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Injectable Natural Biomaterials for Cartilage Tissue Engineering

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Abstract

Osteoarthritis (OA) has been a common disease among the elderly people but there are no existing solutions clinically efficient for OA. Therefore, tissue engineering has developed in the field of cartilage regeneration. As we all know, tissue engineering has three basic elements: cell, scaffold and growth factor. Injectable scaffolds which can form a gel with original liquid form at the site of defect through a syringe are an emerging scaffold for cartilage engineering. Scaffold can be composed of many materials such as natural materials, synthetic materials, composite materials as well as hybrid materials. Here we mainly discuss the natural materials for cartilage regeneration.

Keywords: Cartilage Tissue; Osteoarthritis; Injectable scaffold

Background

A novel and promising treatment has been proposed for improving cartilage regeneration in cartilage tissue engineering [1-3]. Extracellular matrix (ECM) could not only provide physical cues for chondrocytes but also biological cues which can direct cellular behavior. Also, the chondrocytes are encapsulated within the ECM, balancing the information inside and outside the chondrocytes [4]. Therefore, providing a good circumstance for cartilage is the most important thing for cartilage engineering up to now [5,6]. Chondrocytes live in a three dimensional (3D) extracellular matrix (ECM) microenvironment where the basic components such as glycosaminoglycans (GAGs) and collagen type II can be secreted. The content of GAGs and collagen II plays an important role in the physiological and mechanical strength of the implanted scaffold-cell constructs. The glycosaminoglycans (GAGs) (glycosaminoglycans) resemble chondroitin sulfate (CS), heparin sulphate (HS) and usually form proteoglycans by attaching to ECM proteins [2,3,7].

A suitable cartilage tissue engineering (CTTE) scaffold used to repair cartilage defects needs the following properties. First of all, the scaffold should be easy to handle, as well as keeping stable and reproducible under physiological conditions; secondly, it needs to be completely biocompatible and cytocompatible and as well as could help for tissue remodeling; thirdly, the defect sites can be easily filled, and the scaffolds must have excellent integration property which means it can strongly adhere and integrate well with the surrounding host cartilage tissue; finally it is supposed to better imitate cartilaginous ECM properties and promote chondrogenic potential of cells [8].

The significance of tissue engineering cartilage

Cartilage is highly specialized, and is composed of chondrocytes embedded in an extracellular matrix which mainly consists of collagen, proteoglycans, and water. Cartilage has no internal vascular network unlike bone with great regenerative potential, thus cartilage's innate ability is limited for repair and regeneration. Therefore, cartilage injury always leads to scar formation, resulting in structure and function losing permanently. Injury of the joint surface can not heal spontaneously and always develop to degenerative joint disease. Suspending chondrocytes in a three-dimensional matrix, which is similar to their natural environment, could make the cells maintain the native phenotype and produce their extracellular components [9].

Articular cartilage damage mechanism

Trauma, the change of joint biomechanical properties and long-term immobilization are the mechanism of articular cartilage damage. The first two mechanisms are related to the destruction of collagen fiber structure, while as prolonged immobility causing the degeneration of cartilage injury is considered to be due to the effect of the pressure pump cartilage loss that leads to joint soft tissue contractures, synovial infiltration or adhesions with cartilage. In consequence, articular surface pressure increased which prevents the mesenchymal cells in the synovial fluid from permeation.

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Nutritional deficiencies or cartilage damage or synovial fluid fibrinogen interaction could suppress the effect of chemokine factors and mitogenic factor.

Cell sources

The basic principle of cartilage tissue engineering involves isolation of cells that may be expanded in vitro and then either encapsulated in a three-dimensional matrix for proliferation or mixed with in situ gelling systems and subsequently implanted/injected into the site of injury. Different sources of cells have been explored for cartilage tissue engineering which includes primary autologous/heterologous chondrocytes, fibroblasts, stems cells (mesenchymal stem cells and embryonic stem cells. For tissue engineering, articular cartilage have limited ability to regenerate in vivo which we have to expand the chondrocytes prior to use, often causing dedifferentiation of cells. The materials we choose to use must have the capacity to preserve the cell phenotype, up regulating the expression of collagen type II, aggrecan, the transcription factor Sox 9, and at the same time down regulating the expression of collagen type I.

