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Review Article

In Vitro Study of Hyaluronic Acid Based Scaffolds and Its Effect on Cartilage Regeneration

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ABSTRACT

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Recently, it has been proven that cartilage healing is difficult. The most commonly used treatments are autogenously cartilage grafting and allogeneic bone grafting, but grafts cannot fully meet treatment goals because of source, price, safety, and other concerns. Thus, a combination of biological materials and tissue engineering technology has become a recent trend in studies. Among the studies performed on tissue engineering cartilage materials are hydrogels that exhibit biological activity, post-decomposition adsorption, flexibility, and easy preparation. Cell-containing hydrogels are often used in cartilage tissue engineering because of their biocompatibility, ease of use, and ability to adapt to different defects. Hydrogels are used to mimic extracellular matrices. Although multiple materials can configure and form hydrogels, hyaluronic acid and its derivatives are distinguished. Hyaluronic acid (HA) is an extracellular molecule with several physical and biological functions found in many tissues, including cartilage. HA is formed in several biomaterial systems and scaffolding. HA hydrogels have many interests, including increased adhesion, cell proliferation, and wound healing. In addition, they represent adequate biological acting for stimulating a microenvironment for the survival of cells. However, their disadvantages include a slow degradation rate and low mechanical properties. Here, HA-based hydrogels and their applications in cartilage tissue engineering are briefly reviewed.

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Introduction

Articular cartilage has limited potential for spontaneous healing. This property often leads to osteoarthritis, pain, and dysfunction of the affected joint [1, 2]. Although many accepted treatments are accessible to repair damaged articular cartilage structures, including micro-fractures, cell implants, and tissue transplants, these methods often do not repair strong, well-repaired articular cartilage [3-5]. In recent years, cartilage tissue engineering (CTE) has prepared a prospective strategy for articular regeneration by combining cells with scaffolds.

Fakhari et al. developed this scaffold in tissue engineering (TE) applications by researching hyaluronic acid (HA) hydrogels. HA is a non-sulfated glycosamino-glycan (GAG) and is a significant component of the extracellular matrix of cartilage. HA provides a native microenvironment for mesenchymal stem cells and can increase functional cartilage formation compared to other synthetic hydrogels such as polyethylene glycol (PEG) [6, 7].

HA has several biomedical applications due to cellular interactions and its presence and role in the extracellular matrix of many tissues [8]. Among the applications mentioned for HA are drug delivery and tissue bulking [6, 9].

One of the significant goals of TE approaches with HA hydrogels is cartilage tissue repair [10]. Since HA is abundant in healthy cartilage (such as the matrix around cartilage cells) and is involved in cartilage homeostasis, it has been extensively studied as part of hydrogels and scaffolds for cartilage repair [11, 12]. Mesenchymal stem cells enclosed in HA-based hydrogels show higher expression of cartilage markers in both *in vitro* and *in vivo* than those compared to ineffective PEG hydrogels [13].

CTE is a promising way to repair cartilage tissue damage. The most common methods used in CTE include the proper combination of granule cells, biocompatible scaffolds, and biological agents that support the formation of new cartilage [14]. Success in cartilage tissue regeneration depends on individual or combination characteristics of cells, biological agents, and scaffolding [15]. We investigated appropriate cells and biological agents and a convenient scaffold for CTE. We also studied HA scaffolds and HA-based composite scaffolds in CTE and cells and growth factors used with this scaffold to induce and enhance chondrogenesis.

Cells in CTE

Chondrocytes are the most common cells used in CTE. Besides, they play an essential role in cartilage regeneration. On the other hand, stem cells can achieve self-renewal and differentiate into multiple lineages. They can be taken from donor cartilage such as the meniscus, the nose, and the trachea. They can construct, maintain, and regenerate cartilage tissue *in vitro* [16]. Autologous cartilage is difficult to access, and the cells collected from the patient's joints are relatively inactive. Chondrocyte proliferation in monolayer culture leads to disintegration and is presented as decreased proteoglycan synthesis and type II collagen expression and type I collagen overexpression [17]. Young donor chondrocytes are more metabolically active and have higher chondrogenic potential and fast

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expansion compared to cells taken from adult donors [18, 19]. To dominate and control the limited storage of primary cells, the application of multipotent stem cells is recommended, which are mainly isolated from bone marrow, adipose tissue, and before implantation [20, 21]. Adult mesenchymal stem cell (MSC) sources are available in various tissues, including trabecular bone, bone marrow, deciduous teeth, periosteum, articular cartilage, adipose tissue, muscle, and synovial membrane [21-23].

