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Advances of Chitosan-based Hydrogel Scaffolds for Cartilage Tissue Engineering: Preparation, Modification, and Future Perspective

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Abstract

A cutting-edge field of study to create biological substitutes to maintain, restore, or enhance cartilage function is articular cartilage (AC) tissue engineering. Employing this innovative approach, polymer composites that mimic the structure and function of real cartilage tissues are created by carefully mixing biomaterials of scaffolds, cells, and biochemical components. Naturally polymers have recently gained attention as biomaterials to fabricate scaffolds, for instance, chitosan. Despite its shortcomings such as poor mechanical strength and low stiffness, chitosan-based hydrogel scaffolds have been cross-linked with other synthetic polymers, namely hybrid scaffolds, for future perspective of AC tissue engineering. To mimic AC compartment design, additive manufacturing closely resembles native tissue, and its flexibility is possibly tailored.

Keywords: Natural polymer, Composite, Biodegradable, Hybrid scaffold, Tunable, Regenerative medicine

Introduction

Human cartilage is a crucial tissue to support and move the joints its major structures are matrices, fibers, and chondrocytes. It surrounds articulating bones due to its property of high resiliency and deformability to protect from compressive joint load [1]. It has the main role of allowing joints to move smoothly with minimum friction and helps them handle the pressure while protecting the underlying bone. Therefore, articular cartilage (AC) would constantly face physical strain, making it prone to wear, tears, and sports-related damage [2]. On the other hand, AC possesses restricted regenerative capabilities because of its avascular and lymphatic-deficient nature [3,4]. Consequently, stem cells from blood and bone marrow are unable to infiltrate the damaged area to facilitate the repair of AC defects. Therefore, resident chondrocytes fail to migrate to the injury site, leading to the absence of a provisional extracellular matrix and the defect persists permanently and remains unrepaired [1].

Present strategies for regenerating AC, including microfracture, cell-based treatments like autologous chondrocyte implantation (ACI) and ACI combined with biomaterials (MACI), osteoarticular autografts, allograft, and surgical stimulation, are the most common clinical therapies to enhance intrinsic repair potential [5-7]. However, they have several drawbacks such as relapse possibility, poor effect on elder patients, long recovery time, cell and limited graft, immune rejection possibility, biomechanical problem,

and pathogen rejection [8-12]. Therefore, the development and breakthrough of regenerative medicine and tissue engineering are urgently needed to overcome those drawbacks.

Articular cartilage tissue engineering represents an innovative area of research dedicated to creating biological replacements to repair, uphold, or improve the performance of cartilage [13]. This novel method entails strategically combining biomaterials, scaffolds, cells, and biochemical elements to construct materials that replicate the form and role of natural cartilage tissues [14-16]. In cartilage tissue engineering, the scaffold plays a pivotal role as a structural framework crucial for supporting cell attachment, proliferation, and differentiation [17,18]. There are abundant efforts to mimic the biological extracellular matrix of native AC and to optimize the success of AC tissue engineering. Scaffolds must possess specific physical properties, including suitable porosity, mechanical resilience, adhesiveness, and degradability, which are essential for promoting cell proliferation and migration, facilitating cell adhesion, and ultimately enhancing the efficacy of the tissue regeneration process [19-21]. Natural polymers have been explored as scaffolds in tissue engineering as they mimic extracellular matrix. One of the most natural polymers used for scaffold fabrication in AC tissue engineering is chitosan [22,23]. Chitosan's positive charge facilitates interaction with negatively charged glycosaminoglycans (GAGs) in the cartilage matrix, promoting cell adhesion and proliferation. Its porous structure allows for nutrient and waste exchange, crucial for tissue regeneration [24-27].

Chitosan, a natural polymer derived from chitin, is found abundantly in the exoskeletons of Crustaceans such as shrimp, crab, and lobster [24,25,28]. Additionally, it is also present in the cell walls of certain fungi, the exoskeletons of insects and mollusks, as well as in the scales and fish bones [29]. Chitosan exhibits closely structural similarities to GAGs found in the cartilage extracellular matrix (ECM). This resemblance makes chitosan a promising candidate for cartilage tissue engineering due to its advantages in biocompatibility, biodegradability, biomimetic, and controlled release of growth factors [24-29]. The extraction of chitosan involves the deacetylation of chitin, a process that removes acetyl groups from the chitin molecule, resulting in chitosan [30]. This extraction process can be achieved through chemical or biological methods, with each approach offering specific advantages in terms of yield, purity, and environmental impact [31-33]. Chitosan has several unique characteristics that gained attention in AC tissue engineering. Overall, chitosan is a straightforward yet effective option for repairing damaged cartilage.

Chitosan-based hydrogel scaffolds have widespread interest as a candidate for AC tissue engineering [34,35]. However, chitosan hydrogel scaffolds have their shortcomings such as poor mechanical strength and low stiffness [35-37]. Therefore, some studies have modified chitosan-based hydrogel scaffolds into a hybrid scaffold by combining chitosan with another synthetic polymer to improve the mechanical strength of the scaffold. The hybrid chitosan-based hydrogel scaffolds are possibly engineered by physical cross-linking, such as the freeze-thawing method, and chemical cross-linking with the help of cross-linkers or chemical cross-linking *via* photopolymerization [38]. Previous studies have fabricated chitosan- polyvinyl alcohol (PVA) hydrogel scaffold, chitosan- poly (lactic acid) (PLA) hydrogel scaffold, and chitosan-Poly(N-isopropyl acrylamide) (PNIPAAm) hydrogel scaffold, modifying chitosan-based hydrogels become promising and considered as a scaffold for AC tissue engineering [34,39,40]. PLA-chitosan scaffolds have shown mechanical property improvement, but it is still not excellent for promoting cell adhesion, proliferation, and migration [36]. In addition, a combination of chitosan and PNIPAAm performs thermoresponsive characteristics which enhance ease of application and therapeutic success; hence this combination has the potential to be developed for future perspective of AC tissue engineering [34,41-43].

A challenge in scaffold design is the replication of the osteochondral compartment. To address this, researchers have developed and assessed gradients or composite multilayer scaffolds that closely resemble

osteochondral tissue, often in combination with cells [18,44]. Various strategies, such as the integration of multiple layers, the establishment of gradients, and the incorporation of elements like minerals, growth factors, and cells, have been employed to generate a multiphasic scaffold [44]. Advances in mechanobiology and bioreactor design are expected to play a crucial role in shaping the future of cartilage tissue engineering. Innovative technologies, including additive manufacturing or 3-dimensional (3D) printing, hold promise for further advancements. Chitosan and partially synthetic polymer materials can be transformed into hydrogel inks with properties comparable to biological tissues, offering the flexibility to tailor mechanical characteristics and create stable 3D structures [45,46]. However, challenges persist in achieving well-defined structures, along with adequate mechanical strength and biological properties for effective tissue regeneration, as observed in 3D printing investigations of chitosan-based biopolymers [47]. *In situ*, 3D bioprinting, driven by mechanical and biochemical signals, emerges as a promising avenue to create functional tissues at anatomically relevant scales within a bioreactor setting. The continuous progress in these technologies is poised to significantly impact the landscape of cartilage tissue engineering in the coming years.

Chitosan

Structure of chitosan

Chitosan, a polymer derived from chitin, consists of varying proportions of *N*-acetyl-D-glucose amine and D-glucose amine interconnected through β-(1–4) glycosidic linkages [25,48]. Chitosan can be produced by partial deacetylation of chitin; thus, the degree of deacetylation of chitosan is commonly expressed as the proportion of *N*-glucosamine units within its molecular structure and generally falls within the range of 50 - 95 % [49,50]. Chitosan differs from chitin by the presence of amino groups in place of the acetamido group at position C-2 (**Figure 1**). Chitosan has 3 types of reactive groups, namely primary amino group, primary and secondary hydroxyl groups located at C2, C6, and C3, respectively (**Figure 1**) [51]. The variations in chitosan structures and physicochemical properties are primarily influenced by the amino contents. The distribution of amino groups is random, facilitating the formation of both intra- and inter-molecular hydrogen bonds [52].

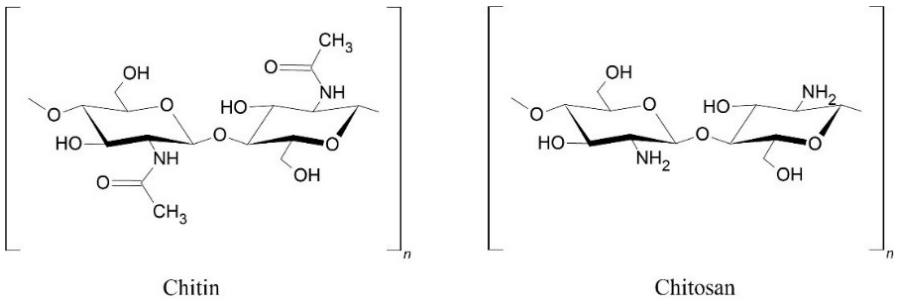

Figure 1 Chemical structure of chitin and chitosan.

Additionally, the N-acetyl glucosamine group of chitosan structurally resembles the glycosaminoglycans (GAGs) found in the extracellular matrix (ECM) of cartilage. Consequently, chitosan's application in cartilage regeneration holds significant promise for cartilage tissue engineering due to its biocompatibility, biomimetic properties, and ability to regulate growth factor release [26,27]. The ionizable groups, such as sulfates and carboxylates on hexuronic acids, bestow polyionic characteristics upon GAGs, which are crucial for functions like water retention, cell adhesion, ion flux regulation, and neuronal

signaling. Leveraging its polycationic nature, chitosan enables cell adhesion and fosters electrostatic interactions with anionic GAGs [53,54].

Physicochemical properties of chitosan

The physicochemical properties of chitosan relevant to articulate cartilage engineering include its biocompatibility, biodegradability, and ability to form hydrogels. Chitosan's positive charge facilitates interaction with negatively charged GAGs in the cartilage matrix, promoting cell adhesion and proliferation [24-27]. Its porous structure allows for nutrient and waste exchange, crucial for tissue regeneration. Chitosan's tunable mechanical properties and ability to mimic the ECM make it an excellent scaffold material for cartilage tissue engineering. Additionally, its thermoresponsive behavior can allow for the controlled release of bioactive molecules for enhanced regeneration [34,41-43].

Solubility

Chitosan is soluble in acidic solvents but remains insoluble in neutral or alkaline solutions [25,29,50]. In contrast to chitin, which is generally insoluble, the deacetylation process converts chitin into soluble chitosan due to the high presence of protonated -NH_2 groups [25]. This makes chitosan soluble in acidic aqueous environments, with a pKa value of around 6.5. As a result, chitosan forms water-soluble salts in both organic and inorganic acids [50]. When chitosan dissolves, the free amine groups become protonated, acquiring a positive charge and enhancing solubility in acidic solvents. However, at a pH of 6 or higher, these amine groups lose their positive charge, causing chitosan to become insoluble [25,50]. In addition to pH, chitosan's solubility is influenced by factors such as polymer molecular weight, temperature, degree of deacetylation, and polymer crystallinity [51].

Degree of deacetylation

The degree of deacetylation (DD) in chitosan is a vital parameter that determines many of the physicochemical and biological properties of this biopolymer. It is typically represented as the percentage of N-glucosamine units within the molecular structure of chitosan [50]. Additionally, the degree of deacetylation specifies the number of free amino groups in the chitosan macromolecule, which in turn influences the polymer's functionality, polarity, and water solubility [55]. The degree of deacetylation of chitosan can be described by the following equation [25]:

$$DD = \frac{n(GlcN)}{n(GlcN)+n(GlcNaC)}\ x\ 100\% \quad (1)$$

where DD is the degree of deacetylation, n(GlcN) is the average number of N-glucosamine units; n(GlcNAc) is the average number of N-acetylglucosamine units.

The DD of chitosan is crucial in AC tissue engineering applications. The degree of deacetylation (DD) of chitosan is crucial in defining its biocompatibility, mechanical properties, degradation rate, and its capacity to facilitate cell adhesion and proliferation. These factors are essential in articular cartilage (AC) tissue engineering applications [29]. Optimization studies help determine the most suitable DD range for a particular tissue engineering approach. The optimum DD for AC tissue engineering can vary depending on specific requirements and conditions, such as the type of cells used, the desired mechanical properties of the scaffold, and the intended application. However, generally, a DD range of 50 to 95 % is commonly considered suitable for AC tissue engineering [50].

There are categories based on the degree of deacetylation that have a substantial impact on both solubility and viscosity levels. A deacetylation degree ranging from 55 - 70 % is considered a low degree of chitosan deacetylation, which is practically non-soluble in water. A deacetylation degree of 70 - 85 % falls within the medium range, allowing partial dissolution in water. Furthermore, 85 - 95 % represents a high degree of chitosan deacetylation, ensuring good solubility in water. Lastly, a deacetylation degree of 95 - 100 % is termed ultrahigh deacetylation of chitosan, which is challenging to attain [56]. A deacetylation degree exceeding 50 % commonly indicates the successful transformation of chitin into chitosan [25,57].

Molecular weight

The molecular weight of chitosan constitutes a vital parameter impacting both the physicochemical and biological characteristics of this biopolymeric substance. This parameter determines the strength of the chitosan fiber or film and the viscosity of the chitosan solution [58,59]. Chitosan can be categorized into high (310 - 375 kDa), medium (190 - 310 kDa), or low (50 - 90 kDa) molecular weight based on its range of molecular weights [60,61]. Chitosan with lower molecular weight typically shows increased water solubility and less viscous properties when compared to its higher molecular weight counterpart [25,62]. In AC engineering, the choice between high molecular weight (HMW) and low molecular weight (LMW) chitosan depends on the specific requirements of the application. For instance, if the goal is to mimic the native cartilage matrix in load-bearing regions, HMW chitosan may be preferred for its mechanical properties [61]. Conversely, in applications focusing on promoting cell infiltration and matrix deposition in less load-bearing areas, LMW chitosan could be more suitable [25,62]. Optimization studies can help determine the most appropriate molecular weight range to achieve the best properties for AC engineering tissue applications.