How to choose an injectable scaffold

Currently, a lot of materials have been used as an injectable scaffold clinically to repair cartilage defects, including natural materials, synthetic materials, composite materials and hybrid materials and this review mainly focuses on the latest developments of natural materials. Physical and chemical properties of materials such as swelling ratio, mechanical properties and the ability to integrate with the surrounding tissue is of vital importance for cartilage defects repair. Followings are the recent researches of natural materials and their respective introductions.

Natural Materials

Natural polymers derived from biological macromolecules and could be used to fabricate scaffolds for cartilage defect repair. Natural polymers should possess inherent biocompatibility and bioactivity as well except for providing a suitable 3-D environment for encapsulated and/or infiltrating cells. Natural polymers in common us include HA, collagen [10,11], alginate[12,13], chitosan [14-16], heparin, fibrin [17,18], cellulose [17] and so on.

HA

HA, also called hyaluronan, is a glycosaminoglycan existed widely in many tissues. HA plays a significant role in cell proliferation and migration as one of the main components of the extracellular matrix.

Direct transplantation of mesenchymal stem cells into the knee joints: Sato et al. [18]. Set up a model of spontaneous osteoarthritis in the Hartley strain guinea pigs knee joints of, aiming to prove if intra-articular transplantation of MSCs in hyaluronic acid (HA) has the favorable result. After five weeks partial cartilage repair was observed in the HA-MSC group but not in control groups. In the HA-MSC group, after examination the cells showcased the ability of proliferation, differentiation and migration of labeled MSCs. In addition, in the OA cartilage, they observed strongly positive immumohistochemical staining for type II collagen of both native chondrocytes as well as transplanted MSCs. These findings approved intra-articular injection's validity in of HA-MSC hybrid in the application for OA.

Modulation of MSCs chondrogenesis in a tunable HA hydrogel: Wei Seong Toh et al. [19] used covalent cross-linking in vivo by adding peroxidase and hydrogen peroxide (H_2O_2) to form an injectable and biodegradable hydrogel system made up of hyaluronic acid-tyramine (HA-Tyr) conjugate. These observations suggest that cellular density could be modulated by the changeable 3-D microenvironment of the HA-Tyr hydrogels during chondrogenesis. It also has a surprising effect on spatial organization of cells and matrix biosynthesis [19].

Collagen

Collagen accounting for about 25% to 35% of the whole-body protein content is the main component of the extracellular matrix in many kinds of connective tissues. Whereas and Meanwhile, the fibroblasts are the main cells producing collagen.

In this research, Pulkkinen HJ et al. [20] isolated bovine chondrocytes (6×106) which were seeded into rhCII gels (rhCII-cell) and subsequently injected subcutaneously into the back of nude mice. The results demonstrated that extracellular matrix stained heavily with toluidine blue and had strongly positive collagen type II immunohistochemical staining. No evidence showed vascular invasion or mineralization. These observations demonstrated that rhCII-gel had good ability to promote cell proliferation and mechanical strength for the formation of cartilage neotissue. RhCII material might be available in repairing cartilage defects.

Alginate

An anionic polysaccharide alginic acid, known as alginate as well, is distributed extensively in the cell walls of brown algae, which could form a viscous gum by combining with water [21]. What's more, it also can be considered as a material for micro-encapsulation [22]. After combined with calcium, alginate can gain an effect on various types of medical products such as skin wound dressings to promote healing [23], having better outcomes than conventional ones.