Growth factors in CTE

Growth factors and chemical stimuli such as transforming growth factor-β (TGF-β) conversion, insulin such as growth factor-1, and bone morphogenic protein-6 are required [24]. However, using the chemical inducers mentioned, the researchers found that neo-cartilage tissues were not similar to native hyaline cartilage due to having more type I collagen and type X collagen and less type II collagen. Therefore, researchers are trying to find an alternative to MSC induction to produce better quality cartilage tissue and lower costs [25].

Scaffolds in CTE

Scaffolds acting as the artificial extracellular matrix (ECM) also have pivotal roles in determining cartilage reconstruction. The scaffold is a three-dimensional construct in which cells can attach and migrate. Fibers, meshes, sponges, and hydrogels scaffolds have been administered as carriers for chondrocytes and stem cells in CTE. The ideal scaffold should be biocompatible, non-toxic, non-stimulatory for inflammatory cells and non-immunogenic [26]. It must also have specific characteristics that lead to cell adhesion, proliferation, differentiation into specific phenotypes such as mechanical support of CTE and porosity, leading to the release and exchange of nutrients and the excretion of waste products [26, 27]. In addition, scaffold components must be resistant to decay at physiological pH and body temperature, be biodegradable and allow new cartilage to regenerate and replace the original structure [28]. A suitable scaffold for CTE is a scaffold with high porosity and the ability to connect pores to pores. High porosity provides a good environment for cell adhesion, growth, and regeneration. The interconnected porous organization facilitates cell migration, exchange of nutrients and physiological gases into the cell, and metabolic of cells [29]. Mechanical stimulation can certainly boost the mechanical features of CTE [30]. CTE studies have focused on two loading regimes: direct or unbound compression and hydrostatic pressure. Direct dynamic compression applied to cartilaginous scaffolds typically increases the production and/or proliferation of the ECM and improves the compressive properties of the engineered tissue [31].

The main goal of TE is to create implant-like structures that can replace damaged tissue. Scaffolds with good porosity provide a suitable environment for cell migration, cell proliferation, and other activities [32-34].

HA as scaffold for CTE

HA also known as hyaluronan, is a linear, anionic, non-sulfated GAG with a combination of saccharide gland units: β-1,4-D and β-1 glucuronic acid, 3 - N -acetyl- D-glucosamide [35]. It is a high molecular weight (105-107 kDa) natural biopolymer that can contain 5000-30000 sugar molecules in the backbone

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structure [36]. HA is one of the main components of ECM and cartilage tissue. HA is synthesized in the inner cell membrane by the synthesis of hyaluronan. After synthesis, it is transferred to the ECM through the membrane after 3-5 days, where it is destroyed by the family of hyaluronidase enzymes [37]. HA is found in the ECM of all living tissues, with varying concentrations and molecular weights, and is more prevalent in mechanically loaded tissues such as cartilage, dermis, and vocal folds [38, 39]. Due to many carboxyl and hydroxyl groups, HA is a highly hydrophilic compound that creates a gel-like structure as a result of the intermolecular interaction of macromolecules in the aqueous medium [40]. It acts as a protection against the penetration of microorganisms and toxic agents [41], lubricant due to its viscoelastic properties in the synovial liquid [42], and the transparent aqueous solution is a filler of eye structures [43]. HA is commonly known to play an essential role in cell division and migration, angiogenesis, wound healing, and tissue regeneration, and its effects are related to molecular weight [44]. Due to its biocompatibility, biodegradation, and chemical modification, HA is of potential interest in the TE field [45]. The use of cells, scaffolds, and growth factors promote tissue regeneration, which can overcome the development of autologous and allogeneic transplant-related immunological responses [21]. HA can interact with stem cell surface receptors, transmit signals within the cell, and affect cell activity, such as proliferation, survival, motility, and differentiation [45]. In various studies of HA and HA-based materials such as biological scaffolds and injectable hydrogels, *in vitro* and *in vivo* tests have been used that show positive results for tissue regeneration. HA does not promote cell adhesion, but it can be modified by motifs such as Arginine-Glycine-Aspartic Acid (RGD) to increase cell attachment [46]; HA is a polymer of disaccharides composed of repeating units of β-1,4-d-glucuronic acid and β-1,3-N-acetyl-d-glucosamine (Fig. 1) [47]. One of the unique properties of hyaluronic acid is its rheological property, which is also observed in low concentrations, characterizing the intertwined chains of this hydrogel (Fig. 2) [48]. The binding of HA can be divided into two incomplete and complete groups, which causes HA to form a polymer network by covalent bonding with the interfaces and is insoluble in water by forming a polymer which is due to the complete bonding. Incomplete bonding of part of the covalent bond of HA molecules is stimulated, and a small amount of water solution remains (Fig. 3) [49].

