

Biomaterials for Cartilage Tissue Engineering

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Abstract

One of the most challenging issues of musculoskeletal medicine is represented by injuries of the articular cartilage due to the poor regenerative properties of this tissue. A consequence of these injuries is represented by osteoarthritis. Osteoarthritis is the most common chronic condition of the joints, caused because of the progressive wear and tear on articular cartilage. A solution to prevent progressive joint degeneration in osteoarthritis is represented by a surgical intervention which offers the advantage of the success of total joint replacement, but also offers several disadvantages such as slower remodeling, immune reaction and disease transmission. In the last years, the researchers have found a solution to avoid surgical intervention by using biomaterials. This study aims to provide an updated survey of the major progress in the field of biomaterials for cartilage tissue engineering, including biomaterials (natural, synthetic or composites), their advantages or disadvantages and the main seeding cell sources. Also, this review focuses on the progress made in the field of biomaterials for cartilage tissue repair and/or regeneration over the last years.

Keywords: Cartilage; Hydrogels; Scaffolds; Stem cells; Tissue engineering

Introduction

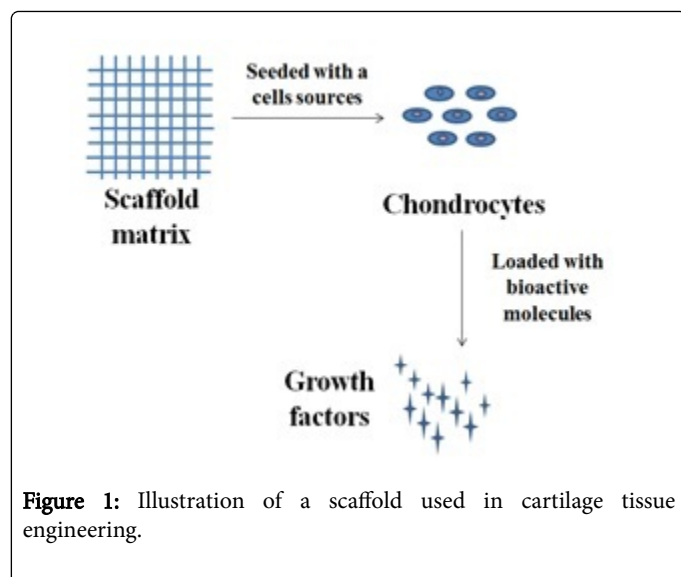
One of the big problems for cartilage tissue engineering is represented by the poor regenerative properties of this tissue and in the last time the researchers have tried to developed solutions to regenerate, repair and/or improve injured or diseased articular cartilage functionality. It was reported that each year over 6 million people visit hospitals in the U.S.A. for various wrist, knee and ankle problems. Osteoarthritis is the most common chronic condition of the joints, caused because of the progressive wear and tear on articular cartilage. This disease affects over 12.4 million people older than 65 years [1]. A solution to prevent progressive joint degeneration in osteoarthritis is represented by a surgical intervention which offers the advantage of the success of total joint replacement. But, the big disadvantage of surgical intervention refers to the fact that the treatments for repair of cartilage damage are often less than satisfactory and not always restore the full function or return the tissue to its native normal state [2]. Also, other disadvantages such as slower remodeling, immune reaction and disease transmission are represented by the use of the allograft [3].

Because of the fact that the surgical intervention presents a lot of disadvantages, in 1977 it was carried out the first experiment for cartilage repair by Green. They had grown chondrocytes in an *ex vivo* environment and the cells proliferated through many generations and then the cells were transplanted into a cartilage defect of a rabbit. It was reported that after 10 days it was observed a large repair of the articular defect [4]. In cartilage tissue engineering, scaffolds can provide a 3D structure (Figure 1) for cartilage cells and help to the cell adhesion and proliferation, but on the entire cartilage repair process

the physical and biochemical properties are crucial for these scaffolds [3].

The repair and the regeneration of a damaged tissue involve the presence of cells able of proliferation, differentiation, which can give a functional contribution to the regenerative processes. In the case of biomaterials, these have to accomplish many problems such as:

- The suitable reparative cells should be chosen to form a functional tissue (chondrocytes);
- The scaffold must be ideal for support and transplantation (biocompatible, good mechanical properties);
- It must exist bioactive molecules (growth factors, cytokines) to support the formation of the tissue;
- Grafting and safety studies [5,6].



