CURRENT CONCEPTS REVIEW

Biomolecules-Loading of 3D-Printed Alginate-Based Scaffolds for Cartilage Tissue Engineering Applications: A Review on Current Status and Future Prospective

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Abstract

Osteoarthritis (OA) can arise from various factor including trauma, overuse, as well as degeneration resulting from age or disease. The specific treatment options will vary based on the severity of the condition, and the affected joints. Some common treatments for OA include lifestyle modifications, medications, physical therapy, surgery and tissue engineering (TE). For cartilage tissue engineering (CTE), three-dimension (3D) scaffolds are made of biocompatible natural polymers, which allow for the regeneration of new cartilage tissue. An ideal scaffold should possess biological and mechanical properties that closely resemble those of the cartilage tissue, and lead to improved functional of knee. These scaffolds are specifically engineered to serve as replacements for damaged and provide support to the knee joint. 3D-bioprinted scaffolds are made of biocompatible materials natural polymers, which allow for the regeneration of new cartilage. The utilization of 3D bioprinting method has emerged as a novel approach for fabricating scaffolds with optimal properties for CTE applications. This method enables the creation of scaffolds that closely mimic the native cartilage in terms of mechanical characteristics and biological functionality.

Alginate, that has the capability to fabricate a cartilage replacement customized for each individual patient. This polymer exhibits hydrophilicity, biocompatibility, and biodegradability, along with shear-thinning properties. These unique properties enable Alginate to be utilized as a bio-ink for 3D bioprinting method. Furthermore, chondrogenesis is the complex process through which cartilage is formed via a series of cellular and molecular signaling. Signaling pathway is as a fundamental mechanism in cartilage formation, enhanced by the incorporation of biomolecules and growth factors that induce the differentiation of stem cells. Accordingly, ongoing review is focusing to promote of 3D bioprinting scaffolds through the utilization of advanced biomolecules-loading of Alginate-based that has the capability to fabricate a cartilage replacement tailored specifically to each patient's unique needs and anatomical requirements.

Level of evidence: III

Keywords: 3D-bioprinted, Alginate, Biomolecule, Cartilage tissue engineering, Scaffolds

Introduction

Knee osteoarthritis (OA) is known as a progressive joint disease, and an average estimation of about 30 million Americans are currently affected by this disease. The OA develops when the normal synovial joint structure is damaged and its function is impaired. Treatment of articular cartilage lesions is associated with significant challenges due to the inherent limitations in the ability of the tissue to repair and regenerate itself.[1]

currently, clinical approaches for OA treatment encompass autologous chondrocyte transplantation and periosteum transfer, as well as auto-chondral and allograft osteochondral transplantation.

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Recently, new strategies for cartilage tissue engineering (CTE), including the use of progenitor cell compounds, bioactive factors, and artificial matrices and devices have been studied.[2] In tissue engineering (TE), a synergistic combination of various cells, bioactive molecules, and biomaterials is used to facilitate the regeneration or repair of damaged tissue. For successful treatment with tissue engineering methods, the scaffolds need to have general properties, including biocompatibility, biodegradability, suitable surface properties, and suitable physical, chemical, and mechanical properties.[3]

Moreover, the scaffold must serve as a unique substrate for the promotion of cell adhesion and regulation of critical cellular behavior, such as migration, proliferation, differentiation, gene expression, apoptosis, and provision of essential mechanical support. In this regard, various materials and methods are used in order to develop scaffolds that mimic the function of the native cartilage tissue for CTE. The three-dimensional (3D) scaffolds required for a successful clinical trial must be made with a similar shape, structure, strength, and biological properties to the natural cartilage tissue.

Among various methods, the utilization of the 3D bioprinting method has emerged as a novel approach for the fabrication of scaffolds with optimal structural, mechanical, and biological properties for CTE applications. This method enables the creation of scaffolds that closely mimic the native cartilage in terms of both mechanical characteristics and biological functionality.[4] Moreover, the 3D bioprinting method was employed to create a unique porous structure with a controlled internal structure that facilitates the natural lubrication mechanisms of the knee for the cartilage scaffolds. Therefore, 3D-bio-printed scaffolds can be produced in the desired shape and architecture with suitable mechanical properties for medical applications.

