International Journal of Bioprinting

RESEARCH ARTICLE

3D-printed gradient scaffolds for osteochondral defects: Current status and perspectives

Jianhang Du[1†], Ziqing Zhu[2†], Jia Liu[3†], Xiaogang Bao[3], Qian Wang[1], Changgui Shi[3], Chaoqian Zhao[4*], Guohua Xu[3*], Dejian Li[1*]

[1]Department of Orthopedics, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai, China

[2]Department of Orthopedics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[3]Department of Orthopedics, Spine Center, Second Affiliated Hospital of Naval Medical University, Shanghai, China

[4]State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai, China

(This article belongs to the *Special Issue: Advances in 3D bioprinting for regenerative medicine and drug screening*)

Abstract

Articular osteochondral defects are quite common in clinical practice, and tissue engineering techniques can offer a promising therapeutic option to address this issue.The articular osteochondral unit comprises hyaline cartilage, calcified cartilage zone (CCZ), and subchondral bone.As the interface layer of articular cartilage and bone, the CCZ plays an essentialpart in stress transmission and microenvironmental regulation.Osteochondral scaffolds with the interface structure for defect repair are the future direction of tissue engineering. Three-dimensional (3D) printing has the advantages of speed, precision, and personalized customization, which can satisfy the requirements of irregular geometry, differentiated composition, and multilayered structure of articular osteochondral scaffolds with boundary layer structure.This paper summarizes the anatomy, physiology, pathology, and restoration mechanisms of the articular osteochondral unit, and reviews the necessity for a boundary layer structure in osteochondral tissue engineering scaffolds and the strategy for constructing the scaffolds using 3D printing. In the future, we should not only strengthen the basic research on osteochondral structural units, but also actively explore the application of 3D printing technology in osteochondral tissue engineering. This will enable better functional and structural bionics of the scaffold, which ultimately improve the repair of osteochondral defects caused by various diseases.

Keywords: 3D printing, Scaffold, Osteochondral defect

1. Introduction

Osteochondral defects are lesions that involve both the articular cartilage (AC) and its underlying subchondral bone (SB). The location, size, and degree of osteochondral defects in clinical practice vary from one cause to another, including traumatic osteochondral injury, exfoliative osteochondritis, osteonecrosis, and osteoarthritis

†These authors contributed equally to the work.

***Corresponding authors:**
Chaoqian Zhao (mpo456@163.com)

Guohua Xu (xuguohuamail@smmu.edu.cn)

Dejian Li (lidejian880820@163.com)

Citation: Du J, Zhu Z, Liu J, *et al*., 2023, 3D-printed gradient scaffolds for osteochondral defects: Current status and perspectives. *Int J Bioprint*. https://doi.org/10.18063/ijb.724

Received: November 18, 2022
Accepted: January 18, 2023
Published Online: March 31, 2023

(OA). In addition, their pathological characteristics are also different. Osteochondral lesions usually require surgical treatment. If fibrocartilage is formed with different biomechanical properties from hyaline cartilage, it will cause degeneration of the adjacent normal cartilage and subchondral bone, eventually leading to severe pain, joint deformity, and mobility loss[1]. The different causes of osteochondral defects contribute to the complexity of their treatment. Significant progress has been made in the repair of articular cartilage defects in recent decades, but osteochondral defects deep into subchondral bone have not gained much attention.

The current treatment options for osteochondral defects include nonsurgical and surgical treatments, such as joint debridement, microfracture, autologous osteochondral grafting or mosaic inlay, matrix-associated autologous chondrocyte implantation (MACI), and autologous chondrocyte implantation (ACI)[2]. Although conventional osteochondral repair strategies have their corresponding advantages, their inherent disadvantages are also evident. For example, arthroscopic debridement is not effective; microfracture repair tends to form fibrocartilage rather than normal hyaline cartilage[3]; autologous or allogeneic osteochondral implantation have a limited source of graft tissue[4] and high incidence in the area of the graft origin; the defective area repair does not fit the surrounding articular cartilage; and among other problems. Therefore, their clinical practical restorative results are not satisfactory. Cell-based treatments, such as ACI and MACI, also involve the possibility of fibrocartilage production in the repair area, incomplete filling of the repair, and poor integration with the surrounding tissues, and their actual results have not been uniformly accepted. As a result, there is a lack of practical and effective treatment for osteochondral defects in clinical practice.

The development of tissue engineering techniques offers a novel approach to the treatment of osteochondral defects. Long-term restorative results can be achieved through the use of an integrated tissue-engineered osteochondral bionic scaffold, combined with the relevant advantages of existing treatment methods and a systematic and personalized postoperative rehabilitation program. Tissue engineering technology aims to repair the structure and function of damaged tissue by combining seed cells and growth factors with material scaffolds. The articular cartilage is relatively homogeneous in composition and simple in structure with no complex vascular system[5]. Due to its low difficulty of tissue engineering, it is considered the most promising alternative for osteochondral defect treatment. Since the 1990s, research into articular osteochondral tissue engineering has made significant progress. However, there are also disadvantages, such as excessive tissue fibrosis, grafts sinking, abnormal bone formation, excessive cartilage growth, and scaffold separation[6]. Compared to articular osteochondral tissue, single- or double-layered scaffolds lack a "boundary structure" between cartilage and bone, that is, calcified cartilage zone (CCZ)[7]. This usually causes an imbalance in the microenvironmental homeostasis of the articular cartilage and subchondral bone, as well as altered stress transmission patterns, ultimately leading to repair failure. In order to achieve complete biomimicry, researchers have designed a strategy for constructing a multilayered osteochondral scaffold with a boundary layer structure. With the advent of additive manufacturing technology in recent years, 3D printing has developed rapidly, providing new tools and technical methods to solve this challenge. As shown in Figure 1, this paper summarizes the anatomy, physiology, pathology and restoration mechanisms of the articular osteochondral unit, and reviews the necessity for a boundary layer structure in osteochondral tissue engineering scaffolds and the strategy for constructing the scaffolds using 3D printing.

2. Osteochondral tissue: Anatomy and physiology, pathology, and restoration mechanisms

2.1. Anatomy and physiology

The articular osteochondral structure can be roughly divided into five layers according to the biological differences in fiber orientation, cell morphology and density, content of glycosaminoglycans (GAGs), collagen and water, and their corresponding mechanical gradients. As shown in Figure 2A and C, the five layers are superficial layer, intermediate layer (transitional layer), deep layer (radial layer), calcified cartilage layer, and subchondral bone layer. The first three layers are generally referred to as hyaline cartilage layer. There is a tidemark structure that connects the relatively soft articular cartilage to hard calcified cartilage. The subchondral bone lies beneath the calcified cartilage layer, and the structure formed by the interlocking of these two layers is called cement line[8].

2.1.1. Hyaline cartilage layer

The superficial layer comprises 10%–20% of the total thickness of hyaline cartilage layer and is characterized by thin and densely arranged collagen fibers that run parallel to the cartilage surface. This layer has a high density of chondrocyte distribution with a long, thin, and flattened morphology[9]. The chondrocytes in this area are primarily associated with the outward tissue growth and are referred to as persistent chondrocytes. The uppermost area of the superficial layer is overlaid with a thin layer