HA composite hydrogels consisting of two or more natural/synthetic biopolymers have the advantages of biopolymers, while they enhance some of the disadvantages through improved biodegradation and adjustable mechanical strength. Using HA and modified several methods and other covalent bonding materials, various hybrid hydrogels have been increased for administration in CTE [45].

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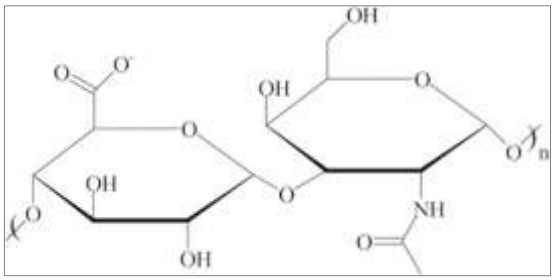

Fig. 1. The disaccharide repeat unit of hyaluronic acid

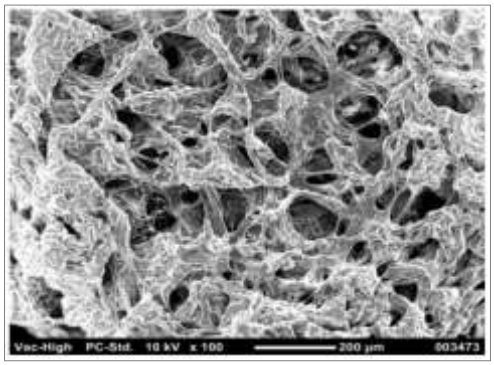

Fig. 2. The morphology of hyaluronic acid (RESTYLANE®) using scanning electron microscopy [48]

Fig. 3. The gross view of cross-linked hyaluronic acid using BDDE at the concentration of 0.8% [49]

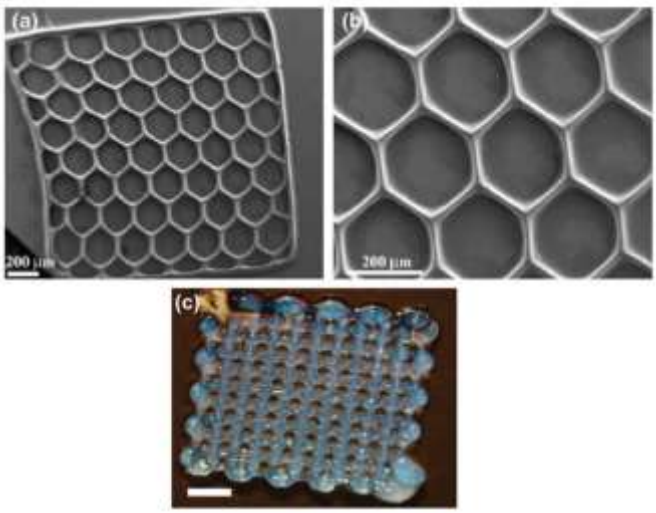

Fig. 4. a and b) Scanning electron microscope micrograph of single-layered hyaluronic acid -based scaffolds with hexagonal patterns reproduced with permission and c) hyaluronic acid -based scaffold produced from bioprinting [50]

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The purpose of this study is to review the effect of HA hydrogels as a scaffold to help repair cartilaginous lesions. Scientific Information Database (SID), MEDLINE, PubMed, OVID, and Scopus databases from 2010 to 2021 were used for this study.

