Hindawi
Advances in Materials Science and Engineering
Volume 2019, Article ID 3027303, 12 pages
https://doi.org/10.1155/2019/3027303

Review Article

The Application of Hyaluronic Acid-Based Hydrogels in Bone and Cartilage Tissue Engineering

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Received 20 April 2019; Revised 28 October 2019; Accepted 26 November 2019; Published 20 December 2019

Academic Editor: Antonio Gloria

At present, the healing of osteopathy, especially the healing of cartilage, has been proven to be difficult. Commonly used treatment methods are autogenous bone grafts and allogeneic bone grafts, but grafts cannot fully meet the clinical treatment requirements due to problems related to the source, price, immunity, and other concerns. Thus, the combination of biomaterials and tissue engineering technology has become a new direction in research. Among studies on tissue engineering bone and cartilage materials, hydrogels that show biological activity, absorbability after degradability, plasticity, and easy preparation have become the focus. Hydrogels are used as extracellular matrix mimics. Although various materials are able to form hydrogels, hyaluronic acid and its derivatives are prominently used. Hyaluronic acid hydrogels have many advantages, such as promoting cell adhesion and proliferation and wound healing. They also demonstrate sufficient biological activity for stimulating a microenvironment for cell survival. However, their disadvantages require further modification and include a poor degradation rate and insufficient mechanical performance. In this paper, hyaluronic acid-based hydrogels, their modifications, applications, and mechanisms, as well as new techniques for processing hyaluronic acid hydrogels in bone and cartilage tissue engineering, are briefly reviewed, and their future prospects and directions for future work are discussed.

1. Introduction

Bone is a basic body structure that constitutes parts of the motor system and supports routine human activities. In bone diseases, bone defects appear as the major clinical manifestation. Generally, external interventions are required when bone defects occur because irreversible damage to the self-healing capabilities has already occurred in most bone defect areas and because bone defects lack blood vessels [1]. Cartilage also does not contain blood vessels, nerves, or lymph, and the growth and proliferation of cartilage cells is slow; thus, cartilage healing is also a challenge [2] in regenerative medicine. Furthermore, problems with autografts and allografts include the source and price as well as immunity and ethics concerns [3]. Currently, bone and cartilage tissue engineering materials consist of stem cells and biological materials; thus, more research is needed [4, 5]. Hydrogels, which are three-dimensional hydrophilic polymer chain systems, contain the same water content as soft tissue, and their composition, structure, and dimensions have been proven to be suitable for tissue engineering research [6]. Hydrogel materials are divided into natural polymers and synthetic polymers. Natural polymers include hyaluronic acid (HA), fibrin, chitosan, alginate, and pullulan [7]. They possess unique biological activities and osteoconductivity but are often lacking in mechanical strength and immunogenicity. Synthetic polymers are under development and include polyethylene oxide (PEO), polyvinyl alcohol (PVA), polyacrylic acid, and polyacrylic-fumaric-ethylene glycol copolymer [8]. The biocompatibility of synthetic polymer hydrogels needs improvement. There have been several in-depth studies of the natural polymer HA [9–13]. As a constituent of the natural cytoplasmic matrix, favorable biological activity of HA and its ability to communicate with cells are undisputed. Though appropriate modifications have been applied to the physicochemical

properties of HA hydrogels, desired constructions with better properties are still needed. In this paper, we review hydrogels composed of HA and their derivates in bone and cartilage tissue engineering, providing a reference for the future development of better hydrogels based on HA and its derivates.

1.1. Bone and Cartilage Injury. Bone defects can be caused by various pathological processes, including trauma, cancer, congenital deformities, and surgical reconstruction. Bone healing is complex because multiple reactions such as inflammation, hematoma organization, cell proliferation, and calcification usually appear in injury areas. Bone loss, infection, insufficient blood supply, and soft tissue injury all influence bone healing. Additionally, the self-healing capabilities of bone are limited by the cell source and complexities of osteogenesis [14]. Therefore, external intervention is needed. Presently, application of the "gold standard," namely, autologous bone grafts, is restricted by inadequate or inappropriate bone stock, particularly for the treatment of extensive bone defects [15, 16]. Currently, the critical dimension necessary for bone self-healing is only 2.5 cm [17].

