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Innovative Tissue-Engineered Strategies for Osteochondral Defect Repair and Regeneration: Current Progress and Challenges

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Clinical treatments for the repair of osteochondral defects (OCD) are merely palliative, not completely curative, and thus enormously unfulfilled challenges. With the in-depth studies of biology, medicine, materials, and engineering technology, the conception of OCD repair and regeneration should be renewed. During the past decades, many innovative tissue-engineered approaches for repairing and regenerating damaged osteochondral units have been widely explored. Various scaffold-free and scaffold-based strategies, such as monophasic, biphasic, and currently fabricated multiphasic and gradient architectures have been proposed and evaluated. Meanwhile, progenitor cells and tissue-specific cells have also been intensively investigated in vivo as well as ex vivo. Concerning bioactive factors and drugs, they have been combined with scaffolds and/or living cells, and even released in a spatiotemporally controlled manner. Although tremendous progress has been achieved, further research and development (R&D) is needed to convert preclinical outcomes into clinical applications. Here, the osteochondral unit structure, its defect classifications, and diagnosis are summarized. Commonly used clinical reparative techniques, tissue-engineered strategies, emerging 3D-bioprinting technologies, and the status of their clinical applications are discussed. Existing challenges to translation are also discussed and potential solutions for future R&D directions are proposed.

1. Introduction

The morphological change of a localized gap in articular cartilage and subchondral bone usually ends up with osteochondral defects (OCD) mostly due to trauma-related injuries or osteoarthritis (OA).[1] OCD might lead to joint pain, deformity, limited range and level of movements, joint stiffness, and even dysfunction.[2] Structurally, articular cartilage is able to be stratified to form four different zones based on a special constitution as well as the arrangement of chondrocytes, collagen fibrils, and proteoglycan. And the unique arrangement could further affect various properties of each zone markedly.[3] Located beneath a thin layer of calcified cartilage, subchondral bone maintains sufficient biomechanical support for the upper articular cartilage, playing a vital role in the homeostasis of cartilage. Subchondral bone, one type of dynamic and sophisticated hard tissue, is formed through endochondral ossification of the cartilage template during growth. It is composed of the subchondral bone plate (SBP) as well as trabecular bone (TB), consisting of water and extracellular

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matrix (ECM). The ECM of subchondral bone includes organic matrix (e.g., collagen, noncollagenous proteins) which contributes elasticity, and hydroxyapatite (HAp) crystals which contributes structural support and material stiffness to the tissue.[4] In contrast to articular cartilage, the subchondral bone is highly vascularized to facilitate the recruiting of progenitor cells. Therefore, unlike avascular cartilage, it has an extraordinary intrinsic capability to remodel and regenerate spontaneously.[4] Previously, the role of the subchondral bone was purely considered as one with mechanical support. More recent evidence suggests there is a much more complex interplay between the cartilage and the underlying bone. And specifically secreted factors produced by bone has been shown to modulate the responses of overlying chondrocytes.[5,6] However, the true challenges for OCD repair and regeneration exactly lie in the avascular and aneural characteristics of cartilage and the complexity of the interface between bone and cartilage.

At present, there are numerous methods utilized for chondral lesions and OCD management for the sake of offering symptomatic relief and improving function. They include nonsurgical strategies with physical immobilization together with nonsteroidal anti-inflammatory drugs, as well as surgical strategies such as osteotomy, abrasion arthroplasty, autografts implantation, mosaicplasty, microfracture, autologous chondrocyte implantation (ACI), and matrix-assisted autologous chondrocyte implantation (MACI).[7] Arthroscopic debridement and microfracture are commonly used as a first-line treatment, and ACI and osteochondral grafting can be used as second-line measures for symptomatic focal chondral lesions or OCD. However recent evidence suggests ACI after a failed microfracture leads to a worse outcome than ACI as a first intervention.[8] ACI is a two-steps procedure which has been established in 1994.[9] ACI implants the patient's autologous chondrocytes into chondral lesions and OCD. MACI, the next generation of ACI, is a surgically convenient delivery method in which autologous chondrocytes are expanded and placed onto the surface of a purified film that is implanted into OCD and absorbed by the surrounding tissues.[10] The above treatment methods are clinically well developed and efficacious to some degree, for minimizing pains and ameliorating the quality of life of our patients. Nevertheless, all of the above described clinical approaches would be ineffective over the long-term. ACI, MACI, and microfracture frequently lead to the generation of fibrocartilage rather than articular cartilage, thus harming the normal function of the patient's joint.[11] Alternatively, both cell- and tissue-based allografts, have been proposed as one of material-free repair strategies. However, the associated risk of transmitted diseases from allograft tissue is a cause of concern.

Articular cartilage has poor regeneration ability, requiring more effective and favorable alternatives for thorough healing of osteochondral lesions. Thus, more advanced treatment, namely specific structurally and functionally biomimetic tissue-engineered strategies, have emerged as promising options for the repair and/or regeneration of both subchondral bone damage and cartilage lesions by considering their totally different architecture and regeneration potential.[12] To date, many different strategies based upon the progress of regenerative medicine and tissue engineering have been proposed and implemented for OCD repair and regeneration, 1) scaffold-free strategies, including tissue-specific cells, genetically modified cells (e.g., induced pluripotent stem cells-iPSCs), and progenitor cells (e.g., bone marrow-derived mesenchymal stem cells (MSCs), BMSCs, adipose-derived mesenchymal stem cells-ADSCs, tendon-derived mesenchymal stem cells-TDSCs); 2) scaffold-based strategies, such as monophasic scaffolds, biphasic and multiple-phasic scaffolds, and gradient-designed scaffolds; and 3) incorporation with and controlled release of various types of bioactive factors and drugs using different delivery systems (**Figure 1**). Among these methods, two layers of biphasic scaffolds are popular, mimicking upper articular cartilage, and underlying subchondral bone separately. These biphasic scaffolds-based strategies have attracted attention both in academia and industry. Separate culture of cells in different differentiation medium has been proposed as a mechanism by which bilayered constructs can be primed prior to implantation. A proof-of-concept study has demonstrated the hypertrophic and cartilaginous layer construct within a subcutaneous defect model.[13] Some have been at the stage of preclinical research and some even in clinical trials.[14] In general, often unique composition, organization, mechanical strength, and biological cues were employed to these types of scaffolds. Synthetic polymers and natural polymers, such as glycosaminoglycans (GAGs), proteins as well as polysaccharides are utilized for repair of the cartilaginous layer.[15,16] For subchondral bone defect repair, a combination of polymers with bioresorbable and bioactive inorganic materials have been designed and fabricated. Polymers often hold some advantages that they could be designed flexibly with high strength. Moreover, sharing intrinsic similarities with ECM, natural polymers have desirable biomedical and chemical versatility with minimal inflammatory reactions and immunological responses.[16,17] By contrast, ceramics such as calcium phosphates (CaPs)[18] (e.g., β-tricalcium phosphate[19]), HAp,[20] and bioglass[21] are beneficially bioresorbable, osteoconductive, and biocompatible.

However, many studies regarding these bi-layered scaffolds reveal that they are sometimes unstable, and incapable of providing sufficient integration with the host tissues. To overcome these shortcomings, several multiphasic and gradient scaffolds with distinct integrated layers have been designed and fabricated.[22,23] These concepts lie in the utilization of various materials, stratification of ECM contents, mineral components, and pore parameters, such as pore diameter, connectivity, and porosity. Some research groups have already yielded promising preliminary results by incorporating one interfacial layer with homogeneous intermediate properties. Herein, we update the current progress and challenges based on tissue-engineered strategies of OCD repair and/or regeneration.

2. Structure and Properties of Osteochondral Unit

The upper articular cartilage, intermediate cartilage–bone interface, and underlying subchondral bone together form an intact osteochondral unit structurally and functionally (**Figure 2**). Without perichondrium, the cartilaginous part could be further divided into four different zones, including the superficial zone (SZ, also known as tangential layer), middle zone (MZ, also known as transitional layer), deep zone (DZ, also known as radial layer), and calcified zone (CZ). Noncalcified and calcified areas could be clearly distinguished by the tidemark. This layered composition and architecture of articular cartilage determine its

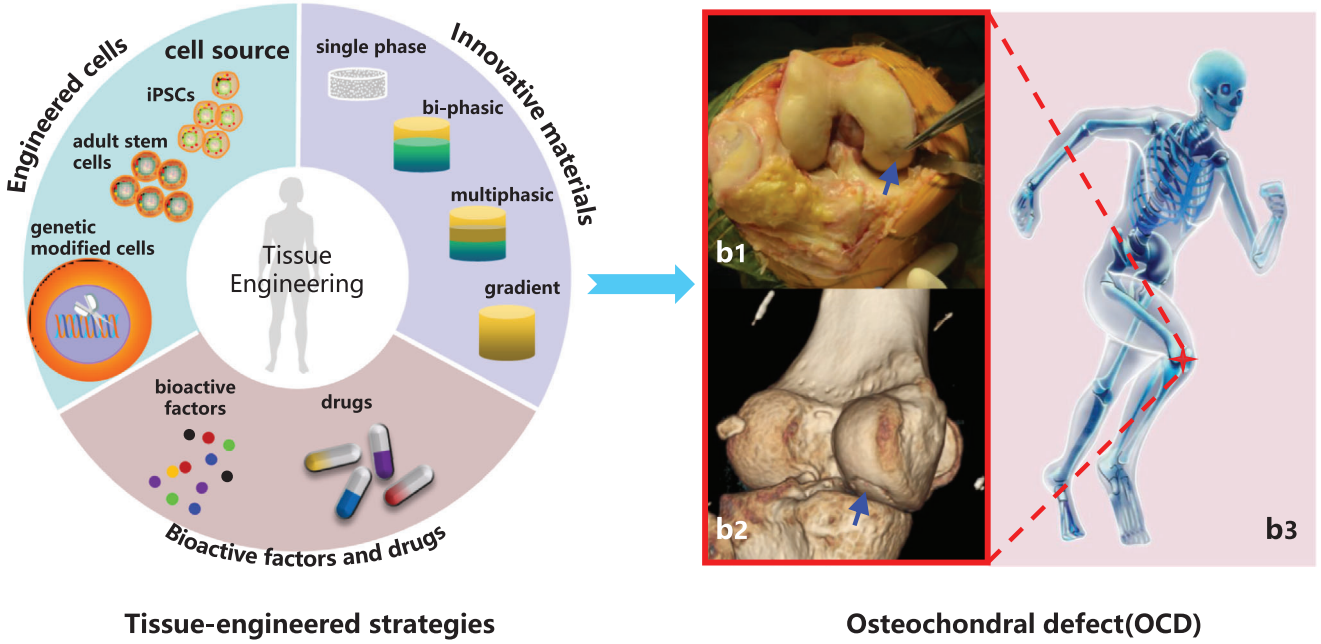


Figure 1. Schematic diagrams of tissue-engineered strategies of OCD repair and regeneration. a) Graphical illustration of emerging tissue-engineered strategies, including innovative regenerative materials, engineered cells, and bioactive factors and drugs. b) Two patients with OCD at the knee joint. b1) OCD in the weight-bearing parts of the medial femoral condyle of a 54-year-old male from the corresponding authors' institution; b2) CT rendering of an OCD in the weight-bearing parts of the medial femoral condyle of a 71-year-old male from the corresponding authors' institution.

special biomechanical properties, thereby endowing it with wear-resistance, load-bearing as well as low-friction.[24] Subchondral bone could be spilt into the subchondral bone plate and trabecular bone. As a load-sharing and nutrition-supporting part as well as warehouses of chondrocytes and various bioactive factors, subchondral bone plays a vital role in cartilage healing. Originally subchondral bone was considered to be important within the context of its load-bearing and load-sharing functionality. However, more recent studies have revealed the critical interaction between the underlying bone and the overlying cartilage from a nutritional standpoint.[6,25] Soluble signals generated from the bony layer are required to maintain cartilage homeostasis. Therefore, the secretory profile of the underlying bone is increasing in importance.

2.1. Zonal Articular Cartilage

The functional characteristics of articular cartilage are intrinsically connected to their biochemical components. Unlike elastic cartilage and fibrocartilage, as one kind of hyaline cartilage, the ECM inside articular cartilage principally consists of type II collagen fibers (15–25%, wet weight), proteoglycans (PGs) (5–10%, wet weight), and water (70–80%, wet weight).[26] Apart from abundant amounts of type II collagen, articular cartilage contains a small amount of type IX, XI, and VI collagens. The proteoglycan is predominantly formed by Aggrecan aggregate, which is ≈300 MDa. It consists of strands of hyaluronan and Aggrecan monomer (200–400 nm, ≈3 MDa).[27] The GAG chains such as keratan sulfate and chondroitin sulfate (≈25 KDa), and some core proteins as well as link proteins are bonded on the monomer.[27] Large amounts of the negative charge derived from these GAG chains on the proteoglycan results in water retention in ECM, providing the sufficient stiffness of articular cartilage.[28] The structure of articular cartilage is unique from other hyaline cartilage. It is highly organized for low-friction load bearing. Four different regions can be identified with different matrix compositions, and shapes and numbers of chondrocytes (Figure 2). The superficial zone, with parallelly oriented flattened chondrocytes and the highest concentration of collagen, provides smooth joint articulation. Below the superficial zone, the middle zone has randomly distributed chondrocytes and collagen fibers, allowing it to resist compression. The deep zone is characterized by spherical chondrocytes which are surrounded by aligned collagen fibers. Transition from cartilaginous part to subchondral bony part is marked by a thin region of calcified cartilage. The calcified area is merged with the underlying subchondral bone plate. Basically, no blood vessels, nerves, or perichondrium exist in healthy mature articular cartilage. Instead, it is nourished by the synovial fluid, which provides oxygen and partial nutrients supply.

