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OSTEOCHONDRAL EXPLANTS FOR DIARTHRODIAL JOINT DISEASES: BRIDGING THE GAP BETWEEN BENCH AND BEDSIDE

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Abstract

Diarthrodial joint diseases, affecting hundreds of millions of people worldwide, mainly include osteoarthritis and cartilage injuries. No consensus on joint disease models has been achieved so far owing to the complex aetiologies, pathophysiological mechanisms and heterogeneity of disorders. The disease models established using isolated chondrocytes or small animals have the weaknesses of lacking native extracellular matrix and inter-species differences in anatomical and biomechanical cartilage properties. Osteochondral explants (OCEs) from large-animal or human joints present characteristics of native articular cartilage, showing promising potential for application in research on joint diseases. The present review focuses on OCEs and highlights the OCE sources, harvesting techniques, culture systems, applications and future developments. The OCE-centred *ex vivo* system has the potential to develop into preclinical models mimicking human joint diseases to help elucidate disease mechanisms, prompt therapeutic strategies and facilitate the clinical translation of findings in basic research.

Keywords: Articular cartilage defect, articular cartilage repair, *ex vivo* model, osteoarthritis, osteochondral explant.

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List of Abbreviations

AC	articular cartilage
ADAMTS	a disintegrin and metalloprotease with thrombospondin motifs
ATP	adenosine triphosphate
BM-MSC	bone-marrow-derived mesenchymal stem cell
COMP	cartilage oligomeric matrix protein
CT	computed tomography
DMEM	Dulbecco's modified Eagle medium
DMMB	dimethylmethylene blue assay
DMOAD	disease-modifying osteoarthritis drug
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
ERK	extracellular signal-regulated kinase
GAG	glycosaminoglycan
HA	hyaluronan
hADSC	human adipose-derived stem cell
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
IL	interleukin
IPFP	infrapatellar fat pad
IPN	interpenetrating polymer network
ITS	insulin-transferrin-selenium
LDH	lactate dehydrogenase
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein-1
MEK	MAPK kinase
MMP	matrix metalloproteinase
MMT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MRI	magnetic resonance imaging
OA	osteoarthritis
OCE	osteochondral explant
OCT	optical coherence tomography
PGE_2	prostaglandin E_2
PRG4	proteoglycan 4
pro-COL I	pro-collagen type I

qPCR	quantitative polymerase chain reaction
ROCK	Rho-associated protein kinase
ROS	reactive oxygen species
RUNX2	runt-related transcription factor 2
SOX9	SRY-box transcription factor 9
TEM	transmission electron microscopy
TGF	transforming growth factor
TNF-α	tumour necrosis factor-alpha

Introduction

As the most prevalent degenerative diarthrodial joint disease, OA predominantly involves the knees, hips, spines and hands. OA affects approximately 250 million people worldwide and is a leading cause of disability, imposing a remarkable burden on individuals and healthcare systems (Hunter and Bierma-Zeinstra, 2019; Safiri *et al.*, 2020; Vina and Kwoh, 2018).

Various risk factors have been reported to contribute to OA development, including increasing age, female gender, obesity, previous joint trauma and genetic factors (Martel-Pelletier *et al.*, 2016; Silverwood *et al.*, 2015; Vina and Kwoh, 2018). OA is pathologically characterised by progressive AC breakdown, subchondral bone remodelling and synovitis (Hunter and Bierma-Zeinstra, 2019; Martel-Pelletier *et al.*, 2016). Among the clinical manifestations, OA features recurrent joint pain, swelling and deformity. Currently, treatment for OA mainly focuses on pain relief, while no DMOADs have been approved yet to inhibit OA development. For patients with end-stage OA, joint replacement becomes an indispensable choice.

Insufficient understanding of the pathophysiological mechanisms underlying OA has largely hindered the development of novel OA therapies. OA is no longer considered a chronic disease solely related to AC but rather a whole-organ disorder, involving not only AC, subchondral bone and synovium but also adipose tissue, meniscus, tendons and ligaments (Coleman *et al.*, 2016; Hunter and Bierma-Zeinstra, 2019; Robinson *et al.*, 2016). During OA progression, the crosstalk between subchondral bone and AC is enhanced due to increased vascularisation and microcracks at the bone-AC interface; hence, potential therapeutic targets may be identified to intervene in these aberrant signalling pathways (Findlay and Kuliwaba, 2016; Yuan *et al.*, 2014). Synovitis, occurring at both early and late stages of OA, promotes OA development by producing pro-inflammatory and catabolic mediators responsible for AC breakdown (Sellam and Berenbaum, 2010). In turn, AC wear particles can amplify synovitis, thus forming a vicious circle (Estell *et al.*, 2019; Sellam and Berenbaum, 2010). The involvement of IPFP, an intra-articular fat tissue, in OA development has also been proposed (Zeng *et al.*, 2020). Although this interplay within joint tissues has been discovered, the complex underpinning molecular pathways still need to be elucidated to develop effective candidate therapeutics (Robinson *et al.*, 2016).