Once bone disease involves the joints, cartilage injury inevitably occurs. Cartilage does not contain blood vessels, nerves, or lymph, and it is maintained and nourished by chondrocytes and plasmids in its extracellular matrix (ECM) [18]. Cartilage ECM is composed of a collagen network and proteoglycans; hyaluronic acid (HA) is a proteoglycan. Proteoglycans consist of a core protein with several glycosaminoglycan (GAG) chains. The mechanical properties of various types of cartilage differ in terms of the composition of collagen and GAG [19, 20]. The cartilage ECM is only sustained by chondrocytes, which account for 1% to 5% of the total volume of cartilage. The low cell density of chondrocytes in cartilage and the lack of blood vessels cause low tissue regeneration capacity. Fibrous cartilage, which possesses higher tissue hardenability, is always generated during the healing process, resulting in long-term performance issues [21–23]. To increase the joint function, the formation of natural joint or hyaline cartilage should be promoted instead of fibrous cartilage [23, 24]. Disadvantages (i.e., the lack of cartilage cell sources, long harvest times, difficulties with fixing, periosteum hypertrophy and ablation, and poor effects in elderly patients [25–27]) remain in autogenous chondrocyte grafts, which have been used for many years. Therefore, investigations into tissue engineering technology, including stem cells and biological materials, are urgently needed [27, 28].

1.2. Bone and Cartilage Tissue Engineering. Bone and cartilage tissue engineering involves the use of cultures and in vitro amplification of autologous high-concentration osteoblasts, bone marrow stroma stem cells, or chondrocytes, which are then implanted into a natural or synthetic cell scaffold or ECM that shows good biocompatibility (BC) and absorbability after degradation. The cells grow in this precast biomaterial three-dimensional scaffold because it is capable of providing nourishment, gas exchange, and waste removal. This cell hybrid material is then implanted into the bone defect area. Combined with growth factors and drugs, the seeded bone cells demonstrate continuous proliferation and differentiation, thus repairing the bone tissue defect while exhibiting gradual degradation of the biological materials (Figure 1) [29].

Three aspects critical to bone tissue engineering (BTE) are osteoconductivity, osteoinductivity, and osseointegration [30], which are the required properties for biological scaffolds. The ideal scaffold materials for bone or cartilage tissue engineering include materials with a natural ecology, BC, the capability to seed osteoprogenitor cells (with the priority being mesenchymal stem cells (MSCs)), differentiation and proliferation capabilities, a natural ECM that can promote cell growth, morphogenesis signals, and adequate nutrition. The above conditions can guarantee the success of the healing phase [31, 32]. As a representative material, hydrogels are being studied in-depth and are expected to industrialize the production of BTE or CTE.

2. Use of Hydrogels in BTE and CTE

Hydrogels are three-dimensional solids that consist of hydrophilic polymer chains held together by cross-links. Hydrogels are a promising material in bone and cartilage tissue engineering due to unique advantages including adjustable physicochemical properties, biological nature, BC, versatility, and high similarity to the natural ECM. The requirements for hydrogel use in BTE and CTE are widely believed to include the following:

(1) Maintaining the phenotype of the osteoblasts or chondrocytes

(2) Rebuilding the interface of bone and cartilage and lessening the generation of fibrous cartilage

(3) Mimicking the natural ECM to create an ecological microenvironment

(4) Providing a sufficient vascularization or secretory matrix to sustain nutrition and metabolism

(5) Displaying a suitable degradation rate and no biological hazard after degradation

(6) Possessing the ability to deliver growth factors and drugs [6, 15, 27, 33–36]

At present, the polymers used for hydrogel generation are divided into natural polymers and synthetic polymers. Natural polymers include HA, chitosan, fibrin, gelatin, alginate, gellan gum, amylopectin, self-assembling peptides, and respective derivatives. Representative synthetic polymers are acrylate-fumarate copolymer (PPF), polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), polyethylene glycol (PEG), poly-*N*-isopropylacrylamide (PNIPAAM), triblock segmented copolymers of polyethylene oxide-b-polypropylene oxide-polyethylene oxide (PEO/PPO/PEO), and amphiphilic triblock-segmented copolymers of polyethylene oxide-ε-caprolactone (PEO/PCL/PEO). The above materials have been used in bone and cartilage tissue engineering [15, 27]. Although there are

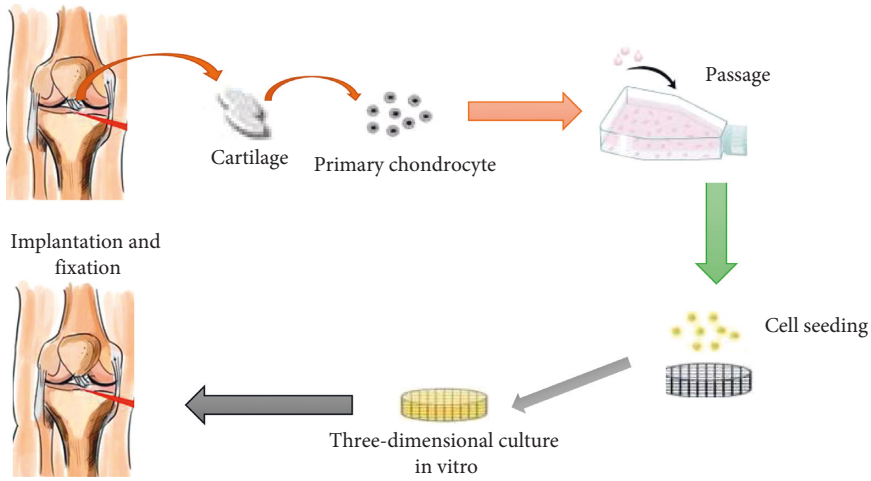

Figure 1: Schematic of cartilage tissue engineering.

