Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review

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Abstract

Once damaged, articular cartilage has very little capacity for spontaneous healing because of the avascular nature of the tissue. Although many repair techniques have been proposed over the past four decades, none has successfully regenerated long-lasting hyaline cartilage tissue to replace damaged cartilage. Tissue engineering approaches, such as transplantation of isolated chondrocytes, have recently demonstrated tremendous clinical potential for regeneration of hyaline-like cartilage tissue and treatment of chondral lesions. As such a new approach emerges, new important questions arise. One of such questions is: what kinds of biomaterials can be used with chondrocytes to tissue-engineer articular cartilage? The success of chondrocyte transplantation and/or the quality of neocartilage formation strongly depend on the specific cell-carrier material. The present article reviews some of those biomaterials, which have been suggested to promote chondrogenesis and to have potentials for tissue engineering of articular cartilage. A new biomaterial, a chitosan-based polysaccharide hydrogel, is also introduced and discussed in terms of the biocompatibility with chondrocytes. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In 1743, Hunter stated, “From Hippocrates to the present age, it is universally allowed that ulcerated cartilage is a troublesome thing and that once destroyed, is not repaired” [1]. Since then, a substantial amount of research has been conducted on articular cartilage, with significant advances in our understanding of the biological repair process being made over the past four decades. It has recently been demonstrated that articular cartilage has a spontaneous repair response in the case of full-thickness cartilage defects [2]. However, this repair response is limited in terms of form and function. In contrast, there have been no meaningful descriptions of repair processes in partial-thickness lesions limited to the cartilage itself [3].

While many repair techniques have been proposed over the past four decades, none have successfully regenerated long-lasting hyaline cartilage tissue to replace damaged cartilage [4]. In fact, most of the surgical interventions to repair damaged cartilage have been directed toward the treatment of clinical symptoms rather than the regeneration of hyaline cartilage, such as pain relief and functional restoration of joint structures and articulating surfaces [5]. The concept of a tissue engineering approach to cartilage repair was first proposed by Green in 1977 [6]. In this scenario, chondrocytes grown in an ex vivo environment would be transplanted into a cartilage defect. Clinical application of such a tissue engineering approach was first attempted by a Swedish group [7] with fair-to-excellent clinical results in their clinical subjects. Since then, chondral repair techniques utilizing laboratory-grown cells have attracted significant attention [4].

2. Historical background in cartilage injury and repair

An initial surgical attempt to restore the normal articulating surface of joint cartilage was made with the introduction of Pridie’s resurfacing technique [8,9]. This chondral repair technique utilized the disruption of subchondral bone to induce bleeding from the bone marrow, thus promoting the regular wound-healing mechanism in
the cartilage defect site. Since Pridie’s abrasion arthroplasty, several subchondral disruption techniques have been introduced in an attempt to improve the healing mechanisms of repaired tissue. They include subchondral drilling [10], arthroscopic abrasion [5,11,12], and microfracture technique [13]. Several experimental animal studies on full-thickness cartilage repair have revealed that subchondral breaching techniques created a fibrin clot formed from bleeding in the region of the cartilage defect. This clot was subsequently infiltrated by mesenchymal cells, and acted as a three-dimensional scaffold for migrating progenitor cells [2]. Within six weeks, these cells gradually differentiated and completely filled the defect region with a hyaline-like repair cartilage containing significantly less proteoglycan than the normal hyaline cartilage [10]. The repair tissue at this stage was also more cellular than the adjacent normal cartilage. Furthermore, the repair tissue showed no structural integration with residual cartilage. As a result, there was evidence of degeneration, fissuring in the cartilage tissue, and even a subsequent failure to maintain the normal appearance at a later stage [14,15]. It appears that, in the full-thickness defect, a spontaneous repair mechanism was present for the first several weeks after surgical repair, but later failed due to inadequate mechanical and biochemical conditions in the repaired tissue [16].

