

Osteochondral Repair Using a Hybrid Implant Composed of Stem Cells and Biomaterial

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53.1 Introduction

Osteoarthritis (OA) is a common disease causing joint pain, joint deformity, and functional disability and affects the quality of life of both young and elderly patients worldwide [1]. Current treatment strategies can be divided into nonsurgical (conservative) and surgical therapies according to the severity of OA [2–4]. In the early stage of OA, pharmacologic and/or physical therapies as conservative treatments are typically selected for the purpose of reducing pain and, in some cases, attempting to delay the progressive structural deterioration in affected joints. Surgical therapies such as joint replacement and osteotomy are available for patients who fail to respond to more conservative measures. These treatments are well established and effective for reducing pain and improving quality of life. Regardless of these

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therapeutic options, however, there is no method available that facilitates complete healing of the articular cartilage [5–10]. Recently, several biological approaches, such as the use of tissue-engineered materials, have been tested to overcome such potential problems. This chapter will focus on the feasibility of tissue-engineered materials in osteochondral repair and highlight recent advances in the biological repair of osteochondral lesions.

53.2 Anatomy of Osteochondral Tissue

The osteochondral complex consists of both the articular cartilage and underlying subchondral bone. The conditions of articular cartilage and its supporting bone are tightly coupled, and they interact with each other biologically as well as biomechanically [11, 12]. Therefore, these structures could be considered as one osteochondral unit.

Biochemically, cartilage tissue is largely comprised of water, chondrocytes, type II collagen, and proteoglycan [13–15]. Cartilage can be differentiated into four distinct zones: the superficial, middle, deep, and calcified cartilage zones (Fig. 53.1) [16]. Each zone is defined by a particular composition and organization of cells and extracellular matrix (ECM) molecules. The

differential proportions in ECM composition influence the mechanical properties of each zone of the cartilage. For example, the superficial zone is strong in tension along the alignment of its collagen fibrils and thereby assists in the resistance of shear forces at the surface. By comparison, the deep zone has more compressive strain.

Subchondral bone is a complex tissue consisting of water, collagen type I, and hydroxyapatite, with the two latter components providing the tissue's stiffness and compressive strength [14, 15, 17]. The compressive modulus of subchondral bone is higher than that of cartilage. The different morphological compositions and mechanical properties of subchondral bone and cartilage indicate the complexity of the tissue interface.

The osteochondral interface is described by the interaction of calcified cartilage and the underlying subchondral bone [18]. Structurally, collagen fibers extend from the deep zone to calcified cartilage through a wavy tidemark, which enables the dispersal of force through the vertical orientation of collagen fibrils [19]. However, despite the fact that calcified cartilage is mineralized tissue, its mechanical strength is lower than that of the subchondral bone [20]. Calcified cartilage is interdigitated with subchondral bone, but fibers do not extend across the zone into the bone [19, 21]. The wavy tidemark and vertically oriented fibers at the tidemark, as well as interdigitations present at the interface, may allow for reducing stress concentrations, as well as better integration with the underlying subchondral bone [14, 19].

Osteoarthritic degenerative changes, such as articular cartilage loss, subchondral bone thickening, and osteophyte formation, may be developed, triggered by a multitude of factors including aging, trauma, obesity, mechanical overload, congenital disorder, and infection [22–26]. The primary morphologic changes include thinning, fissuring, and fragmentation of articular cartilage. With progression of the disease comes a continuous loss of articular cartilage, accompanied with the decrease of collagen type II and aggrecan [27, 28], leading to exposure of subchondral bone. Secondary changes are frequently seen in the underlying bone, such as sclerosis, cystic change, and new bone formation (Fig. 53.2).

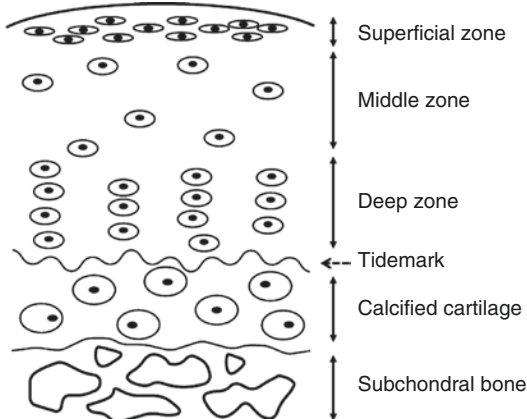


Fig. 53.1 Schematic drawing of the different zones of articular cartilage and subchondral bone



Fig. 53.2 Radiography of osteoarthritic knee joint. In osteoarthritis, the loss of cartilage (joint space narrowing) and subchondral bone change such as sclerosis, cystic change, and new bone formation (osteophyte) are frequently seen (*arrows*)

53.3 Strategy for Osteochondral Repair

For an ideal repair of osteochondral lesions, it is important to regenerate subchondral bone and to facilitate zonal restoration of cartilage and subchondral bone, layer by layer, mimicking the natural articular structure [11, 29–34]. As a strategy to regenerate these structures in a layer-by-layer fashion, biphasic or triphasic constructs have been developed due to both mechanical and biological reasons, including the acquisition of

initial mechanical strength, mimicking a natural articulate structure, a uniform tidemark at the osteochondral junction, and integration of the biphasic implant with host tissue to sustain biological function [9, 35–44]. For satisfying the biological requirements, an osteochondral implant should ideally have a rigid osseous layer (to support the overlying cartilage and integrate with the native bone) and a chondral layer (to allow the seeding and proliferation of chondrocytes or mesenchymal stem cells (MSCs) and subsequent deposition of cartilaginous ECM).

53.4 Choice of Cells

The most direct cell source may be the biopsy specimens taken from the patients, from which mature osteoblasts and chondrocytes may be obtained. However, as the number of cells obtained is usually limited, it is typically not enough to allow seeding onto the scaffolds. Also, the expansion of primary cells may result in a loss of differentiation capacity; for example, the expansion of articular chondrocytes can lead to dedifferentiation into fibroblast [45–47]. To overcome such potential problems with respect to dedifferentiation, a three-dimensional (3D) culture can be used to retain the cellular phenotype and avoid dedifferentiation [48]. The most common method is the use of various scaffolds to produce a 3D culture condition [49, 50] and may be combined with the supplementation of growth factors [51], the use of bioreactor [52], mechanical stimulation of the cells [53, 54], and the use of low oxygen tension [55, 56] during cultivation. Also, even if chondrocytes lose their differentiated phenotype, dedifferentiated chondrocytes can regain their differentiated phenotype through the redifferentiation process of cultivation in a 3D scaffold combined with growth factors [57, 58].