many polymer choices, natural polymers with biological activity are preferred due to their unique characteristics, such as total BC, nonimmunogenicity, and nontoxicity. However, considering the instability, short half-life, and water content of biopolymers, natural polymers need to be modified before use. In particular, their water content, soft nature, and porous structure makes them able to mimic biological tissue and suitable for accommodating cells and encapsulating and releasing water-soluble compounds such as proteins in a controlled manner [37–39].

3. HA-Based Hydrogels

HA is a nonsulfated GAG consisting of repeating disaccharide units of glucuronic acid and *N*-acetylglucosamine, which are widely distributed in cartilage [40, 41]. BTE and CTE have shown remarkable potential for HA-based hydrogels. The bioactivity of HA in cell signaling, wound repair, morphogenesis, and matrix composition depends on its structure and biological properties [42, 43]. Reviews assessing the promotion of the chondrocyte metabolism by HA [44] show that it can increase the synthetic amount of chondroitin-6-sulfate, collagen II, GAG, hydroxyproline, and DNA [45]. When delivering MSCs, HA-based hydrogels induce greater morphological differentiation of stem cells and help rebuild cartilage tissue. Meanwhile, it also provides lubrication and buffering effects for recovering the viscosity and elasticity of synovia [44, 46]. More importantly, through minimally invasive surgery, injectable HA-based hydrogels are capable of completely repairing small-scale bone and cartilage defects of any size. However, HA has been primarily used in early CTE efforts due to its special influence on cartilage tissue and poor mechanical properties.

HA plays a significant role in promoting cell migration and proliferation. However, its use in vivo is limited by the endogenous degradation of hyaluronidase, reactive oxygen, and active nitrogen. Interactions between receptors, namely, CD44, hyaluronic acid-mediated motion receptor (RHAMM), and intercellular adhesion molecule-1 (ICAM-1), and HA, have been identified in early studies. Further studies have been carried out to determine HA's effects on cell formation, wound repair, inflammation, and metastasis. For example, when modifying HA to synthesize HA macromolecule monomers, the interaction between CD44 and HA can be changed. This change depends on the degree of modification, but the exact mechanism requires further study. HA conjugate delivery nanoplatforms for the targeted treatment of CD44-positive metastatic tumors have also emerged. Recent research has further explored the interaction between HA and cell surface receptors, i.e., CD44, RHAMM, and ICAM-1 [47], which confirmed that these receptors influence cellular activities such as cell formation, wound repair, inflammation, and migration [48–51]. Due to the development of material manufacturing technologies, including various cross-linking methods, biological molecular binding [52], and 3D bioprinting, HA-based hydrogels have been increasingly used in both BTE and CTE applications.

4. Modification of HA-Based Hydrogels for Application in BTE and CTE

Due to disadvantages such as a hydrophilic nature and lack of mechanical integrity, HA requires chemical modification and cross-linking to alter it for use in BTE and CTE applications [53–55]. The most commonly used covalent modification sites of HA are the hydroxyl group and the carboxylic acid group (Figure 2). The hydroxyl group can be modified by an ester bond, whereas the carboxylic acid group is modified by hydrazide and then cross-linked with an ester bond. To improve its physicochemical properties, other functional groups can be added during modification. The partial deacetalization of HA via treatment with alkaline or acidic substances degrades HA into free amino groups that can be cross-linked by amides, imino groups, or secondary amine bonds. The bond energy of imino groups is recovered with amine bonds. Divinyl sulfone, glycol diglycidyl ether, carbodiimide, glutaric dialdehyde, sulfide, and polyfunctional epoxide can produce cross-linking reactions in acidic, neutral, or alkaline conditions. In addition,

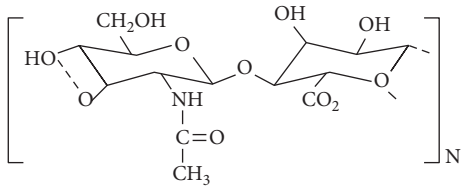

Figure 2: Structure of hyaluronic acid.

