Osteochondral tissue engineering-based subchondral bone plate repair (Review)

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Abstract. Osteochondral defects are a series of pathological changes from the chondral surface to the deeper trabecular bone caused by trauma or degenerative changes; they typically induce serious joint dysfunction. Over the past few decades, various techniques have been attempted to repair these defects. Tissue-engineered osteochondral grafts (TEOGs) with sophisticated architecture have been extensively explored for osteochondral regeneration. However, controversies persist regarding standards for clinical application of TEOGs. The present review focused on the design of TEOGs, emphasizing their capacity to repair the subchondral bone plate (SBP). The effect of animal models on techniques to repair osteochondral defects was also reviewed. To improve the evaluation of SBP regeneration, four typical histological characteristics (abnormal height, uneven surface, poor integration and loose internal structure) are summarized based on cases of unsatisfactory SBP regeneration. Incorporating mesenchymal stem cells with appropriate growth factors into trilayer or multilayer tissue-engineered scaffolds is a promising strategy to avoid unsatisfactory SBP regeneration. Large animal models are recommended for translation to the clinic and there is a need to establish detailed and comprehensive osteochondral defect models in the future.

Contents

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1. Introduction

Articular cartilage is a durable tissue capable of load transmission and articulation of joints. It is primarily composed of hyaline cartilage, which provides low friction and shock absorption in synovial joints (1). Hyaline cartilage benefits from the presence of collagen (Col)-II-based dense extracellular matrix (ECM), which provides resistance against complex loading patterns including compression, shear and friction (2). Together, the upper articular cartilage, subchondral bone plate (SBP) and underlying trabecular bone create an intact structural and functional osteochondral unit (Fig. 1) (3).

There are a number of ways to evaluate osteochondral regeneration, including the International Cartilage Repair Society, Wakitani and O'Driscoll scoring systems (4,5), all of which place greater emphasis on cartilage regeneration. However, the evaluation of subchondral bone repair has been less thoroughly investigated (4,5). Subchondral bone consists of two anatomical entities, the SBP and trabecular bone. The SBP is a thin cortical bone plate with a permeable porous structure located beneath the calcified cartilage (3). The cancellous bone structure within the trabecular bone is located beneath the SBP. The porous structure in subchondral bone provides nutritional support to the osteochondral unit through numerous nerves and vessels. Currently, osteoarthritis-related subchondral bone damage is a highly prevalent pathological condition. The supply of blood and nutrients is limited after injury and osteoarthritis has a major adverse impact on the preservation of osteochondral unit function. Thus, subchondral bone regeneration requires further recognition and investigation.

Osteochondral defects are a considerable symptomatic and functional burden to patients and lead to decreased quality of life. In particular, younger patients lack long-term treatment solutions, may require numerous surgeries and may experience unwanted effects throughout their lives because of the inevitable progression of osteoarthritis (6). Various clinical strategies and techniques have been developed to improve

repair efficacy. These can be classified into bone marrow-stimulating techniques (drilling, abrasion and microfracture), direct chondral replacement (mosaicplasty and osteochondral allograft transplantation), cell culture-based treatment [autologous chondrocyte implantation (ACI), matrix-induced autologous chondrocyte implantation (MACI)] and total joint arthroplasty (7,8) (Fig. 2B). Total joint arthroplasty is considered to be the most useful strategy for both cartilage and bone repair (9). Although the aforementioned treatments are common clinical procedures, obvious drawbacks and limitations remain for long-term joint preservation (7,8).

Microfracture treatment involves drilling tiny holes that permit the cartilage and subchondral bone to take blood and bone marrow components from the underlying tissues (7). This induces cartilage and bone regeneration/remodeling following the introduction of stem cells and biomolecules at the defect site (9). However, the procedure may lead to the formation of fibrocartilage, which has inferior biofunctional and mechanical properties compared with hyaline cartilage (10). Similarly, use of ACI and MACI commonly results in the production of fibrocartilage rather than hyaline cartilage, which hinders the joint from recovering normal function (3). For 20 years, articular cartilage has been successfully regenerated using ACI, with positive surgical results. Nevertheless, drawbacks remain, including a shortage of chondrocyte sources, long chondrocyte harvesting time, difficulty of chondrocyte solution fixation, periosteal hypertrophy and ablation (10), as well as limited effectiveness in aged patients. Also, osteochondral lesions require simultaneous healing of the subchondral bone, which ACI cannot repair (8,11). Allografts have numerous disadvantages, including limited tissue supply, immune rejection, insufficient host-graft integration, low cell viability due to graft storage and potential for disease transmission (12). Osteochondral autografts may be able to overcome the shortcomings of allografts; however, insufficient integration and a deficient tissue source, additional surgery and donor site morbidity limit extensive clinical application (10). For cell culture-based treatment, technical disadvantages such as poor preparation of cell sources, donor site morbidity, inadequate time for cell expansion, poor retention and de-differentiation of cultured cells, decline of intrinsic activity and functionality of senescent cells, as well as inconsistent quality control for large-scale cell production may block progression of mesenchymal stem cell or chondrocyte transplantation (12). Consequently, more sophisticated treatments that take different architecture and regeneration potential into account, namely structurally and functionally biomimetic tissue-engineered strategies, have emerged as promising options for the simultaneous regeneration of subchondral bone and cartilage lesions (Table I).

Significant progress has been made in the field of tissue engineering over the last two decades with numerous studies demonstrating the construction of *de novo* cartilage and bone both *in vitro* and *in vivo* (13). The three important components involved in the tissue engineering of osteochondral grafts are biomaterials, cells and growth factors (Fig. 2A). Currently, very few studies focus specifically on the reconstruction of the SBP during osteochondral regeneration. However, a growing body of research suggests that creating the right microenvironment using tissue engineering approaches and further stimulating SBP regeneration are essential for regenerating the entire osteochondral unit (14-16). Incorporating the three components aforementioned into the process should provide the conditions required to establish the ideal microenvironment and heal the osteochondral lesion.

Achieving satisfactory osteochondral regeneration in small animal models does not guarantee success in large animal models. As both the joint size and the burden increase, osteochondral regeneration must meet the mechanical demands of the new tissues. SBP regeneration, an important indicator of recovery from osteochondral stiffness, may predict the performance of tissue-engineered osteochondral grafts during the translational process. However, very few studies have examined SBP regeneration and almost all have only evaluated the methodology descriptively.

The present study reviewed *in vivo* osteochondral repair with tissue-engineered osteochondral grafts. This includes i) summarizing the design of tissue-engineered osteochondral grafts to demonstrate their role in promoting osteochondral regeneration in terms of cells, scaffolds and growth factors, with a focus on the regeneration of SBPs. ii) Discussing both normal histology and SBP reconstruction. iii) Summarizing current limitations and challenges associated with SBP regeneration during osteochondral defect repair to guide future translational research and accelerate the 'bench to bedside' process.

2. Evaluating SBP regeneration

Defining satisfactory SBP repair. Histological assessment is commonly employed to evaluate osteochondral tissue regeneration. The regenerated tissue, including the SBP, is compared with corresponding host tissue to further assess repair quality. In addition, microcomputed tomography (μ-CT) is an acknowledged 'gold standard' for SBP evaluation, with its global view of bone architecture providing a detailed assessment. However, criteria for satisfactory SBP regeneration have not been established. Typical SBPs share common features, such as a suitable height, a flat and smooth surface, good interface integration and dense texture (17); SBP regeneration is not considered successful unless all four features are present. Other literature has summarized the histological scores of SBP repair by scoring abnormal activity following the repair (18). The standard for scoring abnormal activity is that a SBP with a low score has good mobility and good mechanical properties. However, a low score for repaired SBP is not representative (18), because few studies have used this scoring system and it cannot quantify performance appropriately.

