



Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

journal homepage: <http://ees.elsevier.com/jot>



REVIEW ARTICLE

# Bioactive scaffolds for osteochondral regeneration

Cuijun Deng, Jiang Chang, Chengtie Wu\*

State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Biomaterials and Tissue Engineering Research Center, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, PR China

Received 28 September 2018; received in revised form 13 November 2018; accepted 26 November 2018  
Available online 26 December 2018

## KEYWORDS

Bioactive scaffolds;  
Bioceramics;  
Cartilage repair;  
Osteochondral  
regeneration;  
Subchondral bone  
regeneration

**Abstract** Treatment for osteochondral defects remains a great challenge. Although several clinical strategies have been developed for management of osteochondral defects, the reconstruction of both cartilage and subchondral bone has proved to be difficult due to their different physiological structures and functions. Considering the restriction of cartilage to self-healing and the different biological properties in osteochondral tissue, new therapy strategies are essential to be developed. This review will focus on the latest developments of bioactive scaffolds, which facilitate the osteogenic and chondrogenic differentiation for the regeneration of bone and cartilage. Besides, the topic will also review the basic anatomy, strategies and challenges for osteochondral reconstruction, the selection of cells, biochemical factors and bioactive materials, as well as the design and preparation of bioactive scaffolds. Specifically, we summarize the most recent developments of single-type bioactive scaffolds for simultaneously regenerating cartilage and subchondral bone. Moreover, the future outlook of bioactive scaffolds in osteochondral tissue engineering will be discussed. This review offers a comprehensive summary of the most recent trend in osteochondral defect reconstruction, paving the way for the bioactive scaffolds in clinical therapy.

*The translational potential of this article:* This review summarizes the latest developments of single-type bioactive scaffolds for regeneration of osteochondral defects. We also highlight a new possible translational direction for cartilage formation by harnessing bioactive ions and propose novel paradigms for subchondral bone regeneration in application of bioceramic scaffolds.

© 2018 The Authors. Published by Elsevier (Singapore) Pte Ltd on behalf of Chinese Speaking Orthopaedic Society. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

\* Corresponding author. State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Biomaterials and Tissue Engineering Research Center, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, PR China.  
E-mail address: [chentiewu@mail.sic.ac.cn](mailto:chentiewu@mail.sic.ac.cn) (C. Wu).

## Introduction

Osteochondral defect is a common disease caused by severe traumas, athletic injuries or physical diseases (osteoarthritis), which results in joint pain and deformity, as well as functional disability [1]. Injuries of joint caused by traumas and sports are always developing into osteoarthritis (OA); thus, OA is the major cause of osteochondral defect [2]. It was reported that there were about 31 million Americans who suffered from OA by 2012, among them, 9.2% had osteochondral defects [3]. As the number of OA patients is growing, it is estimated that by 2030, approximately 67 million of Americans will suffer from OA [4]. At the early stage of cartilage lesion in OA, it is characterized by pain and swelling of articular joint. In the natural structures of osteochondral tissue, the articular cartilage is well integrated with subchondral bone, and the defects of cartilage always extend deeply into subchondral bone and further develop into osteochondral defects [5]. Although cartilage has no lymphatic and vasculature, it possesses a complex hierarchical structure including both of microstructure and nanocomposites [6]. Thus, the treatment of cartilage lesion is a great challenge in clinics. Biochemically, cartilage is a complex tissue that consists of water, chondrocytes and protein network (proteoglycan and type II collagen), whereas subchondral bone comprises water, hydroxyapatite (HA) and collagen fibre bundles (type I collagen) [7]. Moreover, cartilage and subchondral bone possess different physiological properties and natural structures, and thus, osteochondral regeneration remains a significant challenge in clinics [8]. Previously, the regeneration of osteochondral defects was mainly concentrated on cartilage layer. There were few studies focused on both the cartilage and subchondral bone [6]. Based on the depth of understanding of osteochondral structure, scientists have a new perspective on osteochondral defects regeneration. Currently, several surgical options, such as microfracture, auto/allograft and joint replacement, are available for the treatment of osteochondral defect [9,10]. Although these therapies can reduce pain and improve the quality of patients' lives, there is no surgical therapy available which could facilitate complete healing of osteochondral defects, especially for complete cartilage regeneration [11,12]. Recently, several tissue engineering materials, such as biochemical factors-incorporated biomaterials and structure mimic biomaterials, have been raised to address this issue. Previously, most of the reviews were focused on the biomimetic multi-phasic structures for the regeneration of osteochondral tissue [7]. This review will concentrate on the latest developments of bioactive scaffolds for regenerating both of cartilage and subchondral bone and highlight the most recent advances in biological regeneration of osteochondral defects.

## Anatomy of osteochondral structure

With the advances of scientific technologies, more and more details of osteochondral structure were discovered. Structurally, the osteochondral structure consists of cartilage, osteochondral interface and subchondral bone [6]. Articular cartilage is a translucent tissue in

appearance, which appears smooth and exhibits light blue morphology [7]. The main component of cartilage contains chondrocytes and extracellular matrix (ECM), and the ECM consists of water, proteoglycan and collagen fiber network. Physiologically, cartilage can be divided into four different zones: the superficial, middle, deep and calcified cartilage zones [13]. Each zone has a unique composition and organization of chondrocytes as well as ECM proteins [14]. With well-organized collagen fibrils, the superficial zone has a strong tension that could resist powerful shear forces. The chondrocytes in this zone exhibit a small and circular morphology. With increasing depth, morphology of chondrocytes changes from round sphere to oval sphere at the deep zone [15]. Furthermore, the proteins in superficial and deep layers have notable differences in both of quantitative and qualitative means. The matrix protein network in the interterritorial zone composes of type II, IX and XI collagen, and the protein components in the pericellular matrix consist of type VI collagen, matrilin 3 and fibromodulin [13]. Thus, as compared with the superficial zone, the deep zone possesses a higher compressive strain.

Beneath the calcified zone of articular cartilage is the subchondral bone. The underlying subchondral bone is an important part of osteochondral tissue, which is responsible for maintaining the outline shape of articular bone and creating an appropriate biomechanical environment for the differentiation and development of cartilage [16]. It is different from cartilage in that the main components of subchondral bone include HA and glycoproteins (type I and V collagen, fibronectin and laminin) [7]. The considerable amount of HA and type I collagen fibres provides powerful compressive strength and strong stiffness for subchondral bone [17]. As compared with cartilage, subchondral bone possesses a higher compressive modulus and a lower elasticity modulus. The significantly distinct biomechanical properties, morphological compositions and physiological functions of subchondral bone and cartilage demonstrate the complexity of cartilage–bone interface.