HA can undergo automatic cross-linking and photo-cross-linking in the presence of methacrylic acid ester. In addition to the abovementioned chemical modification methods, the combination of HA with other materials to form compound hydrogels has become a popular research topic.

4.1. Chemical Modification and Application of HA Hydrogels

4.1.1. Amidation Modification with Carbodiimide (EDC). The modification of HA reduces its degradation rate and solubility in water, guarantying its stability in BTE and CTE applications. The cross-linking of HA with carbodiimide promotes the formation of an ester bond between the hydroxyl group and the carboxyl group, which reacts with other functional groups (), increasing the porosity, rigidity, and antidegradation properties of the HA hydrogel scaffold. Therefore, carbodiimide has been used for the early modification of HA. La Gatta and colleagues [56] found that carbodiimide modification, HA scaffolds cross-linked with lysine (Lys), improves BC. Cross-linking of the amino bond improves the stability of HA scaffolds in the physiological environment, which is a major requirement for cartilage regeneration scaffolds. HA modification by carbodiimide can produce coupled reactions with mercaptamine to generate HA-hyaluronate thiol (HA-SH), which is then blended with a nanogel of another material to form a porous three-dimensional structure. Nanogel can be distributed uniformly. With high selectivity, speed, BC, and in situ polymerization, this newly biodegradable scaffold can be used as an injectable matrix coated with cells and proteins for tissue engineering and drug delivery applications [57].

Additionally, HA modified by both adipic dihydrazide (ADH) and carbodiimide reacts with *N*-acryloxysuccinimide (NAS) to generate HA-acrylic acid (HA-AC), which can be used for gene delivery in vivo during the tissue regeneration process [58]. HA-AC cross-linking with 4-arm PEG thiol (PEG-SH4) has been used to create scaffolds of bone morphogenetic protein-2 (BMP-2) and bone MSCs (hMSCs) for the regeneration of rat skull defects [59]. To increase HA resistance, Pitarresi and colleagues [60] cross-linked HA modified by carbodiimide with α, β-poly(*N*-2-ethoxy) (2-amino/ethyl/carbamate)-d, l-asparaginate (PHEA-EDA) to form a proteoplast polymer gel membrane. The product features not only high hydratability and swelling resistance but also hydrolysis resistance and drug resistance against hyaluronidase. Photocurable hydrogels, which will be discussed later, also need early modification by carbodiimide.

4.1.2. Hydrazide Modification with Adipic Dihydrazide (ADH). Hydrazide modification is used in the abovementioned preparation of HA-AC (Figure 3) [58, 59], which allows for further cross-linking to develop scaffolds for BTE and CTE applications. Scaffolds of porous HA with hydrazide modification have also been used to deliver BMP-2 for bone growth, with in vitro applications showing the continuous controlled release time of BMP-2 for up to a month. Histological analysis and staining after implantation of BMP-2 scaffolds into rats have revealed active bone regeneration and controlled BMP-2 release [61]. Further cross-linking with ADH derivatives can deliver macromolecule bifunctional reagents that can improve the swelling properties and drug resistance of HA. One example is methoxy PEG-propionaldehyde [62], which can be used for drug delivery after the formation of porous microstructural hydrogel membranes. ADH derivatives can also enhance cartilage differentiation by further binding with polypeptides to produce BMP-2 [63]. HA modified with hydrazide can be combined with aldehyde-modified HA through hydrazone cross-linking and be used to BMP-2, which strengthens the regeneration of cartilage tissue [64].

4.1.3. Aldehyde Modification. In glutaric dialdehyde- (GTA-) induced acid conditions, 2-(2-aminophenyl) acetaldehyde dimethyl acetal reacts with HA to form hemiacetal groups or ether bonds. Cross-linking neighboring chains of acetal or hemiacetal groups forms an insoluble gel. However, the complete removal of GTA must be guaranteed through a later process due to its toxicity. HA modification by GTA can be used to prepare compound hydrogels. For example, resorbent scaffolds made from HA modified by polyglycolic acid (PGA) and GTA are available for the meniscus repairs, and experiments have found that the expression of a specific marker is increased on the meniscus via HA-PGA scaffolds [65]. HA modification by 2-(2-aminophenyl) acetaldehyde dimethyl acetal has been verified to improve BMP-2 delivery. At the same time, aldehyde-modified hydrogels combined with acylhydrazide polyvinyl alcohol (PVA) can effectively deliver nontoxic siRNAs that can be applied to MSCs to block the inhibition of the clinically related gene PLEKHO1. This effect was demonstrated in siRNA release experiments and through the rheological properties of siRNA-encapsulated hydrogels [66].