As an additional option, stem cells may represent promising alternatives [59, 60]. Specifically, mesenchymal stem cells (MSCs) have the capability to differentiate into a variety of connective tissue cell types, including bone, cartilage, tendon, muscle, and adipose tissue [10, 61]. These cells may be isolated from various tissues, such as bone

marrow, skeletal muscle, synovial membrane, adipose tissue, and umbilical cord blood [5, 6, 61–64]. Moreover, allogeneic MSCs [10, 65] or induced pluripotent stem (iPS) cells [66, 67] may also be considered. However, there have not been much evidence using these cells forth coming in terms of preclinical and clinical safety, and thus further studies with such cells are likely necessary.

53.5 Choice of Materials

Several methods have been proposed to develop biphasic scaffolds with the hybridization of two distinct biomaterials, each of which being adequate to integrate with the respective surrounding tissue [68]. Many specific material types have been developed for both cartilage and bone regeneration, which are typically made of biocompatible and biodegradable polymers. For the cartilage layer, natural or synthetic polymer-based scaffolds are commonly used. On the other hand, for a scaffold of the subchondral bone layer, it is important to choose materials with initial mechanical strength, good bone ingrowth, and integration of native surrounding bone. Ceramics, glasses, and metallic materials are commonly used. Also, natural or synthetic polymers, similar to cartilage layer, could be used alone or combined with ceramics [36, 37, 69–72].

The natural polymers could provide a naturally occurring environment for the cells and tissues and thereby potentially facilitate cell proliferation and differentiation [73, 74]. Moreover, natural polymers usually contain specific molecular domains that can support and guide cells at various stages of their development [14, 68]; thus biological interaction of the scaffold with the host tissue can be enhanced. However, they are, in general, biomechanically weak and less stiff than other materials [14]. As a source of materials, collagen, gelatin, glycosaminoglycan, chitosan, starch, hyaluronic acid, alginate, and bacterial-sourced polymers (hydroxyalkanoates) are commonly used.

Biodegradable synthetic polymers offer several advantages over other materials for developing scaffolds in tissue engineering. The main advantages are being able to control mechanical proper-

ties (i.e., strength and stiffness) and degradation speed [75]. Synthetic polymers are also attractive because they can be fabricated into various shapes with a desired pore according to the speed of cell migration or tissue ingrowth [76]. Moreover, the progression of current techniques such as electrospinning methods and the 3D printer have enabled the simple design and fabrication of scaffolds, which mimic the original tissue structure [77–79]. On the other hand, synthetic polymers have limitations in bioactivity due to their hydrophobic surface not supporting cell attachment and proliferation [80–83]. Surface treatment with chondroitin sulfate [84], silicate [85], and alkaline [81] could increase hydrophilicity and provide a suitable scaffold for tissue engineering. As a source of biodegradable synthetic polymers, poly(glycolic acid) (PGA), poly(D,L-lactic-co-glycolic acid) (PLGA), poly(L-lactic acid) (PLLA), poly(caprolactone) (PCL), and poly(ethylene glycol) (PEG) have been commonly used.

Ceramics, such as hydroxyapatite (HA) or other calcium phosphates, such as tricalcium phosphate (TCP), are widely used for bone tissue engineering [86–89]. These materials promote the formation of a bone-like tissue and enhance integration of the scaffold to the host tissue due to excellent osteoconductivity and osteoinductivity. On the other hand, these scaffolds have low structural integrity being brittle and unsuitable for applications under mechanical stress, although they exhibit suitable stiffness [14]. The degradation behavior of these scaffolds can be controlled by changes in the porous structures, which can be tailored in terms of their degradation kinetics appropriate for bone tissue engineering. It is also well known that increasing porosity impairs further the mechanical properties of bioceramic scaffolds. This problem can be solved by modifying any porous scaffolds with infiltration or coating by biodegradable polymers [90–92].

53.6 Current Status and Issues

There have been many therapeutic procedures investigated to biologically repair damaged cartilage, some of which are already at the stage of

clinical application. On the contrary, considering the higher incidence of OA, which involves subchondral bone pathology, by comparison to isolated chondral injury [3, 93–97], there is an urgent need to develop novel therapeutic methods for osteochondral repair with clinical relevance. In this regard, the number of animal experiments and clinical trials to treat osteochondral lesions has been recently increased [40, 98–102]. Also, we have originally developed a hybrid implant composed of artificial bone coupled with a mesenchymal stem cell (MSC)-based scaffold-free tissue-engineered construct (TEC) and demonstrated its feasibility for osteochondral repair in a rabbit osteochondral defect model [12, 103]. Our experimental details are described below.

53.7 Our MSC-Based Hybrid Implant for Osteochondral Repair

Currently, artificial bones generated from hydroxyapatite (HA) or beta-tricalcium phosphate (bTCP) have been widely used for clinical treatment of bone defects after fractures or after resection of bone tumors [88, 104, 105]. We have developed a novel fully interconnected HA artificial bone with a sufficient initial strength, as well as an excellent bone formation capacity [88, 89] and previously reported the feasibility of this implant to repair subchondral bone [88]. In addition, we have developed a scaffold-free three-dimensional tissue-engineered

construct (TEC) composed of MSCs derived from the synovium and extracellular matrices (ECMs) synthesized by the cells [6] and demonstrated the feasibility of the resultant TEC to facilitate cartilage repair in a large animal model [5, 10]. These TECs are developed without an artificial scaffold, and, thus, their implantation could eliminate or minimize the risk of potential side effects induced by extrinsic chemical or biological materials. Furthermore, such TECs are highly adherent to cartilage matrix, and secure integration of the TEC to adjacent cartilage tissue is observed after implantation. Therefore, we hypothesized that combined constructs of TEC and the fully interconnected HA-based artificial bone would effectively repair an osteochondral lesion and test this hypothesis using a rabbit osteochondral defect model (Fig. 53.3). At 6 months post implantation, osteochondral defects treated with the hybrid implants exhibited more rapid subchondral bone repair coupled with the development of cartilaginous tissue with good tissue integration to the adjacent host cartilage (Fig. 53.4). Conversely, the control group, in which HA alone was implanted into the osteochondral defect, exhibited delayed subchondral bone repair (Fig. 53.4). In addition, the repair cartilaginous tissue in this group had poor integration to adjacent cartilage and contained clustered chondrocytes, suggesting an early OA-like degenerative change at 6 months post implantation. Biomechanically, the osteochondral repair tissue treated with the combined implants at 6 months restored tissue stiffness,

