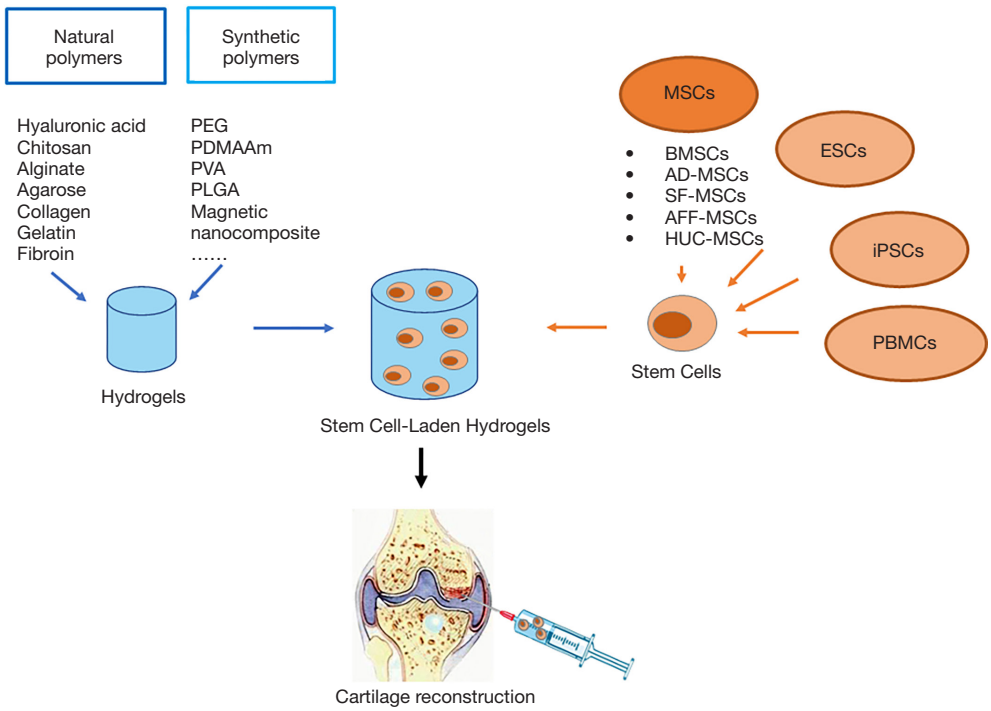

Figure 3 Types of the hydrogels and stem cells used for cartilage tissue engineering. The left part summarizes various types of hydrogels used for cartilage reconstruction, including natural and synthetic polymers. The right part summarizes various sources of stem cells used in cartilage regeneration, including MSCs, ESCs, iPSCs and PBMCs. Due to the extensive use of MSCs, the subgroups of MSCs suitable for cartilage regeneration are also listed. The middle part illustrates the applications of stem cell-laden hydrogels, which are minimally invasive after administration by an injection syringe.

of some bone-like tissues (112). The addition of factors to inhibit endochondral ossification (113) or a preliminary coculture of MSCs and chondrocytes to promote cartilage ECM formation and induce hypertrophy inhibitory factors can be used to overcomes this problem (114). Second, the degradation of hydrogels influences the function of the encapsulated stem cells and the quality of cartilage repair (115). Additional studies are required to develop hydrogels with controllable biodegradability that match the bioactivity of stem cells and the growth rate of cartilage. Third, only hydrogels with a simple composition, such as HA and collagen, have been tested in a clinical setting for cartilage repair, while the alternative, more complex hydrogels have only been investigated at the preclinical stage. Certain barriers to the clinical translation of more complex hydrogel-based constructs remain. Advanced biofabrication technologies, such as 3D bioprinting, should be investigated in depth to provide better biocompatibility and a lower manufacturing cost, as well as personalized customized regeneration strategies (116), which may highlight the future direction of customized cartilage repair.

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