2.2. Subchondral Bone

Lying beneath the hyaline articular cartilage, the subchondral part is composed of dense bone. It connects upper calcified cartilage and lower trabecular bone. Based on the different structural and physiological characteristics by region, the subchondral part could be divided into two areas: SBP which is more compact and closer to the upper layer of calcified cartilage, and TB near the bone medullary cavity. The normal SBP is a thin layer. It ranges from 10 μm to 3 mm in depth at different body regions.[4] TB

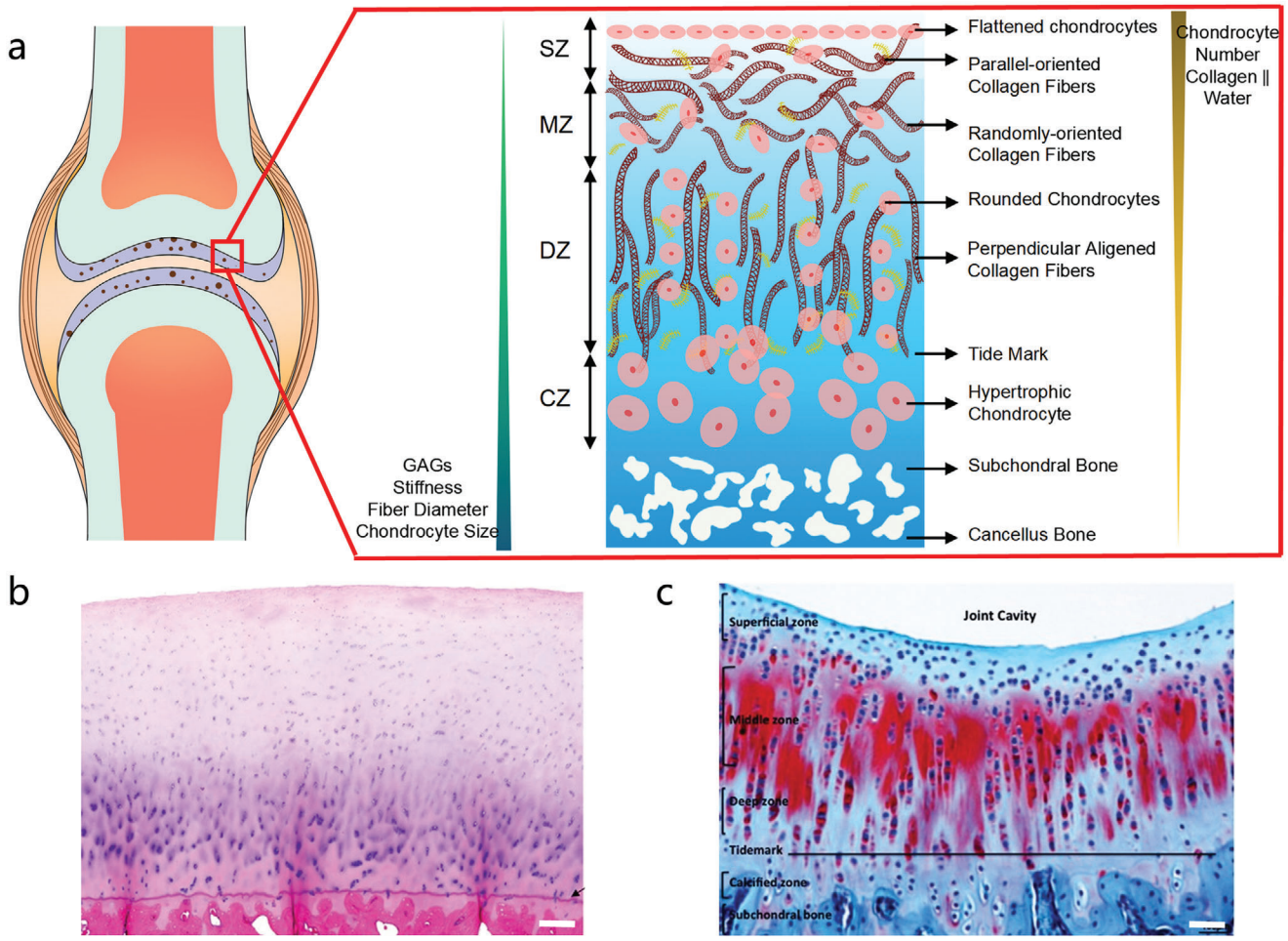


Figure 2. Graphical illustration and representative histological images of an osteochondral unit structure. a) Graphical illustration of the zonal structure of chondrocytes, collagen fibers, proteoglycans (PGs), and the subchondral bone in the osteochondral unit of the knee joint. Please note that this diagrammatic drawing does not represent the actual dimensions. From top to bottom, they are SZ-superficial zone (10–20%), MZ-middle zone (40–60%), DZ-deep zone (30–40%), CZ-calcified zone, subchondral bone plate, and cancellous bone as depicted in the text. Also, the chondrocyte numbers, Collagen II, and water content decrease, whereas the GAG, stiffness, fiber diameter, and chondrocyte size increase gradually. In general, articular cartilage thickness of human knees is ≈2 mm, and for Sprague–Dawley (SD) rats and rabbits, it is about 0.1 and 0.21–0.56 mm separately. b) H&E staining identifies the zonal structure of healthy osteochondral tissues from the medial femoral condyle of an adult SD rat (scale bar = 100 μm). Reproduced with permission.[29] Copyright 2014, Springer. c) Safranin O staining shows that rat's articular cartilage is rich in proteoglycans in the middle-zone and deep zone (scale bar = 100 μm). Reproduced with permission.[30] Copyright 2014, PAGEPress.

(also known as cancellous or spongy bone) is highly porous, with well-organized trabeculae within a 3D structure. The interspaces are usually occupied by bone marrow and vessels. The thickness of subchondral bone in different areas of joint surfaces varies, leading to different characters. The thinner regions are principally appositional stratum connected with trabeculae as well as a few Haversian canals; however, the thicker regions primarily consisted of well-organized osteons.[31] The major function of subchondral bone is to transmit the load from the joint into the bone and support the cartilage. During this process, the compact SBP offers sufficient support, and TB provides elasticity to absorbing and reducing the effects of shock.[31] Maintaining such kind of inherent joint elasticity is of great significance for our body movements.

The subchondral part is biphasic, consisting of organic and inorganic biomaterials. The organic component is mainly comprised of Col I, PGs, GAG, and water content, thus providing elasticity as well as flexibility; while the inorganic part is largely made up of HAp crystals which afford rigidity.[4,31] The special constitution and architecture of subchondral bone contribute greatly to attenuating axial forces, protecting the upper layer of articular cartilage.[32] With an inherent capability to provide feedback on certain conditions, subchondral bone can display both adaptive (longer period) and acute reflections of joints. On the one hand, subchondral bone can disperse loads derived from motion, and it is more deformable in comparison with the cortical bone; on the other hand, with the changes of forces on joints, it can physically adjust its morphology, following Wolff's Law.[33] The adjustment capability is largely promoted by bone resorption and formation processes related to osteoclastogenesis as well as osteoblastogenesis, separately. The abundant vascularization and innervation inside the subchondral bone are conducive to give

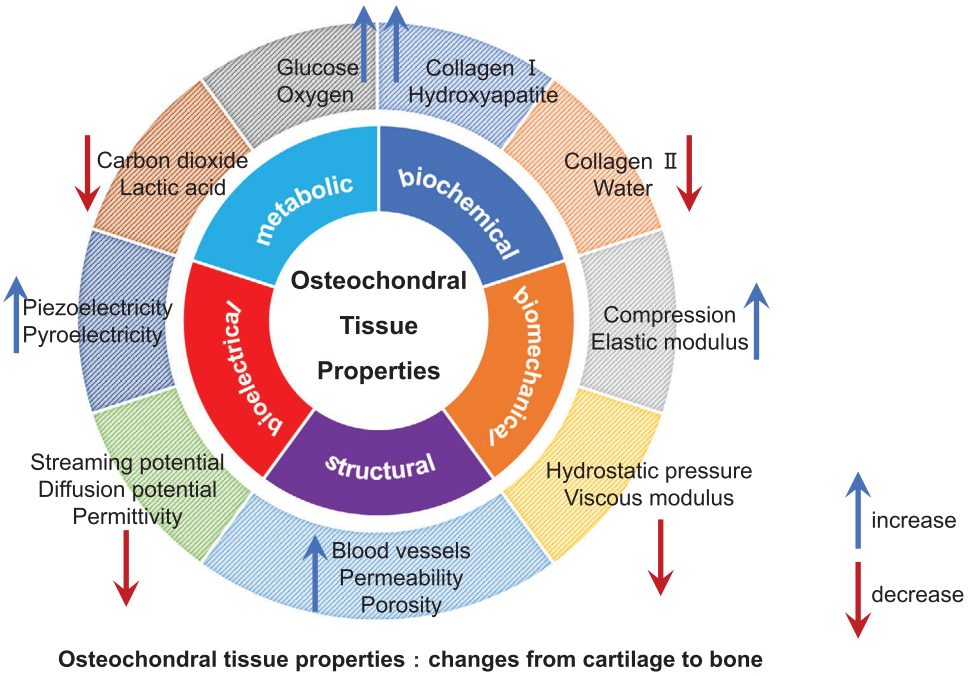


Figure 3. Osteochondral tissue properties: arrows indicate changes from cartilage to bone.

comprehensive and extensive local feedback to pathological and physiological changes within the bone.[34]

2.3. Osteochondral Tissue Properties

The unique-designed gradient structure of the osteochondral unit determines numerous functional properties of joints. From top (articular cartilage) to bottom (bone) the following occurs (indicates as Figure 3): for its biochemical properties, the content of collagen I and hydroxyapatite increase gradually, the content of collagen II and water decrease gradually; for its biomechanical properties, the compression and elastic modulus increase, while the hydrostatic pressure and viscous modulus decrease; for its structural properties, blood vessels, permeability, and porosity increase; for its bioelectrical properties, the piezoelectricity as well as pyroelectricity increase, while the streaming potential, permittivity, and diffusion potential decrease; for its metabolic properties, glucose and oxygen increase, while carbon dioxide and lactic acid decrease.

3. OCD

Based on parameters, such as the severity degree of defects diameter and depth, the defects or lesions of cartilage can be classified into several categories using different approaches. Among them, OCD is the most severe type, requiring more integrated approaches for its repair and regeneration.