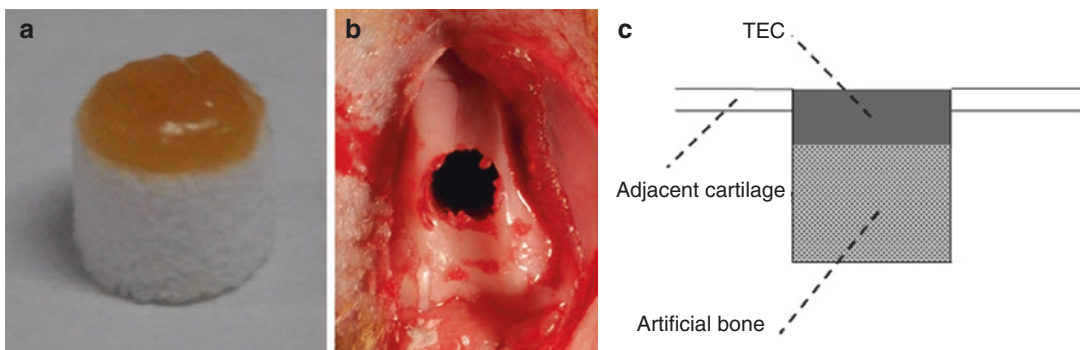


Fig. 53.3 (a) The hybrid implant generated with a tissue-engineered construct (TEC) and an artificial bone. (b) Osteochondral defects in the femoral groove of the rabbit

knee. (c) Schematic representation of implantation of TEC and artificial bone. Quoted and modified from [103] (Shimomura et al., *Tissue Eng Part A*, 2014)

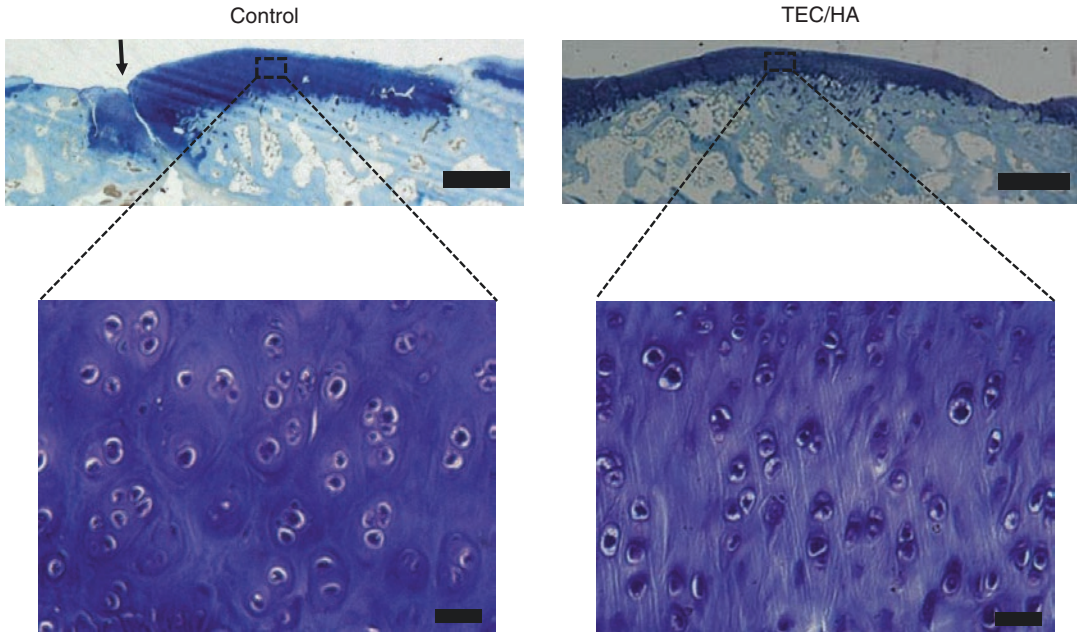


Fig. 53.4 Toluidine blue staining of repair tissues in control (HA alone) and TEC/HA group. Note that the repair tissue in defects treated with the TEC/HA implant sustained good tissue integration to the adjacent host tissue, while that of control group showed poor integration (arrow). Cellular morphology in defects treated with the

TEC/HA implant showed round-shaped cells in lacuna, while that in control group showed cell clustering in lacuna. Bar = 1 mm (upper images). Bar = 20 μ m (lower images). Quoted and modified from [103] (Shimomura et al., *Tissue Eng Part A*, 2014)

similar to normal osteochondral tissue. Therefore, we concluded that the hybrid implants significantly accelerated and improved osteochondral repair [103].

To further improve the repair quality and shorten the maturation time, additional options for the artificial bone component should be evaluated and compared with outcomes with HA. bTCP is an alternative to consider, as it is a highly biocompatible material that provides a resorbable interlocking network to implants and is resorbed more rapidly than HA in vivo [89]. Therefore, the use of bTCP might be advantageous and may result in more efficient and rapid subchondral bone remodeling after implantation. We hypothesized that a bTCP-based hybrid implant coupled with a synovial MSC-derived TEC would exhibit superior osteochondral repair when compared with an HA-based hybrid implant and test this hypothesis using a rabbit osteochondral defect model mentioned above.

Osteochondral defects treated with the TEC/bTCP implants showed more rapid subchondral bone repair at 1 month, but the cartilaginous tissue deteriorated over time out to 6 months post implantation (Fig. 53.5a, b). On the other hand, osteochondral defect treated with TEC/HA showed the delayed subchondral bone repair at 1 month but similar quality of subchondral bone repair to TEC/bTCP at 2 months (Fig. 53.5a, b). Notably, the repair tissue maintained good histological quality out to 6 months after implantation and also exhibited better biomechanical properties at 6 months as compared with the TEC/bTCP implants (Fig. 53.6). Contrary to our hypothesis, the TEC/HA hybrid implant facilitated better osteochondral repair than did the TEC/bTCP implant. The results of the present study suggest the importance of a stable restoration of subchondral bone for long-term effective osteochondral repair rather than rapid remodeling of subchondral bone.

