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Biodegradable Scaffolds for Cartilage Tissue Engineering

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Abstract

Being a connective tissue, the cartilage is present in almost all parts of the body like the rib cage, joints, nose, and ear. Its essential function in body is to serve as a cushion between the joints and prevent the bones friction against each other. In some areas like the rib cage, the cartilage keeps the bones together and creates a shockproof area. Osteoarthritis and traumatic rupture of the cartilage are among the related diseases. Damaged cartilage tissue can be only limitedly repaired because of the low density of chondrocyte and slow metabolism in the tissue. Previous studies achieved different outcomes for the joint-preserving treatment programs such as debridement, mosaicplasty, and perichondrium transplantation; however, the average long-term result is still unsatisfactory. The restriction of clinical success is mainly attributed to the long time required in most treatments for the regeneration of new cartilage at the site of defect. The mechanical conditions of these sites makes the repair process unflavored of the original damaged cartilage. Such problems can be permanently treated by using tissue engineered cartilage. Hence, the limitations can be defeated by using appropriate scaffolds, cell sources, and growth factors. This review dealt with the advances in cartilage tissue engineering, with the focus on cell sources, scaffold materials and growth factors used in cartilage tissue engineering.[**GMJ.2017;6(2):70-80] DOI: 10.22086/GMJ.V6I2.696**

Keywords: Cartilage; Cell Sources; Growth Factors; Scaffold Materials; Tissue Engineering

Introduction

Cartilage is an avascular connective tissue with various significant functions like keeping some bones together, making the joint part shockproof and preventing the bones from rubbing against each other. The constituents of cartilage are chondrocytes specialized cells. These cells create the cartilaginous matrix, which is mainly made of collagen and proteoglycans. Development of cartilage occurs quite slowly. The chondrocytes are fixed in a small space called lacunae, which do not allow their migration to the damaged areas. Any blood vessels do not supply the cartilage tissues; instead, the cartilage compression induces a pumping action leading to a diffusion that feeds the chondrocytes. Since the cartilage tissue has the low intrinsic regenerative ability, its self-heal is restricted, and the trauma- or disease-induced lesions tend to progressively degrade [1, 2]. Improper function or loss of cartilage causes diseases, namely, osteoarthritis and achondroplasia.

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Despite the different levels of success achieved through various clinical therapeutic methods such as microfracture, mosaicplasty, and autologous chondrocytes injection, the durable outcomes are not good enough usual [3]. A common reason for the failure of all the treatment mentioned above strategies is that compared with the natural cartilage, the newly-formed tissue has neither the structural organization of the cartilage nor any mechanical feature in common [4]. Therefore, it is strongly essential to find the solution to these problems.

The emerging technology of tissue engineering speeds up the repair of the damaged tissue if the self-heal fails. The studies dedicated to this field of research mainly aim to develop a replacement tissue with native cartilage organization, composition, and similar mechanical property, which can absolutely restore the joint functionality. Forming the building blocks of tissue engineering, the scaffolds, cells and growth factors are known as the tissue engineering triad.

Some features of cartilage allow its reconstruction through tissue engineering. One of them is the simplicity of this tissue which is made of a single type of cell (chondrocytes). Moreover, its nutrition and excretion of wastes are done through diffusion instead of a vascular network; thus, the cell-scaffold constructs do not require being neovascularized.

The present review elaborates on the issues related to the methods of engineering the cartilage by using a composite polymeric scaffold, chondroprogenitor cells, and various growth factors. The debate goes on with the paramount factors for cartilage tissue engineering such as cell source, scaffolds, and mechanical stimulation. The current condition of cartilage tissue engineering will also be discussed. The study concludes with mentioning the common limitations and accommodating recommendations for further approaches to cartilage engineering.

Search Strategies

This study was a structured literature review of articles published from 1998 to 2016. The keywords were searched on PubMed, Scopus, and Wiley Inter Science databases. The search was limited to English-language publications. Searching the keywords yielded 92 articles. To be included in the study, exact relation to the keywords was required for the publications. The editorials and manufacturer-supported publications were excluded from the review process. Finally, 73 citations remained as the basis for this review.

Different Types of Cartilage

The human body has various types of cartilage including hyaline cartilage (e.g., tracheal and articular), elastic cartilage (e.g., ear) and fibrocartilage (e.g., meniscus and intervertebral disc) [5]. Hyaline cartilage is located in the joints and facilitates their articulation. It is mostly composed of collagen type II fibers. The most flexible cartilage is elastic cartilage due to the more elastin fibers content. Its collagen content is mostly of type I collagen, but it also contains type II collagen. Fibrocartilage is found in the intervertebral discs. It attaches the tendons and ligaments to the bones. It is located in high-stress parts and guards the joints against shocks. Damaged hyaline cartilage is commonly taken over by fibrocartilage, whose rigidity does not let it bear the weight.

Main Factors for Cartilage Tissue Engineering

1. Cell Sources

The ideal cell source for cartilage tissue engineering should be easy to isolate and expand, and secretes abundant cartilage-specific extracellular matrix components. Being potential in cartilage tissue engineering has promoted the chondrocytes and stem cells to the most investigate cell sources [6].

