Journal of Orthopaedic Translation 30 (2021) 93–102

Contents lists available at ScienceDirect

Journal of Orthopaedic Translation

journal homepage: www.journals.elsevier.com/journal-of-orthopaedic-translation

Osteochondral tissue engineering: Perspectives for clinical application and preclinical development

Chengchong Ai [a,b], Yee Han Dave Lee [c], Xuan Hao Tan [a,b], Si Heng Sharon Tan [c], James Hoi Po Hui [c,d,e], James Cho-Hong Goh [a,b,d,e,*]

[a] Integrative Sciences and Engineering Programme, NUS Graduate School, National University of Singapore, Singapore
[b] Department of Biomedical Engineering, National University of Singapore, Singapore
[c] Department of Orthopaedic Surgery, National University Health System, Singapore
[d] NUS Tissue Engineering Programme, Life Sciences Institute, National University of Singapore, Singapore
[e] Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

ARTICLE INFO

Keywords:
Osteochondral regeneration
Subchondral bone
Scaffold
Animal experiment

ABSTRACT

The treatment of osteochondral defects (OCD) remains challenging. Among currently available surgical treatments for OCDs, scaffold-based treatments are promising to regenerate the osteochondral unit. However, there is still no consensus regarding the clinical effectiveness of these scaffold-based therapies for OCDs. Previous reviews have described the gradient physiological characteristics of osteochondral tissue and gradient scaffold design for OCD, tissue engineering strategies, biomaterials, and fabrication technologies. However, the discussion on bridging the gap between the clinical need and preclinical research is still limited, on which we focus in the present review, providing an insight into what is currently lacking in tissue engineering methods that failed to yield satisfactory outcomes, and what is needed to further improve these techniques. Currently available surgical treatments for OCDs are firstly summarized, followed by a comprehensive review on experimental animal studies in recent 5 years on osteochondral tissue engineering. The review will then conclude with what is currently lacking in these animal studies and the recommendations that would help enlighten the community in developing more clinically relevant implants.

The translational potential of this article: This review is attempting to summarize the lessons from clinical and preclinical failures, providing an insight into what is currently lacking in TE methods that failed to yield satisfactory outcomes, and what is needed to further improve these implants.

1. Introduction

Osteochondral defects (OCD) refer to focal areas of articular cartilage damage accompanying the injury of the adjacent subchondral bone. Early diagnosis and treatment of OCDs is important for better prognosis of motor function and osteoarthritis prevention [1]. However, because cartilage tissue lacks intrinsic healing capabilities, the treatment of OCDs is challenging in orthopaedic surgery.

Tissue-engineering (TE) approaches, which use combinations of cells, scaffolds, and biomolecules to regenerate diseased or damaged tissues, are promising methods that can yield satisfactory clinical outcomes [2]. TE approaches have the added advantage of not requiring another part of the patient's body for grafting to regenerate damaged tissues. In recent decades, the field of osteochondral tissue engineering has seen significant development in biomaterials and fabrication technologies, providing feasible approaches to develop implantable constructs that have the potential to achieve better clinical outcomes. Previous reviews have described the gradient physiological characteristics of osteochondral tissue and gradient scaffold design for OCDs [3], tissue engineering strategies, biomaterials and fabrication technologies [4–7]. However, the discussion on bridging the gap between the clinical need and preclinical research is still limited, on which we focus in the present review, providing an insight into what is currently lacking in TE methods that failed to yield satisfactory outcomes, and what is needed to further improve these techniques.

Currently available surgical treatments for OCDs are firstly summarized, followed by a comprehensive review on animal experimental studies in recent 5 years on osteochondral tissue engineering. The review

* Corresponding author. Tissue Repair and BioFab lab, 2 Engineering Drive 3, 117581, Singapore.
E-mail address: biegohj@nus.edu.sg (J.C.-H. Goh).

https://doi.org/10.1016/j.jot.2021.07.008
Received 19 January 2021; Received in revised form 15 June 2021; Accepted 28 July 2021

will then conclude with what is currently lacking in these animal studies and the recommendations that would help enlighten the community in developing more clinically relevant implants.

2. Current clinical therapies for OCDs

Early diagnosis and treatment of OCD is of great importance for better recovery of motor function and preventing osteoarthritis [8]. For OCD patients with symptoms such as joint pain and weakness, a surgical treatment is needed since the outcomes of conservative treatments are rarely satisfactory [9]. There are a variety of surgical treatments for OCDs [10–12] (Fig. 1), such as osteochondral autologous transplantation (OAT), osteochondral allograft (OCA) transplantation including fresh OCA and off-the-shelf OCA, autologous chondrocyte implantation (ACI) sandwich technique, autologous matrix-induced chondrogenesis (AMIC) sandwich technique, and repairing with osteochondral scaffold.

Osteochondral autograft transplantation (OAT) fills the defect site with osteochondral autograft collected from less weight-bearing portions of the joint, which may lead to donor site lesion [13]. In the case of large osteochondral lesion, in which multiple osteochondral autografts are necessary to fill the defect, the risk of donor site morbidity might increase with the number of used autologous osteochondral plugs [14].

For large OCD, osteochondral allograft (OCA) transplantation could be used as an effective procedure [15]. However, the use of osteochondral allografts raises concerns about cost, availability of fresh allograft tissue, immunogenic reaction, and disease transmission [16]. Off-the-shelf allograft products emerged as an alternative to the fresh OCA, which are processed to be sterile and ready for implantation, such as Chondrofix® osteochondral allograft (Zimmer, US), whereas the potential limits include the absence of viable cells and the graft size is only up to 15 mm.