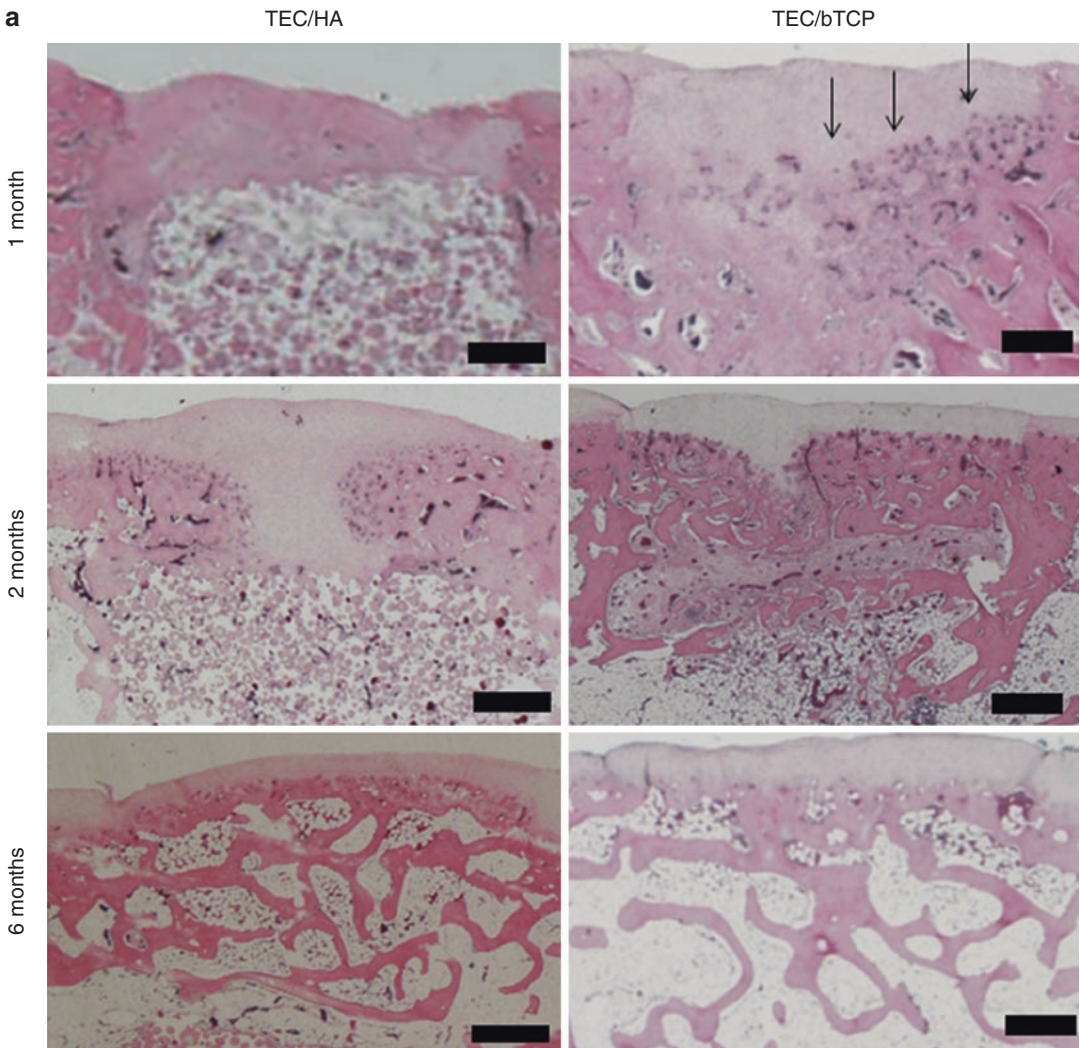


Fig. 53.5 (a) Hematoxylin and eosin (H&E) staining of repair tissue resulting from the implantation of TEC/HA or TEC/bTCP hybrid implants. The osteochondral defect treated with TEC/bTCP showed rapid subchondral bone repair at 1 month. The osteochondral defect treated with TEC/HA showed the delayed subchondral bone repair at 1 month but similar quality of subchondral bone repair to TEC/bTCP at 2 months. Bar = 1 mm. (b) Toluidine blue staining of repair tissue resulting from the implantation of

TEC/HA or TEC/bTCP hybrid implants at 6 months after surgery. The repair tissue exhibited fibrocartilaginous-like tissue with weak toluidine blue staining in the TEC/bTCP group, while that did in hyaline cartilage-like features, and the chondrocytes were arranged in longitudinal columns in TEC/HA group. Bar = 20 μm (*lower images*). Quoted and modified from [12] (Shimomura et al., *Am J Sports Med*, 2017)