Viscosity

Another parameter of chitosan is viscosity, which is a significant concern from a technological perspective, as solutions with high viscosity pose challenges in terms of handling and management. The viscosity of chitosan is affected by the degree of deacetylation and the molecular weight of the chitosan [25]. Indeed, viscosity serves as an indicator for assessing the stability of the polymer in a solution, showing a decrease as the polymer undergoes degradation during storage [63]. The viscosity of chitosan solutions plays a significant role in determining the stability of AC engineering tissue properties by influencing scaffold formation, cell distribution, mechanical support, retention of bioactive molecules, and degradation kinetics [42,44,47]. Chitosan solutions with higher viscosity tend to form more stable scaffolds during fabrication processes such as gelation or freeze-drying with excellent mechanical properties [61,63]. Thus, it preserves bioactive molecules such as growth factors or signaling molecules within the scaffold matrix. Moreover, higher viscous chitosan solution may result in scaffolds with slower degradation kinetics [56]. Overall, the optimization study of chitosan solution helps to achieve optimum scaffold for AC tissue engineering.

Biological properties of chitosan

Biodegradability

Chitosan has gained attention in tissue engineering, including applications in cartilage regeneration. Its biodegradability is a key feature contributing to its suitability for such purposes. Studies have explored the biodegradation of chitosan and its potential in cartilage tissue engineering [27,64-66]. Chitosan undergoes enzymatic degradation within the body through the action of lysozyme, an enzyme found in the extracellular matrix of human bone tissue. Lysozyme hydrolyzes the chitosan chain, cleaving the glycosidic

bonds between polysaccharide units within the polymer [67]. This process leads to a reduction in the molecular weight of the polymer, ultimately resulting in solubility and the removal of degradation products. The by-products of degradation, primarily composed of glucosamine and saccharide, are non-toxic and can be easily extracted from the body without causing interference with organs [68]. Chitosan degradation is inversely related to the molecular weight and the DD. A higher molecular weight of chitosan is associated with a reduced degradation rate, while an increased degree of deacetylation (DD) corresponds to elevated polymer crystallinity and, consequently, slower degradation rates [50,69]. However, the correlation between DD, polymer crystallinity, and degradation rate is more complex depending on various factors [69]. A study suggested that chitosan-based scaffolds provide a favorable environment for cell attachment, proliferation, and differentiation, promoting cartilage regeneration. The controlled biodegradability of chitosan allows for the gradual release of bioactive molecules, which play an important role in tissue regeneration [70]. It is essential to consider the balance between the degradation rate of the chitosan scaffold and the rate of tissue formation. Overall, the biodegradability of chitosan in cartilage tissue engineering appears promising, with ongoing studies focusing on optimizing its properties for enhancing tissue regeneration outcomes.

Biocompatibility

The biocompatibility of biomaterials is one of the most crucial considerations in cartilage tissue engineering. This is attributed to the composition of chitosan, which consists of glucosamine (GlcN) and N-acetylglucosamine (GlcNAc), natural constituents found in mammalian tissues [71]. Some studies supported the biocompatibility of chitosan-based systems using chondrocyte cells. The results have shown that chitosan supports high cell viability and proliferation, demonstrating compatibility and having no cytotoxic effects [72]. The influence of chitosan characteristics on biocompatibility is a key aspect in understanding its applicability in various biomedical applications. Previous studies suggested there is an optimal range for deacetylation, striking a balance between biocompatibility and mechanical properties [73]. These findings contribute to the comprehensive understanding of how chitosan characteristics affect its biocompatibility, crucial information for its successful utilization in biomedical and tissue engineering endeavors.

Source of chitosan and its extraction

Chitin and chitosan are extracted from shells of Crustaceans like crabs, shrimps, and lobsters, as well as the exoskeletons of insects and mollusks. Additionally, it is derived from the cell walls of certain fungi and fish scales [24,25]. Crustaceans contain a chitin content ranging from 15 to 40 %, embedded in a matrix comprising proteins (20 - 40 %), calcium carbonate, and calcium phosphate (30 - 50 %) [32,74,75]. Insects exhibit a chitin content of approximately 10 - 25 %, with a matrix composed of proteins (30 - 60 %) and lipids (25 - 40 %) [33,76,77]. Thus, Mollusks (for example squid, octopus, cuttlefish, clams, oysters, and snails) contain about 23 % chitosan, embedded in a matrix rich in proteins and calcium carbonate [78,79]. Thus, shrimp shells consist of 3 primary constituents: α-chitin (15 - 40 %), proteins (20 - 40 %), and $CaCO_3$ (20 - 50 %). Additionally, there are minor components such as pigments and other metal salts present [31]. Moreover, fungi with chitin concentrations of 2 - 44 %, feature a matrix comprising glucans and proteins [78]. Additionally, fishery by-products contribute chitin at levels ranging from 15 - 30 %, often accompanied by proteins and minerals [80]. Notably, chitosan derived from Crustaceans, particularly shrimp and crab shell, stands out as a promising and extensively researched biomaterial in the biomedical field. Its abundance and unique properties, including biocompatibility and antimicrobial activity, have led

to widespread investigation for applications in drug delivery systems, wound healing, and tissue engineering [81].

Chitin and chitosan are commonly extracted using both chemical and biological methods. Chemical methods involve the use of strong acids or alkalis to dissolve minerals and proteins, followed by deacetylation to convert chitin into chitosan [76,82]. Chitosan's solubility and viscosity characteristics are mainly influenced by the amino groups (-NH2) formed during the deacetylation process. These amino groups give chitosan molecules a positive charge in acidic solutions, making them soluble [82]. The solubility of chitosan allows for the formation of solutions with varying viscosities, depending on factors such as concentration and molecular weight. On the other hand, biological methods utilize enzymes, such as proteases for deproteinization, to break down the complex structure of chitin into chitosan [83]. Before extraction, selecting the right shells is crucial for ensuring high-quality chitin. This involves choosing shells of the same size and species, particularly from crustaceans like lobsters and crabs. Once selected, the shells are cleaned, dried, and ground into small pieces, preparing them for the chitin extraction process. This careful preparation is vital to yield chitin with the desired purity for diverse applications [84].

Chemical extraction of chitosan

The chemical extraction method offers efficiency and scalability in obtaining chitosan, making it suitable for large-scale industrial applications [85]. There are 3 primary stages in chemical methods: deproteinization, demineralization, and deacetylation [86]. In the study on the chemical extraction of chitosan from mangrove crab shells (*Scylla serrata*) conducted by Setiawati and colleagues [87], the initial stage involves deproteination to remove proteins from the sample. Deproteination involves breaking the chemical bonds between proteins and chitin through alkaline treatment which makes it a crucial step in purifying polysaccharides [88]. Subsequently, demineralization occurs to eliminate minerals from the deproteinated shell powder. Demineralization plays an important role in the extraction of chitosan from Crustaceans due to their elevated mineral content compared to fungi and insects [89]. In demineralization, the deproteinated powder was treated in an acidic condition. The final step, deacetylation, transforms chitin into chitosan under alkaline conditions. During deacetylation, chitin undergoes structural modification, resulting in the formation of chitosan. This conversion is essential as it removes acetyl groups from the chitin molecules, altering their properties and rendering them soluble in acidic solutions [90]. While chemical methods are effective, they often require harsh conditions and may produce chemical waste [91]. In the case of shrimp shells, demineralization with acid treatment is often conducted before deproteination. Subsequently, unlike the chitosan extraction process from crab shells, decoloration or bleaching is commonly carried out to remove undesired pigments such as melanin and carotenoids from shrimp shells using hypochlorite, hydrogen peroxide, or oxidizing agents [31]. After decoloration, deacetylation is conducted to produce chitosan with 80 % degree of deacetylation [57]. A study by William and Wids demonstrated that better quality chitosan with a higher percentage of deacetylation was yielded when the extraction process began with deproteination. However, lower-quality chitosan was produced if the extraction process started with demineralization [86]

Biological extraction

In the extraction process using chemicals, strong chemical substances interact with the biomass at high temperatures over an extended duration, leading to alterations in the physicochemical traits and functionality of chitosan [89]. Additionally, these chemicals pose significant environmental hazards [92]. Consequently, the biological extraction method is extensively adopted to address these concerns. The biological extraction methods improve the consistency of chitin production, providing a greener substitute

for the rigorous chemical methods [93]. In the biological method, 3 primary stages are conducted, like the chemical process: demineralization, deproteinization, and deacetylation [32]. Demineralization is the initial stage, wherein shells are treated to remove minerals, often using lactic acids-producing bacteria (for example: *Lactobacillus plantarum*) or lactic acid [94,95]. This process is critical to dissolve the inorganic components, primarily calcium carbonate, and prepare the shells for subsequent stages [96]. Subsequently, deproteinization is carried out by employing enzymatic or microbial methods. Proteolytic enzymes, including proteases like pepsin, papain, and trypsin break down proteins and separate them from the chitin-rich matrix of the crab shells [84,89]. Hamdi *et al.* [81] reported that utilizing crude digestive alkaline proteases derived from *Portunus segnis* effectively extracts chitin through the deproteinization process of both blue crabs (*P. segnis*) and shrimps (*P. kerathurus*). The final step, deacetylation, involves treating the chitin with an enzyme called chitin deacetylase which was initially discovered and partially purified from extracts of the fungus *Mucor rouxii*. This process removes acetyl groups, resulting in the transformation of chitin into chitosan [97]. After deacetylation, chitin undergoes a structural modification into chitosan, altering its properties and rendering it soluble in acidic solutions [90]. When dissolved, the unbound amino groups within chitosan become protonated, resulting in a positive charge and the creation of a soluble chitosan compound in acidic solutions. However, when the pH rises to 6 or higher, these amino groups lose their positive charge, causing chitosan to precipitate and become insoluble [25,50]. Aside from pH, various factors affect the solubility of chitosan, including the molecular weight of the polymer, degree of deacetylation, temperature, and polymer crystallinity [51]. Biological methods offer an environmentally friendly approach to chitosan extraction, maintaining the integrity of the natural material and facilitating its potential applications in various fields, including biomedicine and biotechnology [98,99]. Although biological methods are eco-friendly, they come with limitations, including extended processing times and the requirement for increased interventions to accommodate industrial-scale adaptation [100].

Cartilage tissue engineering

Articular cartilage composition

AC is an elastic and supportive tissue surrounded by hyaline cartilage on the joint surface, bearing in conjunction with adjacent bones to decrease friction during physical movement [4,46]. Before and during birth, articular cartilage (AC) is a dense tissue with many cells that are evenly distributed but have low amounts of the extracellular matrix (ECM) [101]. Only a small number of adult bones, such as the outer ear, the tip of the nose, the tip of the ribs, and so on, are cartilage. Hyaline, elastic, and fibrous cartilage are the 3 types of cartilage based on the composition of the intercellular matrix. Among them, hyaline cartilage, occasionally referred to as AC, is mostly found on the surfaces of bones and joints, as well as in the sternal end of ribs [4]. Unlike most other tissues, cartilage has a single cell type of specialized cell, the chondrocyte, and a large amount of specialized and plentiful extracellular matrix (ECM) secreted by themselves [102]. AC is mostly composed of water (70 - 80 %), collagen (12 - 14 %), proteoglycan (6 - 8 %), mineral (4 %), and matrix protein as presented in **Table 1**. Collagen, other proteins, proteoglycans, and water create a fibrous network [2]. Water is the most prominent component of AC, attributing up to 80 % of its wet weight. A minor portion of this water exists in the intracellular space, whereas most of it is linked to the intrafibrillar space within the collagen, making up roughly 30 % of it. In the deep zone, the relative water content drops from roughly 80 % at the superficial zone to 65 %. Water flows through chondrocytes and over the articular surface, supplying chondrocytes with nutrients and serving as a transport mechanism for those nutrients [103].

Table 1 General compositions of AC [104].

Components	Percentage
Chondrocytes	1-10
Water	70-80
Collagen	
Type II	10-12
Type IX	~1
Type XI	~1
Proteoglycans	
Hyaluronic acid-proteoglycan-aggregates	6-8
Other proteoglycans	~1
Mineral materials	< 4
Matrix proteins	< 1

Approximately 60 % of the dry weight of cartilage is composed of collagen, the most common structural macromolecule in extracellular matrix (ECM). 90 to 95 % of the collagen found in the extracellular matrix (ECM) is type II collagen, which combines with proteoglycan aggregates to create fibrils and fibers (**Table 1, Figure 2**). Although they are present, collagen types I, IV, V, VI, IX, and XI make up very little of the total. The type II collagen fibril network is formed and stabilized by the minor collagens [103]. Cartilages are distinguished tissue by harboring a substantial amount of glycosaminoglycans, especially chondroitin sulfate and keratan sulfate, linked to proteoglycan namely aggrecan (**Figure 2**) [105]. Within AC, proteoglycans constitute the second-largest group of macromolecules in the ECM, making up approximately 10 to 15 % of the cartilage's wet weight [103]. Aggrecan is a proteoglycan containing over 100 chondroitin sulfate and keratin sulfate chains. It is distinguished by its capacity to bind with hyaluronan (HA) and create substantial proteoglycan aggregates through link proteins [105,106]. Occupying the interfibrillar space within the cartilage extracellular matrix (ECM), aggrecan plays a crucial role in imparting osmotic properties to the cartilage, essential for its ability to withstand compressive loads, and increases hydraulic permeability [103,107]. Another essential proteoglycan is decorin, a small leucine-rich proteoglycan, which has a role in the structure and biomechanical function of the cartilage by elevating aggrecan retention in newly formed ECM of chondrocytes, rather than interfering with aggrecan biosynthesis [107]. It improves intermolecular binding between aggrecan and aggrecan molecules, as well as aggrecan molecules and collagen II fibrils (**Figure 2**) [107].

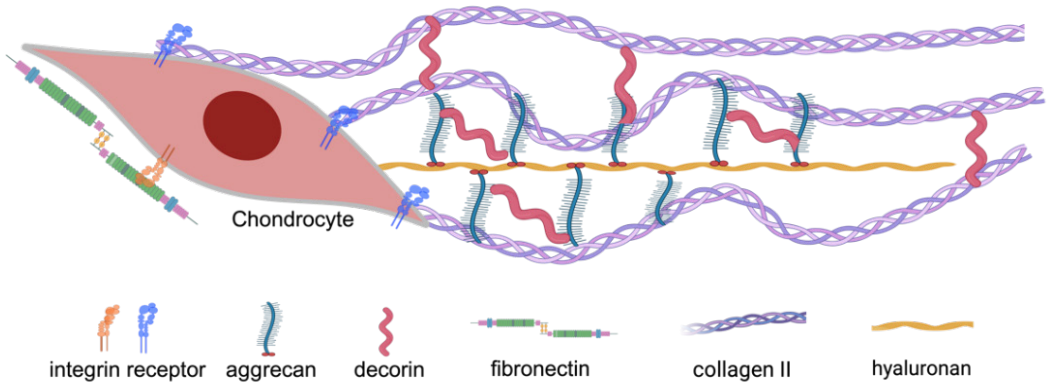

Figure 2 Extracellular Matrix Organization in AC Tissue (modified from Chen *et al*. [108]). This figure was created using BioRender.