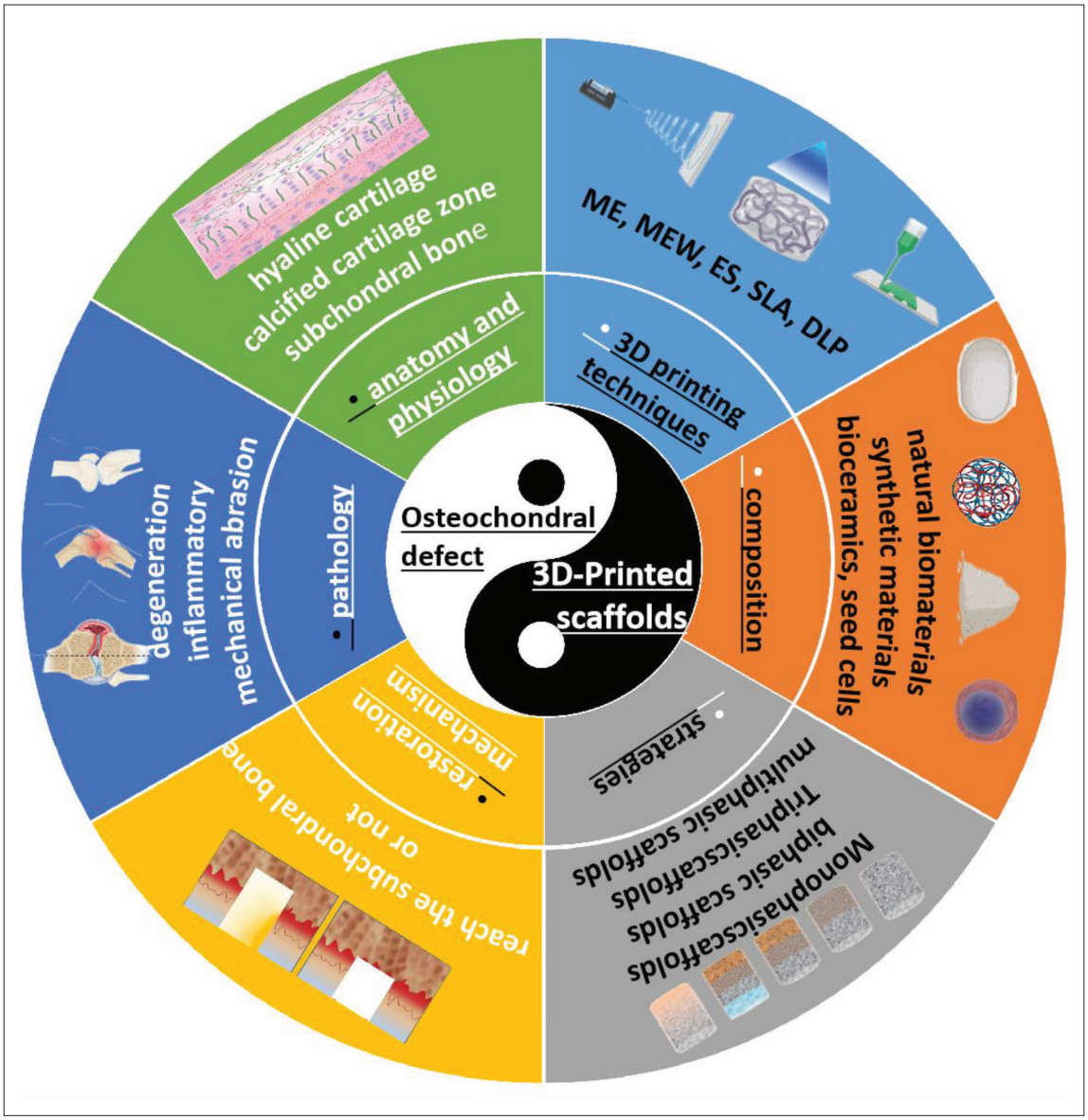

Figure 1. Schematic illustration of different aspects of 3D-printed gradient scaffolds for osteochondral defects in this review. (Some of the icons used in this figure are derived from Biorender.com.)

of noncellular structures, usually only a few hundred nanometers, whose primary function is to reduce the surface friction on articular cartilage[10]. This protective layer contains high levels of glycoproteins, also known as mucosal proteoglycans. Type II collagen and water content are the highest, while type I collagen and GAGs content are the lowest in the superficial layer. The arrangement and density of the collagen fibers, water, and GAGs content in the superficial layer allow for the highest permeability of this layer and the effective dispersion of shear stresses from the joints. The middle layer comprises 40%–60% of the total hyaline cartilage layer thickness, with thick collagen fibers (9–60 nm) that are unevenly aligned and cross the articular cartilage surface. The cells in this layer are round and randomly arranged chondrocytes, which are usually known as the proliferating chondrocytes[11]. From superficial to deep layers, there is an increasing trend in type I collagen and GAGs, and a decreasing trend in type II collagen and water content. Due to its high content of GAGs, the permeability of the intermediate layer is low compared to the superficial layer, thus moderating and supporting the compressive stress from the joint[12]. The radial layer (deep layer) of articular cartilage accounts for 20%–50% of the hyaline cartilage layer thickness, with thick collagen fibers (60–140 nm) aligned perpendicular to the articular cartilage surface. The chondrocytes in this

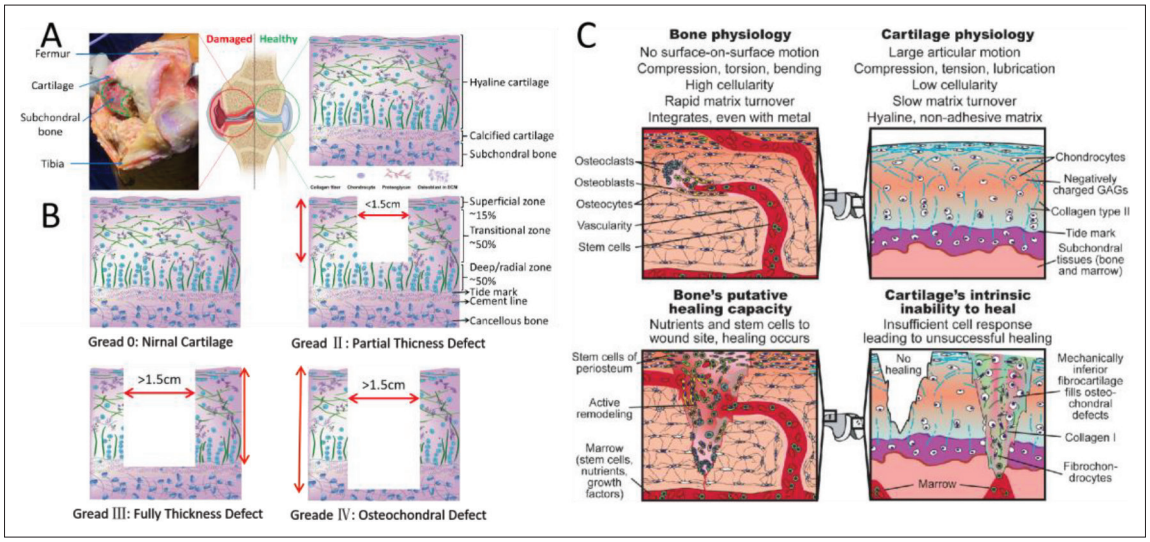

Figure 2. Physiological and pathological occurrence and repair of bone and cartilage defects. (A) Diseased joint and osteochondral units including cartilage, calcified cartilage, and subchondral bone. (B) Categoriesofosteochondral defect. (C) The schematic diagram of the osteochondral physiologic environment and healing capacities in different conditions [92] (Reproduced with permission from Huey DJ, Hu JC, Athanasiou KA, *Science*, 2012, 338(6109):917–921).

layer are elongated and subglobular, and the cell density is lower than that of the superficial and intermediate layers[13]. Type I collagen and GAGs content are the highest, while type II collagen and water content are the lowest. The permeability of the deep layer is so low that almost no fluid flow can pass through this layer. This layer is subjected to the maximum interfacial shear stress[14]. The partial cartilage defect involves only the hyaline cartilage layer, and the tidemark is intact (Figure 2B).

2.1.2. Tidemark and CCZ

The tidemark lies between the deep layer and CCZ. It was found that the number of tidemarks increased correspondingly with age as the tissue is reconstructed. The CCZ is located below the tidemark and contains abundant apatite and alkaline phosphatase, with marked tissue mineralization and low cell density, mostly rounded and hypertrophied chondrocytes[15]. Some thick collagen fibers from deep layer connect CCZ to hyaline cartilage layer through tidemark. Tidemarks and CCZ serve as an interface between soft hyaline cartilage layer and hard subchondral bone. Biologically, this layer acts as a barrier against vascular invasion of the subchondral bone and prevents mineralization of hyaline cartilage layer. Mechanically, this layer is subjected to extreme and variable shear stresses during joint movement, providing cushioning mechanical support to the upper and lower layers it connects. In addition, the modulus of elasticity varies considerably among the layers, with the superficial, deep, calcified cartilage, and subchondral bone layers having a compressive modulus of approximately 0.079, 2.1, 320 MPa, and 5.7 GPa, respectively[16,17]. Thus, it can be seen that the tidemark and CCZ, as the interface between bone and cartilage, play an important role in the integrated osteochondral structure and function. Total cartilage damage reaches the level of CCZ, but not yet below the cement line, with varying degrees of tidemark loss[18] (Figure 2B).

2.1.3. Subchondral bone layer

Subchondral bone is located below the cement line and is mainly composed of collagen, laminin, fibronectin, and other types of glycoproteins and hydroxyapatite with a thickness of 2–5 nm and length of 20–80 nm[18]. This layer can be divided into two parts based on the distribution of blood vessels and porosity. The upper cortical bone is adjacent to CCZ with minimal vascularity and low porosity. The lower part is spongy cancellous bone, which contains abundant blood vessels and randomly arranged trabecular porous structures[19]. Disruption of the subchondral bone layer integrity is a sign of osteochondral damage.

2.2. Pathology

Articular cartilage is an elastic, smooth tissue that encases the surface of articular bone to form the joint structure. Due to its unique physical properties, articular cartilage provides a force cushioning effect in the weight-bearing area and significantly reduces friction during joint movements[20,21]. Many diseases including OA, rheumatoid arthritis, and sports injuries can cause damage to articular cartilage. Their pathophysiological factors leading to cartilage damage vary: it is mainly articular cartilage degeneration in OA, inflammatory erosion in rheumatoid arthritis (RA), and mechanical abrasion in sports injury[22].

2.2.1. Cartilage degeneration in OA

Although many tissue cells are involved in the pathological process of OA, chondrocytes are thought to be a key factor in the development and progression. Aging of articular chondrocytes causes impaired synthesis and secretion of type II collagen and proteoglycan, and imbalances in the anabolic and catabolic processes of the extracellular matrix (ECM). This can cause further cartilage degradation and destruction, ultimately leading to the onset of OA[23-25].

It was found that the number of senescent chondrocytes in OA cartilage was significantly higher than in healthy control cartilage of the same age[26-28]. Senescent chondrocytes are predominantly located around damaged cartilage in OA and are rarely detected in intact cartilage, further suggesting an intrinsic link between chondrocyte senescence and OA[27,29]. Senescent chondrocytes secrete senescence-associated secretory phenotype (SASP) factors that inhibit ECM synthesis and activate matrix protein hydrolases to promote the development of OA[30]. Notably, although the accumulation of senescent chondrocytes (chronic cellular senescence) can lead to joint tissue dysfunction and OA, acute cellular senescence plays a positive role in embryonic development and tissue regeneration[31]. For example, senescent cells derived from the wound can release platelet-derived growth factor AA (PDGF-AA) to promote wound healing[32]. Research shows that PDGF-AA promotes chondrocyte proteoglycan secretion and cartilage repair, and that PDGF-AA from subchondral bone can alleviate cartilage degeneration in an OA mouse model[33].