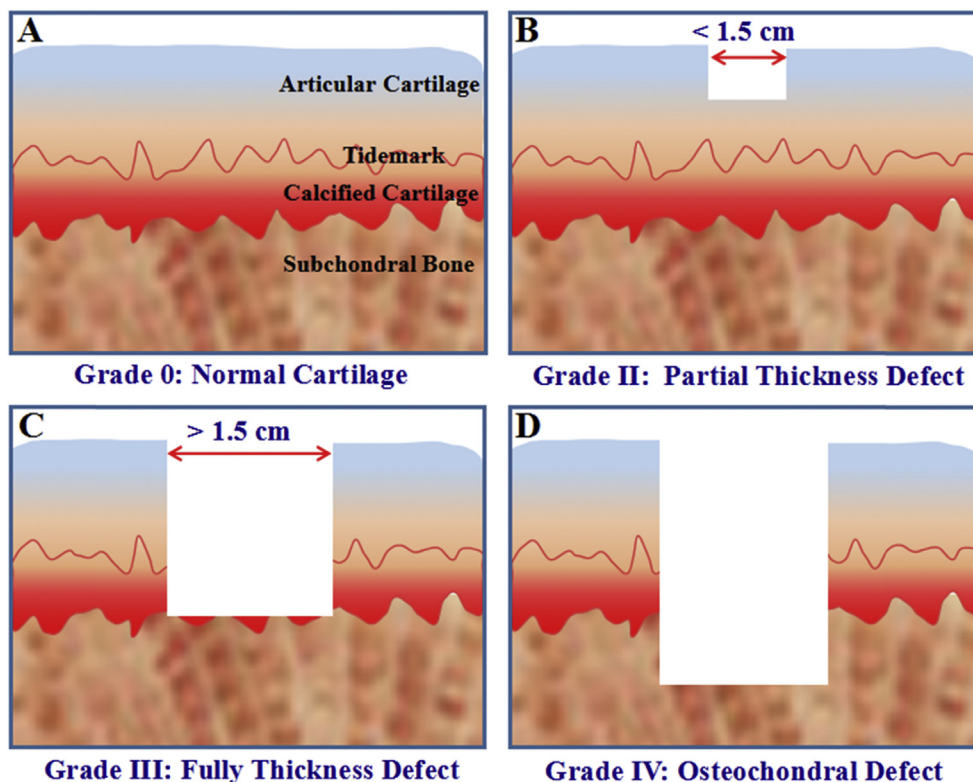
Anatomically, the cartilage–bone interface is the connection hub of articular cartilage and subchondral bone, which plays a key role in the load transfer between these two tissues. As the cartilage–bone interface is a complex structure with multicomponents, the wavy tidemark, a golden line, is used to divide cartilage and subchondral bone in anatomy [18]. The tidemark is a complex three-dimensional structure, which intersects between articular cartilage and calcified cartilage and prevents the articular cartilage from the invasion of vasculature and nerves [19]. Calcified cartilage is the transition between cartilage and subchondral bone, which acts as a physiological barrier between these two tissues [20]. As the main component of osteochondral interface, calcified cartilage possesses considerable amount of HA and collagen. The vertical orientation of collagen fibrils has a positive effect on dispersal of shear force and compressive strength [21]. Furthermore, calcified cartilage has a lower permeability than hyaline cartilage, which allows only the molecules lower than 500 Da move unidirectionally from subchondral bone to cartilage [22]. With the existence of calcified cartilage and tidemark, subchondral bone and cartilage maintain independent physiological environments. Hence,

osteochondral interface plays a crucial role in balancing the structures and functions of cartilage and subchondral bone.

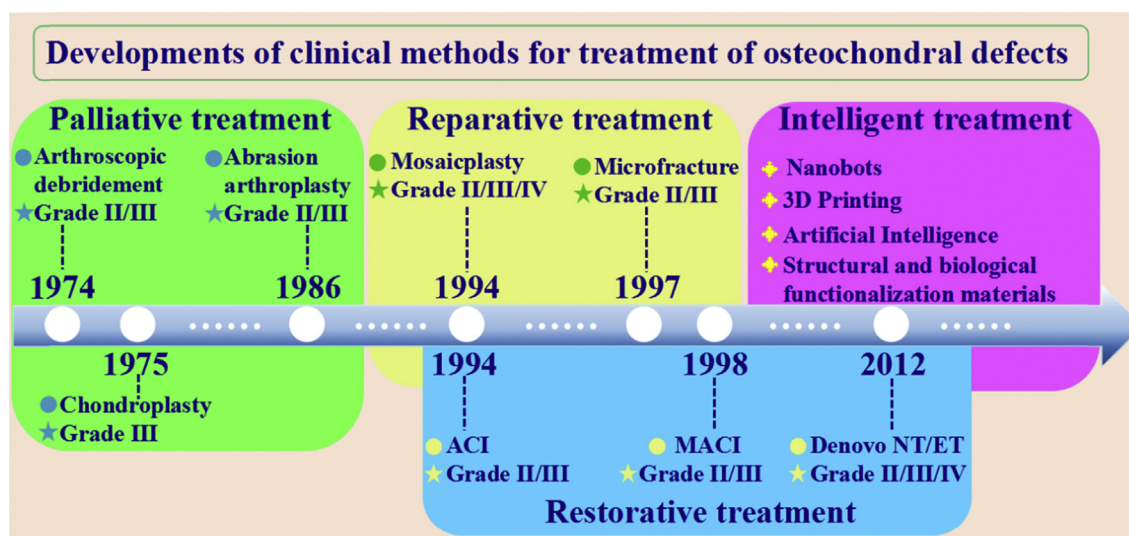
### Current strategies and challenges for treating osteochondral defects

Osteochondral defects involve articular cartilage, osteochondral interface and subchondral bone, and three tissues have significantly different bioactive properties. On the basis of the Outerbridge classification system, cartilage lesions are classified as Grade 0 (normal cartilage), Grade II (partial thickness defect), Grade III (fully thickness defect) and Grade IV (osteochondral defect) (Figure 1). Histologically, articular cartilage has no vasculature, nervous and lymphatic systems, whereas subchondral bone is rich in blood supply, nerves and lymphatics [23,24]. Nutritionally, articular cartilage has a hypoxic environment, and chondrocytes obtain energy via glycolysis; subchondral bone exchanges nutrients through blood vessels and lymphatic vessels, and aerobic respiration is the primary way for the energy intake of subchondral bone [20]. Furthermore, articular cartilage and subchondral bone have different mechanical and biological properties [25]. In addition, there is an interdependent and interact relationship between articular cartilage and subchondral bone. Consequently, the most difficult challenge for osteochondral regeneration is to regenerate articular cartilage, subchondral bone and osteochondral interface simultaneously.

Current strategies for osteochondral treatment can be categorized as palliative treatment, reparative treatment and restorative treatment (Figure 2). In clinics, palliative treatment, such as arthroscopic debridement, abrasion arthroplasty and chondroplasty, was developed for the minimally invasive treatment of osteochondral defects [12,26,27]. However, there are some limitations. Debridement and arthroplasty are not suitable for patients who have lesions smaller than 0.0127–0.0254 m<sup>2</sup>. The high temperature during chondroplasty treatment may result in the death of chondrocytes. As compared with palliative treatment, the reparative treatment methods, such as microfracture, autografts and allografts, are much more invasive. The reparative treatment attends to regeneration of osteochondral defects by drilling subchondral bone or transplant-donated bone to reach the defect regions [28,29]. However, there are several disadvantages, including postoperative rehabilitation, slower remodelling, immune reaction and transmission. Superior to the palliative treatment and reparative treatment, the restorative treatment, such as autologous chondrocyte implantation, matrix-assisted chondrocyte implantation and DeNovo natural tissue/engineered tissue (NT/ET), is performed via transplanting autologous chondrocytes or cartilage tissue to generate neotissue to heal the defects [30,31]. Although these strategies are typical engineering methods for osteochondral defects regeneration, the secondary traumatization, long recovery time and slow tissue maturation time limit their application in clinics. Hence, there is no



**Figure 1** Osteochondral defect category. Visual representation for cartilage defects by using an Outerbridge classification system: (A) Grade 0, normal cartilage; (B) Grade II, partial thickness defect; (C) Grade III, full-thickness defect; (D) Grade IV, osteochondral defect.



**Figure 2** Developments of clinical methods for treatment of osteochondral defects. Current strategies for osteochondral treatment can be categorized as palliative treatment methods, reparative treatment methods and restorative treatment methods. The future strategies for treatment of osteochondral defects will be intelligent methods, which may involve nanobots, 3D printing, artificial intelligence and structural and biological functionalization materials. Structural and biological functionalization strategies, which apply 3D printing technique and artificial intelligence, are promising methods for regeneration of osteochondral defects. 3D = three-dimensional.

proper method available in clinics which facilitates complete healing of articular cartilage, osteochondral interface and subchondral bone simultaneously.