AC structure

The direction of collagen fibers and the form of the chondrocytes define the 3 zones of AC [2]. Thus, chondrocytes are spread all over the adult human AC, but they make up only about 1 % of the whole tissue volume [102]. The outermost layer of the articular cartilage (AC), making up about 10-20% of its thickness, features collagen fibrils and chondrocytes aligned parallel to the joint surface. In this layer, the chondrocytes are flattened and elongated to preserve the integrity of the zone, which is crucial for protecting the deeper layers. This zone contributes to the tensile properties of the AC, allowing it to withstand the shearing forces exerted on the joint. The middle layer, or transitional zone, comprises 40-60% of the AC's thickness and is characterized by somewhat larger chondrocytes at a lower density and thicker collagen fibrils arranged obliquely. The first line of defense against the compressive stresses that the articulation imposes is the transitional zone. The collagen fibrils are organized perpendicular to the articulating surfaces and are thickest in the deep zone. Normally, the enormous chondrocytes are positioned in columns parallel to the collagen fibrils. The deep zone, which accounts for approximately 30% of the AC volume, provides the strongest protection against compressive pressures (**Figure 3**) [103].

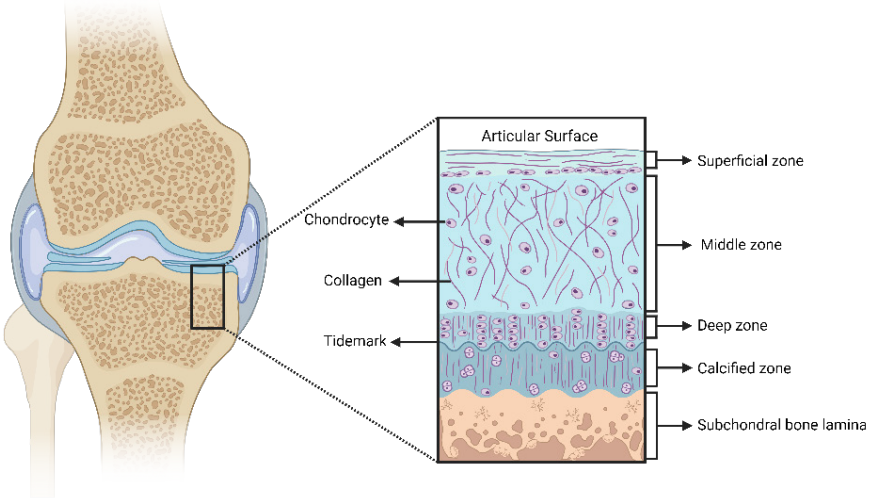

Figure 3 Structure of AC (modified from Fox *et al*. [103]). This figure was prepared using BioRender.

Articular cartilage regeneration

Articular cartilage (AC) has a limited regeneration capacity due to its avascular and lack of lymphatics [4]. Consequently, stem cells in blood and bone marrow cannot enter the injured region to heal the defect AC. Thus, resident chondrocytes do not migrate to the wound, and no provisional matrix is formed. Hence, the defect remains permanent and unrepaired [1]. Endogenous AC restoration is generally thought of as an internal natural process of restoring damaged AC to its original composition and capabilities. There is a significant relationship between cartilage repair and the healing of wounds. The initial phase of acute inflammation and cell apoptosis, the intermediate phase, and the remodeling and matrix formation phase are the 3 almost overlapping phases of the healing process [109]. AC regeneration involves stem cells which are important for self-renewal of injured tissue [110]. Osteochondral cartilage defect causes blood vessel rupture and ingress into bone marrow, causing hematoma. The fibrin network in hematoma traps platelets, which secrete platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β that attract mesenchymal stem cells (MSC) [1]. There are several stages: first, molecular signals recruit and transform naïve MSC into an active proliferation state during AC trauma. Second, MSCs undergo directed chondrogenic differentiation, and the MSCs maintain microenvironment homeostasis and support chondrocytes. Thus, mature chondrocytes bind to the ECM network and reciprocally interact to be functional new cartilage tissue [110].

Current treatment strategies

Once an AC finishes growing around age 20, it remains unchanged. Unlike any other tissues in our body, the cartilage that was built during childhood will stay the same throughout our lives. The problem is a mature AC lacks a direct supply of circulating stem cells and nutrients; thus it relies only on synovial fluid to get its nutrients [111]. On top of that, it has very limited cells and low metabolic activity making it barely capable of regenerating itself. By this, a deep understanding of the tissue's structure and composition will pave the way for tissue engineering strategies that usually aim to replicate the complex chemical and physical landscape of the target tissue's ECM [6]. There are 4 treatment strategies for AC deflects that are employed globally: microfracture, autologous chondrocyte implantation (ACI), matrix-induced autologous chondrocyte implantation (MACI), and autologous matrix-induced chondrogenesis (AMIC). Microfracture is the most popular surgical procedure for treating chondral abnormalities in the knee, which produces fibrocartilage repair by stimulating the bone marrow [112]. Microfracture adheres to the concept of stimulating bone marrow by fabricating perforations in the subchondral bone plate, the natural repair processes are triggered. This action induces medullary bleeding, transporting proteins and pluripotent cells to the cartilage defect. Consequently, a series of physiological cell differentiations ensue, forming a clearly defined clot over the damaged area [113,114].

In addition to microfracture, there is an alternative method for the repair of AC called autologous chondrocyte implantation (ACI). ACI technique was introduced in 1987 as an improvement on microfracture. This technique is a 2-step surgical procedure that repairs cartilage using our cells [115]. In the first step, autologous chondrocytes will be collected using an arthroscopic technique from a low-stress area of the joint and these cells will be grown *in vitro* to obtain 12 - 48 million cells. In the second surgery, the damaged area is debrided, and the cell suspension is placed and confined to the defect by a periosteal patch [116]. This technique shows positive results with hyaline-like tissue formation early on and because it uses the patient's cells, it could potentially lead to better cartilage formation. However, the new tissue is not always identical to healthy cartilage, possibly due to cell changes during the *in vivo* culturing stage and it requires a long recovery period for the new tissue to form fully [117]. In this technique, patients also suffer from more pain since it requires 2 major surgeries and the patches that were placed could cause

overgrowth and pain to the patients. In the US, these patches might come from animal sources that potentially could cause immune reactions [118].

A derivative of the ACI technique is matrix-induced MACI, which is a tissue engineering approach that uses a 3D scaffold to assist cartilage repair. It starts with harvesting and expanding our cartilage cells, but the cells are seeded onto a collagen or hyaluronic acid scaffold creating a 3D environment for growth. This scaffold is implanted into the defect, and it helps cells to attach, spread, and multiply, which guides a new formation of the cartilage matrix [6]. There are many benefits of using scaffold-based treatment like an improved graft fit and stability in the defect, reduced risk of cells losing their cartilage-forming ability due to the 3D environment, and less fibrocartilage formation. However, like ACI, it still requires 2 surgeries and a long recovery period, this technique also has not shown to be superior to other techniques yet [119].

Alternatively, AMIC could be an option to repair chondral and subchondral defects. Unlike ACI and MACI, this single-step procedure avoids harvesting and culturing cells *in vitro*, therefore it only requires one surgery, and no donor site is used, reducing any potential issues [120]. After removing damaged cartilage and bone, tiny holes will be drilled in the underlying bone to trigger a blood clot rich in stem cells. A biocompatible membrane, usually composed of collagen I/III derived from porcine sources, would cover the area to create an optimal environment for regenerating and forming hyaline-like cartilage [121]. Research indicates that using an AMIC technique with a collagen membrane enhances joint function and stability over a 5-year period compared to microfracture alone, but some patients still develop a less ideal fibrocartilage instead of hyaline cartilage [6]. Finally, there is bone marrow aspirate concentrate (BMAC) which is a concentrated mix that is rich in hematopoietic stem cells, bone marrow-derived MSCs, platelets, and growth factors harvested from bone marrow. This concentrated mix would either be injected directly into the damaged area or used with other techniques like microfracture or scaffolds, the concentrated mix would attract new cells and blood vessels, encouraging cartilage and bone healing [122]. Clinical trials have shown that this procedure is safe, minimally invasive, does not require additional surgery, and is applicable alone or combined with other techniques for an extra boost. Nevertheless, there is still no one-size-fits-all approach since the quality of the concentration would vary between individuals, affecting the outcome. Besides that, the new cartilage that was created is still not as strong as the original cartilage [6].

Despite advancements in cartilage repair techniques, most successes fade after 5 years and full restoration of both cartilage and underlying bone remains elusive, leaving patients and healthcare systems facing limitations. Accordingly, new approaches in tissue engineering are aiming for faster weight-bearing and a neo-tissue that seamlessly integrates and matures into healthy and functional cartilage. These new approaches are required to improve the patient's quality of life and reduce the economic burden on healthcare costs. In conclusion, the recent advances in biotechnology, nanotechnology, and nanomaterials will be a promising future for regenerative medicine of AC [123].

Cell based-tissue engineering for cartilage repair

Tissue engineering is a cutting-edge field that focuses on the development of biological substitutes to restore, maintain, or enhance tissue function [13]. This innovative approach involves the strategic combination of biomaterials, cells, and biochemical factors to create constructs that mimic the structure and function of native tissues [14,15]. The properties of tissue-engineered constructs aim to replicate the natural microenvironment, fostering cell growth, differentiation, and ultimately, the formation of functional tissues [124,125]. Precision in designing these constructs allows for tailored solutions, addressing specific tissue needs [126]. Tissue engineering holds great promise in revolutionizing regenerative medicine by offering solutions for damaged or degenerated tissues, potentially providing new therapeutic avenues for various medical conditions. As the AC is an avascular and aneural tissue, AC of healing has a low tendency to self-

heal and hardly transfer nutrients to the cells, thus they are incapable of healing naturally. The inability to repair itself leads to a gradual erosion of the cartilage due to cartilage damage, fracture, or injury [34,127]. There are several clinical treatments available for repairing AC. However, these treatments have some limitations and show poor long-term regeneration quality.

Cell-based tissue engineering techniques are used to repair cartilage by creating tissue that closely mimics natural cartilage. This involves using a scaffold or matrix for support, adding cells to promote tissue growth, and using chemical or physical signals to promote cartilage or bone development [110, 117,122,128]. The implanted constructs must be porous to allow for nutrient flow and waste removal, promote mature tissue organization, have the correct biochemical composition, and seamlessly integrate with surrounding tissue for smooth joint movement and load distribution [26,67,116].

Scaffold for cell based-tissue engineering for cartilage regeneration

The scaffold that is used for cartilage tissue replacement must provide a proper environment by mimicking the extracellular matrix for cell adherence, proliferation, and migration. The scaffold must be designed to be biocompatible and biomimetics to support chondrogenesis by facilitating cell adhesion, proliferation, and extracellular matrix (ECM) production. Moreover, the scaffold must be mechanically strong and resistant to an applied bearing load. Thus, the scaffold degradation must match the tissue formation to ensure the function. Therefore, biodegradable polymers are preferable for scaffold synthesis due to their degradability and similarity to native ECM. Natural biodegradable polymers such as protein (collagen, keratin, gelatin, elastin, silk fibroin), glycosaminoglycan (hyaluronic acid), and polysaccharides (chitin, chitosan, alginate, gellan gum) [128].

Nowadays, 3D scaffolds are more widely used for tissue engineering rather than 2D scaffolds because of their effectiveness. The 3D scaffolds prevent the differentiation of the chondrocytes into fibroblast-like cells and allow chondrocytes to generate protein to form the native cartilage [34]. A 3-dimensional (3D) scaffold mimics the natural extracellular matrix (ECM) environment, providing spatial cues for cell growth and interaction. On the other hand, 2D scaffolds are typically flat surfaces where cells adhere and grow in a monolayer. 3-dimensional (3D) cultured cells exhibit more pertinent behaviors and conditions compared to the 2D cell model. These include enhanced occurrences of cell adhesion, migration, mechanical properties, proliferation, differentiation, and reactions to signaling molecules [129].

Numerous techniques exist for scaffold fabrication, such as 3D printing, hydrogels, supercritical fluid technology, electrospinning, and weaving offering the advantage of precise cell and biomolecule placement within scaffolds made of diverse materials, enabling predefined designs and geometries [128]. Hydrogel scaffolds have received widespread interest in cartilage tissue engineering due to their properties and ability to mimic the ECM of the cartilage. Hydrogel scaffolds are 3D network structures with water as their dispensing medium and have a high swelling rate, excellent hydrophilicity, and good biocompatibility. Hence, they effectively absorb wound exudate, reduce infection in damaged tissue, and hasten the healing of cartilage tissue [34,35,130,131]. Additionally, hydrogel scaffolds exhibit self-healing and self-recovery abilities that make them a promising option [132]. The hydrogel scaffolds will load seeded cells and possibly be transferred to the patient's body by injection or implantation. Then the scaffold will finally degrade gradually and induce cell and tissue regeneration [34,133]. To promote cell proliferation and migration, cell adhesion, and increase the success of AC tissue engineering, hydrogel scaffolds must have some appropriate specific physical properties, including porosity, mechanical strength, adhesion, and biodegradability.

Scaffold porosity

The porosity of hydrogel scaffolds for tissue engineering possibly be tailored based on the requirements of each intended application. Several studies have described how porosity and pore sizes affect cell activity and the effectiveness of repairing damaged tissue. In addition, adequate porosity is closely related to nutrient transfer and gas exchange [134]. High porosity and large pore sizes give results in inducing cell migration, proliferation, and spreading, enhancing gene expression, and ECM secretion [34,36,131]. On the other hand, small pore sizes help cell differentiation [131]. Previous studies show that the range of pore size between 250 - 500 μm in the scaffold is appropriate for cell proliferation, differentiation, and secretion of the ECM [131]. Cartilage hydrogel scaffolds require microporous structure with 300 μm size pores to maximize chondrogenic gene expression, chondrocyte cell differentiation, and chondrocyte cell proliferation [34,36]. For cartilage tissue engineering, an optimal pore size range is typically desired to balance cell infiltration, nutrient diffusion, and mechanical stability. Pore sizes in the range of 150 - 300 μm are often considered suitable for cartilage regeneration, as they can support cell migration, nutrient exchange, and tissue ingrowth while maintaining scaffold integrity [34,36,131]. While chitosan scaffolds exhibit microporous structures with promising pore sizes (50 - 200 μm) and porosity (75 - 80 %) according to Ikeda *et al.* [134] they may not meet the ideal criteria for certain applications. Ideally, scaffolds should possess a porosity exceeding 80 %. Therefore, enhancing chitosan scaffold porosity and pore size while simultaneously improving its mechanical strength is crucial for optimal performance in tissue engineering applications.