2.2.2. Inflammatory erosion in rheumatoid arthritis

Inflammation, one of the most remarkable pathological features of rheumatoid arthritis, plays a crucial role in its development. Macrophages are the main cells that promote the inflammatory response in RA. A large number of macrophages have been found in the cartilage and synovial tissue of patients with rheumatoid arthritis[34]. The study found that the synovial macrophage activation caused overexpression of major histocompatibility complex II (MHC II) molecules, chemokines, and inflammatory factors, and that the number of macrophages and the levels of interleukin (IL)-1β and tumor necrosis factor alpha (TNF-α) correlated with the degree of joint damage and clinical symptoms in patients[35].

On the other hand, T cells play a crucial part in the development of RA. Its development is caused by the interplay of $CD4^+$ cells and antigen-presenting cells (APCs). The binding of T cells with MHC II and antigenic peptides activates macrophages to release inflammatory cytokines including TNF-α and IL-1. These cytokines further stimulate chondrocytes and synovial fibroblasts to release various enzymes that break down collagen and glycoproteins, thereby destroying the tissue[36,37]. In addition, helper T cells (Th17) and regulatory T cells (Tregs) are related T cells. Th17 are able to release pro-inflammatory cytokines, which further activate immune cells and induce inflammation. In contrast, regulatory T cells secrete immunosuppressive cytokines that effectively suppress the activity of T cells and other immune cells. Studies have shown that decreased Tregs function in RA patients induces the initiation of harmful autoimmunity by Th1 cells, which further leads to chronic inflammation[38,39].

2.2.3. Mechanical abrasion in sports injury

Traumatic osteochondral lesions are related to the high-intensity violence to bones, including bone bruises, cartilage fractures, subchondral fractures, and osteochondral fractures. Traumatic osteochondral lesions are "outside-in" lesions that affect articular cartilage first and then destroy the subchondral bone when sufficient force is applied. Articular cartilage provides a smooth and load-bearing surface. Synovial fluid in the joint cavity contains hyaluronic acid and lubricating hormones secreted by superficial chondrocytes[40], which lubricate the cartilage surface. The synovial fluid is one of nutrition sources for chondrocytes and contains electrolytes, oxygen and glucose. Due to the lack of directly nourishing blood vessels and nerves in the cartilage, its nutrition source is mainly derived through matrix penetration of the subchondral bone vessels. Therefore, articular cartilage has a limited ability to repair itself. Studies have shown that the cartilage tissue surrounding the damaged area has a lower cell density compared to healthy tissue in articular osteochondral lesions. The animal model of osteochondral lesions further demonstrates that a decrease in cell density within 100 μm of the defect edge can be observed for weeks and even months after surgical treatment. Some chondrocytes undergo apoptosis, with the highest apoptosis rate on day 4 after trauma[41].

2.3. Restoration mechanisms

Mature articular cartilage will make some attempts to repair itself when damaged, although the ultimate effect is rather limited. When articular cartilage is impaired by factors such as trauma or repeated abrasion, the repair mechanism varies depending on the lesion category. The cartilage tissue at the edge of the lesion is barely capable of repairing itself when the lesion depth does not reach the subchondral bone level. Although a few fibroblasts and mesenchymal stem cells (MSCs) from the synovium or synovial fluid migrate to the lesion site, this is not sufficient to repair the cartilage defect[42], whereas in complete lesions (when the lesion reaches the level of subchondral bone), a large amount of blood and even bone marrow will rush into the defect and

form a thrombus. As a result, mobilized MSCs and blood cells form hematomas to repair the cartilage defects[43]. In this case, only fibrocartilaginous scar tissue is generated, which has more type I collagen and less type II collagen in the ECM. Additionally, its mechanical properties are far inferior to those of hyaline cartilage, and it does not form an effective and long-lasting bond with the surrounding tissue. Bone marrow stimulation techniques, including microfracture and deep drilling, use similar principles to penetrate the subchondral bone to the bone marrow cavity and mobilize cells to the cartilage defect site to achieve regenerative repair. However, the regenerative repair by these techniques ultimately only generates fibrocartilage tissue in the defect and does not completely regenerate the articular cartilage to restore its original functional state[44].

3. 3D-printed osteochondral repair materials

The articular osteochondral tissue units are an ordered and integrated whole. In normal tissue, articular cartilage polysaccharide chains have a pore size of approximately 6 nm between them and the collagen fibril network has a pore size of 60–200 nm and extends vertically to the CCZ[45]. Unlike hyaline cartilage layer, the internal structure of CCZ and SB is much denser. This structural difference poses a major challenge for the bionic fabrication of CCZ-containing osteochondral scaffolds, particularly in the selection of the scaffold raw material and its design strategies.

3.1. 3D printing techniques in osteochondral tissue engineering

Currently, the most common 3D printing techniques for articular osteochondral scaffolds include electrospinning (ES), material extrusion (ME), stereolithography (SLA), digital light processing (DLP), and melt electrowriting (MEW). However, every technique has their own advantages and limitations, as well as their appropriate printing materials. In terms of material selection, there is no evidence to date that one material is definitely better than another. In general, hydrogels are mostly used for the printing of hyaline cartilage layer; bioceramics, hyaluronic acid, tricalcium phosphate (TCP), and metallic materials are more suitable for the printing of SB[46]. In addition, the development of new materials with better biocompatibility, plasticity, and modifiability is one of the most important issues in the future.

ME technology involves depositing material via nozzles on a print bed in the *X*-*Y* plane and then stacking it layer-by-layer in the *Z*-axis plane[47]. It is suitable for a wide range of materials, including thermopolymers, bioceramics, and hydrogels. Each material requires fine-tuning of printing parameters, such as temperature, extrusion pressure, print speed, speed of hydrogel crosslinking or gelation[48,49]. ME can be used to fabricate scaffolds with relatively high porosity, which facilitates seed cell adhesion, proliferation, chondrogenesis and osteogenic differentiation. Porous structures similar to SB can be printed with thermopolymer-based ME technology to promote bone growth. The pore size for the SB section of the multiphase osteochondral scaffold is usually 0.3–1.0 mm, with a porosity of 70%–80%[50,51]. ME printing technology based on bioceramics is mainly used in the CCZ and SB sections of osteochondral scaffolds[52]. In general, the printed bioceramic scaffolds achieve low porosity (20%–60%) and small pore sizes (0.1–0.4 mm). Many scaffolds failed to produce pore sizes >0.3 mm to promote bone growth in the SB section[53,54]. Although hydrogel ME printing is commonly used for the AC section, Gao *et al.*[55] fabricated a biphasic osteochondral scaffold using this technique. The addition of β-tricalcium phosphate (β-TCP) to the SB section of the hydrogel increases the mechanical stiffness and osteoinductive properties of the hydrogel, while transforming growth factor-beta 1 (TGF-β1) is incorporated into the AC section to enhance cartilage formation.

MEW and ES technologies allow long filaments to be deposited layer-by-layer through a nozzle[56]. Its fiber diameters range from microns to nanometers. In addition, ES is a solvent-based printing technique that deposits material fibers randomly on a collector bed, whereas MEW is a solvent-free method that regulates where and how the fibers are deposited, thus controlling the final pattern. Polycaprolactone (PCL) is the most used material in MEW, as well as gels, chitosan, polyvinyl alcohol (PVA), hyaluronic acid, and collagen[57]. Despite the increased availability of suitable materials in ES, the solvents used are often biotoxic and require significant attention[58]. When applying MEW and ES to the construction of articular cartilage scaffolds, the main challenge is the limited total thickness of the structure printed in the *Z*-axis direction[59]. The current solution is to print the material onto various collectors and body beds in order to increase the structure height in the *Z*-axis direction[60]. On the other hand, given the limited height and strength of the micro/nanofibers, MEW and ES often produce soft scaffolds that are well suited for the AC section manufacture of articular osteochondral scaffolds.

SLA and DLP technologies are used to achieve 3D-printed shapes by depositing material layer-by-layer. However, these technologies are not based on a nozzle approach, but rather on a liquid material in a resin bath. The difference between SLA and DLP technology is the light source used; SLA uses a laser while the light source of DLP comes from projection[61]. The accuracy of SLA/

DLP conventional printing is up to 50 μm, which is between MEW/ES and ME[62]. The basic materials used in these techniques are compatible with many of the above-mentioned materials, but usually require extensive modification[63]. SLA and DLP printing technologies are not as widely used in scaffold preparation as ME, probably due to the high upfront investment and maintenance costs of these systems. To date, SLA- and DLP-printed scaffolds have no porosity advantage (50%–65%) over other technologies[50]. These techniques can be used to construct multilayered articular osteochondral scaffolds. For example, Zhu *et al.*[64] prepared a multilayered osteochondral scaffold by combining poly(ethylene glycol) (PEG) material with natural bovine cartilage ECM using the DLP technique.

Of various 3D printing solutions, ME is the most commonly used due to its wide availability, material versatility and low cost. Second, MEW and ES are mainly used in the cartilage phase and their achievable scaffold thickness is limited. This so-called "limitation" makes it suitable for the fabrication of thin and dense boundary structures, but its technical potential needs to be further developed. The delicate connection of CCZ to the adjacent structures provides excellent mechanical properties of the entire articular osteochondral unit. In order to achieve its maximum bionic potential, the imitation of this connection should also be a key direction to be considered. Therefore, 3D printing technology with higher precision should be a major priority in the future.