1.1. Chondrocytes

Chondrocytes play the key role in the regeneration of cartilage. They can be taken from donor organs including cartilaginous tissues like the menisci of the knee joint, trachea, and nose. They have the capability to create, maintain and remodel the cartilage tissue in vitro. However, the autologous chondrocytes are scarcely accessible, and the cells gath-

ered from diseased joints are rather inactive. The expansion of chondrocyte in monolayer culture results in dedifferentiation and is presented as reduced proteoglycan synthesis and expression of the type II collagen, and overexpression of the type I collagen [7, 8]. Chondrocytes from younger donors are more metabolically active *in vitro*; whereas, those taken from adult donors have higher chondrogenic potential and rapid expansion [9]. Another drawback with the extracted articular chondrocytes is the morbidity at the donor site and loss of joint function.

1.2. Stem Cells

To overcome the limited supply of primary chondrocytes, it is suggested to use multipotent stem cells which are mainly isolated from the bone marrow, adipose and pre-implantation embryo tissue [10]. The supplies of adult mesenchymal stem cells can be accessed in different tissues namely trabecular bone, bone marrow, deciduous teeth, periosteum, articular cartilage, adipose tissue, muscle and synovial membrane.

Some specific signaling molecules (e.g., transforming growth factor-β (TGFβ) can inspire the differentiation of mesenchymal stem cells in diverse 3-dimensional (3D) culture environments [10-12].

The cartilage tissue engineering restrictedly benefit from the bone marrow extracted stem cells since the subsequent builds would have lower matrix accumulation and mechanical properties compared with the chondrocyte-seeded constructs [13, 14].

Adipose-isolated stem cells are capable of differentiating into chondrocytes in 3D culture systems in the presence of ascorbate, dexamethasone, and TGF-β [15]. However, their chondrogenic potential is lower than that of the bone marrow-derived stem cells. Additional researches are needed to better comprehension the chondrogenic potential of these cells. Studies are also needed on other sources for cartilage tissue engineering like muscle, synovium, and periosteum, all of which have shown chondrogenic potential but still restrictedly compared to bone marrow-derived and/or adipose-derived stem cells [16].

2. Growth Factors for Cartilage Regenertion

Growth factors are mainly the signaling molecules which trigger the differentiation of cells into certain phenotype.

The chondrocytes anabolic and catabolic processes are affected by some growth factors, cytokines, and hormones.

The most prominent factors that assist the regeneration of chondrocytes are polypeptide growth factor, TGF-β, basic fibroblast growth factor (FGF-2), insulin growth factor I (IGF-I), and bone morphogenetic growth factors (BMPs).

They are employed either per se or in combination to improve the chondrogenesis. Polypeptide growth factors significantly affect the regulation of cell activities, like that of chondrocytes [5]. Besides, it hinders the transcription of cartilage-specific matrix genes in long-term cultures [17]. The growth and repair of cartilage is generally determined by the TGF-β superfamily members. The TGF-β1, 2 and 3 isoforms promote the proliferation of chondrocyte and the synthesis of extracellular matrix content by chondrocytes [18-20]. The two isoforms of IGF are IGF-1 and IGF-2, the former of which is the most investigated form in cartilage restoration. It inspires the anabolic activity of chondrocytes and induces chondrogenesis in bone marrow-derived stem cells [21-23]. The IGF-2 is an effective mitogen for articular chondrocytes and assists the differentiation of chondrocytes in 3D culture system. The FGF-18 is said to support the cartilage repair [24].

Another kind of agents is a group of growth factors identified as bone morphogenetic proteins (BMPs), also known as cytokines or metabologens. Some BMPs are recognized as cartilage-derived morphogenetic proteins (CDMPs). So far, 20 BMPs have been recognized. The chief BMPs incorporating in cartilage repair are BMP-1, BMP-2, BMP-4, BMP-5, BMP-7, BMP-8a, BMP-9 and BMP-12. Proper cartilage formation highly depends on BMP activity [25-27]. The BMPs are thoroughly involved in stages of chondrogenesis and directly control the expression of some chondrocyte definite genes. Hence, the chondrocyte proliferation and matrix synthesis are considerably influenced by this category of

signaling molecules. The BMPs trigger the chondrogenic differentiation that is actually mediated through gap junction-mediated intercellular contact [28]. *In vitro* addition of a combination of growth factors to chondrocyte and bone marrow-derived stem cell cultures is likely to increase their efficacy (Table-1). For instance, combinations of IGF-1/TGF-β1, IGF-1/TGF-β2, IGF-1/BMP-2 and IGF-1/bFGF/TGF-β2 increased the anabolic effects on chondrocytes and stimulated extracellular matrix synthesis [21, 29].

The differentiation of the cartilage tissue is profoundly affected by the dosage of different growth factors. For instance, transient, rather than continuous, use of TGF-β3 yielded higher compressive properties and increased the glucose aminoglycan content of chondrocyte loaded hydrogels and bone marrow-derived stem cell-laden constructs [30, 31]. Almost all cartilage tissue engineering investigations applied 10 ng/ml of growth factors (e.g., TGF-β, FGF-2 and BMPs) [32].