3.1. Defect Classifications

As no continuous collagen fibrils exist between the SBT layer and calcified cartilage layer, the bone-cartilage interface is mechanically more fragile compared with the middle zone in the osteochondral unit. Different from immature cartilage, mature cartilage does not exist blood vessels and is inclined to elevated activities of apoptosis.[26] The above-mentioned characteristics can lead to some negative results, for example, the relatively mature articular cartilage has the very limited potential of self-healing. Additionally, diabetes, menopause, and anti-inflammatory therapeutics have been proven to deteriorate the quality of cartilage, destroying its fundamental framework.[35] There are different types of articular cartilage lesions and among them, the four most common types of knee cartilage damage encountered in clinical practice include osteochondritis dissecans, patellofemoral defects, incidental chondral defects, and defects encountered after meniscectomy.[36] The OCD is the next stage of cartilaginous defects, which contain partial-thickness and full-thickness defects just in the cartilage. If someone's subchondral bone is exposed due to accidental trauma or arthritis, then such a defect is known as an OCD. For both clinical and preclinical purposes, plenty of various classification systems have been established for the evaluation of cartilage defects. Among them, the "Outerbridge Classification System," the most broadly used method, is well described in **Figure 4**.[37] This method contains five continuous levels (Grade 0–IV), ranking the severity of chondral lesions and OCD gradually. Healthy cartilage is defined as Grade 0. When the cartilage becomes relatively soft as well as swelling, it can be then marked as Grade I. When the diameter of partial-thickness chondral defect is smaller than 1.5 cm, it is denoted as Grade II, which is often diagnosable clinically. Grade III represents a full-thickness defect with a size larger than 1.5 cm. The thorough exposure of subchondral bone can be considered as Grade IV, known as OCD. Apart from this classification system, there exist several other types of the classification systems for accurately describing and evaluating lesions, such as the Noyes and Stabler

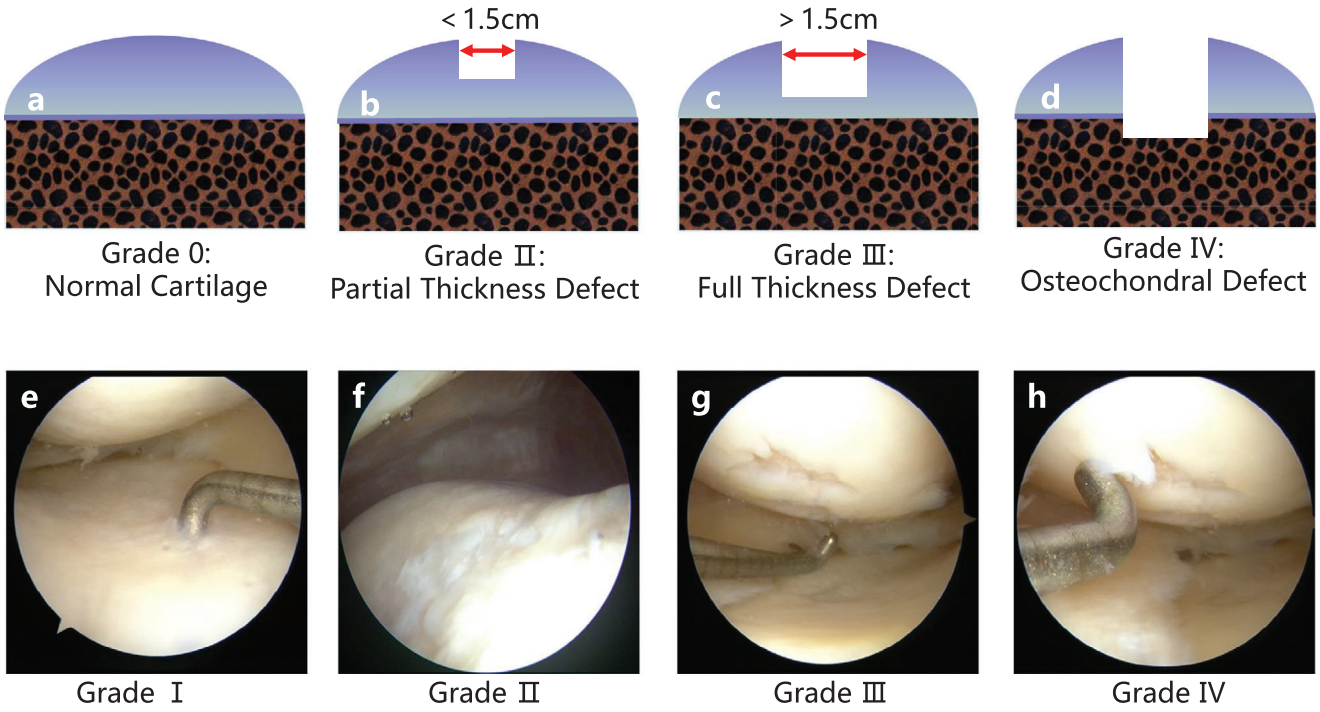


Figure 4. Schematic diagram of a modified "outerbridge classification system" and arthroscopic clinical images of human cartilage. a) Grad 0 indicates "healthy cartilage" with no damage in upper cartilage layer or underlying subchondral bone. b) Grade II indicates "partial-thickness defect" with the diameter smaller than 1.5 cm only in the cartilage layer. c) Grade III indicates "full-thickness defect" with the diameter larger than 1.5 cm only in the cartilage layer. d) Grade IV indicates "osteochondral defect" with damage both in cartilage and underlying subchondral bone layer. e–h) The arthroscopic clinical images of human cartilage from Grade I to Grade IV, gradually.

method,[38] the histological and histochemical grading system,[39] the Osteoarthritis Research Society International (OARSI) Cartilage Histopathology Assessment System (OOCHAS),[40] and International Cartilage Repair Society (ICRS) Grading System.[41]

3.2. Diagnosis of the OCD

A proper diagnostic method is a good start to treat OCD properly. In general, clinical doctors choose treatments based on each actual situation, which means that they rely on defect location, symptoms, severity, and so on. Clinically, X-ray imaging is broadly utilized for the diagnosis of fractures or OA. It can acquire information about narrowing joint space, sclerosis, osteophytosis, and cystic lesions for OA patients' joints. That means this technique primarily displays the late pathological alterations of bone rather than the small pathological alterations of cartilage during the early period[42] (**Figure 5**a1). That is because of its drawbacks in sensitivity of detecting cartilage.[42] Typically, a case of OCD can be verified by images which reveal the detached bone surrounded with radiolucency. In order to generate more comprehensive and detailed images, computed tomography (CT) is often utilized, but it also has similar limitations. By producing many X-ray images, it can have cross-sectional images of one tissue or organ, thus having a relatively high level of sensitivity as well as specificity. It can detect OCD more effectively; however, it still cannot visualize cartilage. It can provide predicted results of cartilage only depending on image analysis of lower subchondral bone.[43] As a noninvasive approach for precise diagnosis of cartilage conditions, several magnetic resonance imaging (MRI) techniques (Figure 5b) have been well developed by using cartilage-specific pulses.[44] Compared with primary radiographic approaches, MRI can obtain notably augmented information of the region of interest (ROI), and surrounding tissues, especially cartilage and vasculature.[45] It has been reported that the specificity and sensitivity of MRI could reach a range of 95–100%.[46] However, high cost and long scanning time hinder its broad application. Arthroscopy for diagnostics and treatments (Figure 5c) is a low invasive method to offer details regarding cartilage surface directly. However, this technology cannot visualize the variations in the layers of the deep cartilaginous region and the subchondral bone.[47] Despite this, it serves as the most comprehensive approach to the clinical detection and therapy of chondral defects and OCD. Additionally, clinically used ultrasound techniques are also used with a wide spectrum of frequencies to visualize the cartilage and subchondral bone. When using a relatively low frequency, it is not sensitive enough for assessing articular cartilage with early degeneration. High-frequency ultrasound (HF-ultrasound) assessment (Figure 5d) (usually larger than 20 MHz) provides higher resolutions. Hence, HF-ultrasound can assess cartilage and bone simultaneously.[48] Huang et al. used several ultrasound parameters (e.g., surface reflection coefficient, backscattering coefficient, and roughness index) to evaluate the quality of articular cartilage and distinguished normal articular cartilages and degenerated ones at OA's early stage.[48]

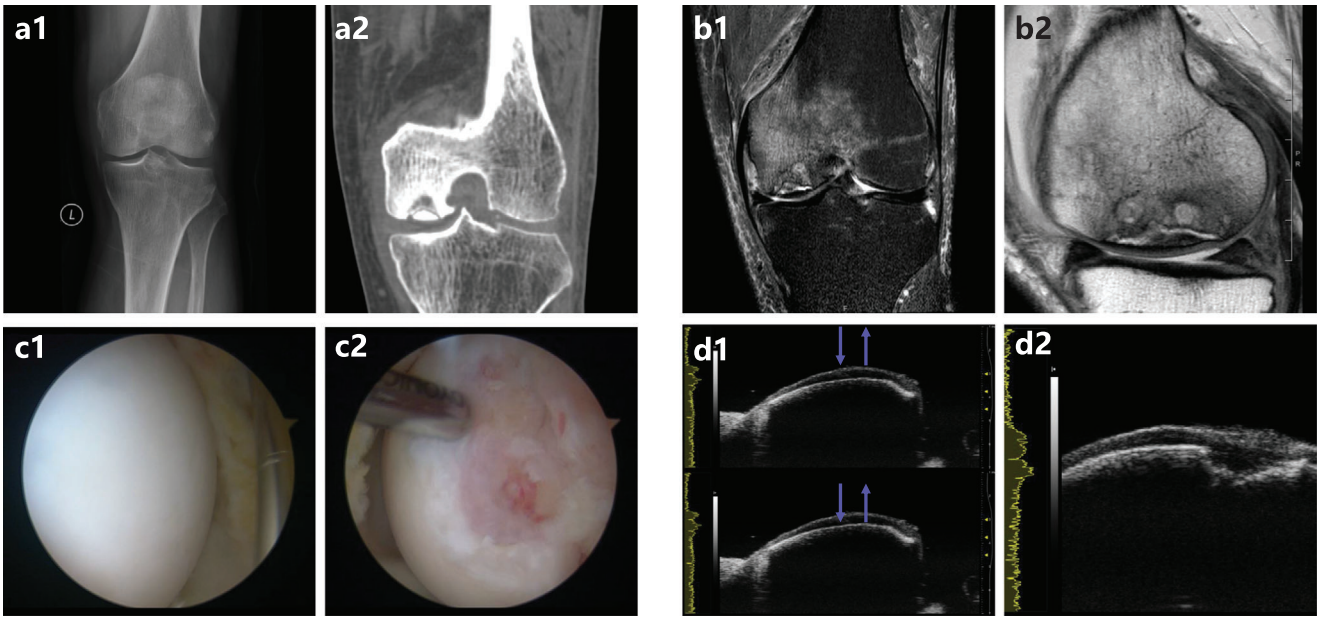


Figure 5. Diagnosis of the osteochondral defect (OCD) using various imaging modalities. a1) Coronal plain radiograph image of a 65-year-old gentleman with left knee pain, the OCD of the medial femoral condyle is not visible. a2) Coronal CT image of the same knee showing the sizable OCD. b1) Coronal T2 MR image of the same knee showing OCD with substantial marrow edema and cartilage irregularity. b2) Sagittal T1 MR image of the same knee showing the OCD with high signal intensity in the subchondral bone. c1) Knee arthroscopic clinical photograph of a healthy knee with pristine hyaline cartilage. c2) Knee arthroscopic clinical photograph of an advanced stage of OCD with 1 cm in diameter. d1) High-frequency ultrasound image of the osteochondral unit (cartilage surface and cartilage-bone interface) locating at lateral femoral condyle of a 24-week-old New Zealand rabbit. d2) High-frequency ultrasound image of the osteochondral unit of a 24-week-old New Zealand rabbit with an implanted multilayered construct for a 3 × 3 mm OCD at week 12.

4. Osteochondral Defect Repair

A study revealed more than 60% of patients with knee arthroscopic assessments endured the pain of Grade III or Grade IV defects.[49] Usually, articular cartilage damage occurs when patients are young, and without efficacious and effective therapies and interventions, these small damages will further develop into joint OA.[50] There are ≈0.9 million reported cases of cartilaginous damage in the US annually, and 22.22% of them receive surgical treatment.[51] These case numbers are quickly increasing under the circumstances of prolonged average life expectancy. Conventional clinical utilized techniques provide various options, however, still with huge challenges. Meanwhile, both the preclinical and clinical studies of advanced tissue engineering-based strategies for OCD repair and regeneration are flourishing. Therefore, in this section, we will summarize some clinical therapeutic approaches for OCD and state-of-the-art tissue-engineered strategies.

4.1. Clinical Treatment Options

Currently, numerous treatments are being used clinically, but none of them have demonstrated a complete functional repair of OCD with durable hyaline cartilage. Clinical utilized treatment options are classified into several types based on the OCD repair results. For palliative treatment methods, they often cannot replace the damaged regions. Reparative treatment methods attempt to replace either the chondral lesions or the full OCD often with some additional biomaterials. Due to the unique architecture of the osteochondral unit, more ideal strategies are restorative treatments, which aim to reconstruct the natural tissues. **Figure 6** summarizes the currently used treatment options. These treatments are selected depending on the defect size, location, severity, and patient conditions. There are conservative treatments of immobilization, stabilization of loose body by screw or pin fixation, and debridement of damaged tissues. For small chondral lesions or OCD less than 2 cm, marrow-stimulation techniques could be applied by drilling (microfracture or nanofracture) in the subchondral parts to stimulate an influx of MSCs from the bone marrow into the OCD.[52] Numerous grafts that originate from periosteum[53] and perichondrium[54] have been used since they contain progenitor cells, but the outcomes, to date, are not so optimal. Mosaicplasty, or osteoarticular transfer system (OATS), is performed by transplanting autologous osteochondral plugs. These plugs contain both the upper hyaline cartilage and the lower subchondral bone from nonweight-bearing areas of the patient's joints.[55] In recent decades, tissue-engineered approaches have been adopted. Autologous chondrocytes implantation (ACI) has been used via expanding chondrocytes from nonweight-bearing regions in monolayer culture followed by transplantation with a periosteal flap.[56] This method has been modified to MACI through seeding chondrocytes into various scaffolds.[57] However, it is worth noting that many treatments applied to chondral defects generate an injury to the underlying bone (e.g., microfracture), thus creating a de novo bone defect and by proxy an OCD. In general, all of these methods only achieve partial or temporary success.