In addition, the 3D bioprinting method was used to fabricate unique porous structures for the cartilage scaffolds.[5,6] A variety of bio-ink polymers are utilized in the fabrication of 3D-bioprinted scaffolds for CTE.[7] Alginate is frequently employed as a biomaterial in the field of TE due to its appropriate biocompatibility (low toxicity and immunoprotective properties) and biodegradability.[8] Furthermore, Alginate is ideal for CTE application as it has the ability to replicate the extracellular matrix (ECM) found in various tissues, creating a conducive 3D environment for cellular growth. Alginate can be used to encapsulate cells or stimuli factors and deliver them to the site of injury or damage, where they can promote tissue regeneration.[9]

Based on the findings of previous studies, a wide range of strategies are utilized for the promotion of chondrocyte proliferation and facilitation of the chondrogenic differentiation of stem cells. These include the implementation of cytokines, biomolecules and growth factors, 3D culture systems, mechanical stimulation, co-culture systems, hypoxic culture conditions, bioreactors, and microRNAs (miRNAs).[10] In this condition, the usage of biomolecules has been approved since it has several advantages over other factors, such as induced chondrogenesis differentiation. Firstly, these compounds possess well-defined chemical structures and can be fabricated with a high degree of efficiency.[11] Secondly; the effects of biomolecule compounds on the biology of the body are typically dose-dependent, rapid, and reversible. Thirdly, in comparison to other methods, biomolecule compounds are relatively simple to handle and administer, which makes them more practical for *in vivo* and *in vitro* applications.[8,12,13]

Hence, the usage of 3D bioprinting scaffolds imbued with biomolecules for inducing chondrogenic differentiation presents a favorable approach due to its advantageous features, such as customizable designs and the optimization of structural, mechanical, and biological properties for CTE. This review focused on the continuous improvement of a 3D-bioprinted scaffold based on Alginate loaded with biomolecules to achieve the best effect of cartilage regeneration.

Pure and Composite Alginate

Three-dimensional-bioprinted scaffolds based on Alginate loaded with biomolecules are used as 3D scaffolds for CTE. Alginate is a highly versatile polysaccharide that is derived from seaweed. Its characteristics make it an ideal biomaterial for bio-fabrication methods, owing to its excellent printability (shear-thinning properties), biodegradability, and biocompatibility. Moreover, Alginate is readily available and has a low degree of toxicity, which further enhances its appeal as a material of choice in this field.[8,14] In addition, the rapid rate of gelation of Alginate in the presence of divalent Ca^{2+} ions provides several advantages. This property facilitates the encapsulation of cells within the 3D scaffold and promotes interlayer adhesion during the layer-by-layer 3D bioprinting process. This property is essential for ensuring the structural integrity and mechanical stability of the scaffold during the bioprinting process.[15]

In order to improve the properties of Alginate scaffolds, such as mechanical strength, biological value, biocompatibility, and bioactivity, researchers have fabricated composite scaffolds by blending Alginate with other natural polymers, including Chitosan, Collagen, Gelatin, Hyaluronic acid, and Cellulose. In the following, the kind of scaffolds based on Alginate loaded with biomolecules are explained for CTE.

3D-printed pure Alginate

In order to fabricate 3D scaffolds for CTE, the 3D-bioprinted Alginate scaffolds were fabricated by 3D bioprinting method. The 3D bioprinting method enables the suspension of cells in a controlled environment, allowing for the simulation of complex conditions mimicking those found within the human body. Cell biology is greatly influenced by physical and chemical cues in the environment, particularly those presented by the ECM.[16]

Previous research has demonstrated the effectiveness of bio-inks Alginate for 3D scaffold fabrication through the extrusion of bioprinting methods. Alginate is widely used in the 3D bioprinting method due to its remarkable printability and biocompatibility, along with its nearly low cost, low toxicity, and one-step gelation process with rapid rate (in the presence of a Ca^{2+} cross-linker).[17]