3.2. Composition

The materials used in osteochondral tissue engineering scaffolds are mainly categorized into the following groups, such as natural biomaterials, synthetic materials and bioceramics. Due to their composition and structure, various types of osteochondral scaffolds have different biological and mechanical properties.

Natural biological scaffold materials have the advantages of excellent biocompatibility, high degradability, and favorable cell attachment and proliferation for subsequent recruitment and infiltration. However, they also have disadvantages, including excessive degradation rates, poor mechanical properties, and limited sources[65]. Collagen is the major constituent of osteochondral ECM and its role is to maintain the structural integrity of ECM[66]. Studies have shown that chondrocytes in 3D collagen gels maintain their normal phenotype and that collagen also plays a crucial part in tissue repair and wound healing. The chemical structure of chitosan is similar to that of GAGs in the cartilage ECM and its biomimetic structure is highly conducive to the morphogenesis, differentiation, and proliferation of chondrocytes.

Despite their advantages of better mechanical properties, higher plasticity, controlled degradation rate, and availability of a wide range of sources, synthetic scaffold materials are poorly biocompatible and less hydrophilic and their degradation products may be toxic[67]. Bioceramics such as bioglass, hydroxyapatite (HA), and TCP have been common scaffold materials in bone tissue engineering because of their high mechanical strength, but they also have the disadvantage of brittleness.

A combination of two or more materials is used to design the ideal osteochondral scaffold in order to overcome the disadvantages of a single material. Composite scaffolds incorporate the advantages of each constituent material: controlled degradation rate, good cytocompatibility and hydrophilicity, and suitable biomechanical strength. Natural biomaterials, including ECM, are enriched with favorable molecules for cells (e.g., GAGs, collagen, and GAGs-like polysaccharides), and therefore, these materials can be incorporated into composite scaffolds to enhance their affinity for the host tissue[68]. Inspired by the collagen fiber structure and ECM composition gradients in osteochondral tissue, Qiao *et al.*[69] prepared a layered scaffold composed of MSCs-laden GelMA hydrogel with zone-specific growth factor delivery and melt electro-written triblock polymer of poly (e-caprolactone) and poly (ethylene glycol) (PCEC) networks with depth-dependent fiber organization. It was found that the introduction of PCEC fibers into GelMA hydrogels significantly improved the mechanical strength. Considering the osteochondral anatomy and physiology and the properties and functions of various scaffold materials, the cartilage layer prefers hydrogels derived from natural or synthetic polymers (because their hydration properties and viscoelasticity are similar to natural ECM), reinforcing materials favor the subchondral bone layer, such as bioceramics and hard polymers, and the combination of cartilage and bone layer materials with a specific ratio is suitable for the intermediate layer (osteochondral interface).

3.3. Seed cells for the osteochondral tissue engineering

The seed cells are an important basis for osteochondral tissue engineering to achieve clinical translation. Currently, the most researched seed cells are various types of stem cells, including MSCs, cartilage stem/progenitor cells, embryonic stem cells, skeletal stem cells, and induced pluripotent stem cells.

Bone marrow-derived MSCs (BMSCs), which have a strong proliferative capacity, can easily differentiate into chondrocytes and maintain their phenotype *in vitro*. In addition, large numbers of cells can be obtained from many different bone marrow sites, making them

widely used in cartilage regeneration[70,71]. However, the BMSCs also shows certain limitations. Their tendency to differentiate toward chondrocyte phenotype on the growth plate *in vitro* or *in vivo* results in chondrocyte hypertrophy or death and endochondral bone formation, ultimately affecting the articular cartilage regeneration[72]. A series of recent studies have used different approaches to improve the ability of BMSCs to differentiate into chondrocytes. Studies have shown that the addition of stimulating factors, such as insulin-like growth factor 1 (IGF-1) and bone morphogenetic protein (BMP)-7, can promote the differentiation of BMSCs into chondrocytes, maintain the target phenotype, and inhibit the hypertrophic phenotype[73]. In addition, due to its advantages of natural components and the unique structure of cartilage matrix and good biocompatibility, the decellularized cartilage matrix could significantly promote the BMSCs maintenance and differentiation[74]. BMSCs have been widely used in the articular osteochondral tissue engineering, but further research is needed to improve their application effects.

Adipose stem cells are MSCs of adipose origin. They are easy to obtain and have broad sources and good immunomodulatory properties, while being less invasive to the donor, having fewer postoperative complications and possessing the potential to be seed cells for osteochondral tissue engineering. Studies have shown significant improvements in clinical symptoms and imaging in patients with OA following implantation of adipose stem cells alone or in combination with a scaffold[75]. Although studies have shown positive short-term effects, the long-term recovery and histological regeneration characteristics after use of adipose stem cells in cartilage defects need to be further investigated.

Chondral progenitors/stem cells are found in the superficial layer of the articular cartilage, which account for 0.1%–1% of the total articular cartilage tissue. These cells are capable of self-renewal *in vitro* and maintain the potential to differentiate into osteoblasts, chondrocytes and adipocyte lines even after 60 generations of expansion[76]. They play an important role in the formation, growth and maturation of articular cartilage, and also respond to chondral damage by migrating to the injury site and proliferating and differentiating to repair the defect[77]. Compared to MSCs, chondrocytes derived from chondrogenic stem/progenitor cells are more likely to maintain their phenotype rather than progress to chondrocyte hypertrophy and calcification[78]. These results show the possible prospect of chondrogenic stem/progenitor cells as a source of chondral tissue-engineered seed cells, but the limitations of their scarcity and safety issues of aberrant karyotypes after many passages need to be further investigated.

There have been many attempts to use highly differentiated embryonic stem cells in articular cartilage repair. Wakitani *et al.*[79] successfully repaired cartilage damage in the knee joint of mice using a combination of chondrocytes differentiated from embryonic stem cells and hyaluronic acid-based hydrogel. McKee *et al.*[80] successfully induced the differentiation of embryonic stem cells into chondrocytes on a 3D scaffold material under stress-stimulated conditions. However, embryonic stem cells have many limitations, including the risk of tumorigenicity, disease transmission, immune rejection, and ethical issues, all of which restrict their widespread use to some extent. Nevertheless, an in-depth exploration of the mechanisms of articular cartilage repair during the fetal period may provide valuable information for chondral regenerative repair.

The presence of skeletal stem cells was first confirmed by Chan *et al.*[81] in mice. Unlike conventional MSCs, these stem cells merely differentiate into osteoblasts, chondrocytes, and stromal cells, but not into adipocytes, hematopoietic cells, or myoblasts. Murphy *et al.*[82] demonstrated that one of the main mechanisms of cartilage repair of microfracture techniques is the proliferation of skeletal stem cells. In addition, BMP and vascular endothelial growth factor (VEGF) inhibitors were used to induce differentiation of skeletal stem cells into hyaline cartilage tissue, which was comparable to the native tissue in terms of mechanical properties, composition, and degree of integration with the surrounding tissue.

3.4. Monophasic and biphasic scaffolds

Monophasic scaffolds use a homogeneous single material or composite to repair the entire articular osteochondral defects. This means that the scaffold needs to meet the structural and functional requirements of each of these tissue areas with the same porosity and mechanical properties. Studies have been reported on the preparation of single-phase scaffolds using a range of materials and bio-fabrication techniques, including ES based on ZnO–PCL composites and ME based on bio-ceramics[83,84]. Herein, the material solution concentration and its surface characteristics are optimized and modified to enhance chondrogenic or osteogenic differentiation potential of cells. Furthermore, confocal laser scanning microscope (CLSM) images display that micro/nanostructured surface significantly promoted the attachment of both chondrocytes and rBMSCs. Most importantly, the *in vivo* study has shown that the micro/nanostructures on the surface of the 3D-printed scaffolds significantly promote the regeneration of cartilage and subchondral bone tissues (Figure 3C). However, monophasic scaffolds cannot mimic the biological microenvironment well due to the lack of