For OCDs with small lesion area (<1.5 cm^2) and shallow depth (5–8 mm), ACI alone showed promising results to restore the lesion [17]. Regarding deep OCDs (>8–15 mm), surgeons have sought to restore the osseous phase and cartilaginous surface together as a functional osteochondral unit [17,18]. As such, the ACI "sandwich" technique was developed, combining the autologous bone grafting and ACI technique [19]. ACI technique was initially developed for cartilage repair. For the procedure of ACI technique, a full-thickness cartilage tissue is taken from a low-weight-bearing region of the joint, and then the autologous chondrocytes are extracted and expanded *in vitro*. During the second surgery, periosteum or a scaffold is used to cover the lesion, and the expanded chondrocytes are injected under the graft to fill the lesion site [20]. In newer generation ACI, the harvested and expanded chondrocytes are seeded onto the scaffold to be delivered on the cartilage lesion [21]. In the ACI "sandwich" technique, autologous cancellous bone chips are impacted to fill the subchondral lesion, followed by covering with a periosteum or collagen membrane. After a second membrane was fixed on the articular surface, autologous chondrocytes were then injected between two membranes to regeneration the cartilage layer [19]. The other way is to cover the bone grafts with a scaffold loaded with autologous chondrocytes [21].

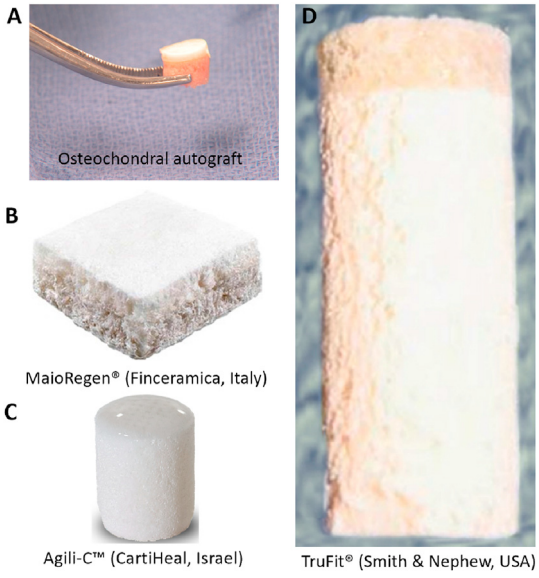

Figure 1. (A) Images of osteochondral autograft [32], (B-D) osteochondral scaffolds [33].

As ACI technique requires two-step procedure and is expensive, the interest in one-step repair approaches increases, such as autologous matrix induced chondrogenesis (AMIC) [22]. In AMIC sandwich technique, surgeons tried to use the membrane or gel only to cover on bony chips, such collagen I/III bilayer matrix membrane (Chondro-Gide®; Geistlich Pharma AG, Switzerland), collagen type I gel CaReS®-1S (Arthro Kinetics, Austria) [23,24]. In some cases, the membrane is infiltrated with bone marrow aspirate concentrate (BMAC) to promote cartilage regeneration [25,26].

Furthermore, with biomaterial exploration and attempts to achieve cell-free approaches even without bone grafting, the last decade witnessed many scaffold products emerging for the treatment of OCDs, aiming to promote tissue repair with physiological properties similar to native osteochondral unit, such as TruFit® (Smith & Nephew, USA) [27,28], MaioRegen® (Finceramica, Italy) [29], Agili-C (CartiHeal, Israel) [30], and BioMatrix™ Cartilage Repair Device (CRD) (Arthrex, USA) [31]. In this review, the surgical treatments using osteochondral scaffolds are called scaffold-based therapies. As shown in Table 1, these scaffolds are designed to mimic the characteristics of osteochondral unit. This review will focus on discussing the current development of osteochondral scaffolds.

3. Review of preclinical studies on osteochondral TE

A literature search on the basic research of OCD scaffolds in recent 5 years was conducted in PubMed through May 2020, using the keywords "osteochondral" and "scaffold" with the search string ((osteochondral [Title/Abstract]) AND scaffold [Title/Abstract] AND "last 5 years" [PDat] AND Animals [Mesh:noexp]). After screening by abstract and title, all studies performed animal experiment to investigate the tissue repairing outcome of scaffolds were summarized. Fifty-two studies were included, and the scaffold composition, cell source, features of animal experiment are listed in the supplementary table. In the body of the

Table 1
Characteristics of osteochondral scaffolds.

Product name	TruFit® (Smith & Nephew, USA)	MaioRegen® (Finceramica, Italy)	Biomatrix™ CRD (Arthrex, USA)	Agili-C™ (CartiHeal, Israel)
Phase design	Biphasic	multiphasic	biphasic	biphasic
Chondral phase	poly D-L-lactic-co-glycolic acid	type I collagen (Col I)	type I collagen	aragonite and hyaluronic acid
		Intermediate layer: Col I (60 %) and magnesium-enriched hydroxyapatite (Mg-HA; 40 %)		
Bony phase	calcium sulfate	Col I (30 %) and Mg-HA (70 %).	β-tricalcium-phosphate (80 %) with polylactic acid (PLA) (20 %)	calcium carbonate in the aragonite crystalline form

present review, most common issues are summarized and discussed.

3.1. Scaffold design

All reported scaffolds were categorized into three types: monophasic scaffolds (supplementary table 1), biphasic scaffolds (supplementary table 2), multiphasic (supplementary table 3). In monophasic scaffolds, the biomaterial composition, structural porosity, and mechanical property are homogenous through the whole construct. To mimic the specific microenvironment for cartilage and underlying bone regeneration, biphasic and multiphasic scaffolds emerged to better guide the hierarchical structure of osteochondral tissue regeneration. Biphasic scaffolds were made of two kinds of scaffold design, in terms of biomaterial composition, fabrication process or architectural characteristics. The two layers in biphasic scaffolds is to mimic the bone and cartilage ECM, respectively. Furtherly, particular design aimed to regenerate the osteochondral interface and gradient structural and compositional change of the native osteochondral tissue inspire the design of multiphasic scaffold. In the preclinical studies of multiphasic studies, three-layer design is commonly used, which consists of three different chemical compositions in each layer to mimic the cartilage phase, osteochondral interface, and subchondral phase. The middle osteochondral interface layer is compact to separate the microenvironment for chondral and subchondral tissue regeneration. In addition, another method is to design a gradient throughout the whole osteochondral scaffold, such as pore size or scaffold component [34].

There is still no consensus on which design is optimal for OCD repair. Based on the results of the included studies, no conclusion could be drawn on which design is the optimal one because most of the published studies reported positive outcomes no matter which design was used and it is difficult to compare across different studies. It could be publication bias that hinders an objective narration comparing three kinds of scaffold designs, but some pros and cons of each design could be summarized based on the published data.