Scaffolds

The scaffold is a 3D construction whereupon the cells can attach properly, and grow potentially. Different kinds of biomaterial are used for constructing the scaffolds. The ideal biomaterial should be biocompatible, non-toxic, non-attractive, non-stimulatory of inflammatory cells, non-immunogenic. It should also have some particular features that aid adequate cell adhesion, proliferation, differentiation into specific phenotype like the mechanical support of the cartilage engineered tissue and having porosities that permit diffusion of nutrients and waste products. Moreover, these materials should be biodegradable and allow remodeling as the new cartilage forms and substitute the original build. They should be decay-resistant at physiological pH and body temperature [32].

The perfect scaffold for cartilage tissue engineering is the one with high porosity and pore-to-pore interconnectivity. High porosity (normally>90%) provides adequate space for in vitro cell adhesion, ingrowth, and restructuring of cells. Interconnected porous organization facilitates the cell migration, spread of physiological nutrients and gasses to the cells, and discharge of metabolic waste and side-products from cells [33].

Mechanical stimulation can be certainly used for boosting the mechanical features of tissue-engineered cartilage. Bioreactors have been made to subject the cell-seeded constructs to mechanical loading regimes [34]. Investigations of cartilage tissue engineering

Table 1. The Growth Factors Evaluated For Their Effects on Chondrocyte Growth and Matrix Production

Growth factors	Chondrocyte growth	Matrix production
IGF-1	Mitogenic differentiation	Matrix synthesis
FGF-α	Mitogenic differentiation	Matrix synthesis
PDGF(platelet derived growth factors)	Mitogenic differentiation	Matrix synthesis
TGF-β	Promotes differentiation	Proteoglycan synthesis
BMP-1	Cartilage proliferation	Collagen synthesis
BMP-2	Promotes cartilage formation by inducing production of cartilage matrix.	Collagen synthesis
BMP-4	Promotes cartilage formation by inducing MSCs to become chondroprogenitor and chondrocyte maturation	Matrix synthesis
BMP-5	Chondrocyte proliferation	Matrix synthesis
BMP-9	Potent anabolic factor for juvenile cartilage	Matrix synthesis
BMP-12 (GDF7)	Modulates in vitro cartilage formation in a similar fashion as BMP-2 does	Collagen synthesis

have been mostly focused on two loading regimes including straight confined or unconfined compression and hydrostatic pressure. The direct dynamic compression administered in chondrocyte-seeded scaffolds usually generate enhanced extracellular matrix production and/or proliferation and improves the compressive characteristics of the engineered tissue [35].

Types of Scaffold

The scaffolds needed for cartilage repair has been made by using several types of materials with both natural and synthetic polymer basis in a variety of forms.

Synthetic polymers are mainly favored because they are quite flexible in modifying the physical, mechanical and chemical properties; consequently, the ultimate scaffold can be simply processed into the desired form and dimensions. An enormous group of synthetic polymers has been already incorporated in cartilage tissue engineering successfully. The synthetic polymeric scaffolds used in cartilage tissue engineering are most frequently made of poly-α-hydroxy esters, especially polylactic acid (PLA) and polyglycolic acid (PGA). The reason is the biodegradability of these materials, besides being approved by the US Food and Drug Administration (FDA) for clinical applications [36].

A small number of synthetic polymers are beeing currently clinically evaluated for their potential use in cartilage repair. The main disadvantage which occurs by using the synthetic polymers is their cells frequently do not keep the chondrocyte phenotype and make an extracellular matrix with lower properties [37]. On the other side, the natural polymers are not only cost-effective, environment-friendly, highly biodegradable, less toxic, and renewable but also take low manufacturing and disposal costs [38]. Furthermore, they have important controlling features that highly determine the success of cartilage tissue regeneration including remodeling, biological signaling, cell responsive degradation, and cell adhesion.

The most important drawback of natural polymers is their rapid degradation. Moreover, the scaffold making procedures might geopardize their biological properties.

The threats of immune rejection and disease transmission demand strict monitoring and purification of the natural polymer [38, 39].

The defects of natural and synthetic polymers can be compensated by using composite scaffolds made of two or more polymers, and functionalization of the polymers that provide proper conditions for cartilage regeneration. Composites create and amalgamation of different features of various polymers to control the biodegradation, cell adhesion, proliferation, and differentiation [40].

A previous study reported the use of a composite scaffold composed of gelatin, hyaluronic acid, chondroitin-6-sulfate, and fibrin to improve the chondrogenesis, and the use of a composite scaffold of hydroxyapatite mixed with chitosan for the healing of osteochondral defects [41].

Functionalization can create new functional groups in the polymer, which might supply particular cues to the cells for cartilage regeneration. Functionalized polymers are currently being employed to make up for the defects of natural and synthetic polymers. The integrin combining activity of adhesion proteins can be replicated by introducing short synthetic peptides, containing the Arg-Gly-Asp (RGD) or other like adhesion serieses in the polymer, which increases cell adhesion [42].

Peptide ligands have modified several materials to support chondrogenesis. According to Hwang *et al*., human embryonic stem cell-derived cells can be enveloped in RGD-modified hydrogels and be used to enhance the cartilage creation [43]. Attempts were made to improve in vitro construction of cartilage by rabbit chondrocytes through adding chitosan-alginate-hyaluronate complexes modified with RGD-containing proteins [44]. In another study, RGD-coupled alginate hydrogels containing a co-transplantation of osteoblasts and chondrocytes, the formed growing tissues had structure and function similar to a growth plate cartilage [45].