3. Current concepts in cartilage tissue engineering

Recently, tissue engineering concepts have been introduced to develop cell-based repair approaches for articular cartilage [6,17,18]. Tissue engineering of articular cartilage involves the isolation of articular chondrocytes or their precursor cells that may be expanded in vitro and then seeded into a biocompatible matrix, or scaffold, for cultivation and subsequent implantation into the joint. Certainly, the type of cell used to engineer cartilage is critical to the long-term outcome. Different cell populations that have been investigated in the experimental studies include matured articular chondrocytes [6,19], epiphyseal chondrocytes [20,21], mesenchymal stem cells [22], bone marrow stromal cells [23–25], and perichondrocytes [26].

The choice of biomaterial is also critical to the success of such tissue engineering approaches in cartilage repair. A variety of biomaterials, naturally occurring and synthetic, biodegradable and non-biodegradable, have been introduced as potential cell-carrier substances for cartilage repair [27]. The naturally occurring biomaterials include various forms of types I and II collagen-based biomaterial, in the form scaffold matrices [28–31], gels [22,32–34], or collagen–alginate composite gels [34]. The synthetic polymer-based biomaterials include polyglycolic acid (PGA) and poly-L-lactic acid (PLLA), or their composite mixture [35]. In cartilage tissue engineering, PGA [18,27,36], PLLA [17,26], and PGA–PLLA copolymers [27,37–39] have been studied for their efficacy as chondrocyte-delivering scaffolds in vitro and in vivo. Several investigators have also found that some non-biodegradable polymer substances, such as polytetrafluoroethylene [40], polyethylmethacrylate [41], and hydroxyapatite/Dacron composites [42], also facilitate the restoration of an articular surface. However, further studies are needed to objectively evaluate their merits in comparison with other biomaterials.

The ideal cell-carrier substance should be the one which most closely mimics the naturally occurring environment in the articular cartilage matrix. It has been shown that cartilage-specific extracellular matrix (ECM) components such as type II collagen and glycosaminoglycan (GAG) play a critical role in regulating expression of the chondrocytic phenotype and in supporting chondrogenesis both in vitro and in vivo [43,44]. Otherwise, chondrocytes may undergo de-differentiation and produce an inferior fibrocartilaginous matrix rich in type I collagen [45]. This inferior matrix then leads to a failure to form hyaline cartilage [46]. Thus, we can assume that the criteria for the choice of biomaterial in cartilage tissue engineering include biological friendliness and biomechanical strength [27,47]. These features may provide a biochemically and biomechanically appropriate environment necessary for engineered cells to regenerate a long-lasting hyaline cartilage in the defect site.

4. Polysaccharide-based hydrogels

Polysaccharides form a class of materials which have generally been underutilized in the biomaterials field. Recognition of the potential utility of this class of materials is however growing and the field of polysaccharide biomaterials is poised to experience rapid growth. Three factors have specifically contributed to this growing recognition of polysaccharide-based biomaterials. First is the large and growing body of information pointing to the critical role of saccharide moieties in cell signaling schemes and in the area of immune recognition in particular. Second has been the recent development of powerful new synthetic techniques with the potential for automated synthesis of biologically active oligosaccharides. These techniques may allow us to finally decode and exploit the language of oligosaccharide signaling. The third factor is the explosion in tissue engineering research and the associated need for new materials with specific, controllable biological activity and biodegradability.

Apart from their biological activity, one of the more important properties of polysaccharides in general is their ability to form hydrogels. Hydrogel formation can occur by a number of mechanisms and is strongly influenced by the types of monosaccharide involved, as well as the presence and nature of substituent groups. Polysaccharide gel formation is generally of two types: hydrogen bonded and ionic. Hydrogen-bonded gels are typical of molecules such as agarose (thermal gellation) and chitosan (pH-dependent gellation), whereas ionically
bonded gels are characteristic of alginates and carrageenans. However, the distinction is limited, since some charged polysaccharides exhibit hydrogen-bonded gel formation under neutral conditions.

