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Research Article

Research Progress of Osteochondral Composite Scaffolds in Tissue Engineering Cartilage Repair

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Abstract: Repair and regeneration of articular cartilage has always been a major challenge in the medical field due to its peculiar structure (e.g. sparsely distributed chondrocytes, no blood supply). Cartilage tissue engineering is one promising strategy for cartilage repair, however, one critical issue for cartilage tissue engineering is the integration between tissue-engineered and native cartilage. In recent years, osteochondral tissue engineering has attracted growing interest for overcoming this problem. Herein, we review the development of osteochondral tissue engineering. Firstly, currently used seed cells in osteochondral tissue engineering will be described. Secondly, several types of scaffolds and their (dis)advantage for osteochondral tissue engineering will be introduced. Thirdly, the growth factors currently used in osteochondral tissue engineering will be presented and discussed.

Keywords: *Articular cartilage; repair; seed cells; biological scaffolds; growth factors; cartilage tissue engineering*

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1 Introduction

Articular cartilage is the hyaline cartilage that covers the bone end of synovial joint in articular cavity. The articular cartilage in normal body has good elasticity. It exists as an important part of buffer system. It plays a role of conduction decompression and alleviates joint injury. Therefore, the integrity of articular cartilage plays a crucial role in facilitating body movement and locomotion. However, adult human articular cartilage lacks vasculature, without blood supply; besides, chondrocytes distribute sparsely in cartilage tissue. Therefore, damaged cartilage has limited self-repair capacity. Significantly, articular cartilage injury is usually accompanied by the lesions of the sub articular bone, whose aggravation will cause serious osteoarthropathy, greatly affecting the daily life of patients. However, the therapeutic effects of current clinical treatments of osteochondral injury, such as drilling and grinding of cartilage, periosteal and perichondral transplantation, subchondral bone drilling and artificial joint replacement, are not satisfactory. In recent years, the rapid development of tissue engineering has provided great hope for the damage of cartilage and subchondral bone.

The technological process of tissue engineering is that the seed cells are culture-expanded in vitro, incubate in a scaffold form a cell-scaffold construct, and then the construct is implanted into the tissue defect in vivo, wherein the scaffold is gradually degraded with tissue formation in situ. This review summarizes the advantages of osteochondral tissue engineering on the three key elements-cell, scaffold, and growth factors.

2 Seed cells

2.1 Autologous or allogeneic chondrocytes and osteocytes

At present, the main cell for osteochondral tissue engineering research is obtained from autologous or allogeneic bone and articular cartilage in vitro by the combination of mechanical separation and enzymatic digestion. From the point of cell proliferation and adherence, the subcultured cells are superior to the primary cells, but from the autologous or allogeneic bone and articular cartilage cell sources are limited, and

the proliferation ability is low, metabolism is slow, in vitro culture depends on high density, after subcultured to about the sixth generation, a series of changes in cell function, such as chondrocyte markers. The synthesis and secretion of type II collagen decreased, while the synthesis and secretion of type I and type III collagen increased, gradually losing the original characteristics of high differentiation, that is, the tendency of dedifferentiation, phenotype (especially type II collagen) is difficult to maintain[1]. With the emergence and development of three-dimensional scaffolds, many experiments have proved that three-dimensional environment is conducive to maintaining the phenotypic stability of chondrocytes cultured in vitro, maintaining the highly differentiated state of chondrocytes, and even restoring dedifferentiated chondrocytes in monolayer culture. Artificial extracellular matrix provides a continuous microenvironment for cells to proliferate and differentiate. Therefore, autologous or allogeneic osteochondral cells are still the main cells in the study of osteochondral tissue engineering.