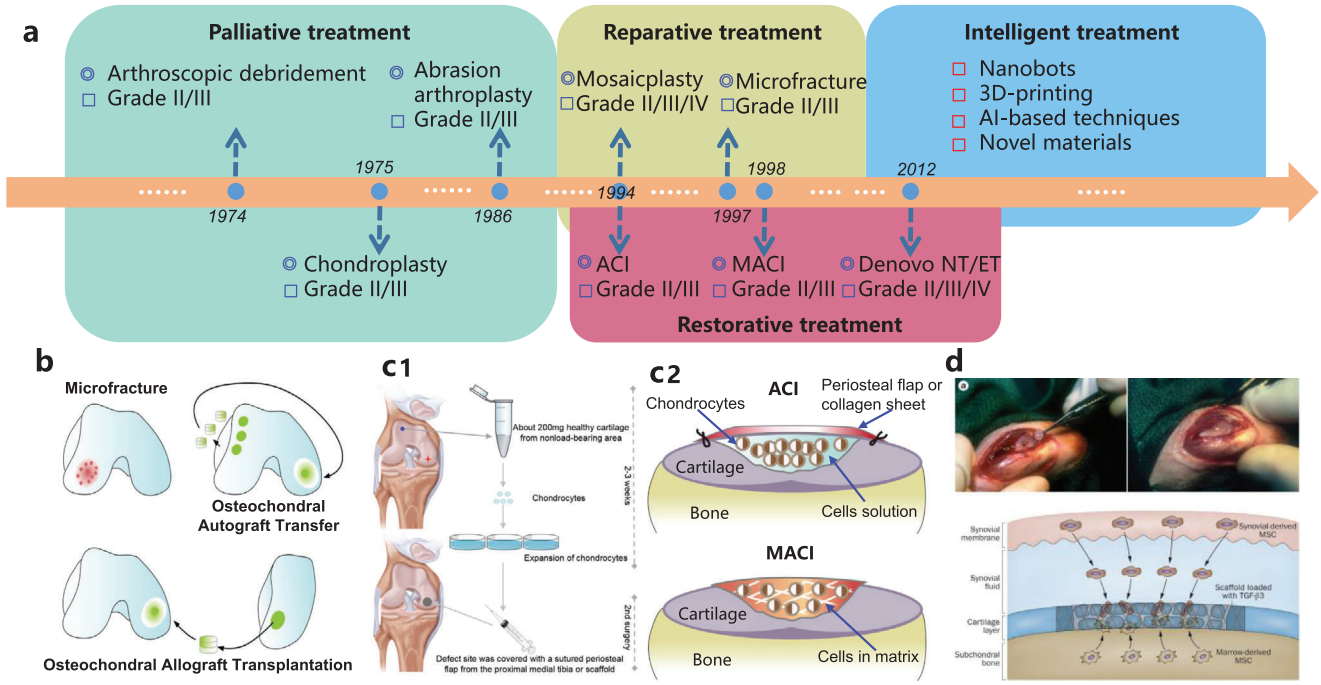


Figure 6. Clinical treatment approaches for the repair and regeneration of cartilage lesions and OCD. a) Summary of the development history of clinically utilized methods for the repair or/and regeneration of cartilage lesions and OCD. b) Graphical illustration of clinical techniques: microfracture, osteochondral autograft transfer, and osteochondral allograft transplantation. c1) Brief clinical procedures of ACI for repairing chondral defects or OCD. c2) Graphical illustration of ACI and MACI and their differences. d) Use of scaffolds with local delivery of growth factors (i.e., TGF-β3) to enable direct endogenous cell homing for OCD repair. Reproduced with permission.[62] Copyright 2015, Springer Nature.

Durable and long-lasting repaired osteochondral tissue cannot currently be regenerated. Some approaches even exhibit side effects. Debridement and subchondral drilling are known to cause fibrocartilage formation,[58] and perichondral or periosteal grafting do not consistently yield hyaline cartilage.[53] Autologous chondrocyte transplantation involves multiple operations and may cause problems brought by different types of scaffolds and biomaterials.[59] Autologous osteochondral transplantation is the only clinically used surgical treatment targeting at restoring both hyaline cartilage and the subchondral bone. However, it is sometimes associated with donor site morbidity,[60] graft apoptosis,[61] and contour mismatch, regardless of the availability of the grafts. In addition to the above limitations, in prospective randomized clinical trials, both mosaicplasty and ACI are reported to have variable clinical outcomes.

4.2. Proposed Tissue-Engineered Strategies

During the past several decades, tissue-engineered strategies (**Figure 7**) emerged as promising options for osteochondral repair and regeneration. At present, numerous important advances, including 3D-bioprinting, gene-editing technology (i.e., CRISPR/Cas9), induced pluripotent stem cells (iPSCs), immunomodulation, and mechanobiology, make a great contribution to the progress of tissue engineering. In general, cells, scaffolds, and bioactive factors are fundamental components of tissue-engineered strategies for OCD repair and regeneration. Herewith, we update various innovative scaffolds, engineered cell sources, and numerous bioactive factors.

4.2.1. Scaffold-Free Strategies

Comparing with scaffold-based strategies, scaffold-free strategies (**Table 1**) hold some advantages, in terms of preparation procedures, optimization of the construct, time, cost, and minimization of the risks of negative effects caused by extrinsic materials . The core of scaffold-free tissue-engineered strategies is engineered cells, ranging from tissue-specific cells[63] and progenitor cells, such as BMSCs,[64] ADSCs,[65] articular cartilage progenitor cells (ACPCs),[66] synovial membrane-derived MSCs (S-MSCs)[67] and induced pluripotent stem cells (iPSCs).[68] These strategies utilize some techniques, e.g., centrifugation, to acquire initial high density of cells for the formation of cell sheets or cell pellets, thus enhancing cell-to-cell interactions, mimicking the embryonic development process, and producing native extracellular matrix derived from these cells.[69]

Among various cell types utilized to produce cartilage-like constructs, as tissue-specific cells, chondrocytes hold advantages in several aspects. Chondrocytes are abundant, restricted to chondrogenesis, and without severe clinical safety issues related to ACI technology. Cheuk et al.[63] established a method by using allogeneic scaffold-free chondrocyte pellets fabricated from rabbit costal cartilage for OCD repair. The results revealed that the scaffold-free chondrocyte pellets could only enhance cartilage repair at an early stage without immune rejection,

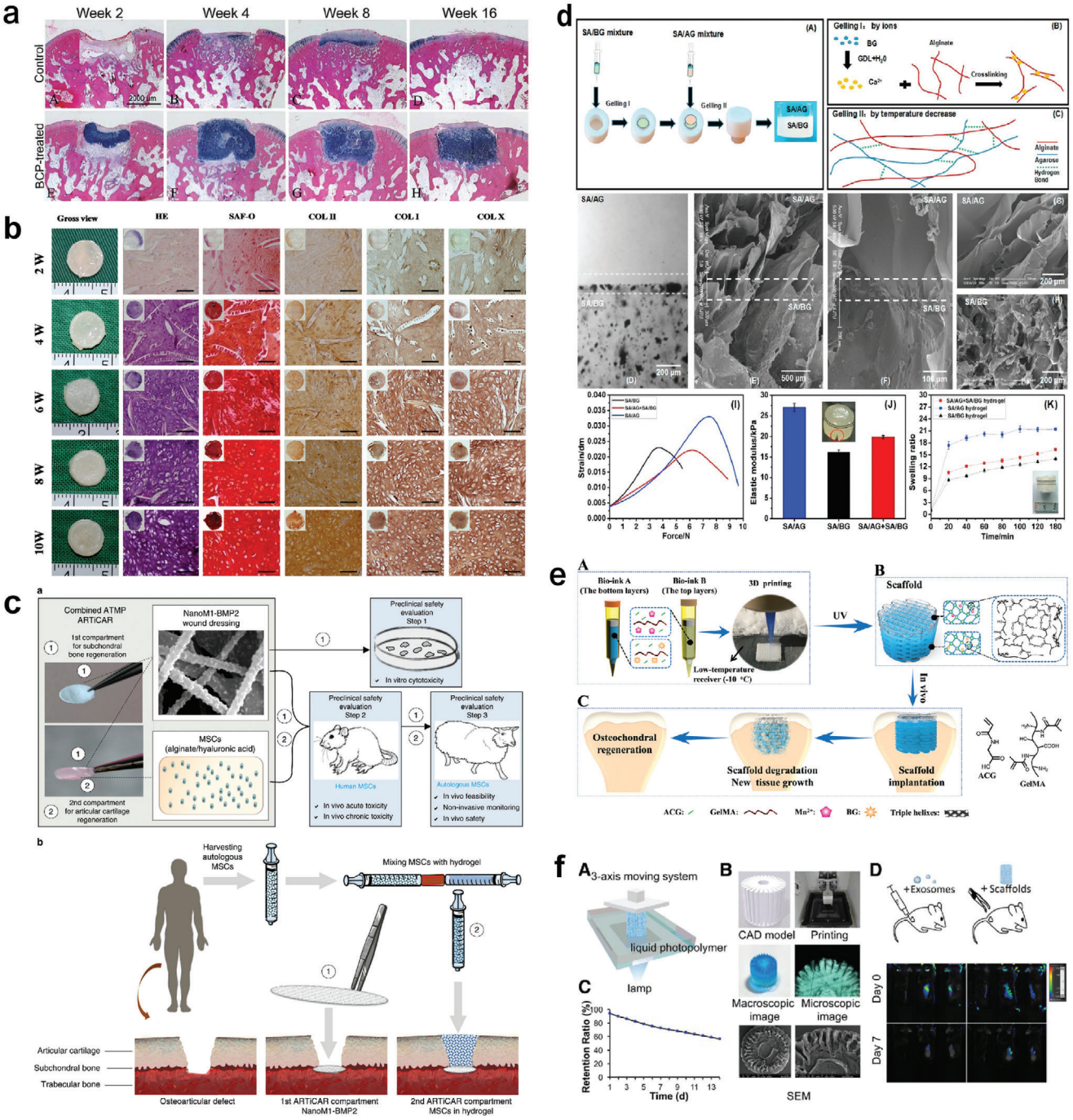


Figure 7. Representative methods and results of proposed tissue-engineered strategies for OCD repair and regeneration. a) Utilization of allogeneic scaffold-free chondrocyte pellets for OCD repair in rabbit model. Morphological results of H&E staining demonstrated the advancement of subchondral bone in the control group (without chondrocyte pellets) at week 16. While in the pellets-treated group, these tissue-engineered pellets increased in size and filled up the defect. Reproduced with permission.[63] Copyright 2011, John Wiley and Sons. b) In vitro tissue-engineered cartilage formed by autologous BMSCs for OCD repair. The ex vivo chondrogenesis and maturation of BMSCs revealed a time-dependent manner. The expression of the hypertrophy-related proteins such as collagen type I and X (COL I, COL X) was detected in all samples at different time points, suggesting that in vitro BMSC-engineered cartilage maintained the potential of endochondral ossification even in a chondrogenic culture environment. Reproduced with permission.[64] Copyright 2017, Springer Nature. c) The development of composite advanced therapy medicinal product (ATMP) based on a polymeric nanofibrous bone wound dressing and BMSCs for osteoarticular regeneration. Reproduced with permission. [90] Copyright 2019, Springer Nature. d) Preparation and characterization of an injectable continuous stratified structurally and functionally biomimetic scaffold (SA/AG+ACs/BMSCs-SA/BG+BMSCs) for OCD repair. Reproduced with permission.[94] Copyright 2019, Elsevier. e) 3D printed biodegradable biohybrid gradient and high-strength supramolecular polymer reinforced-gelatin hydrogel for repairing OCD. Reproduced with permission.[106] Copyright 2019, Wiley-VCH GmbH. f) 3D-bioprinting of radially oriented ECM/GelMA/exosome construct for OCD repair. Both the in vitro and in vivo data showed this 3D-printed composite scaffold could successfully control the release of exosomes for at least 7 days. Reproduced with permission.[108] Copyright 2019, Ivyspring.

Table 1. Recent preclinical results of tissue-engineered scaffold-free strategies for OCD repair and regeneration. BM-MSCs: bone marrow-derived mesenchymal stem cells; AD-MSCs: adipose-derived mesenchymal stem cells; S-MSCs: synovial membrane-derived mesenchymal stem cells; TD-MSC: tendon-derived mesenchymal stem cells; iPSCs: induced pluripotent stem cells.