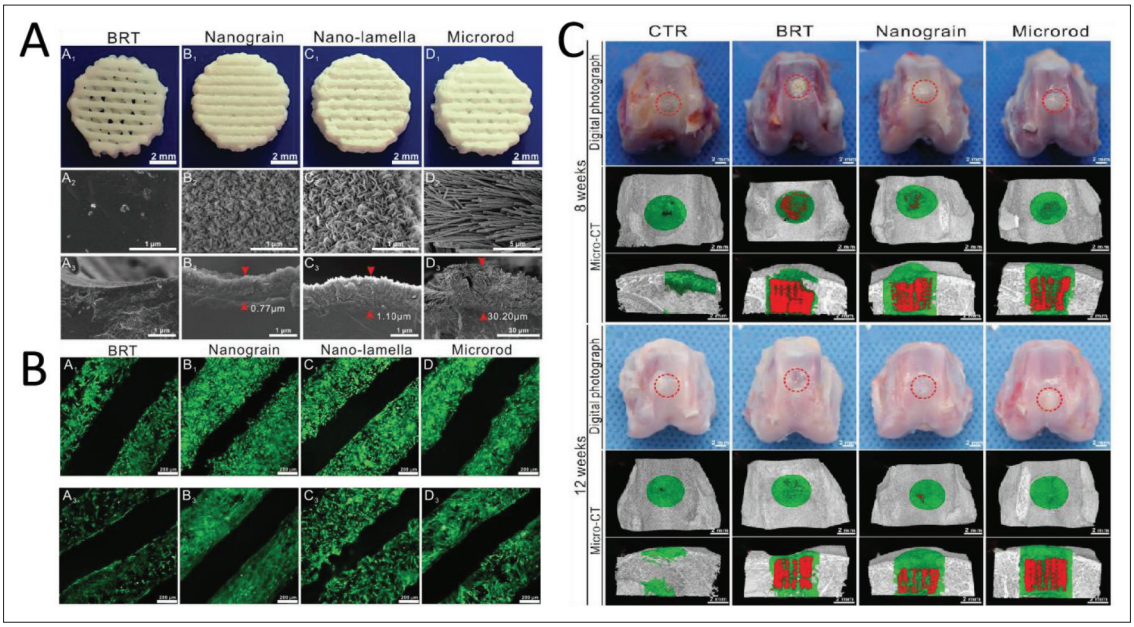

Figure 3. 3D-printed monophasic scaffolds for osteochondral tissue engineering. (A) Morphology of bredigite (BRT) scaffolds before and after covering with micro/nanostructured surface. (B) Cell adhesion of 3D-printed monophasic scaffolds. (C) Photographs and micro-CT analysis of the osteochondraldefectsinrabbits at weeks 8 and 12 after surgery. CTR means control[84] (Reproduced with permission from Deng C, Lin R, Zhang M, *et al*, *Adv Funct Mater*, John Wiley & Sons).

intrinsic physical structure and properties of osteochondral tissue. Therefore, they are not a good treatment option for osteochondral defects.

Biphasic scaffolds usually have a cartilage and bone phase, more similar to natural osteochondral tissue than monophasic scaffolds.Their two layers are fabricated separately, which gives researchers more space to control and optimize their materials, design, porosity, mechanical function and unit type.Biphasic scaffolds have the following advantages: (i) the scaffolds can promote the differentiation of cartilage and bone tissue respectively by the addition of appropriate growth factors; (ii) they can provide suitable chemical, mechanical, and biological stimuli to proliferation and differentiation of different cells[85]; and (iii) they can also provide the appropriate microenvironment to direct cell–cell and cell–matrix communications[86]. For example, the biphasic scaffold of GM + SF-MA/GM + SF-PTH was fabricated via 3D bioprinting and implanted into the osteochondral defects of rabbits (Figure 4A and D). The results showed that the GM + SF-MA bio-ink had good mechanical properties, while the GM + SF-PTH bio-ink inhibited chondrocyte hypertrophy and promoted the ECM production in hyaline cartilage[87]. Moreover, the cell viability of the three groups of scaffolds was high, as shown in Figure 3D. However, as an integral part of the osteochondral unit, the CCZ was neglected in biphasic scaffolds. Furthermore, the scaffolds did not show all the gradient characteristics of osteochondral tissues.

3.5. Triphasic scaffolds

Considering that osteochondral units are composed of gradient regions with different compositions and structures, triphasic and multiphasic scaffolds with CCZ simulation have been designed and fabricated. As a narrow transition layer between cartilage and subchondral bone, CCZ facilitates converting shear stresses into compressive and tensile ones during joint loading and kinematics[88,89]. This zone not only forms a physical barrier against vascular invasion into the cartilage to prevent the full cartilage layer ossification, but also serves to support the articular cartilage load to facilitate the integration of the implant with the host tissue at the interface[90]. For example, the mechanical interface bonding strength of the triphasic SA/MBG scaffold is superior to biphasic scaffolds. The results showed that the scaffolds immersed in simulated body fluid (SBF) and cell culture medium induced apatite formation and had weak compressive and tensile strengths without layer dislocation or delamination[91]. Due to the unique hierarchical, biological, and mechanical properties of the osteochondral tissue[92], the triphasic scaffolds cannot still meet its full complexity.

3.6. Multiphasic and continuous gradient scaffolds

Natural osteochondral tissue has a more complex gradient of heterogeneity rather than a direct stratification of three

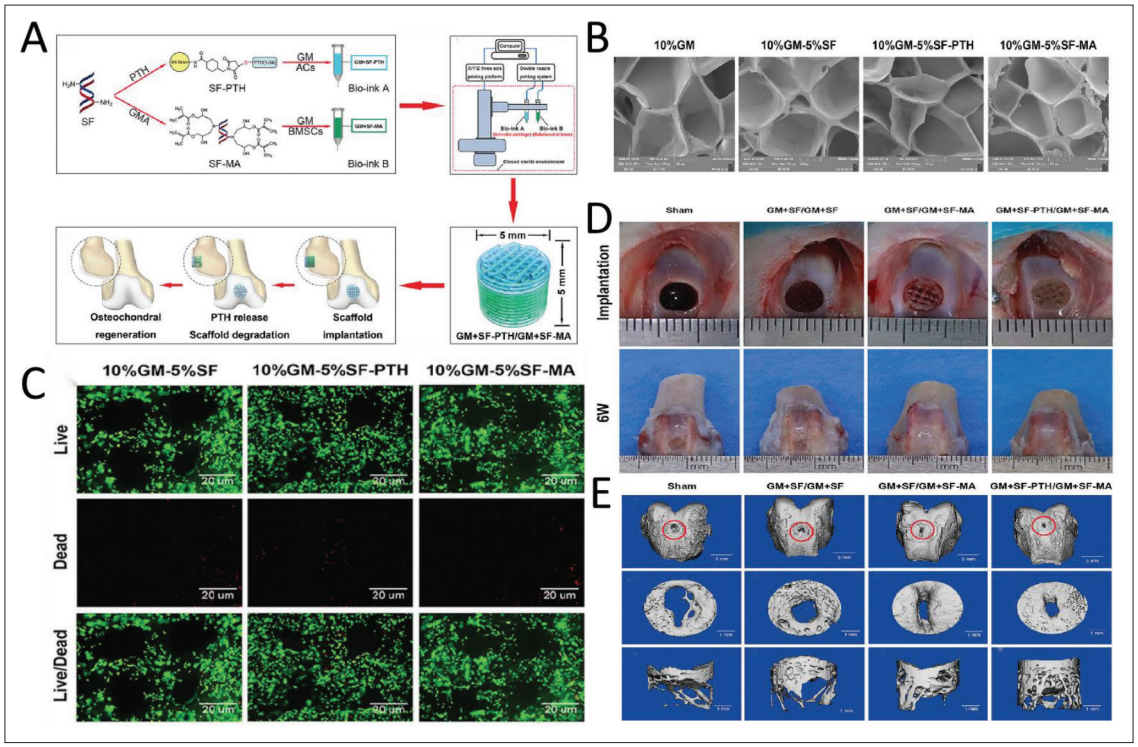

Figure 4. 3D-printed biphasic scaffolds for osteochondral tissue engineering. (A) Schematic diagram of the preparation of 3D-printed biphasic scaffold and its applications. (B) Microscopic morphology of 3D-printed biphasic scaffolds. (C) The viability of cellswithin the scaffolds on the first day after printing 24h. (D) Gross observation of the repaired joints in different groups. (E) Micro-CT images at week 12 after implantation (Reproduced from ref.[87] with permission from The Royal Society of Chemistry).

separate regions. Therefore, multiphasic and continuous gradient scaffolds with gradient physical and chemical properties are necessary for a smooth transition between different layers of the osteochondral unit. The multilayered osteochondral scaffold has at least four different layers. Increasing the number of phases from three to four or more usually means dividing the AC section into different zones. For example, Mancini *et al.*[93] proposed an osteochondral scaffold consisting of four different layers, with the aim of better simulating the properties of the different regions of AC. This multiphasic scaffold has a different collagen arrangement from top to bottom: the PCL scaffold is based on a 0°, 90° cross-alignment pattern with decreasing porosity until the texture is dense, with the CCZ-like structure acting as the interface zone; the third layer is that PCL extending along the hydrogel containing mesenchymal stromal cells with a 70% porosity; the PCL is removed from the fourth layer and the hydrogel is retained, but the MSCs are replaced by articular cartilage progenitor cells (ACPCs)[94]. Therefore, it is easy to see how complex this construction is, and finer 3D printing technology is required to make it easy to achieve. As shown in Figure 5C, multiphasic scaffolds with discrete gradients are prepared by stitching, gluing and press-fitting different phases into a single structure[95-98]. It is noted that there is no clear boundary between layers of the scaffolds[98].