Typical indicators of insufficient/unsatisfactory SBP repair. In contrast to the aforementioned four criteria for adequate SBP regeneration, the four typical indicators of a failed repair are abnormal height, uneven surface, poor integration and loose lacunae structure (Fig. 3). When three or more indicators are present, defects filled with fibrous tissue have been reported, along with either tissue resembling collapsed cartilage or scarcely any cartilage regeneration. When one or two indicators were found in the repaired SBP, regenerated hyaline cartilage with sufficient integration with surrounding host cartilage was observed (Fig. 3). while a plate with one or two

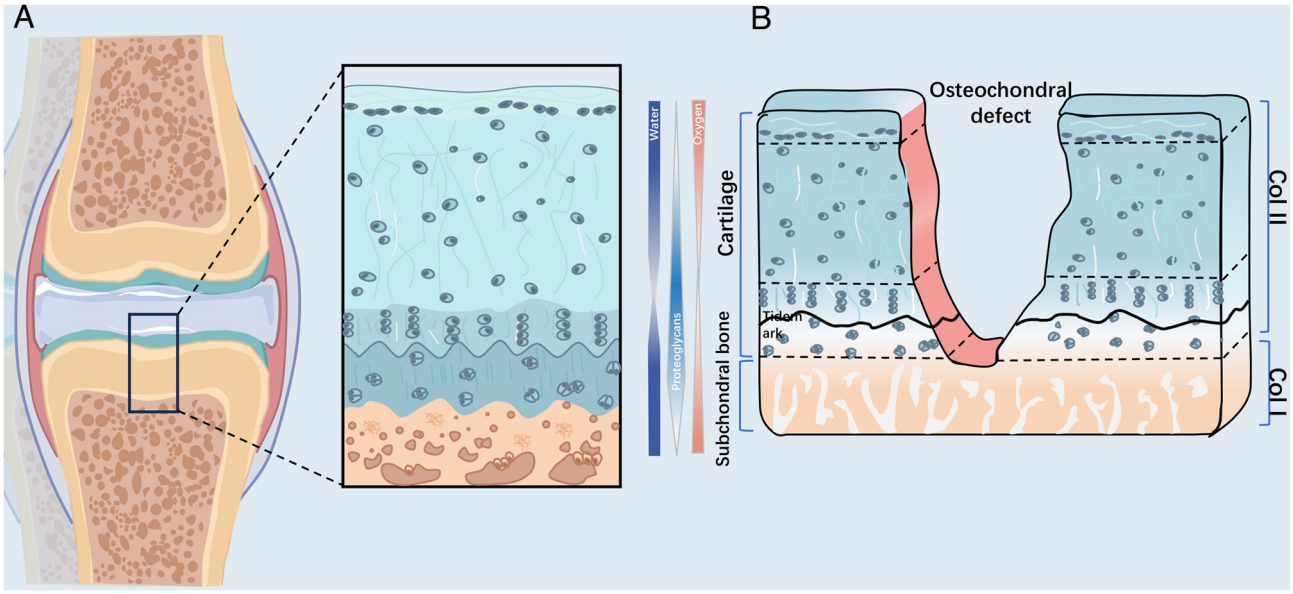

Figure 1. Schematic of osteochondral unit structure and osteochondral defect definition. (A) The osteochondral unit is composed with articular cartilage and subchondral bone, (B) Schematic diagram of defect is exhibited and the different type of collagens are shown in the figure. As the depth of the defect deepens, the changes in the contents of water, oxygen and proteoglycan are also shown in the figure. Col, collagen.

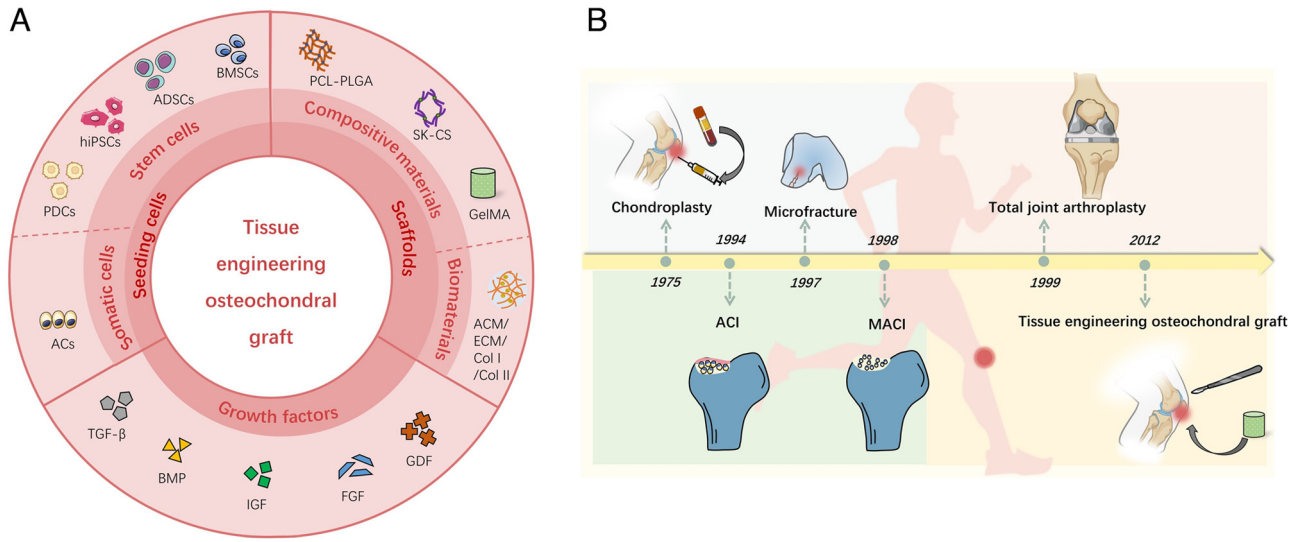

Figure 2. Overview of composition of tissue engineering osteochondral graft and clinical treatments for osteochondral lesion. (A) Graphical illustration of tissue engineering graft, including seeding cells, scaffolds and growth factors. Moreover, the cells are divided into two types, stem cells and somatic cells. Scaffolds are also divided into composite scaffolds and biomaterial scaffolds. (B) Summary of the development history of clinically utilized methods for the repair or/and regeneration of osteochondral lesions. PDCs, periosteum-derived cells; hiPSCs, human-induced pluripotent stem cells; ADSCs, adipose-derived stem cells; BMSCs, bone marrow stem cells; ACs, articular cells; PCL-PLGA, polycaprolactone-poly lactic-co-glycolic acid; SK-CS, silk-chitosan; GelMA, gelatin methacryloyl; ACM, acellular cartilage matrix; ECM, extracellular matrix; GDF, growth differentiation factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; BMP, bone morphogenetic protein; TGF, transforming growth factor.

indicators of failure was defined as a moderate repair. Among these four indicators of failed repair, abnormal height was the most frequent. Moreover, when abnormal height was apparent, it was often accompanied by two or three other indicators, which suggests that abnormal height has a clear influence on SBP repair and that SBP may be required to reach an appropriate height during regeneration. These four histological findings provide a path to understanding the underlying causes of inadequate osteochondral regeneration from the perspective of the SBP.