Mechanical strength

Mechanical strength is an important characteristic that must be maintained to increase the effectiveness of the hydrogel scaffolds. Chitosan-based hydrogel scaffolds are breakable and must be modified to ensure the stability of the scaffolds [34]. The tensile strength of cartilage before failure ranges between 0.8 and 25 MPa, while the peak force applied to human cartilage, typically associated with a 70 kg individual, usually falls between 0.84 and 3 MPa [131,135]. The mechanical strength and properties of hydrogel scaffolds depend on their intended use, whether *in vitro* or *in vivo*. If the scaffold is used to support cell growth *in vitro* before implantation, it may not need to match the mechanical strength of natural cartilage. The main point is hydrogel scaffold for AC should have sufficient mechanical strength comparable to natural AC. Changes in mechanical properties will affect the scaffold's degradation rate and profile [131]. Scaffolds made of chitosan demonstrated poor mechanical properties, low tensile strength, and low fracture stiffness as chitosan is a natural biodegradable polymer [136]. Compressive modulus and mechanical strength of chitosan scaffolds range between 0.0038 - 2.56 MPa and 0.059 - 0.125 MPa, respectively which are significantly lower than cartilage tensile strength [68]. The mechanical properties of chitosan scaffolds must be improved by tuning chitosan concentration [134]. Mechanical strength also depends on pore diameter and overall scaffold porosity [50]. Therefore, balanced porosity and mechanical strength are required in fabricating chitosan-based hydrogel scaffolds.

Cell adhesion on scaffold

Cell adhesion to the hydrogel scaffold requires careful attention because it significantly affects cell migration, proliferation, and differentiation. There are some receptors on the surface of ECM, such as integrins, selectins, Cluster of Differentiation 44 (CD44), and syndecan that will make a specific interaction with the scaffolds. This interaction plays a central role in cell migration, cell function, and tissue development. Chondrogenic gene expression, cell proliferation, and migration will be stimulated by high

cell adhesion. Previous studies have conjugated the hydrogel scaffolds with cell adhesive peptides, including arginine-glycine-aspartate peptide (RGD) to mimic the ECM phenotypes and maximize cell adhesion [34]. RGD is a tri-amino acid sequence that has great potential to improve cell attachment to a material with structural, mechanical, or other properties that are beneficial for specific tissue repair [137]. RGD peptides enhance cell bioactivity, activate the local adhesion signaling pathways, and will be a primary binding site to the integrin receptor, hence RGD enhances cell adhesion [138]. Furthermore, the hydrogel crosslinking network affects cell adhesion, so the composition of the scaffold composite must be considered to tune the cell adhesion. Chitosan scaffolds have good adhesion as chitosan is a bioadhesive material and has a hydrophilic surface that promotes cell adhesion and proliferation [50,136]. Adding RGD into chitosan scaffolds makes it more compatible as it will improve the adhesion of the scaffolds [138]. Moreover, cell adhesion in chitosan scaffolds is related to the chitosan deacetylation degree. Therefore, chitosan DD must be tailored to maximize chitosan scaffold cell adhesion.

Scaffold biodegradability

Ideally, the hydrogel scaffolds must be temporarily supported and degraded to provide tissue regeneration and tissue adaptation to the new physiological environment. However, the scaffold's degradation rate should coincide with the new tissue formation rate to guarantee the formation of functional new tissue [34,131,139]. Degradability of the scaffold is commonly influenced by the material behaviors and the crosslinked network of the hydrogel. Hence, the desirable degradation rate of the hydrogel scaffolds must be customized by controlling the ratio of the based material, copolymer composition, and combination. Some factors also play an important role in accelerating scaffold degradation rate, including pH and temperatures. The pH and temperature directly affect the degradation rate, influencing their biodegradability in the body. In acidic conditions (inflamed and infected tissue), enhanced hydrolysis of glycosidic bonds within the polymer chain. While, in alkaline conditions (wound healing), the degradation rate of chitosan may be slower due to reduced protonation and decreased susceptibility to hydrolysis. Therefore, these factors must be considered in designing hydrogel scaffolds for AC tissue engineering [132]. Chitosan is a suitable natural material for fabricating hydrogel scaffolds as it is biodegradable and can degrade gradually [134,136]. However, the chitosan biodegradability rate must match the new cartilage turnover.

Chitosan-based hydrogel scaffold for cartilage tissue engineering

Hydrogel is a highly hydrated polymer that has a 3D network structure and the ability to absorb water or biological fluids. Hydrogel network structure is obtained from the cross-linking reaction of natural and synthetic biomaterial. The presence of hydrophilic groups within the polymers and the extent of the crosslinked network will influence the affinity of the hydrogel for water absorption and affect the properties of the hydrogel scaffolds [36,38,140].

Preparation of chitosan hydrogel

Chitosan is a cationic polysaccharide that establishes hydrogen bridges, hydrophobic interactions, ionic interactions, or covalent bonding to create crosslinked hydrogel scaffolds and enhance the scaffold's mechanical and physicochemical properties [38,141,142]. In a conventional 2-dimensional (2D) culture, cells undergo distinct polarization as they adhere to a plastic surface on one side and establish contact with a liquid medium. In a typical 2D culture setup, cells primarily interact with neighboring cells along a flat surface, with minimal to no interaction occurring vertically. Moreover, the intricate 3-dimensional physical cues provided by the interstitial extracellular matrices are completely absent in this setup. Since cellular

behavior is largely governed by interactions with both the extracellular matrix and neighboring cells, the absence of these cues logically leads to cellular dysfunction [129,143]. Consequently, placing a greater emphasis on 3D culture platforms for chondrocyte cultivation could yield more precise insights into *in vivo* cellular physiological functions. This strategy also facilitates the development of more sophisticated applications, including AC tissue engineering (**Figure 4**). In addition, chitosan provides a mimic natural cartilage microenvironment as the N-acetylglucosamine group in chitosan has a comparable structure with glycosaminoglycans that are present in the cartilage extracellular matrix [144].

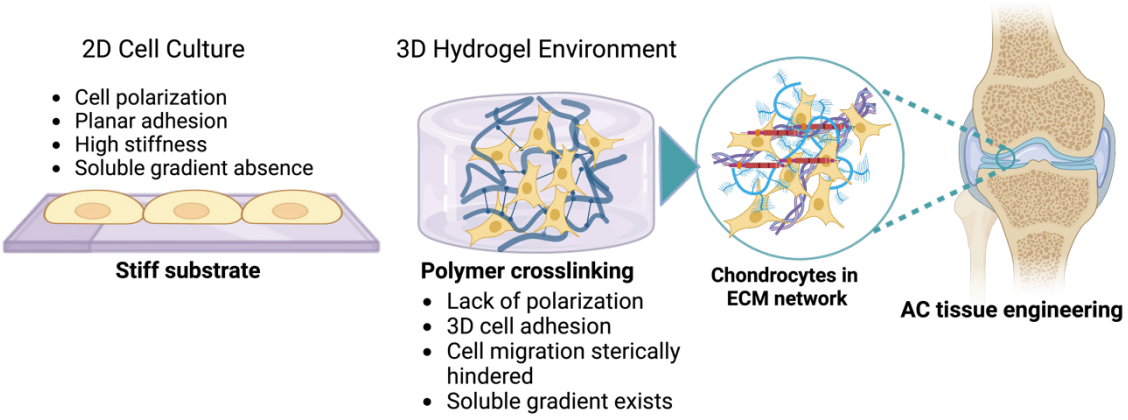

Figure 4 Cells behaviors on a 2D stiff substrate and 3D hydrogel environment formed by crosslinking polymer which closely mimics chondrocyte in ECM network and apply for AC tissue engineering. This figure was drawn using BioRender.

The preparation of chitosan-based hydrogel is carried out by physical cross-linking and chemical cross-linking, between natural and synthetic polymer. First, biomaterial for hydrogels is chosen based on their compatibility with the intended application and desired properties of the chitosan-based hydrogel scaffold. Subsequently, cross-linking by covalent or physical bonds between chitosan and another polymer to create a 3D network structure [141,142]. Chemical agents like glutaraldehyde, genipin, or carbodiimide are used to form covalent bonds between polymer chains [38]. On the other hand, physical bonds such as hydrogen bonding, hydrophobic interactions, or electrostatic interactions, can also be utilized to cross-link polymer chains [141]. Chitosan physical cross-linking through hydrogen interaction is a preferred method considering its safety as it does not use toxic agents. Physical and chemical cross-linking is influenced by the amino groups, the molecular weight, and the ionic strength [38,141].

Physical cross-linking

The fabrication method uses physical cross-linking, including ionic interaction (ionic complexes and polyelectrolyte complexes), hydrogen bonds, or molecular entanglement. However, it has disadvantages to produce weak, temporary, and reversible interactions as they will be affected by changes in pH, temperature, and ionic strength. On the other hand, this method provides advantages to produce scaffolds with higher biocompatibility [141,145]. This cross-linking formed hydrogen bonds through the freeze-thawing method (**Figure 5**) [146]. Chitosan-based hydrogel scaffolds are made with physical cross-linking by combining chitosan and PVA (CS/PVA). PVA facilitates the rapid formation of scaffolds into a 3D hydrogel network, leading to improvements in mechanical strength, increased stability in aqueous environments, and inherent self-repair capabilities [35,37,40]. The optimal ratio of chitosan to polyvinyl alcohol (PVA) for cartilage

tissue engineering applications can vary depending on several factors, including the specific requirements of the tissue being engineered, the desired mechanical properties of the scaffold, and the intended method of fabrication. However, ratios in the range of 1:1 to 1:3 (chitosan: PVA) have been commonly reported as suitable for cartilage tissue engineering [37].

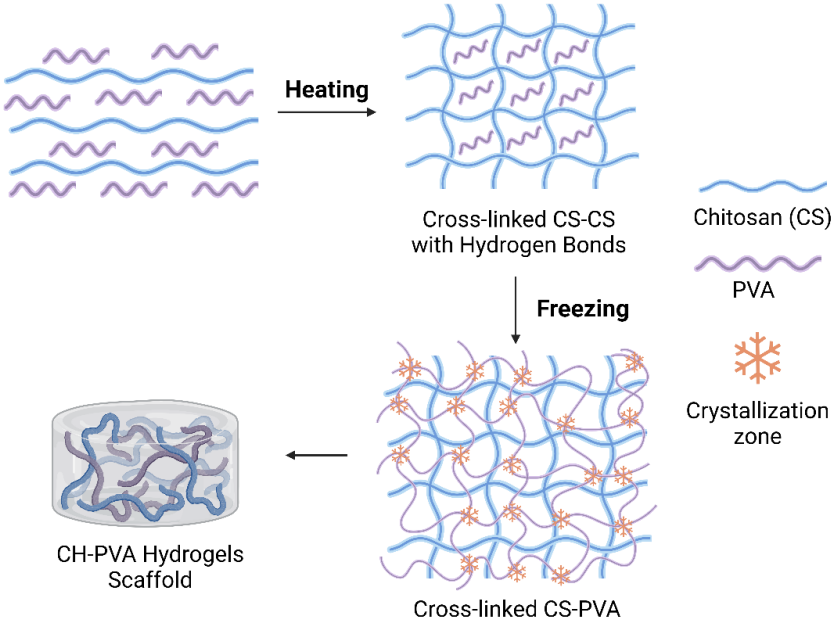

Figure 5 Preparation of CS/PVA hydrogel scaffold (modified from Zhu *et al*. [146]). This figure was prepared using BioRender.

Ionically cross-linked chitosan hydrogel

Chitosan is a cation polysaccharide and has ionizable amine groups that act as ionic cross-linkers to form ionic complexes and create a cross-linking network of the hydrogels (**Figure 6**(**a**)). These ionic complexes are made without the need for cross-linkers. The protonated amino groups of chitosan facilitate the ionic interaction with negatively charged molecules [38,141]. Chitosan ionic complexes possess a pH-responsive, depending on the degree of deacetylation (DD), size, and charge density of the anionic amine group of the chitosan [141].

Polyelectrolyte complexed chitosan hydrogel

Polyelectrolytes are defined as a polymer that has a relatively large number of charged or with suitable conditions become charged functional groups [147]. Polysaccharides, such as chitosan are polyelectrolytes as they have an amine functional group. The formation of polyelectrolyte complexes is based on an ionic interaction between the cationic polyelectrolyte of chitosan with anionic charge polymers (**Figure 6**(**b**)). Polyelectrolyte complexes are formed spontaneously without the addition of organic catalysts or chemical covalent cross-linkers [38,141,147]. Ionic interaction between opposite charges of polyelectrolyte provides strong interactions yet reversible and non-toxic. These interactions include electrostatic interaction, hydrogen interaction, and hydrophobic interaction. In cartilage tissue engineering, chitosan forms a polyelectrolyte complex through the interaction with collagen as a negative charge polyelectrolyte [38,147].

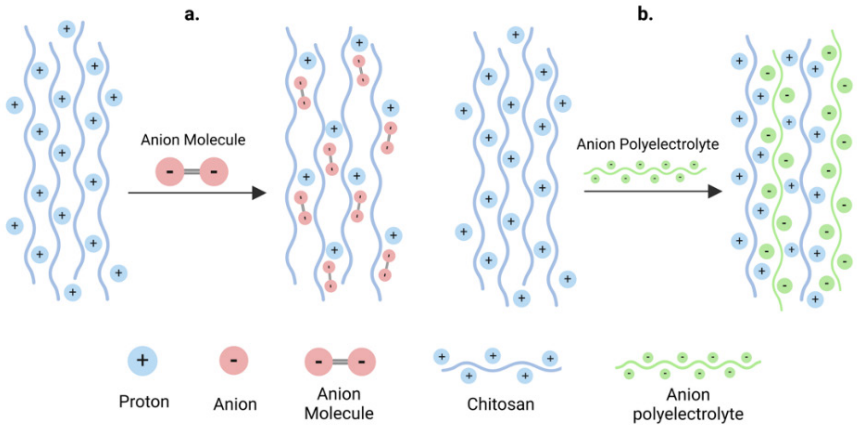

Figure 6 Physical cross-linking of chitosan. (a) Ionically cross-linked chitosan hydrogel and (b) polyelectrolyte complexed chitosan hydrogel (modified from Aminabhavi and Dharupaneedi [141]). This figure was drawn using BioRender.