Cell category	Advantages	Disadvantages	Applications	Results	References
Tissue-specific cells: chondrocytes	1) Promising cell options for cartilage repair. 2) Restricted to chondrogenic lineage. 3) Limited severe clinical issues with ACI. 4) More sufficient than progenitor cells.	1) Donor site morbidity. 2) Dedifferentiation sometimes occurs during cell expansion. 3) Limited cells available; many surgery steps involved to hinder the usage of chondrocytes.	Allogeneic scaffold-free chondrocyte pellets for OCD repair (rabbit model).	It could only enhance cartilage repair at an early stage without immune rejection.	[63]
			Transplanted scaffold-free chondrocyte sheets for cartilage repair (minipig model).	It facilitated the repair of full-thickness cartilaginous defects in the knee joints of the minipig model.	[129]
Progenitor cells: a) BM-MSCs b) AD-MSCs c) S-MSCs d) TD-MSCsd) iPSCs...	1) Easily and readily available from bone marrow, fat tissue, and synovial membrane, etc. 2) Higher differentiation capability and rapid expandable. 3) With the capability of resistance to cellular senescence. 4) No requirements for obtaining these cell types for autologous cartilage grafts.	1) Cell quality is associated with the age and diseases of donors. 2) Potential risks of bringing on tumorigenesis. 3) Not restricted to osteogenic and chondrogenic lineages. 4) Formation of fibrocartilage rather than hyaline-like cartilage in defects and terminal hypertrophic differentiation as well as mineralization results in the replacements of cartilage by bone. 5) ADSCs possess limited chondrogenic potential.	Ex vivo tissue-engineered cartilage from autologous BMSCs for OCD repair (swine model).	It repaired OCD by regenerating the cartilage and subchondral bone.	[64]
			Allogeneic construct from AD-MSCs for OCD repair (rabbit model).	It could increase the stainability of Col II gradually with the passage of time as well as promote histologic healing.	[65]
			S-MSCs transplantation for OCD repair (rabbit model).	Using S-MSCs transplantation could facilitate the repair of appropriate tissue texture.	[67]
			Spherical aggregated human BM-MSCs for OCD repair (rabbit model).	Implantation of aggregated spherical hBM-MSCs was superior to culture single cells in monolayer for improving OCD repairing.	[77]
			Ex vivo chondrogenesis evaluation and in vivo OCD repair by iPSCs.	Implantation of hiPSCs under the chondrogenesis induction indicated superior cartilage quality than the control group. And the composition of newly formed cartilage was mainly implanted hiPSCs.	[68]

suggesting that scaffold-based chondrocyte implantation was more effective for the repair of chondral defects than OCD. Also, these tissue-specific cells sometimes undergo dedifferentiation during expansion ex vivo, resulting in losing their appealing cartilaginous characters.[70]

With regard to stem cells, they could self-renew and differentiate into several lineages, e.g., chondrogenesis,[71] osteogenesis[72] and adipogenesis.[73] Although different origins of mesenchymal stem cells hold some similar properties, these kinds of cells cannot possess identical phenotypes, nor differentiation potentials.[74] Apart from the above points, usually, the selection of cell types is vastly dependent on the accessibility, isolation yield, and procedures. Since being first isolated by Friedenstein,[75] BMSCs have wide utilizations for skeletal tissue repair or regeneration. He et al.[64] utilized in vitro engineered cartilage formed by autologous BMSCs for OCD repair in a swine model and the data demonstrated that this scaffold-free BMSCs-based method could improve tissue-specific repair of OCD. To be noted, compared with bone marrow origination, ADSCs have some merits, on account of that they are less invasive for isolation and expansion, as well as requiring simple harvest procedures.[76] Besides, many preclinical applications of synovial membrane-derived MSCs (S-MSCs)[67] and iPSCs[68] have achieved successful preliminary results of improved repair of both cartilage and subchondral bone.

However, these scaffold-free strategies might fail due to the mechanical instability of implants inserted into the defect site. An anchorage system (e.g., the utilization of fibrin) or a cellular agglomerates-based strategy is needed for adequate fixation when implanting monolayers or cell sheets of MSCs into OCD. Lee et al.[77] fabricated spherical aggregated hBM-MSCs and indicated that implantation of spherical hBM-MSCs was superior to single cells cultured in monolayer for facilitating OCD regeneration. In other words, through distinct methods, cells created spheroid-like or columnar structures with enough size and thickness to fill into the defect site. Nonetheless, the tight connection between agglomerates and the subchondral bone is still a problem. Needless to say, it is essential to provide fixation with these implanted scaffold-free cells and to afford sufficient and proper loading transfer from the top to bottom.

4.2.2. Scaffold-Based Strategies

Unlike scaffold-free strategies, scaffold-based strategies (**Table 2**) could provide 3D microenvironments for cells (endogenous or exogenous cells) to augment cell adhesion, proliferation, migration, and differentiation. In this part, we would like to discuss the development of different types of materials and scaffolds for repairing and regenerating OCD.

Materials for Articular Cartilage: Various different materials, typically synthesized by biocompatible and biodegradable polymers, such as natural and synthetic polymers as well as composite fibers have been commonly investigated the formation of cartilaginous constructs.

Natural Polymers: Natural-derived polymer fibers are well-known candidates for tissue-engineered cartilage, owing to the merits of cytocompatibility and biocompatibility, non-antigenicity, biofunctionality, and biodegradability. They include gelatin, glycosaminoglycan, collagen chitosan, alginate, hyaluronic acid (HA), starch, and bacterial-sourced polymers (i.e., hydroxy alkanoates). They confer naturally occurring environments, thus largely expediting cell migration, proliferation, and differentiation.[17] Some of their specific molecular domains can support and guide cells during their different periods of development. Meanwhile, unfortunately their characteristics of generally being mechanically weak and having low stiffness are deeply rooted in their chemical structure.[17] Collagen fibers or gels, mainly exist in ECM and bone and to date, have been successfully applied for cartilage constructs. As one type of non-adhesive glycosaminoglycan, HA usually is encapsulated and crosslinked with cells as well as other materials respectively in the form of a hydrogel for various applications of cartilage repair. Chitosan belongs to one type of linear biodegradable polysaccharide. 3D chitosan naturally exists within human body, acting as a lubricant. Due to its multifunctional structure and crosslinking capability, chitosan is oftentimes blended with some other bioactive materials to amend the properties of scaffolds.

Synthetic Polymers: Compared with natural polymers, the mechanical properties (e.g., stiffness and strength) and degradation speed of synthetic polymers have been flexibly regulated. Their tailored multiple shapes and size with desirable porosity according to cell migration speed or tissue in-growth, makes synthetic polymers attractive.[15,17] Additionally, the huge technical progress in electrospinning approaches together with 3D-printing has enabled the fabrication of scaffolds faster. However, synthetic polymers still possess drawbacks of bioactivity, since they generally possess a hydrophobic surface which can affect cell adhesion, and proliferation.[15,17] Therefore, chondroitin sulfate, silicate, and alkaline have been applied to surface treatment, for the purpose of improving hydrophilicity and offering suitable constructs. Besides, by incorporating growth factors, e.g., TGF-β or/and BMP proteins, these polymers show a certain role in supporting cell proliferation and differentiation, thus augmenting the repair and regeneration process.[78] Recently, as a kind of biodegradable synthetic polymers, poly(D,L-lactic-co-glycolic acid), poly(glycolic acid), poly(caprolactone), poly(ethylene glycol), and poly(L-lactic acid) have been regularly employed.[15,17]

Composite Polymers: Composite polymer materials consist of different polymers (natural polymers or/and synthetic polymers) and can avoid the shortcomings of each single material. Composite polymers combine their merits together, maximizing the whole comprehensive performance.

Bone Materials: For scaffold fabrication of subchondral bone, material selection is very important. Original biomechanical strength, desirable bone ingrowth, and integration with host adjacent bone tissues are aspects that should be taken into account. Ceramics, bioglass, and metallic materials are three common candidates. For polymers, both natural, and synthetic ones, could be used alone or combined with ceramics.

Ceramics and Glasses: Ceramics (e.g., HA, CaPs) as well as bioactive glasses (i.e., bioglass) are broadly used for skeletal tissue engineering. Because of their excellent osteoconductivity and osteoinductivity, the formation of a bone-like tissue, as well as the integration of a scaffold to the host tissue, are improved by such materials. At the same time, inclusion and controlled release of bioactive factors in these scaffolds may contribute to the maturation of subchondral bone. For example, the inclusion of TGF-β1

Table 2. Current preclinical results of tissue-engineered scaffold-based strategies for OCD repair and regeneration. PCL: poly (ϵ-caprolactone); BMP-2: bone morphogenic protein 2; PVA-MA: poly(vinyl alcohol) (PVA)-methacrylate(MA); PVA-MA/CS-MA: poly(vinyl alcohol) (PVA)-methacrylate(MA)/chondroitin sulfate (CS)-methacrylate(MA); PLA: polylactic acid; Col I: type I collagen; HA: hydroxyapatite; TCP: tricalcium phosphate; Yarn CH-TCP: nanofiber yarn-collagen type I/hyaluronate/TCP hybrid scaffold; BMSCs: bone marrow-derived stem cells; SA/BG-SA/AG: alginate(SA)/bioglass (BG)-alginate(SA)/agarose (AG); PLGA: poly (lactic-co-glycolic acid); PEGS/MBG: PEGylated poly(glycerol sebacate) (PEGS) /mesoporous bioactive glass(MBG); EDAC/NHS: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC)/*N*-hydroxysuccinimide (NHS); nHA+ChS-NPs: nanohydroxyapatite (nHA)+ chondroitin sulfate nanoparticles (ChS-NPs); hMSCs: human mesenchymal stem cells; PACG-GelMA: poly(*N*-acryloyl 2-glycine) (PACG)-methacrylated gelatin (GelMA); PLGA/HAp: poly (lactide-*co*-glycolide) (PLGA)/hydroxylapatite (HAp); TGF-1: transforming growth factor-1.

Scaffold phase(s)	Materials	Cells and growth factors	Results	References
Monophasic scaffolds	PCL	BMP-2, chondrocytes	After 5 weeks of implantation, cartilaginous tissues were generated significantly. However relatively higher expressions of markers of hypertrophic chondrocytes suggested the newly formed cartilage might undergo endochondral ossification.	[86]
	PVA-MA, PVA-MA/CS-MA	n.a.	Nanofiber scaffolds enhanced chondrogenic differentiation and cell proliferation ex vivo, and cartilaginous tissue formation in the rat model.	[130]
	PLA	Perichondrocytes	One year later, from these macroscopic pictures, the repair results were grossly successful. However, further results demonstrated inconsistent subchondral bone formation in the rabbit model.	[87]
Bi-phasic and multiphasic scaffolds	Col I/HA Sponge-TCP, Yarn CH-TCP	BMSCs	The repairing scores and compressive modulus were largely promoted by this biphasic construct for repairing OCD in the rabbit model.	[92]
	Aragonite-HA	n.a.	The acellular Ar-HA-based bi-phasic scaffold could help to repair the cartilage and subchondral bone at 12-months after implantation in the goat model.	[93]
	SA/BG-SA/AG	BMSCs, chondrocytes	This biomimetic construct helped to facilitate the regeneration of cartilage as well as subchondral bone and promote them to integrate with host tissue.	[94]
	PLGA	BMSCs	The histological scores of neo-tissues were comparable with healthy joints tissue. Even though the mechanical characters of newly regenerated tissues were inferior to healthy joints tissue, yet no obvious differences were discovered.	[95]
	PEGS/MBG	n.a.	At week 12 of postimplantation, the PEGS/MBG biphasic scaffold successfully reconstructed well-integrated articular hyaline cartilage and subchondral bone, showing desirable regenerative capability.	[91]
	CAN-PAC hydrogel	n.a.	The results revealed newly formed hyaline-like cartilage and regenerated subchondral bone, suggesting that this hydrogel was capable of enhancing OCD repair in the rabbit model.	[97]
	Col I/Col II/HyA-Col I/Col II HA-Col I/HA	n.a.	This scaffold could lead to OCD regeneration with zonal organization, by forming new subchondral bone, cartilaginous layer, as well as intermediate tidemark in the rabbit model.	[100]
	Col I/Col II/HyA-Col I/HA-Col I/HA-EDAC/NHS	n.a.	At 12 months postimplantation, histological analysis of the scaffold group confirmed the OCD repair, by forming a well-structured subchondral trabecular bone and hyaline-like cartilage as well as the restoration of the anatomical tidemark.	[99]
	PCL/Col I/HA/TCP	BMSCs	Ex vivo results showed that the orientation of BMSCs was largely improved by OEM, and these BMSCs could penetrate this scaffold. After combining the BMSCs, the construct could successfully regenerate the OCD in the rabbit model.	[98]
Gradient scaffolds	nHA + ChS-NPs	n.a.	The results showed that the regeneration of hyaline-like cartilage and mineralization of subchondral bone was significantly enhanced by this gradient hydrogel. And the newly formed tissue could integrate well with the host.	[23]
	PCL+ HA/PCL	n.a.	Ex vivo studies showed that cell adhesion and proliferation were supported by SLS-derived gradient scaffolds, which displayed desirable biocompatibility. And this scaffold could promote the formation of articular cartilage through the early regeneration of subchondral bone. The newly regenerate parts could integrate with host tissues.	[105]
	PCL	hMSCs	The 3D scaffold with a gradient pattern of pore shapes was fabricated. Pores with squared shapes could mainly promote chondrogenesis, while pores with rhomboidal shapes could mainly improve osteogenic differentiation of cells in vitro.	[104]
	PACG-GelMA hydrogel-Mn^{2+}, PACG-GelMA hydrogel-bioglass	n.a.	The results showed this biohybrid gradient hydrogel with superior performance could accelerate the repair of cartilage and subchondral bone simultaneously in rat knees.	[106]
	PLGA/HAp	TGF-1, BMP-2	The continuous gradient of materials(PLGA/HAp) and growth factors(TGF-1, BMP-2) lead to complete bone ingrowth, with the overlying hyaline-like cartilage with high GAGs contents, the proper thickness, as well as integration with adjacent host tissues.	[107]

and BMP-2 into a bi-layered alginate-poly(lactic-co-glycolic acid) (PLGA) construct was designed and fabricated to enhance subchondral bone and chondral layer repair.[79] Alternatively, by using gene activated matrix approaches, researchers could combine two different lentiviral constructs in a woven composite scaffold to form a bilayer osteochondral implant. As one proof of concept study, the results demonstrated that this scaffold-mediated lentiviral delivery approach could resurface entire hip joints in dogs.[80] Besides, some features of being fragile as well as inadaptable for applications under mechanical stress result in the low structural integrity of these scaffolds, despite that they show appropriate stiffness.[81] The degradation rate of these scaffolds is dependent on variations of porous architecture apart from their composition and fabrication technologies. Their structures can be designed and tailored based on degradation kinetics. Although increased porosity can further impair the biomechanical characters of ceramic-based scaffolds, modifying them by infiltration or coating with biodegradable polymers could assist in solving this problem.[81]

Metallic Materials: Clinically widely used metallic materials include Mg alloys, stainless steel, titanium (Ti), Ti alloys, and cobalt-chrome alloys. When employed as orthopedic implants for subchondral bone, metallic materials are capable of withstanding high mechanical loading. As permanent metals, stainless steel, cobalt-chrome, Ti and its alloys are not degradable and also the possible formation of wear particles are their limitations.