Properties of the Alginate as a novel shear-thinning bio-ink play a crucial role in determining its printability.[18] These properties include the viscosity and rheology of the bio-ink, as well as its mechanical strength, crosslinking behavior, and

stability over time.[19] Therefore, its printability is influenced by several key parameters. Among these, the molecular weight and M/G ratio of its composition as well as the concentration and used gelling agent have been identified as critical factors.[20] Optimization of these parameters can significantly improve the quality and fidelity of printed structures, making them more clinically relevant for TE and regenerative medicine applications.[21] Therefore, understanding and controlling Alginate-related parameters is essential for successful bioprinting with this material.[22] These factors make them essential parameters for the achievement of successful and high-resolution 3D structure fabrication.

3D-printed Alginate/Natural Polymer Composite

As mentioned before, an approach that has gained traction is the utilization of the 3D bioprinting method to fabricate 3D scaffolds that closely resemble the structure of natural cartilage tissue. Alginate used in 3D bioprinting must possess the necessary viscosity to maintain its shape during the bioprinting method. Additionally, it should have crosslinking abilities that enable the material to retain its 3D structure post-printing.[23] One common challenge encountered in 3D bioprinting scaffolds is that the printed structures often collapse under the weight of the layers above, owing to their low viscosity.[24] While it is possible to increase the viscosity of Alginate by adjusting its concentration and molecular weight, such measures have not proven sufficient for achieving shape stability during bioprinting method.[25,26]

In order to enhance the structural stability of 3D-bioprinted structures, bio-inks are often used in combination with other biomaterials, like chitosan, Collagen, Gelatin, Hyaluronic acid, and Cellulose.[27-30] Moreover, the 3D-bioprinted composite based on Alginate with different natural polymers could be for the fabrication of complex structural and highly functional biomedical devices with tailored mechanical and biological properties and functionalities.[31]

In the following, the kind of 3D-bioprinted scaffolds based on Alginate combined with natural polymer loaded with biomolecules are explained. Chondroinductive biomolecules include growth factors, such as transforming growth factor-beta (TGF-β), bone morphogenetic proteins (BMPs), insulin-like growth factor (IGF), and fibroblast growth factors (FGFs). The structural similarity between insulin and IGF-1 enables insulin to activate the IGF-1 receptor. Consequently, research has demonstrated that insulin possesses significant efficacy in the promotion of ECM production within chondrocytes.

In addition, kartogenin (KGN) is recognized as a promising chondroinductive biomolecule, presenting a viable novel therapeutic compound for the improvement of the healing process of osteoarthritic joints. Johnson et al. have recently employed a low molecular weight compound called KGN to selectively induce the differentiation of mesenchymal stem cells (MSCs) into chondrocytes. Notably, KGN exhibits a lack of cytotoxicity and demonstrates a dose-dependent effect as a differentiation-inducing agent. Sadaf et al. developed beta cyclodextrin (β-CD)-modified Alginate/ECM-based interpenetrating polymer network (IPN) hydrogel for release of KGN.

The β-CD has been found to have extensive utilization in drug delivery systems due to its hydrophobic inner cavity and hydrophilic outer surface.[32] Given various factors, such as water swelling, degradation rate, mechanical properties, cell cytotoxicity, and gene expression, the β-CD-modified hydrogel, reinforced with a 3D-printed structure facilitating the controlled release of KGN, emerges as a highly promising candidate for articular cartilage repair applications.[33]

In addition, Ascorbic acid (vitamin C) has been proposed as a clinically translatable mediator of hyaline cartilage repair as it facilitates the transcription of Collagen genes in MSC.34 These molecules can stimulate the proliferation and differentiation of chondrocytes, which are the cells responsible for cartilage formation. Moreover, they can be incorporated into 3D scaffolds. More details regarding the usage of various methods for the fabrication of the Alginate-based 3D scaffolds for cartilage repair are summarized in.