Proteoglycans are one of the major macromolecules found in articular cartilage. These molecules consist of a core protein and covalently attached GAG chains. The GAGs are long, unbranched heteropolysaccharides, consisting of repeated disaccharide units, with the general structure: \( \text{\text{uronic acid–amino sugar}}_n \) (Fig. 1). The cartilage-specific GAGs include chondroitin 4-sulfate (glucuronic acid and \( N \)-acetyl-galactosamine with an \( \text{SO}_4^- \) on the 4-carbon position), chondroitin 6-sulfate (glucuronic acid and \( N \)-acetyl-galactosamine with an \( \text{SO}_4^- \) on the 6-carbon position) and keratan sulfate (galactose and \( N \)-acetyl-glucosamine with an \( \text{SO}_4^- \) on the 6-carbon position) [48,49]. The high charge density and lack of crystallinity make the sulfated GAGs highly water soluble. In solution, these GAGs assume a flexible rod conformation, and their generally low molecular weight (5–40 kDa) results in poor intrinsic gel formation properties. Because of this deficiency, the sulfated GAGs have not been extensively studied as biomaterials for cartilage repair. However, as described later, these molecules are capable of forming hydrogel complexes with oppositely charged ionic polymers, particularly the cationic polysaccharide chitosan. This interaction may form the basis of a new materials approach to cartilage tissue engineering.

The other important cartilage GAG is hyaluronic acid (glucuronic acid and \( N \)-acetyl-glucosamine). This molecule is one of the major components in synovial fluid. Hyaluronic acid molecules are also present in cartilage matrix as the backbone structure in proteoglycan aggregates. In general, hyaluronic acid plays a major role as an organizer of the ECM. Furthermore, its importance in wound healing is indicated by its transient increase during the granulation phase [50]. Purified hyaluronic acid has been employed as a structural biomaterial because of its high molecular weight and gel forming ability. The properties of the molecule may be broadly altered by chemical modification. For example, partial esterification of the carboxyl groups reduces the water solubility of the polymer and increases its viscosity. Extensive esterification generates materials that form water-insoluble films or swellable gels [51]. Ethyl and benzyl esterified hyaluronate membranes have demonstrated excellent healing responses and biodegradability in vivo. The fully esterified membranes have in vivo lifetimes of several months, whereas the partially esterified forms have been shown to degrade within a few weeks [52].

Hyaluronic acid and derivatives have been used as therapeutic aids in the treatment of osteoarthritis as a means of improving lubrication of articulating surfaces and thus reducing joint pain [53,54]. Several in vitro culture studies have also demonstrated that hyaluronic acid has a beneficial effect by inhibiting chondrocytic chondrolysis mediated by fibronectin fragment [55]. Hyaluronic acid has also been shown to have anti-inflammatory effects [56–58], as well as inhibitory effects on prostaglandin synthesis [59,60], and proteoglycan release [61] and degradation [62]. Based on these findings, it has been suggested that hyaluronic acid may be a good candidate for a cell-carrier material in chondrocyte transplantation therapy [63]. Bone-marrow-derived mesenchymal cells, delivered in a hyaluronic acid hydrogel, demonstrated good potential for cartilage resurfacing in animal models [23,64]. However, further studies are needed to evaluate cell–material interactions and the mechanical characteristics of the cell–material constructs in order to determine the efficacy of hyaluronic-acid-based hydrogels as cell carriers for cartilage repair.

5. Chitosan as a biomaterial

Given the importance of GAGs in stimulating the chondrogenesis [43], use of GAGs or GAG analogs as components of a cartilage tissue scaffold appears to be a logical approach for enhancing chondrogenesis. One such candidate is chitosan, a partially de-acetylated derivative of chitin, found in arthropod exoskeletons. Structurally, chitosan is a linear polysaccharide consisting of \( \beta \) (1 \( \rightarrow \) 4) linked D-glucosamine residues with a variable number of randomly located \( N \)-acetyl-glucosamine groups. It thus shares some characteristics with various GAGs and hyaluronic acid present in articular cartilage, as shown in Fig. 1 [65]. Depending on the source and preparation procedure, chitosan’s average molecular weight may range from 50 to 1000 kDa. Commercially available preparations have degrees of deacetylation ranging from 50 to 90%.

Chitosan is a semi-crystalline polymer and the degree of crystallinity is a function of the degree of deacetylation. Crystallinity is maximum for both chitin (i.e. 0% deacetylated) and fully deacetylated (i.e. 100%) chitosan. Minimum crystallinity is achieved at intermediate degrees of deacetylation. Because of the stable, crystalline structure, chitosan is normally insoluble in aqueous solutions above pH 7. However, in dilute acids, the free amino groups are protonated and the molecule becomes fully soluble below \( \sim \) pH 5. The pH-dependent solubility of chitosan provides a convenient mechanism for processing under mild conditions. Viscous solutions can be extruded and gelled in high pH solutions or baths of non-solvents such as methanol. Such gel fibers can be subsequently drawn and dried to form high-strength fibers. The polymer has been extensively studied for industrial applications based on film and fiber formation, and the preparation and mechanical properties of these forms have been reviewed previously [66–68].