A broad range of materials have been developed and are accessible in the form of injections, microspheres, and thermoreversible hydrogels, which can be applied for in situ tissue

regeneration [46].
Injectable biodegradable chitosan-hyaluronic acid-based hydrogels are also applicable for in situ cartilage tissue engineering [47].

Scaffold Architecture, Porosity, Stiffness and Biodegradability

The porosity, pore size and interconnectivity of scaffold materials can affect the cell migration and diffusion of nutrients, signaling molecules, oxygen and waste products [48]. For instance, inhomogeneous oxygen delivery from the periphery towards the center of the cell-seeded structure may result in cell death in the inner areas but not in the periphery [49]. Moreover, when the material is porous, the mechanical interlocking developed between the implant and the surrounding natural cartilage creates a better mechanical stability at the interface. The proliferation and phenotype of chondrocytes are significantly impressed by the porosity and permeability features [50, 51]. The proper pore size for the better proliferation of scaffolds should be optimized between 100 and 500 μm [52].
The rigidity of scaffolds affects the mechanical properties of the seeded cell's surroundings, which can in turn impress the cell differentiation and tissue growth in culture. Enhancing the substrate rigidity affects the chondrocyte morphology; it can be transformed from a rounded shape to elongated shape on weaker substrates to a mainly flat morphology with actin stress fibers on more rigid substrates [53, 54].
Production and deposition of new tissue would be influenced by the 3D structure of the scaffold and its degradation rate.
Optimal degradation kinetics guarantee the primary stability and shape of the scaffold. Comparison of the rapid and slow degrading scaffolds revealed that the latter yields elevated and more homogeneous deposition of extracellular matrix [55]. When the scaffold is degraded, the new tissue is permitted to integrate and reform into the adjacent cartilage after implantation.
Ng *et al*. reported that supervised degradation of agarose scaffold via agarase enzyme improved the collagen content and dynamic mechanical characteristics related to control over time in culture. They attributed it to the facilitated transportation of nutrition and enlarged room for collagen fibril increase with time of culture [56-59].

Tissue Engineered Cartilage: Content, Structure and Functionality

The mechanical conditions of joints are quite challenging. The constituent material of tissue-engineered cartilage implant should be strong enough to survive or appropriately function under normal joint loading. An engineered tissue is not necessarily a precise duplication of the original natural tissue; it is rather likely to get and obtain features after implantation.

1. Collagen
The low level of collagen content and the subsequent weak tensile characteristics are considered the main deficiency of tissue-engineered cartilage. The maximum collagen level gets up to 15–35% of the natural amount within five to twelve weeks [60, 61].
The *in vitro* synthesis of collagen is highly affected by some culture features including the cell source, cell seeding density, growth factors, mechanical stimulation, and scaffold properties [62, 63]. The low level of collagen contents might be due to the rapid synthesis of glycosaminoglycans (GAGs) that prevents the increase of collagen.
The self-assembly of collagen might be adjusted by modified transport of synthesized products or altered extracellular biochemical surroundings. Studies announced that in vitro degradartion of type I and II collagen was strain dependent [64, 65]. The tissue-engineered cartilage contains more GAGs than collagens; it has negative effects on the tensile characteristics of the tissue.
The plausible reason can be the higher or changed crosslinking, the larger size or altered direction of fibril [66, 67].

2. Proteoglycan
The GAGs content and compressive characteristics enhanced with increasing the culture duration, densifying the cell seeding, adding

anabolic growth factors, and/or raising the serum supplementation. Reports also mentioned the considerable increase in deposition of GAGs by applying dynamic loads to chondrocytes-seeded constructs [63, 68].

Future Perspectives in Cartilage Regeneration

Up to date, numerous studies have been performed on cartilage tissue engineering, and cartilage regeneration has been greatly progressed.

According to the previous studies, *in vivo* restoration of damaged articular cartilage through mosaicplasty, microfracture, and autologous chondrocytes injection effectively alleviated pain and recovered joint function; however, the long-term results were not satisfying. The main problem of these techniques is the development of mechanically weak fibrocartilage, which is expected to degrade over time due to its poor load bearing capacity. The main benefits of *in vitro* engineering of cartilage are the precise supervision of the culture and evaluation of the material properties during culture, unlike the *in vivo* methods that highly rely on the status of the donor site. Implantation of a construct that can bear the *in vivo* loads is more likely to succeed.

The path of cartilage repair still has several obstacles to overcome to reach the desired excellence. These obstacles are related to the three pillars of cartilage tissue-engineering; cells, scaffold, and growth factors. What matters is how to increase chondrogenesis, and how to support it by introducing the biophysical, chemical and mechanical stimuli to the cells.