4.1.4. Divinyl Sulfone Modification. Cross-linking divinyl sulfone (DVS) and the hydroxyl group of HA generates an ether bond. The rapid cross-linking of DVS and HA in alkaline conditions generates a hydrogel with strong mechanical properties, high BC, and high biological affinity, which can then be used in BTE and CTE applications [67, 68]. For example, Xu and colleagues [69] developed fixed heparin (HP)-HA granule (HGP) through inverse emulsion polymerization using DVS as the cross-linking agent. This spherical carrier containing nanoscale micropores can be used for coating growth factors. HP specifically binds to BMP-2, and its release kinetics can be adjusted by regulating the granulometric composition. DVS cross-linked

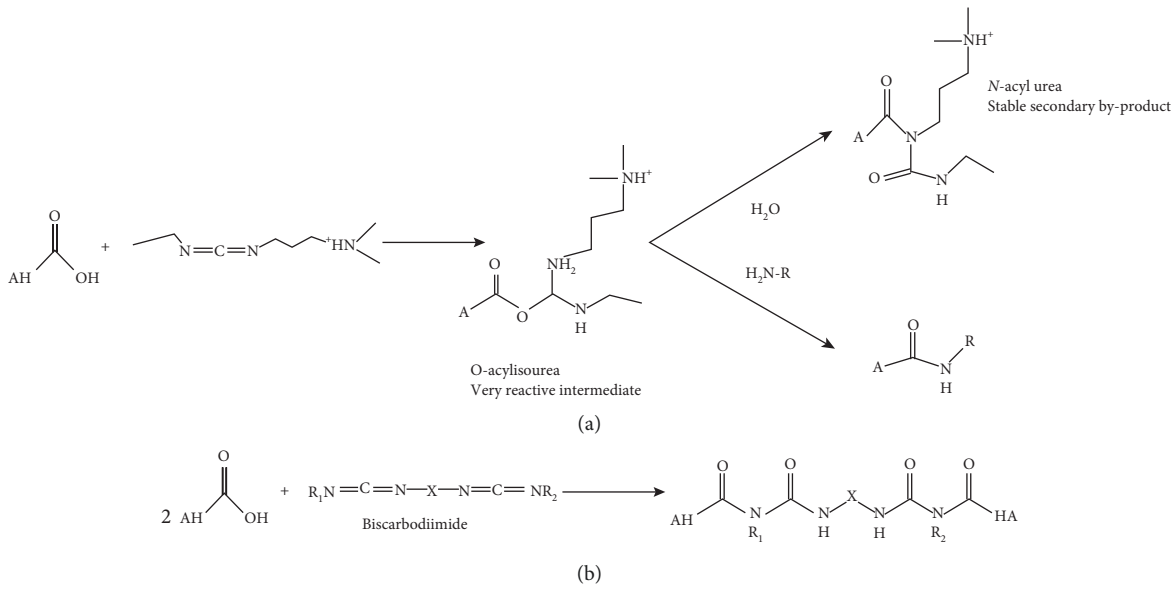

Figure 3: The HA amidation mechanism with EDC (a) and the cross-linking of HA with biscarbodiimides (b) [47].

hydrogels are also compatible with other materials such as carbon nanotubes [70].

4.1.5. Polyfunctional Epoxide Modification. The reaction of the epoxy group and hydroxide group of HA with hydroxide can generate an ester bond and ether bond. Kim and colleagues [71] used ethylene glycol diglycidyl ether to produce HA-collagen composite hydrogels for application in cartilage regeneration. An evaluation of its mechanical properties, degradation, and BC have concluded that this porous scaffold containing collagen can be effectively used in cartilage regeneration. Similarly, HA can react with 1,4-butanediol diglycidyl ether (BDDE) in alkaline conditions to generate a highly swelling hydrogel whose original shape remains unchanged [72].

Adding optical cross-linking to these hyperdistended hydrogels can increase the cross-linking density and limit swelling. After evaluation, they have been shown to be capable of being used for bone and cartilage tissue engineering scaffolds and molecular delivery.

4.1.6. Methacrylic Acid Modification. HA modified with methacrylic acid possess enhanced cellular affinity. Magalhaes and colleagues [73] found that chondrocytes adhere and present spherical morphology in the construction of HA hydrogels following methacrylic acid modification. Chondrocytes therein are able to proliferate and synthesize a hyaline matrix containing abundant glycosaminoglycans and collagen II. A common way to produce hydrogels is to use glycidyl methacrylate (GMA) to introduce photopolymerizable methacrylic acid ester on the molecule of HA to form GMHA [72], which can then be use to form HA-based hydrogels after photopolymerization. Irgacure 2959 is widely used as a photoinitiator in *N*-vinylpyrrolidone, which can not only maintain cell activity but also support the formation of hydrogels. Photo-cross-linking of HA methacrylic acid ester hydrogels (HAMAs) can also provide the 3D microenvironment that will allow MSCs to differentiate into the cartilage phenotype [74].