3. Cell selection for tissue-engineered osteochondral grafts

Cells with specific differentiation and proliferation capacities are used in osteochondral tissue engineering. Bone marrow-derived mesenchymal stem cells (BMSCs) are the most common seed cells used in both monolayer and multilayer grafts. BMSCs can differentiate into various cell types, including osteocytes and chondrocytes, which then form osteochondral units (19). Adipose-derived stem cells (ADSCs) are another important source of stem cells for osteochondral

Table I. Clinical studies for cartilage/subchondral repair.

First author/s, year	Clinical strategy	Basic process	Advantages	Limitations	Phase	Corresponding accession number	(Refs.)
Kwon *et al*, 2019; Maia *et al*, 2018	Microfracture	Creating small holes in the subchondral bone and stimulating bone marrow	Minimally invasive	Cause the formation of fibrocartilage	III	NCT03696394	(7,8)
Yang *et al*, 2017	ACI	Implant the patient's autologous chondrocytes, harvested from healthy patients, into chondral lesions	Enhance the probability of hyaline-like cartilage compared with microfracture	Long chondrocyte harvesting time, periosteal hypertrophy and ablation	III	NCT01947374	(10)
Maia *et al*, 2018; Campos *et al*, 2018	MACI	Autologous chondrocytes placed onto the surface of a purified film and then the same implantation	More sufficient source of autologous chondrocytes than ACI	Inevitable fibrocartilage formation and poor maintenance for long-term evaluation	III	NCT00719576	(8,11)
Kim *et al*, 2020	Osteochondral allograft transplantation	Osteochondral tissue from a donor is transplanted into osteochondral defect	Suitable for large defects, avoids donor site morbidity	Immune rejection, poor host-graft integration and risk of disease transmission	III	NCT04236492	(12)
Zhao *et al*, 2019	Tissue engineering graft	Combines cells, biomaterials and growth factors to promote the regeneration of both cartilage and bone	Offer the prospect of the both functional and structural regeneration of osteochondral defect	Lack of actual clinical application, challenge in scaffold integration	II	NCT06163573	(13)

ACI, autologous chondrocyte implantation; MACI, matrix-induced autologous chondrocyte implantation.

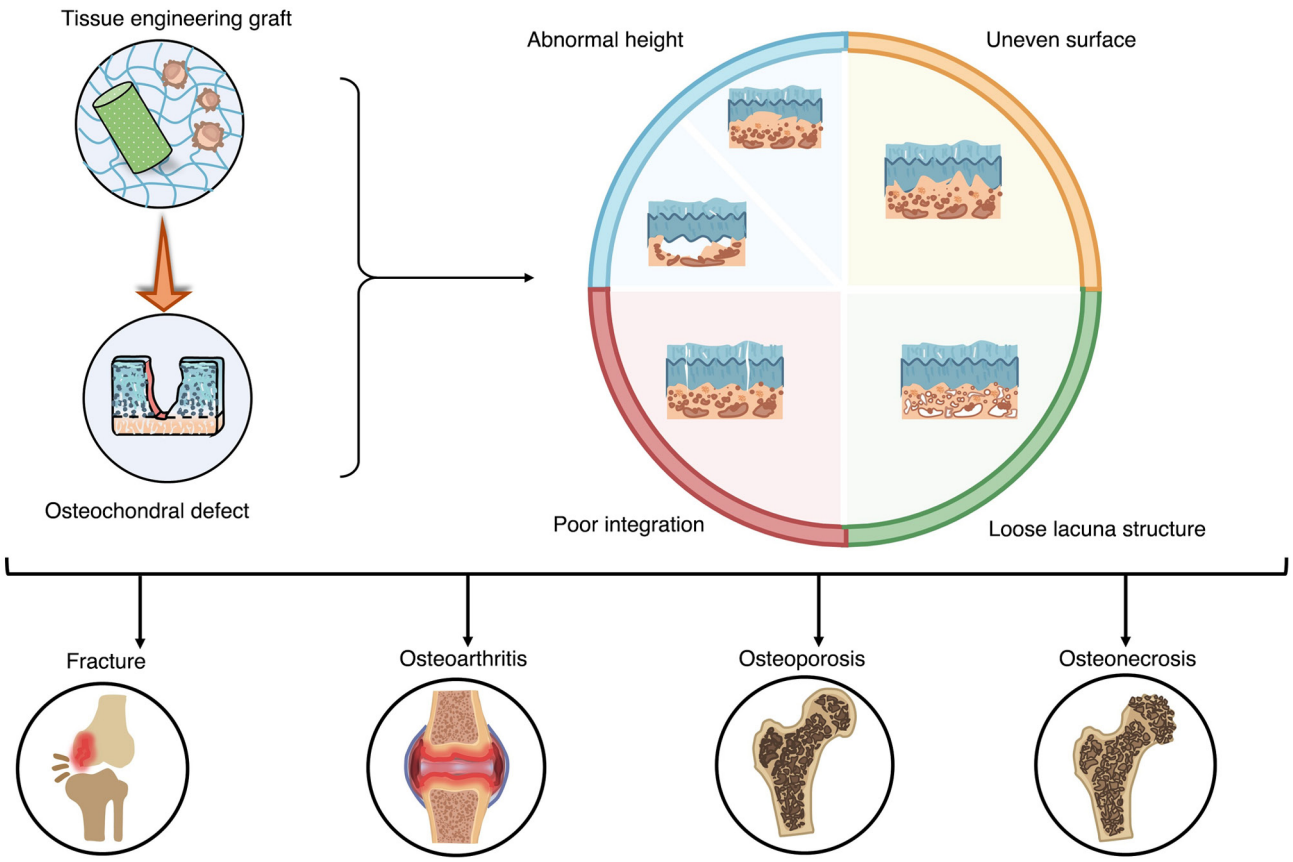

Figure 3. Natural history and typical schematic of unsatisfied subchondral bone plate regeneration in osteochondral defect repair. The following poor repair outcomes may occur after implantation of the graft into the defect, including abnormal height, uneven surface, poor integration and loose subchondral bone structure. These poor repair outcomes may eventually lead to serious problems, including fractures, osteochondritis, osteoporosis and bone necrosis. ACI, autologous chondrocyte implantation; MACI, matrix-induced autologous chondrocyte implantation.

transplants. Along with chondrogenic potential, ADSCs have demonstrated extraordinary potential for invasion, migration and proliferation (19) and provide a suitable microenvironment for osteochondral regeneration. In addition to stem cells, somatic cells are also used. Chondrocytes are the primary option as they promote chondral portion regeneration in osteochondral grafts (8,11,14) (Fig. 4). Recently, genetically modified cells were introduced in osteochondral tissue engineering (13,20). These cells upregulate gene expression, thus promoting cell proliferation or differentiation.

Cells for cartilage repair. One of the most significant functions of seeding cells is to promote the regeneration of cartilage. Research has indicated that BMSCs have a distinct advantage over ADSCs in cartilage repair because of their greater capacity for cartilage differentiation (21,22). Compared with chondrocytes, BMSCs can produce ECM with higher mechanical strength (21,22). However, the aforementioned advantages do not consider the effect of the graft itself. When widely applied to osteochondral grafts, BMSCs occasionally result in the formation of fibrocartilage instead of hyaline cartilage (23). This unsatisfactory repair should not be attributed to the cell type since the same limited cell types are used in bilayer and trilayer grafts. The bilayer graft, which is usually composed of chondrogenic and osteogenic layers, resembles a normal osteochondral unit, with the top layer encouraging cartilage repair. BMSCs can be inserted into the cartilage layer to restore the ECM. Alternatively, chondrocytes can be implanted in the chondrogenic layer to improve chondrogenesis because of their outstanding ability to synthesize cartilage matrix (22). Similarly, BMSCs and chondrocytes are utilized in trilayer or multilayer grafts for cartilage regeneration. Under these circumstances, the neocartilage is always reconstructed with firm and compact cell lineage. BMSCs and chondrocytes have significant potential to promote cartilage regeneration in both bilayer and multilayer grafts.