3D-printed Alginate/Chitosan

Articular cartilage has a limited ability for self-regeneration due to its low capacity for self-renewal, which becomes apparent when it is injured.[35] Chitosan is a promising biomaterial for cartilage regeneration due to its unique characteristics, including biocompatibility, hydrophilicity, biodegradability, and low immunological response.[36,37] The pre-gel solution serves as the ink for 3D bioprinting scaffolds and its viscosity and transition from a sol to a gel state play a critical role in determining the shape fidelity and overall 3D structure of the printed scaffold.[38]

As mentioned before, Alginate crosslinking refers to the process of creating a 3D scaffold of Alginate by forming ionic bonds between the COO^- groups of Alginate and positively charged ions.[39] Moreover, Alginate, as a biocompatible polyanion, can be crosslinked with a polycation, including Ca^{2+}, to create a polyion complex (PIC). The mechanical properties of a PIC scaffold, which is made by the combination of two oppositely charged polyelectrolytes, can be adjusted by the modification of the reactive ion pairs.[40]

Qiongqiong Liu et al. developed a 3D-bioprinted Alginate/chitosan PIC for TE application.[41] Due to the enhanced formation of ion pairs, the 3D-bioprinted Alginate/Chitosan PIC scaffolds exhibited superior mechanical strength and improved compression stress, compared to the 3D-bioprinted Alginate scaffold. However, the limited viscosity of Alginate ink poses a challenge when attempting to fabricate scaffolds with complex 3D structures. This is primarily due to the vulnerability and instability of the deposited layers during the 3D bioprinting process, which can often result in their collapse.

The Chitosan powder swelled but remained insoluble in utilized for this purpose must effectively emulate the biomechanical and biochemical properties of native tissue. An integral aspect of 3D bioprinting cell-laden the Alginate solution. Consequently, the addition of chitosan powders to the Alginate bio-ink developed the viscosity of the 3D bioprinting bio-ink.[40,42,43] The scaffolds

scaffolds is to optimize the printing factors that regulate biomaterial properties, improve efficient printing, and preserve cell viability and phenotype.[44] Hence, the use of growth factors and mechanical stimulation in conjunction with these 3D scaffolds is anticipated to facilitate growth and differentiation toward the suitable chondrocyte phenotype [Table 1].[45-47]

Table 1. 3D Alginate-based scaffolds for cartilage repair

Materials	Method	Bioactive Agent	Reported result
Alginate/chitosan	Bioactive Agent	Non-loaded	Adhesion behavior of rabbit chondrocytes onto an Alginate/chitosan polymer improved *in vivo*.
Alginate/chondroitin sulfate/silk fibroin	Porous hydrogel	PRP	Fabricated hydrogel based on Alginate/PRP shows higher glycosaminoglycan deposition, expression of Collagen type II, and aggrecan.
Alginate	Ionic gelation	BMP-7, TGF-β2	Encapsulation of growth factors into hydrogel based on Alginate is commonly formed in $CaCl_2$ solution for crosslinking agents. In the context of cartilage repair, the control over gelation rates is exceptionally beneficial, especially when considering its applicability as an injectable system for delivering cells or growth factors directly into damaged tissues.
PLA/Alginate	3D construct	TGF-β1	Stable 3D PLA/Alginate amalgam represents a novel scaffold system of mesenchymal chondrogenesis.
Calcium/cobalt Alginate	Bead synthesis	Non-loaded	An innovative and cost-effective method is proposed for inducing *in vitro* chondrogenic differentiation of MSCs that are enclosed within Alginate beads.
Alginate/Gelatin	Electrospray	hBMSC-embedded microsphere	HBMSCs–embedded Alginate–Gelatin microspheres loaded with hBMSCs have suitable mechanical strength and biological properties for CTE.
Alginate	Bead synthesis	IGF-1	Chondrocytes from equine stifle joints were expanded and either encapsulated in Alginate beads or maintained in monolayer culture for 10 days. This result showed the capacity of beads for cartilage repair.
Alginate	Bead synthesis	TGF-β1	Growth factor appeared to be released focally at the site of repair at a relatively slow speed. This system was proposed for the treatment of osteochondral defects in the rabbit knee model.
Alginate/ HA	Hydrogel	IGF-1	Bovine chondrocytes were embedded in Alginate hydrogels containing varying HA concentrations and structure were cultured with exogenous IGF-1. The HA construct with the delivery of IGF-1 indicated the chondrocyte phenotype.
Alginate/ β-CD/ pluronic-amine	Injectable hydrogels	KGN	KGN facilitated encapsulated MSCs differentiation towards chondrocytes.