Chemical cross-linking

Chemically crosslinked hydrogels exhibit irreversible, persistent, and strong interaction as they are formed by covalent bonds within the polymer chain [145]. Chemical cross-linking, including photopolymerization reaction and cross-linkers interaction, occurs due to the presence of several cross-linked structures as shown in **Figure 7**. The chemical cross-linked structure of chitosan-based hydrogels forms through a cross-linking reaction with its chain (chitosan cross-linked system) (**Figure 7**(**a**)), cross-linking with a different polymer (hybrid polymer network) (**Figure 7**(**b**)), cross-linked chitosan entangles with another cross-linked polymer (interpenetrating polymer network) (**Figure 7**(**c**)), and cross-linked chitosan entangle with another polymer (semi-interpenetrating polymer network) (**Figure 7**(**d**)) [38]. A covalent bond in the hydrogel cross-linking is required as the mechanical strength of physically cross-linked hydrogel is low. Hence, chemical cross-linking is generally preferred to form the cross-linking chitosan-based hydrogel scaffolds [148].

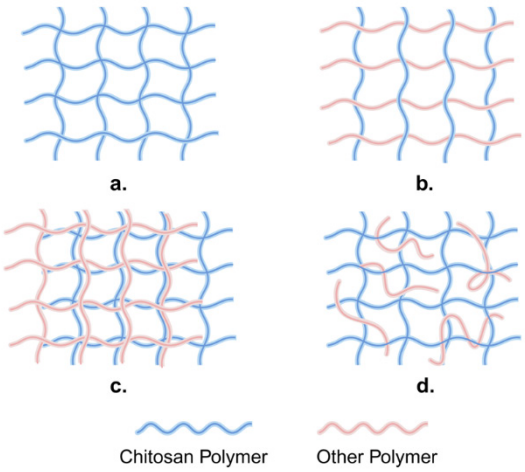

Figure 7 Structure of chemical cross-linked chitosan-based hydrogel (modified from Ahmadi *et al*. [38]). (a) Chitosan cross-linked system, (b) Hybrid polymer network, (c) interpenetrating polymer network, and (d) Semi-interpenetrating polymer network. This figure was prepared using BioRender.

Cross-linking *via* cross-linkers

Chitosan-based hydrogels are engineered by an interaction with dialdehyde compounds, such as glutaraldehyde and genipin. In this condition, dialdehyde compounds act as cross-linkers which will result in the formation of the cross-linking network in the hydrogel. Cross-linker that is widely used for AC tissue repair and replacement is genipin (**Figure 8**) [38]. Genipin has attracted attention to replace glutaraldehyde primarily due to the expanding biochemical significance of genipin-chitosan cross-linked hydrogel, as well as due to its advantages of stability, biocompatibility, and general safety as glutaraldehyde is considered highly toxic [149]. Chitosan and genipin crosslinking forms a hydrogel scaffold with a slower biodegradation rate, higher biocompatibility, and larger mechanical strength [38,149].

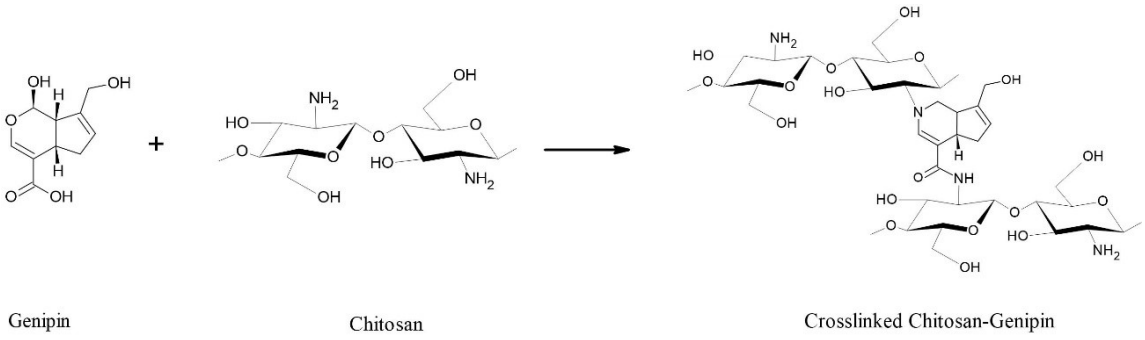

Figure 8 Chitosan and genipin cross-linking (modified from Ahmadi *et al*. [38]).

Cross-linking *via* photopolymerization

Photopolymerization forms a covalent bond in chitosan-based hydrogels. This method involves converting a liquid percussor solution into a gel using a photo initiator and visible light irradiation or ultraviolet light with an appropriate wavelength through free radical polymerization to form cross-linked hydrogels [38,150]. Many chitosan-based hydrogel scaffolds for tissue engineering are created by photopolymerization as this method avoids the degradation of molecules that are sensitive to high temperatures since photopolymerization is commonly carried out at room temperature [150]. Moreover, cross-linking density and the reaction of the polymer are adjusted by the UV exposure distance, UV exposure time, and photoinitiator concentration [38,150]. In the photopolymerization process, the photoinitiator plays an important role in determining scaffold biocompatibility and the reaction rate [151]. The toxicity of photopolymerize chitosan hydrogels is reduced by using a non-toxic photoinitiator [148,151]. Based on previous studies, the major advantage of forming photopolymerize chitosan is the possibility of creating a new hybrid hydrogel scaffold by combining natural polymer with biocompatible and degradable synthetic polymer [150]. A prior study by Zhu *et al*. [146] tried to synthesize chitosan-based hydrogels using the photopolymerization method by combining conjugated chitosan with maleic anhydride (MCS) and thiol-terminated PVA (TPVA) (**Figure 9**). In this process, the presence of a biocompatible photoinitiator, such as D-2959, and appropriate UV light wavelength are required to form a cross-linking network from a free radical photopolymerization. This formulation results in scaffolds with better stiffness, and higher mechanical strength, and provides cell attachment and proliferation.

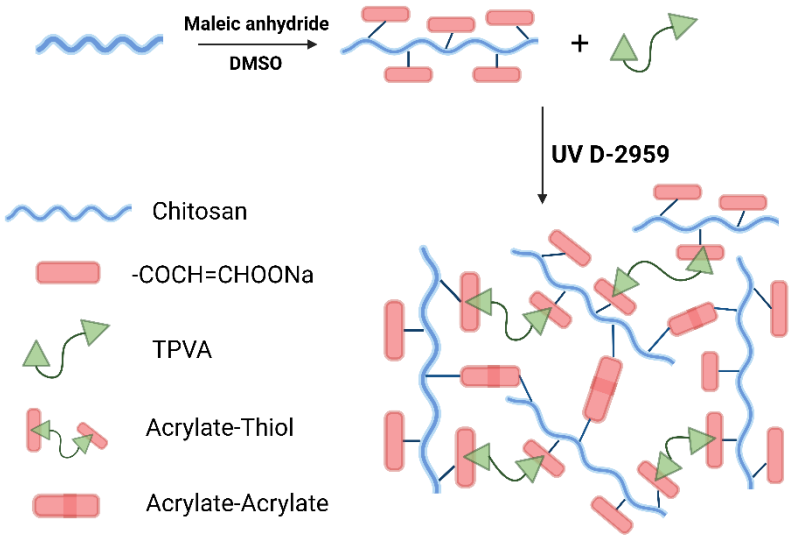

Figure 9 Photopolymerization of MCS/TPVA hydrogels (adapted from Zhu *et al*. [146]). This figure was prepared using BioRender.

Modification of chitosan hydrogel for cartilage tissue engineering

An appropriate polymeric biomaterial should be selected rightly for producing the hydrogel scaffolds as it affects cell activity and function [34,131]. Chitosan-based hydrogel scaffolds combine the advantages of chitosan and hydrogel, making them a captivating strategy for cartilage tissue engineering. They are an ideal candidate for cartilage tissue engineering owing to their good biocompatibility, biodegradable, and good encapsulability which is proven by their effectiveness in repairing cartilage tissue [34,43,131]. Nevertheless, chitosan-based hydrogel scaffolds have their limitations in losing the properties required for the scaffold structure, including poor mechanical properties, low toughness, and short *in vivo* duration because of their high solubility in aqueous conditions [35-37]. Previous studies have carried out a strategy to overcome chitosan-based hydrogel shortcomings and achieve the desired behaviors of the scaffolds by fabricating hybrid hydrogel scaffolds. Hybrid hydrogel scaffolds are fabricated by combining chitosan as a natural polymer with a synthetic water-soluble material. As AC biomechanical characteristic depends on its capacity to reconstitute and replenish lost fluids into the structure, a hybrid scaffold made up of a very hydrophilic natural polymer and a fibrous synthetic polymer with a higher mechanical strength gives an essential and ideal property for cartilage hydrogel scaffolds [39].

Chitosan - polyvinyl alcohol (PVA) hydrogel scaffold

Some studies prepared hybrid chitosan-based hydrogel scaffolds by combining chitosan as a natural polymer and polyvinyl alcohol as a synthetic polymer to improve the mechanical strength due to its capacity for water retention and porous structure. Chitosan and PVA construct a hydrogen bond and form unique crosslink network properties (**Figure 10**) [35,37,40]. Chitosan-based hydrogel scaffolds are engineered into a 3D network hydrogel scaffold that consists of chitosan, polyvinyl alcohol, graphene, and agar [152]. In addition, some studies prepared 2D network hydrogel with physical cross-linking methods, such as repeated freeze-thawing method. This method is a great option due to its low cost and high safety [35,145,146,152]. Through this process, PVA accelerates the scaffolds to form a 3-dimensional hydrogel network that indicates mechanical strength improvement, increases stability in aqueous environments, possesses self-healing properties, and meets the requirements for cartilage tissue replacement [35,37,40,152]. PVA is

copolymerized with another compound to create a scaffold that is closely like human cartilage [35]. Adding nanocrystal hydroxyapatite by *in situ* mineralization to overlay and coat the surface of the hydrogel scaffold effectively enhances cartilage healing [35,37]. Furthermore, collagen II as a composition of AC ECM is integrated into the scaffold to mimic the ECM, promote better cartilage healing, and develop cartilage regeneration [35]. Formulated Chitosan/PVA hydrogel scaffolds with hydroxyapatite/collagen II (HAP/COL II) exhibited high porosity, high mechanical strength, biodegradable, and non-toxic. Therefore, this composite becomes a potential option for AC tissue replacement as it promotes the repair of the cartilage.

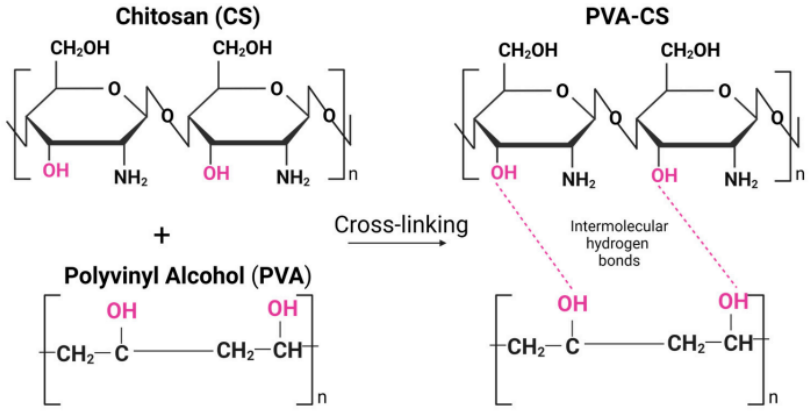

Figure 10 Chitosan PVA cross-linking [40].

Chitosan - PLA hydrogel scaffold

Some synthetic polymers have been employed to get beyond the mechanical drawback of chitosan. Specifically, the Food and Drug Administration (FDA) has certified poly (lactic acid) or PLA as a biodegradable synthetic polymer for various biomedical applications, especially for cartilage tissue regeneration [36,141,153,154]. PLA has large stiffness and tensile strength to increase the scaffold's mechanical strength [155]. Thus, the PLA-chitosan scaffolds have shown mechanical enhancement, but it is still not good enough to promote cell adhesion, proliferation, and migration as the scaffolds are too hard. Therefore, previous studies have combined PLA-chitosan with collagen II to tune the mechanical strength and closely mimic the chondrocytes microenvironment as cartilage ECM consists of collagen II [36,141]. PLA, chitosan, and collagen II (C2C1H) scaffolds have interconnected pores and appropriate properties that make them a promising candidate for cartilage tissue regeneration [36]. The macroporosity and highly porous structure of the composite guarantee good porosity for cell migration and maintain high pore volume after hydrogel impregnation [153]. C2C1H hydrogel scaffolds are fabricated using freeze-drying and melt-spun, which form physical cross-linking within the polymers [36]. Collagen II and chitosan, 2 hydrophilic polymers, were added to the scaffold composite to improve their capacity to absorb water and to resemble the natural ECM in cartilage. PLA was combined to stabilize the mechanical strength and properties of the hydrogel scaffold. This composite is a considerable option as it shows appropriate properties that support cell migration, proliferation, and differentiation for cartilage replacement.

Chitosan - PNIPAAm hydrogel scaffold

Chitosan-based hydrogels are designed and developed into smart hydrogels, such as thermoresponsive injectable hydrogels [34,42,43]. This fabrication garnered a lot of interest lately due to its distinct physical characteristics that are similar to the original cartilage ECM and its capacity for reversible phase transition

by a slight changing of temperature [42,43]. In comparison to other injectable hydrogels, thermoresponsive hydrogels have numerous benefits for cartilage tissue engineering, such as they are easily embeddable in the gel, fill irregular cartilage defects, and readily triggered to gel under mild physiological conditions without the need for harsh environments or organic solvents [42]. Chitosan-based thermoresponsive hydrogels form a solution state at room temperature, hence they are easily transferred into the human body by injection. The hydrogels transform into their gel state in the human body due to temperature changes. Therefore, the material used for this hydrogel scaffold must be a lower critical solution temperature (LCST) polymer [34,43]. Once the threshold temperature (T > TLCST) is exceeded, the phase transition of the hydrogel is achieved [41]. The biggest drawback of chitosan-based thermoresponsive hydrogels is their low mechanical strength. Therefore, some studies incorporated chitosan with synthetic thermoresponsive polymers, such as poly N-Isopropylacrylamide or PNIPAAm.