One advantage of monophasic scaffolds is the relatively easier fabrication process. Although monophasic scaffolds are theoretically unable to mimic the two divergent microenvironments for bone and cartilage regeneration, some animal studies which repaired OCD with monophasic scaffolds reported histological results showing a clear tidemark zone with cartilage and bone formation [35–38]. With regards to biphasic and multiphasic scaffolds, all of them used different biomaterial composition for the chondral phase and bone phase respectively, hence a complicated fabrication process was needed. In the animal studies with biphasic scaffolds, some studies set a control group in which is a single phasic scaffold was used [39–45]. Compared to the hyaline cartilage and porous bone regeneration with a clear tidemark observed in the group pf biphasic scaffolds, the inferior outcomes in the group of single chondral phase scaffolds included: subchondral bone formation and disordered fibrocartilage [39]; Disorganized fibrous tissue in both bone and chondral phase [40]; Lower ICRS II scores without regeneration of subchondral bone [41]; Slower cartilage and bone regeneration and integration with the native tissue [42]; A mixture of fibrocartilage and cartilage tissue [43]; Slower regeneration of bone layer [44]; fibrotic tissue separated from the surrounding bone [45]. In the studies of triphasic scaffolds, the corresponding control group of monophasic scaffolds also resulted in limited bone and cartilage repair with poor quality [34,46]. All the evidence supported the superiority of biphasic design and multiphasic design to monophasic scaffolds.

In addition, the superiority of multiphasic design to biphasic design also showed up in some studies. In multiphasic scaffolds one advantage of the interposed dense layer showed in Zhai's study is to prevent the cartilage downgrowth as the samples of bilayered scaffolds showed conspicuous cartilage downgrowth [47]. Without the interposed layer, the interference of these two kinds of microenvironment resulted in uncalcified cancellous bone and immature cartilage formation. Levingstone et al. [48] compared a multilayered scaffold with a bilayered scaffold in a caprine model. At 12 months after surgery, the anatomical tidemark in multilayered scaffold has been restored, but in the bilayered scaffold, the subchondral bone was barely regenerated, displaying many cavities and the cartilage phase was predominantly filled with fibrocartilaginous tissue. Of note, in this study, the multilayered scaffold is made of natural polymers including collagen I, collagen II, hyaluronic acid, and hydroxyapatite, however, the bilayer scaffold is composed of polyglycolic acid (PLGA) and calcium sulfate. The difference on repairing outcome may be attributed to differences in biomaterials, degradation properties and inflammatory acidic degradation products of PLGA.

3.2. Biomaterial selection

Careful selection of appropriate biomaterial is an important aspect for tissue engineering research. Biomaterials, fabrication process, and growth factors for OC TE have been comprehensively summarized in the previous reviews on osteochondral tissue engineering [6,7]. Various biomaterials, natural or synthetic, have been tested and resulted in positive histological outcomes for OCD repair as summarized in supplementary tables, hence based on current published data it's difficult to give recommendations on biomaterials selection, and fabrication method. In this review, we tried to evaluate the translational value of the current preclinical studies from the perspective of authority. The experimental workflow summarized from preclinical studies of osteochondral tissue engineering was compared with the biocompatibility evaluation endpoints recommended by U.S Food and Drug Administration (FDA) as guidance to assist industry in preparing premarket applications (Table 2). According to the framework to assess the medical device [49,50], osteochondral scaffolds belong to an invasive medical device with long contact (> 30 days *in vivo*). The biological evaluation should include cytotoxicity, sensitization, irritation, acute systemic toxicity/pyrogenicity, subacute/subchronic toxicity, genotoxicity, implantation, chronic toxicity, and carcinogenicity. One animal study with proper experimental design is capable to address multiple aspects of these assessments together, such as implantation, acute, subchronic, and chronic toxicity. However, through the comparison (Table 2) it was found that some issues haven't been fully addressed in current animal studies, such as genotoxicity, and degradation assessment.

The biocompatibility of the biomaterials that have been used in a legally US-marketed medical device is relatively well-established. Repurposing usages of these biomaterials in a new medical device is easier to get approval from the authority. However, as long as the chemical composition, manufacturing, or processing is changed in the new medical device, additional testing would be requested because the biological response of the final device may have changed as well. In the study from Perdisa group [51], they used a chitosan-based biphasic scaffold to repair OCD on sheep. Even though there is much evidence in the literature supporting the osteogenic and chondrogenic capability of chitosan [52], and chitosan-based gel has been applied to treat chondral defects in clinics [53], the chitosan-based biphasic scaffold in this study did not provide osteochondral regeneration in both rabbit and sheep model. The authors of this study attributed the negative outcomes to the crosslinking treatment used on the material. The high degree of crosslinking used in this study reduced active charged sites for water adsorption on chitosan molecular surface, thus hampering the deposition of proteins for stem cell adhesion and affecting the biodegradability properties of chitosan. The contradiction between this finding and previous studies highlights the influence of different processing methods on the biologically repairing performance of materials. In this study, only *in vivo* results were reported, and there was no *in vitro* data. This reminds us of the importance of *in vitro* test, which is in line with the recommendation from ISO 10993–1 that biocompatibility testing should start with *in vitro* test. Early screening candidate materials in their finished form through *in vitro* tests is helpful to reduce the high cost of animal experiment.

On the other hand, to maximize the value of animal studies, more

Table 2
Comparison between the experimental workflow from the preclinical studies and the framework for biocompatibility evaluation recommended by FDA [49,50].