The reaction of HA and methacrylic acid generates MeHA containing methacrylic acid ester or binding functional groups containing acrylic ester (AHA), which can also permit photoinitiation cross-linking [75]. In the research of Erickson and colleagues [76], HA hydrogel construction based on hMSCs has been developed to repair articular cartilage defects through engineering approaches, and the highest modulus of compressibility has exceeded 1 MPa during testing. 2-aminoethyl methacrylate (AEMA) can also cross-link as described above through amido bonds. As the substitution degree of AEMA has increased, research has shown [75] that the mechanical properties and swelling ratio of the HA hydrogels have improved. These two properties are keys for influencing the drug loading capacity and the proliferation, migration, and differentiation of cells. After methacrylic acid modification, the changes in the molecular weight of HA allow macromonomers such as polyethylene glycol diacrylate (PEGDM), in different concentrations, to mix in precursor solutions, forming a high cross-linking density network [77]. These networks have been used in animal experiments to maintain cell viability and generate new cartilage tissue.

Recently, there has been new research on improving compression stress, that is, employing the concept of double networks (DBs) to prepare high-intensive HA hydrogels using a two-step method [78]. First, HA is cross-linked to ethylene glycol diglycidyl ether (EGDE) in water; in the second step, the DB method is used to generate a DB hydrogel comprised of high-intensive HA/*N*, *N*-dimethylacrylamide (PDMA) containing 84–94% water. When compression stress increases to 19.4 MPa, these new hydrogels can be selected for BTE applications.

In addition to photo-cross-linking, hydrogel granules of covalent integration (HA-c-HGP) [79] have been developed, which possess a higher tenacity and compression stress compared to traditional photo-cross-linked hydrogels. Cell adhesion peptides in connective tissue can be conjugated with acrylate or methacrylic acid ester functional groups on HA using the Michael addition reaction. A HA-based hydrogel has recently been developed by conjugating HA modified with acrylic acid to two different peptides. This HA-based hydrogel is sensitive to matrix metalloproteinase (MMP) and can be used as the carrier of cells and growth factors for bone formation [80, 81]. HA hydrogels modified with methacrylic acid and photo-cross-linking have also been used in 3D bioprinting [82], which will be introduced in Section 4.3.

4.1.7. Modification with Other Compounds. In addition to the abovementioned cross-linking modifications, other modifications adaptive to BTE and CTE have been studied. Lin and colleagues [83] prepared thiolated HA through disulfide bonds and cross-linking the former to PEGDM. When used to repair defects in the thighbone cartilage of the articularis patellar groove of rabbits, the results showed that bone and cartilage recovered well after 12 weeks. Cell adhesion peptide can also be conjugated to thiolated HA [84]. HA can also covalently cross-link tyramine in peroxide conditions, and the degree of hydrogel cross-linking can also adjust the matrix synthesis and cartilage histogeny. This injectable hydrogel possesses an adjustable three-dimensional microenvironment that is able to alter cell condensation during chondrogenesis and improve the histogeny of the overall cartilage tissue [85]. Kaderli and colleagues [86] found that HA-4-aminoresorcinol (HA-4AR) hydrogels have protective effects on cartilage and can reduce the generation of synovium hypertrophy. Unterman and colleagues [87] used an HA-binding peptide (HABPep) to specifically bind HA to restrain HA-mediated delivery of hemameba. Hydrogel scaffolds of HABPep bound to polyethylene glycol diacrylate (PEGDA) are able to enhance cartilage repair in vivo, and this effect has been confirmed in a rat model of cartilage defects. Additionally, the hydroxyl group and carboxyl group (via the intermolecular bonds and intramolecular bonds that exist between them) can form self-cross-linking polymers, such as ACPTM, after esterification in vivo. These hydrogels have been used to reduce postoperative adhesions and have served as scaffolds for cell growth and repair in bone tissue defects [11].

4.2. Composite HA Hydrogels and Their Application. Composite HA hydrogels that consist of two or more natural/synthetic biopolymers incorporate the advantages of biopolymers, while eliminating some of the disadvantages through improved BC and biodegradability and adjustable mechanical strength. Therefore, research on composite HA hydrogels is appealing to the field of bone cartilage regeneration [88]. Using HAs modified by multiple methods and with other covalent binding materials, diversified composite HA hydrogels have been developed for use in BTE and CTE applications.