2.2 Osteo chondrogenic cells (such as periosteal cells)

Periosteum is a tissue that can grow in cell and whole tissue culture. It is one or several necessary conditions for tissue-engineering osteochondral repair. Periosteum contains pluripotent hematopoietic stem cell and mesenchyma stem cell, which has the potential to form cartilage and bone; Periosteum can be used as a scaffold for whole tissue transplantation or as a target for adhesion of other cells and growth factors. It can produce biological stimulants that promote cartilage formation, including TGF-beta 1, IGF-I and BMP-2, as well as receptors of these molecules. Therefore, periosteal cells can also be used as a cell source for repairing articular cartilage[2]. Wang Yurong et al.[3] have achieved good results in repairing articular cartilage defects with autogenous periosteum transplantation in clinic. Hommigna et al.[4] used autologous perichondrium transplantation to repair articular cartilage defect of knee joint, and achieved good results, suggesting that periosteum or perichondrium derived chondrocyte can be used as a cell source for repairing articular cartilage.

2.3 Mesenchymal cells (Such as synovial cells, embryonic stem cells and bone marrow stromal cells)

The research of bone marrow mesenchymal stem cells (BMSCs) has been a hot topic in recent years. MSCs have attracted wide attention because of their super self-renewal, high reproductive capacity and multi-directional differentiation ability. It can synthesize ECM components, including collagen type I, fibronectin, collagen type IV and laminin of basement membrane, which can secrete cytokines such as interleukin-7, IL-8, IL-11 and stem cell factor. Some studies have shown that MSCs obtained from mice, rabbits and humans can stably differentiate into bone marrow tissue[5], osteoblasts, chondrocytes, adipocytes and even myocytes[6-9] under the action of dexamethasone, VitD3 and cytokines such as BMP-2. Paulette et al.[10] found that about 20% of MSCs cultured without hematopoietic cells and proliferative stimulator were in G0 phase and seldom proliferated, which had strong self-renewal ability. It is considered that these cells are sufficient to supply differentiated osteoblasts, chondroblasts, adipocytes and myocytes. Shan Yuxing et al.[11] adopted the cultured rabbit MSCs to make the artificial cartilage culture. Mature cartilage tissue was developed in vitro and vivo, which confirmed that MSCs could be used as functional cells in cartilage tissue engineering. Caplan et al.[12] attempted to isolate MSCs from the bone marrow of a degenerative arthritis patient and injected them directly into the articular cavity to repair the articular surface after amplification in the culture medium. Experiment results showed that local injection of MSCs could promote the repair of knee joint damage in rabbits. People have tried to repair articular cartilage injury with chondrocyte and apply chondrocyte to the reconstruction and repair of osteoarthritis patients. It shows that MSCs is feasible to repair articular cartilage defect, but the normal chondrocyte obtained from patients is very limited. Therefore, MSCs can differentiate into chondrocytes, making them an alternative source of chondrocytes for tissue engineering cartilage.

3 Composite bio-scaffolds:

3.1 Natural materials

Natural materials can be obtained from animals or plants, which are widely used in cartilage tissue engineering because of their good biocompatibility, easy absorption of degradation products, and little adverse reactions with growth factors contained. At present, collagen, chitosan, hyaluronic acid, alginate, agarose and silk protein are commonly used as natural materials. Ochi et al.[13] used collagen gel to culture autologous

chondrocytes to treat articular cartilage defects of knee joint, and achieved good results. Chitosan can cross-link with chondroitin sulfate to form a thermo sensitive hydrogel, which can be converted into gel by liquid injection into the body, and is widely used in cartilage tissue engineering[14]. Toh et al.[15] adopted hyaluronic acid as scaffold material. Under the action of selective growth factor, human embryonic stem cells successfully differentiate into cartilage tissue. Wang et al.[16] adopted highly organized alginate scaffolds to be the porcine chondrocytes of stent inoculation, and cartilage-like tissue formation was found after transplantation. Kawakami et al.[17] still used silk protein with three pore sizes as scaffolds, and chondrocyte inoculation resulted in cartilage formation. The main shortcomings of natural materials have poor mechanical properties, which can't provide strong support in the process of cartilage tissue formation. For large-scale preparation, the material sources are limited, and there's the risk of spreading disease.