Much of the potential of chitosan as a biomaterial stems from its cationic nature and high charge density in
solution. The charge density allows chitosan to form insoluble ionic complexes or complex coacervates with a wide variety of water-soluble anionic polymers. Complex formation has been documented with anionic polysaccharides such as GAGs and alginates, as well as synthetic polyanions such as poly(acrylic acid) [69–73]. Because the chitosan charge density is pH dependent, transfer of these ionic complexes to physiological pH can result in dissociation of a portion of the immobilized polyanion. This property can be used as a technique for local delivery of biologically active polyanions such as the GAGs and DNA. For example, heparin released from ionic complexes may enhance the effectiveness of growth factors released by inflammatory cells in the vicinity of an implant [74]. In the case of DNA, complexation with chitosan has been shown to protect plasmids from degradation by nucleases and also facilitates cellular transfection by poorly understood interactions with cell membranes [75–77].

The N-acetylglucosamine moiety in chitosan is a structural feature also found in the GAGs. Since GAG properties include many specific interactions with growth factors, receptors and adhesion proteins, this suggests that the analogous structure in chitosan may also have related bioactivities. In fact, chitosan oligosaccharides have been shown to have a stimulatory effect on macrophages, and the effect has been linked to the acetylated residues [78]. Furthermore, both chitosan and its parent molecule, chitin, have been shown to exert chemotactic effects on neutrophils in vitro and in vivo [79–81].

In vivo, chitosan is degraded by enzymatic hydrolysis. The primary agent is lysozyme, which appears to target acetylated residues [82]. However, there is some evidence that some proteolytic enzymes show low levels of activity with chitosan. The degradation products are chitosan oligosaccharides of variable length. The degradation kinetics appear to be inversely related to the degree of crystallinity which is controlled mainly by the degree of deacetylation. Highly deacetylated forms (e.g. > 85%) exhibit the lowest degradation rates and may last several months in vivo, whereas samples with lower levels of deacetylation degrade more rapidly. This issue has been addressed by derivatizing the molecule with side chains of various types [83–86]. Such treatments alter molecular chain packing and increase the amorphous fraction, thus allowing more rapid degradation. They also inherently affect both the mechanical and solubility properties.

A number of researchers have examined the tissue response to various chitosan-based implants [65,73,83,87–96]. In general, these materials have been found to evoke a minimal foreign body reaction. In most cases, no major fibrous encapsulation has been observed. Formation of normal granulation tissue, often with accelerated angiogenesis appears to be the typical course of healing. In the short term (≤7 d), a significant accumulation of neutrophils in the vicinity of the implants is often seen, but this dissipates rapidly and a chronic inflammatory response does not develop. The stimulatory effects of chitosan and chitosan fragments on immune cells mentioned above may play a role in inducing local cell proliferation and ultimately integration of the implanted material with the host tissue.

One of chitosan’s most promising features is its excellent ability to be processed into porous structures for use in cell transplantation and tissue regeneration. Porous chitosan structures can be formed by freezing and lyophilizing chitosan–acetic acid solutions in suitable molds [97]. During the freezing process, ice crystals nucleate from solution and grow along the lines of thermal gradients. Exclusion of the chitosan acetate salt from the ice crystal phase and subsequent ice removal by
Fig. 2. SEM images of various forms of porous chitosan scaffolds.

lyophilization generates a porous material whose mean pore size can be controlled by varying the freezing rate and hence the ice crystal size (Fig. 2). Pore orientation can be directed by controlling the geometry of thermal gradients during freezing.