Recently, Mg and Mg alloys seem to be considered as suitable biodegradable, cytocompatible and biocompatible (in certain cases) and osteopromotive metallic biomaterials, however their fast degradation in vivo at an early stage retards their performance of providing sufficient mechanical support and reduces their biocompatibility with hydrogen production.[82] Therefore, considering the strengths and weaknesses of Mg, researchers proposed a hybrid fixation system with parts composed of Mg and Ti, Ti alloys or stainless steel to maximize the biological benefits of Mg itself.[83] Tian et al. established a novel Mg/Ti hybrid fixation system to provide sufficient mechanical support.[84] Also, the underlying molecular mechanism of promoting calcitonin gene-related peptide (CGRP) mediated osteogenic differentiation induced by implant-derived Mg ions had been well elaborated.[85]

Monophasic Scaffolds: Monophasic scaffolds are usually referred to those with single-phase and homogeneity in architecture and composition, regardless of containing one material, or different materials. Namely, structure and porosity are spatially uniformly distributed throughout such kind of constructs. For monophasic scaffolds, different layers rely on different invading cells and the depth-dependent mechanical stimulus. It has been suggested that this kind of single-phase scaffolds could augment the recruitments and proliferation of chondrocytes as well as stem cells. Nevertheless, without addressing these prevalent differences of microenvironments from cartilage to underlying bone, there exist some intrinsic limitations of boosting the activities of site-specific cell differentiation and matrix depositions. Thus, this kind of scaffold has the lowest capability of regenerating respective layers in osteochondral lesions.

When implanted with monophasic scaffolds, the newly regenerated tissues are usually homogeneous and incomplete. Jeong et al. fabricated PCL scaffolds loaded with BMP-2, and at 5 weeks of postimplantation, cartilaginous tissues were generated significantly by seeding these bio-inspired constructs. However relative higher expressions of the markers from hypertrophic chondrocytes induced by this scaffold in vitro suggested that these seeded primary chondrocytes underwent endochondral ossification.[86] Chu et al. conducted a relatively long-term in vivo evaluation of monophasic PLA scaffolds in the rabbit OCD model. He found that one year after implantation, the defect area had mostly been filled up with regenerated cartilage-like tissue, yet with inadequate GAG in the regenerated subchondral bone layer.[87] The above findings indicate that single-phase scaffolds without inherent physical architecture cannot guide the regeneration of this complicated tissue, usually leading to a kind of newly regenerated tissue throughout its entirety.

Bi-Phasic and Multiphasic Scaffolds: Conventional monophasic scaffolds are incompetent of repairing deficient interfacial cartilage-to-bone tissue with anisotropic functional and structural characteristics. Plenty of bi-phasic as well as multiphasic scaffolds thus have been designed and tested. Biphasic scaffolds utilize up to two different material types or two respective architectural arrangements with the structural disparity in spite of being consisted of only one material. These designs could help to form two opposing regions with distinct structural and mechanical properties. Numerous soft polymers and hydrogels are used for the cartilaginous part because cartilage tissue is soft. For the subchondral bone layer, stiff matrices are desirable candidates as discussed above. At present, the synthesis and utilization of bi-phasic and multiphasic scaffolds have been investigated broadly both in vivo and ex vivo, and some of them have demonstrated promising results. With various special designs, some of the two-layered scaffolds are advancing to the clinical trial stage (Table 4).

Recently, Keller et al. summarized and updated the emerging concept of "smart implants" combining double compartments and triple-3D technology for regenerating well-founded cartilage in the field of regenerative nanomedicine.[88] In this concept, the triple-3D microenvironment included 1) BMSCs well-formed microtissues, 2) nanofibrous membrane functionalized with nanoreservoirs (e.g., BMP), 3) alginate/HA hydrogel. The double compartments were the mineralization capability of BMSCs microtissues on a nanofibrous membrane and the chondrogenic capability of BMSCs microtissues in alginate/HA hydrogels. They reported this bi-layered and hybrid bioimplant outfitted with well-organized 3D BMSCs for OCD repair.[89] In their study, BMSCs microtissues were developed to mimic embryonic endochondral development, and nanofibrous collagen membrane enhanced mineralization of subchondral bone, and alginate/HA hydrogel improved cartilage regeneration. This hybrid compartmented implant could facilitate subchondral bone regeneration by supporting the cartilage layer. More recently, the same group developed a double-layered implant for the treatment of OA.[90] The first compartment included NanoM1-BMP2 wound dressing for subchondral bone regeneration, and the second compartment was MSCs embedded into alginate/HA hydrogel for articular cartilage regeneration. Such a unique strategy demonstrated the following strengths, 1) the feasibility of treating OCD in large animal models, 2) the possibility of monitoring the healing process noninvasively, and 3) the overall safety in two animal models under preclinical standards of Good Laboratory Practices (GLP). These data indicated the preclinical safety of this

new technology based on the international regulatory guidelines and requirements for phase I clinical trials.[90]

Lin et al. produced a biphasic construct based on viscoelastic PEGylated poly(glycerol sebacate) (PEGS).[91] The lower layer was the mesoporous bioactive glass (MBG) scaffold, which improved osteogenesis. The upper region of the construct was low crosslinked PEGS. 12 weeks after implantation into rabbit knee joints, histological results illustrated hyaline cartilage formation on the top with low potential of hypertrophic indications and mineralization in the subchondral bone defect area. Meanwhile, the newly regenerated bone integrated well with adjacent tissues. A study from Liu et al. demonstrated biphasic scaffolds consisting of oriented nanofiber yarn-Col I/HA hybrid/TCP after seeded with BMSCs were press-fit into OCD of patellar grooves in rabbits.[92] The results demonstrated improved repair scores and a compressive modulus. The acellular Aragonite-HA bi-phasic scaffold[93] and the biomimetic alginate/bioglass-alginate/agarose (SA/BG-SA/AG) construct[94] were effective for regenerating hyaline-like articular cartilage as well as underlying subchondral bone and promoting them to integrate with host tissues. Duan et al. synthesized a graft consisted of PLGA for OCD restoration in rabbit knees.[95] In this graft, the chondral layer with the pore diameter of ≈100–200 µm was seeded with BMSCs and the pore diameter of the osseous layer was about 300–450 µm. Finally, these histological sores of neo-tissues repaired were comparable with that in healthy host tissues. Even though the mechanical characters of neo-tissues were inferior to the healthy host tissues, yet without obvious differences. This clearly shows the weakness of only using histological scores for evaluation, as mechanical properties are very important. Frenkel et al. fabricated a bilayer graft and the in vivo results illustrated that there existed Col II and GAG in the regenerated hyaline-like articulating surface. However, they observed an abnormal spatial dissociation of the expression of Col II and GAG, suggesting that regenerated hyaline-like cartilage was still unsatisfactory.[96] The above results revealed that it could not be decided conclusively whether these bi-phasic constructs exerted profound effects on the healing procedures or not. It should be noted that the biphasic design hindered its capability of generating structural microenvironments favorable for cartilage-bone interface regeneration that existed in native tissues.

To date, multifarious multi-layered scaffolds with particular designs regard to cartilage-bone interface have drawn great attention. These kinds of special designs could offer favorable environments for directing cells-to-cells as well as cells-to-matrices communications. Besides, they could be suitable for transferring the physical and chemical events from chondral layer to osseous layer, as this interface zone was exposed to shear forces during joint locomotion. Liao et al. designed and constructed a multiphasic scaffold with a seamless interfacial layer through biomimetic CAN-PAC-based hydrogel.[97] The hydrogel exhibited optional compositions, spatially controlled porosity, and excellent biomechanical characters. After implantation into OCD of a rabbit model, the in vivo results revealed newly formed translucent cartilage and subchondral bone, suggesting that this hydrogel could be an appealing option for enhancing OCD repair. A research group made use of oriented electrospinning fibrous membranes for developing Col-I/HA sponge triphasic scaffolds.[98] The results demonstrated that the oriented poly(ϵ-caprolactone) fibrous membrane (OEM) could enhance BMSCs orientation for reproducing environmental cues, particularly in the superficial zone of articular cartilage. After the combination of BMSCs, the construct successfully regenerated the OCD in the rabbit model. Additionally, collagen-based multi-layered scaffolds from Levingstone et al.[99,100] indicated that histological analysis confirmed the regeneration with a zonal organization, including hyaline-like cartilaginous layer, subchondral bone, and restoration of intermediate anatomical tidemark in rabbits in 12 months.

Gradient-Designed Scaffolds: With a more comprehensive understanding of biological sciences of osteochondral unit, the research paradigm of tissue-engineered constructs for OCD repair has shifted from original scaffold-free strategies to monolayer scaffold-based strategies, to biphasic, multiphasic, and even gradient scaffold-based strategies in recent years. As mentioned above, the natural osteochondral unit displays the gradient properties of biochemistry, biomechanics, structure, bioelectricity, and metastasis. Following these features and also with the advancement of biotechnology and biomaterial engineering, many researchers tried to simulate the complicated gradient architecture more appropriately by designing gradient scaffolds. Gradient-designed scaffolds mainly focused on the gradual variations of physical structure, composition, as well as the doses of numerous growth factors. For physical gradient scaffolds, the features of architecture and biomechanics might alter along the axis for simulating these transitions from soft cartilaginous tissues to calcified zones, and ultimately subchondral bone. Controllable stiffness of local environments could significantly affect and guide cell behavior.[101] Pore parameters of scaffolds can be applied in various gradient patterns to enhance site-specific differentiation. Previous research indicated that larger pore sizes could drastically improve chondrogenesis,[102] whereas the subchondral phase favors smaller pore sizes, due to the constraints from the need for mechanical stability.[103] Also, pore shapes could be an appealing strategy. Di Luca et al. synthesized 3D scaffolds with a gradient of pore shapes, and he found that pores with square shapes could mainly facilitate the chondrogenesis of stem cells, while pores with rhomboidal shapes could strengthen osteogenesis of residing cells in vitro.[104] Apart from the physical structure, strategy based on gradient components is also an option. Researchers utilized plenty of chondroinductive, as well as osteoinductive materials to form constructs with gradient compositions, significantly driving the related residing cell sources to proliferate and differentiate. A type of nHA/ChS-NPs gradient hydrogel from Radhakrishnan et al. demonstrated its desirable healing potential by regenerating hyaline cartilage and the mineralization of subchondral bone.[23] And both the newly formed cartilage and subchondral bone could integrate well with the host. SLS-derived gradient scaffolds from Du et al.[105] and hybrid gradient hydrogel from Gao et al.[106] showed favorable biocompatibility of supporting cells adhesion and proliferation in vitro, and those innovative gradient designs could boost the formation of hyaline cartilage through the acceleration of regenerating early subchondral bone and integrating tightly with host surrounding parts.

Lastly, the gradient doses of one single or a cluster of growth factors could be an effective strategy, which could directly guide cell behavior. However, the actual challenges relevant to this strategy lie on the fact that it is quite difficult to fabricate

Table 3. Summary of bioactive factors used in tissue-engineered methods for OCD repair. SDF: stromal cell-derived factor; TGF: transforming growth factor; IGF: insulin-like growth factor; BMP: bone morphogenic protein; PECE: poly(ϵ-caprolactone)–poly(ethylene glycol)–poly(ϵ-caprolactone); FPSCs: fat-pad-derived stem cells; OPF: oligo(poly(ethylene glycol) fumarate); PLGA: poly (lactic-*co*-glycolic acid);PCL-POEGMA: poly(ϵ-caprolactone)-poly(oligo (ethylene glycol) methacrylate).