The major scaffold associated issue is the fabrication of scaffold in a way that precisely replicate the native features of the tissue. Several natural and synthetic materials have been studied so far, but none has met all the required conditions. The matrix formation and tissue construction require proper mechanical and biochemical triggers. It is still a challenge to develop optimal stimuli capable of supporting the cells proliferation and differentiation, synthesizing appropriate and enough extracellular matrix components, and secreting enzymes that can modify the produced extracellular matrix. A combination of allogenous chondrocytes and gelatin–chondroitin–hyaluronan tri-copolymer scaffold was used for cartilage repair in a porcine model and yielded satisfactory outcome [69]. Human polymer-based cartilage tissue engineering grafts prepared of human autologous fibrin, PGA and human chondrocytes were reported to be clinically helpful in regenerating the articular cartilage damages [70]. A different investigation used chitosan hydrogels to repair a sheep articular cartilage damage, and the outcome was satisfactory successful [71]. Since a few years ago, polymeric nanofibers have been employed by many scientists for cartilage regeneration [33].

Finding an ideal cell source is of paramount importance. Despite the optimal performance of primary native chondrocytes, their utilization is almost impractical due to inadequate availability. The main challenge of developing the chondrocytes is to be cautious not to lose the phenotype. Seemingly, the stem cells can be a promising substitute; however, their cartilage tissue has weaker properties than those of the chondrocytes. In the near future, studies will prove if stem cells are the optimal cell supply for cartilage tissue engineering.

Quite little is known about the effects of sequences and concentrations of growth factors on the cartilage regeneration. Although cartilage regeneration is multifactorial and influenced by multiple growth factors, the majority of investigations engaged a single growth factor. A limited number of systems have been established with the privilege of the biphasic release of dual growth factors. Double growth factor-releasing alginate-based nanoparticle/hydrogel system was just utilized to deliver BMP-7 and TGFβ-2 to improve chondrogenesis [72]. Therefore, a study should be conducted to focusing on the release of several growth factors at a time, to favor the making of more natural cartilage tissue.

The extracellular matrix content is undoubtedly so significant, but the extent to which the native matrix components should be reproduced is vague in pre-implantation of engineered cartilage implants. It is probable to acquire the GAGs is possible to reach its native

amount in engineered cartilage, but the collagen content is almost always far less than the natural level. Future studies are recommended to investigate methods that enhance collagen content, which is highly essential for the proper mechanical functioning of the tissue. Meanwhile, most studies on cartilage tissue engineering take cells from young adults and even fetal animals, not from elderly osteoarthritis patients. There is a need for a comprehensive study on use of the cells from elderly osteoarthritis patients to broaden the outcomes for treating human cartilage defects.

The final and maybe most challenging issue is the translation of the findings of *in vitro* and animal studies to be marketed and employed in clinical conditions. Despite the development of numerous cartilage products and growth factor carrier materials, only a limited number are approved for clinical application. It can be attributed to the cost of production and materials, manufacturing scale-up, sterility, and patent subjects. Besides, there are regulatory barriers including quality control and quality assurance for reliable manufacturing, comparability researches required for component and procedure changes, the establishment of shipping and storage states, and proper shelf life [73].

Conclusion

Currently, both the existing and new cartilage engineering products need to be approved by the corresponding organizations so that they can be available for clinical uses. To bring the obtained laboratory results into the use for treating human cartilage defects, cost-effective tissue engineered products needs to be produced. It will encourage the patients with cartilage defects to seek tissue engineering solution instead of other surgical options and prosthetics. Undoubtedly, despite the extensive progress achieved in regenerative medicine, developments are still highly required in cartilage tissue engineering to find the ideal economical states for cartilage regeneration. The authors wish that the progress in this field will find more great application in therapeutic strategies in regenerative medicine to solve the problem of the aging population of the world.

Conflict of Interest

None to declare.

References

1. Hunziker E. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthritis Cartilage. 2002;10:432–63.
2. Buckwalter JA, Mankin HJ. Articular cartilage repair and transplantation. Arthritis Rheum. 1998;41(8):1331-42.
3. Kreuz PC, Steinwachs MR, Erggelet C, Krause SJ, Konrad G, Uhl M, et al. Results after microfracture of full-thickness chondral defects in different compartments in the knee. Osteoarthritis Cartilage. 2006;14(11):1119-25.
4. Hunziker EB. The elusive path to cartilage regeneration. Adv Mater. 2009;21(32-33):3419-24.
5. Kinner B, Spector M. Smooth muscle actin expression by human articular chondrocytes and their contraction of a collagen-glycosaminoglycan matrix in vitro. J Orthop Res. 2001;19(2):233-41.
6. Chung C, Burdick JA. Engineering cartilage tissue. Adv Drug Deliv Rev. 2008;60(2):243-62.
7. Darling EM, Athanasiou KA. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. J Orthop Res. 2005;23(2):425-32.
8. Goessler UR, Bieback K, Bugert P, Naim R, Schafer C, Sadick H, et al. Human chondrocytes differentially express matrix modulators during in vitro expansion for tissue engineering. Int J Mol Med. 2005;16(4):509-15.
9. Pestka JM, Schmal H, Salzmann G, Hecky J, Sudkamp NP, Niemeyer P. In vitro cell quality of articular chondrocytes assigned for autologous implantation in dependence of specific patient characteristics. Arch Orthop