Cells for trabecular bone repair. Although bone regeneration appears easier than cartilage regeneration over an extended period, bone tissue reconstruction must be relatively rapid and provide early support for superficial chondral tissue regeneration (24,25). For this purpose, cells were incorporated into tissue-engineered grafts to adjust the speed of bone reconstruction. BMSCs have been widely used in this process because of their self-renewal and differentiation capacities (1). Other studies have reported the use of periosteum-derived progenitor cells, which can induce the formation of subchondral bone. These cells can also stimulate differentiation of mineralized bone tissue and bone marrow (Fig. 4) (19,26). Furthermore, osteoblasts in the underlying layer of bone can promote the growth, differentiation and infiltration of new bone (27) and are mainly responsible for the synthesis, secretion and mineralization of bone matrix (19). When cultured on osteochondral tissue

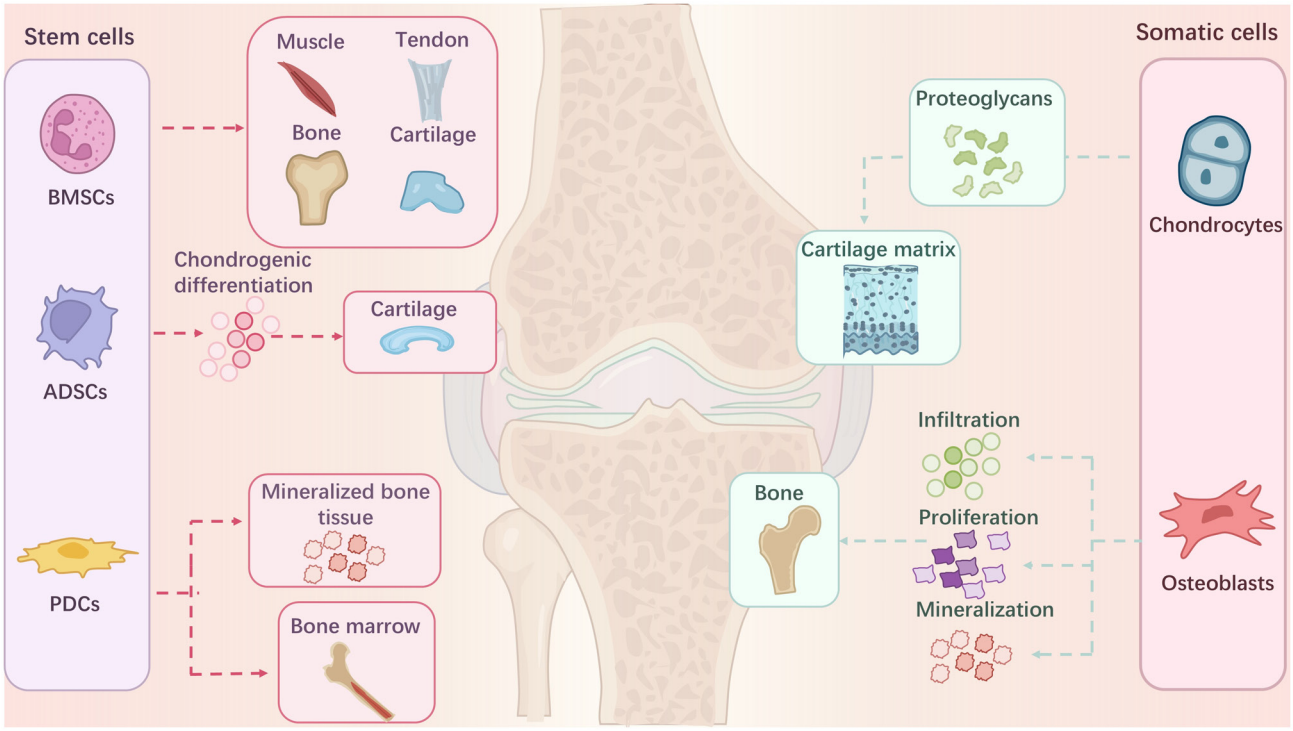

Figure 4. Diagram of seeding cells for tissue engineering osteochondral strategy. A schematic listing of the most commonly used seeding cell types, divided into stem cells and somatic cells, briefly simulates how they promote cartilage and/or subchondral bone regeneration in tissue engineered grafts. BMSCs, bone marrow stem cells; ADSCs, adipose-derived stem cells; PDCs, periosteum-derived cells.

grafts, the regenerated bone tissue stimulated by osteoblasts is firm with good mechanical properties.

Cells for SBP repair. No existing literatures discuss the function of a certain cell to promote the regeneration of SBP exclusively, as SBP is often regarded as part of subchondral bone. Previous studies viewed that cells which can regenerate bone certainly can repair SBP (28-30). However, the SBP can be satisfactorily rebuilt when specific cell types are incorporated into a well-integrated graft that contains growth factors. It has been found in previous studies that BMSCs embedded in bi-layer or tri-layer grafts can obtain flat and compact SBP regeneration (28-30), which no is doubt related to the polypotent and powerful differentiation potential of BMSCs (22). Others have shown that ADSCs can achieve good SBP regeneration after implantation in bi-layer grafts (28-30). Besides these pluripotent stem cells, chondrocytes may have the similar function to repair SBP (24). A flat and neat SBP structure well integrated with surrounding tissues can be detected. This is also closely related to the chondrocyte's function of secretion, synthesis and induction of calcified cartilage matrix (22). Previous research revealed that the regeneration of the SBP could not be independently stimulated by any one cell type, including stem cells or somatic cells. In general, SBP, cortical bone above trabecular bone, can usually achieve ideal repair results right after subchondral trabecular bone is well repaired (Table II).

Subsequent research revealed that the biocompatibility of the three-layer/multi-layer graft with the typical osteochondral unit structure gave it unique benefits in the repair of osteochondral lesions. Three-layer graft, distinguished from the two-layer graft, adds a transition layer between the chondrogenesis layer and the osteogenesis layer. It was expected that the SBP would undergo good regeneration when the cells were grown into the transition layer (30). Some stem cells with osteogenic potential, such as BMSCs and ADSCs, are commonly applied into the translational layer. For instance, these investigations all share the construction of the calcified cartilage layer (CCZ) and the successful SBP regeneration that results from the addition of BMSCs to the CCZ (31-33). On the other hand, the SBP typically heals poorly if these cells are denied access to the intermediate transition layer (30). These results can prove that osteogenesis is one of the necessary factors for the SBP. The trabecular bone structure can provide a stable biomechanical environment and mechanical support for the regeneration of the SBP. Also, translational layer of grafts provides enough space to accommodate neo SBP.

4. Scaffold design for osteochondral repair

Monolayer scaffold design. As a pivotal part of tissue engineering strategy, seeding cells can promote regeneration of the osteochondral unit, but cannot be effective without supportive scaffolds. The critical concept of the supportive scaffold design is to induce cell growth, proliferation and differentiation at the defect sites (34). As research rapidly progressed, monolayer scaffolds were the first to be designed and studied. Owing to their inherent limitations, monolayer grafts cannot regenerate the entire osteochondral structure; instead, their primary goal is to stimulate cartilage regeneration (35-37). The most recent tissue engineering approaches for the development of monolayer scaffolds include acellular cartilage matrix (ACM)

Table II. Cell types for subchondral bone plate repair.