PNIPAAm is a non-biodegradable, non-toxic, synthetic LCST thermoresposive polymer, that hydrates and swells at lower temperatures [34,41-43]. The glass transition temperature (Tg) of poly(N-isopropylacrylamide) (PNIPAAm) is around 32 - 34 °C. Recently, PNIPAAm has been broadly used in tissue engineering as it forms a solution state at room temperature and a gel state above its LCST temperatures (33 - 34 °C), which is close to human body temperature [34,42]. Despite better biocompatibility of PNIPAAm thermoresponsive hydrogels, a combination of chitosan and PNIPAAm hydrogel scaffolds still has not provided an appropriate mechanical property [34]. Therefore, Chitosan-PNIPAAm hydrogel scaffold properties are tuned by using the incorporation of PNIPAAM and other monomers [34,43]. Poly(N-isopropylacrylamde-co-acrylic acid) (PNIPAAm-co-AAc) is preferred as it is more stable and provides better cell proliferation. In addition, providing vitamin C and some growth factors, such as Transforming Growth Factor Beta 3 (TGF- β3) and glucocorticoids, are essential to promote cell proliferation and differentiation [34]. Some studies tried to fabricate chitosan-PNIPAAm thermoresponsive hydrogel with another synthetic polymer, including polyethylene glycol (PEG) and polycaprolactone (PCL) to enhance its mechanical strength [34,42,43]. Chitosan-PNIPAAm hydrogel scaffolds are fabricated by free radical polymerization to form chemical cross-linking. This method requires the need for crosslinkers, initiators, and a synthesis solvent, which plays an important role in tuning the hydrogel properties [34]. Chitosan-PNIPAAm injectable thermos-responsive hydrogel is an advanced scaffold that gives a better option as it provides better application and better cell replacement in future cartilage tissue engineering. However, this scaffold design requires further research and studies to better understand its biocompatibility and mechanical properties through the composite composition, biomaterials, synthesis solvents, cross-linking methods, and fabrication strategies.

Conclusion and future perspective of chitosan for cartilage regeneration platform

The emerging technologies in biomaterials and biofabrication will undoubtedly have a significant impact on the field of cartilage tissue engineering in the coming years [156]. One of the best approaches for AC regenerative therapy is to attempt to develop regenerative tissue that closely resembles the original AC's zonal organization, histology, and metabolic components [3]. Because of its structural similarity to glycine aminoglycan, which is extensively present in connective tissues, chitosan has been widely utilized as a scaffold in cartilage tissue engineering [157]. Its key advantage lies in its ability to be highly customized by leveraging the reactivity of glucosamine residues. to form physically or chemically crosslinking with biodegradable or non-biodegradable polymer [42,43,154].

An obstacle in the design and construction of scaffolds is osteochondral compartment replication. To do this, gradients or composite multilayer scaffolds that resemble osteochondral tissue were developed and evaluated in conjunction with cells [44]. Various approaches were employed to generate multiphasic

scaffold configurations, encompassing the integration or fabrication of multiple layers, the establishment of gradients, or the incorporation of elements such as minerals, growth factors, and cells [44]. In the past, cells were seeded onto already manufactured scaffolds either manually, statically, or automatically, dynamically [158]. Uneven cell distribution across the biomaterial's width is rendered feasible through dynamic seeding [158,159]. By applying tremendous progress in mechanobiology and bioreactor design, to produce a crucial mark on cartilage tissue engineering over the next years.

Innovative innovations such as additive manufacturing in the treatment should be possible with the development of 3-dimensional (3D) printing. Chitosan and partially synthetic polymer materials are readily transformed into hydrogel inks possessing hydrophilic properties and biocompatibility comparable to biological tissues [45]. Their mechanical characteristics are tailored to create stable 3-dimensional structures [46]. In this context, the 3D printing investigation of a chitosan-based biopolymer revealed that despite extensive research, significant challenges persist in achieving hydrogels with well-defined structures and adequate mechanical strength and biological properties to promote tissue regeneration [47]. Moreover, *in situ,* 3D bioprinting offers a bioreactor and is stimulated by mechanical and biochemical signals to achieve functional tissues at anatomically relevant scales.

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References

[1] AF Steinert, SC Ghivizzani, A Rethwilm, RS Tuan, CH Evans and U Noth. Major biological obstacles for persistent cell-based regeneration of articular cartilage. *Arthritis Res Ther*. 2007; **9**, 213.

[2] Y Liu, KM Shah and J Luo. Strategies for articular cartilage repair and regeneration. *Front. Bioeng. Biotechnol.* 2021; **9**, 770655.

[3] M Iwamoto, Y Ohta, C Larmour and M Enomoto-Iwamoto. Toward regeneration of articular cartilage. *Birth Defects Res. C. Embryo Today* 2013; **99**, 192-202.

[4] X Guo, L Xi, M Yu, Z Fan, W Wang, A Ju, Z Liang, G Zhou and W Ren. Regeneration of articular cartilage defects: Therapeutic strategies and perspectives. *J. Tissue Eng.* 2023; **14**, 1-27.

[5] FJ O'Brien. Biomaterials & scaffolds for tissue engineering. *Mater. Today*. 2011; **14**, 88-95.

[6] C Vyas, H Mishbak, G Cooper, C Peach, RF Pereira and P Bartolo. Biological perspectives and current biofabrication strategies in osteochondral tissue engineering. *Biomanuf. Rev*. 2020; **5**, 2.

[7] Z Yang, H Li, Z Yuan, L Fu, S Jiang, C Gao, F Wang, K Zha, G Tian, Z Sun, B Huang, F Wei, F Cao, X Sui, J Peng, S Lu, W Guo, S Liu and Q Guo. Endogenous cell recruitment strategy for articular cartilage regeneration. *Acta Biomater*. 2020; **114**, 31-52.

[8] K Mithoefer, T Mcadams, RJ Williams, PC Kreuz and BR Mandelbaum. Clinical efficacy of the microfracture technique for articular cartilage repair in the knee: An evidence-based systematic analysis. *Am. J. Sports Med.* 2009; **37**, 2053-63.

[9] A Richter, G Hauschild, HM Escobar, I Nolte and J Bullerdiek. Application of high-mobility-group-a proteins increases the proliferative activity of chondrocytes *in vitro*. *Tissue Eng. Part A* 2009; **15**, 473-7.

[10] EA Makris, AH Gomoll, KN Malizos, JC Hu and KA Athanasiou. Repair and tissue engineering techniques for articular cartilage. *Nat. Rev. Rheumatol.* 2015; **11**, 21-34.

[11] RA Whiteside, RP Jakob, UP Wyss and P Mainil-Varlet. Impact loading of articular cartilage during transplantation of osteochondral autograft. *J. Bone Surg. Brit.* 2005; **87**, 1285-91.

[12] EH Kang, YJ Lee, TK Kim, CB Chang, JH Chung, K Shin, EY Lee, EB Lee and YW Song. Adiponectin is a potential catabolic mediator in osteoarthritis cartilage. *Arthritis Res. Ther*. 2010; **12**, R231.

[13] NC Negrini, AA Volponi, CA Higgins, PT Sharpe and AD Celiz. Scaffold-based developmental tissue engineering strategies for ectodermal organ regeneration. *Mater. Today Bio.* 2021; **10**, 100107.

[14] ME Carnes and GD Pins. Skeletal muscle tissue engineering: Biomaterials-based strategies for the treatment of volumetric muscle loss. *Bioengineering* 2020; **7**, 85.

[15] GG Flores-Rojas, B Gomez-Lazaro, F Lopez-Saucedo, R Vera-Graziano, E Bucio and E Mendizabal. Electrospun scaffolds for tissue engineering: A review. *Macromol* 2023; **3**, 524-53.

[16] AE Eldeeb, S Salah and NA Elkasabgy. Biomaterials for tissue engineering applications and current updates in the field: a comprehensive review. *AAPS PharmSciTech* 2022; **23**, 267.

[17] J Liang, P Liu, X Yang, L Liu, Y Zhang, Q Wang and H Zhao. Biomaterial-based scaffolds in promotion of cartilage regeneration: Recent advances and emerging applications. *J. Orthop. Transl.* 2023; **41**, 54-62.

[18] G Rahman, TP Frazier, JM Gimble and OA Mohiuddin. The emerging use of ASC/Scaffold composites for the regeneration of osteochondral defects. *Front. Bioeng. Biotechnol.* 2022; **10**, 893992.

[19] M Hafezi, SN Khorasani, M Zare, RE Neisiany and P Davoodi. Advanced hydrogels for cartilage tissue engineering: recent progress and future directions. *Polymers* 2021; **13**, 4199.

[20] Z Cao, C Dou and S Dong. Scaffolding biomaterials for cartilage regeneration. *J. Nanomater*. 2014; **2014**, 489128.

[21] M Wang, Y Wu, G Li, Q Lin, W Zhang, H Liu and J Su. Articular cartilage repair biomaterials: Strategies and applications. *Mater. Today Bio*. 2024; **24**, 100948.

[22] X Zhao, DA Hu, D Wu, F He, H Wang, L Huang, D Shi, Q Liu, N Ni, M Pakvasa, Y Zhang, K Fu, KH Qin, AJ Li, O Hagag, EJ Wang, M Sabharwal, W Wagstaff, RR Reid, MJ Lee, JM Wolf, ME Dafrawy, K Hynes, J Strelzow, SH Ho, T He and A Athiviraham. Applications of biocompatible scaffold materials in stem cell-based cartilage tissue engineering. *Front. Bioeng. Biotechnol*. 2021; **9**, 603444.

[23] D Yuan, Z Chen, T Lin, X Luo, H Dong and G Feng. Cartilage tissue engineering using combination of chitosan hydrogel and mesenchymal stem cells. *J. Chem*. 2015; **2015**, 530607.

[24] D Izzo, B Palazzo, F Scalera, F Gullotta, V lapesa, S Scialla, A Sannino and F Gervaso. Chitosan scaffolds for cartilage regeneration: influence of different ionic crosslinkers on biomaterial properties. *Int. J. Polym. Mater. Polym. Biomater*. 2019; **68**, 936-45.

[25] MA Shamekhi, H Mirzadeh, H Mahdavi, A Rabiee, D Mohebbi-Kalhori and MB Eslaminejad. Graphene oxide containing chitosan scaffolds for cartilage tissue engineering. *Int. J. Biol. Macromol*. 2019; **127**, 396-405.

[26] Y Pang, A Qin, X Lin, L Yang, Q Wang, Z Wang, Z Shan, S Li, J Wamg, S Fan and Q Hu. Biodegradable and biocompatible high elastic chitosan scaffold is cell-friendly both *in vitro* and *in vivo*. *Oncotarget* 2017; **8**, 35583-91.

[27] A Khan and KA Alamry. Recent advances of emerging green chitosan-based biomaterials with potential biomedical applications: A review. *Carbohydr. Res*. 2021; **506**, 108368.

[28] F Hisham, MH Maziati Akmal, F Ahmad, K Ahmad and N Samat. Biopolymer chitosan: Potential sources, extraction methods, and emerging applications. *Ain Shams Eng. J.* 2024; **15**, 102424.

[29] D Alemu, E Getachew and AK Mondal. Study on the physicochemical properties of chitosan and their applications in the biomedical sector. *Int. J. Polym. Sci*. 2023; **2023**, 5025341.

[30] M Kozma, B Acharya and R Bissessur. Chitin, chitosan, and nanochitin: extraction, synthesis, and applications. *Polymers* 2022; **14**, 3938.

[31] M Pakizeh, A Moradi and T Ghassemi. Chemical extraction and modification of chitin and chitosan from shrimp shells. *Eur. Polym. J.* 2021; **159**, 110709.

[32] M Muthu, J Gopal, S Chun, AJP Devadoss, N Hasan and I Sivanesan. Crustacean waste-derived chitosan: antioxidant properties and future perspective. *Antioxidants* 2021; **10**, 228.

[33] KU Rehman, C Hollah, K Wiesotzki, V Heinz, K Aganovic, RU Rehman, J Retrusan, L Zhang, J Zhang, S Sohail, MK Mansoor, CI Rumbos, C Athanassiou and M Cai. Insect-derived chitin and chitosan: a still unexploited resource for the edible insect sector. *Sustainability* 2023; **15**, 4864.

[34] MM Rana and HDLH Siegler. Tuning the properties of pnipam-based hydrogel scaffolds for cartilage tissue engineering. *Polymers* 2021; **13**, 3154.

[35] Q Ding, S Zhang, X Liu, Y Zhao, J Yang, G Chai, N Wang, S Ma, W Liu and C Ding. Hydrogel tissue bioengineered scaffolds in bone repair: A review. *Molecules* 2023; **28**, 7039.

[36] M Wasyłeczko, W Sikorska and A Chwojnowski. Review of synthetic and hybrid scaffolds in cartilage tissue engineering. *Membranes* 2020; **10**, 348.

[37] S Bi, P Wang, S Hu, S Li, J Pang, Z Zhou, G Sun, L Huang, X Cheng, S Xing and X Chen. Construction of physical-crosslink chitosan/PVA double-network hydrogel with surface mineralization for bone repair. *Carbohydr. Polym*. 2019; **224**, 115176.

[38] F Ahmadi, Z Oveisi, M Samani and Z Amoozgar. Chitosan based hydrogels: Characteristics and pharmaceutical applications. *Res. Pharm. Sci.* 2015; **10**, 1-16.

[39] AM Haaparanta, E Järvinen, IF Cengiz, V Ella, HT Kokkonen, I Kiviranta and M Kellomaki. Preparation and characterization of collagen/PLA, chitosan/PLA, and collagen/chitosan/PLA hybrid scaffolds for cartilage tissue engineering. *J. Mater. Sci. Mater. Med*. 2014; **25**, 1129-36.

[40] KG Nathan, K Genasan and T Kamarul. Polyvinyl alcohol-chitosan scaffold for tissue engineering and regenerative medicine application: A review. *Mar. Drugs* 2023; **21**, 304.