Experimental workflow in preclinical studies for osteochondral repair		Biocompatibility evaluation endpoints	
		Biocompatibility endpoints	Related standards
Physical property	Morphology;Surface morphology and roughness;Compressive modulus;Chemical component analysis	Cytotoxicity	ISO[a] 10993-5
Cellular response	Cytocompatibility;Osteogenesis or chondrogenesis analysis	Sensitization	ISO 10993-10ASTM[b] F2148
Cellular response	Cytocompatibility; osteogenesis or chondrogenesis analysis.	Irritation or intracutaneous reactivity	ISO 10993-10
		Acute systemic toxicity/ material-mediated pyrogenicity	ISO 10993-11ISO 10993-12
Osteo-chondral repair in animal model	Tissue regeneration evaluated by:Macroscopic evaluation;Histology;Immunohistochemical analysis;Micro-CT;MRI;Biochemical analysis	Subacute/subchronic toxicity	ISO 10993-1ISO 10993-11
		Genotoxicity	OECD[c] 471 (1997); OECD 476 (1997); OECD473 (2014); OECD 487 (2014); OECD 474 (2014); OECD 475 (1997)
		Implantation	ISO 10993-6
Subcu-taneous implan-tation in nude mice	Tissue regeneration evaluated by: histology; immunohistochemical analysis.	Chronic toxicity	ISO 10993-11
		Implantation	ISO 10993-6
		Degradation assessment	ISO 10993-1

[a] ISO, International Organization for Standardization
[b] ASTM, American Society for Testing and Materials.
[c] OECD, Organisation for Economic Co-operation and Development.

attention should be paid to the scaffolds' degradability. As shown in Table 2, the current animal studies usually focus on tissue regeneration, but didn't evaluate the biodegradable and bioabsorbable process of the scaffold. Use of a detailed histological evaluation system to score the biodegradability and bioabsorption, such as the scoring matrices for residual implant materials and bioabsorption features by Rousselle et al. [54], could help speed up the optimization of biomaterial selection for osteochondral repair.

Other than the main biomaterials of scaffolds, many studies have tried to add some components into the scaffold to increase its osteoinductivity or chondro-inductivity. Stronium(Sr) incorporated calcium silicate (CS) improved the osteochondral regeneration compared to the CS scaffold alone because stronium could benefit both the osteogenic and chondrogenic differentiation of MSCs, and also help to suppress synovial inflammatory response [55]; TGF-β1 promoted early chondrogenesis by attracting progenitor cells from the subchondral bone marrow into the cartilaginous repair tissue [56,57]; Silicon-calcium-phosphate scaffold showed better osteochondral repair compared to the calcium-phosphate scaffold as the Si ion affects the biological process related to early osteogenesis in BMSCs and cartilage development [58]. Exploring the potentials of new biomaterials for OCD repair, or combination of the advantages from different biomaterials is proved to be an efficient way to improve favored biological characteristics of biomaterials. Meanwhile, it is worth noting that biomaterials that haven't been used in a legally US-marketed medical device would require more effort to establish a comprehensive file of biocompatibility. The addition of growth factors would make the product classified as a biological rather than a medical device, requiring a more complicated regulatory clearance process.

3.3. Cell-laden versus cell-free scaffolds

Most animal studies for OCD repair support preloading of chondrocytes in the chondral layer could benefit cartilage repair. However, it is still controversial whether preloading cells is necessary in the subchondral bone layer and what cell type should be used. Many positive results have been reported using the combination of chondrocytes in the chondral layer and MSCs or osteoblasts in the bony layer for OCD repair, but several studies showed preloaded cells in the bone layer might lead to fibrocartilage formation due to excessive blood vessels formation. Perez-Silos group [40] found the scaffolds in which both osteoblasts and chondrocytes were loaded in the bone layer and chondral layer respectively (group 3) resulted in fibrocartilage repair. However, the group in which only chondrocytes were loaded on to the chondral layer (group 2) showed organized collagen II fibers and proteoglycans at the chondral layer and trabeculae in the bone layers. In this study, a great number of newly formed blood vessels and organized trabeculae with osteocytes were observed in group 2, indicating this non-cellularized scaffold in the bone phase is capable of attracting cells from the bone marrow environment, initiating vascularization and osteogenesis. However, the preloaded osteoblasts in the bone layer induced excess blood vessels protruding into the chondral layer, which compromised the avascular environment needed for hyaline cartilage formation and maturation. The nude mice model also showed preloaded cells could promote angiogenesis [59]. Although no histological difference on tissue regeneration was found between the hMSCs-loaded scaffold and plain scaffold in a subcutaneous implantation model in nude mice, new blood vessel formation in the hMSCs-loaded scaffold group is more evident at 4 weeks [59].

Recent research with regard to cell-free scaffolds indicate vesicles such as exosome, microvesicles, or stem cell-derived conditioned medium as promising cell-free approaches for regenerative medicine [60,61]. Veronesi et al. [62] loaded surnatants(SN) from bone marrow concentrate(BMC) or mesenchymal stromal cells(MSC) on scaffolds to treat OCDs in a rabbit model. The histological results of SN groups were superior to that of the scaffold alone group. As development of cell-free approaches for OCD repair is currently favored to reach clinical application [63], it is expected that more preclinical studies to explore scaffold properties and other biological sources to stimulate the regenerative capability of the body could be available in the near future.

3.4. Animal model for OCD repair: difficulties in evaluating the results of preclinical studies

As summarized in the supplementary tables, animals of different species, including rat, rabbit, beagle, mini-pig, pig, goat, sheep, and equine, have been used for OCD repairing model. In addition, subcutaneous implantation in nude mice was used before OCD repairing model as a preliminary test. Commonly used locations for OCD repair are patellar groove, femoral trochlea, femoral condyles on the knee, and few studies used hip joint [64]. As shown in Table 3, the OCD dimension and postoperative time point for histological evaluation in different species are widely varied. In addition, the heterogeneity in methods and outcome measures among preclinical animal studies hinders effective comparisons across different studies. These parameters include the lesion site, microfracture, scaffold fixation, follow-up time.

Table 3
OCD dimension and postoperative time point in animal studies.

Species		Rat	Rabbit	Large animals
Range of OCD dimension	Diameter(mm)	1–2	3–6	3–11
	Depth(mm)	1–3	1.5–8	5–12
Range of postoperative time point		4w-12w	3w-36w	4w-12m

Different lesion sites showed different repairing outcome. Trochlear groove and femoral condyles are usually chosen to drill the defect, but the mechanical loading condition is different at these two sites. Levingstone et al. [48] tested a multilayered scaffold in a goat model. They compared the tissue regeneration in two different lesion sites, i.e., trochlear ridge (TR) and medial femoral condyle (MC), and found the histological staining at the TR defect site showed smooth hyaline cartilage formation at 12 months after surgery, but there is a conspicuous cleft on the repairing cartilage tissue at the MC lesion site.