First author/s, year	Cell types	Mechanism	Application	(Refs.)
Mendes *et al*, 2020	BMSCs	Multipotent differentiation into osteoblasts, secretion of growth factors, angiogenesis promotion	Bilayered PLGA/PLGA-HAp composite scaffold	(19)
Nie *et al*, 2019	ADSCs	Chondrogenic differentiation and provision of settlement for SBP	Cartilage-dECM-decorated nanofibrils	(24)
Lu *et al*, 2018	iPSCs	Capable of differentiating into osteoblasts, improving the tissue repair micro-environment, promoting angiogenesis	-	(23)
Kim *et al*, 2020	Chondrocytes	Secretion of extracellular matrix components (such as chondroitin sulfate and type II collagen) and support of the foundation of SBP	Sole graft of tissue-engineered hyaline cartilage	(29)
Xu *et al*, 2019	Osteoblasts	Secretion of bone matrix proteins (such as type I collagen) and mineralization to form new bone, which indirectly improves the formation of SBP	Bi-layered composite chitosan/chitosan-tricalcium phosphate (CS/CS-s-TCP) scaffold	(27)

BMSCs, bone marrow-derived mesenchymal stem cells; ADSCs, adipose-derived stem cells; iPSCs, induced pluripotent stem cells.

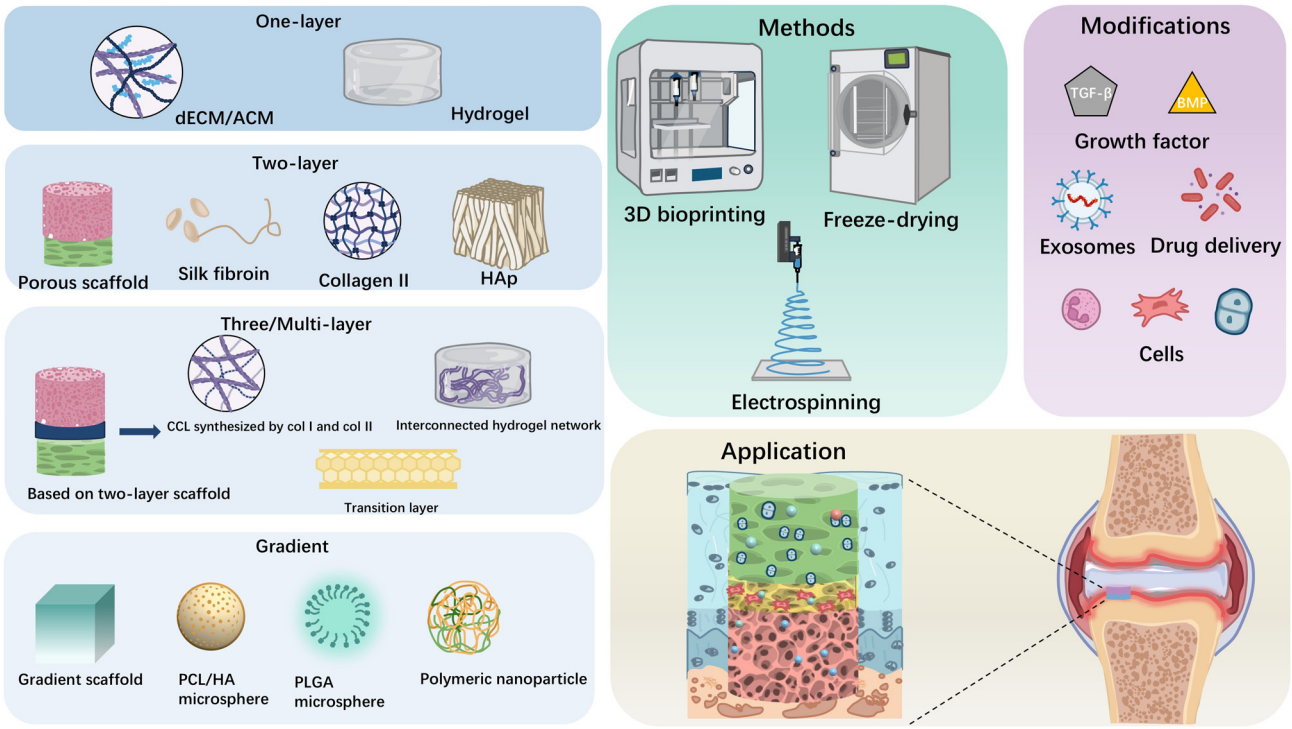

Figure 5. Preparation methods, materials types, structures and modifications of scaffolds for osteochondral repair. The illustration of preparation methods including 3D-printing, freeze-drying and electrospinning, scaffold designs including single, double, triple/multilayer and gradient and the main materials used in them respectively, as well as the functional modifications including growth factors, cells and exosomes. ECM, extracellular matrix; ACM, acellular cartilage matrix; HAp, hydroxyapatite; CCL, calcified cartilage layer; PCL/HA, polycaprolactone/hyaluronic acid; PLGA, poly lactic-co-glycolic acid.

and hydrogels, which are shown in Fig. 5 (38-41). Of these, ACM is the most frequently used material (38-41) because cartilage matrix clearly promotes cartilage regeneration and has demonstrated the ability to mimic various distinctive

requirements of an ECM-like microenvironment. Hydrogels can be made from natural or synthetic polymers, depending on their unique characteristics and specific functions (36). They both offer conditions suitable for cell proliferation and differentiation. Hydrogels based on natural polymers including silk fibroin protein, sodium alginate, porous chitosan and hyaluronic acid have been extensively documented (41-45). Widely reported synthetic polymers include polycaprolactone (PCL), poly lactic-co-glycolic acid (PLGA) and polyurethane (43). These hydrogels are often a good choice for scaffold design due to good biocompatibility and biodegradability (46). In addition to these single-component hydrogel scaffolds, other scaffolds using combinations of two or more biomaterials, such as sodium alginate-gelatin, collagen-fibroin, hyaluronic acid-chitosan, have been used (23,29,47-49). These composite scaffolds are not only more beneficial to cartilage reconstruction but also markedly enhance the biomechanical properties of scaffolds (47). However, only a portion of cartilage tissue may be restored by monolayer grafts and subchondral bone is frequently disregarded (35,50). Although monolayer grafts do not appear to improve the repair of osteochondral lesions, their creation provides a foundation for additional study and the development of bi- and trilayer grafts.

Bilayer scaffold design. Typically, monolayer grafts only partly repair the defect and cannot achieve overall regeneration of the osteochondral unit. Due to the distinct mechanical strengths and biological environments of the cartilage and bone layers, the design of bilayer scaffolds is much more complicated. The cartilage layer of bilayer scaffolds shares a common structure with monolayer scaffolds, that is, polymer and ACM hydrogels (18,47,51). Due to the robust mechanical properties, excellent biocompatibility and slow biodegradability, cartilage regeneration was evident (38) and the arrangement of newly formed chondrocytes was similar to natural cartilage (52,53). The design of the osteogenesis layer of the bilayer scaffold is also important. To provide a suitable microenvironment and ensure bone regeneration, hydrogels and some inorganic materials became the main source for the osteogenesis layer (54-56). Commonly used materials, including bioceramics and bioglass (54-56), have been improved over the past few decades, with excellent osteoconductive and inductive properties, stiff mechanical strength and extraordinary biodegradability, allowing these materials to demonstrate superiority for subchondral bone repair (54). The osteogenesis layer of scaffolds constructed with hydroxyapatite (HAp) (57) show good bioplasticity (58) and a pattern similar to trabecular bone structure can be shaped using three-dimensional (3D) printing and other technologies. Bilayer scaffolds tend to achieve better repair results for both the cartilage layer and the subchondral bone layer compared with monolayer scaffolds.