Mechanical properties of HA

HA undergoes multiple degradation procedures because of hydrolysis and enzymatic hydrolysis by naturally arising hyaluronidase. Non-enzymatic reactions can degrade HA. These include acid and alkali hydrolysis, ultrasonic decomposition, thermal decomposition, and oxidant degradation [51]. Achieving mechanical properties is important in the design of HA-based hydrogels. In principle, they must have ECM-like mechanical properties in normal tissues, be sufficiently resistant to enzymatic and non-enzymatic degradation, and not deform against the compressive forces of the surrounding tissues. The mechanical properties of artificial substrates *in vitro* environments significantly influence some cell functions such as adhesion, proliferation, migration, and differentiation [52]. Due to disadvantages such as hydrophilic nature and lack of mechanical integrity, HA needs chemical modification and cross-linking to change it for CTE applications [53, 55]. In order to control the degree of degradation and improve its mechanical properties, in designing HA-based scaffolds for cartilage tissue, various strategies such as cross-linking or using a composite structure to create a stable material are used [53, 54].

The results of the studies are summarized in Table 1.

Table 1. Hyaluronic Acid scaffolds in cartilage tissue engineering

Author	Year	Scaffold Type	Growth Factor	Cell	Outcome
Yuan et al. [55]	2015	HA+ collagen	chondrogenic medium + icariin	Rabbit chondrocytes	Expression of sox9, aggrecan, collagen type II genes from seed cartilage is increased. Production of glycosaminoglycans and collagen type II was much higher in HA-Ica/ collagen hydrogels.
Mondal et al. [56]	2016	HA+ divinyl sulfone	chondrogenic medium	Adipose-derived stem cells	Cytotoxicity analysis showed that all hydrogels are cytotoxic and can be used to deliver AMSCs. Hydrogels have been shown to aid in forcing various AMSC differentials, and Thoms may be potential support in repairing articular cartilage in osteoarthritis.
Chen et al. [57]	2016	Glucosamine in gelatin/HA cryogel	chondrogenic medium	Rabbit articular chondrocytes	Cryogel scaffolds containing 9% glucosamine showed better efficacy in maintaining cartilage phenotype by affecting cell proliferation increasing the secretion of GAGs and COL II.
Mahapatra et al. [58]	2016	Alginate + Hyaluronic acid + Collagen type I (Alg-HA-Col)	chondrogenic medium	Rat articular chondrocytes	The mRNA levels of chondrodite phenotypes, including SOX9, type II collagen, and aggregates, are significantly regulated when cells are cultured in Alg-HA-Col gel compared to those cultured in Alg-HA be. The secretion of glycosaminoglycan sulfate, a