Microfracture and subsequent cell infiltration from bone marrow should be described clearly in the method. In animal models, most of the studies did not mention whether the microfracture procedure was performed before graft implantation. As per the definition of OCD, the osteochondral lesions drilled during animal surgery reach the subchondral bone part, so there should be some bone marrow infiltration into the injury site, which means some endogenous reparative cells are available in the OCD animal model. However, due to the unclear description of this procedure, it is hard to determine the effect of microfracture on mobilizing bone marrow stem cells in each study. For example, some studies mentioned the irrigation of saline at the lesion site before implantation [65,66], which may affect the repairing effect.

Scaffold fixation methods will also affect the outcome of repair [67]. In most animal studies, a description of how to fix the scaffold in the lesion site is usually omitted. Generally, scaffolds are fixed by a press-fit technique [68]. In some big animal models, the fixation method includes glue Tisuacryl (Tisuacryl®,BIOMAT) [40], porcine fibrin sealant (Guangzhou Bioseal Biotech, China) [69].

Follow-up time is crucial in determining the repairing effect. Bolanos et al. [70] tested a decellularized cartilage-derived matrix (CDM) scaffold in a horse OCD model. In the 8-week pilot study, the repaired cartilage phase was stained positively for proteoglycans and collagen II and integrated at the margins with native cartilage. Even though some clefts were exhibited, regenerated bone tissue was integrated well with the native subchondral bone. However, at 6 months after surgery, the repaired tissue was turned out to be fibrotic tissue containing a small quantity of GAGs and collagen II but rich in collagen I. This study highlights the need for long-term studies for OCD repair even the *in vitro* or short-term *in vivo* studies had shown promising outcomes.

4. Lessons from previous preclinical studies

4.1. Subchondral bone repair to support hyaline cartilage regeneration

In the study from Kon group [51], the biphasic scaffold composed of chitosan/Col I in the chondral layer and MgHA/chitosan/Col I in the bone layer failed to repair the OCD on the sheep model. The histological results showed fibrocartilage tissue or no cartilage regeneration in the cartilage part. Meanwhile, in the subchondral part cysts encapsulated in a thick connective membrane were observed instead of normal subchondral bone healing in all experimental samples. In a rabbit model to test the osteogenic capability of MgHA/chitosan/Col I scaffolds, a clear separation between native bone and scaffold could be identified from Micro-CT images and the histological staining showed the interposed connective tissue was infiltrated by inflammatory cells. Overall, the reason for the poor performance on OCD repair might be the lack of bone regeneration in this biphasic scaffold, as the subchondral bone layer is essential to support the overlying chondral regeneration.

Both physiological and mechanical support from the underlying subchondral bone is essential for OCD repair. A biphasic scaffold composed of a polyetherketoneketone(PEKK) bone anchor and a polyurethane elastomer failed to repair OCD in an equine model [71]. The repaired tissue was almost negative for GAGs and collagen II, but stained extensively positive for collagen I, indicating the formation of fibrocartilage instead of hyaline cartilage. Furthermore, the native cartilage surrounding the defect showed degeneration. PEKK, as a non-degradable and bio-inert polymer, is often used for permanent orthopaedic implantable devices [72]. In this study, PEKK was used to anchor the elastomer cartilage layer. In this case, the subchondral bone part cannot be regenerated and there is a layer of fibrous connective tissue at the interface of PEKK and native bone. This indicated mechanical support alone is insufficient to support cartilage regeneration.

Many studies verified that better subchondral bone repair benefits cartilage repair. In Zhu et al.'s study [42], the addition of bioglass in the subchondral bone could stimulate subchondral bone regeneration, thus achieving a bony tissue much more similar to the adjacent normal subchondral bone. In the group the scaffold made of sodium alginate and agarose without bioglass in the subchondral layer, the regeneration of subchondral bone is slower, and the whole regenerated repair tissue is inferior in terms of structures, components, thickness, and integration with the surrounding tissue. This study indicates that mature subchondral bone regeneration is crucial for OCD repair. Du et al. [34] designed a gradient PCL-based scaffold throughout which the hydroxyapatite (HA) content increased from 0 on the top to 30 % at the bottom. The OCD repair in HA-gradient PCL scaffold group is superior to that in PCL scaffold group, partly because the HA gradient benefited the early osteogenesis in the subchondral bone part and enhanced the integration between the newly-formed bone and adjacent native bone.

Shimomura et al. [73] compared hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP) in the subchondral part for OCD repair in a rabbit model. At 2 months after surgery, in both groups observed a comparable hyaline cartilage-like repair in which the chondrocytes were organized in longitudinal columns, and progressive new bone formation. But at 6 months after surgery, the distribution of chondrocytes in the cartilage part in β-TCP group became disorganized instead of remaining arranged in longitudinal columns in HA group. Meanwhile, the newly formed bone tissue in β-TCP group displayed more porosity. This study indicated high-quality restoration of subchondral bone is important for long term effective OCD repair. In addition, mature subchondral bone repair also favors the integration of repaired cartilage with the surrounding native cartilage [42]. The integration of the newly-formed bone with adjacent subchondral bone may involve many processes, such as bone phase activates cell migration, connection of collagen fibers between newly formed bone tissue and the recipient site, network of vessels at the defect site linking with the surrounding vascular network. As a result of such processes, integration of subchondral bone with native bone could help provide cell source, nutrition, growth factors, and mechanical support for chondral phase regeneration.