Among bilayer graft studies, only a few reported that the SBP regenerated with an uneven surface or anomalous tissue formation between the cartilage and subchondral bone layer (58-60). At present, however, no phase of bilayer scaffolds has been developed that can precisely repair the SBP. Nonetheless, it has been demonstrated that offering a somewhat stable 3D microenvironment allows the bilayer scaffold to accomplish SBP regeneration (58). Proper porosity is one of the key conditions for creating a microenvironment and is also important for scaffold design (61,62). Relevant studies have reported that bilayer scaffolds with specific porosities can enhance SBP regeneration (55,63-65). Using different porosities, the microenvironment can support the growth and multiplication of encapsulated seeding cells as well as osteogenic and chondrogenic differentiation and can also improve structural stability and enhance mineralization in the subchondral bone region (66,67). For example, a bilayer scaffold with an upper porosity of 200 μm and a lower porosity of 400 μm (66,67) provided clear evidence of improving SBP regeneration (68). Although it is not entirely convincing to rely solely on designing different porosity to achieve SBP regeneration (55), these designs provide insight into how best to repair the SBP. If trilayer or multilayer scaffolds achieve unsatisfactory reconstruction, porosity may be a potential target to improve the SBP regeneration.

Trilayer/multilayer scaffold design. Trilayer grafts are designed based on bilayer grafts. The biggest advantage of the trilayer design is that it addresses the structural flaws in bilayer grafts by incorporating more structure into the graft. A transitional layer between the cartilage and bone layers often plays a pivotal role, with its functions including isolation of components and provision of a physicochemical barrier, cross-linking network and mechanical support (69). These grafts have two types of components; one that is similar to the composition of cartilage and another similar to the composition of bone (31,34). For the cartilage-like phase, Col-II-based scaffolds have been the most used. A dense isolated layer produced by Col-II/PLGA, with a small enough pore size and porosity, prevents excessive downward cartilage growth (34) and at the same time inhibits bone hyperplasia and hypertrophy (70). Others synthesized the calcified cartilage layer with Col-II/HAp and Col-I/HAp as a transitional layer (71) that served as a vital physical barrier, separating nutrients and cells within their respective spaces, avoiding crosstalk and interference between the cartilage and subchondral bone layers and ensuring a distinction between the cartilage and bone environments (72). For the bone-like phase, bioceramic was the most common material. PCL-β-tricalcium phosphate is a prepared transitional layer that has been reported to match the local Young's modulus in the middle and also provides steady support with excellent mechanical properties (31). Additionally, this transitional layer may offer sufficient space to regenerate the SBP. Trilayer scaffolds reported in the literature thus distinguish themselves from bilayer scaffolds. Together with the creation of stable cartilage and trabecular bone structures, trilayer tissue-engineered osteochondral grafts yield good repair of the SBP.

Compared with trilayer grafts, multilayer grafts could provide a more sophisticated structure to promote SBP regeneration (70,73). Typically, multilayer grafts are multifunctional, with each layer capable of performing a distinct function. Of those reported, the most common type includes a top layer promoting cartilage regeneration, a second calcified cartilage layer, a third transitional layer and a bottom layer that mainly mimics the porous structure of trabecular bone (33). This complex architecture creates a biomimetic environment for the entire osteochondral tissue (74). The calcified cartilage layer not only balances the differential physical load between upper cartilage and lower subchondral bone (34),

but also helps stabilize the overall mechanical properties of the scaffold. In addition to the isolation and barrier function aforementioned (34), the intermediate transitional layer can release growth factors to the upper and lower layers of the scaffold (75). Notably, in both small and large animals, SBP regeneration results were good with a flat and even surface achieved (30,33,76). In general, the closer the structure of the artificial scaffold is to normal osteochondral structure, the more improved the repair efficacy. Multilayer grafts may thus result in improved osteochondral unit regeneration compared with bilayer grafts.

Given the complex structure of multilayer scaffolds, fabrication techniques have limited their progress. 3D printing has become a standard technique for fabricating biomimetic scaffolds. To date, a variety of 3D printing methods such as fused deposition modeling (FDM), inkjet printing, light-assisted bioprinting (digital light processing), stereolithography and laser-based printing have been used to engineer different tissue repair scaffolds (67,68) (Fig. 5). To mimic the structural and mechanical characteristics of subchondral bone and improve the hydrophilicity of the bone scaffold, some previous researchers fabricated the core-sheath structure bone layer using FDM (30). Other researchers have produced the chondral and osteochondral phases with 3D bioplotting; these have been designed with a cross-linking network and high porosity (71,73). These structures had surprising biocompatibility and could be adjusted according to the lesion position. Moreover, with 3D printing technologies, the fabrication of patient-specific scaffolds that perfectly match the size and shape of the defect would come true. And targeting porosity, layers, integration would be designed by 3D-printing technology, all these provide the possibility for future customized strategies. Consequently, multilayer scaffolds are unquestionably of higher quality because of 3D printing. Moreover, additional techniques for multiphase scaffolds are anticipated in the future.

Non-layer/gradient scaffold design. Gradient scaffolds have gained increasing popularity over the past decades. Gradient scaffolds involved two types: Compositional gradient scaffolds and structural gradient scaffolds (77). First, as with other kinds of stratified scaffolds, the materials applied in gradient component scaffold included collagen or extracellular matrix (77-79) and inorganic polymers such as PCL, PLGA and gelatin methacryloyl (GelMA) (80,81): With their suitable biodegradability and mechanical properties, scaffolds physicochemical stability could be sustained continuously (77). In contrast to stratified scaffolds, the primary characteristic of gradient scaffolds was the smooth transition between layers. Gradient scaffolds can prevent sudden component changes in distinct zones since the components in the osteochondral micro-environment vary gradually as depths increased (81). A gradient component scaffold was designed with PLGA/TCP and the upper cartilage layer was constructed of a microtubule-like structure. These microtubules were interconnected and parallel arranged in perpendicular plane (diameter, 84.2±20.7 μm). The bone layer had a highly interconnected porous structure with large pores (450.5±47.2 μm) (76). The whole osteochondral structure was repaired integrally and both cartilage layer and trabecular bone layer showed perfect reconstruction. Other studies have also proposed a biodegradable hydroxyapatite-collagen (HAp/Coll) gradient distribution scaffold with collagen matrix synthesized in four different weight ratios as follows: 0:100, 10:90, 30:70 and 50:50 to simulate the normal amount of cartilage and bone components (82). As well as cartilage and trabecular bone regeneration, regeneration of SBP could be also detected (82). The special benefits of the compositional gradient scaffold, structural stability, a biocompatible interior environment and successive compositional alteration, were the main reason of all these satisfactory results (77,82).