Gradient scaffolds consistently outperform monophasic and biphasic ones in osteochondral defect repair[99,100]. The gradient can be described in terms of variations in the chemical composition and structural characteristics of the basic units, further including several basic forms of arrangement, distribution, size, and orientation[101]. The combined incorporation of different patterns of chemical and structural gradients in a monolithic osteochondral scaffold have been explored in orthopedic research as well. For example, with regard to the hydrogel-based scaffolds, sequential addition of different solutions into a cylindrical container layer-by-layer before the complete gelation allows for the formation of a gradient interface. Utilizing the silk protein-based composites coupled with biosilica selective peptide-R5, Guo *et al.* fabricated a bioinspired gradient protein/biosilica analog by layering three regions with high, medium, and low concentrations of the R5 peptide along the longitudinal direction[102]. This gradient silicified silk/R5 system showed continuous transitions in composition, structure, and mechanical properties and could promote the osteogenic differentiation of MSCs *in vitro* in a gradient manner[102]. Multiphasic gradient

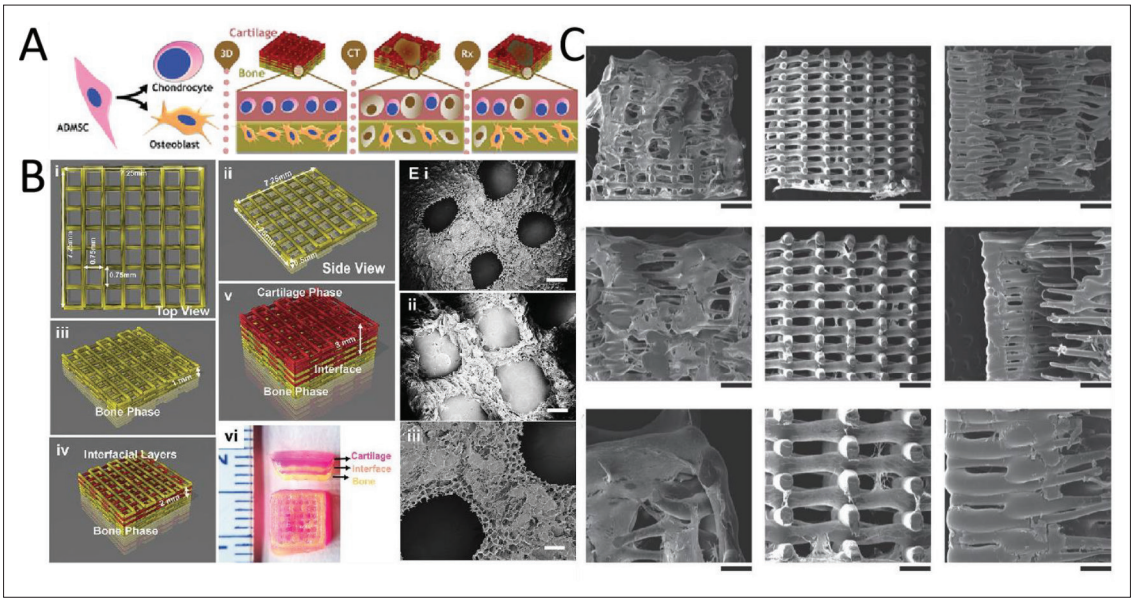

Figure 5. 3D-printed triphasic and multiphasic scaffolds for osteochondral tissue engineering. (A) The graphical abstract of 3D-printed triphasic scaffolds. (B) The constructdesign and printing. (Bi–Bv) The designing and development of the grid structure, and (Bvi) representative images of the printed structures showing the threelayers. (C) Scanning electron microscope (SEM) images of 3D-printed multiphasic scaffolds[98]. (Reproduced with permission from Di Luca A, Lorenzo-Moldero I, Mota C, *et al*., 2016, *Adv Healthc Mater*, John Wiley and Sons). (Di–Diii) Field emission scanning electron microscope (FESEM) images of the printed structure showing the porous grid structure. Scale bar = 400 μm[103]. (Reproduced with permission from Singh YP, Moses JC, Bandyopadhyay A, *et al*., *Adv Healthc Mater*, John Wiley and Sons).

and continuous gradient scaffolds prepared by emerging technologies and traditional methods have achieved success in both chemical composition and structural properties, as summarized in this section. However, the studies on developing gradient scaffolds imitating the osteochondral heterogeneities in anatomical, biological, physicochemical, and mechanical properties are still limited and in the infancy stage.

4. Conclusion and prospects

Osteochondral defects have been a widespread and serious osteoarticular disease in clinical practice. The effective osteochondral defect repair has been a pressing challenge in the field of tissue engineering. This paper systematically reviews the current problems faced by the conventional treatment of osteochondral defects and the current status of research on osteochondral integrated bionic scaffolds. The osteochondral tissue-engineered scaffold imitates not only the normal osteochondral structure, but also the natural osteochondral composition, ultimately achieving effective repair of osteochondral defects. However, the complex anatomy and composition of osteochondral tissue and the dynamic changes of time and space in the defect area indicate that the osteochondral repair is not a simple filling of new tissue, but the formation of an integrated bone–cartilage interface coupled with the simultaneous osteochondral regeneration.

The osteochondral integrated scaffold is a good solution to some problems in conventional treatments, but it also has its corresponding shortcomings. For example, there are currently no clinical trials using 3D-printed osteochondral scaffolds to repair osteochondral defects in joints. Compared to other tissue engineering solutions, 3D printing allows for the construction of a personalized scaffold that matches the geometry of the defect on the basis of magnetic resonance imaging (MRI) and computed tomography (CT) scans. Furthermore, the repair and regeneration mechanism of the osteochondral integrated scaffold has not been investigated in depth, and it cannot still be elucidated at a microscopic cellular and molecular level. Although the osteochondral integrated scaffold is structurally and compositionally biomimetic, it is not comparable to normal osteochondral tissues at either the biological or mechanical level. No special materials that resemble natural osteochondral tissues have been found. Finally, the CCZ and tideline play a crucial role in the osteochondral structure, which is not yet fully imitated by the integrated bionic scaffold. Therefore, the problems of cartilage layer calcification and easy separation between layers of triphasic and multiphasic scaffolds have not yet been well resolved. Nevertheless, it is believed that more suitable scaffold materials can be discovered or synthesized through novel fabrication technologies and methods including 3D printing and electrostatic spinning. Furthermore, multiple disciplines can be combined to

solve the clinical problem of osteochondral defects, such as materials, biostructures, and biomechanics.

Acknowledgments

Some of the images in this article are created using Biorender.com.

Funding

This work was supported by the Young Medical Talents Training Program of Pudong Health Committee of Shanghai (Grant No. PWRq 2021-08), the Health Industry Clinical Research Project of Shanghai Health Commission (Project no. 20224Y0393), the Youth Science and Technology Project of Health Commission of Shanghai Pudong New Area (Project no. PW2020B-5), the Outstanding Clinical Discipline Project of Shanghai Pudong (Grant No. PWYgy2021-04), the Outstanding Leaders Training Program of Pudong Hospital affiliated to Fudan University (Grant No. LX202201), the Project of Key Medical Specialty and Treatment Center of Pudong Hospital of Fudan University (Project no. Tszb2023-05), the Scientific Research Project for Agricultural and Social Undertakings in Jiading District (Grant No. Shanghai JDKW-2019-W08), the Talents Training Program of Pudong Hospital affiliated to Fudan University (Project no. PX202001), and the Scientific Research Foundation provided by Pudong Hospital affiliated to Fudan University (Project no. YJRCJJ201906), the Shanghai Scientific and Technology Committee, China (grant no. 201409003700).

Conflict of interest

The authors declare no conflict of interests.

Author contributions

Conceptualization: Dejian Li

Writing – original drafts: Jianhang Du, Ziqing Zhu, Jia Liu, Xiaogang Bao, Qian Wang, Changgui Shi, Chaoqian Zhao

Writing – review & editing: Guohua Xu, Dejian Li

All the authors read and approved the final form of the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Armiento AR, Alini M, Stoddart MJ, 2019, Articular fibrocartilage—Why does hyaline cartilage fail to repair? *Adv Drug Deliv Rev*, 146:289–305.
2. Yasui Y, Wollstein A, Murawski CD, *et al.*, 2017, Operative treatment for osteochondral lesions of the talus: Biologics and scaffold-based therapy. *Cartilage*, 8(1):42–49.
3. Gobbi A, Lane JG, Dallo I, 2020, Editorial commentary: Cartilage restoration—What is currently available? 36(6): 1625–1628.
4. Assenmacher AT, Pareek A, Reardon PJ, *et al.*, 2016, Long-term outcomes after osteochondral allograft: A systematic review at long-term follow-up of 12.3 years. *Arthroscopy*, 32(10):2160–2168.
5. Hoshi K, Fujihara Y, Yamawaki T, *et al.*, 2018, Biological aspects of tissue-engineered cartilage. *Histochem Cell Biol*, 149(4):375–381.
6. Kok A, van Bergen C, Tuijthof G, *et al.*, 2015, Macroscopic ICRS poorly correlates with O Driscoll histological cartilage repair assessment in a goat model. *Clin Res Foot Ankle*:1–7.
7. Galperin A, Floreani RA, Florczyk SJ, *et al.*, 2013, Integrated bi-layered scaffold for osteochondral tissue engineering. *Adv Healthc Mater*, 2(6):872–883.
8. Camarero-Espinosa S, Cooper-White J, 2017, Tailoring biomaterial scaffolds for osteochondral repair. *Int J Pharm*, 523(2):476–489.
9. Duraine G, Hu J, Athanasiou K, 2011, Bioengineering in the oral cavity: Insights from articular cartilage tissue engineering. *Int J Oral Maxillofac Implant*, 26(Suppl):11.
10. Grogan SP, Miyaki S, Asahara H, *et al.*, 2009, Mesenchymal progenitor cell markers in human articular cartilage: Normal distribution and changes in osteoarthritis. *Arthritis Res Ther*, 11(3):1–13.
11. Boushell MK, Hung CT, Hunziker EB, *et al.*, 2017, Current strategies for integrative cartilage repair. *Connect Tissue Res*, 58(5):393–406.
12. Kuyinu EL, Narayanan G, Nair LS, *et al.*, 2016, Animal models of osteoarthritis: Classification, update, and measurement of outcomes. *J Orthop Surg Res*, 11(1):1–27.
13. Fujioka R, Aoyama T, Takakuwa T, 2013, The layered structure of the articular surface. *Osteoarthritis Cartilage*, 21(8):1092–1098.
14. Hunziker EB, Michel M, Studer D, 1997, Ultrastructure of adult human articular cartilage matrix after cryotechnical processing. *Microsc Res Tech*, 37(4):271–284.
15. Hoemann CD, Lafantaisie-Favreau C-H, Lascau-Coman V, *et al.*, 2012, The cartilage-bone interface. *J Knee Surg*, 25(02):085–098.