Cartilage Structure and Function

Articular cartilage is a thin layer with unique viscoelastic properties which facilitates the transmission of loads to the underlying subchondral bone and provides a lubricated and smooth surface for low friction articulation [7,8]. Its thickness and composition vary depending on the species and age. In the case of human articular cartilage, the average thickness is at most a few millimetres. This tissue has low metabolic activity and it consists of chondrocytes (which are responsible for the maintenance of a stable and abundant extracellular matrix (ECM)) and a dense ECM composed of 75-80% water, a 50-70% collagen II and 15-30% proteoglycan macromolecules [7]. The chondrocytes interact with ECM with the help of integrins, these molecules serve as mechanical links between the chondrocytes and ECM and aid in cell homeostasis [8-10].

The cartilage can be divided according to his functions in four zones: the superficial zone, the middle zone, the deep zone and the zone of calcified cartilage.

The superficial zone is the articulating surface than makes up approximately 10% to 20% of articular cartilage thickness and provides a smooth gliding surface. Also, this zone has the highest collagen content and the fibrils are densely packed and have a highly ordered alignment parallel to the articular surface, but the disadvantage of this zone is represented by the fact that has the lowest compressive modulus and will deform approximately 25 times more than the middle zone. The chondrocytes found in this zone preferentially express proteins that have protective functions and secrete relatively little proteoglycan.

The middle zone is represented by the articular cartilage in proportion of 40-60%, its compressive modulus is higher than the superficial zone and the chondrocytes are more rounded than in the superficial layer. The collagen fibrils in this zone are thicker fibers and are aligned obliquely to the surface.

The deep zone represents 30% of the cartilage and consists in collagen fibrils with large diameter and oriented perpendicular to the articular surface. This zone contains the lowest water concentration and has the highest quantity of proteoglycan and compressive modulus. In this zone, the chondrocytes are arranged parallel to the

collagen fibers and perpendicular to the joint line. The calcified cartilage is separated from the deep zone by the tidemark which rests directly on the subchondral bone. This zone contains small cells in a chondroid matrix [11-13].

Articular cartilage is one of the most exposed tissues every day because of its ability to reduce surface friction and joint stress. For example, in the case of moderate walking this tissue has the ability to deform and enlarge its surface contact area to lessen the effect of direct loads by decreasing applied stress [7].

Biomaterials in Cartilage Tissue Engineering

Due to their limited potential of regeneration, the articular cartilage damage represents as one of the most challenging tasks in musculoskeletal therapeutics and a way to solve this problem is represented by biomaterials. These biomaterials have to present some advantages such as biocompatibility and bioresorbability, it must support cell growth, proliferation and differentiation, provide ideal mechanical properties and it must transport of nutrients and cell waste. In the last years, the researchers have tested both natural and synthetic biomaterials to design a suitable environment to provide necessary biological signals to control cellular behavior towards cartilage repair and cell support [14,15].

These biomaterials can be dividing into two classes: natural and synthetic materials. Natural materials such as collagen, chitosan, alginate, fibrin, cellulose, etc. present weak mechanical properties and their physical properties can vary from source to source. Synthetic materials such as poly (lactic-co-glycolic acid) (PLGA), polymer of lactic acid (PLA), polycaprolactone (PCL), etc., presents the advantages that provide consistent, controllable and precise mechanical properties like stiffness, porosity and elasticity, but present the disadvantages that some of these materials can induce cytotoxicity [16,17]. To overcome the disadvantages of single materials the researchers have applied composite materials because these materials can be modified by biochemical and physical methods to retain their advantages and overcome their disadvantages.

Biochemical methods: To solve the problems of natural (the weak mechanical properties) or synthetic materials (poor hydrophilicity and weak cell adhesive ability) the scaffolds were combined with a biological modifier. Once with the introduction of this modifier in the original material the scaffolds will present better tissue compatibility and provide an appropriate microenvironment for cell growth and proliferation [3].

Physical methods: Physical modification of scaffolds occurs by methods like filtration, UV light irradiation and compression to improve the porosity and biomechanical property of materials which lead to the cartilage repair. Also, the manipulation of scaffolds can produce a significant influence on the functions of macrophages [18].

In a study it was reported that after the cartilage-derived matrix (CDM) scaffold was treated with dehydrothermal (DHT) or UV light irradiation, it was observed that the scaffold not only prevent cell-mediated contraction, but also can support cell attachment [3]. In another study, it was demonstrated that glutaraldehyde crosslinking of collagen scaffold have increased the vascularization in a murine subcutaneous implantation model [18].