Microsphere scaffolds, another common construction prototype as gradient scaffolds, organize a three-dimension and porous structure for cells proliferation and tissue formation. In addition to the inherent advantages such as biocompatible structure and smooth translation between layers, the microsphere scaffolds show unique features to repair osteochondral defect. First is the architectural and mechanical variations throughout their 3D structure. Chitosan/mesoporous silica nanoparticles was designed as microsphere scaffold by Yuan *et al* (83). The nanoparticles possess excellent bio-remodeling activity, different patterns can be arranged in the chondrogenesis zone and osteogenesis zone. With their 3D structure and porous formation, the differentiation of chondrocytes can be obviously promoted (83) (Fig. 5). By adjusting the arrangement of nanoparticles, the mechanical properties of the scaffolds can mimic the transition from soft cartilage tissue to the calcified cartilage and ultimately subchondral bone (84). Along with the feasibility of nanoparticles, osteochondral defects could gain regeneration. The second characteristic of microsphere scaffolds is their ability to create interfacial cohesiveness between several layers. PCL/HA was used to construct a composite microsphere scaffold by Gu *et al* (85). Due to their tiny spherical 3D structure, these microspheres have the potential to improve scaffold transitions while also strengthening the interface integration (77). Regeneration of cartilage and bone tissue was observed following implantation of these microsphere scaffolds (84,85). With their unlimited and promising prospect, the design of gradient grafts will be markedly improved. After long-term observation and evaluation, the satisfactory result of osteochondral repair would be achieved with the gradient grafts application.

5. Growth factors for osteochondral repair

Incorporation of therapeutic growth factors into tissue-engineered grafts allows modulation of the local microenvironment (making it chondro- or osteo-inducive), which improves differentiation and increases matrix production (86,87). The transforming growth factor-β (TGF-β) superfamily includes TGF-β, bone morphogenetic protein (BMP) and growth differentiation factors. Of these, TGF-β3, TGF-β1 and BMP-2 are the three most widely used (86-88). TGF-β has a stimulatory effect at the early stage of chondrogenesis, promoting cartilage matrix synthesis, cell proliferation and upregulation of chondrogenic-specific genes (86,87). Moreover, it inhibits the terminal differentiation of hypertrophic chondrocytes (5). BMP-2 can stimulate bone regeneration by promoting the deposition of Col-I, inducing osteocyte differentiation and initiating angiogenesis in trabecular bone (89). In addition to the TGF superfamily, other frequently used growth factors

Table III. Summary of growth factors about their function and application for osteochondral repair.

First author/s, year	Growth factor	Function	Influence on tissue regeneration			(Refs.)
			Cartilage	Trabecular bone	Subchondral bone plate	
Qasim *et al*, 2019; Chen *et a*l, 2020	TGF-β3	Provocation of glycosaminoglycan deposition; Assistance in chondrogenesis; Induction of chondrocyte proliferation	Promotion	No influence	No influence	(86,87)
Spencer *et al*, 2018	TGF-β1	Provocation of glycosaminoglycan deposition; Assistance in chondrogenesis; Induction of chondrocyte proliferation; Stimulation of mineralized bone tissue synthesis	Promotion	Promotion	Indirect promotion	(5)
Sun *et al*, 2020	BMP-2	Promotion of the deposition of type I collagen; Induction of osteocyte differentiation; Induction of subchondral bone tissue integration	No functioning	Promotion	Indirect promotion	(89)
Zhai *et al*, 2018; Xue *et al*, 2018	FGF	Stimulation of chondrocytes proliferation	Promotion	Unclear	Unclear	(34,36)
Zhai *et al*, 2018; Xue *et al*, 2018	GDF	Regulation of apoptosis Promotion of cartilage differentiation	Promotion	Unclear	Unclear	(34,36)

TGF, transforming growth factor; BMP, bone morphogenetic protein; FGF, fibroblast growth factor; GDF, growth differentiation factor.

include insulin-like growth factor-1, fibroblast growth factor and platelet-derived growth factor (PDGF) (19,26,36,50,90). Their functions include stimulating cell proliferation, triggering chondrogenesis gene expression and regulating apoptosis (36,50). Additionally, PDGF is important for vascularization, since it induces angiogenesis, regulates cell migration and supports vessel maturation and stabilization.

These growth factors can encourage the regeneration of cartilage or trabecular bone, but their specific efficacy in promoting SBP regeneration has not yet been reported. There are circumstances in which the SBP may be repaired. The plate is composed of cortical bone tissue (91). Growth factors, which can stimulate the repair of trabecular bone, can similarly regenerate the SBP. BMP encapsulated into the osteogenic layer contributes to the regeneration of the SBP (19) not only because of its excellent osteogenic differentiation properties, but also because of its ability to maintain homeostasis in the joint (19,33,36). Furthermore, SBP repair can be indirectly accomplished if the bone and cartilage layers are repaired together. Multilayer scaffolds encapsulating TGF-β1 and BMP-2 induce more uniform osteochondral tissue regeneration than scaffolds without growth factors (19,33,75). This indirectly stimulates the regeneration of the SBP. Use of growth factors may prove to be a useful strategy for SBP regeneration, despite the lack of evidence of a direct role for growth factors in SBP repair (Table III).

6. Translating animal models for SBP repair

Animal studies are an important stage between *in vitro* studies and clinical application. The choice of an appropriate animal model is fundamental to making appropriate conclusions (92). Animal studies usually include two groups according to size, small animal models and large animal models (Table IV). Small animal models, including rabbit, rat, dog and mouse models, are widely used in preclinical studies. These smaller models are low-cost, easy to handle and house and studies are easy to implement (5). Rabbits are the most used small animal model. Short- and long-term evaluations can be performed easily due to their light weight, robust exercise capacity and low load on the defect location (5). Compared with small animal models, large animal models, including pig, sheep, goat and horse models, have the advantage of similarity to humans in joint size, cartilage thickness and lesions (5). In addition, their bone tissue macro- and microstructure, composition, biochemical properties and mineral density are closer to humans (4,7,93).

Table IV. Overview and characterization of experimental studies with a focus on alterations of the subchondral bone plate and osteochondral unit.

First author/s, year	Model			Defect information		Subchondral bone plate alterations	Osteochondral unit alterations	Untreated defect alterations	(Refs.)
	Animal	Animal age	Animal type	Location	Geometry (diameter x depth)				
Liu *et al*, 2017	Rabbit	4-5 months	Small animal	On the patellar groove	4x3	Sufficient thickness regenerated and seamless interface in the sub-chondral bone plate region	Satisfying regeneration of osteochondral tissue	The repair of cartilage defect failed but trabecular bone tissue slightly grew in the defect location	(47)
Nie *et al*, 2019	Rabbit	16 weeks	Small animal	On the patellar groove	3x2	Subchondral bone plate layers were revealed with compact and flat surface	The osteochondral defects were completely healed. The traumatic dents have vanished	Huge fibrous tissue filled in the defect location	(24)
Zhang *et al*, 2019	Rabbit	12 weeks	Small animal	On the medial femoral condyle	4x3	The surface appeared relatively flat and thickness increased	Entire structure was regenerated with orderly and compact components	The newly repaired tissue was thinner than treatment group but cartilage and subchondral bone were reshaped initially	(97)
Critchley *et al*, 2020	Sheep	1.5-2 years	Large animal	In the condyle of femur	6x6	Deep clefts and obvious blank interface space were detected	Nearly blank fill in the defect location	Entire defect location was filled with tangled fibrous tissue or worse no tissue filled in with deep clefts	(18)
Zhai *et al*, 2018	Goat	10 months	Large animal	In the condyle of femur	6x9	A clearly visible transition in the interface could be detected	Whole structure tended to be collapsed	Deep clefts and huge interface in the defect location, big hollow can be detected in the depths	(34)
Xiao *et al*, 2019	Miniature pig	6 months	Large animal	In the femoral trochlea	7x3	The thickness was under expectation and nearly no subchondral bone plate existed	The defect location was filled with amount of fibrous tissue	A large amount of fibrous tissue filled in the site	(98)
Korthagen *et al*, 2019	Shetland pony	7.3±3.2 years	Large animal	In the trochlea of femur	5.9x7.5	A clearly visible transition in the interface could be detected	Merely slight repair of osteochondral tissue	Only fibrous tissue occupied into the site and the thickness was thinner than surrounding tissue	(99)

Of the large animal models, pigs are used most. As they are heavier than sheep and goats, the injury site bears a larger load and requires a longer evaluation period (17); however, satisfactory repair of osteochondral lesions is rarely achieved in large animals. Therefore, achievement of satisfactory osteochondral regeneration in large animal models could indicate potential strategies for further clinical work.