Trauma Surg. 2011;131(6):779-89.
10. Chen FH, Rousche KT, Tuan RS. Technology insight: adult stem cells in cartilage regeneration and tissue engineering. Nat Clin Pract Rheumatol. 2006;2(7):373-82.
11. Buxton AN, Bahney CS, Yoo JU, Johnstone B. Temporal exposure to chondrogenic factors modulates human mesenchymal stem cell chondrogenesis in hydrogels. Tissue Eng Part A. 2011;17(3-4):371-80.
12. Alves da Silva ML, Martins A, Costa-Pinto AR, Costa P, Faria S, Gomes M, et al. Cartilage tissue engineering using electrospun PCL nanofiber meshes and MSCs. Biomacromolecules. 2010;11(12):3228-36.
13. Thorpe SD, Buckley CT, Vinardell T, O'Brien FJ, Campbell VA, Kelly DJ. The response of bone marrow-derived mesenchymal stem cells to dynamic compression following TGF-beta3 induced chondrogenic differentiation. Ann Biomed Eng. 2010;38(9):2896-909.
14. Vinardell T, Buckley CT, Thorpe SD, Kelly DJ. Composition-function relations of cartilaginous tissues engineered from chondrocytes and mesenchymal stem cells isolated from bone marrow and infrapatellar fat pad. J Regen Med Tissue Eng. 2011;5(9):673-83.
15. Estes BT, Guilak F. Three-dimensional culture systems to induce chondrogenesis of adipose-derived stem cells. Methods Mol Biol. 2011;702:201-17.
16. Li Q, Tang J, Wang R, Bei C, Xin L, Zeng Y, et al. Comparing the chondrogenic potential in vivo of autogeneic mesenchymal stem cells derived from different tissues. Artif Cells Blood Substit Immobil Biotechnol. 2011;39(1):31-8.
17. Sandell LJ, Daniel JC. Effects of ascorbic acid on collagen mRNA levels in short term chondrocyte cultures. Connect Tissue Res. 1988;17(1):11-22.
18. Puetzer JL, Petitte JN, Loboa EG. Comparative review of growth factors for induction of three-dimensional in vitro chondrogenesis in human mesenchymal stem cells isolated from bone marrow and adipose tissue. Tissue Eng Part B Rev. 2010;16(4):435-44.
19. Schulz RM, Zscharnack M, Hanisch I, Geiling M, Hepp P, Bader A. Cartilage tissue engineering by collagen matrix associated bone marrow derived mesenchymal stem cells. Biomed Mater Eng. 2008;18(1 Suppl):S55-70.
20. Xu J, Wang W, Ludeman M, Cheng K, Hayami T, Lotz JC, et al. Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. Tissue Eng Part A. 2008;14(5):667-80.
21. Seifarth C, Csaki C, Shakibaei M. Anabolic actions of IGF-I and TGF-beta1 on Interleukin-1beta-treated human articular chondrocytes: evaluation in two and three dimensional cultures. Histol Histopathol. 2009;24(10):1245-62.
22. Yoon DM, Fisher JP. Effects of exogenous IGF-1 delivery on the early expression of IGF-1 signaling molecules by alginate embedded chondrocytes. Tissue Eng Part A. 2008;14(7):1263-73.
23. Vinatier C, Bouffi C, Merceron C, Gordeladze J, Brondello JM, Jorgensen C, et al. Cartilage tissue engineering: towards a biomaterial-assisted mesenchymal stem cell therapy. Curr Stem Cell Res Ther. 2009;4(4):318-29.
24. Martin I, Vunjak-Novakovic G, Yang J, Langer R, Freed LE. Mammalian chondrocytes expanded in the presence of fibroblast growth factor 2 maintain the ability to differentiate and regenerate three-dimensional cartilaginous tissue. Exp Cell Res. 1999;253(2):681-8.
25. Reddi AH, Reddi A. Bone morphogenetic proteins (BMPs): from morphogens to metabologens. Cytokine Growth Factor Rev. 2009;20(5-6):341-2.
26. Bleuming SA, He XC, Kodach LL, Hardwick JC, Koopman FA, Ten Kate FJ, et al. Bone morphogenetic protein signaling suppresses tumorigenesis at gastric epithelial transition zones in mice. Cancer Res. 2007;67(17):8149-55.
27. Pan Q, Wu Y, Lin T, Yao H, Yang Z, Gao G, et al. Bone morphogenetic protein-2 induces chromatin remodeling and modification at the proximal promoter of Sox9 gene. Biochem Biophys Res Commun. 2009;379(2):356-61.
28. Zhang W, Green C, Stott NS. Bone morphogenetic protein-2 modulation of chondrogenic differentiation in vitro involves gap junction-mediated intercellular communication. J Cell Physio. 2002;193(2):233-43.
29. Elder BD, Athanasiou KA. Systematic assessment of growth factor treatment on biochemical and biomechanical properties of engineered articular cartilage constructs. Osteoarthritis Cartilage. 2009;17(1):114-23.
30. Huang AH, Stein A, Tuan RS, Mauck RL. Transient exposure to transforming growth factor beta 3 improves the mechanical properties of mesenchymal stem cell-laden cartilage constructs in a density-dependent