Natural scaffolds have greater biological interaction with the cells due to their bioactive properties which allow them to have better performance in the biological system [19]

Collagen: Collagen represents the principal component of ECM; it's a natural protein with a triple-helix structure, which can form a reversible gel. This protein presents excellent tissue compatibility, facile biodegradation and its degradation products are absorbed facily without inflammation. In several studies the collagen gel was used as substrates for articular cartilage substitutes, but its biomechanical property is still not satisfying [3,20,21]. It was reported that different collagen scaffold structures may provide different immunogenicity [22]. Also, it was demonstrated that collagen-hyaluronic acid (HA) scaffolds own great potential as appropriate matrices for promoting cartilage tissue repair, but a higher molecular weight of HA let to greater collagen fiber maturation which can increase cartilaginous matrix expression [3,23].

In several studies have been demonstrated that a combination of collagen with chondrocytes and stem cells facilitated cartilage tissue growth *in vitro* and *in vivo*. For example, Chen et al. used bone marrow mesenchymal stem cell-seeded type II collagen scaffolds to repair cartilage defect of the rabbits. It was observed after 8 weeks that chondrocyte-like cells with lacuna structure were found in the newly formed tissue with no signs of inflammation and after 24 weeks it was observed that the affected tissue was almost completely repaired [24]. In another study, isolated bovine chondrocytes seeded into rhCII gels were injected subcutaneously into the back of nude mouse model. It was observed that this complex offered the ability to promote cell proliferation and mechanical strength for the formation of cartilage [25]. In a recent study, Wang et al. used core-shell nanofibrous scaffold fabricated by collagen and poly(L-lactic acid-co-epsilon-caprolactone) to encapsulate rhTGF-β3 and bovine serum albumin into the core of the nanofibers for tracheal cartilage regeneration. It was reported that the proliferation and morphology analyses have indicated the good biocompatibility of the fabricated nanofibrous scaffold and it was concluded that this scaffold could be an effective delivery and serve as a promising tissue engineered scaffold for cartilage regeneration [26].

Chitosan: Chitosan is a partially de-acetylated derivative of chitin, found in arthropod exoskeletons and a semi-crystalline polymer. His structure consists of β(1,4) linked D-glucosamine residues with a variable number of randomly located N-acetyl-glucosamine groups [27]. Like the native cartilage, this polymer contains glycosaminoglycan (GAG) and hyaluronic acid, presents properties such as biocompatibility, biodegradability and non-toxicity and in the last years it was very used in the cartilage tissue engineering field. The main disadvantage of this polymer refers to the lack of gelling properties, leading to the possibility that it will flow out of the joint when applied, forming cartilage-like tissue ectopically [19,20,28].

Hoemann et al. created defects in the distal part of the femur of sheep and a group was treated with chitosan-glycerol phosphate/autologous whole blood and the implants were allowed to solidify and the other group doesn't received any further treatment (control group). At six months, in the case of the defects that had been treated with chitosan-glycerol phosphate/blood it was observed significantly more hyaline repair tissue ($p < 0.05$) and a much larger amount of cells and collagen compared with control [29]. In another study, chitosan was used in combination with glycerol phosphate (GP) and tested for cartilage repair in adult rabbits. Comparing with control (no treatment) it was observed that the treatment with chitosan-GP

improved the vascularization and this led to the establishment of more hyaline repair cartilage [30]. Also, when it was used a chitosan scaffolds enriched with D-(+) raffinose in osteochondral defects in rabbits (the cartilage defects were created in distal femurs) it was reported that after 4 weeks the defects are not completely healed [31]. Because of its ideal properties in the future studies, chitosan will be used as a growth factor delivery system to promote tissue regeneration [32].