[41] M Lemanowicz, A Gierczycki, W Kuznik, R Sancewicz and P Imiela. Determination of lower critical solution temperature of thermosensitive flocculants. *Miner. Eng*. 2014; **69**, 170-6.

[42] Y Zhang, J Yu, K Ren, J Zuo, J Ding and X Chen. Thermosensitive hydrogels as scaffolds for cartilage tissue engineering. *Biomacromolecules* 2019; **20**, 1478-92.

[43] S Li, W Wang, W Li, M Xie, C Deng, X Sun, C Wang, Y Liu, G Shi, Y Xu, X Ma and J Wang. Fabrication of thermoresponsive hydrogel scaffolds with engineered microscale vasculatures. *Adv. Funct. Mater.* 2021; **31**, 2102685.

[44] R Chen, JS Pye, J Li, CB Little and JJ Li. Multiphasic scaffolds for the repair of osteochondral defects: outcomes of preclinical studies. *Bioact. Mater.* 2023; **27**, 505-45

[45] D Fan, Y Liu, Y Wang, Q Wang, H Guo, Y Cai, R Song, X Wang and W Wang. 3D printing of bone and cartilage with polymer materials. *Front. Pharmacol*. 2022; **13**, 1044726.

[46] Z Wang, H Le, Y Wang, H Liu, Z Li, X Yang, C Wang, J Ding and X Chen. Instructive cartilage regeneration modalities with advanced therapeutic implantations under abnormal conditions. *Bioact. Mater*. 2022; **11**, 317-38.

[47] F Pahlevanzadeh, R Emadi, A Valiani, M Kharaziha, SA Poursamar, HR Bakhsheshi-Rad, AF Ismail, S RamaKrishna and F Berto. Three-dimensional printing constructs based on the chitosan for tissue regeneration: state of the art, developing directions and prospect trends. *Materials* 2020; **13**, 2663.

[48] EM Yahia and A Carrillo-Lopez. *Postharvest physiology and biochemistry of fruits and vegetables.* 1st ed. Elsevier, Amsterdam, Netherlands, 2018.

[49] T Jiang, R James, SG Kumbar and CT Laurencin. *Chitosan as a biomaterial: Structure, properties, and applications in tissue engineering and drug Delivery*. *In*: SG Kumbar, CT Laurencin and M Deng (Eds.). Natural and Synthetic Biomedical Polymers. Elsevier, Amsterdam, Netherlands, 2014.

[50] SL Levengood and M Zhang. Chitosan-based scaffolds for bone tissue engineering. *J. Mater. Chem. B Mater. Biol. Med.* 2014; **2**, 3161-84.

[51] I Aranaz, AR Alcantara, MC Civera, C Arias, B Elorza, AH Caballero and N Acosta. Chitosan: An overview of its properties and applications. *Polymers* 2021; **13**, 3256.

[52] D Ngo, T Vo, D Ngo, K Kang, J Je, HN Pham, H Byun and S Kim. Biological effects of chitosan and its derivatives. *Food Hydrocoll*. 2015; **51**, 200-16.

[53] N Hassan, T Krieg, M Zinser, K Schroder and N Kroger. An overview of scaffolds and biomaterials for skin expansion and soft tissue regeneration: Insights on zinc and magnesium as new potential key elements. *Polymers* 2023; **15**, 3854.

[54] H Sodhi and A Panitch. Glycosaminoglycans in tissue engineering: A review. *Biomolecules* 2021; **11**, 29.

[55] B Li, J Elango and W Wu. Recent advancement of molecular structure and biomaterial function of chitosan from marine organisms for pharmaceutical and nutraceutical application. *Appl. Sci.* 2020; **10**, 4719.

[56] F Pacheco-Torgal, V Ivanov, N Karak and H Jonkers. *Biopolymers and biotech admixtures for eco-efficient construction materials. In*: SH Lv (Ed.). High-performance superplasticizer based on chitosan, Woodhead Publish, Cambridgeshire, 2016.

[57] M Eddya, B Tbib and K EL-Hami. A comparison of chitosan properties after extraction from shrimp shells by diluted and concentrated acids. *Heliyon* 2020; **6**, e03486.

[58] H Zhang, Y Li, X Zhang, B Liu, H Zhao and D Chen. Directly determining the molecular weight of chitosan with atomic force microscopy. *Front. Nanosci. Nanotechnol*. 2016; **2**, 123-7.

[59] J Qian, X Wang, Y Chen, C Mo, C Liang and H Guo. The correlation of molecule weight of chitosan oligomers with the corresponding viscosity and antibacterial activity. *Carbohydr. Res*. 2023; **530**, 108860.

[60] MA Matica, FL Aachmann, A Tondervik, H Sletta and V Ostafe. Chitosan as a wound dressing starting material: antimicrobial properties and mode of action. *Int. J. Mol. Sci*. 2019; **20**, 5889.

[61] S Grobler and V Perchyonok. Cytotoxicity of low, medium and high molecular weight chitosan's on balb/c 3t3 mouse fibroblast cells at a 75 - 85 % de-acetylation degree. *Mater. Sci. Eng. Adv. Res*. 2018; **2**, 27-30.

[62] J Jiao, J Huang and Z Zhang. Hydrogels based on chitosan in tissue regeneration: How do they work? a mini review. *J. Appl. Polym. Sci*. 2019; **136**, 47235.

[63] L Budai, M Budai, ZE Fulopne Papay, Z Vilimi and I Antal. Rheological considerations of pharmaceutical formulations: Focus on viscoelasticity. *Gels* 2023; **9**, 469.

[64] CEG Garcia, B Lardy, F Bossard, FAS Martinez and M Rinaudo. Chitosan based biomaterials for cartilage tissue engineering: chondrocyte adhesion and proliferation. *Food Hydrocoll. Health* 2021; **1**, 100018.

[65] P Haghighi and A Shamloo. Fabrication of a novel 3D scaffold for cartilage tissue repair: *In-vitro* and *in-vivo* study. *Mater. Sci. Eng. C* 2021; **128**, 112285.

[66] SP Mallick, K Pal, A Rastogi and P Srivastava. Evaluation of poly(L-lactide) and chitosan composite scaffolds for cartilage tissue regeneration. *Des. Monomers Polym*. 2016; **19**, 271-82.

[67] SB Qasim, S Husain, Y Huang, M Pogorielov, V Deineka, M Lyndin, A Rawlinson and IU Rehman. In-vitro and in-vivo degradation studies of freeze gelated porous chitosan composite scaffolds for tissue engineering applications. *Polym. Degrad. Stab*. 2017; **136**, 31-8.

[68] A Ressler. Chitosan-based biomaterials for bone tissue engineering applications: A short review. *Polymers* 2022; **14**, 3430.

[69] H Li, C Hu, H Yu and C Chen. Chitosan composite scaffolds for articular cartilage defect repair: A review. *RSC Adv*. 2018; **8**, 3736-49.

[70] A Rogina, M Pusic, L Stefan, A Ivkovic, I Urlic, M Ivankovic and H Ivankovic. Characterization of chitosan-based scaffolds seeded with sheep nasal chondrocytes for cartilage tissue engineering. *Ann. Biomed. Eng*. 2021; **49**, 1572-86.

[71] M Rodriguez-Vazquez, B Vega-Ruiz, R Ramos-Zuniga, DA Saldana-Koppel and LF Quinones-Olvera. Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. *BioMed Res. Int*. 2015; **2015**, 821279.

[72] S Saladino, ERD Leonardo, M Salamone, D Mercuri, F Segatti and G Ghersi. Formulation of different chitosan hydrogels for cartilage tissue repair. *Chem. Eng. Trans*. 2014; **38**, 505-10.

[73] T Hao, N Wen, JK Cao, HB Wang, SH Lu, T Liu, Q Lin, C Duan and C Wang. The support of matrix accumulation and the promotion of sheep articular cartilage defects repair *in vivo* by chitosan hydrogels. *Osteoarthr. Cartil*. 2010; **18**, 257-65.

[74] K Mohan, T Muralisankar, R Jayakumar and C Rajeevgandhi. A study on structural comparisons of α-chitin extracted from marine crustacean shell waste. *Carbohydr. Polym. Tech. Appl*. 2021; **2**, 100037.

[75] A Ibram, AM Ionescu and E Cadar. Comparison of extraction methods of chitin and chitosan from different sources. *Eur. J. Med. Nat. Sci.* 2019; **2**, 23-36.

[76] BT Iber, NA Kasan, D Torsabo and JW Omuwa. A review of various sources of chitin and chitosan in nature. *J. Renew. Mater.* 2022; **10**, 1097-123.

[77] AI Saenz-Mendoza, PB Zamudio-Flores, MC Garcia-Anaya, CR Velasco, CH Acosta-Muniz, M Espino-Diaz, JM Tirado-Gallegos, M Hernandez-Gonzalez, G Vela-Gutierrez, R Salgado-Delgado, J R Rendon-Villalobos and A Ortega-Ortega. Insects as a potential source of chitin and chitosan: Physicochemical, morphological and structural characterization. -a review. *Emir. J. Food Agr*. 2023; **35**, 388-407.

[78] A Pellis, GM Guebitz and GS Nyanhongo. Chitosan: Sources, processing and modification techniques. *Gels* 2022; **8**, 5-25.

[79] D Karthick Rajan, K Mohan, J Rajarajeswaran, D Divya, R Kumar, S Kandasamy, S Zhang and AR Ganesan. β-Chitin and chitosan from waste shells of edible mollusks as a functional ingredient. *Food Front*. 2024; **5**, 46-72.

[80] VC Roy, MR Islam, S Sadia, M Yeasmin, JS Park, HJ Lee and B Chun. Trash to treasure: An up-to-date understanding of the valorization of seafood by-products, targeting the major bioactive compounds. *Mar. Drugs* 2023; **21**, 485.

[81] M Hamdi, A Feki, S Bardaa, S Li, S Nagarajan, M Mellouli, T Boudawara, Z Sahnoun, M Nasri and R Nasri. A novel blue crab chitosan/protein composite hydrogel enriched with carotenoids endowed with distinguished wound healing capability: *In vitro* characterization and *in vivo* assessment. *Mater. Sci. Eng. C* 2020; **113**, 110978.

[82] H Ehrlich, LA Shaala, DTA Youssef, S Żołtowska-Aksamitowska, M Tsurkan, R Galli, H Meissner, M Wysokowski, I Petrenko, KR Tabachnick, VN Ivanenko, Nicole Bechmann, Y Joseph and T

Jesionowski. Discovery of chitin in skeletons of non-verongiid Red Sea demosponges. *PLoS One* 2018; **13**, e0195803.

[83] I Younes, S Hajji, V Frachet, M Rinaudo, K Jellouli and M Nasri. Chitin extraction from shrimp shell using enzymatic treatment. antitumor, antioxidant and antimicrobial activities of chitosan. *Int. J. Biol. Macromol.* 2014; **69**, 489-98.

[84] I Younes and M Rinaudo. Chitin and chitosan preparation from marine sources. structure, properties and applications. *Mar. Drugs* 2015; **13**, 1133-74.

[85] T Hahn, E Tafi, A Paul, R Salvia, P Falabella and S Zibek. Current state of chitin purification and chitosan production from insects. *J. Chem. Tech. Biotechnol*. 2020; **95**, 2775-95.

[86] W William and N Wid. Comparison of extraction sequence on yield and physico-chemical characteristic of chitosan from shrimp shell waste. *J. Phys. Conf. Ser.* 2019; **1358**, 012002.

[87] A Setiawati, K Tricahya, FDO Riswanto and Y Dwiatmaka. Towards a sustainable chitosan-based composite scaffold derived from Scylla serrata crab chitosan for bone tissue engineering. *J. Biomater. Sci. Polym. Ed*. 2024; **35**, 146-63.

[88] VP Santos, NSS Marques, PCSV Maia, MAB Lima, LDO Franco, GMD Campos-Takaki. Seafood waste as attractive source of chitin and chitosan production and their applications. *Int. J. Mol. Sci*. 2020; **21**, 4290.

[89] N Desai, D Rana, S Salave, R Gupta, P Patel, B Karunakaran, A Sharma, J Giri, D Benival and N Kommineni. Chitosan: a potential biopolymer in drug delivery and biomedical applications. *Pharmaceutics* 2023; **15**, 1313.

[90] M Mathaba and MO Daramola. Effect of chitosan's degree of deacetylation on the performance of pes membrane infused with chitosan during AMD treatment. *Membranes* 2020; **10**, 52.

[91] A Hosney, S Ullah and K Barcauskaite. A review of the chemical extraction of chitosan from shrimp wastes and prediction of factors affecting chitosan yield by using an artificial neural network. *Mar. Drugs* 2022; **20**, 675.

[92] MB Kaczmarek, K Struszczyk-Swita, X Li, M Szczesna-Antczak and M Daroch. Enzymatic modifications of chitin, chitosan, and chitooligosaccharides. *Front. Bioeng. Biotechnol*. 2019; **7**, 243.

[93] MK Lagat, S Were, F Ndwigah, VJ Kemboi, C Kipkoech and CM Tanga. Antimicrobial activity of chemically and biologically treated chitosan prepared from black soldier fly (*Hermetia illucens*) pupal shell waste. *Microorganisms* 2021; **9**, 2417.

[94] AC Neves, C Zanette, ST Grade, JV Schaffer, HJ Alves and MK Arantes. Optimization of lactic fermentation for extraction of chitin from freshwater shrimp waste. *Acta Sci. Tech.* 2017; **39**, 125-33.

[95] BHC Greene, KN Robertson, JCOC Young and JAC Clyburne. Lactic acid demineralization of green crab (*Carcinus maenas*) shells: Effect of reaction conditions and isolation of an unusual calcium complex. *Green Chem. Lett. Rev*. 2016; **9**, 1-11.

[96] Y Kim and R Park. Progress in bioextraction processes of chitin from crustacean biowastes. *J. Korean Soc. Appl. Biol. Chem*. 2015; **58**, 545-54.

[97] K Thongdonson, A Boonmahitthisud and S Tanpichai. Effect of sodium hydroxide on properties of shrimp-shells-extracted chitin nanofibers. *J. Phys. Conf. Ser*. 2022; **2175**, 012019.