manner. Tissue Eng Part A. 2009;15(11):3461-72.
31. Byers BA, Mauck RL, Chiang IE, Tuan RS. Transient exposure to transforming growth factor beta 3 under serum-free conditions enhances the biomechanical and biochemical maturation of tissue-engineered cartilage. Tissue Eng Part A. 2008;14(11):1821-34.
32. Ahmed TA, Hincke MT. Strategies for articular cartilage lesion repair and functional restoration. Tissue Eng Part B Rev. 2010;16(3):305-29.
33. Stoop R. Smart biomaterials for tissue engineering of cartilage. Injury. 2008;39 Suppl 1:S77-87.
34. Schulz RM, Bader A. Cartilage tissue engineering and bioreactor systems for the cultivation and stimulation of chondrocytes. Eur Biophys J. 2007;36(4-5):539-68.
35. Bian L, Fong JV, Lima EG, Stoker AM, Ateshian GA, Cook JL, et al. Dynamic mechanical loading enhances functional properties of tissue-engineered cartilage using mature canine chondrocytes. Tissue Eng Part A. 2010;16(5):1781-90.
36. Yoon DM, Fisher JP. Chondrocyte signaling and artificial matrices for articular cartilage engineering. Adv Exp Med Biol. 2006;585:67-86.
37. Chen G, Sato T, Ushida T, Hirochika R, Shirasaki Y, Ochiai N, et al. The use of a novel PLGA fiber/collagen composite web as a scaffold for engineering of articular cartilage tissue with adjustable thickness. J Biomed Mater Res A. 2003;67(4):1170-80.
38. Fabbri P, Bondioli F, Messori M, Bartoli C, Dinucci D, Chiellini F. Porous scaffolds of polycaprolactone reinforced with in situ generated hydroxyapatite for bone tissue engineering. J Mater Sci Mater Med. 2010;21(1):343-51.
39. He Y, Lu F. Development of synthetic and natural materials for tissue engineering applications using adipose stem cells. Stem Cells Int. 2016;2016:5786257.
40. Oliveira JM, Rodrigues MT, Silva SS, Malafaya PB, Gomes ME, Viegas CA, et al. Novel hydroxyapatite/chitosan bilayered scaffold for osteochondral tissue-engineering applications: Scaffold design and its performance when seeded with goat bone marrow stromal cells. Biomaterials. 2006;27(36):6123-37.
41. Chou CH, Cheng WT, Kuo TF, Sun JS, Lin FH, Tsai JC. Fibrin glue mixed with gelatin/hyaluronic acid/chondroitin-6-sulfate tri-copolymer for articular cartilage tissue engineering: the results of real-time polymerase chain reaction. J Biomed Mater Res A. 2007;82(3):757-67.
42. Garcia AJ, Keselowsky BG. Biomimetic surfaces for control of cell adhesion to facilitate bone formation. Crit Rev Eukaryot Gene Expr. 2002;12(2):151-62.
43. Hwang NS, Varghese S, Zhang Z, Elisseeff J. Chondrogenic differentiation of human embryonic stem cell-derived cells in arginine-glycine-aspartate-modified hydrogels. Tissue Eng. 2006;12(9):2695-706.
44. Hsu SH, Chang SH, Yen HJ, Whu SW, Tsai CL, Chen DC. Evaluation of biodegradable polyesters modified by type II collagen and Arg-Gly-Asp as tissue engineering scaffolding materials for cartilage regeneration. Artif Organs. 2006;30(1):42-55.
45. Alsberg E, Anderson KW, Albeiruti A, Rowley JA, Mooney DJ. Engineering growing tissues. Proc Natl Acad Sci U S A. 2002;99(19):12025-30.
46. Chen JP, Cheng TH. Thermo-responsive chitosan-graft-poly(N-isopropylacrylamide) injectable hydrogel for cultivation of chondrocytes and meniscus cells. Macromol Biosci. 2006;6(12):1026-39.
47. Tan H, Chu CR, Payne KA, Marra KG. Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for cartilage tissue engineering. Biomaterials. 2009;30(13):2499-506.
48. Nuernberger S, Cyran N, Albrecht C, Redl H, Vecsei V, Marlovits S. The influence of scaffold architecture on chondrocyte distribution and behavior in matrix-associated chondrocyte transplantation grafts. Biomaterials. 2011;32(4):1032-40.
49. Volkmer E, Drosse I, Otto S, Stangelmayer A, Stengele M, Kallukalam BC, et al. Hypoxia in static and dynamic 3D culture systems for tissue engineering of bone. Tissue Eng Part A. 2008;14(8):1331-40.
50. Stenhamre H, Nannmark U, Lindahl A, Gatenholm P, Brittberg M. Influence of pore size on the redifferentiation potential of human articular chondrocytes in poly(urethane urea) scaffolds. J Regen Med Tissue Eng. 2011;5(7):578-88.
51. Jeong CG, Hollister SJ. Mechanical and biochemical assessments of three-dimensional poly(1,8-octanediol-co-citrate) scaffold pore shape and permeability effects on in vitro chondrogenesis using primary chondrocytes. Tissue Eng Part A. 2010;16(12):3759-68.
52. Lien SM, Ko LY, Huang TJ. Effect of pore size on ECM secretion and cell growth in