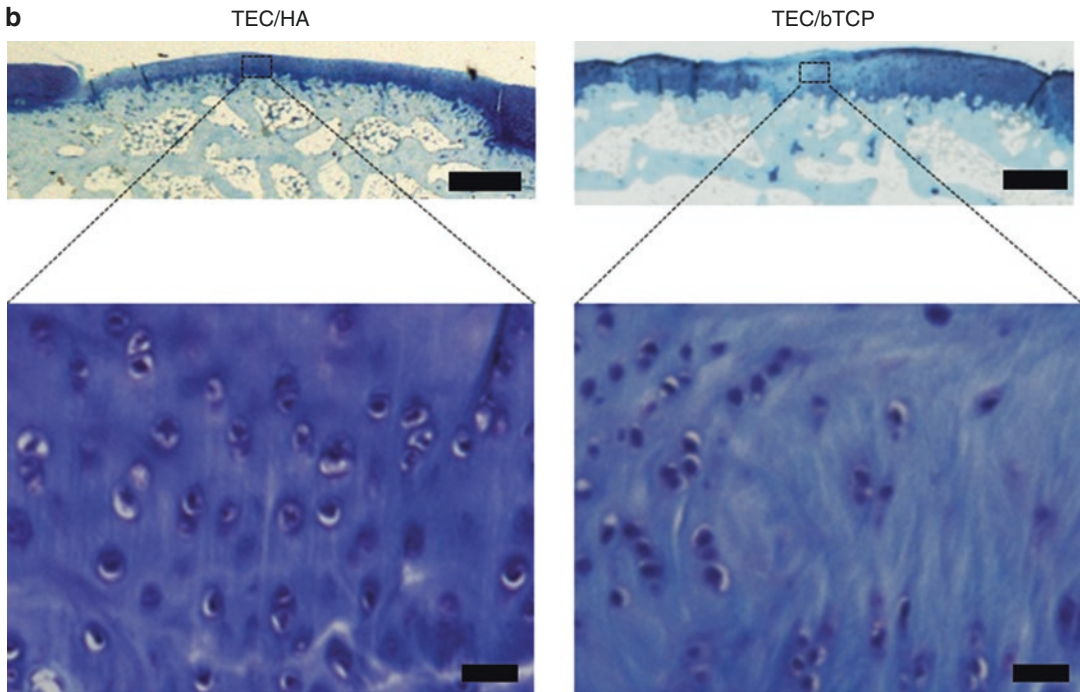


Fig. 53.5 (continued)

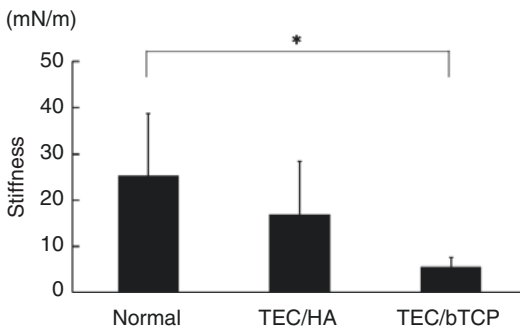


Fig. 53.6 The cartilage tissue stiffness of a healthy rabbit and after implantation of a TEC/HA or TEC/bTCP hybrid implant at 6 months after surgery. The TEC/HA hybrid implant exhibited enhanced mechanical properties compared with the TEC/bTCP hybrid implant. * $p < 0.05$. Quoted and modified from [12] (Shimomura et al., Am J Sports Med, 2017)

53.8 Future Directions

The management of OA remains challenging and controversial. Considering the steady progression of tissue engineering and cell-based technologies over the past decade, we may have new therapeutic options for osteochondral repair in

clinical practice. In this chapter, we have focused on the current techniques and recent advances of tissue-engineered biomaterial scaffolds as well as our novel MSC-based hybrid implant for the treatment of osteochondral lesion. There have been many promising scaffolds developed, some of which contribute to good osteochondral repair in vivo. In addition, the recent work has been focused on not only investigating the effectiveness of materials or cells but also to apply several new concepts and techniques such as mechanical [100], microstructural [76], and local microenvironment modification [106] for the design and fabrication of scaffolds. Therefore, the application of additional new implants to osteochondral lesions could be expected in the near future. On the other hand, the most suitable biomaterials for the cartilage or subchondral bone layers have not been fully investigated, while there are many biomaterials available for osteochondral repair. Therefore, the comparison of these materials should be performed to ultimately determine the ideal material. Further studies will be needed and should be conducted in a methodologically rigorous fashion.

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References

- Harris Jr ED. The bone and joint decade: a catalyst for progress. *Arthritis Rheum.* 2001;44(9):1969–70.
- Zhang W, Moskowitz RW, Nuki G, et al. OARSI recommendations for the management of hip and knee osteoarthritis, part I: critical appraisal of existing treatment guidelines and systematic review of current research evidence. *Osteoarthr Cartil.* 2007;15(9):981–1000.
- Zhang W, Moskowitz RW, Nuki G, et al. OARSI recommendations for the management of hip and knee osteoarthritis, part II: OARSI evidence-based, expert consensus guidelines. *Osteoarthr Cartil.* 2008;16(2):137–62.
- Zhang W, Nuki G, Moskowitz RW, et al. OARSI recommendations for the management of hip and knee osteoarthritis: part III: changes in evidence following systematic cumulative update of research published through January 2009. *Osteoarthr Cartil.* 2010;18(4):476–99.
- Ando W, Tateishi K, Hart DA, et al. Cartilage repair using an in vitro generated scaffold-free tissue-engineered construct derived from porcine synovial mesenchymal stem cells. *Biomaterials.* 2007;28(36):5462–70.
- Ando W, Tateishi K, Katakai D, et al. In vitro generation of a scaffold-free tissue-engineered construct (TEC) derived from human synovial mesenchymal stem cells: biological and mechanical properties and further chondrogenic potential. *Tissue Eng Part A.* 2008;14(12):2041–9.
- Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med.* 1994;331(14):889–95.
- Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthr Cartil.* 2002;10(6):432–63.
- Kandel RA, Grynopas M, Pilliar R, et al. Repair of osteochondral defects with biphasic cartilage-calcium polyphosphate constructs in a sheep model. *Biomaterials.* 2006;27(22):4120–31.
- Shimomura K, Ando W, Tateishi K, et al. The influence of skeletal maturity on allogenic synovial mesenchymal stem cell-based repair of cartilage in a large animal model. *Biomaterials.* 2010;31(31):8004–11.
- Gomoll AH, Madry H, Knutsen G, et al. The subchondral bone in articular cartilage repair: current problems in the surgical management. *Knee Surg Sports Traumatol Arthrosc.* 2010;18(4):434–47.
- Shimomura K, Moriguchi Y, Nansai R, et al. Comparison of 2 different formulations of artificial bone for a hybrid implant with a tissue-engineered construct derived from synovial mesenchymal stem cells. *Am J Sports Med.* 2017;45(3):666–75.
- Keeney M, Pandit A. The osteochondral junction and its repair via bi-phasic tissue engineering scaffolds. *Tissue Eng Part B Rev.* 2009;15(1):55–73.
- Nooeaid P, Salih V, Beier JP, Boccaccini AR. Osteochondral tissue engineering: scaffolds, stem cells and applications. *J Cell Mol Med.* 2012;16(10):2247–70.
- Yang PJ, Temenoff JS. Engineering orthopedic tissue interfaces. *Tissue Eng Part B Rev.* 2009;15(2):127–41.
- Madry H, van Dijk CN, Mueller-Gerbl M. The basic science of the subchondral bone. *Knee Surg Sports Traumatol Arthrosc.* 2010;18(4):419–33.
- Arvidson K, Abdallah BM, Applegate LA, et al. Bone regeneration and stem cells. *J Cell Mol Med.* 2011;15(4):718–46.
- Castro NJ, Hacking SA, Zhang LG. Recent progress in interfacial tissue engineering approaches for osteochondral defects. *Ann Biomed Eng.* 2012;40(8):1628–40.
- Oegema Jr TR, Carpenter RJ, Hofmeister F, Thompson Jr RC. The interaction of the zone of calcified cartilage and subchondral bone in osteoarthritis. *Microsc Res Tech.* 1997;37(4):324–32.
- Mente PL, Lewis JL. Elastic modulus of calcified cartilage is an order of magnitude less than that of subchondral bone. *J Orthop Res.* 1994;12(5):637–47.
- Clark JM, Huber JD. The structure of the human subchondral plate. *J Bone Joint Surg Br.* 1990;72(5):866–73.
- Barr RJ, Gregory JS, Reid DM, et al. Predicting OA progression to total hip replacement: can we do better than risk factors alone using active shape modelling as an imaging biomarker? *Rheumatology (Oxford).* 2012;51(3):562–70.
- Haverkamp DJ, Schiphof D, Bierma-Zeinstra SM, Weinans H, Waarsing JH. Variation in joint shape of osteoarthritic knees. *Arthritis Rheum.* 2011;63(11):3401–7.
- Lynch JA, Parimi N, Chaganti RK, Nevitt MC, Lane NE. Study of osteoporotic fractures research G. The association of proximal femoral shape and incident radiographic hip OA in elderly women. *Osteoarthr Cartil.* 2009;17(10):1313–8.
- Mosher TJ, Walker EA, Petscavage-Thomas J, Guermazi A. Osteoarthritis year 2013 in review: imaging. *Osteoarthr Cartil.* 2013;21(10):1425–35.
- Roemer FW, Guermazi A. Osteoarthritis year 2012 in review: imaging. *Osteoarthr Cartil.* 2012;20(12):1440–6.
- Duan Y, Hao D, Li M, et al. Increased synovial fluid visfatin is positively linked to cartilage degradation biomarkers in osteoarthritis. *Rheumatol Int.* 2012;32(4):985–90.

