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REVIEW

A Review on the Reconstruction of Articular Cartilage Using Collagen Scaffolds

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Abstract

The reconstruction of articular cartilage has a great importance since more and more people are affected by osteoarticular diseases. Among these, osteoarthritis occupies a central place and affects mostly mature patients. Cartilage contains a significant quantity of collagen, so a first step in osteochondral reconstruction is represented by the developing of new materials based on collagen that can be used as implants in hard tissue engineering. There are many possibilities to construct biomaterials containing collagen, depending on the purpose. The collagen scaffolds prepared must have a series of characteristics such as biocompatibility and osseointegration. Before use, those scaffolds have to be well chemically characterized and tested in vitro experiments on cell cultures (chondrocytes are responsible for the cartilage regeneration) and in vivo studies on animal models (especially rats). The most important ways to prepare collagen scaffolds that can be used in the articular cartilage reconstruction are reviewed here.

Keywords: *cartilage, collagen, scaffolds, osteoarthritis, reconstruction, cells, chondrocytes*

1. Introduction

More than 10% of the population having over 60 years is affected by osteoarthritis in USA and the number is increasing due to the obesity risk and the modern life style (K.E. Keenan & al [1]).

There are three different types of cartilage: hyaline, fibrocartilaginous and elastic. Cartilage contains interstitial fluids (75%) and has a macromolecular network containing collagen (20% of the cartilage volume) and proteoglycans (glycosaminoglycans). Chondrocytes are the cells accountable for the synthesis and maintaining the cartilage, and represent about 1% of its volume (E.B. Hunziker & al [2]). The progress of osteoarthritis is associated with the changes in structure and orientation of collagen and with the loss of proteoglycans (A. Hanifi & al [3]).

The cartilage matrix can be determined using specific methods such as: T2 mapping, delayed gadolinium-enhanced MR imaging of cartilage (dGEMRIC), sodium imaging, T1p imaging, diffusion-weighted imaging, (M.D. Crema & al [4]), radiography, CT arthrography, ultrasonography (V. Jurans & al [5]). The collagen content extracted from cartilage can be evaluated using different techniques: high performance liquid chromatography (HPLC), mass spectroscopy (MS), and Fourier transform infrared spectroscopy (FTIR) (A. Hanifi & al [3]).

The reparation of the cartilage affected by osteoarthritis can be accomplished using biological products such as: mesenchymal stem cells namely mature adipocytes (J. Bushmann & al [6], A.M. Rogriguez & al [7], M.E. Fernyhough & al [8], L. Danisovic & al [9], Z. Alharbi & al [10], D. Zheng & al [11], M. Maumus & al [12]) or platelet-rich plasma, an autologous blood plasma injected in cartilage having the capacity to regenerate the cartilage (N.A. Smyth &

al [13], B.A. Tinsley & al [14], K. Akeda & al [15], Y. Mifune & al [16], B.J. Cole & al [17], J. De La Mata & al [18]).

2. Collagen scaffolds for cartilage reconstruction

One of the causes for the weak tendency of the defects in articular cartilage to heal spontaneously is the absence of a sufficient number of chondrocytes to get into the lesion and the lack of a temporary fibrin scaffold to accommodate the relocation of cells into the defect. This situation can lead to osteoarthritis and to the necessity of a total joint replacement.

The cartilage repair (S. N. Redman & al [19], A. Matsiko & al [20]) can be enhanced by using the methods of tissue engineering, namely the use of cell seeded scaffolds (S. Frenkel & al [21]). Scaffolds in cartilage tissue engineering are used in the shape of either hydrogels (C. G. Williams & al [22], D. Bosnakovski & al [23]) or porous matrices (W.J. Li & al [24], E. Steck & al [25], Y. Wang & al [26], M. Liu & al [27]). The 3D scaffolds implanted in cartilage are made of porous materials, and besides being a delivery vehicle for cells, growth factors or genes, they reinforce structurally the defect and prevent neighboring tissue to access it (S.M. Vickers & al [28]).

The scaffold's construction defines the ultimate shape of the newly grown tissue. Since the thickness of human articular cartilage is in the range from less than 500 μm up to7.1 mm (B. Kladny & al [29]), the thickness of the scaffold for cartilage should fit the thickness of defects, to allow easy cell seeding and have good mechanical properties as an initial cell support (G. Chen & al [30]).

The biomechanical properties of articular cartilage imply the capability to restore its shape and recover into its structure the lost liquids, after loading. This is therefore a crucial quality for a scaffold (B. Kinner & al [31], C. Vinatier & al [32]). For the construction of the scaffolds (Z. Cao & al [33]) both natural materials: collagen, gelatin, fibrin, chitosan, alginate, hyaluronan, agarose, silk (H.A. Awad & al [34], Y. Wang & al [26], D. Bosnakovski & al [23], G.R. Ragetly & al [35]) and synthetic biodegradable macromolecules (polylactic acid, polyglicolic acid, polycaprolactone (G. Chen & al [36]) were used.