gelatin scaffold for articular cartilage tissue engineering. Acta Biomater. 2009;5(2):670-9.
53. Kelly DJ, Prendergast PJ. Prediction of the optimal mechanical properties for a scaffold used in osteochondral defect repair. Tissue Eng. 2006;12(9):2509-19.
54. Velasco MA, Narvaez-Tovar CA, Garzon-Alvarado DA. Design, materials, and mechanobiology of biodegradable scaffolds for bone tissue engineering. BioMed Res Int. 2015;2015:729076.
55. Solchaga LA, Temenoff JS, Gao J, Mikos AG, Caplan AI, Goldberg VM. Repair of osteochondral defects with hyaluronan- and polyester-based scaffolds. Osteoarthritis Cartilage. 2005;13(4):297-309.
56. Ng KW, Kugler LE, Doty SB, Ateshian GA, Hung CT. Scaffold degradation elevates the collagen content and dynamic compressive modulus in engineered articular cartilage. Osteoarthritis Cartilage. 2009;17(2):220-7.
57. Chung C, Beecham M, Mauck RL, Burdick JA. The influence of degradation characteristics of hyaluronic acid hydrogels on in vitro neocartilage formation by mesenchymal stem cells. Biomaterials. 2009;30(26):4287-96.
58. Matsiko A, Levingstone TJ, Gleeson JP, O'Brien FJ. Incorporation of TGF-beta 3 within collagen-hyaluronic acid scaffolds improves their chondrogenic potential. Adv Healthc Mater. 2015;4(8):1175-9.
59. Matsiko A, Gleeson JP, O'Brien FJ. Scaffold mean pore size influences mesenchymal stem cell chondrogenic differentiation and matrix deposition. Tissue Eng Part A. 2015;21(3-4):486-97.
60. Eyrich D, Wiese H, Maier G, Skodacek D, Appel B, Sarhan H, et al. In vitro and in vivo cartilage engineering using a combination of chondrocyte-seeded long-term stable fibrin gels and polycaprolactone-based polyurethane scaffolds. Tissue Eng. 2007;13(9):2207-18.
61. Dong C, Lv Y. Application of collagen scaffold in tissue engineering: recent advances and new perspectives. Polymers. 2016;8(42):1-20.
62. Kock LM, Ravetto A, van Donkelaar CC, Foolen J, Emans PJ, Ito K. Tuning the differentiation of periosteum-derived cartilage using biochemical and mechanical stimulations. Osteoarthritis Cartilage. 2010;18(11):1528-35.
63. Elder BD, Athanasiou KA. Effects of confinement on the mechanical properties of self-assembled articular cartilage constructs in the direction orthogonal to the confinement surface. J Orthop Res. 2008;26(2):238-46.
64. Saeidi N, Sander EA, Ruberti JW. Dynamic shear-influenced collagen self-assembly. Biomaterials. 2009;30(34):6581-92.
65. Flynn BP, Bhole AP, Saeidi N, Liles M, Dimarzio CA, Ruberti JW. Mechanical strain stabilizes reconstituted collagen fibrils against enzymatic degradation by mammalian collagenase matrix metalloproteinase 8 (MMP-8). PloS ONE. 2010;5(8):e12337.
66. Bian L, Crivello KM, Ng KW, Xu D, Williams DY, Ateshian GA, et al. Influence of temporary chondroitinase ABC-induced glycosaminoglycan suppression on maturation of tissue-engineered cartilage. Tissue Eng Part A. 2009;15(8):2065-72.
67. Natoli RM, Revell CM, Athanasiou KA. Chondroitinase ABC treatment results in greater tensile properties of self-assembled tissue-engineered articular cartilage. Tissue Eng Part A. 2009;15(10):3119-28.
68. Bastiaansen-Jenniskens YM, Koevoet W, de Bart AC, van der Linden JC, Zuurmond AM, Weinans H, et al. Contribution of collagen network features to functional properties of engineered cartilage. Osteoarthritis Cartilage. 2008;16(3):359-66.
69. Chang CH, Kuo TF, Lin CC, Chou CH, Chen KH, Lin FH, et al. Tissue engineering-based cartilage repair with allogenous chondrocytes and gelatin-chondroitin-hyaluronan tri-copolymer scaffold: a porcine model assessed at 18, 24, and 36 weeks. Biomaterials. 2006;27(9):1876-88.
70. Endres M, Neumann K, Schroder SE, Vetterlein S, Morawietz L, Ringe J, et al. Human polymer-based cartilage grafts for the regeneration of articular cartilage defects. Tissue Cell. 2007;39(5):293-301.
71. Hao T, Wen N, Cao JK, Wang HB, Lu SH, Liu T, et al. The support of matrix accumulation and the promotion of sheep articular cartilage defects repair in vivo by chitosan hydrogels. Osteoarthritis Cartilage. 2010;18(2):257-65.
72. Lim SM, Oh SH, Lee HH, Yuk SH, Im GI, Lee JH. Dual growth factor-releasing nanoparticle/hydrogel system for cartilage tissue engineering. J Mater Sci Mater Med. 2010;21(9):2593-600.
73. Chen JP, Su CH. Surface modification of electrospun PLLA nanofibers by plasma treatment and cationized gelatin immobilization for cartilage tissue engineering. Acta Biomater. 2011;7(1):234-43.