### Cells and biochemical factors for osteochondral repair

In tissue engineering method, cells and growth factors are important components for osteochondral tissue engineering strategies. Previously, the autologous chondrocytes were applied in autologous chondrocyte implantation treatment. However, during the expansion process, chondrocytes may be dedifferentiated into fibroblasts. Beside chondrocytes, umbilical stem cells and specifically mesenchymal stem cells (MSCs) are used for osteochondral regeneration. It is well known that MSCs have the capability to differentiate into articular cartilage, subchondral bone, tendon and adipose tissues [11]. Previously, bone marrow mesenchymal stem cells (BMSCs) were embedded in biomaterials, such as collagen gel and platelet-rich fibrin glue, and then transplanted into osteochondral defects to reconstruct articular cartilage and subchondral bone [32,33]. Although the osteochondral defect symptoms were improved, the defect regions were filled with fibro cartilage instead of hyaline cartilage tissue. Furthermore, BMSC treatment required donor sites which can cause secondary trauma and pain, as well as infection. Besides the MSCs derived from the synovial membrane, the adipose stem cells (ASCs) obtained from adipose tissue are another choice for osteochondral regeneration. It was reported that ACSs could differentiate along distinct tissues, such as cartilage, bone, muscle, nerve and adipose tissue [34]. In long-term cultures, ASCs were proved to be immune privileged and more genetically stable than BMSCs [35]. There are several challenges, such as low cell yields and slow adipogenesis, before ASCs can be

applied in clinics. Another alternative source to obtain stem cells is the umbilical cord blood (UCB). As compared with BMSCs and ACSs, UCB-derived stem cells were reported to have more chondrogenic potential [36]. However, there is no global standard for the isolation, purification and amplification of UCB-derived stem cells in clinics.

Moreover, growth factors and cytokines are used to facilitate the formation of osteochondral tissue. Previous studies reported that the application of bone morphogenetic protein (BMP-2 and BMP-7), insulin-like growth factor-1 (IGF-1) and fibroblast growth factor-2 (FGF-2) can support the maturation of cartilage and that platelet-derived growth factor, IGF-1, IGF-2, transforming growth factor beta and BMP (BMP-2, BMP-4, BMP-6 and BMP-7) have the capability to induce osteogenic differentiation [5,37–39]. Generally, the common administration routes of growth factors include direct injection and systemic administration. However, many growth factors have a short half-life in blood circulation. The scaffolds that utilize physical or chemical properties to control the release of growth factors were developed to address this issue [40]. There are several challenges, such as burst release, difficult storage and easy inactivation.

### Bioactive materials for osteochondral regeneration

For a long time, auto/allograft was a golden standard for osteochondral regeneration [41]. However, there are some drawbacks such as insufficient donor, donor-site pain, secondary traumatization and immune rejection, although artificial bone materials, such as polymers, metallic materials, inorganic materials and the mixture of the three kinds of materials, were prepared to overcome the challenge [42]. There was few artificial materials that could offer dual

bioactivities for both of cartilage and subchondral regeneration. With the development of scientific technology, the osteochondral tissue engineering appeared and brought new hopes for patients with osteochondral defects [43].

## Materials for cartilage regeneration

Articular cartilage is a highly organized tissue, which can be regarded as an organic solid matrix [44]. Furthermore, cartilage possesses a hierarchical structure including both of micro and nanoscale structures. In view of the components and structures of cartilage, most of the materials designed for cartilage layer regeneration were organic matters, including polymers and ECM-based materials. Considering cartilage is saturated with water and bioactive ions, bioceramics that could constantly release bioactive ions were developed to repair cartilage lesions.

### Polymers

Many specific materials for cartilage regeneration are made up of biocompatible and biodegradable polymers. The polymers used in the cartilage layer can be divided into natural polymers and synthetic polymers. Natural polymers can be sourced from gelatin, chitosan, hyaluronic acid, collagen, alginate, glycosaminoglycan, starch and bacterial polymers. Most of the natural polymers contain specific molecular domains that could facilitate the proliferation and differentiation of chondrocytes [7]. However, as compared with other materials, natural polymers possess low stiffness and weak biomechanics [45]. Superior to natural polymers, the synthetic polymers possess controlled degradation kinetics and regulated mechanical properties. The poly (D, L-lactic-co-glycolic acid), poly (caprolactone), poly (ethyleneglycol) and poly (glycolic acid) are commonly used as a source of synthetic polymers for cartilage regeneration [46]. Although some of the synthetic polymers can be developed into different shapes for cartilage tissue regeneration, their hydrophobic surface is not beneficial for attachment and proliferation [47]. Surface treatments with bioactive factors, such as growth factors (BMP and transforming growth factor beta), alkaline, chondroitin sulphate and silicate, could enhance hydrophilicity and provide an appropriate environment for cell differentiation and tissue ingrowth [48–51].

### ECM-based materials

ECM is an important part of cartilage which could provide nutrient and mechanical support for chondrocytes. After decellularization or devitalization, cartilage ECM can serve as a structural foundation and biosignals for cartilage regeneration [52]. Generally, chemical or physical methods were applied to remove DNA and cell components, whereas cellular membranes and nuclei may not be fully devitalized [53]. Currently, both physical and chemical methods are effective ways to devitalize ECM [54]. As a source of ECM, native cartilage matrix and cell-derived matrix (CDM) are commonly used. Native cartilage matrix comes from human or animal joints, and their compositions largely depend on the species, health status, age and other genetic factors of the donors. Furthermore, certain disease states and zonal variations in cartilage structure are also key factors that

affect the composition of the harvested ECM. The *in vitro* and *in vivo* results revealed that ECM has the possibility to facilitate the chondrogenic differentiation of stem cells and stimulate cartilage defect repair. However, decellularization may reduce the content of glycosaminoglycan (GAG), and the effect of residual cells and nuclei components on ECM is unknown. Superior to native cartilage matrix, CDM can be generated from an autologous source that could reduce the immunological response [55]. The CDM is obtained from cell grown in monolayer or three-dimensional (3D) culture *in vitro*, and the CDM from synovium-derived stem cells has shown significant favourable responses on stem cell proliferation and differentiation [56]. Owing to the limitations of being time-consuming and expensive and resulting in impure products, the clinical application of CDM is restricted.

### Bioceramics

It is well known that bioceramics possess excellent osteoconductivity and bioresorbability. Over the past few years, bioceramics and the mixture of polymer/bioceramics have been developed for cartilage regeneration. Considering the poor elasticity and high stiffness of bioceramics, polymers were used to improve their elasticity modulus. Previously, a nonmineralized collagen/tricalcium phosphate scaffold was prepared for cartilage regeneration, and the *in vivo* results demonstrated that considerable amount of hyaline-like cartilage was formed in the defect region [57]. As compared with poly(lactic acid) poly(glycolic acid) copolymer (PLGA) scaffold, PLGA/nano hydroxyapatite hybrid scaffold distinctly improved cartilage regeneration [58]. Furthermore, incorporation of nanoscale hydroxyapatite (HAp) notably improved the bioactivity, osteoconductivity and osteoinductivity of poly-L/D-lactide scaffolds [59]. As bioactive ions, such as lithium, zinc, strontium, manganese and silicon, are essential factors for tissue regeneration, more and more studies are focused on the development of bioactive ions-doped bioceramics for osteochondral regeneration. Recently, our study showed that manganese-incorporated  $\beta$ -tricalcium phosphate (Mn-TCP) was efficient to regenerate cartilage and subchondral bone by harnessing the bioactivity of the released Mn ions from Mn-TCP scaffolds [60].

## Materials for subchondral bone reconstruction

Subchondral bone supports the main compressive strength to the joint and has a lower modulus of elasticity; thus, it is important that the materials for subchondral bone regeneration possess initial mechanical strength. Furthermore, subchondral bone is a highly vascularized tissue that could provide nutrients for both of itself and articular cartilage. Hence, materials that could facilitate bone ingrowth and promote the integration of bone and cartilage are preferred. Considering the requirements of mechanics in subchondral bone, materials with adequate compressive strength, such as metallic materials, bioglass and bioceramics, are commonly applied. Moreover, natural or synthetic polymers, which have appropriate load-bearing capacity, could be combined with metallic materials and bioceramics or used alone in subchondral bone tissue engineering [61–63].