[98] M Ul-Islam, KF Alabbosh, S Manan, S Khan, F Ahmad and MW Ullah. Chitosan-based nanostructured biomaterials: Synthesis, properties, and biomedical applications. *Adv. Ind. Eng. Polym. Res*. 2024; **7**, 79-99.

[99] RAI Reshad, TA Jishan and NN Chowdhury. Chitosan and its broad applications: a brief review. *J. Clin. Exp. Investig*. 2021; **12**, em00779.

[100] SM Joseph, S Krishnamoorthy, R Paranthaman, JA Moses and C Anandharamakrishnan. A review on source-specific chemistry, functionality, and applications of chitin and chitosan. *Carbohydr. Polym. Tech. Appl*. 2021; **2**, 100036.

[101] A Weiss, E Livne, J Bernheim and M Silbermann. Structural and metabolic changes characterizing the aging of various cartilages in mice. *Mech. Ageing Dev.* 1986; **35**, 145-60.

[102] D Correa and SA Lietman. Articular cartilage repair: Current needs, methods and research directions. *Semin. Cell Dev. Biol*. 2017; **62**, 67-77.

[103] AJS Fox, A Bedi, and SA Rodeo. The basic science of articular cartilage: structure, composition, and function. *Sports Health*. 2009; **1**, 461-8.

[104] J Eschweiler, N Horn, B Rath, M Betsch, A Baroncini, M Tingart and F Migliorini. The biomechanics of cartilage - an overview. *Life* 2021; **11**, 302.

[105] BK Connizzo, L Han and RL Sah. *Electromechanobiology of cartilage and osteoarthritis: a tribute to alan grodzinsky on his 75th birthday. In*: AH Plaas, MM Moran, JD Sandy and VC Hascall (Eds.). Aggrecan and hyaluronan: The infamous cartilage polyelectrolytes - then and now. Springer, Cham, Switzerland, 2023.

[106] PJ Roughley, Y Geng and JS Mort. The non-aggregated aggrecan in the human intervertebral disc can arise by a non-proteolytic mechanism. *Eur. Cells Mater*. 2014; **28**, 129-36.

[107] B, Han, Q Li, C Wang, P Patel, SM Adams, B Doyran, HT Nia, R Oftadeh, S Zhou, CY Li, XS Liu, XL Lu, M Enomoto-Iwamoto, L Qin, RL Mauck, RV Iozzo, DE Birk and L Han. Decorin Regulates the Aggrecan Network Integrity and Biomechanical Functions of Cartilage Extracellular Matrix. *ACS Nano* 2019; **13**, 11320-33.

[108] FH Chen, KT Rousche and RS Tuan. Technology insight: adult stem cells in cartilage regeneration and tissue engineering. *Nat. Clin. Pract. Rheumatol.* 2006; **2**, 373-82.

[109] D McGonagle, TG Baboolal and E Jones. Native joint-resident mesenchymal stem cells for cartilage repair in osteoarthritis. *Nat. Rev. Rheumatol.* 2017; **13**, 719-30.

[110] S Zhang, B Hu, W Liu, P Wang, X Lv, S Chen, H Liu and Z Shao. Articular cartilage regeneration: The role of endogenous mesenchymal stem/progenitor cell recruitment and migration. *Semin. Arthritis Rheum*. 2020; **50**, 198-208.

[111] R Chijimatsu and T Saito. Mechanisms of synovial joint and articular cartilage development. *Cell. Mol. Life Sci*. 2019; **76**, 3939-52.

[112] JM Case and JM Scopp. Treatment of articular cartilage defects of the knee with microfracture and enhanced microfracture techniques. *Sports Med. Arthrosc. Rev*. 2016; **24**, 63-8.

[113] C Erggelet. Enhanced marrow stimulation techniques for cartilage repair. *Oper. Tech. Orthop*. 2014; **24**, 2-13.

[114] MF Sommerfeldt, RA Magnussen, TE Hewett, CC Kaeding and DC Flanigan. Microfracture of articular cartilage. *JBJS Rev*. 2016; **4**, e6.

[115] E Vinod, K Padmaja, B Ramasamy and S Sathishkumar. Systematic review of articular cartilage derived chondroprogenitors for cartilage repair in animal models. *J. Orthop.* 2023; **35**, 43-53.

[116] T Minas, T Ogura and T Bryant. Autologous chondrocyte implantation. *JBJS Essent. Surg. Tech*. 2016; **6**, e24.

[117] P Niemeyer, S Porichis, M Steinwachs, C Erggelet, PC Kreuz, H Schmal, M Uhl, N Ghanem, NP Sudkamp and G Salzmann. Long-term outcomes after first-generation autologous chondrocyte implantation for cartilage defects of the knee. *Am. J. Sports Med*. 2014; **42**, 150-7.

[118] L Roseti, G Desando, C Cavallo, M Petretta and B Grigolo. Articular cartilage regeneration in osteoarthritis. *Cells* 2019; **8**, 1305.

[119] BJ Erickson, SM Strickland and AH Gomoll. Indications, techniques, outcomes for matrix-induced autologous chondrocyte implantation (MACI). *Oper. Tech. Sports Med.* 2018; **26**, 175-82.

[120] L Gao, P Orth, M Cucchiarini and H Madry. Autologous matrix-induced chondrogenesis: A systematic review of the clinical evidence. *Am. J. Sports Med*. 2019; **47**, 222-31.

[121] CHB Tan, XYO Huang, ZQ Tay and HRBA Razak. Arthroscopic and open approaches for autologous matrix-induced chondrogenesis repair of the knee have similar results: A meta-analysis. *J. ISAKOS* 2023; **9**, 192-204.

[122] EJ Cotter, KC Wang, AB Yanke and S Chubinskaya. Bone marrow aspirate concentrate for cartilage defects of the knee: From bench to bedside evidence. *Cartilage* 2018; **9**, 161-70.

[123] GB Kim, MS Seo, WT Park and GW Lee. Bone marrow aspirate concentrate: Its uses in osteoarthritis. *Int. J. Mol. Sci*. 2020; **21**, 3224.

[124] TT Tran, ZA Hamid and KY Cheong. A review of mechanical properties of scaffold in tissue engineering: Aloe vera composites. *J. Phys. Conf. Ser*. 2018; **1082**, 012080.

[125] P Nitti, A Narayanan, R Pellegrino, S Villani, M Madaghiele and C Demitri. Cell-tissue interaction: the biomimetic approach to design tissue engineered biomaterials. *Bioengineering* 2023; **10**, 1122.

[126] A Eltom, G Zhong and A Muhammad. Scaffold techniques and designs in tissue engineering functions and purposes: A review. *Adv. Mater. Sci. Eng*. 2019; **2019**, 3429527.

[127] B Huang, P Li, M Chen, L Peng, X Luo, G Tian, H Wang, L Wu, Q Tian, H Li, Y Yang, S Jiang, Z Yang, K Zha, X Sui, S Liu and Q Guo. Hydrogel composite scaffolds achieve recruitment and chondrogenesis in cartilage tissue engineering applications. *J. Nanobiotechnol*. 2022; **20**, 25.

[128] A Zelinka, AJ Roelofs, RA Kandel and CD Bari. Cellular therapy and tissue engineering for cartilage repair. *Osteoarthr. Cartil.* 2022; **30**, 1547-60.

[129] MC Ratri, AI Brilian, A Setiawati, HT Nguyen, V Soum and K Shin. Recent advances in regenerative tissue fabrication: Tools, materials, and microenvironment in hierarchical aspects. *Adv. NanoBiomed Res.* 2021; **1**, 2000088.

[130] Q Wang, X Wang and Y Feng. Chitosan hydrogel as tissue engineering scaffolds for vascular regeneration applications. *Gels* 2023; **9**, 373.

[131] Z Izadifar, X Chen and W Kulyk. Strategic design and fabrication of engineered scaffolds for articular cartilage repair. *J. Funct. Biomater*. 2012; **3**, 799-838.

[132] MH Ghanian, H Mirzadeh and H Baharvand. In situ forming, cytocompatible, and self-recoverable tough hydrogels based on dual ionic and click cross-linked alginate. *Biomacromolecules* 2018; **19**, 1646-62.

[133] N Olov, S Bagheri-Khoulenjani and H Mirzadeh. Injectable hydrogels for bone and cartilage tissue engineering: A review. *Prog. Biomater*. 2022; **11**, 113-35.

[134] T Ikeda, K Ikeda, K Yamamoto, H Ishizaki, Y Yoshizawa, K Yanagiguchi, S Yamada and Y Hayashi. Fabrication and characteristics of chitosan sponge as a tissue engineering scaffold. *BioMed Res. Int*. 2014; **2014**, 786892.

[135] CJ Little, NK Bawolin and X Chen. Mechanical properties of natural cartilage and tissue-engineered constructs. *Tissue Eng. Part B Rev*. 2011; **17**, 213-27.

[136] R Adamski and D Siuta. Mechanical, structural, and biological properties of chitosan/ hydroxyapatite/silica composites for bone tissue engineering. *Molecules* 2021; **26**, 1976.

[137] SL Bellis. Advantages of RGD peptides for directing cell association with biomaterials. *Biomaterials* 2011; **32**, 4205-10.

[138] MA Kafi, K Aktar, M Todo and R Dahiya. Engineered chitosan for improved 3D tissue growth through Paxillin-FAK-ERK activation. *Regen. Biomater.* 2020; **7**, 141-51.

[139] FA Szaman, K Zhou, MDM Blanes-Martinez, MB Fournet and DM Devine. Bioresorbable chitosan-based bone regeneration scaffold using various bioceramics and the alteration of photoinitiator concentration in an extended UV photocrosslinking reaction. *Gels* 2022; **8**, 696.

[140] B Kaczmarek-Szczepanska, O Mazur, M Michalska-Sionkowska, K Łukowicz and AM Osyczka. The preparation and characterization of chitosan-based hydrogels cross-linked by glyoxal. *Materials* 2021; **14**, 2449.

[141] JA Jennings and JD Bumgardner. *Chitosan based biomaterials. In*: TM Aminabhavi and SP Dharupaneedi. (Eds.). Production of chitosan-based hydrogels for biomedical applications, Woodhead Publishing, Cambridgeshire, 2017.

[142] A Vishwakarma and JM Karp. *Biology and engineering of stem cell niches. In*: JL Wilson and TC McDevitt (Eds.). Biofunctional hydrogels for three-dimensional stem cell culture. Academic Press, London, 2017.

[143] K Wegrzynowska-Drzymalska, P Grebicka, DT Mlynarczyk, D Chelminiak-Dudkiewicz, H Kaczmarek, T Goslinski and M Ziegler-Borowska. Crosslinking of chitosan with dialdehyde chitosan as a new approach for biomedical applications. *Materials* 2020; **13**, 3413.

[144] Y Kim, Z Zharkinbekov, K Raziyeva, L Tabyldiyeva, K Berikova, D Zhumagul, K Temirkhanova and A Saparov. Chitosan-based biomaterials for tissue regeneration. *Pharmaceutics* 2023; **15**, 807.

[145] ML Pita-Lopez, G Fletes-Vargas, H Espinosa-Andrews and R Rodriguez-Rodriguez. Physically cross-linked chitosan-based hydrogels for tissue engineering applications: A state-of-the-art review. *Eur. Polym. J*. 2021; **145**, 110176.

[146] Y Zhu, Y Zhang and Y Zhou. Application progress of modified chitosan and its composite biomaterials for bone tissue engineering. *Int. J. Mol. Sci.* 2022; **23**, 6574.

[147] Y Luo and Q Wang. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. *Int. J. Biol. Macromol.* 2014; **64**, 353-67.

[148] SR Batool, MA Nazeer, E Yildiz, A Sahin and S Kizilel. Chitosan-anthracene hydrogels as controlled stiffening networks. *Int. J. Biol. Macromol*. 2021; **185,** 165-75.

[149] RAA Muzzarelli, ME Mehtedi, C Bottegoni, A Aquili and A Gigante. Genipin-crosslinked chitosan gels and scaffolds for tissue engineering and regeneration of cartilage and bone. *Mar. Drugs* 2015; **13**, 7314-38.

[150] N Alemdar. Synthesis of chitosan-based hydrogel by using photopolymerization technique. *Anadolu Univ. J. Sci. Tech. A Appl. Sci. Eng*. 2016; **17**, 391-400.

[151] A Sun, X He, X Ji, D Hu, M Pan, L Zhang and Z Qian. Current research progress of photopolymerized hydrogels in tissue engineering. *Chin. Chem. Lett.* 2021; **32**, 2117-26.

[152] N Samadi, M Sabzi and M Babaahmadi. Self-healing and tough hydrogels with physically cross-linked triple networks based on Agar/PVA/Graphene. *Int. J. Biol. Macromol*. 2018; **107**, 2291-7.

[153] A Rogina, L Pribolsan, A Hanzek, L Gomez-Estrada, G Gallego Ferrer, I Marijanovic, M Ivankovic and H Ivankovic. Macroporous poly(lactic acid) construct supporting the osteoinductive porous chitosan-based hydrogel for bone tissue engineering. *Polymer* 2016; **98**, 172-81.

[154] M Chen, R Jiang, N Deng, X Zhao, X Li and C Guo. Natural polymer-based scaffolds for soft tissue repair. *Front. Bioeng. Biotechnol.* 2022; **10**, 954699.

[155] M Ferdinand, R Vardai, J Moczo and B Pukanszky. Poly(lactic acid) reinforced with synthetic polymer fibers: Interactions, structure and properties. *Composites Part A* 2023; **164**, 107318.

[156] T Stampoultzis, P Karami and DP Pioletti. Thoughts on cartilage tissue engineering: A 21st century perspective. *Curr. Res. Transl. Med.* 2021; **69**, 103299.

[157] R Zhang, SJ Chang, Y Jing, LY Wang, C Ching-Jung and JT Liu. Application of chitosan with different molecular weights in cartilage tissue engineering. *Carbohydr. Polym.* 2023; **314**, 120890.

[158] C Weinand, WX Jian, GM Peretti, LJ Bonassar and TJ Gill. Conditions affecting cell seeding onto three-dimensional scaffolds for cellular-based biodegradable implants. *J. Biomed. Mater. Res. B* 2009; **91B**, 80-7.

[159] J Schmid, S Schwarz, R Meier-Staude, S Sudhop, H Clausen-Schaumann, M Schieke and R Huber. A perfusion bioreactor system for cell seeding and oxygen-controlled cultivation of three-dimensional cell cultures. *Tissue Eng. C* 2018; **24**, 585-95.