4.2.1. Binding to Collagen. Collagen is an abundant ingredient of bone, cartilage, and ECM [89]. It is believed that collagen is capable of facilitating the growth, proliferation, and conglutination of MSCs, as well as the formation and differentiation of cartilage [90]. Attempts to bind together collagen and HA have demonstrated better effects than using either one alone. For example, Liu [91] cross-linked activated formyl group-modified HA with collagen to form a new collagen-HA (COL/HA) matrix. Experimental results indicated that this matrix exhibited improved BC and osteoconduction in the skull of rats. Modified HA bound to collagen has also shown good performance [92] for inducing the osteogenic differentiation of hMSCs and assisting mineralization. It can also promote the gene expression of some bone components in order to promote bone formation. In CTE, collagen-HA scaffolds have shown high MSC infiltration, stimulating the motility of MSCs and their capacity for cartilage formation [71, 93]. To enhance mechanical strength, different concentrations of HA-dialdehyde (HAD), produced by periodate oxidation, combined with collagen to prepare collagen-HAD composite (CH) gels have been verified as a bionic matrix that promotes cell migration and morphology changes [94].

4.2.2. Binding to Gelatin. Gelatin is the hydrolysate of collagen and is composed of many cell adhesion peptide (RGD) sequences and MMP target sequences that promote cell remodeling [41]. Therefore, it has received significant attention from the tissue engineering field. The combination of HA modified with mercaptan (Glycosil), gelatin modified with mercaptan (Gelin-S), and PEGDA has been used to form cell construction matrices that have shown good effects in CTE [46]. Chang and colleagues [95] prepared scaffolds using a trinal copolymer of porcine chondrocytes, gelatin/chondroitin, and sulfate/HA to mimic the natural cartilage matrix to treat through thickness joint injuries in pigs with good efficacy. Similar experiments have also been conducted in rabbits [83]. We showed that the combination of photo-cross-linked GelHA hydrogels and ASCs enables ASC to differentiate into nucleus pulposus tissue and enhances the efficacy of ASCs in disc repair by activating the integrin $\alpha v\beta 6$ 6-TGF-beta 1 pathway [96].

4.2.3. Binding to Fibrin. Fibrin containing RGDs can not only induce the aggregation of polymers but also have commendable BC [97]. Because they can be cross-linked or bound to reinforcing materials, HA bound to fibrin has been shown to increase mechanical strength and cell adhesion. Fibrin/HA-MA hydrogels [98]/HA-AC hydrogels [58]/PCL/PLGA scaffolds coated with fibrin and HA admixtures [99] and PGA-HA scaffolds mixed with fibrin [65] have, respectively, been used in BTE and CTE applications (Table 1).

Table 1: Binding to fibrin.

Modification	Conformation	Biochemical function/applications	References
MA	Fibrin/HA-MA hydrogel	Increased mechanical strength, BMSC proliferation, and chondrogenic potential in vitro	[98]
EDC, ADH, AHA	Fibrin/HA-AC hydrogel	DNA delivery	[58]
PCL/PLGA	PCL/PLGA stent coated with a mixture of fibrin and HA	Used as a carrier for the delivery of BMP-2 and ASCs, resulting in better bone formation and mineralization	[99]
PGA	Fibrin-stabilized 3D PGA-HA scaffold	Cultures meniscus cells, promotes the re-expression of meniscus-specific markers, and forms meniscus matrix components	[65]

4.2.4. Binding to Alginate. Mahapatra and colleagues [100] prepared alginate-HA hydrogels mixed with collagen I. These Alg-HA-Col composite hydrogels demonstrated higher secretion of cartilage matrices, which can not only enhance mechanical strength and maintain biological stability and the biochemical environment but also contribute to the maintenance of the cell phenotype. Research on coating with both alginate microspheres containing TFGF-β3 and HA hydrogels containing human MSCs has demonstrated optimized MSC differentiation [101].

4.2.5. Binding to Chitosan. In addition to the abovementioned materials, chitosan is also a favorable biological binding material. Due to Schiff base reactions between the amidogen groups and aldehyde groups of polysaccharide derivatives, gelation does not require a cross-linking agent that shows toxicity on living cells. *N*-succinyl-chitosan (S-SC) and aldehyde-HA (A-HA) are used to prepare composite injectable hydrogels. Its hydraulic time, microstructure, surface morphology, equilibrium swelling, compression modulus, and degradation in vitro are suitable, and the effects of coating chondrocytes are obvious [102]. In experiments in rabbits, HA-acetylation chitosan hydrogels have surprisingly augmented subchondral bone remodeling and cartilage formation [86]. The porosity of lyophilized chitosan-HA-dialdehyde gels increases with the degree of oxidation, and chondrocytes coated in CHDA gel retain their viability and special phenotypic characteristics [102].