### Metallic materials

Metallic materials are widely used in orthopaedic transplants because of their excellent mechanical properties. As a source of metallic materials, titanium, titanium alloys, cobalt-chromium alloy and stainless steel are commonly used. As the first generation of metallic materials for bone substitute, they have a common feature of biological inertness. Titanium and its alloys are common metallic materials in orthopaedic applications because of their good compatibility and integration with bone tissue [64]. Although sufficient in mechanical strength, most of the metallic materials are inert. By the mid-1980s, the study of metallic materials began to shift its focus from inertness to bioactivity and biodegradability. Previously, nanosized bioceramic particles, such as calcium phosphate, TCP and HA, and bioceramic coatings on metallic materials facilitated formation of apatite mineralization and further helped implant fixation with subchondral bone [65]. Recently, magnesium-based alloys have been successfully used in orthopaedic implants because of their excellent mechanical properties and good bioactive and biodegradable properties [66]. However, inappropriate degradation rate, possibility of corrosion and wear particle release are the limitations.

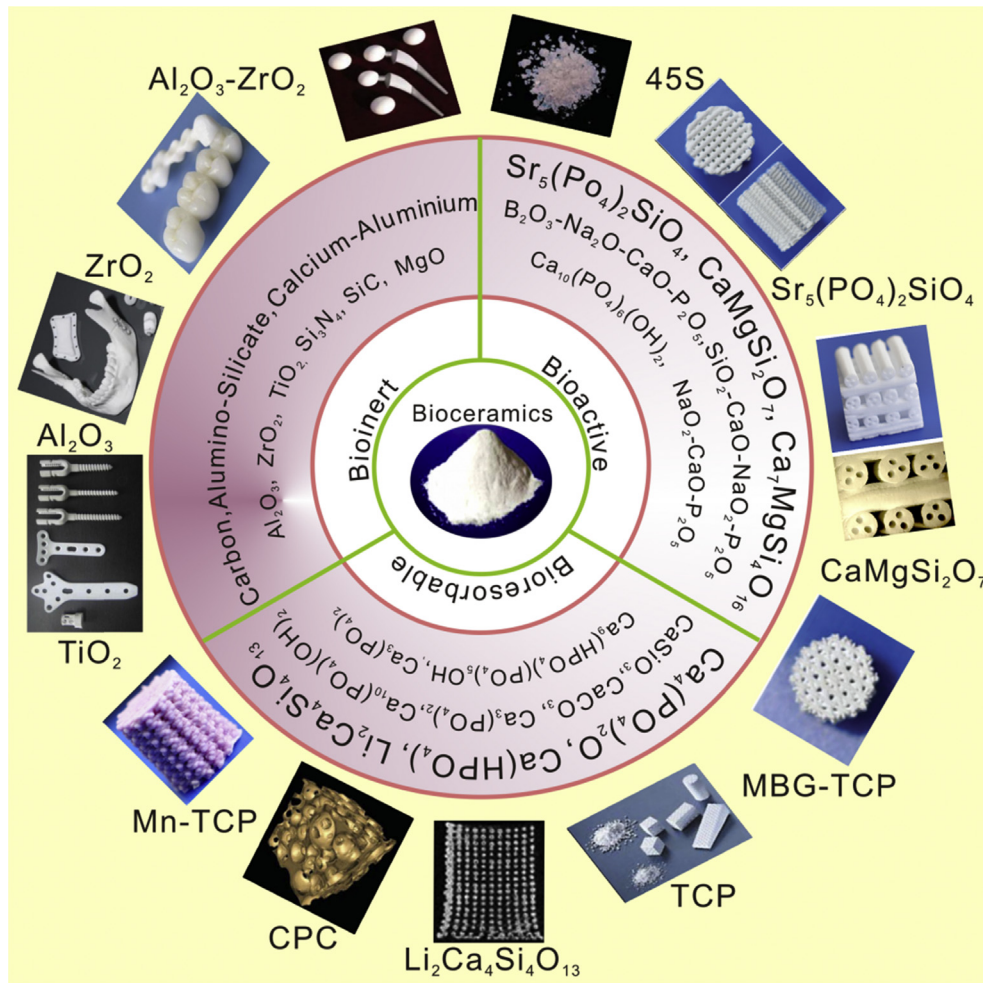
### Bioceramic and bioglass

It is well known that bioceramics and bioglasses could promote biomineralization because of their excellent osteoconductivities. According to the ability to bond with surrounding living tissue after surgery, bioceramics can be divided into three different categories (Figure 3). The first category is bioinert ceramics, such as alumina and zirconia. Although bioinert ceramics could provide adequate mechanical properties for subchondral bone regeneration, they have no distinct biological interaction with the surrounding living tissue. As bioactive ceramics, bioglasses and glass-ceramics bond directly with their surrounding tissue in a bonding osteogenesis way. Moreover, bioactive ceramics offer reasonable fracture toughness and chemical corrosion or wearing resistance after implantation. The third category is bioresorbable ceramics, which include calcium phosphates, calcium carbonates, calcium phosphate cements and calcium silicates. With increasing time, bioresorbable ceramics are gradually absorbed and substituted by neobone tissue *in vivo*. HA, TCP and 45 S bioglass are commonly used for bone tissue engineering, and the *in vivo* and *in vitro* studies indicated that these materials could stimulate the formation of bony tissue and promote integration of materials to surrounding tissue [67–69]. However, there are some drawbacks, such as low elasticity, heavy brittleness, extremely high stiffness and poor fracture toughness. The incorporation of ductile materials, such as gelatin, collagen, chondroitin sulphate and polylactide acid, may enhance the mechanical properties of bioceramics. Previously, collagen/HA scaffolds possessed better mechanical properties and osteoinductivity than HA or collagen scaffolds [70]. To fulfil significantly different biological and mechanical requirements of cartilage and subchondral bone, biphasic and multilayered scaffolds have been developed. In most of the biphasic and multilayered scaffolds, polymers such as gelatin, collagen and polylactide acid serve as the cartilage layer, and

bioceramics including HA and TCP serve as the subchondral bone layer. Although biphasic and multilayered constructs may provide a similar mechanical environment for osteochondral regeneration, it is difficult to biologically mimic the natural microstructure and physiological properties of cartilage and subchondral bone with currently available biotechnologies. Furthermore, the adhesive strength between the adjacent two layers in biphasic or multilayered scaffolds is often insufficient, which leads to the delamination of the adjacent two layers. Hence, a smart single-phase scaffold that possesses bilineage functions for simultaneously regenerating both of cartilage and subchondral bone was developed [60]. The Mn-TCP scaffolds were fabricated by a 3D printing method, and an *in vitro* study demonstrated that ionic products from Mn-TCP distinctly promoted the proliferation and differentiation of chondrocytes and MSCs. The *in vivo* study indicated that Mn-TCP scaffolds significantly facilitated the regeneration of both cartilage and subchondral bone tissues in a rabbit osteochondral defect model. Furthermore, the  $Mn^{2+}$  ions released from the bilineage scaffolds could protect chondrocytes from inflammatory osteoarthritis environment by activating autophagy. Based on the aforementioned study, lithium- and silicon-containing biomaterials scaffolds, which could continuously release of Li and Si ions, were prepared for osteochondral regeneration [71]. *In vitro*, lithium-calcium-silicate extracts distinctly facilitated the proliferation and maturation of chondrocytes, as well as stimulated the osteogenic differentiation of rabbit bone mesenchymal stem cells (rBMSCs). Moreover, Li and Si ions synergistically protected chondrocytes via inhibiting the Hedgehog pathway in an osteoarthritis model. In a rabbit osteochondral defect model, lithium-calcium-silicate scaffolds significantly stimulated osteochondral regeneration by harnessing the synergistic effect of Li and Si ions. It is well known that the interface between cartilage and subchondral bone is quite complicated, and the regeneration of osteochondral interface remains a great challenge in clinics. Recently, 3D printing  $Sr_5(PO_4)_2SiO_4$  (SPS) scaffolds were successfully developed for the complex osteochondral interface regeneration [72]. The *in vivo* study showed that SPS scaffolds distinctly promoted the regeneration of cartilage and subchondral bone via activating hypoxia-inducible factor (HIF) pathway and Wingless/Integrated (Wnt) pathway, respectively. Furthermore, SPS significantly reconstructed the osteochondral interface and preserved chondrocytes from osteoarthritis via activating autophagy and inhibiting the Hedgehog pathway by harnessing the synergistic effect of Sr and Si ions.