PRP: platelet-rich plasma, BMP: bone morphogenetic proteins, TGF: transforming growth factor, PLA: polylactic acid, MSC: mesenchymal stem cells, hBMSCs: human bone marrow-derived mesenchymal stem cells, CTE: cartilage tissue engineering, IGF: insulin-like growth factors, HA: Hyaluronic acid, β-CD: beta-cyclodextrin, KGN: kartogenin

3D-printed Alginate/Collagen

Collagen has enticed significant attention as a promising matrix for bio-ink due to its biocompatibility, low cytotoxicity, and high water content which mimics the natural ECM.[48] Collagen is a major component of the ECM in the human body and is responsible for the provision of structural support to various tissues, such as cartilage.[49] Collagen also contains specific peptide sequences, such as RGD (Arg-Gly-Asp), which are known to play a critical role in cell adhesion and cell migration.[50] Therefore, the combination of Alginate and Collagen prepares cell-binding motifs and promotes biological properties.[51,52]

In addition, Collagen crosslinking is a process that occurs naturally in the body and involves the formation of chemical bonds between Collagen molecules. This process helps to

increase the strength and stability of tissues that contain Collagen, such as cartilage. Collagen crosslinking enhances tensile strength and viscoelastic properties, making it a suitable biomaterial for the 3D bioprinting method.[53-55]

Overall, 3D bioprinting of Alginate or Collagen loaded with chondrogenic biomolecules has the potential to create highly customized scaffolds for CTE, with applications in both research and clinical trials. Hyeong Jin Lee et al. improved a scaffold based on Collagen/ECM and Alginate for 3D bioprinting of porous cell blocks. The in vitro studies of the scaffold, which were loaded with pre-osteoblasts and human adipose stem cells, have revealed that the cells within the bio-inks remained viable.[56] Therefore, 3D-bioprinted Alginate/Collagen is suitable for TE application.

Xingchen Yang et al. developed 3D bioprinting inks by mixing Collagen type I or agarose with sodium Alginate and incorporating chondrocytes to construct in vitro 3D-printed cartilage tissue.[52] Their findings showed that the composites of Alginate/Agarose and Alginate/Collagen improved the mechanical properties with a significantly lower swelling ratio and water content, depending on the characteristics of the base polymer. Furthermore, these additives had little impact on the gelling behavior of the bio-inks, demonstrating their suitability to be used as bio-inks in 3D bioprinting methods.[52]

3D-printed Alginate/Gelatin

Gelatin is a commonly used biocompatible and biodegradable biomaterial in TE. Additionally, the mechanical strength of Gelatin can be tailored by adjusting the degree of cross-linking, enabling its use in various TE applications.[57] Gelatin and Alginate have been extensively studied as bio-inks due to their biocompatibility and structural properties similarities to the native ECM.[58,59] In addition, methacrylated Gelatin (GelMA) is a widely used material that overcomes the limitation of reversible thermal cross-linking by allowing covalent cross-linking in the presence of ultraviolet light.[60]

Stiffness of GelMA can be regulated by adjusting the degree of MA during the fabrication process. Owing to its ability to support cell proliferation, migration, and ECM production, GelMA is widely used in TE applications. Moreover, GelMA has shown promise as a bio-ink for CTE due to its ability to promote the growth and differentiation of chondrocytes, which are the cells responsible for the production of cartilage tissue. In addition, GelMA has been shown to promote a fibrochondrogenic phenotype, by the production of Collagen type I and Collagen type II, which are important structural proteins in cartilage tissue. Therefore, GelMA scaffold is able to support the formation of cartilage tissue with improved mechanical properties, particularly in load-bearing applications. Overall, GelMA is considered a promising biomaterial for CTE.[61]

Bin Wang et al. developed an Alginate-GelMA-based bioink to fabricate IPN scaffold akin to the structure of cartilage using 3D bioprinting.[62] Based on the results of their study, the composition of Alginate sulfate and GelMA did not significantly affect the viscosity, shear-thinning, and thixotropic properties of the bio-ink. Stiffness of the 3D-bioprinted IPN scaffold was found to be significantly higher than that achieved by 3D bioprinting Alginate or GelMA alone. Additionally, this IPN scaffold exhibited maintained resilience and toughness, indicating its potential for use in medical applications, such as TE.