28. Mobasheri A. Osteoarthritis year 2012 in review: biomarkers. *Osteoarthr Cartil.* 2012;20(12):1451–64.
29. Jiang CC, Chiang H, Liao CJ, et al. Repair of porcine articular cartilage defect with a biphasic osteochondral composite. *J Orthop Res.* 2007;25(10):1277–90.
30. Kon E, Delcogliano M, Filardo G, Busacca M, Di Martino A, Marcacci M. Novel Nano-composite multilayered biomaterial for osteochondral regeneration: a pilot clinical trial. *Am J Sports Med.* 2011;39(6):1180–90.
31. Minas T, Gomoll AH, Rosenberger R, Royce RO, Bryant T. Increased failure rate of autologous chondrocyte implantation after previous treatment with marrow stimulation techniques. *Am J Sports Med.* 2009;37(5):902–8.
32. Orth P, Cucchiari M, Kohn D, Madry H. Alterations of the subchondral bone in osteochondral repair—translational data and clinical evidence. *Eur Cell Mater.* 2013a;25:299–316. discussion 314–296
33. Orth P, Meyer HL, Goebel L, et al. Improved repair of chondral and osteochondral defects in the ovine trochlea compared with the medial condyle. *J Orthop Res.* 2013b;31(11):1772–9.
34. Schek RM, Taboas JM, Segvich SJ, Hollister SJ, Krebsbach PH. Engineered osteochondral grafts using biphasic composite solid free-form fabricated scaffolds. *Tissue Eng.* 2004;10(9–10):1376–85.
35. Ahn JH, Lee TH, Oh JS, et al. Novel hyaluronate-atelocollagen/beta-TCP-hydroxyapatite biphasic scaffold for the repair of osteochondral defects in rabbits. *Tissue Eng Part A.* 2009;15(9):2595–604.
36. Alhadlaq A, Mao JJ. Tissue-engineered osteochondral constructs in the shape of an articular condyle. *J Bone Joint Surg Am.* 2005;87(5):936–44.
37. Chen J, Chen H, Li P, et al. Simultaneous regeneration of articular cartilage and subchondral bone in vivo using MSCs induced by a spatially controlled gene delivery system in bilayered integrated scaffolds. *Biomaterials.* 2011;32(21):4793–805.
38. Gao J, Dennis JE, Solchaga LA, Goldberg VM, Caplan AI. Repair of osteochondral defect with tissue-engineered two-phase composite material of injectable calcium phosphate and hyaluronan sponge. *Tissue Eng.* 2002;8(5):827–37.
39. Hung CT, Lima EG, Mauck RL, et al. Anatomically shaped osteochondral constructs for articular cartilage repair. *J Biomech.* 2003;36(12):1853–64.
40. Marquass B, Somerson JS, Hepp P, et al. A novel MSC-seeded triphasic construct for the repair of osteochondral defects. *J Orthop Res.* 2010;28(12):1586–99.
41. O’Shea TM, Miao X. Bilayered scaffolds for osteochondral tissue engineering. *Tissue Eng Part B Rev.* 2008;14(4):447–64.
42. Oliveira JM, Rodrigues MT, Silva SS, 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.
43. Sherwood JK, Riley SL, Palazzolo R, et al. A three-dimensional osteochondral composite scaffold for articular cartilage repair. *Biomaterials.* 2002;23(24):4739–51.
44. Shimomura K, Moriguchi Y, Murawski CD, Yoshikawa H, Nakamura N. Osteochondral tissue engineering with biphasic scaffold: current strategies and techniques. *Tissue Eng Part B Rev.* 2014b;20(5):468–76.
45. Benya PD, Shaffer JD. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell.* 1982;30(1):215–24.
46. Schnabel M, Marlovits S, Eckhoff G, et al. Dedifferentiation-associated changes in morphology and gene expression in primary human articular chondrocytes in cell culture. *Osteoarthr Cartil.* 2002;10(1):62–70.
47. Takahashi N, Knudson CB, Thankamony S, et al. Induction of CD44 cleavage in articular chondrocytes. *Arthritis Rheum.* 2010;62(5):1338–48.
48. Takahashi T, Ogasawara T, Asawa Y, et al. Three-dimensional microenvironments retain chondrocyte phenotypes during proliferation culture. *Tissue Eng.* 2007;13(7):1583–92.
49. Marcacci M, Berruto M, Brocchetta D, et al. Articular cartilage engineering with Hyalograft C: 3-year clinical results. *Clin Orthop Relat Res.* 2005;435:96–105.
50. Zheng MH, Willers C, Kirilak L, et al. Matrix-induced autologous chondrocyte implantation (MACI): biological and histological assessment. *Tissue Eng.* 2007;13(4):737–46.
51. Chubinskaya S, Segalite D, Pikovsky D, Hakimiyan AA, Rueger DC. Effects induced by BMPs in cultures of human articular chondrocytes: comparative studies. *Growth Factors.* 2008;26(5):275–83.
52. Forsey RW, Tare R, Oreffo RO, Chaudhuri JB. Perfusion bioreactor studies of chondrocyte growth in alginate-chitosan capsules. *Biotechnol Appl Biochem.* 2012;59(2):142–52.
53. El-Ayoubi R, DeGrandpre C, DiRaddo R, Yousefi AM, Lavigne P. Design and dynamic culture of 3D-scaffolds for cartilage tissue engineering. *J Biomater Appl.* 2011;25(5):429–44.
54. Kawanishi M, Oura A, Furukawa K, et al. Redifferentiation of dedifferentiated bovine articular chondrocytes enhanced by cyclic hydrostatic pressure under a gas-controlled system. *Tissue Eng.* 2007;13(5):957–64.
55. Kurz B, Domm C, Jin M, Sellckau R, Schunke M. Tissue engineering of articular cartilage under the influence of collagen I/III membranes and low oxygen tension. *Tissue Eng.* 2004;10(7–8):1277–86.
56. Yasui Y, Chijimatsu R, Hart DA, et al. Preparation of scaffold-free tissue-engineered constructs derived from human synovial mesenchymal stem cells under low oxygen tension enhances their chondrogenic differentiation capacity. *Tissue Eng Part A.* 2016;22(5–6):490–500.