### Preparation strategies of bioactive scaffolds for osteochondral regeneration

Over the past few decades, a variety of technologies for 3D scaffolds manufacturing have sprung up in osteochondral tissue engineering research. Traditional methods such as electrospinning, phase separation, gas-foaming, template, freeze-dry, sol-gel method and space holder method were successfully developed. However, the traditional methods for the regeneration of both cartilage and subchondral bone remain to be complex and of low efficiency because



**Figure 3** The category of bioceramic materials applied in bone tissue engineering. Based on the ability to bond with living tissue after surgery, bioceramics can be divided into three different categories: bioinert ceramics, bioactive ceramics and bioresorbable ceramics [70,71,84–86].

TCP = tricalcium phosphate.

they cannot biologically mimic the original microstructure of cartilage and subchondral bone. These issues may be solved with the application of 3D printing technology, which can highly mimic the anisotropic nature of ECM and heterogeneity of osteochondral tissue.

### Traditional methods

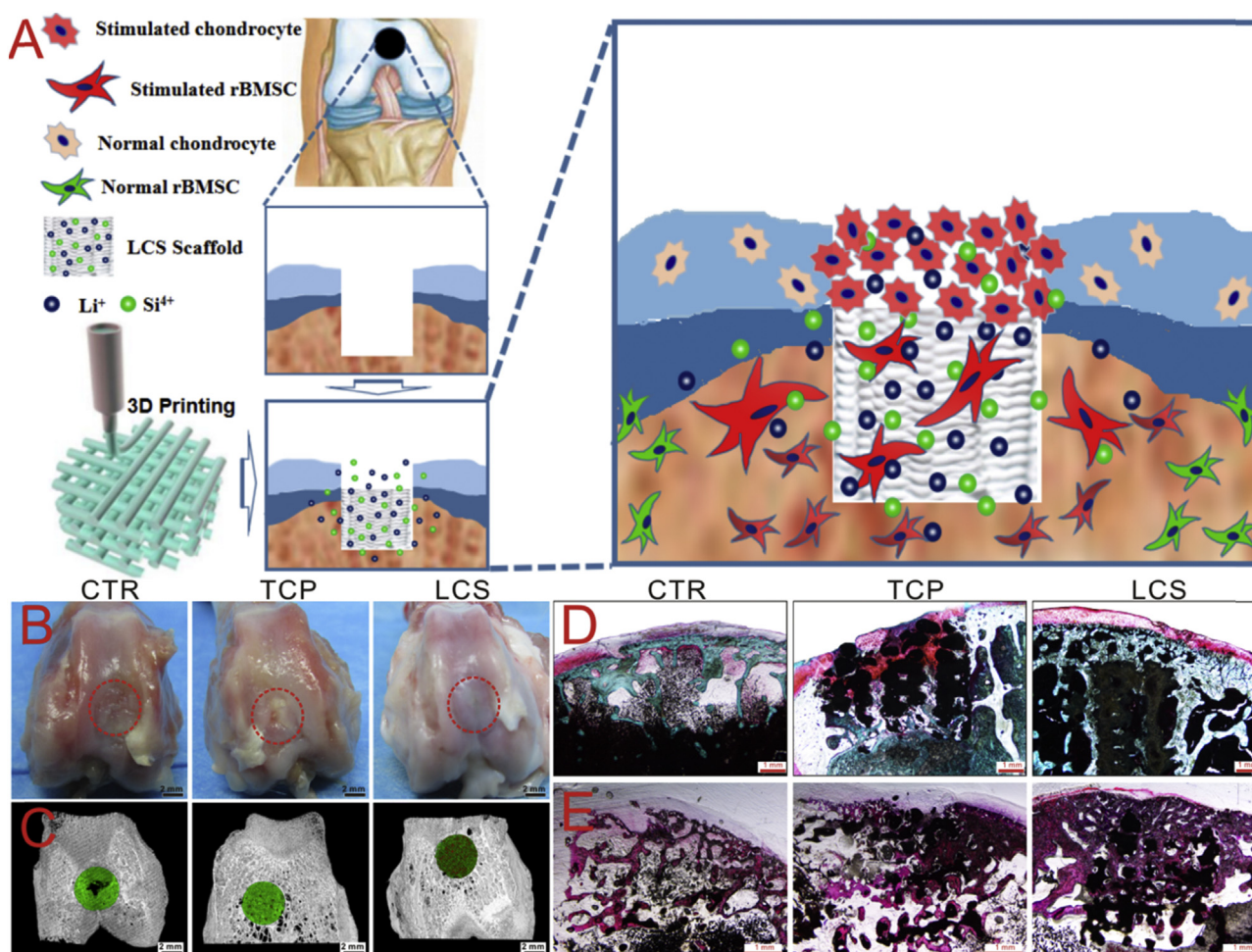
Because it is an inexpensive and versatile method for forming ECM-mimicking scaffolds, electrospinning has been proved to be a powerful technique for preparing osteochondral tissue engineering scaffolds [73]. Considering the insufficient mechanical properties and cell infiltration hindrance of electrospinning structure, a variety of methods for addressing these issues have been proposed. The phase separation is a process of thermally inducing or using a nonsolvent to fabricate porous scaffolds with uniform pore structures [74]. As compared with electrospinning, phase separation has a great potential for development of 3D nano/micrometer structures. Although phase separation has the advantages of rapid prototyping and solid freeform fabrication, limitations such as

inadequate resolution and limited material selection remain. During a gas-foaming method, chemical foaming or mechanical foaming was used to generate gas to obtain porous scaffold [75]. Gas-foaming method takes the advantages of being simple and inexpensive and the shape and density of scaffolds being controllable, whereas the disadvantages such as low porosity, disconnected pores and poor mechanical property hinder their application in osteochondral tissue engineering. The template and space holder are methods used to fabricate porous scaffolds [76]. Although the prepared scaffolds possess controlled and interconnected pores size, they lack adequate mechanical properties for bone regeneration. With the advantages of environmental protection and easygoingness, freeze-drying is suitable for biomedical application [77], whereas it remains a great challenge to prepare hierarchical structures that mimic osteochondral tissue via freeze-drying. Sol-gel is a method that involves gentle reaction temperature, and it is suitable for new scaffold manufacturing [78]. However, small size and low porosity of scaffolds limit their application in osteochondral tissue area [69,79,80].

### 3D printing

3D printing technique is a convenient and promising method to develop scaffolds with controlled macroporous structures, and it has represented a milestone for cartilage and subchondral bone tissue engineering. The technology is categorized as scaffold-based printing and scaffold-free printing. And, scaffold-based printing has been subclassified into cellular and acellular 3D printing, depending on whether the ink contains living cells or not. Furthermore, based on different printing processes, cellular 3D printing can be subclassified into extrusion-based printing, laser-based printing, droplet-based printing and stereolithography, and acellular 3D printing can be divided into fused deposition modelling, melt electrospinning writing and selective laser sintering [81–84]. Previously, most of the 3D printing biomaterials were fabricated for the regeneration of skin, tracheal splints, cardiovascular structures, hard tissues and cartilaginous structures. Because 3D printing has the unique capacity to mimic the anisotropic and heterogeneous properties of ECM, much attention has been paid in treating osteochondral defects

[85]. Recently, 3D printing has been used to prepare scaffolds with uniform pores for cartilage and subchondral bone regeneration, and MSCs on these scaffolds demonstrated a chondrogenic and osteogenic differentiation to osteochondral structures [86–88]. The *in vivo* studies showed the potential of 3D printing scaffolds in the regeneration of osteochondral defects by generating both of cartilage and subchondral bone tissues in the defect regions [89–93]. As compared with the gold standard treatment for osteochondral defects, 3D printing scaffolds can be easily designed into specific shape for the different types of osteochondral defects. Furthermore, 3D printing scaffolds provide interconnected macroporosity and microporosity, as well as heterogeneity and anisotropy, to fulfil the constituent and mechanical requirements for osteochondral tissue engineering. However, the cost of specialized equipment and fee for experienced personnel are too high for middle-class and poor patients. Before this technology can be used in clinics, some problems such as mass fabrication, sterilization, quality control and high medical expenditure should be solved.