4.2.6. Binding to Other Materials. Chiari et al. [103] used new biological materials composed of HA and polycaprolactone as substitutions for sheep menisci. Solid-free form (SSF) preparations of polylactic-co-glycolic acid-grafted HA (HA-PLGA) polymers and polymers of ethylene glycol, and were then coated with BMP-2 for use on skull defects in rats. The effects were obvious [61]. Sheu [104] prepared oxydic HA/resveratrol (Oxi-HA/Res) hydrogels that were capable of BC, allowed the formation of the ECM, and reduced the inflammations and injury induced by LPS. It has been verified that HA cross-linked to aggrecan and cartilage-linked proteins are better lubricants than Simplex HA coatings [105]. HA modified by adipic dihydrazide (ADH-HA) and HA modified by aldehyde (a-HA), which are enhanced by cellulose nanocrystals (α-CNCs) with different contents of aldehyde modifications, have been used to form new injectable hydrogels. hASCs coated in HA-CNC hydrogels show diffusion and active proliferative activities in these hydrogels [106]. Hydroxyapatite nanoparticles blended with polyvinyl derivatives react with HA in situ to form a hydrogel, which then can be used to release BMP-2 for bone remodeling [68]. Pentosan polysulfate (a highly sulfating semisynthesis polysaccharide, PPS) shows chondrocyte induction potential. PEG/HA hydrogels have been used to deliver mesenchymal progenitor cells (MPCs), and covalently bound HA-PPS hydrogels can promote cartilage regeneration in treatments for multiple diseases, including intervertebral disc degeneration (IVD) [107]. Collagen/chitosan/HA hydrogels cross-linked by genipin have excellent mechanical properties and extended degradation times. These hydrogels display a compact structure, which shows advantages in ossicle regeneration [108]. Heparin modified with mercaptan (Heprasil™) and combined with HA modified by mercaptan hybridized PEDGA can adequately control the release of BMP-2 [109].

4.3. Other Modifications of HA Hydrogels. Though the abovementioned research on HA-based delivery of stem cells, growth factors, and drugs have shown promise and effects in bone regeneration, more work is needed to meet the functionality and complexity of mature tissue or organ construction in tissue engineering applications. With the continuous development of materials technology, research on the directional assembly of hydrogels is imperative. Electrostatic spinning, centrifugal casting, template leaching, and phase separation technologies are examples of newly emerged technologies [47]. These technologies have shown advantages for regulating and controlling the macroscopic pore size and shape but have limited benefits in terms of porosity and the exchange of biological information on a microscopic level. At present, the combination of 3D bioprinting and micropatterning has been the most studied [110].

With "biology ink" (cell aggregations or spheroids) and "biology paper" (scaffold materials), 3D bioprinting fabricates layers that are later stacked in the computer to create functional living tissues [111]. The technology two-photon polymerization (TPP) under femtosecond laser irradiation generates 3D submicron patterns on hydrogel scaffolds that have been gradually introduced into HA hydrogels and 3D bioprinting [112]. The methods for selecting biological ink and biological paper vary.

Experiments have shown that the three kinds of composite material preparations of HA-g, HA-pHEA, and HA-gelatin hydrogels have stable rheology, excellent BC, and printability, showing their potential for use in tissue engineering in the future.

Methacrylic acidizing and photo-cross-linking are used to form hydrogels and scaffold materials that are then used for tubulose construction printing of HA-gelatin hydrogels [82]. New semi-interpenetrating polymer networks (semi-IPNs) of dextran are derived from HA and ethoxyl metacrylic acid ester [113]. Furthermore, 24 nm gold nanoparticles (AuNPs) are dynamically cross-linked to HA modified with thiol and gelatin to form sECM hydrogels [114]. The abovementioned materials are conducive for 3D bioprinting.

5. Conclusions and Prospects

Despite the above advantages and modification methods, HA-based scaffolds still face challenges. HA-based scaffolds used in vivo can induce foreign body reactions. Various proteins can be absorbed onto the surfaces of implanted HA-based scaffolds and induce a series of degeneration effects. Moreover, scaffolds can induce an inflammatory reaction [115]. The generation and activation of hyaluronidase remains a problem to be solved for controlled release. In conclusion, with the support of a series of cross-linking technologies and materials technologies, HA hydrogels and their derivatives have shown great potential in BTE and CTE applications. It is believed that bionic matrices capable of highly mimicking the cellular environment will be important in the further refinement of bioreactors and scaffold construction.

Conflicts of Interest

There are no conflicts of interest to declare.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (no. 31572217) and the Jilin Provincial Educational 135 Science and Technology Project (nos. JJKH20180224KJ and JJKH20180209KJ).

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