3.2 Synthetic material

Compared with natural materials, synthetic materials have better mechanical properties, unlimited sources that can regulate their degradation rate, but their biocompatibility is not as good as natural materials. At present, the commonly used synthetic materials are polylactic acid (PIA), polyglycolic acid (PGA) and PLA-PGA compound. Guo et al.[18] adopted polylactic acid to cultivate mesenchyma stem cell under the action of TGIF-beta, and a large number of hyaline cartilage has been formed after 24 weeks' transplantation. El et al.[19] adopted polyglycolic acid as the scaffold materials, and inoculate nasal septum and auricular cartilage cells, which successfully cultured the cartilage tissue. Cohen et al.[20] used the PLA-PGA compound support to cultivate the chondrocyte, which formed the cartilage 12 weeks after it transplanted in rabbits. One of the major drawbacks of synthetic materials is that they can't provide specific biological functions. To promote cell attachment and stimulate matrix formation, synthetic materials need to be functionalized with motifs or bioactive molecules[21].

3.3 Hydrogel

As the most abundant component of articular cartilage, water accounts for 65-80%[22] of arthrodial cartilage's wet weight[22]. Therefore, the ideal support materials should be able to replicate the physiological environment of articular cartilage. However, as a three-dimensional polymer network structure rich in water, hydrogels are widely used in cartilage regeneration and cartilage tissue engineering. Hydrogels are highly modular that can be specially adjusted in fields of polymer types, cross-linking methods, degradation products, degradation rates, and combined growth factors, etc.[23]. Vinatier et al.[24] transplanted rabbit nasal chondrocyte with self-curing hydrogel to cartilage defect. The immune-histochemical analysis of II-type collagen protein showed that there's the formation of transparent cartilage. Hydrogel combines the advantages of natural materials and synthetic materials that can be implanted by injection, so it has right application prospects.

4 Growth factors

4.1 TGF-βfamily

The members of TGF-β superfamily include TGF-β, bone morphogenetic protein (BMP) and growth differentiation factor (GDF). Members of the TGF-βsuperfamily are combined to type I and type I receptors, activate downstream signaling pathways and participate in the development and metabolic balance of various tissues[25]. It is widely expressed in chondrocytes and plays an important role in the formation and maintenance of cartilage phenotype. TGF-β1, TGF-β3, BMP-2, BMP-4, BMP-6, BMP-7 and GDF-5 are closely related to cartilage development and tissue engineering[26]. (1) TGF-β: In vitro, TGF-βstimulates chondrocyte to produce extracellular matrix and articular cartilage, and inhibits the metabolic activity of inflammatory mediators such as interleukin-1 (IL-1)[27]. TGF-β1 can promote bone marrow mesenchymal stem cells to differentiate into chondrocyte, resulting in the down-regulation of type I collagen gene expression and the up-regulation of type I collagen and proteoglycan gene expression. TGF-β2 and TGF-β3 have the same effect, and are more effective than TGF-β1 in cartilage formation promotion, early accumulation of type II collagen and accumulation of proteoglycan. The results showed that TGF-βalso played an important role in inhibiting the maturation of mast chondrocyte and stabilizing the phenotype of chondrocyte[28].

4.2 FGF family

There're many members in fibroblast growth factor family, and many researches on the soft tissue engineering, such as fibroblast growth factor-2, fibroblast growth factor-9, fibroblast growth factor-18

and so on. FGF-9 is closely related to FGF-18. They mainly play a role through FGFR3, and FGF-2 mainly plays a role through FGFRl[29]. FGF-2 inhibits the synthesis of proteoglycan and up-regulates the expression of matrix metalloproteinase-13, which has a negative effect on chondrocyte differentiation[30]. FGF-9 and FGF-18 can selectively stimulate the secretion of chondrocyte's extracellular matrix by stimulating the secretion of fibroblast growth factor R3, delay chondrocyte differentiation to hypertrophic chondrocyte, but this role can only be played in a certain period of time[31].