**Figure 4** The LCS scaffolds significantly promoted osteochondral regeneration. (A) Abstract graphic; (B) knee samples at 12 weeks; (C) micro-CT analysis; (D) Safranin O staining; (E) HE staining [70]. CT = computed tomography; HE = haematoxylin and eosin; LCS = lithium-calcium-silicate.



## Summary and outlook

As mentioned previously, osteochondral tissue is quite complex, and the regeneration of osteochondral defects remains a great challenge in tissue engineering and orthopaedic surgery. In osteochondral tissue, articular cartilage and subchondral bone are well-organized tissues with multiple-scale microstructures. Hence, ideal bioactive scaffolds prepared to facilitate and enhance osteochondral regeneration should have the capability to replicate the natural architecture and physiological properties of articular cartilage and subchondral bone tissues. To mimic the hierarchical structures of osteochondral tissue, biphasic and multilayered scaffolds were fabricated. However, it is difficult to biologically mimic the original structures of osteochondral tissue, and the application of biphasic and multilayered scaffolds in clinics is limited by nonhomogeneous responses of mechanics and low adhesive strength between adjacent two layers. Until now, the approaches for the fabrication of bioactive scaffolds are including phase separation, gas-foaming, template method, freeze-drying, sol-gel method and space holder method. The traditional methods are commonly used to regulate and control the micro/nano structures and mechanic properties of scaffolds. The appearance of 3D printing technique provides a feasible strategy to prepare hierarchical structures for osteochondral tissue regeneration.

Currently, most of the materials used in cartilage layer are polymers and ECM, and materials applied in subchondral bone layer are metallic materials, bioceramics and bioglasses. To improve the specific biofunctionality for osteochondral regeneration, the bioactivators, such as growth factors and bioactive ions, were incorporated into scaffolds. Furthermore, specific cells including stem cells and chondrocytes were seeded in scaffolds to enhance regenerate efficiency. Although cells and growth factors are important for osteochondral tissue regeneration, the same good regeneration efficiency is obtained without them in many studies [60,72,92,94]. In the process of commercialization, growth factors—incorporated and cells-incorporated scaffolds are difficult to be stored and delivered because of the low survival rate of cells and instability of growth factors. Thus, most of the commercial scaffolds are cell and growth factor free. Recently, a series of smart 3D scaffolds incorporating bioactive ions were prepared for osteochondral regeneration by harnessing the synergistic effect of multiple inorganic ions (Figure 4) [60,72,92,94,95]. However, the problems of large-scale fabrication, exactly controlled release of bioactive ions and high medical expenditure still need to be solved.

In brief, the major challenges are developing bioactive scaffolds or advanced strategies, which could completely replicate the native architecture and function of osteochondral tissue and establish an interface that structurally and physiologically mimic cartilage and subchondral bone, as well as prevent the phenotypic drift of neocartilage and subchondral bone. Hence, a promising bioactive scaffold will not only structurally and biologically regenerate osteochondral tissue but also provide a satisfactory postoperative follow-up. Overall, structural and biological functionalization strategies will become a well-deserved focus in osteochondral tissue engineering.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

This research was supported by the National Key Research and Development Program of China (2018YFC1105201), the National Natural Science Foundation of China (5171101275, 81771985, 81601612), Key Research Program of Frontier Sciences CAS (QYZDB-SSWeSYS027), Science and Technology Commission of Shanghai Municipality (17441903700) and Key Research Program of Science & Technology Support Program of Jiangsu Province (BE2016763).

## References

- [1] Li X, Ding J, Wang J, Zhuang X, Chen X. Biomimetic biphasic scaffolds for osteochondral defect repair. *Regen Biomater* 2015;2(3):221–8.
- [2] Lee WY, Wang B. Cartilage repair by mesenchymal stem cells: clinical trial update and perspectives. *J Orthop Transl* 2017;9(C):76.
- [3] Murphy L, Helmick CG. The impact of osteoarthritis in the United States: a population-health perspective: a population-based review of the fourth most common cause of hospitalization in U.S. adults. *Orthop Nurs* 2012;112(1):85–91.
- [4] Cheng YJ, Hootman JM, Murphy LB, Langmaid GA, Helmick CG. Prevalence of doctor-diagnosed arthritis and arthritis-attributable activity limitation - United States, 2007–2009. *Mmwr Morb Mortal Wkly Rep* 2010;59(39):1261–5.
- [5] Re'Em T, Witte F, Willbold E, Ruvinov E, Cohen S. Simultaneous regeneration of articular cartilage and subchondral bone induced by spatially presented TGF-beta and BMP-4 in a bilayer affinity binding system. *Acta Biomater* 2012;8(9):3283–93.
- [6] Huey DJ, Hu JC, Athanasiou KA. Unlike bone, cartilage regeneration remains elusive. *Science* 2012;338(6109):917.
- [7] Nooaeid P, Salih V, Beier JP, Boccaccini AR. Osteochondral tissue engineering: scaffolds, stem cells and applications. *J Cell Mol Med* 2012;16(10):2247–70.
- [8] Magill P, Byrne DP, Baker JF, Mulhall KJ. Review article: osteochondral reconstruction and grafting. *J Orthop Surg* 2011;19(1):93–8.
- [9] Das RK, Gocheva V, Hammink R, Zouani OF, Rowan AE. Stress-stiffening-mediated stem-cell commitment switch in soft responsive hydrogels. *Nat Mater* 2016;15(3):318.
- [10] Liu X, Jin X, Ma PX. Nanofibrous hollow microspheres self-assembled from star-shaped polymers as injectable cell carriers for knee repair. *Nat Mater* 2011;10(5):398–406.
- [11] Shimomura K, Ando W, Tateishi K, Nansai R, Fujie H, Hart DA, 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.
- [12] 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.
- [13] Madry H, Dijk CNV, Muellergerbl M. The basic science of the subchondral bone. *Knee Surg Sports Traumatol Arthrosc* 2010;18(4):419–33.
- [14] Goldring MB. Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. *Ther Adv Musculoskel Dis* 2012;4(4):269–85.