Some researchers made preparations of calcium alginate microcapsules, which was made up with by human subchondral cortico spongious progenitor cells to and the chondrogenic differentiation potential was analyzed by RT-PCR. When encapsulated in calcium alginate microcapsule stimulated with TGF–β3, these cells can differentiated into chondrocytes. In the course of culture, the mechanical stability and permeability of calcium capsules were analyzed and found to be suitable for stable cell immobilization.

Therefore, from the cell biology perspective, Ca-alginate is extremely suitable for regenerative medicine of cartilage defects combined with progenitor cells [24].

Moreover, alginates are anionic linear polysaccharides with 1, 4-linked b-d-mannuronate (M) and 1, 4-linked a-L-guluronate (G) residues, forming ionic hydrogels in the presence of divalent ions (calcium ions) Biji Balakrishnan et al. [25] believe that alginate-based hydrogels have been shown to have better properties than poly (ethylene glycol)-based scaffolds by detecting the expression of collagen type II and aggrecan and GAG deposition [26] and also can induce re-differentiation of chondrocytes, which are de-differentiated into fibroblast phenotype in monolayer cultures and this is of great importance [27]. Nevertheless, unmodified alginate hydrogels are non-biodegradable [28] and can cause foreign body cell reactions and immunological responses [29,30]. Furthermore, there are no special interactions between alginates and cells and only by incorporating cell-adhesive peptides [31], gelatin [32], collagen type I and beta-tricalcium phosphate [33] can this hydrogel have cell-adhesive properties.

This kind of scaffolds can also act as a cellular matrix, promoting

healing by attracting cells from the surrounding native tissue towards the matrix and can function within the matrix towards regeneration of new tissue [25].

Chitosan

Chitosan has biocompatiblity and biodegradability, which as a result has been extensively applied as an injectable scaffold in reparing cartilage defect. In Hao T's [34] research, chondrocytes were incorporated into chitosan hydrogel scaffold, aiming to obtain tissue-engineered cartilage and repair sheep's articular cartilage defects. Chitosan owns specific interactions with many growth factors. Tissue-engineered cartilage scaffolds were fabricated by mixing sheep chondrocytes within a chitosan hydrogel in vitro, from which the chondrocytes survived and maintained their ability to secrete matrix. In vivo, within 24 weeks, sheep's cartilage defects were completely repaired. Besides, the chitosan-based hydrogel could also support matrix accumulation of chondrocytes In conclusion, this study demonstrates the success of a new approach in its ability to reconstruct a kind of chitosan-based scaffold to repair articular cartilage defects and it may be clinically significant in the future. [34]

In addition, there does another chitosan-beta glycerophosphate-hydroxyethyl cellulose (CH-GP-HEC) scaffold own biocompatible, biodegradable and cytocompatible properties that could make a transition from sol to gel at 37°C. similar to the physiological temperature. In Naderi-Meshkin H's. study, incorporated human mesenchymal stem cells (hMSCs) chondrogenic differentiation capacity of the was also ensured after inducted with TGF-β3. During the 28-day investigation, MSCs showed excellent survival and proliferative rates within CH-GP-HEC hydrogel which indicate the material's good cytocompatibility [35].

Fibrin

Fibrin, a component of blood clots, can actually a fabricate a kind of protein-based scaffold which could support a variety of human tissues. Therefore, it also has a wide application in repairing cartilage defects due to its biodegradability and biocompatibility.

Fibrin gel, a natural biomaterial, shows excellent biocompatibility, promoting cell attachment and migration, and can be degraded in a controllable way has many advantages better than synthetic materials as a tissue-engineered scaffold. Additionally, fibrin gel imitates the natural blood-clotting process and can have the ability to self-assembles into a polymer network, with already existing reports for fibrin gel to repair the damaged cardiac and cartilage tissues in situ. Moreover, we could also use fibrin gel as a cell carrier to reduce the loss of cells due to the forces during cell delivery processes so as to guarantee certain number cells to enhance the cell viability and tissue regeneration. [36].