4.3 BMP family

BMP-2, BMP-4, BMP-6 and BMP-7 play an important role in the formation of cartilage in vivo. BMP-2 has the same effect on progenitor cells as TGF-1, which can promote the generation of extracellular matrix in chondrocytes, and reduce the expression of I type collagen protein; BMP-4 and BMP-6 can induce progenitor cells to differentiate into chondrocytes, but the effect of BMP-4 and BMP-6 on the generation of proteoglycan and II type collagen protein is not as good as BMP-2[32]. BMP-7 is generated by chondrocyte and promotes the generation of II type collagen protein and cartilage proteoglycan with TGF-beta 1[33]. However, although BMP plays these roles in chondrocyte formation, long-term exposure to BMP can also lead to chondrocyte differentiation into mast chondrocyte[34]. (3)GDF-5: GDF plays an important role in joint development. As one of the most important factors in the GDF subfamily, GDF-5 can promote the differentiation of bone marrow mesenchymal stem cells into chondrocytes. GDF-5 can also up-regulate the expression of sex-determining and region-related high mobility group box protein (Sox)-9 gene, and promote the differentiation of adipose-derived stem cells into chondrocytes[35].

4.4 IGF-1 family

The structure of IGF-1 is similar to that of insulin, which reaches articular cartilage through synovial fluid, mediates cell metabolism and maintains the balance of proteoglycan metabolism. IGF-1 stimulates the generation of proteoglycan in a dose-dependent manner and reduces the metabolism of proteoglycan, but does not regulate the generation and degradation of collagen[23]. Besides, many studies have shown that the combination of IGF-1 and BMP-7 or TGF-beta 1 has greater repair potential than any single growth factor[36].

5 Conclusions

Tissue engineering technology has great potential in articular cartilage repair. In recent years, great progress has been made in cartilage tissue engineering, but there're still many problems to be solved. The best way to obtain ideal seed cells is to combine primitive seed cells with genetic engineering to form immortalized seed cells and genetically modified seed cells. From single polymer materials to composite functional composites, the development of support materials is more conducive to cell proliferation and tissue engineering culture. The role of various growth factors is gradually clear, which eliminates some unfavorable growth factors. Basic experiment of cartilage tissue engineering technology and the study of clinical treatment is a painful and long process. On the one hand, we must vigorously study the intermediate treatment methods and constantly improve their clinical application. On the other hand, we must continue to seek the best treatment method combining material engineering, molecular biology and nanotechnology research to effectively solve the problem of articular cartilage repair.

References

[1] Yang Zhi-ming. Basic and Clinical Aspects of Tissue Engineering. 2000, 152-156.

[2] O' Driscoll SW, Fitzsimmons JS. The Role of Periosteum in Cartilage Repair. Clin Orthop, 2001, 391:190.

[3] Wang Yurong, Han Fawu, Zhao Jianning, et al. Autogenous Periosteum Transplantation for Repairing Articular Cartilage Defects, Chinese Journal of Repair and Reconstruction Surgery, 2000, 14(2):67.

[4] Hommigna GN, Bulstra SK, Bousmesstr PS, et al. Perichondral Grafteing for Cartilage Lesions of the Knee. J Bone Joint surg(Br), 1990, 72(6):1003.

[5] Galmiche MC, Koteliansky VE, Briere J, et al. Stromal Cells from Human Long-term Marrow Cultures are Mesenchymal Cells that Differentiate Following a Vascular Smooth Muscle Differentiation Ppathway. Blood, 1993, 82(1):66.

[6] Caplan AI. Mesenchymal Stem Cells. J Orthop Res, 1991; 9(5):641.

[7] Pereira RF, Halford KE, O' Hara MD, et al. Cultured Adherent Cells from Marrow Can Serve as Long-lasting Precursor Cells for Bone, Cartilage and Lung in Irradiated Mice. Proc Natil Acad Sci USA, 1995, 92(11):4857.

[8] Prockop DJ. Marrow Stromal Cells as Stem Cells fpr Nonhematopoietic Tissues. Science, 1997, 276(5390):71.