Alginate: Alginate is a polysaccharide extracted from brown algae, but the main disadvantage refers to its inferior biomechanical properties. This polymer has extensively investigated as a cartilage substitute; serving as supporting scaffold for cell growth and it was reported in several studies that the alginate interacts with the cells via specific surface receptors, facilitating cell migration and the proliferation [2,20]. For example, Wang et al. prepared a 3D alginate scaffold using a microfluidic device and then the scaffold was seeded with porcine chondrocytes and implanted in the dorsal subcutaneous site of SCID mice. After 4 weeks the cartilage structures were formed and the authors concluded that this scaffold presents the ability to maintain functional phenotypes for chondrocytes [33]. In several studies, it was reported that the size of microcavity hydrogel would affect the growth and the function of chondrocytes. Zeng et al. studied the effect of microcavitary alginate hydrogel by using porcine chondrocytes encapsulated into alginate hydrogel with various sizes of gelatin microspheres (80–120 μm/150–200 μm (250–300 μm). It was reported that the cells cultivated on in the small microcavitary hydrogel (80–120 μm) presented the higher capacity of proliferation and expression of cartilaginous markers [34]. In the present experiments, to resolve the main disadvantage of alginate (biomechanical properties) the researchers have focused on creating a hybrid structure. For example, in a study it was used alginates hydrogels modified with low molecular weight hyaluronate via carbodiimide chemistry using ethylenediamine as a linker. After the ATDC5 cells were encapsulated in hydrogels, it was observed that the alginate-hyaluronate hydrogels promoted chondrogenic differentiation of ATDC5 cells compared with control (alginate hydrogels). Also, the ability to promote chondrogenic differentiation of ATDC5 cells was dependent on the amount of hyaluronate in the hydrogels. The ideal hydrogel from the point of view of the mechanical stiffness and chondrogenic differentiation were the hydrogels with hyaluronate/alginate ratio (wt/wt) reached 0.5 and 1.0. In contrast, hyaluronate/alginate ratio (wt/wt) reached 2.0 hydrogels presented low mechanical stiffness [35].

Fibrin: Fibrin is a component of blood clots which presents properties such as biocompatibility and biodegradability and in the last years was widely used in applications for cartilage repair. A big advantage of human fibrin gels is represented by the fact that they are approved by the Food and Drug Administration (FDA) [20,36].

In some studies it was reported that in cartilage application this biomaterial is not as chondro-permissive as other well developed hydrogels. For example in the case of bone marrow mesenchymal stem cells (BMSCs) encapsulated in fibrin the results confirm a diminished chondrogenic potential [37]. At the moment, this subject is still studied. Promising results were reported when the researchers tried to use fibrin hydrogels functionalized with cartilage extracellular matrix (ECM). Almeida et al. developed an injectable fibrin hydrogel functionalized with cartilage ECM microparticles and to enhance the chondrogenesis it was added exogenous TGF-β3. It was reported that the *in vivo* tests confirmed a larger amount of cartilage-like tissue

formed compared with control (constructs loaded with gelatin microspheres) [38].

Synthetic scaffolds

Due to the main disadvantage of natural materials (low mechanical properties) the researchers have tried to use synthetic materials which present good mechanical properties, elastic modulus and degradation rate (Young modulus of native cartilage is approximately 0.2-0.3 GPa). In cartilage tissue engineering has been studied polyglycolic acid (PGA), poly(ethylene glycol) (PEG), polyurethane (PU), poly-L-lactic acid (PLLA) and PGA-PLLA copolymers due to their efficacy as chondrocyte-delivering scaffolds *in vitro* and *in vivo* [27].

- Poly (ethylene glycol) (PEG) is a polyether extensively used as material support in cartilage tissue engineering. In several studies it was reported that when PEG is used in combination with other natural or synthetic materials it was observed an improvement strength and compression modulus [20]. For example, in a study it was reported that human chondrocytes encapsulated into the PEG-albumin hydrogel and subcutaneously implanted in immunodeficient mice resulted to be a beneficial implant support for chondrocytes because the cells maintained their characteristic genotype expressing type I and II collagen and aggrecan [39]. Neumann et al. obtained PEG hydrogels by photo-clickable reactions and encapsulated juvenile bovine chondrocytes in the hydrogels. It was observed that chondrocytes deposited increasing amounts of sulfated glycosaminoglycans and collagens (especially collagen type II) in the hydrogels and it was confirmed the degradation of the hydrogels [40].

Hydrogels prepared from PEG are widely applied in cartilage tissue engineering, but their poor mechanical strength still represents a big problem. So, the future applications will perform to overcome this disadvantage. For example, in a recent study, Wang et al. fabricated an injectable high strength hydrogel based on 4-arm star PEG for cartilage tissue engineering and it was followed the relationship between the dynamics of the pre-gel solution and the mechanical property of the resultant hydrogel. It was observed that when gelation takes place at the overlap concentration, the resultant hydrogel has a local maximum compressive strength approx. 20 MPa, while still keeps ultralow mass concentration and Young's modulus. The hydrogel loaded with chondrocytes was transplanted into the subcutaneous pocket and an osteochondral defect model in SCID mice and it was observed that the cells can proliferate and maintain their phenotypes in the hydrogel [41].