4.2. To rebuild a proper crosstalk between the subchondral phase and chondral phase

The interface between the calcified cartilage and the articular cartilage is histologically defined as tidemark [74]. The early studies indicated calcified cartilage and tidemark act as an impermeable barrier preventing the transport of soluble molecules between the articular cartilage and subchondral bone [75]. However, recent studies found there are indirect and direct crosstalk between the articular cartilage and subchondral bone [76] (Fig. 2). Some small molecules, such as water, glucose, nitric oxide, prostaglandin E2 (PGE2), insulin-like growth factor, could diffuse the calcified cartilage, which is so called indirect crosstalk [75,77]. There are many arterial terminal branches in the subchondral region, and some tiny vessels even invade the calcified cartilage zone up to the tidemark. The blood flow perfusion could provide at least 50 % of

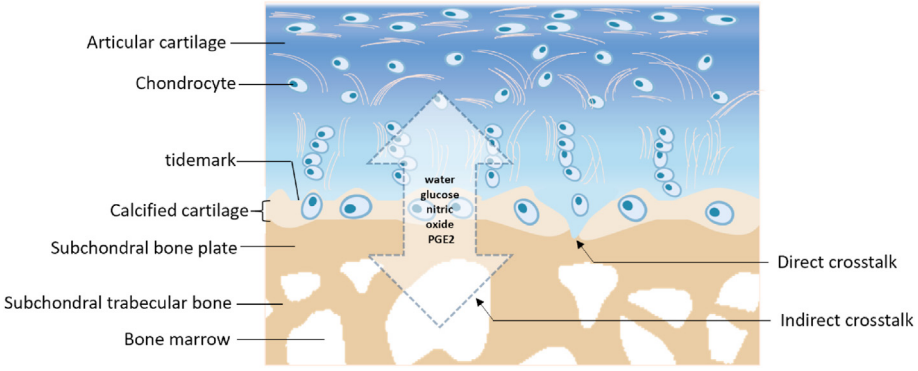

Figure 2. the diagram of direct and indirect crosstalk in the osteochondral unit.

the glucose, oxygen, and water requirement of cartilage [78], and some small molecules from blood vessels can perfuse in to the cartilage [79]. In addition, large molecules may transport through the osteocyte lacuno-canalicular network to the overlaying cartilage [79]. On the other hand, direct bone-cartilage contact is also observed histologically. Some uncalcified cartilage prolongations passing through the calcified cartilage to adjoin the subjacent bone or marrow space [78,80]. As part of the crosstalk network in the osteochondral unit, some biochemical signaling pathways, such as TGF-β/Smad, Wnt/β-catenin, RANK/RANKL/OPG, and MAPK pathways have been proved contribute to the pathogenesis of osteoarthritis [81]. The detailed chondro-osseous crosstalk molecular mechanism has not been fully elucidated but plays a crucial role in the physiological or pathological development in the osteochondral unit [77,82].

Corresponding to the physiological crosstalk between subchondral bone and cartilage [76], the crosstalk design between each layer in a scaffold is essential for tissue regeneration. For example, angiogenesis is necessary for osteogenesis, but vessels invading the chondral layer may lead to fibrocartilage formation instead of desired hyaline cartilage. In the pig OCD model testing a biphasic scaffold from Perez-Silos group [40], it was observed excessive blood vessels in the chondral layer resulted in fibrocartilage formation. Lin et al. [83] found the normal cartilage-derived ECM inhibited angiogenesis, which is advantageous to the capacity of osteochondral ECM scaffold to promote hyaline cartilage regeneration. As demonstrated in many osteoarthritis studies [84], the avascularity and resistance to the invasion of vascular networks are crucial for the physiological and mechanical function of articulating hyaline cartilage.

Some studies have tried to control the crosstalk between bone layer and cartilage layer by specific scaffold design. Seong et al. [85] fabricated a biphasic scaffold with aligned channels in the subchondral bone part and found the channel diameter significantly affect the tissue regeneration. Channels that are too large in diameter (600 μm) may lead to excessive blood supply, resulting in fibrocartilage tissue containing loosely aligned collagen fibers and disordered chondrocytes in the chondral layer; if the channel diameter is too small (140 μm), chondrocytes were irregularly distributed, and cell density becomes insufficient because the channels were blocked before fully repairing. Hyaline cartilage was achieved in the group with a channel diameter of 270 μm, in which BMSCs and other ECM migrate upward sufficiently for cartilage regeneration. Too many blood vessels protruding into the chondral layer will damage the microenvironment for chondrocyte phenotype maintenance and organization, but if the support from the subchondral bone is totally absent, the chondral repair will fail.

In animal experiment, the gradual transition of neo-cartilage band toward the joint surface is observed in both monophasic and multiphasic studies [34,58,86,87] (Fig. 3). It is postulated that the repairing process begins with a rapid influx of host MSCs and growth factors into the lesion

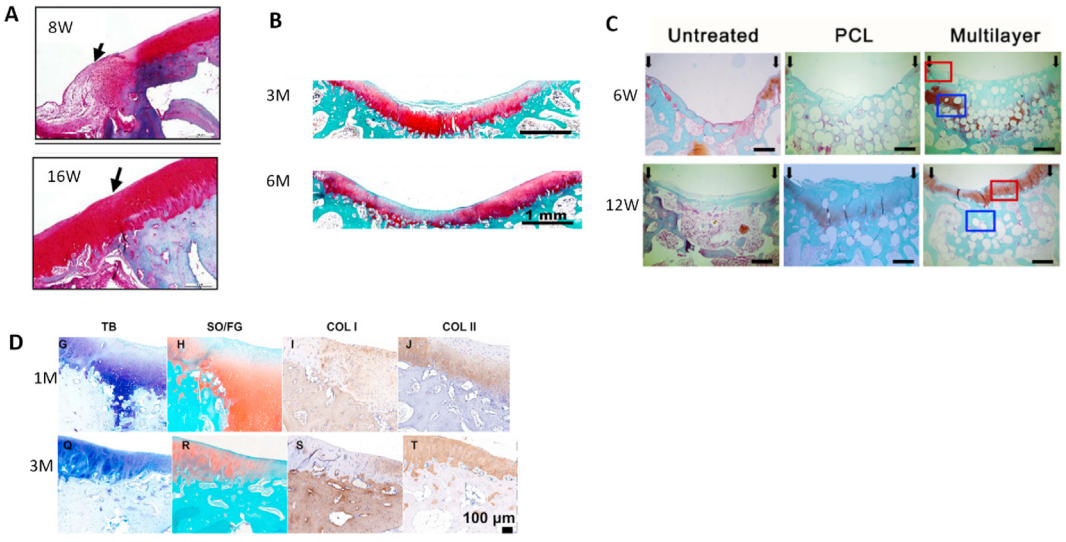

Figure 3. Histological results of studies showing the neo-cartilage band moving up toward the surface. (A) Silicate-based monophasic scaffold [58]; (B) A monophasic scaffold composed of decellularized cartilage matrix (DCM) and functionalized self-assembly Ac-(RADA) $_4$ -CONH $_2$ /Ac-(RA-DA) $_4$ GGSKPPGTSS-CONH $_2$ (RAD/SKP) peptide nanofiber hydrogel [86]; (C) a monophasic scaffold made of icariin (Ica) conjugated hyaluronic acid/collagen (Ica-HA/Col) hydrogel [87]; (D) a multiphasic scaffold consisted of the poly(ε-caprolactone) (PCL) and the hydroxyapatite (HA)/PCL microspheres [34].