[9] Martin I, Padera RF, Vunjak-Novakovic G,et al. In Vitro Differentiation of Chick Embryo Bone Marrow Stromal Cells into Cartilaginous and Bone-like Tissues. J Orthop Res, 1998, 16(2).

[10] Paulette AC, Jose JM. Phenotypical and Functional Properties of Human Bone Marrow Mesenchymal Progenitor Cells. J Cell Physiol, 1999, 181(1):67.

[11] Shan Yuxing, Liu Yi, Xu Xinxiang. Rabbit Bone Marrow Mesenchymal Stem Cells for Construction of Soft Tissue Engineering preliminary report of bone tissue. Chinese Journal of Repair and Reconstruction Surgery, 2001, 15(1):49.

[12] Caplan AI. Stem Cell Delivery Vehicle. Biomaterials, 1990, 11:44.

[13] Ochi M, Uchio Y, Kawasaki K, et al. Transplantation of Cartilage-Like Tissue Made by Tissue Engineedng in the Treatment of Cartilage Defects of the Knee[J]. J Bone Joint surg Br, 2002, 84(84):571-578. DOI:10.1302/0301-620X/8484.11947.

[14] Chenite A, Chaput C, Wang D, et a1. Novel Injectable Neutml Solutions of Chitosan form Biodegradable Gels in Situ[J]. Biomaterials, 2000, 21(21):2155-2161. DOI:10.1016/S0142-9612(00)00116-2.

[15] Toh WS, Lee EH, Guo XM, et a1. Canilage Repair Using Hyalumnan Hydrogel-encapsulated Human Embryonic Stem Cell-derived Dhondro-genic CellsI[J]. Biomaterials, 2010, 3l(27):6968-6980. DOI:10.1016/j.biomaterials.2010.05.064.

[16] Wang CC, Yang KC, Lin KH, et al. Cartilage Regeneration in SCID Mice Using a Highly Organized Three-dimensional Alginate Scaffold[J]. Biomaterials, 2012, 33(1):120. DOI:10.1016/i.biomaterials.2011.09.042.

[17] Kawakami M, Tomita N, Shimada Y, et al. Chondmcyte Distribution and Cartilage Regeneration in Silk Fibroin Sponge[J]. Biomed Master Eng, 2011, 21(1):53.DOI:10.3233/BME.2011-0656.

[18] Guo X, Zheng Q, Yang S, et al. Repair of Full-thickness Anicular Carti-lage Defects by cultured Mesenchymal Stem Cells Transfected with the Transfoming Growth Factor Betal Gene[J]. Biomed Mater, 2006, 1(4):206.DOI: 10.1088/1748-6041/1/4/006.

[19] El SK, Marzahnu. John T, et a1. PGA-associated Heterotopic Chondro-cyte Cocultures: Implications of Nasoseptal and Auricular Chondrocytes in Articular Cartilage Repair[J]. J Tissue Eng Regen Med, 2013, 7(1):61-72.DOI :1 10.1002/term.496.

[20] Cohen SB, Meirisch CM, Wilson HA, et al. The Use of Absorbable Copolymer Pads with Alginate and Cells for Articular Canilage Repair in Rabbits[J]. Biomatedals, 2003, 24(15):2653.DOI:10.1016/S0142-9612(03)O00589.

[21] Vinatier C, Mrugala D, Jorgensen C. Cartilage Engineering: A Crucial Combination of Cells, Biomaterials and Biofactors[J]. Trends Biotechnol. 2009, 27(5):307.314.DOI:10.1016/j.tibtech.2009.02.005.

[22] Muir H. The Chondmcvte, Architect of Cartilage. Biomechanics, Structure, Function and Molecular Biology of Cartilage Matrix Macromolecules[J]. Bioessays, 1995, 17(12):1039-1048. DOI:10.1002/bies.950171208.

[23] Spiller KL, Maher SA, Lowman AM. Hydmgels for the Repair of Articular Cartilage Defects[J].Tissue Eng Part B Rev, 2011, 17(4):281. DOI:10.1089/ten.TEB.2011.0077.