The mechanical properties of chitosan scaffolds are mainly dependent on the pore sizes and pore orientations. Tensile testing of hydrated samples shows that porous membranes have greatly reduced elastic moduli (0.1–0.5 MPa) compared to non-porous chitosan membranes (5–7 MPa). The extensibility (maximum strain) of porous membranes varied from values similar to non-porous chitosan (∼30%) to greater than 100% as a function of both pore size and orientation. The highest extensibility was obtained with a random pore orientation structure frozen rapidly at −78°C to give pores 120 μm in diameter. Porous membranes exhibited a stress–strain curve typical of composite materials with two distinct regions: a low-modulus region at low strains and a transition to a 2–3-fold higher modulus at high strains. The tensile strengths of these porous structures were in the range of 30–60 kPa.

Chemical derivatization of chitosan provides a powerful means to promote new biological activities and to modify its mechanical properties. The primary amino groups on the molecule are reactive and provide a mechanism for side group attachment using a variety of mild reaction conditions. The general effect of addition of
a side chain is to disrupt the crystal structure of the material and hence increase the amorphous fraction. This modification generates a material with lower stiffness and often altered solubility. Of course, the precise nature of changes in chemical and biological properties depends on the nature of the side group. For example, N-alkyl derivatives can exhibit reduced solubility and micellar aggregation in solution for alkyl lengths greater than five carbons. In addition, the characteristic features of chitosan, such as cationic, hemostatic and insoluble at high pH, can be completely reversed by a sulfation process which can render the molecule anionic and water soluble, and also introduce anticoagulant properties. The variety of groups which can be attached to chitosan is almost unlimited, and side groups can be chosen to provide specific functionality, alter biological properties, or modify physical properties.

6. Chitosan–GAG composite material and its biological interaction with articular chondrocytes

Use of chitosan as a biomaterial or drug delivery agent has recently drawn a considerable attention in the applications for the repair of articular cartilage. Lu et al. [98] has demonstrated that the chitosan solution injected into the knee articular cavity of rats caused a significant increase in the density of chondrocyte in the knee articular cartilage, suggesting that the chitosan could be potentially beneficial to the wound healing of articular cartilage. Mattioli-Belmonte [99] has shown that the bone morphogenetic protein (BMP)-7, associated with N,N-dicarboxymethyl chitosan induces or facilitates the repair of articular cartilage lesions.

As mentioned above, chitosan is capable of forming insoluble ionic complexes with the negatively charged GAGs. This ionic cross-linking mechanism can be used to immobilize chondroitin sulfates within hydrogel materials which mimic the GAG-rich ECM of the articular chondrocyte. A recent study by Denuziere [73] has demonstrated that, when associated with various polyelectrolytic GAGs, the chitosan has a protective effect against GAGs hydrolysis by their specific enzymes. Recent preliminary studies performed in our laboratory have demonstrated the biocompatibility and the chondrogenic characteristics of such GAG-augmented chitosan hydrogels [100–102]. When chondrocytes were cultured on the chondroitin 4-sulfate (CSA)-augmented chitosan hydrogel surfaces, the cells maintained many morphological and functional features characteristic of chondrocytes in normal cartilage. These promising results are briefly summarized below.

6.1. Cell growth kinetics

Chondrocytes seeded at low density (6 x 10^4 cells/ml) onto either the standard polystyrene culture plates or the CSA-chitosan membrane surfaces attached to the substrate within 2h. Over the course of one week, the majority of cells attached to the polystyrene surface assumed a fibroblast-like morphology, and the cell population rapidly expanded. In contrast, primary chondrocytes seeded at the same low density onto the CSA-chitosan surface assumed a spherical morphology after attachment. After one week, chondrocytes attached to the CSA-chitosan had largely maintained a rounded or polygonal morphology and undergone only a modest degree of mitosis (Fig. 3). The increase in cell population of chondrocytes cultured on the polystyrene and CSA-chitosan surfaces were 313 ± 61 and 66 ± 28%, respectively.

6.2. Scanning electron microscopic morphology

Scanning electron microscopic evaluation of the CSA-chitosan membrane revealed a matrix material with an irregular surface. We found no evidence that chondrocytes were embedded or partially embedded in the hydrogel membrane. Rather, the chondrocytes adherent to the CSA-chitosan had formed discrete points of attachment with the biomaterial through pseudopodial extensions (Fig. 4). These microscopic findings suggest that maintenance of the phenotypic morphology is due to an interaction between cell-membrane receptors and the CSA-rich ECM. A similar phenomenon was previously described with primary murine articular chondrocytes cultured on a collagen type II membrane [103].