But fibrin is difficult to attach to the graft (D.H. Hendrickson & al [37]), and hyaluronan with chondrocytes (D. Robinson & al [38]) also has no adhesive qualities and little stiffness to assure their fastening on large defects. Collagen seems to be the most promising biomaterial for scaffolds, since it presents a natural adhesion to the cells surface, and contains biological information able to direct the activity of cells. Collagen is the main component of the articular cartilage extracellular matrix, which plays an important role in maintaining the chondrocyte phenotype and supporting the chondrogenesis, both in vitro and in vivo. (T. Minas & al [39], S. Nehrer & al [40], S. Wakitani & al [41], C.J. Hunter & al [42]). Moreover, collagen is known for its excellent biocompatibility and low antigenicity (C.H. Lee & al [43]) and its degradation products are not dangerous for the organism.

In the preparation of scaffolds, collagen fibrils have to be cross-linked, in order to improve its mechanical properties and to slow its degradation (L. Ma & al [44]). Physical methods, such as ultraviolet irradiation (K.S. Weadock & al [45]) or gamma rays irradiation (B.C. Liu & al [46]) are less effective. So cross linking is achieved mostly by using different compounds as chemical agents such as glutaraldehyde (L. Ma & al [47]), diisocianate, diepoxide, formaldehyde, N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (S. N. Park & al [48]).

For instance, this last agent was used for the chemical cross-linking of refibrillized jellyfish collagen to give porous scaffolds (B. Hoyer & al [49]). There is an increasing

tendency to use as cross-linker genipin – a natural product much less toxic than glutaraldehyde and epoxides (F.L. Mi & al [50], Y. Yuan & al [51], V. Chiono & al [52], L.P. Yan & al [53], L. Bi & al [54]).

Chondrocyte allografts were used in combination with collagen (S. Wakitani & al [55]) to resurface articular cartilage defects. A fibrin matrix with chondrocytes was also applied and produced repair tissues with 62% type II collagen content (D.H. Hendrickson & al [37]).

There are many examples for the use of different collagen scaffolds with chondrocytes (S. Mizuno & al [56], M. Ochi & al [57], C.R. Lee & al [58], P. Cherubino & al [59], S. Andereya & al [60]). On bovine type I collagen scaffolds, murine chondroprogenitor cells were cultured *in vitro*, and after being embedded with chondrocytes were implanted in articular defects in rabbits (S. Maor & al [61], A. Ben-Yishay & al [62]). A similar procedure was applied with equine chondrocytes embedded in collagen scaffolds and cultured in vitro (A.J. Nixon & al [63]). Collagen scaffolds give the necessary stiffness for the implant to be pressed securely into the defect. Scaffolds made of chondrocyte-collagen composites, obtained by culturing chondrocytes in expanded collagen scaffolds, were tested in articular defects (A.E. Sams & al [64], A.E. Sams & al [65]). They improved the cartilage healing in extensive defects, but their long-term durability is doubtful.

A type II collagen-glycosaminoglycan scaffold was used as a nonviral gene delivery vehicle for the gene transfer to seeded adult articular chondrocytes, to produce an elevated, prolonged and local expression of insulin-like growth factor (IGF)-1 for enhancing cartilage regeneration. (R.M. Capito & al [66]). Chondrocyte-seeded type II collagen scaffolds were used in vitro, and the effects of scaffold cross-link density and bioreactor culture environment on chondrogenesis were evaluated (S.M. Vickers [28]), as well as their mechanical properties (C.R. Lee & al [58]).

Mixtures of collagen with the amine groups containing polysaccharide chitosan, also a natural and biodegradable polymer, were also often used (S.S. Silva & al [67]). Scaffolds made from collagen and chitosan, and cross-linked with genipin were also investigated to find the effects of the preparative method on properties such as porosity, swelling rate, degradation rate, mechanical properties and biocompatibility (L.P. Yan & al [53], L. Bi & al [54]). Hyaluronan was also added to collagen scaffolds, and the obtained 3D sponges were injected with chondrocytes and investigated *in vitro* in order to identify mechanisms by which extracellular matrix molecules influence chondrocyte function (F. Allemann & al [68]).

The effect of pore size in collagen porous scaffolds was investigated (Q. Zhang & al [69]) and no obvious effect on cell proliferation and adhesion was found, but there were different effects on cartilage tissue regeneration. Three cartilage repair techniques were compared experimentally: treatment with a cell-free collagen type-I gel, a collagen type-I gel seeded with autologous chondrocytes, and abrasion arthroplasty (U. Schneider & al [70]). The implantation of the cell-free collagen gel lead to a high-quality repair tissue that equals a cell-based procedure after 1 year postoperatively. The study demonstrates the high chondrogenic potential of the collagen gel.