[24] Vinatier C, Gauthier O. Fatimi A, et al. An Iniectable Cellulose-based Hydrolgel for the Transfer of Autologous Nasal Chondrocytes in Articular Cartilage Defects[J]. Biotechnol Bioeng, 2009, 102(4):1259-1267.DOI10:1002/bit.22137.

[25] Umlauf D, Frank S, Pap T, et al. Cartilage Biology, Pathology, and Repair[J]. Cell Mol Life Sci, 2010, 67(24):4197-4211.DOI :10.1007/s00018-010-0498-0.

[26] DanisovicL, Varga I, Polak S. Growth Factors and Chondmgenic Differentiation of Mesenchvmal Stem Cells[J]. Tiss Cell, 2012, 44(2):69-73.DOI:10.1016/j.tice.2011.11.005.

[27] Van Der Kraan PM, Blaney Davidson EN. Blom A, et al. TGF-beta Signaling in Chondmcyte Terminal Differentiation and Osteoarthritis: Modulation and Integration of Signaling Pathways Through Receptor-smads[J]. Osteoarthritis Cartilage, 2009, 17(12):1539-1545. DOI:10.1016/j joca.2009.06.008.

[28] Fortier LA, Barker Ju, Strauss EJ, et al. The Role of Growth Factors in Cartilage Repair[J]. Clin Orthop Relat Res, 2011, 469(10):2706-2715.DOI:10.1007/s11999-011-1857-3.

[29] Zhang X, Ibrahimi OA, Olsen SK, et al. Receptor Specificity of the Fibroblast Growth Factor Family. The Complete Mammalian FGF Family[J]. J Biol Chem, 2006, 281(23):15694-15700. DOI:10.1074/jbc.M601252200.

[30] Yan D, Chen D, Cool SM, et al. Fibroblast Growth Factor Receptor 1 is Principally Responsible for Fibroblast Growth Factor 2-Induced Catabolic Activities in Human Articular Chondrocytes[J]. Arthritis Res Ther, 2011, 13:R130. DOI:10.1186/ar3441.

[31] Correa D, Lietman SA. Articular Cartilage Repair: Current Needs, Methods and Research Directionsf[J]. Semin Cell Dev Biol, 2017, 62:67-77.DOI:10.1016/j.semcdb.2016.07.013.

[32] Sekiya I, Larson BL, Vuoristo JT, et al. Comparison of Effect of BMP-2, -4, and-6 on in Vitro Cartilage Formation of Human Adult Stem Cells from Bone Marrow Stroma[J]. Cell Tissue Res, 2005, 320(2):269-276.DOI:10.1007/s00441-004-1075-3.

[33] Lietman SA, Yanagishita M, Sampath TK, et al. Stimulation of Proteoglycan Synthesis in Explants of Porcine Articular Cartilage by Recombinant Osteogenic Protein-1 (Bone Morphogenetic Pmtein-7)[J]. J Bone Joint Surg Am, 1997, 79(8):1132-1137.DOI:10.2106/00004623-199708000-0003.

[34] Shintani N, Hunziker EB. Chondrogenic Differentiation of Bovine Synovium: Bone Morphogenetic Proteins 2 and 7 and Transfoming Growth Factor Betal Induce the Formation of Different Types of Cartilaginous Tissue[J]. Arthritis Rheum, 2007, 56(6):1869-1879.DOI:10.1002/art.22701.

[35] Feng G, Wan Y, Balian G, et al. Adenovirus-mediated Expression of Growth and Differentiation Factor-5 Promotes Chondrogenesis of Adipose Stem Cells[J]. Growth Factors, 2008, 26(3):132-142.DOI:10.1080/08977190802105917.

[36] Montaseri A, Busch F, Mobasheri A, et al. IGF-1 and PDGF-bb Suppress IL-1 β-induced Cartilage Degradation Through Down-regulation of NF-KB Signaling: Involvement of src/Pl-3K/AKT Pathway[J]. PLoS One, 2011, 6(12):e28663. DOI:10.137l/journal.pone.0028663.