It is necessary to establish standard criteria for animal models used in translational studies. Generally, the criteria for osteochondral defects include defect location, defect size (depth and diameter) and the age of the animal. At present, the creation of an osteochondral defect is based on the protocol used in small animal models, with the defects mostly located in the femoral trochlea, patellar trochlea and condyle of the femur (92,94). The induced defect is usually 2-4 mm in diameter and 3-6 mm deep; this depth can generally lead to formation of a full-scale osteochondral defect. Selected animals must have mature skeletons (92,94). Although several studies have reported osteochondral tissue regeneration using small animal models, use of these models has been restricted due to the major disparities between human and animal joints (92). By contrast, large animal model-based research is likely to be more useful in the future. Defects in large animal models are always induced in the condyle or trochlea of the femur (17). The defect diameter is usually 6-10 mm and the depth is also 6-10 mm, which generally reaches the depth of a full-scale osteochondral defect (92). Due to the similarities to human disease development and pathophysiological changes, large animal models are ideal for studying osteochondral defects. Future research should establish uniform standards for selecting animal models. First, large animals make more suitable models. Second, large animals are ideal for the choice of defect location; most human knee joint injuries occur on the femoral condyle, which is a suitable option for the defect site in the model (92) and the defect size must reach that of a full-scale osteochondral defect. Furthermore, adult animals should be used, which is consistent with the current trend of human disease; osteoarthritis is more common in elderly adults than in adolescents (8,11).

7. Challenges and perspectives

Previous studies have illustrated various failures in osteochondral transplants (30,33). Such unsatisfactory consequences comprise two main classifications: Functional failure and pathological abnormalities. The most perplexing findings for pathological abnormalities are the collapsed structure of the osteochondral unit (18). Cartilage hypertrophy, fibro tissue hyperplasia, concavity formation and inconsistency among phases (47) are common in osteochondral regeneration studies. Even structural collapsing directly indicates the functional failure of osteochondral transplants. Other pathological changes, such as cartilage hypertrophy, fibro tissue hyperplasia, cause difficulty for long maintenance.

In the complicated progression of osteochondral repair and design of transplants variation, it is difficult to explain a single failure in performance comprehensively. However, inherent deficiency for chondral regeneration and temporal disorder among chondral/osteo-regeneration have been attributed to the aforementioned pathological abnormalities and functional failures. Compositional and structural insufficient for neo formed cartilage are strongly associated with cartilage hypertrophy, fibro tissue hyperplasia and concavity formation. On the other hand, a relative earlier completion of bone tissue repair is equally important for reducing pathological abnormalities. Immunological rejection related insufficient interfacial integration is another potential reason for transplants failure (58).

Neo-formed SBP, with proper location and acceptable architecture, is used as a crucial standard to determine successful osteochondral regeneration. The present review summarized four common SBP pathological performances from previous studies with failed osteochondral regeneration including abnormal height, uneven surface, poor integration and loose internal structure. Abnormal height was the most common presentation of poor repair and markedly affected SBP reconstruction. With the help of our pathological classification, transplants failure interpretation may be possible for future studies.

To achieve satisfactory SBP during osteochondral regeneration, multi-layer TE grafts with a transition layer or calcified layer was introduced into current studies. However, graft layer quantity is limited to a certain extent. With the increase of the number of layers, the manufacturing cost gradually increases, as well as the difficulty of the fabrication techniques. However, the anticipated improvement in the ultimate repair has not been achieved (30,33), which is contrary to the concept of high efficiency and low consumption. The structure of gradient scaffolds is different from that of traditional layered scaffolds. The gradient grafts can obtain satisfactory osteochondral repair by virtue of its unique advantages. However, the shortcomings of the gradient scaffold cannot be ignored. Gradually changed components in the graft could not acquire the distinct regions at the defect site. In addition, the degradation rate of gradient scaffolds is faster than the stratified scaffolds (95) and such rapid degradation rate is not conducive to the load-bearing capacity of neo tissues at the defect site.

Tissue-engineered osteochondral grafts have promise for repairing osteochondral defects. Nonetheless, barriers remain throughout the translational process and must be overcome. First, the restrictive microenvironment *in vitro* may be a crucial factor in cultivating a successful graft, including appropriate culture medium, suitable temperature and sophisticated culture techniques (13). Second, because improper biomaterial selection may result in the premature collapse of the scaffold or, conversely, late degeneration, adaptive biomaterials may potentially be a barrier to further deployment (3). Multilayer graft with proper biomaterial to regenerate SBP is a promising way to obtain reasonable chronological order for chondral/osteo-regeneration. Third, the translational animal model selected also affects the repair results depending on such factors as species, age, defect location and defect geometry (96). Lack of a unified standard for translational animal types will lead to a biased interpretation of regeneration results. Security concerns and expense also limit the repair efficiency of tissue-engineered grafts. Although these grafts show promise for healing osteochondral lesions, several obstacles to successful clinical application remain; these obstacles may guide breakthroughs in the creation of new tissue engineering strategies (97-99).

There are also several limitations in current reviews. As the limited quantity of studies focus on SBP regeneration, the present study merely focused on the local regeneration of SBP, instead of establishing connection between SBP pathological changes and the regeneration of other parts. Second, the evaluation of SBP was based on histological and radiological performance. The summary of poor SBP repair was subjective and additional research is required to address this issue. Third, no conclusion for the TE graft with ideal SBP regeneration was drawn in the present review. Even multilayer scaffold with cocktail growth factors to promote SBP regeneration was suggested. Further study to provide proper biomaterials allowing fine control for chondral and osteo-portion regeneration as well as biocompatibility are required.

8. Conclusion

SBP is a new promising path to understand the osteochondral regeneration and to interpret osteochondral transplants destiny. In the present review, the histology of the SBP was discussed and four common histological manifestations of poor repair were established, including abnormal height, uneven surface, poor integration and loose internal structure. The impact of different tissue engineering graft designs on osteochondral unit repair was also discussed. Incorporating mesenchymal stem cells into trilayer/multilayer scaffolds, supplemented by appropriate growth factors, can produce satisfactory osteochondral unit repair. Moreover, the SBP has also been repaired. Finally, future studies should focus on large animal models given their physical similarities to humans; such models will inspire future clinical research on tissue-engineered grafts. This review has shed light on potential standards for the construction of future animal models of osteochondral defects.

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MC and XZ made substantial contributions to the conception and design of the work. XZ, WJ, QD and YS drafted the manuscript. MC, XZ, WJ, QD and YS revised the manuscript critically for important intellectual content. Data authentication is not applicable. All authors read and approved the final manuscript. All co-authors agree to be accountable for all aspects of the work.

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Competing interests

The authors declare that they have no competing interests

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