Also collagen type I gels or scaffolds without cells, but combined with marrow stimulation techniques have been used successfully for cartilage repair (L. de Girolamo & al [71], J.P. Benthien & al [72], J.P. Benthien & al [73], J. Gille & al [74], A.A. Dhollander & al [75], T. Efe & al [76], T. Kusano & al [77]). Atelocollagen - a highly purified type I collagen from which pepsin and telopeptid were removed to make it nonimmunogenic - mixed with fibrinogen and thrombin, was used in autologous collagen-induced chondrogenesis technique for cartilage repair (A.A. Shetty & al [78]). Along with collagen, chitosan was often used,

single or mixed with other biomacromolecules, such as alginates (P.B. Malafaya & al [79], N. Iwasaki & al [80]). A collagen-glycosaminoglycan (GAG) scaffold was used as an analog of extracellular matrix *in vitro* (J.M. Zaleskas & al [81]).

On the other hand, tissue engineering used also scaffolds made from synthetic - but biocompatible and biodegradable polymers, such as the polyesters: poly(lactic acid) – PLA, poly(glycolic acid) - PGA, or their copolymer poly(dl-lactic-co-glycolic acid) –PLGA, these can be easily formed into designed shapes in which chondrocytes or differentiated stem cells were seeded. Such constructs are then implanted in the defect articulation (D.W. Hutmacher & al [82]).

Other synthetic polymers were also used to this aim, single or in combination with natural compounds (F.T. Moutos & al [83]), for instance as polyurethane (Z. Li & al [84]), poly (ε-caprolactone) – PCL with starch (J.T. Oliveira & al [85]), or PC L and collagen (P. Prabu & al [86]). While synthetic polymers have relatively hydrophobic surfaces, a disadvantage for cell seeding, scaffolds from natural polymers such as collagen have hydrophilic surfaces, beneficial to cell seeding and cell attachment.

But these natural macromolecules present lower mechanical properties in the implants. Therefore combinations of the two kinds of materials, a hybrid scaffold comprising a highly hydrophilic natural component together with a fibrous synthetic component with higher strength should give better results. Such hybrids can contain a two-dimensional synthetic fiber component (G. Chen & al [30], W. Dai & al [87]), or a three-dimensional structure formed form the synthetic component, using a non-fibrous method, for example salt leaching (T. Sato & al [88], G. Chen & al [89]).

A composite made from collagen with PLGA fibers was used for tissue engineering of articular cartilage with the thickness adjustable between 200 μm and 8 mm (G. Chen & al [90], G. Chen & al [30]). The PLGA forms a strong knitted mesh, with the role of a skeleton, including in its openings the web-like collagen microsponges, which facilitate cell seeding, cell distribution, and tissue formation. A blend of poly (L-lactide)-g-chondroitin sulfate with poly (L-lactide) was applied in cartilage tissue engineering (C.T. Lee & al [91]).

Another composite biomaterial made from synthetic and natural polymers was poly (ε-caprolactone)-graft-type II collagen-graft-chondroitin sulfate (PCL-g-COL-g-CS) (K. Y. Chang & al [92]) was used as a biomimetic matrix, which promoted the spreading and growth of chondrocytes. Scaffolds for cartilage tissue engineering were prepared from hybrid materials made of polyester, collagen and chitosan: collagen/PLA, chitosan/PLA, and collagen/chitosan/PLA (A.M. Haaparanta & al [93]).

The use of autologous chondrocytes cells on scaffolds for the repair of cartilage defects is limited by the induction of morbidity at the donor site and the instability in monolayer cultivation (M. Keeney & al [94]). An alternative to these is the use of mesenchymal stem cells from various tissues, such as bone marrow (T. Mimura & al [95]) or adipose tissue [Keeney 2011]. This problem is now intensely investigated in cartilage tissue engineering (C.G. Williams & al [22], W.J. Li & al [24], S.M. Vickers & al [28]). A concentration-gradient collagen scaffold was devised showed enhanced efficiency in the transport of mesenchymal stem cells to the central region of the full-thickness cartilage defects improving its regeneration (T. Mimura & al [95]).

Collagen and mixed scaffolds were also used for the transfer of other therapeutic materials, for the transport of DNA, collagen scaffolds were used for instance (R.E. Samuel & al [96]), but also scaffolds made from synthetic polymers, such as PLGA (L.D. Shea & al [97], J. H. Jang & al [98], Y. K. Luu & al [99]), poly(D,L-lactide)–poly (ethyleneglycol)

(PLA–PEG) [Y.K. Luu & al [99]). A porous collagen scaffold hybridized with PLGA microbeads loaded with insulin was prepared to be used for the release of insulin for application to cartilage tissue regeneration. (H.S. Nanda & al [100]).

3. Conclusions

Nowadays, the use of scaffolds is the principal technique in articular cartilage repair. Both natural (mostly collagen) and synthetic materials are used in the construction of scaffolds, but hybrid materials give the best results. Cross linking and the selection of the suitable agent is important in the manufacture of scaffolds. The scaffolds reinforce structurally the defect and serve as vehicle for the transport of chondrocytes, mesenchymal stem cells or other bioactive agents.

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