16. Mente P, Lewis J, 1994, Elastic modulus of calcified cartilage is an order of magnitude less than that of subchondral bone. *J Orthop Res*, 12(5):637–647.

17. Schinagl RM, Gurskis D, Chen AC, *et al.*, 1997, Depth-dependent confined compression modulus of full-thickness bovine articular cartilage. *J Orthop Res*, 15(4): 499–506.

18. Blumer MJ, Longato S, Fritsch H, 2008, Structure, formation and role of cartilage canals in the developing bone. Ann Anat Anatom Anzeiger, 190(4):305–315.

19. Youn I, Choi J, Cao L, *et al.*, 2006, Zonal variations in the three-dimensional morphology of the chondron measured in situ using confocal microscopy. *Osteoarthritis Cartilage*, 14(9):889–897.

20. Hunziker EB, 2002, Articular cartilage repair: Basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis Cartilage*, 10(6):432–463.

21. Buckwalter J, Mankin H, 1998, Articular cartilage: Degeneration and osteoarthritis, repair, regeneration, and transplantation. *Instr Course Lect*, 47:487–504.

22. Mort JS, Billington CJ, 2001, Articular cartilage and changes in arthritis: Matrix degradation. *Arthritis Res Ther*, 3(6):1–5.

23. Mcculloch K, Litherland GJ, Rai TS, 2017, Cellular senescence in osteoarthritis pathology. *Aging Cell*, 16(2):210–218.

24. Jeon OH, David N, Campisi J, *et al.*, 2018, Senescent cells and osteoarthritis: A painful connection. *J Clin Investig*, 128(4):1229–1237.

25. Di Micco R, Krizhanovsky V, Baker D, *et al.*, 2021, Cellular senescence in ageing: From mechanisms to therapeutic opportunities. *Nat Rev Mol Cell Biol*, 22(2):75–95.

26. Martin JA, Buckwalter JA, 2002, Human chondrocyte senescence and osteoarthritis. *Biorheology*, 39(1-2): 145–152.

27. Price JS, Waters JG, Darrah C, *et al.*, 2002, The role of chondrocyte senescence in osteoarthritis. *Aging Cell*, 1(1):57–65.

28. Loeser RF, 2009, Aging and osteoarthritis: The role of chondrocyte senescence and aging changes in the cartilage matrix. *Osteoarthritis Cartilage*, 17(8):971–979.

29. Gao SG, Zeng C, Li LJ, *et al.*, 2016, Correlation between senescence-associated beta-galactosidase expression in articular cartilage and disease severity of patients with knee osteoarthritis. *Int J Rheum Dis*, 19(3):226–232.

30. Jacob J, Aggarwal A, Aggarwal A, *et al.*, 2022, Senescent chondrogenic progenitor cells derived from articular cartilage of knee osteoarthritis patients contributes to senescence-associated secretory phenotype via release of IL-6 and IL-8. *Acta Histochem*, 124(3):151867.

31. Childs BG, Gluscevic M, Baker DJ, *et al.*, 2017, Senescent cells: An emerging target for diseases of ageing. *Nat Rev Drug Discov*, 16(10):718–735.

32. Demaria M, Ohtani N, Youssef SA, *et al.*, 2014, An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev Cell*, 31(6):722–733.

33. Yao Z, Chen P, Wang S, *et al.*, 2019, Reduced PDGF-AA in subchondral bone leads to articular cartilage degeneration after strenuous running. *J Cell Physiol*, 234(10):17946–17958.

34. Udalova IA, Mantovani A, Feldmann M, 2016, Macrophage heterogeneity in the context of rheumatoid arthritis. *Nat Rev Rheumatol*, 12(8):472–485.

35. Boutet M-A, Courties G, Nerviani A, *et al.*, 2021, Novel insights into macrophage diversity in rheumatoid arthritis synovium. *Autoimmun Rev*, 20(3):102758.

36. Moelants EA, Mortier A, van Damme J, *et al.*, 2013, Regulation of TNF-α with a focus on rheumatoid arthritis. *Immunol Cell Biol*, 91(6):393–401.

37. Romas E, Gillespie M, Martin T, 2002, Involvement of receptor activator of NFκB ligand and tumor necrosis factor-α in bone destruction in rheumatoid arthritis. *Bone*, 30(2):340–346.

38. Yang P, Qian FY, Zhang MF, *et al.*, 2019, Th17 cell pathogenicity and plasticity in rheumatoid arthritis. *J Leukoc Biol*, 106(6):1233–1240.

39. Niu Q, Cai B, Huang Z-C, *et al.*, 2012, Disturbed Th17/Treg balance in patients with rheumatoid arthritis. *Rheumatol Int*, 32(9):2731–2736.

40. Peal BT, Gagliardi R, Su J, *et al.*, 2020, Synovial fluid lubricin and hyaluronan are altered in equine osteochondral fragmentation, cartilage impact injury, and full-thickness cartilage defect models. *J Orthop Res*, 38(8):1826–1835.

41. Costouros JG, Dang AC, Kim HT, 2004, Comparison of chondrocyte apoptosis in vivo and in vitro following acute osteochondral injury. *J Orthop Res*, 22(3):678–683.

42. Bornes TD, Adesida AB, Jomha NM, 2014, Mesenchymal stem cells in the treatment of traumatic articular cartilage defects: A comprehensive review. *Arthritis Res Ther*, 16(5):1–19.

43. Carballo CB, Nakagawa Y, Sekiya I, *et al.*, 2017, Basic science of articular cartilage. *Clin Sports Med*, 36(3):413–425.

44. Steadman JR, Briggs KK, Rodrigo JJ, *et al.*, 2003, Outcomes of microfracture for traumatic chondral defects of the knee: Average 11-year follow-up. *Arthroscopy*, 19(5): 477–484.

45. Bajpayee AG, Grodzinsky AJ, 2017, Cartilage-targeting drug delivery: Can electrostatic interactions help? *Nat Rev Rheumatol*, 13(3):183–193.

46. Niu X, Li N, Du Z, *et al.*, 2023, Integrated gradient tissue-engineered osteochondral scaffolds: Challenges, current efforts and future perspectives. *Bioact Mater*, 20:574–597.

47. O'Connell C, Ren J, Pope L, *et al.*, 2020, Characterizing bioinks for extrusion bioprinting: Printability and rheology, in *3D Bioprinting*, 111–133.