- Poly-L-lactic acid (PLLA) was approved in 2004 by Food and Drug Administration, the L-isomer of this synthetic polymer presents ideal properties such as biocompatibility and biodegradability [42]. In a study, it was compared several polylactides and related polymer scaffolds (PLLA and PLGA) administered with a chondrocyte/atelocollagen mixture and then these scaffolds were implanted subcutaneously in nude mice. After 2 months of implantation all the scaffolds were studied and it was observed that their 3-D shape was maintained throughout the probing period and the higher level for of type I and type II collagen production it was reported in the case of PLLA and PLGA scaffolds [43]. In another study, it was reported a compressive modulus of approximately 6 MPa in PLLA scaffolds with a porous microstructure and it was observed that when PLLA is combined with fibrin gel, the mechanical properties of this complex are increasing and also a higher cell proliferation occurs [44].

Natural/synthetic scaffolds

In the last years, due to the necessity to use an ideal scaffold the researchers have combined a natural material which presents better biocompatibility and cell affinity (than a synthetic material), with a synthetic material which presents good mechanical properties, elastic modulus and degradation rate. In table 1 are presented some of this biomaterials.

Biomaterials	Cell type	Study type	References
Fibrin-PLGA	Chondrocytes	<i>In vitro</i>	[45]
Collagen-PLC	Chondrocytes	<i>In vivo</i>	[46]
Alginate/PVA	Chondrocytes	<i>In vitro</i>	[47]
Collagen/PEG	Human mesenchymal stem cells	<i>In vitro</i>	[48]
Hyaluronan/PEG	Chondrocytes	<i>In vivo</i>	[39]

Table 1: Overview of studied natural/synthetic biomaterials for articular cartilage applications.

The Role of Stem Cells

Numerous cell types have been proposed for cartilage tissue applications such as chondrocytes, bone-marrow derived mesenchymal stem cells (MSCs), stem cells isolated from bone marrow or embryonic stem cells because these cells exhibit a chondrogenic potential under appropriate culture conditions [5].

Chondrocytes

Chondrocytes are the major cell type present in cartilage that synthesizes and turnover a large volume of ECM components like collagen, hyaluronan, glycoproteins and proteoglycans. These cells are derived from MSCs and occupy 1-5% of the total cartilage tissue. It was reported that the *in vitro* expansion of these cells is limited because once removed from their extracellular environment and expanded in monolayer, chondrocytes would rapidly lose their differentiated phenotype. Recently, it was reported a solution for this problem, if these cells are suspending in a 3D environment like collagen gel, alginate beads and agarose gel the chondrocyte phenotype can be retained or re-expressed [5,49,50].

Mesenchymal stem cells (MSCs)

MSCs are multipotent progenitor cells which are able to differentiate into a variety of connective tissues cells like cartilage, bone, ligament fat, and tendon both *in vitro* and *in vivo*. It was reported that the regenerative effects of MSCs are due to their ability to stimulate tissue repair while also providing an anti-inflammatory effect, through direct secretion of bioactive molecules. Currently, MSCs can be isolated from Bone Marrow, but also from other sources like adipose tissue, articular cartilage, umbilical cord blood, dermis, synovial membrane, synovial fluid, muscle, etc., with similar phenotypic characteristics but different proliferation and differentiation potentials [5,51].

Conclusion and Perspective

Articular cartilage is a highly specialized and organized tissue produced by chondrocytes, but its big disadvantage is represented by the poor regenerative properties. Due to the frequent deterioration of the cartilage tissue in adult humans which provoke osteoarthritis (a disease which affects 12.4 million people older than 65 years), the researchers were motivated in the last decades to find suitable ways to treat damaged joints and repair cartilage defects. The ideal scaffold for cartilage tissue should present properties such as biocompatibility, cell affinity and suitable porosity. It has been demonstrated that a variety of materials with suitable properties are being explored to be used in cartilage tissue applications and these materials can be natural (biocompatible, biodegradable, low toxicity, relatively low cost, bioactive), synthetic (porosity, tensile strength, elastic modulus, degradation rate) or composites (which combine the characteristics of both natural and synthetic materials). At this moment, it has been understood the advantages and the disadvantages of both natural and synthetic materials and the researchers are working to overcome these disadvantages. Also, the future studies will be based on understanding the *in vivo* developmental mechanisms that are involved in the specificity of articular cartilage generation and to create a durable cartilage repair tissue.

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