- [15] Quinn TM, Hunziker EB, Häuselmann HJ. Variation of cell and matrix morphologies in articular cartilage among locations in the adult human knee. *Osteoarthritis Cartilage* 2005;13(8):672–8.
- [16] Yang PJ, Temenoff JS. Engineering orthopedic tissue interfaces. *Tissue Eng B Rev* 2009;15(2):127.
- [17] Arvidson K, Abdallah BM, Applegate LA, Baldini N, Cenni E, Gomezbarrena E, et al. Bone regeneration and stem cells. *J Cell Mol Med* 2011;15(4):718–46.
- [18] Keeney M, Pandit A. The osteochondral junction and its repair via bi-phasic tissue engineering scaffolds. *Tissue Eng B Rev* 2009;15(1):55–73.
- [19] Lyons TJ, McClure SF, Stoddart RW, McClure J. The normal human chondro-osseous junctional region: evidence for contact of uncalcified cartilage with subchondral bone and marrow spaces. *BMC Musculoskelet Disord* 2006;7(1):52.
- [20] Ulrich-Vinther M, Maloney MD, Schwarz EM, Rosier R, O’Keefe RJ. Articular cartilage biology. *J Am Acad Orthop Surg* 2003;11(6):421.
- [21] Oegema TR, Carpenter RJ, Hofmeister F, Thompson RC. The interaction of the zone of calcified cartilage and subchondral bone in osteoarthritis. *Microsc Res Tech* 1997;37(4):324–32.
- [22] Arkill KP, Winlove CP. Solute transport in the deep and calcified zones of articular cartilage. *Osteoarthritis Cartilage* 2008;16(6):708–14.
- [23] Hunziker EB, Quinn TM, Häuselmann HJ. Quantitative structural organization of normal adult human articular cartilage. *Osteoarthritis Cartilage* 2002;10(7):564–72.
- [24] Zhang Y, Wang F, Tan H, Chen G, Guo L, Yang L. Analysis of the mineral composition of the human calcified cartilage zone. *Int J Med Sci* 2012;9(5):353–60.
- [25] Poole AR, Kojima T, Yasuda T, Mwale F, Kobayashi M, Laverty S. Composition and structure of articular cartilage: a template for tissue repair. *Clin Orthop* 2001;391(391 Suppl):S26.
- [26] Upmeier H, Brügggenjürgen B, Weiler A, Flamme C, Laprell H, Willich SN. Follow-up costs up to 5 years after conventional treatments in patients with cartilage lesions of the knee. *Knee Surg Sports Traumatol Arthrosc Off J Esska* 2007;15(3):249–57.
- [27] Caffey S, Mcpherson E, Moore B, Hedman Jr T. Effects of radiofrequency energy on human articular cartilage: an analysis of 5 systems. *Am J Sports Med* 2005;33(7):1035.
- [28] Haene R, Qamirani E, Story RA, Pinsker E, Daniels TR. Intermediate outcomes of fresh talar osteochondral allografts for treatment of large osteochondral lesions of the talus. *J Bone Joint Surg Am* 2012;83-A(8):1285.
- [29] Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. *Clin Orthop* 2001;391(391 Suppl):S362.
- [30] Kon E, Verdonk P, Condello V, Delcogliano M, Dhollander A, Filardo G, et al. Matrix-assisted autologous chondrocyte transplantation for the repair of cartilage defects of the knee: systematic clinical data review and study quality analysis. *Am J Sports Med* 2009;37(1\_suppl):156S.
- [31] Bedi A, Feeley BT. Management of articular cartilage defects of the knee. *J Bone Joint Surg Am* 2010;92(4):994–1009.
- [32] Haleem AM, Singergy AAE, Sabry D, Atta HM, Rashed LA, Chu CR, et al. The clinical use of human culture-expanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: a pilot study and preliminary results. *Cartilage* 2010;1(4):253–61.
- [33] Nejadnik H, Hui JH, Feng CE, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am J Sports Med* 2010;38(6):1110–6.
- [34] Rodríguez LV, Alfonso Z, Zhang R, Leung J, Wu B, Ignarro LJ. Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. *Proc Natl Acad Sci U S A* 2006;103(32):12167–72.
- [35] Sukarto A, Yu C, Flynn LE, Amsden BG. Co-delivery of adipose-derived stem cells and growth factor-loaded microspheres in RGD-grafted N-methacrylate glycol chitosan gels for focal chondral repair. *Biomacromolecules* 2012;13(8):2490–502.
- [36] Berg LC, Koch TG, Heerkens T, Besonov K, Thomsen PD, Betts DH. Chondrogenic potential of mesenchymal stromal cells derived from equine bone marrow and umbilical cord blood. *Vet Comp Orthop Traumatol* 2009;22(05):363–70.
- [37] Shintani N, Hunziker EB. Chondrogenic differentiation of bovine synovium: bone morphogenetic proteins 2 and 7 and transforming growth factor beta1 induce the formation of different types of cartilaginous tissue. *Arthritis Rheum* 2014;56(6):1869–79.
- [38] Holland TA, Bodde EW, Cuijpers VM, Baggett LS, Tabata Y, Mikos AG, et al. Degradable hydrogel scaffolds for in vivo delivery of single and dual growth factors in cartilage repair. *Osteoarthritis Cartilage* 2007;15(2):187–97.
- [39] Salgado AJ, Coutinho OP, Reis RL. Bone tissue engineering: state of the art and future trends. *Macromol Biosci* 2010;4(8).
- [40] Niu X, Feng Q, Wang M, Guo X, Zheng Q. Porous nano-HA/collagen/PLLA scaffold containing chitosan microspheres for controlled delivery of synthetic peptide derived from BMP-2. *J Contr Release* 2009;134(2):111–7.
- [41] Urist MR, Dowell TA, Hay PH, Strates BS. Inductive substrates for bone formation. *Clin Orthop* 1968;59(7):59–96.
- [42] García-Gareta E, Coathup MJ, Blunn GW. Osteoinduction of bone grafting materials for bone repair and regeneration. *Bone* 2015;81:112–21.
- [43] Langer R, Vacanti JP. Tissue engineering. *Science* 1993;260(5110):920–6.
- [44] Mow VC, Wang CC, Hung CT. The extracellular matrix, interstitial fluid and ions as a mechanical signal transducer in articular cartilage. *Osteoarthritis Cartilage* 1999;7(1):41.
- [45] 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.
- [46] Shimomura K, Moriguchi Y, Murawski CD, Yoshikawa H, Nakamura N. Osteochondral tissue engineering with biphasic scaffold: current strategies and techniques. *Tissue Eng B Rev* 2014;20(5):468–76.
- [47] Sarasam AR, Krishnaswamy RK, Madhally SV. Blending chitosan with polycaprolactone: effects on physicochemical and antibacterial properties. *Biomacromolecules* 2006;7(4):1131–8.
- [48] Chang KY, Cheng LW, Ho GH, Huang YP, Lee YD. Fabrication and characterization of poly( $\gamma$ -glutamic acid)-graft-chondroitin sulfate/polycaprolactone porous scaffolds for cartilage tissue engineering. *Acta Biomater* 2009;5(6):1937–47.
- [49] Chouzouri G, Xanthos M. In vitro bioactivity and degradation of polycaprolactone composites containing silicate fillers. *Acta Biomater* 2007;3(5):745–56.
- [50] Reyes R, Delgado A, Sánchez E, Fernández A, Hernández A, Evora C. Repair of an osteochondral defect by sustained delivery of BMP-2 or TGF $\beta$ 1 from a bilayered alginate-PLGA scaffold. *J Tissue Eng Regen Med* 2014;8(7):521–33.
- [51] Reyes R, Delgado A, Solis R, Sánchez E, Hernández A, San RJ, et al. Cartilage repair by local delivery of TGF- $\beta$ 1 or BMP-2 from a novel, segmented polyurethane/poly(lactic-co-glycolic) bilayered scaffold. *J Biomed Mater Res* 2013;102(4):1110–20.
- [52] Sutherland AJ, Converse GL, Hopkins RA, Detamore MS. The bioactivity of cartilage extracellular matrix in articular cartilage regeneration. *Adv Healthc Mater* 2015;4(1):29–39.
- [53] Yang Q, Peng J, Guo Q, Huang J, Zhang L, Yao J, et al. A cartilage ECM-derived 3-D porous acellular matrix scaffold for in vivo cartilage tissue engineering with PKH26-labeled chondrogenic bone marrow-derived mesenchymal stem cells. *Biomaterials* 2008;29(15):2378–87.