48. O'Connell CD, Konate S, Onofrillo C, *et al.*, 2020, Free-form co-axial bioprinting of a gelatin methacryloyl bio-ink by direct in situ photo-crosslinking during extrusion. *Bioprinting*, 19:e00087.
49. Sodupe Ortega E, Sanz-Garcia A, Pernia-Espinoza A, *et al.*, 2019, Efficient fabrication of polycaprolactone scaffolds for printing hybrid tissue-engineered constructs. *Materials*, 12(4):613.
50. Gong L, Li J, Zhang J, *et al.*, 2020, An interleukin-4-loaded bi-layer 3D printed scaffold promotes osteochondral regeneration. *Acta Biomater*, 117:246–260.
51. Ginestra P, Pandini S, Ceretti E, 2020, Hybrid multi-layered scaffolds produced via grain extrusion and electrospinning for 3D cell culture tests. *Rapid Prototyp J,* 26(3):593–602.
52. Kilian D, Ahlfeld T, Akkineni AR, *et al.*, 2020, 3D bioprinting of osteochondral tissue substitutes—In vitro-chondrogenesis in multi-layered mineralized constructs. *Sci Rep*, 10(1):1–17.
53. Diloksumpan P, De Ruijter M, Castilho M, *et al.*, 2020, Combining multi-scale 3D printing technologies to engineer reinforced hydrogel-ceramic interfaces. *Biofabrication*, 12(2):025014.
54. Chen L, Deng C, Li J, *et al.*, 2019, 3D printing of a lithium-calcium-silicate crystal bioscaffold with dual bioactivities for osteochondral interface reconstruction. *Biomaterials*, 196:138–150.
55. Gao F, Xu Z, Liang Q, *et al.*, 2018, Direct 3D printing of high strength biohybrid gradient hydrogel scaffolds for efficient repair of osteochondral defect. *Adv Funct Mater*, 28(13):1706644.
56. Saidy NT, Wolf F, Bas O, *et al.*, 2019, Biologically inspired scaffolds for heart valve tissue engineering via melt electrowriting. *Small*, 15(24):1900873.
57. Hejazi F, Bagheri-Khoulenjani S, Olov N, *et al.*, 2021, Fabrication of nanocomposite/nanofibrous functionally graded biomimetic scaffolds for osteochondral tissue regeneration. *J Biomed Mater Res A*, 109(9):1657–1669.
58. Wortmann M, Frese N, Sabantina L, *et al.*, 2019, New polymers for needleless electrospinning from low-toxic solvents. *Nanomaterials*, 9(1):52.
59. Saidy NT, Shabab T, Bas O, *et al.*, 2020, Melt electrowriting of complex 3D anatomically relevant scaffolds. *Front Bioeng Biotechnol*, 8:793.
60. Wunner FM, Wille ML, Noonan TG, *et al.*, 2018, Melt electrospinning writing of highly ordered large volume scaffold architectures. *Adv Mater*, 30(20):1706570.
61. Stansbury JW, Idacavage MJ, 2016, 3D printing with polymers: Challenges among expanding options and opportunities. *Dent Mater*, 32(1):54–64.
62. Derakhshanfar S, Mbeleck R, Xu K, *et al.*, 2018, 3D bioprinting for biomedical devices and tissue engineering: A review of recent trends and advances. *Bioact Mater*, 3(2):144–156.
63. Kumar H, Sakthivel K, Mohamed MG, *et al.*, 2021, Designing gelatin methacryloyl (GelMA)-based bioinks for visible light stereolithographic 3D biofabrication. *Macromol Biosci*, 21(1):2000317.
64. Zhu S, Chen P, Chen Y, *et al.*, 2020, 3D-printed extracellular matrix/polyethylene glycol diacrylate hydrogel incorporating the anti-inflammatory phytomolecule honokiol for regeneration of osteochondral defects. *Am J Sports Med*, 48(11):2808–2818.
65. Mano J, Reis R, 2007, Osteochondral defects: Present situation and tissue engineering approaches. *J Tissue Eng Regen Med*, 1(4):261–273.
66. Galois L, Freyria A, Grossin L, *et al.*, 2004, Cartilage repair: Surgical techniques and tissue engineering using polysaccharide- and collagen-based biomaterials. *Biorheology*, 41(3-4):433–443.
67. Nair LS, Laurencin CT, 2007, Biodegradable polymers as biomaterials. *Progr Polym Sci*, 32(8-9):762–798.
68. Gonçalves AM, Moreira A, Weber A, *et al.*, 2021, Osteochondral tissue engineering: The potential of electrospinning and additive manufacturing. *Pharmaceutics*, 13(7):983.
69. Qiao Z, Lian M, Han Y, *et al.*, 2021, Bioinspired stratified electrowritten fiber-reinforced hydrogel constructs with layer-specific induction capacity for functional osteochondral regeneration. *Biomaterials*, 266:120385.
70. Li L, Duan X, Fan Z, *et al.*, 2018, Mesenchymal stem cells in combination with hyaluronic acid for articular cartilage defects. *Sci Rep*, 8(1):1–11.
71. Huang X, Chen Z, Zhao G, *et al.*, 2020, Combined culture experiment of mouse bone marrow mesenchymal stem cells and bioceramic scaffolds. *Exp Ther Med*, 20(5):19.
72. Zhang L, He A, Yin Z, *et al.*, 2014, Regeneration of human-ear-shaped cartilage by co-culturing human microtia chondrocytes with BMSCs. *Biomaterials*, 35(18): 4878–4887.
73. Peng XB, Zhang Y, Wang YQ, *et al.*, 2019, IGF-1 and BMP-7 synergistically stimulate articular cartilage repairing in the rabbit knees by improving chondrogenic differentiation of bone-marrow mesenchymal stem cells. *J Cell Biochem*, 120(4):5570–5582.
74. Xue J, He A, Zhu Y, *et al.*, 2018, Repair of articular cartilage defects with acellular cartilage sheets in a swine model. *Biomed Mater*, 13(2):025016.
75. Koh Y-G, Kwon O-R, Kim Y-S, *et al.*, 2016, Adipose-derived mesenchymal stem cells with microfracture versus microfracture alone: 2-year follow-up of a prospective randomized trial. *Arthroscopy*, 32(1):97–109.
76. Williams R, Khan IM, Richardson K, *et al.*, 2010, Identification and clonal characterisation of a progenitor cell

sub-population in normal human articular cartilage. *PLoS One*, 5(10):e13246.

77. Fellows CR, Williams R, Davies IR, *et al.*, 2017, Characterisation of a divergent progenitor cell sub-populations in human osteoarthritic cartilage: The role of telomere erosion and replicative senescence. *Sci Rep*, 7(1):1–11.

78. Mccarthy HE, Bara JJ, Brakspear K, *et al.*, 2012, The comparison of equine articular cartilage progenitor cells and bone marrow-derived stromal cells as potential cell sources for cartilage repair in the horse. *Vet J*, 192(3):345–351.

79. Wakitani S, Aoki H, Harada Y, *et al.*, 2004, Embryonic stem cells form articular cartilage, not teratomas, in osteochondral defects of rat joints. *Cell Transplant*, 13(4):331–336.

80. Mckee C, Hong Y, Yao D, *et al.*, 2017, Compression induced chondrogenic differentiation of embryonic stem cells in three-dimensional polydimethylsiloxane scaffolds. *Tissue Eng Part A*, 23(9-10):426–435.

81. Chan CK, Seo EY, Chen JY, *et al.*, 2015, Identification and specification of the mouse skeletal stem cell. *Cell*, 160(1-2): 285–298.

82. Murphy MP, Koepke LS, Lopez MT, *et al.*, 2020, Articular cartilage regeneration by activated skeletal stem cells. *Nat Med*, 26(10):1583–1592.

83. Khader A, Arinzeh TL, 2020, Biodegradable zinc oxide composite scaffolds promote osteochondral differentiation of mesenchymal stem cells. *Biotechnol Bioeng*, 117(1):194–209.

84. Deng C, Lin R, Zhang M, *et al.*, 2019, Micro/nanometer-structured scaffolds for regeneration of both cartilage and subchondral bone. *Adv Funct Mater*, 29(4):1806068.

85. Chen G, Sato T, Tanaka J, *et al.*, 2006, Preparation of a biphasic scaffold for osteochondral tissue engineering. *Mater Sci Eng C*, 26(1):118–123.

86. Seo S-J, Mahapatra C, Singh RK, *et al.*, 2014, Strategies for osteochondral repair: Focus on scaffolds. *J Tissue Eng*, 5:2041731414541850.

87. Deng C, Yang J, He H, *et al.*, 2021, 3D bio-printed biphasic scaffolds with dual modification of silk fibroin for the integrated repair of osteochondral defects. *Biomater Sci*, 9(14):4891–4903.

88. Simkin PA, 2012, Consider the tidemark. *J Rheumatol*: 890–892.

89. Goldring SR, Goldring MB, 2016, Changes in the osteochondral unit during osteoarthritis: Structure, function and cartilage–bone crosstalk. *Nat Rev Rheumatol*, 12(11):632–644.

90. Levingstone TJ, Matsiko A, Dickson GR, *et al.*, 2014, A biomimetic multi-layered collagen-based scaffold for osteochondral repair. *Acta Biomater*, 10(5):1996–2004.

91. Zhu M, He X, Xin C, *et al.*, 2020, 3D printing of an integrated triphasic MBG-alginate scaffold with enhanced interface bonding for hard tissue applications. *J Mater Sci Mater Med*, 31(12):1–8.

92. Huey DJ, Hu JC, Athanasiou KA, 2012, Unlike bone, cartilage regeneration remains elusive. *Science*, 338(6109):917–921.

93. Mancini IA, Vindas Bolaños RA, Brommer H, *et al.*, 2017, Fixation of hydrogel constructs for cartilage repair in the equine model: A challenging issue. *Tissue Eng C* Methods, 23(11):804–814.

94. Mancini I, Schmidt S, Brommer H, *et al.*, 2020, A composite hydrogel-3D printed thermoplast osteochondral anchor as example for a zonal approach to cartilage repair: In vivo performance in a long-term equine model. *Biofabrication*, 12(3):035028.

95. Zhang B, Huang J, Narayan RJ, 2020, Gradient scaffolds for osteochondral tissue engineering and regeneration. *J Mater Chem B*, 8(36):8149–8170.

96. Nooeaid P, Salih V, Beier JP, *et al.*, 2012, Osteochondral tissue engineering: Scaffolds, stem cells and applications. *J Cell Mol Med*, 16(10):2247–2270.

97. Nooeaid P, Roether JA, Weber E, *et al.*, 2014, Technologies for multilayered scaffolds suitable for interface tissue engineering. *Adv Eng Mater*, 16(3):319–327.

98. Di Luca A, Lorenzo-Moldero I, Mota C, *et al.*, 2016, Tuning cell differentiation into a 3D scaffold presenting a pore shape gradient for osteochondral regeneration. *Adv Healthc Mater*, 5(14):1753–1763.

99. Lowen JM, Leach JK, 2020, Functionally graded biomaterials for use as model systems and replacement tissues. *Adv Funct Mater*, 30(44):1909089.

100. Radhakrishnan J, Manigandan A, Chinnaswamy P, *et al.*, 2018, Gradient nano-engineered in situ forming composite hydrogel for osteochondral regeneration. *Biomaterials*, 162:82–98.

101. Liu Z, Meyers MA, Zhang Z, *et al.*, 2017, Functional gradients and heterogeneities in biological materials: Design principles, functions, and bioinspired applications. *Progr Mater Sci*, 88:467–498.

102. Guo J, Li C, Ling S, *et al.*, 2017, Multiscale design and synthesis of biomimetic gradient protein/biosilica composites for interfacial tissue engineering. *Biomaterials*, 145:44–55.

103. Singh YP, Moses JC, Bandyopadhyay A, *et al.*, 2022, 3D bioprinted silk-based in vitro osteochondral model for osteoarthritis therapeutics. *Adv Healthc Mater*:2200209.