Growth factors	Scaffolds	In vivo/in vitro	Cell types	Results	References
SDF-1	Collagen	In vivo	Rabbit BMSCs	Radially oriented collagen scaffold with SDF-1 improves OCD repair by promoting cell homing.	[114]
TGF-β1	PCEC hydrogel	In vivo	Rat BMSCs	Improved chondrogenesis and cartilage regeneration.	[78]
TGF-β3	ECM	In vitro	FPSCs	Promoted superior chondrogenesis of FPSCs.	[131]
TGF-β1+IGF-1	Laminin gel	In vivo	Rabbit BMSCs	Drastically facilitated hyaline-like cartilage formation with the improved cellular arrangement, clear tidemark zone, proteoglycan deposition, and subchondral bone formation.	[115]
TGF-β3+IGF-1	OPF hydrogel	In vivo	Rabbit BMSCs	Dual delivery might not synergistically boost the formation quality of engineered tissue; the delivery of IGF-1 alone positively affects OCD repair.	[132]
BMP-2	Alginate-PLGA	In vivo	Chondrocytes, BMSCs	Combinations of BMP-2 and cells did not lead to additive or synergistic effects. The identically efficient OCD repair was achieved with chondrocytes, stem cells, and BMP-2 treatments.	[133]
BMP-7	Porous tantalum	In vivo	Rabbit BMSCs	More new osteochondral tissue and bone formed at the interface site.	[134]
TGF-β3+BMP-2	PCL-POEGMA	In vitro	hMSCs	Brush-supported growth factors largely affected hMSCs osteochondral differentiation when the scaffolds were homogenously designed and tailored.	[116]
TGF-β1+BMP-2	Alginate–PLGA	In vivo	Rabbit BMSCs	Preserved cartilage integrity from 12 weeks up to at least 24 weeks.	[79]

scaffolds with one continuously and uniformly distributed pattern of growth factors. This strategy needs more advanced manufacturing technologies for precisely controlling these fabrication procedures. Mohan et al. synthesized scaffolds with continuous gradient TGF-1 and BMP-2, and the in vivo studies demonstrated complete bone ingrowth, with the overlying cartilaginous part with relatively high contents of GAG, proper thickness, as well as integration with adjacent host tissues.[107]

4.2.3. Bioactive Factors for Osteochondral Tissue Engineering

Basic research has already identified numerous bioactive factors that are essential for osteogenesis as well as chondrogenesis both in vivo and ex vivo.[109] During the process of skeletal and cartilage repair and regeneration, bioactive factors serve as biomolecular cues of enhancing cellular proliferation, migration, maturation, and differentiation.[110] Broadly speaking, bioactive factors include a series of factors: mineral ions such as Mg^{2+}, biological growth factors, intracellular signaling molecules such as receptors, kinases and transcription factors, and signaling mimetics derived from synthetic or natural compounds.

In a previous study by our group, a relatively high concentration of Mg ions ($2–10 \times 10^{-3}$ M) was shown to facilitate chondrogenesis and osteogenesis instead of adipogenesis of BMSCs and TDSCs under induction conditions respectively ex vivo.[111] By incorporating Mg particles with the 3D-printed PLGA/TCP constructs, Lai et al. found that the coupling osteogenic and angiogenic effects induced by the Mg-based composite scaffold could improve the formation of new bone as well as its quality.[112] Our human bodies are able to generate plenty of types of growth factors intrinsically. Many studies showed that these endogenic growth factors could play critical roles in facilitating both in vivo and in vitro osteochondral repair and regeneration.[113] Therefore, for the purpose of the ultimate regeneration of tissues or organs, a relatively higher dose of such kinds of exogenous growth factors could be locally delivered onto or into these scaffolds. These growth factors could be released in a rigid time-controlled manner to facilitate the healing process. Generally, they include insulin-like growth factors (IGFs), bone morphogenetic proteins (BMPs), basic fibroblast growth factors (b-FGFs), hedgehogs (Hh), Wnts, Sp7, SRY box-containing gene 9 (Sox 9) and transforming growth factors (TGFs). They have already revealed their significant roles in increasing the production of matrix molecules and anabolic cellular effects. Multiple growth factors incorporated with various scaffolds and cells are listed below in **Table 3**. Chen et al. designed and fabricated a novel radially oriented and random collagen-based scaffold, with channels arranged in horizontal and vertical directions. Combined with stromal cell-derived factor-1 (SDF-1) to facilitate cells homing of BMSCs, it could augment the OCD regeneration of rabbits 12 weeks after implantation.[114] Gugjoo et al. implanted laminin gels with MSCs, IGF-1 as well as TGF-β1 into OCD of rabbits. And the results indicated that by combining with growth factors, such as TGF-β1 and IGF-1, the formation of hyaline-like articular cartilage has been largely improved by facilitated proteoglycan deposition, cellular arrangement, and formation of subchondral bone as well as a clear tidemark.[115] Di Luca et al. used polymer brushes as selective linkers of TGF-β3 and BMP-2, which were covalently bound with a kind of poly(ethylene glycol) (PEG)-based brush-functionalized materials. When the materials were homogenously synthesized and tailored, these brush-supported

growth factors tremendously affected the osteochondral differentiation of hMSCs; while yet no influences were observed in the group of gradient materials.[116]

4.3. Emerging Innovative 3D-Bioprinting Technologies for OCD Repair and Regeneration

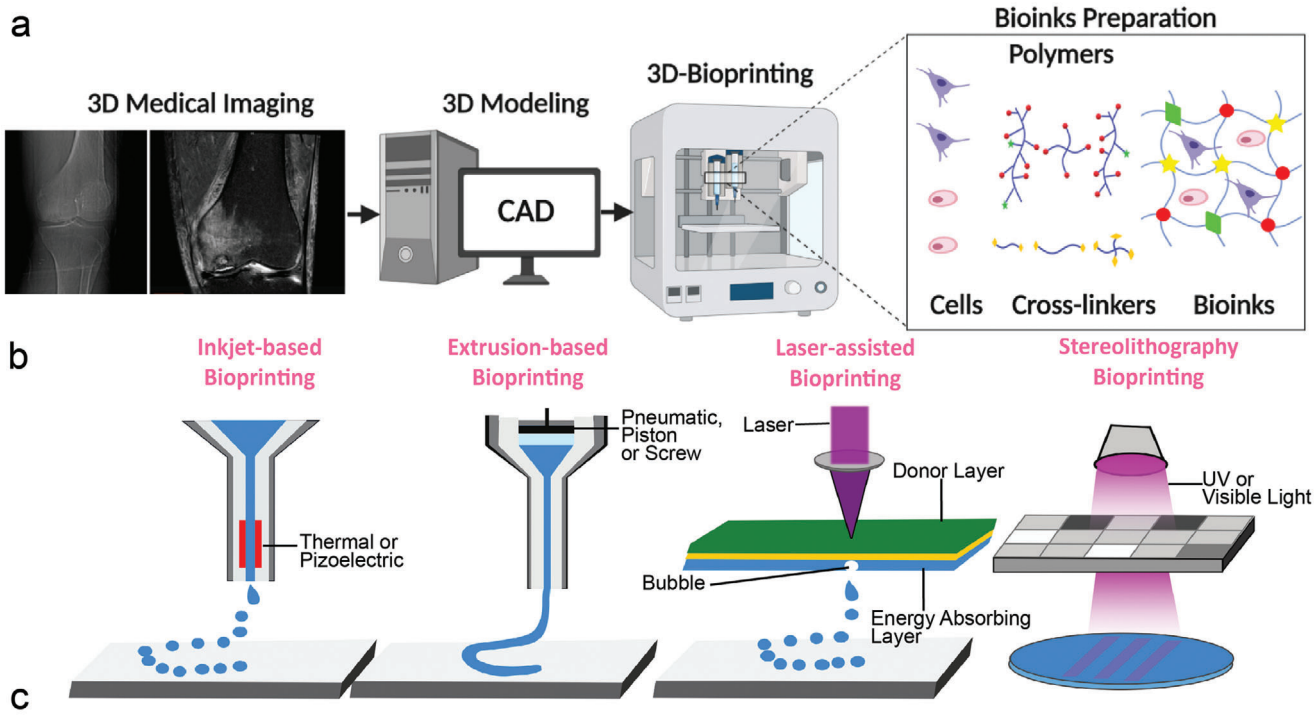
3D-Bioprinting is a form of additive manufacturing that may combine cells, growth factors, biomolecules within biocompatible materials such as "bioink," to precisely fabricate biological constructs layer-by-layer with various complicated hierarchical architectures and compositions which maximally imitate their native counterparts. As a consequence, 3D-bioprinting technologies have been vastly expanded and widely explored in the field of tissue engineering and regenerative biomaterials during the past several decades. This has enabled the rapid progress of the application of osteochondral scaffolds which resemble the multi-material composition and native architecture of natural tissue more precisely, compared with conventional fabrication approaches. At present, based on either inkjet, extrusion, acoustic, or laser technologies, various 3D-bioprinting methods (indicates as Figure 8b and Figure 8c) develop rapidly, such as vat-photopolymerization, inkjet printing method, extrusion-based method, powder-bed fusion method, and melt electrospinning writing (MEW). Although there are many different types, a typical bioprinting process (indicates as Figure 8a) includes a more-or-less standard series of steps: 1) 3D medical imaging (e.g., CT or MRI). These images can provide the exact dimensions of the tissue. 2) 3D modeling generated by AutoCAD software. Then this 3D solid model is tessellated (STL file format) and sliced into thin cross-sections to be printed by an appropriate printing machine. 3) Bioink: they usually combine living cells (e.g., tissue-specific cells or/and progenitor cells) and a cytocompatible base (e.g., gelatin, collagen, silk, hyaluronan, nanocellulose alginate, or their composites). These above mentioned cytocompatible biomaterials provide cells with nutrients to survive on and scaffolding to grow on. 4) 3D-bioprinting: through the accurate control of the printing program, the machine automatically deposits the bioinks in a layer-by-layer manner. Generally, the thickness of each layer is ≈0.5 mm. The bioink, as a highly viscous fluid, comes out of the nozzle. 5) Solidification: the layer is a viscous liquid at the start of deposition and then it solidifies to maintain the structure and shape of itself until more layers deposit continuously. The utilization of UV light, heat or some specific chemicals may conduce to the process of blending and solidification, also known as crosslinking.

Benefiting a lot from the rigid printing program of various emerging 3D-bioprinting technologies, osteochondral scaffolds with complex multi-material architectures can be fabricated precisely and repeatedly. Whereas conventional fabrication technologies are process-dependent rather than design led, and unable to acquire the accuracy requested by biomimetic osteochondral scaffolds with complex architecture and composition. For osteochondral applications, the selection of a suitable and reliable 3D-bioprinting system depends on processing conditions, material types, and scaffold strategies (e.g., cellular or acellular, direct implantation or ex vivo culture). Due to requiring high energy for material processing, some types of 3D-bioprinting technologies, for example, powder-bed fusion techniques, cannot be utilized for linking with biological materials (i.e., cells). As tissue-engineered strategies for OCD repair and regeneration need combine different tissue types, including bone, articular cartilage, and bone-cartilage interface together into a one scaffold, printing techniques such as inkjet-based, extrusion-based, and vat-photopolymerization predominate in the literature. More recently to remove the shear stress damage associated with passing cells through a needle during classical 3D-bioprinting approaches, soundwave patterning technology has been developed to enable cell localization to take place more gently.

Bittner et al. described the manufacture process and mechanical characteristics of dual (porosity/ceramic content) gradient scaffolds (PCL/HA) fabricated by a multi-material extrusion-based 3D-bioprinting system for OCD repair and regeneration.[118] These dual gradient scaffolds were designed and printed to better mimic the simultaneous gradients in the structure and mineralization of a healthy natural osteochondral unit. In this study, results demonstrated that this technology could better address the inherent complexity in heterogeneous tissues, providing a new angle for our further research. By extrusion-based 3D-bioprinting technique, Liu et al. developed a tri-layered osteochondral construct (GelMA/nHA hydrogels), which exhibited appropriate degradation rate, swelling ratio, mechanical properties, and excellent biocompatibility.[117] And the in vivo results of using the tri-layered scaffolds showed better integration with host tissues, smoother joint surface, and more expression of cartilage-specific extracellular matrix and collagen type II. Recently, Daly et al. reported an innovative biofabrication method which enables the engineering of structurally organized tissues by guiding the growth of cellular spheroids within arrays of inkjet-based 3D-bioprinted polymeric microchambers.[119] Based on multi-tool biofabrication, they could print anatomically accurate, human scale, osteochondral templates via fabricating this microchamber system on the top of a hypertrophic cartilage region designed to support endochondral bone formation. Then they kept the entire construct into a bioreactor for long-term culture. This work provided a scalable and versatile method to engineering structurally organized articular cartilage tissues for joint resurfacing applications. Despite significant scientific advancements achieved by 3D-bioprinting technologies, no regulatory bodies have approved the clinical translation of a 3D-bioprinted product to date. It should be noted that this research area is still relatively young, and we still believe 3D-bioprinting technologies will play a significant role in facilitating the repair and regeneration of OCD clinically.