In repairing cartilage defect, there appears evidence showing that fibrin is not as chondro-permissive as other well developed hydrogels [37], with bone marrow mesenchymal stem cells(BMSCs) and adipose derived stem cells(ADSCs) showing a diminished chondrogenic potential when encapsulated in fibrin [38-40]. Therefore, we need to further functionalize this versatile injectable hydrogel system to optimize for cartilage repair therapies. Articular cartilage extracellular matrix (ECM)-derived materials have been previously used to fabricate cartilage grafts with promising results [41-44]. Moreover, ECM particles have also been used to functionalize other biomaterials in order to enhance chondrogenesis [45-47].

Cellulose

Xu Y et al, [17] reported they developed a new method using adipose tissue-derived stromal cells (ADSCs) incorporated into hydroxypropylmethyl cellulose (HPMC) for fabricating injectable tissue-engineered cartilage in vivo. HPMC, a cellulose derivative polymer, is a methylcellulose modified with propylene glycol ether groups attached to the an hydroglucose of the cellulose [47,48]. HPMC has excellent solubility and can transit from liquid of low viscosity at 23°C to a solid gel at 37°C. Therefore, ADSCs isolated from rabbit subcutaneous fat that is and cultured in chondrogenic differentiation medium with TGF-β1 and basic fibroblast growth factor (bFGF) are encapsulated in the HPMC hydrogel. After cultured for a short time, the neocartilage began to secrete cartilage-like components like collagen II as well as GAGs, proved by Masson's trichrome staining, picrosirius red staining and AB-PAS staining. We are not sure if the differentiated chondrocytes maintained their phenotype, although ther examinations like histological, immunohistochemistry and RT-PCR guaranteed that after induction, the ADSCs differentiated into chondrocytes. The induced ADSCs were mixed with 15% HPMC, and then injected into the dorsal subcutaneous tissue of nude mice and after cultured for 8 weeks. Neocartilage can be observed at the site of injection. Therefore, induced ADSCs-formed chondrocytes mixed with HPMC has been proved to offer a feasible solution for cartilage tissue engineering.

Summary

Natural biomaterials great potential for cartilage tissue engineering as we already mentioned in the beginning. On one hand, cartilage cells or MSCs of any kind need a 3-D microenvironment to form into a complete cartilage tissue. It's just the reason why we use scaffolds. Not like osteogenic cells, chondroblasts need an agent that can fully incorporate them like hydrogel. On the other hand, natural material-related scaffold can promote the integration between the scaffold and the native cartilage tissue to achieve s mechanical stability.

The existing techniques like microfracture, mosaicplasty, autologous chondrocyte transplantation, and osteochondral allograft transplantation to treat cartilage defects have solved some problems of patients, but cartilage produced by these methods are often composed of collagen type I, while this kind of collagen is mainly present in scar tissue, as well as tendons, ligaments, the endomysium of myofibrils, the dermis and so on. Thus it is inferior to hyaline cartilage both chemically and mechanically [48].

Compared with conventional methods in tissue engineering, the injectable scaffolds have the advantages of minimal invasiveness and targeted delivery at the preferred site. It also relieves the pain of the patients and is easy for operation. Moreover, it can fill the defects with irregular shape when the hydrogel form from sol to gel in situ. In my opinion, for cartilage tissue engineering, the natural materials should be used to repair cartilage defects because they have the similar components of native cartilage and good biocapability and cytocapability and it can be easier for the natural materials to integrate with the surrounding tissue. Moreover, natural materials could help secrete more extracellular matrix such as collagen II and GAGs. However, the mechanical strength of natural materials is a big issue. The tissue-engineered construct should be able to maintain physiologic loads within the joints over a long term. To solve this kind of problem, many researchers combined natural materials such as

HA with synthetic materials such as PEG. The hybrid material could not only enhance cell proliferation and differentiation but also could enhance the mechanical strength of cartilage. Therefore, I believe the best materials for cartilage tissue engineering in the future should be a combination of these two kinds of materials, not just one of them.

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