site, followed by the formation of immature cartilage formation throughout the defect area. Subsequently, the endochondral ossification occurs with the presence of chondrocyte hypertrophy and vascular invasion. The ossification process began from the deep defect area, resulting in a gradual neo-cartilage band moving up toward the surface. In the regeneration process, the biochemical crosstalk and neo-vascular network between the cartilage and bone regeneration in the lesion site could determine the quality of the repairing tissue.

5. Unmet clinical needs for scaffold-based therapies

As of yet, there is still no consensus regarding the clinical effectiveness of scaffold-based therapies for OCDs repair. For the clinical products in Table 1, one systematic review evaluating the clinical performance of TruFit® for OCD repair found that all included five studies showed improvement at follow-up of 12 months, in which two studies reported deterioration of early improvement; None of the included studies showed evidence for bone ingrowth and the evidence for cartilage regeneration was conflicting [28]. Another systematic review evaluating the clinical performance of MaioRegen® for knee OCD repair reported significant clinical improvement at follow-up of 24 months in 13 studies out of included 16 studies; Available radiological and histological evaluation showed positive results of both subchondral bone and cartilage regeneration [29]. For Biomatrix™ CRD (ClinicalTrials.gov Identifier: NCT02309957) and Agili-C™ (ClinicalTrials.gov Identifier: NCT02423629), data has not been reported yet. Shimozono et al. [88] analyzed the effectiveness of scaffold-based therapies for osteochondral lesion of talus and found it hard to draw a reliable conclusion because of the poor quality of evidence and variability among studies. Here, three challenges to investigate the efficacy of scaffold-based therapies for OCDs were summarized from the literature.

5.1. No clear indication for scaffold-based therapies

The osteochondral defects were not strictly differentiated from deep cartilage defects in clinical practice, and in literature deep cartilage defects were sometimes described as osteochondral defects. However, considering the difference on histological composition and biomechanical property between osteochondral tissue and cartilage tissue, the efficacy of each therapy for osteochondral repair or cartilage repair should be investigated separately. Looking into the outcome of each type of tissue defect is essential to determine the proper and clear indication for different lesions. According to the random clinical trial (RCT) which compared cell-free scaffold therapy with microfracture [89], there was no significant difference on clinical scores after 24-month follow-up if the outcomes of all patients who were diagnosed with chondral or osteochondral defect were analyzed together. However, for the subgroup of patients diagnosed with Outerbridge grade IV and OCD, the scaffold group exhibited a statistically significant better IKDC subjective outcome. This suggested that scaffold-based therapies might be more suitable for deep cartilage lesions and OCDs. Therefore, in future studies, a clear record about the depth of osteochondral defects is needed to differentiate the indication for treatments better.

5.2. Cell usage in OCD repair

In order to regenerate native tissue, resident cells of the target tissue can be embedded in scaffolds, which allow cells to gradually degrade and replace the scaffold and deposit its own extracellular matrix. The initial strategy to regenerate native articular cartilage is to embed autologous primary chondrocytes, such as ACI sandwich technique. However, this strategy involves multiple steps, and the chondrocytes might dedifferentiate, causing some inferior clinical outcomes [90]. Later, other cell sources were applied to avoid secondary operation. For example, bone marrow aspirate concentrate (BMAC) could be used as an effective biologic stimulation to promote tissue regeneration in OAT technique [14]. The addition of platelet-rich plasma (PRP) on OCA could enhance the clot formation in the graft [91]. Cugat et al. [92] combined autologous hyaline cartilage chips obtained from the defect edges with PRP and plasma rich in growth factors (PRGF) to repair OCDs. Vannini et al. [93] used BMAC-infiltrated hyaluronic acid membranes and platelet-rich fibrin gel to treat juvenile osteochondritis dissecans.

On the other hand, with the advancement of biomaterial properties on mimicking the biological and mechanical characteristics of healthy tissues and exploiting the intrinsic tissue regeneration ability, cell-free scaffold therapies have aroused surgeons' interest in recent years [63,94]. Chubinskaya et al. [95] had proved that chondrocytes in fresh human cadaveric articular cartilage tissue could migrate into the Agili-C™ scaffold and produced ECM rich in Col II and GAG in an ex vivo model cultured for 60 days. However, there is still no sufficient research evidence to explain the cell source after implantation for tissue regeneration in cell-free scaffolds. Given the potential negative effects of bone marrow microenvironment on cartilage repair process [96], the mechanism behind osteochondral scaffolds to repair OCDs could be even more complex. In addition, some studies showed that the quality of repaired tissue by cell-free scaffolds was poor, displaying inability to regenerate the associated tissue structures [97,98]. Hence more direct evidence for the justification of cell-free scaffolds is needed, especially a randomized clinical trial to compare cell-laden scaffolds and cell-free scaffolds. Even though some preclinical researches showed positive OCD repairing outcomes using mesenchymal stem cells, chondrocytes or osteoblasts-seeded osteochondral scaffolds [40,42,47,99], so far, no clinical report is available in which combines autologous chondrocytes, BMAC or PRP with osteochondral scaffolds to treat OCD.