5. Tissue-Engineered Osteochondral Grafts: From Bench to Bedside

It is well-known that before finally approved by regulatory bodies of the government, the production of tissue-engineered grafts involves numerous steps of R&D replications. R&D steps intend to guarantee the safety and efficacy of the implanted grafts from tissue-engineered strategies. Over the past decades, based on desirable preclinical results, some tissue-engineered grafts have advanced into the stage of clinical trials. Among them, some have been designed with bi-phase or multiphase and approved for



	Inkjet	Extrusion	Laser-assisted	Stereolithography
Cell density	Low, < 10^6 cells/ml	No limitation	Medium, < 10^8 cells/ml	No limitation
Ink viscosity	3.5-12 mPa/s	Up to 6 × 10^7 mPa/s	1-300 mPa/s	No limitation
Resolution	High	Medium	High	High
Print Speed	Fast	Low	Medium	Fast
Cost	Low	Medium	High	Low

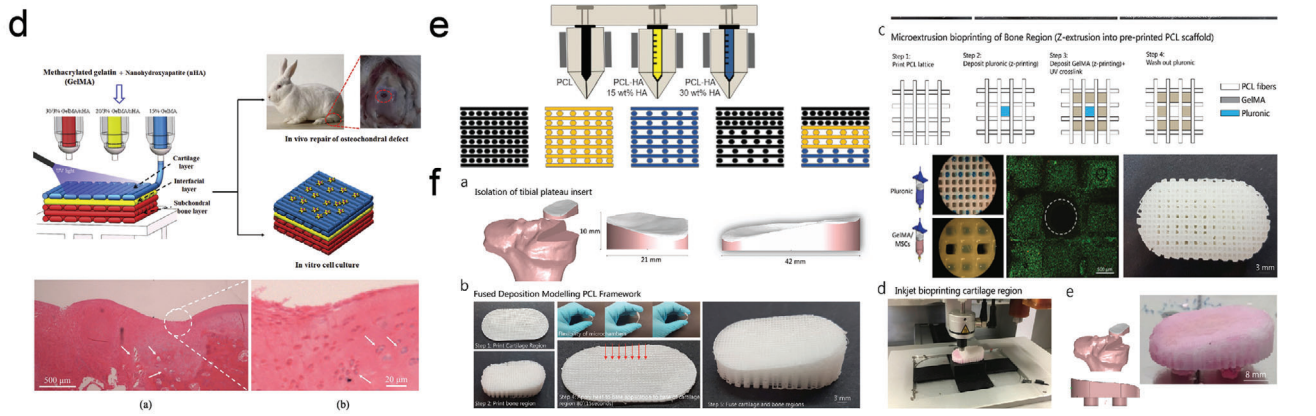


Figure 8. Innovative 3D-bioprinting technologies for OCD repair and regeneration. a) Graphical illustration of the processes involved in 3D-bioprinting technology, including 3D medical imaging, 3D modeling, bioinks preparation, and 3D-bioprinting (created with BioRender.com). b) Different strategies and methods of 3D-bioprinting technologies, such as inkjet-based bioprinting, extrusion-based bioprinting, laser-assisted bioprinting, and stereolithography bioprinting. c) Comparison of 3D-bioprinting modalities in several aspects (e.g., cell density, ink viscosity, resolution, print speed, and cost). d) 3D-bioprinting of a multilayered biomimetic scaffold (GelMA/nHA) for OCD repair, and representative results of H&E staining showed the bone regeneration and lacunae in the regenerated cartilage in the defects by using this tri-layered scaffold. Reproduced with permission.[117] Copyright 2019, Elsevier. e) Fabrication of 3D printed vertical uniform and gradient PCL-HA scaffolds for OCD repair. Reproduced with permission.[118] Copyright 2019, Elsevier. f) Multi-tool bioprinting of osteochondral implants. Reproduced with permission.[119] Copyright 2019, Elsevier.

Table 4. Current commercially available tissue-engineered grafts for the repair and regeneration of OCD. n.d.: not defined; GAG: glycosaminoglycans; CaPs: calcium phosphates; PLGA: poly(lactic-*co*-glycolic) acid; HA: hydroxyapatite; PLA: polylactic acid; PGA: polyglycolic acid.

Brand of products	Company	Material composition	Phase	Bioresorbable	Status
Agili-C	CartiHeal	Aragonite with strontium and magnesium (bone), modified aragonite and hyaluronic acid (cartilage)	Biphasic	Yes	Approved in EU; clinical trials in USA (NCT03299959)
BioMatrix CRD	Kensey Nash	Bovine collagen, β-TCP, PLA	Biphasic	Yes	Approved in the EU
ChondroMimetic	TiGenix NV	Col II/chondroitin sulfate, Col I/GAG with CaPs	Biphasic	Yes	Approved in the EU
MaioRegen	Med & Care	Type I equine collagen and magnesium-enriched HA	Multiphasic	Yes	Approved in the EU
Chondrofix Osteochondral Allograft	Zimmer Biomet	Decellularized human subchondral bone and hyaline cartilage	Biphasic	Yes	Premarket approval not required in the USA
TruFit Plug	Smith & Nephew	PLGA/PGA, calcium sulfate	Biphasic	Yes	Approved in the EU limited approval in the USA
CartiFill	Sewon Cellontech	Modified porcine Col I (stabilized with removal of telopeptides).	Monophasic	Yes	Clinical trials (NCT02685917)
OsseoFit Plug	Kensey Nash	Col I, and 80% β-TCP+20% PLA	Biphasic	Yes	
Hyalonect	Anika Therapeutics	Hyaluronic acid	Monophasic	Yes	
Collagraft	Nuecoll Inc.	Bovine Col I with granules of HA and β-TCP	Multiphasic	Yes	
BST-CarGel	Piramal life Sciences	Polysaccharide chitosan gel, glycerophosphate, and autologous blood	Monophasic	n.d.	Approved in the EU
Bioseed-C	Biotissue	PLA/PGA		n.d.	

use commercially to repair or/and regenerate cartilage and subchondral bone, mostly in the European Union. Generally, there are two commonly used strategies, one is to seed the autologous chondrocytes on the top of these scaffolds, allowing for chondrocyte-scaffold implantation, and the other is to integrate or combine two different scaffolds together for mimicking the cartilage layer and subchondral layer, aiming to assure OCD repair and regeneration. **Table 4** summarizes some commercially available grafts, of which composition varies vastly. Such as different types of collagenase, PLGA, PGA, PLA, β-TCP, aragonite, and chondroitin sulfate. Among them, CartiFill, Hyalonect, and BST-CarGel constructs are monophasic; Agili-C, BioMatrix CRD, ChondroMimetic, Chondrofix Osteochondral Allograft, TruFit Plug, and OsseoFit Plug are biphasic; And MaioRegen and Collagraft are multiphasic. Up to now, Agili-C, BioMatrix CRD, ChondroMimetic, MaioRegen, TruFit Plug, and BST-CarGel have been clinically approved as therapeutics in the European Union.

6. Current Challenges and Future Directions

Basically, the concept of "osteochondral unit repair and regeneration" has already been around for a long period. As nothing clinically available is perfect yet, it still attracts wide attention both preclinically and clinically, especially in the area of tissue engineering and regenerative biomaterials. Now researchers design and fabricate many materials and scaffolds to repair challenging OCD in vivo. Some of them are at the stage of preclinical studies using large animal models, and some are currently in clinical trials. Despite a steady change and improvement in the aspect of scaffold design and fabrication, the material type selection, components, and structure optimization, fabrication approaches, and procedures have not been fully investigated and elucidated. In particular, producing a scaffold that enables cell infiltration, while providing mechanical stability during the early stage of healing is challenging. When developing an osteochondral implant, issues such as aligning the tidemark between the bone and the cartilage still need to be addressed. In addition, the effects of scaffold properties (e.g., morphology, chemical composition, structure, and so on) on cell fate, and the relation between defect repair and scaffold properties or other external cues (e.g., biomechanical, chemical stimulus) are not fully understood. For some "scaffold plus bioactive factors" strategies, the dose spectrum and release profile of bioactive factors should be further optimized and validated in a methodologically rigorous fashion. Furthermore, methods to enhance lateral integration to the surrounding cartilage and techniques to reduce or prevent delamination from the underlying bone need to be developed.

Apart from scaffolds, cell sources are also key issues for valid OCD treatment by tissue-engineered strategies. The research interest of the majority of research groups has a noticeable shift from using tissue-specific cells at the very beginning to using progenitor cells. Although both the usage of tissue-specific cells and progenitor cells have their own pros and cons, some researchers firmly believe that the progenitor cells, specifically MSCs with high proliferative potential, outweigh their negative attributes.[120] Chondrogenic and osteogenic differentiation of MSCs is extensively studied and well established for OCD repair. Nevertheless, the mechanism underlying the process remains unclear. Up to now, no tissue-engineered cartilage with equivalent properties as native cartilage has been developed. Before translation to the clinic with effective and feasible treatment, issues regarding the MSCs source heterogeneity, isolation approaches and differentiation procedures need to be tackled.[121] Furthermore, aging,[122] serial passaging,[123] and

donor parameters[124] can influence MSCs' regenerative potential. The formation of ossified hypertrophic cartilage or fibrocartilage also needs to be avoided.

Additionally, the selection of appropriate animal models plays a vital role in guaranteeing successful clinical translation. For a proof-of-concept study or the evaluation of degradation and biosafety, a small animal or rodent model is highly recommended before validation by using large animal model(s). However for the next stage, considering the differences in natural osteochondral healing potential, matrix structure and composition, gross morphology, and technical complexity of generating identical defects with uniform size and location, the utilization of small animals such as mice, rats, and rabbits might be inappropriate,[125] especially for evaluating tissue-engineered products. Therefore, from ultimate translational perspectives, we shall better develop large animal models, e.g., dogs, pigs, sheep, goats, horses, and emus, in spite of the high cost involved. In particular, the differences of morphological structure between species should be considered, when using animal models for evaluating the function or pathology of human articular cartilage. Studies have suggested a categorization into either a column/fiber-based or a leaf-based arrangement.[126] With respect to gross morphology, pig has the closest collagen structure to human articular cartilage.

Besides, as the replacement of animal models, 3D in vitro tissue models attract more and more attention in academia in recent years, due to their capabilities of mimicking the structure and function of native tissues via precise deposition and assembly of materials and cells. 3D in vitro tissue models can provide the spatiotemporal control over cell-cell as well as cell-extracellular matrix communication, facilitating the regeneration of tissue-like well-organized structures. Therefore, 3D in vitro tissue models offer the opportunity to model biological processes, such as tissue development in various diseases or tissue regeneration.[127]

Finally, other critical challenges are good manufacturing practices (GMP) and regulatory issues before and after clinical approval.[128] During the whole process of clinical translation, scientific and social aspects would face all-round problems, some of which could even hamper or interrupt the normal R&D schedule. The academia, industry, hospitals, and regulatory bodies (e.g., FDA, EMA, and NMPA, previously known as CFDA) are in great need of integrative collaboration, conducing more valuable and potential findings and inventions for translating into safe and efficacious commercial products, ultimately benefiting OCD patients and the society. In brief, currently, some preclinical and clinical results of osteochondral tissue engineering are preliminary and the effects of engineered cells and material characters exerted on the results of repair or regeneration over a long period should be validated. Optimistically, the application of newly fabricated 3D tissue-engineered bio-implants for the repair of clinical osteochondral lesions could be expected shortly within years.

7. Conclusions

Recent clinical treatment strategies for OCD repair have limited success in terms of keeping the structure and function of the regenerated tissues over a long period. Microfracture, ACI, and MACI are usually associated with fibrocartilage formation instead of regeneration of articular cartilage, thereby impairing joint normal functions. As allograft may carry the risk of disease transmission, the innovative tissue-engineered strategies are now emerging. Osteochondral repair and regeneration belong to an interdisciplinary field, thus integrative approaches should be collaboratively employed. With continuous advances in the basic biological science in joint osteochondral unit, we have a more comprehensive understanding of how to establish a relevant disease model and the assessment of repair and regeneration. Concerning the treatment strategies, tremendous advances have been accomplished in the area of the design and fabrication of innovative multiphasic or gradient scaffolds, engineered cells, e.g., genetically modified MSCs, as well as bioactive factors and relevant drugs, thus contributing greatly to their clinical translation. In spite of existing obstacles in our preclinical and clinical work, tissue-engineered strategies still represent the main focus of future directions, especially in OCD repair and regeneration.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

clinical applications, osteochondral defect repair and regeneration, scaffolds, tissue-engineered strategies

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