6.3. Proteoglycan synthesis

The total 35S-sulfate-incorporation rate produced by primary chondrocytes cultured on the CSA-chitosan surface (2580 ± 460 cpm/10^6 cells) was approximately equal.
to that produced by chondrocytes cultured on the standard polystyrene surface (2810 ± 320 cpm/10^6 cells). Thus, CSA-chitosan supports, but does not appear to enhance the total amount of PG synthesized by primary articular chondrocytes.

6.4. Collagen synthesis

After one week, chondrocytes cultured on the control polystyrene surface (P) demonstrated a complex collagen phenotype. The protein migration pattern shown in Fig. 5 indicates that the collagens produced by cells in this culture system were types I and II, type I collagen being the predominant collagen synthesized. The presence of type I collagen is indicated by the presence of α2(I) chains. Also present in smaller amounts were protein bands consistent with α1(V)/α1(XI) and α2(XI) chains. Although the high molecular weight protein bands were not identified, they may represent chains of type III collagen, type IX collagen, and/or β-chains of type I collagen.

For chondrocytes cultured on the CSA-chitosan, on the other hand, the predominant collagen produced by chondrocytes was type II, indicated by the presence of the strongly stained protein band in the α1(II) position with only faint protein bands in the α2(I) position. Chondrocytes in this culture system also demonstrated protein bands consistent with α1(V)/α1(XI) and α2(XI) chains. The high molecular weight protein bands in the CSA-chitosan group were not identified, but may represent pepsin-derived fragments of type IX collagen. These data indicate that the primary chondrocytes cultured on CSA-chitosan maintain the synthesis of cartilage-specific collagens.

7. Future direction and discussion

Despite extensive research on articular cartilage conducted over the past four decades, we still do not have the definitive answer for a successful repair of damaged cartilage. While many new repair techniques have recently emerged and demonstrated promising results in experimental models, few have exhibited long-term clinical efficacy.

Tissue engineering has recently emerged as a new interdisciplinary science to repair injured body parts and restore their functions by using laboratory-grown tissues, materials and artificial implants. It is our hope that tissue engineering approaches with novel materials will provide methods for developing a greater understanding of articular cartilage and associated pathologies. This understanding of the tissue pathogenesis will help us develop new methods to enhance the repair of damaged cartilage.

The biochemical and morphologic results of this study of primary chondrocytes cultured on CSA-chitosan re-emphasize the favorable influence of articular cartilage-specific matrix components on the chondrocytic phenotype in vitro. Our findings, coupled with the favorable biological properties of chitosan, suggest that CSA-chitosan may be well suitable as a carrier-material for the transplant of autologous chondrocytes as described by Brittberg et al. [7] and/or as a scaffold for the tissue engineering of cartilage-like tissue as originally described by Vacanti et al. [104]. Future studies using chondrocytes that have undergone proliferative expansion in vitro will explore this potential. It is also very important that the chitosan scaffold possesses necessary mechanical properties in order to provide seeded chondrocytes with a proper biomechanical environment [105].
studies are necessary to investigate the material and mechanical properties (such as porosity, compressive elastic modulus, permeability, and viscoelastic properties) of the CSA-chitosan scaffold. A development of a chitosan-based material that can support chondrogenesis may be significant not only in terms of the quality of neocartilage produced, but also in terms of the ability of that tissue to integrate with the host matrix.

Along with tissue engineering as a new emerging science for various tissue repairs, gene therapy has also been suggested as a means of delivering sustained therapeutic levels of anti-arthritis gene products to the transplanted cells in diseased joints [58,106,107]. Genes have been successfully introduced locally to the target cells in the knee joints of rabbits by both ex vivo and in vivo methods, with gene expression lasting for several weeks [108,109]. Such gene therapy has been successfully combined with chondrocyte transplantation to investigate the feasibility of ex vivo gene transfer to chondrocytes in full-thickness cartilage defects [32]. Combination of chitosan-based cartilage scaffolds with gene therapy and the use of growth factors will hopefully improve the long-term outcomes of cartilage repair in clinical settings.

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References