57. Albrecht C, Schlegel W, Bartko P, et al. Changes in the endogenous BMP expression during redifferentiation of chondrocytes in 3D cultures. *Int J Mol Med*. 2010;26(3):317–23.
58. Levett PA, Melchels FP, Schrobback K, Huttmacher DW, Malda J, Klein TJ. Chondrocyte redifferentiation and construct mechanical property development in single-component photocrosslinkable hydrogels. *J Biomed Mater Res A*. 2014;102(8):2544–53.
59. Shimomura K, Ando W, Moriguchi Y, et al. Next generation mesenchymal stem cell (MSC)-based cartilage repair using scaffold-free tissue engineered constructs generated with synovial mesenchymal stem cells. *Cartilage*. 2015;6(Suppl 2):13S–29S.
60. Sundelacruz S, Kaplan DL. Stem cell- and scaffold-based tissue engineering approaches to osteochondral regenerative medicine. *Semin Cell Dev Biol*. 2009;20(6):646–55.
61. De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum*. 2001;44(8):1928–42.
62. Koga H, Shimaya M, Muneta T, et al. Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. *Arthritis Res Ther*. 2008;10(4):R84.
63. Martin MJ, Muotri A, Gage F, Varki A. Human embryonic stem cells express an immunogenic non-human sialic acid. *Nat Med*. 2005;11(2):228–32.
64. Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum*. 2005;52(8):2521–9.
65. Dashtdar H, Rothan HA, Tay T, et al. A preliminary study comparing the use of allogenic chondrogenic pre-differentiated and undifferentiated mesenchymal stem cells for the repair of full thickness articular cartilage defects in rabbits. *J Orthop Res*. 2011;29(9):1336–42.
66. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663–76.
67. Tsumaki N, Okada M, Yamashita A. iPS cell technologies and cartilage regeneration. *Bone*. 2015;70:48–54.
68. Mano JF, Reis RL. Osteochondral defects: present situation and tissue engineering approaches. *J Tissue Eng Regen Med*. 2007;1(4):261–73.
69. Deng T, Lv J, Pang J, Liu B, Ke J. Construction of tissue-engineered osteochondral composites and repair of large joint defects in rabbit. *J Tissue Eng Regen Med*. 2014;8(7):546–56.
70. Kon E, Delcogliano M, Filardo G, et al. Orderly osteochondral regeneration in a sheep model using a novel nano-composite multilayered biomaterial. *J Orthop Res*. 2010;28(1):116–24.
71. Zhang S, Chen L, Jiang Y, et al. Bi-layer collagen/microporous electrospun nanofiber scaffold improves the osteochondral regeneration. *Acta Biomater*. 2013a;9(7):7236–47.
72. Zhang W, Chen J, Tao J, et al. The promotion of osteochondral repair by combined intra-articular injection of parathyroid hormone-related protein and implantation of a bi-layer collagen-silk scaffold. *Biomaterials*. 2013b;34(25):6046–57.
73. Hsu FY, Hung YS, Liou HM, Shen CH. Electrospun hyaluronate-collagen nanofibrous matrix and the effects of varying the concentration of hyaluronate on the characteristics of foreskin fibroblast cells. *Acta Biomater*. 2010;6(6):2140–7.
74. Tan W, Twomey J, Guo D, Madhavan K, Li M. Evaluation of nanostructural, mechanical, and biological properties of collagen-nanotube composites. *IEEE Trans Nanobiosci*. 2010;9(2):111–20.
75. Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. *Eur Cell Mater*. 2003;5:1–16. discussion 16
76. Duan P, Pan Z, Cao L, et al. The effects of pore size in bilayered poly(lactide-co-glycolide) scaffolds on restoring osteochondral defects in rabbits. *J Biomed Mater Res A*. 2014;102(1):180–92.
77. Li WJ, Cooper Jr JA, Mauck RL, Tuan RS. Fabrication and characterization of six electrospun poly(alpha-hydroxy ester)-based fibrous scaffolds for tissue engineering applications. *Acta Biomater*. 2006;2(4):377–85.
78. Li WJ, Mauck RL, Cooper JA, Yuan X, Tuan RS. Engineering controllable anisotropy in electrospun biodegradable nanofibrous scaffolds for musculoskeletal tissue engineering. *J Biomech*. 2007;40(8):1686–93.
79. Lin H, Zhang D, Alexander PG, et al. Application of visible light-based projection stereolithography for live cell-scaffold fabrication with designed architecture. *Biomaterials*. 2013;34(2):331–9.
80. Bhattarai SR, Bhattarai N, Viswanathamurthi P, Yi HK, Hwang PH, Kim HY. Hydrophilic nanofibrous structure of polylactide; fabrication and cell affinity. *J Biomed Mater Res A*. 2006;78(2):247–57.
81. Pena J, Corrales T, Izquierdo-Barba I, et al. Alkaline-treated poly(epsilon-caprolactone) films: degradation in the presence or absence of fibroblasts. *J Biomed Mater Res A*. 2006;76(4):788–97.
82. Sarasam AR, Krishnaswamy RK, Madihally SV. Blending chitosan with polycaprolactone: effects on physicochemical and antibacterial properties. *Biomacromolecules*. 2006;7(4):1131–8.
83. Shafiee A, Soleimani M, Chamheidari GA, et al. Electrospun nanofiber-based regeneration of cartilage enhanced by mesenchymal stem cells. *J Biomed Mater Res A*. 2011;99(3):467–78.
84. Chang KY, Cheng LW, Ho GH, Huang YP, Lee YD. Fabrication and characterization of poly(gamma-glutamic acid)-graft-chondroitin sulfate/poly-caprolactone porous scaffolds for cartilage tissue engineering. *Acta Biomater*. 2009;5(6):1937–47.
85. Chouzouri G, Xanthos M. In vitro bioactivity and degradation of polycaprolactone composites containing silicate fillers. *Acta Biomater*. 2007;3(5):745–56.
86. Huttmacher DW. Scaffolds in tissue engineering bone and cartilage. *Biomaterials*. 2000;21(24):2529–43.