Additionally, the sulfated IPN bio-ink, containing Alginate sulfate, has a high affinity for heparin-binding growth factors. This facilitated the consistent and prolonged release of TGF-β3. The TGF-β signaling pathway is a crucial regulator of various cellular behaviors, such as cell differentiation, proliferation, and ECM metabolism. This pathway plays a key role in many different cell types, including chondrocytes in cartilage tissue.[63] As a result, an environment conducive to robust chondrogenesis in vitro was established with minimal indication of hypertrophy or mineralization during extended culture periods.[62,64]

3D-printed Alginate/Hyaluronic Acid

Hyaluronic acid is a type of glycosaminoglycan that consists of N-Acetyl-D-glucosamine and D-glucuronic acid. It serves as a major content of the ECM in connective tissues, with high concentrations found in synovial fluids and glycosaminoglycans (GAGs) found in articular cartilage.[65,66] The binding of the surface receptor to Hyaluronic acid initiates several signaling pathways in chondrocytes, that enable chondrocytes to maintain their original phenotype.[67]

Therefore, Hyaluronic acid was conjugated to Alginate to create an artificial ECM environment for chondrocytes. Larsen et al. investigated whether this environment could stimulate the proliferation of chondrocytes while maintaining their original phenotype. Their findings indicated that the addition of Hyaluronic acid did promote chondrocyte proliferation while preserving their characteristic phenotype. This finding suggested that the use of Hyaluronic acid-conjugated Alginate might be a promising approach for TE applications aimed at the regeneration of cartilage.[68]

Oerther et al. reported that the addition of the polycation polyethyleneimine (PEI) to composite Alginate/Hyaluronic acid resulted in improved mechanical properties when crosslinked with calcium chloride. Furthermore, they found that the interaction between Hyaluronic acid and water significantly reduced the rate of weight loss of Alginate/Hyaluronic acid ink.[69,70] These scaffolds were designed not only to provide structural support but also to perform as vehicles for delivering growth factors to the site of tissue for repair. Incorporation of PEI into Alginate/Hyaluronic acid scaffolds may enhance the entrapment and extend the delivery of these GF, resulting in a more efficient delivery system.

3D-printed Alginate/Cellulose

Cellulose is a naturally occurring polysaccharide composed of repeating units of glucose, making it a homo-polymer. Cellulose is biodegradable and its degradation is mainly

carried out by enzymes produced by microorganisms. Solubility of Cellulose in water is dependent on its chain length; accordingly, shorter chains of Cellulose are more soluble than longer chains.[71] Cellulose is a biocompatible, hydrophilic, and biodegradable biomaterial that has been extensively used for TE applications.[72] One of the properties of Cellulose that makes it suitable for TE includes high tensile strength and the ability to resist deformation under stress.[73,74]

Thomas Moller et al. fabricated a novel 3D-bioprinted scaffold composed of cell-laden nanofibrillated Cellulose/Alginate (NFC-A) and implanted it in a subcutaneous pocket. To ensure a homogeneous distribution of cells within the NFC-A bio-ink at a ratio of 1:11, a cell mixer was utilized. Different groups of printed scaffolds, such as human nasal chondrocytes (hNCs), and human bone marrow-derived mesenchymal stem cells (hBMSCs) were used. The scaffolds exhibited excellent mechanical properties and maintained their structural integrity.[75] An incremental rise in GAGs production and hNC proliferation was observed in this scaffold. The fact that hBMSCs stimulated hNC proliferation and matrix secretion in the co-culture group indicates the promising potential of the 3D bioprinting method for human cartilage tissue fabrication in regeneration surgery.