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					specific cartilage matrix molecule, was observed in collagen composite hydrogels.
Kim et al. [59]	2017	Oxidized hyaluronate + glycol chitosan	chondrogenic medium	ATDC5 cells	These hydrogels are well adapted to physiological conditions and can act as an injectable cell transport system in CTE.
Amann et al. [60]	2017	HA	chondrogenic medium	Human articular chondrocytes /hADSC	Hyaluronic acid stimulates the differentiation of collagen from collagen hydrogel supplementation in a dose-dependent manner. 1% HA showed the best results
La Gatta et al. [61]	2017	Hyaluronan+lysine Methyl-ester cross-linking	chondrogenic medium	Human articular chondrocytes	Primary human chondrocytes cultured hydrogels are viable and maintained in their lineage. They also secrete cartilage-specific matrix proteins. These scaffolds are promising candidates for CTE.
Liu et al. [62]	2018	Glycol chitosan/oxidized hyaluronic acid And Glycol chitosan/oxidized hyaluronic acid+ ECM	chondrogenic medium	BMSCs	To evaluate chondroinductivity induction of ECM in vitro, BMSCs were compared in S1 (G-CS/OHA) and S3 (G-CS/OHA/ECM 2-weight) hydrogels. Higher levels of glycosaminoglycans (GAG) and type II collagen (COL II) were accumulated in the S3 hydrogel.
Lin et al. [63]	2019	Methacrylate gelatin	chondrogenic medium	human BMSCs	mGL/mHA with a ratio of 9: 1 (٪, w/v) leads to the lowest hBMSC hypertrophy and the highest glycosaminoglycan production, with a slight increase in the total volume of the structure.
Sharifian et al. [45]	2019	HA + Fibrin + Polylactic acid-polyglycolic acid	chondrogenic medium	hADSCs	poly(lactide-co-glycolide)/fibrin/HA stimulates cartilage production in hADSCs. Decreased hypertrophic markers and increased characteristic markers of hyaline cartilage were observed in hydrogels.
Jooybar et al. [64]	2019	HA-tyramine (HA+TA)	chondrogenic medium + platelet lysate	Human mesenchymal stem cells	Platelet laser materials have a significant function in supporting human mesenchymal stem cells (hMSCs), acting like cell binding, viability, and proliferation in the three-dimensional hydrogel. When placed in a cartilaginous differentiation medium, hMSCs produce hyaline cartilage produced by HA-TA.
Wang et al. [65]	2019	polypeptides	chondrogenic medium	Rabbit BMSCs	Adhesion and proliferation were represented, and an experimental study of BMSC demonstrated that the PAP-3SF/6.5COL/0.5HA scaffold had good biocompatibility.
Ren et al. [66]	2020	Maleimide-modified hyaluronic acid+ collagen mimetic peptide (GPO)8-CG-RGDS	chondrogenic medium +matrix metalloproteinase	Bone mesenchymal stem cells	A combination of CMP with an MMP-sensitive peptide can have the possibility to differentiate mesenchymal stem cells into cartilage and prevent the hypertrophic phenotype throughout differentiation.
Tsanaktsidou et al. [67]	2020	Methacrylated hyaluronic acid (MeHA)+ chondroitin sulfate	chondrogenic medium + matrix metalloproteinase	human mesenchymal stem cells	Methacrylated hyaluronic acid and chondroitin sulfate hydrogels have been developed to create an environment conducive to the growth and proliferation of human mesenchymal stem cells and promote their differentiation from tubular phenotypes, even if grown in an expansion medium.

HA= Hyaluronic acid; AMSCs= Adipose tissue-derived mesenchymal stem cells; hBMSCs= human bone marrow mesenchymal stem cells; CTE= Cartilage tissue engineering; ADSC= Adipose derived stem cells; BMSC= Bone marrow stem cell

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Conclusion and future trends

Although an extensive range of surgical methods is accessible to treat cartilage injuries and be prosperous in short-term and long-term follow-up, none of them are qualified to fully return the activity and construction of damaged cartilage to its original state. HA is a promising bright spot to help reduce side effects. Its effectiveness is due to many practical methods, including lubrication, anti-inflammatory effects, and cartilage protection. HA treatment demonstrates great potential that we hope will be identified with further research. Further research is needed to obtain a specific HA molecular mass to achieve clinical efficacy and expand its applications to complete control of the disease and its complications.

Although the widespread use of HA hydrogels is important in biomedical applications, the effect of HA on cellular behavior, especially through cell surface receptors such as CD44, has been poorly studied. We reviewed articles that examined hyaluronic acid scaffolds for cell differentiation into cartilage and their effect on surface receptors.

HA adhesion has a large effect on the hMSC response, leading to increased cell proliferation, proliferation, and the formation of focal adhesions. HA parameters have been shown to affect hMSC cartilage formation, as seen through gene expression profiles, potentially by activating cytoskeletal organization and cell ability. HA fibrous hydrogels are a promising alternative to non-fibrous hydrogels for regenerative strategies that can be used in the future. HA can direct articular cartilage. Combining this material with other natural and synthetic scaffolds has been shown to have cartilage induction capabilities. HA supports the migration, survival, and differentiation of stem cells. HA supports the proper formation of the matrix by differentiating stem cells to become articular. It suggests that if used *in vivo*, such a device can be integrated into a joint defect site and healed.

Nevertheless, it is necessary to have appropriate molecular signals to support the repair and healing of joint lesions. Synergistic growth factors must be added to maintain and improve the induction of regenerative articular cartilage, thus preventing fibroids from returning to the cartilage or endochondral ossification. Under these circumstances, the HA matrix with other scaffolds could be a viable alternative to promote better regeneration of articular cartilage.

Conflicts of interest

The authors have no conflict of interest to declare.

Acknowledgment

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