5.3. Durability is a concern

All published reviews investigating the efficacy of scaffold-based therapies for OCDs reported overall positive clinical outcomes [29,88,100,101]. But in most of the reported studies, the follow-up time is less than 2 years, and clinical data showing with long-term outcomes is seldom available. Unfavorable subchondral bone changes after surgery could be a concern, especially in time frames beyond 2 years. Gelber et al. [102] and Verdonk et al. [103] reported unfavorable subchondral bone changes after repairing with TruFit® and MaioRegen® osteochondral scaffolds at 12- or 24-months' follow-up on MRI, such as edema, granulation, sclerosis, or cyst. Although the clinical outcomes at 12 or 24 months after surgery measured by KOOS and VAS showed significant improvement after surgery, the negative subchondral bone change might be problematic for long-term clinical outcomes.

Christensen et al. [104] investigated the outcome of OCD repairing using MaioRegen® scaffolds in the knee and talus, and followed up patients with both MRI and CT. The CT results showed none of the ten patients had complete subchondral bone repair, and the MRI showed no improvement in the MOCART score at 1 year and 2.5 years after surgery. Subchondral bone health has been recognized to play an important role in joint disease [105]. Treatments that only focus on repairing the surface cartilage lesions without support from an intact subchondral bed are less likely to achieve good regeneration [106]. For large and deep OCDs, patients might experience initial clinical improvement which is attributed to the debridement or removal of pathological tissue in the joint. However, if there is no bone ingrowth in the subchondral layer to support cartilage regeneration, the clinical improvement might deteriorate in the long term. Therefore, more clinical evidence on the long-term effectiveness of scaffold-based therapies is needed, with particular focus on the regeneration of both cartilage and subchondral bone regions during follow-up investigations.

In summary, the clinical efficacy of scaffold-based therapies for osteochondral repair is still in doubt, especially in subchondral bone remodeling. The present clinical data is insufficient to compare different scaffold-based therapies or summarize appropriate indications for each scaffold-based therapy. It is still unknown which design of scaffold

product is the best based on available research data. Although a variety of scaffolds have yielded positive outcomes in animal experiments [107], the commercialization and application of medical instruments are restricted by corresponding regulations in different countries and areas, as most papers reporting the effectiveness of scaffold-based therapies are from Europe.

Although a majority of studies using TruFit reported positive results and supported the use of this product [108,109], and two studies reported poor tissue regeneration on MRI [98,102], Trufit® scaffold has been withdrawn from the market at the beginning of 2013. Overall, more high-quality clinical trials are required to determine the efficacy of scaffold-based therapies for OCD repair and to define indications for these techniques.

6. Summary

The product lifecycle of medical devices typically follow five phases: Opportunity phase (where clinical needs are identified), concept and feasibility phase (when the feasibility of innovation development is assessed), verification and validation phase (when the innovation is verified with *in vitro* and/or *in vivo* models), product launch preparation phase (where manufacturing and quality control according to Good Manufacturing Practices are implemented) and product launch phase (where market sales and post market surveillance is conducted) [110]. To ensure that the final product has strong clinical applicability and quality, it is essential that experimental studies in the initial phase to be assessed for quality and integrity [111]. This review found two similar issues surfaced from clinical and preclinical studies on OCD repair, which need further addressing in future experiments: cell loading and subchondral repair.

Cell-free approaches are much more convenient and less expensive to repair OCDs, so the attempt to explore cell-free treatment is on-going. So far, the issue is controversial in clinics as the level of currently available clinical evidence comparing cell-free and cell-laden treatment is low [67]. A randomized clinical trial to compare cell-laden and cell-free scaffolds is needed to provide substantial evidence. In preclinical research, many studies showed positive results using scaffolds preloaded chondrocytes in the chondral layer. It could be summarized preloading chondrocytes in the chondral layer could benefit hyaline cartilage regeneration, so the question is whether cell-free scaffolds could achieve the normal repair. Although in preclinical research many cell-free scaffolds are reported showing good histological results, but in animal studies the osteochondral lesion is freshly drilled followed by implanting the scaffold, so bone marrow could penetrate into the scaffolds, thus working as the cell source to complete chondrogenic differentiation in the chondral layer. In this case, the key to achieve hyaline cartilage regeneration is to ensure a proper amount of stem cells entering the chondral layer. Because in clinics, microfracture technique often results in fibrocartilage, and the ACI technique, in which normally the opening of tidemark and subchondral bone is carefully avoided, results in worse outcome if conducted as the revision surgery after microfracture [112], researchers attributed this to the potential negative effects of bone marrow environment disrupting hyaline cartilage regeneration [96]. As shown in the animal study using cell-free scaffold to repair OCD, the diameter of channels linking the chondral phase and bone phase would affect the outcome of cartilage regeneration [85]. Too large or too small channel diameter resulted in inferior histological results. To summarize, for the attempt to develop cell-free approaches for OCD repair, controlling the proper crosstalk between two layers could be a noteworthy direction. The proper crosstalk in the scaffold structure should be capable of attracting enough stem cells from bone marrow while preventing excessive exposure to a multitude of cells and factors in bone marrow and blood vessel penetration as well.

The significance of subchondral bone regeneration for OCD repair has been widely recognized both in clinics and in preclinical researches. Numerous studies using cell-free scaffolds reported good subchondral bone regeneration, while the animal study in which the bone layer was preloaded osteoblasts showed excessive blood vessels formation penetrating into the chondral layer, indicating that it is not necessary to concern the cell source for bone regeneration in OCD repair because the stem cells in bone morrow are sufficient for bone regeneration. The clinical adverse events in the subchondral phase mainly include edema and cyst formation; in animal studies, the most noticeable effect that subchondral bone repair could exert might come from the speed of regeneration, since many studies that reported inferior cartilage repair usually exhibited incomplete filling or some cavities in the subchondral phase, and superior cartilage repair often accompanied better subchondral bone regeneration. Therefore, regarding the subchondral bone repair, the main attention should be paid to the osteoconductivity and osteoinductivity of the subchondral scaffold.

Besides cell loading and subchondral repair issue, unclear surgical indication and procedure also impede the advancement of scaffold studies for OCD repair. More precise surgical indications should be reported, such as the depth of the lesion. A standard protocol to make the OCD model on varied animal species should be established to maximize the translational value of animal experiments to clinical trials.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

This work was funded by Singapore's Ministry of Education Academic Research Fund Tier 2: MOE2017-T2-2-150 and the National Research Foundation (NRF) Central Gap Funding - TGIIT18may0011.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jot.2021.07.008.

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