Summary and Future Prospective

The OA is a degenerative joint disease that affects the cartilage in joints, causing pain, stiffness, and decreased mobility. Therefore, CTE is a promising and innovative approach for the treatment of cartilage injuries as it involves the use of cells, biomaterials, and growth factors to create neo tissue that can repair or replace damaged cartilage. In CTE, chondrocytes and MSCs are mainly used for repairing the cartilage tissue. In order to promote the differentiation of stem cells into cartilage, the incorporation of specific signaling is essential.

A promising approach involves the utilization of biomolecules and growth factors (TGF, IGF, and BMP) within the signaling pathway for the differentiation of stem cells. The growth factor is a multifunctional cytokine that plays a critical role in the regulation of cell growth, differentiation, and ECM synthesis in many tissues, including cartilage. It has been shown to stimulate chondrogenesis and promote the production of ECM components, such as Collagen and GAGs in cartilage cells. However, these growth factors have a short lifetime of about a few minutes to hours in vivo. Functionality of this feature for cartilage repair decreases over time, which can also lead to high costs.

Recently a biomolecule compound, such as insulin, KGN, and Vitamin C, has been used to induce selective differentiation of MSCs into chondrocytes. These molecules play a significant role in the regeneration of cartilage tissue; however, it is inefficient to inject it in vivo as it can be easily cleaned up. For 3D scaffold design, natural polymers have the advantage of being manufactured with biomechanical and biological properties. Among various methods, 3D bioprinting is an innovative process for CTE that enables the fabrication of complex structures with high resolution and reproducibility.

Alginate is an attractive biomaterial for 3D bioprinting as it can be easily cross linked into a scaffold under mild conditions and has good biocompatibility. The 3D-bioprinted scaffolds based on Alginate can support the growth and differentiation of chondrocytes as well as the cells responsible for cartilage production. Alginate scaffolds have also been shown to promote the formation of cartilage-like tissue both in vitro and in vivo. In order to have mechanical and biological properties, the 3D-bioprinted Alginate-based scaffolds could be conjugated with natural biopolymers, such as chitosan, Collagen, Gelatin, Hyaluronic acid, and Cellulose.

To develop the mechanical properties of Alginate scaffolds, natural polymers can be incorporated into the matrix. These 3D-bioprinted scaffolds based on Alginate with natural polymer loaded with inducing differentiation factors have been shown to enhance cell adhesion, proliferation, differentiation, and the mechanical strength of the scaffold. Among these scaffolds, the 3D-bioprinted Alginate/chitosan scaffolds promote biocompatible, rheological, and mechanical properties that are not observed in pure scaffolds based on Alginate. When Alginate and chitosan are combined, they form a 3D-bioprinted scaffold that takes advantage of the beneficial properties of both biomaterials. Overall, the incorporation of chitosan on 3D-bioprinted Alginate-based scaffolds loaded with biomolecules offers a promising strategy for the enhancement of the performance of CTE.

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Abbreviations:

List of abbreviations			
Abbreviation	**Full form**	**Abbreviation**	**Full form**
OA	Osteoarthritis	GF	Growth factors
CTE	Cartilage tissue engineering	hASCs	Human adipose stem cells
TE	Tissue engineering	IPN	Interpenetrating network
3D	Three-dimensional	GelMA	Gelatin-methacryloyl
ECM	Extracellular matrix	UV	Ultraviolet
KGN	Kartogenin	GAGs	Glycosaminoglycans
TGF	Transforming growth factor	PEI	Polyethyleneimine
BMP	Bone morphogenetic proteins	NFC-A	Nanofibrillated Cellulose/Alginate
IGF	Insulin-like growth factors	hNCs	Human nasal chondrocytes
FGF	Fibroblast growth factors	hBMSCs	Human bone marrow-derived mesenchymal stem cells
IPN	Interpenetrating polymer network	PRP	Platelet-rich plasma
MSC	Mesenchymal stem cells	PLA	Polylactic acid
RGD	Arg-Gly-Asp	HA	Hyaluronic acid
PIC	Polyion complex		