- [54] Cheng NC, Estes BT, Awad HA, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells by a porous scaffold derived from native articular cartilage extracellular matrix. *Tissue Eng* 2009;15(2):231–41.
- [55] Hang L, Yang G, Jian T, Tuan RS. Influence of decellularized matrix derived from human mesenchymal stem cells on their proliferation, migration and multi-lineage differentiation potential. *Biomaterials* 2012;33(18):4480–9.
- [56] Li J, Hansen KC, Zhang Y, Dong C, Dinu CZ, Dzieciatkowska M, et al. Rejuvenation of chondrogenic potential in a young stem cell microenvironment. *Biomaterials* 2014;35(2):642–53.
- [57] Lv YM, Yu QS. Repair of articular osteochondral defects of the knee joint using a composite lamellar scaffold. *Bone Joint Res* 2015;4(4):56–64.
- [58] Xue D, Zheng Q, Zong C, Li Q, Li H, Qian S, et al. Osteochondral repair using porous poly(lactide-co-glycolide)/nanohydroxyapatite hybrid scaffolds with undifferentiated mesenchymal stem cells in a rat model. *J Biomed Mater Res* 2010;94A(1):259–70.
- [59] Żylińska B, StodolakZych E, SobczyńskaRak A, Szponder T, Silmanowicz P, Łańcut M, et al. Osteochondral repair using porous three-dimensional nanocomposite scaffolds in a rabbit model. *In Vivo Athens, Greece* 2017;31(5):895.
- [60] Deng C, Yao Q, Feng C, Li J, Wang L, Cheng G, et al. 3D printing of bilineage constructive biomaterials for bone and cartilage regeneration. *Adv Funct Mater* 2017;27(36):1703117.
- [61] Chen J, Chen H, Li P, Diao H, Zhu S, Dong L, 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.
- [62] Zhang S, Chen L, Jiang Y, Cai Y, Xu G, Tong T, et al. Bi-layer collagen/microporous electrospun nanofiber scaffold improves the osteochondral regeneration. *Acta Biomater* 2013;9(7):7236–47.
- [63] Zhang W, Chen J, Tao J, Hu C, Chen L, Zhao H, et al. The promotion of osteochondral repair by combined intra-articular injection of parathyroid hormone-related protein and implantation of a bilayer collagen-silk scaffold. *Biomaterials* 2013;34(25):6046–57.
- [64] Kang KT, Koh YG, Son J, Jin SY, Park JH, Kim HJ. Biomechanical evaluation of pedicle screw fixation system in spinal adjacent levels using polyetheretherketone, carbon-fiber-reinforced polyetheretherketone, and traditional titanium as rod materials. *Compos B Eng* 2017;130:248–56.
- [65] Bohner M. Physical and chemical aspects of calcium phosphates used in spinal surgery. *Eur Spine J* 2001;10(2):S114–21.
- [66] Yang K, Zhou C, Fan H, Fan Y, Jiang Q, Song P, et al. Biofunctional design, application and trends in metallic biomaterials. *Int J Mol Sci* 2018;19(1):24.
- [67] Tamai N, Myoui A, Hirao M, Kaito T, Ochi T, Tanaka J, 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). *Osteoarthritis Cartilage* 2005;13(5):405–17.
- [68] Martin I, Miot S, Barbero A, Jakob M, Wendt D. Osteochondral tissue engineering. *J Biomech* 2007;40(4):750–65.
- [69] Huttmacher DW. Scaffolds in tissue engineering bone and cartilage. *Biomaterials* 2000;21(24):2529–43.
- [70] Zhou J, Xu C, Wu G, Cao X, Zhang L, Zhai Z, et al. In vitro generation of osteochondral differentiation of human marrow mesenchymal stem cells in novel collagen-hydroxyapatite layered scaffolds. *Acta Biomater* 2011;7(11):3999–4006.
- [71] Deng C, Yang Q, Sun X, Chen L, Feng C, Chang J, et al. Bioactive scaffolds with Li and Si ions-synergistic effects for osteochondral defects regeneration. *Appl Mater Today* 2018;10:203–16.
- [72] Deng C, Zhu H, Li J, Feng C, Yao Q, Wang L, et al. Bioactive scaffolds for regeneration of cartilage and subchondral bone interface. *Theranostics* 2018;8(7):1940–55.
- [73] Castaño O, Eltohamy M, Kim HW. Electrospinning technology in tissue regeneration. *Methods Mol Biol* 2012;811:127–40.
- [74] Witte PVD, Dijkstra PJ, Berg JWAVD, Feijen J. Phase separation processes in polymer solutions in relation to membrane formation. *J Membr Sci* 1996;117(1–2):1–31.
- [75] Garg T, Goyal AK. Biomaterial-based scaffolds-current status and future directions. *Expert Opin Drug Deliv* 2014;11(5):767–89.
- [76] A B, Z J. Fabrication of metallic biomedical scaffolds with the space holder method: a review. *Materials* 2014;7(5):3588–622.
- [77] Sheridan WS, Duffy GP, Murphy BP. Optimum parameters for freeze-drying decellularized arterial scaffolds. *Tissue Eng C Methods* 2013;19(12):981–90.
- [78] Balamurugan A, Rebelo A, Kannan S, Ferreira JMF, Michel J, Balossier G, et al. Characterization and in vivo evaluation of sol–gel derived hydroxyapatite coatings on Ti6Al4V substrates. *J Biomed Mater Res B Appl Biomater* 2007;81B(2):441–7.
- [79] Ben-Nissan B. Natural bioceramics: from coral to bone and beyond. *Curr Opin Solid State Mater Sci* 2003;7(4):283–8.
- [80] Valletregí M, Ruizhernández E. Bioceramics: from bone regeneration to cancer nanomedicine. *Adv Mater* 2011;23(44):5177–218.
- [81] O’Connell G, Garcia J, Amir J. 3D bioprinting: new directions in articular cartilage tissue engineering. *ACS Biomater Sci Eng* 2017;3(11).
- [82] Ozbolat IT. Bioprinting of osteochondral tissues: a perspective on current gaps and future trends. *Int J Bioprint* 2017;3(2).
- [83] Daly AC, Freeman FE, Gonzalez-Fernandez T, Critchley SE, Nulty J, Kelly DJ. 3D bioprinting for cartilage and osteochondral tissue engineering. *Adv Healthc Mater* 2017;6(22):1700298.
- [84] Kang HW, Sang JL, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol* 2016;34(3):312–9.
- [85] Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol* 2014;32(8):773–85.
- [86] Fedorovich NE, Schuurman W, Wijnberg HM, Prins HJ, van Weeren PR, Malda J, et al. Biofabrication of osteochondral tissue equivalents by printing topologically defined, cell-laden hydrogel scaffolds. *Tissue Eng C Methods* 2012;18(1):33.
- [87] Graham AD, Olof SN, Burke MJ, Jpk A, Mikhailova EA, Nicholson JG, et al. High-resolution patterned cellular constructs by droplet-based 3D printing. *Sci Rep* 2017;7(1).
- [88] Zhou X, Nowicki M, Cui H, Zhu W, Fang X, Miao S, et al. 3D bioprinted graphene oxide-incorporated matrix for promoting chondrogenic differentiation of human bone marrow mesenchymal stem cells. *Carbon* 2017;116:615–24.
- [89] Boushell MK, Hung CT, Hunziker EB, Strauss EJ, Lu HH. Current strategies for integrative cartilage repair. *Connect Tissue Res* 2017;58(5):1.
- [90] Bella CD, Fosang A, Donati DM, Wallace GG, Choong PFM. 3D bioprinting of cartilage for orthopedic surgeons: reading between the lines. *Front Surg* 2015;2:39.
- [91] Du Y, Liu H, Yang Q, Wang S, Wang J, Ma J, et al. Selective laser sintering scaffold with hierarchical architecture and gradient composition for osteochondral repair in rabbits. *Biomaterials* 2017;137:37.
- [92] Wu Y, Zhu S, Wu C, Lu P, Hu C, Xiong S, et al. A Bi-lineage conducive scaffold for osteochondral defect regeneration. *Adv Funct Mater* 2014;24(28):4473–83.
- [93] Chen L, Deng C, Li J, Yao Q, Chang J, Wang L, et al. 3D printing of a lithium-calcium-silicate crystal bioscaffold with dual bioactivities for osteochondral interface reconstruction. *Biomaterials* 2018. <https://doi.org/10.1016/j.biomaterials.2018.04.005>.
- [94] Deng C, Yang Q, Sun X, Chen L, Feng C, Chang J, et al. Bioactive scaffolds with Li and Si ions-synergistic effects for osteochondral defects regeneration. *Mater Today* 2017;10.
- [95] Oliveira JM. Osteochondral tissue engineering - nanotechnology, scaffolding-related developments and translation. 2018.