87. Martin I, Miot S, Barbero A, Jakob M, Wendt D. Osteochondral tissue engineering. *J Biomech.* 2007;40(4):750–65.
88. Tamai N, Myoui A, Hirao M, et al. A new biotechnology for articular cartilage repair: subchondral implantation of a composite of interconnected porous hydroxyapatite, synthetic polymer (PLA-PEG), and bone morphogenetic protein-2 (rhBMP-2). *Osteoarthr Cartil.* 2005;13(5):405–17.
89. Yamasaki N, Hirao M, Nanno K, et al. A comparative assessment of synthetic ceramic bone substitutes with different composition and microstructure in rabbit femoral condyle model. *J Biomed Mater Res B Appl Biomater.* 2009;91(2):788–98.
90. Chen QZ, Boccaccini AR. Poly(D,L-lactic acid) coated 45S5 bioglass-based scaffolds: processing and characterization. *J Biomed Mater Res A.* 2006;77(3):445–57.
91. Miao X, Tan DM, Li J, Xiao Y, Crawford R. Mechanical and biological properties of hydroxyapatite/tricalcium phosphate scaffolds coated with poly(lactic-co-glycolic acid). *Acta Biomater.* 2008;4(3):638–45.
92. Ren J, Zhao P, Ren T, Gu S, Pan K. Poly (D,L-lactide)/ nano-hydroxyapatite composite scaffolds for bone tissue engineering and biocompatibility evaluation. *J Mater Sci Mater Med.* 2008;19(3):1075–82.
93. Aroen A, Loken S, Heir S, et al. Articular cartilage lesions in 993 consecutive knee arthroscopies. *Am J Sports Med.* 2004;32(1):211–5.
94. Curl WW, Krome J, Gordon ES, Rushing J, Smith BP, Poehling GG. Cartilage injuries: a review of 31,516 knee arthroscopies. *Arthroscopy.* 1997;13(4):456–60.
95. Dawson J, Linsell L, Zondervan K, et al. Epidemiology of hip and knee pain and its impact on overall health status in older adults. *Rheumatology (Oxford).* 2004;43(4):497–504.
96. Hjelle K, Solheim E, Strand T, Muri R, Brittberg M. Articular cartilage defects in 1,000 knee arthroscopies. *Arthroscopy.* 2002;18(7):730–4.
97. Peat G, McCarney R, Croft P. Knee pain and osteoarthritis in older adults: a review of community burden and current use of primary health care. *Ann Rheum Dis.* 2001;60(2):91–7.
98. Dhollander AA, Liekens K, Almqvist KF, et al. A pilot study of the use of an osteochondral scaffold plug for cartilage repair in the knee and how to deal with early clinical failures. *Arthroscopy.* 2012;28(2):225–33.
99. Kon E, Filardo G, Di Martino A, et al. Clinical results and MRI evolution of a nano-composite multilayered biomaterial for osteochondral regeneration at 5 years. *Am J Sports Med.* 2014;42(1):158–65.
100. Kon E, Filardo G, Robinson D, et al. Osteochondral regeneration using a novel aragonite-hyaluronate biphasic scaffold in a goat model. *Knee Surg Sports Traumatol Arthrosc.* 2014;22(6):1452–64.
101. Miot S, Brehm W, Dickinson S, et al. Influence of in vitro maturation of engineered cartilage on the outcome of osteochondral repair in a goat model. *Eur Cell Mater.* 2012;23:222–36.
102. Sosio C, Di Giancamillo A, Deponti D, et al. Osteochondral repair by a novel interconnecting collagen-hydroxyapatite substitute: a large-animal study. *Tissue Eng Part A.* 2015;21(3–4):704–15.
103. Shimomura K, Moriguchi Y, Ando W, et al. Osteochondral repair using a scaffold-free tissue-engineered construct derived from synovial mesenchymal stem cells and a hydroxyapatite-based artificial bone. *Tissue Eng Part A.* 2014;20(17–18):2291–304.
104. Shen C, Ma J, Chen XD, Dai LY. The use of beta-TCP in the surgical treatment of tibial plateau fractures. *Knee Surg Sports Traumatol Arthrosc.* 2009;17(12):1406–11.
105. Tamai N, Myoui A, Kudawara I, Ueda T, Yoshikawa H. Novel fully interconnected porous hydroxyapatite ceramic in surgical treatment of benign bone tumor. *J Orthop Sci.* 2010;15(4):560–8.
106. Reyes R, Delgado A, Solis R, et al. Cartilage repair by local delivery of TGF-beta1 or BMP-2 from a novel, segmented polyurethane/poly(lactic-co-glycolic) bilayered scaffold. *J Biomed Mater Res A.* 2014;102(4):1110–20.