The abovementioned crosstalks in a native joint cannot be achieved in current *in vitro* models established using isolated chondrocytes. Moreover, present *in vivo* models for OA research predominantly rely on small animals. Due to their anatomical, biomechanical and structural differences compared to human AC, the clinical translation of findings from animal research is often hampered. These limitations could be addressed using OCE models. OCE models are *ex vivo* culture systems established using OCEs extracted mainly from large animal or human diarthrodial joints, which incorporate AC and subchondral bone, and also comply with 3R (replace, reduce and refine) principles of animal experimentation (Cope *et al.*, 2019; Humpenoder *et al.*, 2021; Kleuskens *et al.*, 2021; Schwab *et al.*, 2017). Thus, an OCE model is a useful preclinical tool mainly to aid in exploring OA mechanisms, drug screening and AC regeneration strategies. However, it might be unsuitable to apply an OCE model for demonstrating the pharmacokinetics and systemic side effects of therapeutics. This review is focused on OCE models, while they are also compared with *in vitro* cell models and *in vivo* animal models. Moreover, OCE harvesting and their culture or co-culture systems are explained in detail. Furthermore, the current applications of OCEs are highlighted, followed by suggestions of future directions for OCE development.

Comparison of current OA models

Models for OA research can be classified as cell culture models (*in vitro*), animal models (*in vivo*) and OCE models (*ex vivo*). For *in vitro* models, 2D or 3D cultured chondrocytes are widely used. For *in vivo* models, animals are used to establish spontaneously occurring or induced OA models. As for *ex vivo* OA models, tissues harvested from joints are cultured to emulate *in vivo* OA-like conditions. The advantages and disadvantages of these models are summarised in Table 1.

2D cell culture model

The 2D monolayer culture model is still widely used due to the easy cell harvesting, cell seeding and controlled experimental conditions. Additionally, the cells show a speedy response to altered environmental conditions, the addition of cytokines or potential pharmacological substances (Singh *et al.*, 2021). It is also straightforward to rapidly expand cells to the needed amount.

2D co-culture models with multiple cell types within a shared milieu are also well-developed. Effects of co-cultured osteoblasts on chondrocytes gene expression have been explored (Lin *et al.*, 2010; Sanchez *et al.*, 2005). Synoviocytes co-culture with chondrocytes contribute to a rapid formation

Table 1. Comparison of currently used OA models.

Model type	Characteristics	Advantages	Disadvantages
2D cell culture	Monolayer culture in plastic or glass dishes	• Rapid cell expansion • Reproducibility • High throughput possible	• Prone to dedifferentiation with altered phenotype • Limited growth directions • Do not represent native tissue composition of the joint
3D cell culture	Cell-laden scaffold or cell pellet	• Reproducibility and controllability • Suitable to evaluate material properties for AC repair	• Absence of native tissue or *in vivo* condition • Do not represent AC layers as *in vivo*
AC explants	Full-thickness AC culture	• Easy and cheap to produce • Possess natural cell and ECM components • Maintain cell-cell communication	• Prone to create AC damage • Difficult to get homogeneous explants • Without oxygen and nutrient gradients • More cell outgrowth
Osteochondral explants	Osteochondral plug culture	• Close to natural joint microenvironment • Easy and cheap to produce • Maintain inter-tissue interactions • Conform to 3R principles • Useful preclinical tool for clinical translation • Feasible for screening drugs and cartilage defect repair	• AC matrix degeneration over time • Tissue viability decrease during long-term culture • Limited availability of explants originated from human samples
Animal models	Performed using small or large animals	• Mimic OA initiation and progress in natural joints • Include all local and systemic tissues and cells	• AC thickness too thin in small animals, not representing human AC • High cost with large animals • Long-time expenditure • Species disparity

of chondrocyte sheets for AC repair (Kokubo *et al.*, 2016). However, cells in planar culture are prone to dedifferentiation and lose their morphology after only a few passages (von der Mark *et al.*, 1977). As loss of AC ECM is central to OA, the inability to explore the cell-ECM crosstalk also limits the application of 2D culture models.

3D cell culture model

3D cell culture models include pellet and scaffold-based cultures. The former, also named micromass culture, aggregates cells into a high density using a centrifuge or hanging-drop method (Singh *et al.*, 2021). Caron *et al.* (2012) proved that pellet cultures of human articular chondrocytes exhibit a better chondrogenic potential as well as a decreased expression of hypertrophic markers in comparison with 2D cultured cells. Schlichting *et al.* (2014) successfully established an OA-like model using porcine chondrocyte micromass culture treated with TNF-α.

Scaffolds for 3D culture can be made of natural or synthetic biomaterials and decellularised ECM (Benders *et al.*, 2014; Campos *et al.*, 2019; Xia *et al.*, 2019). Numerous compositions and techniques have been developed to better emulate the microstructure of natural tissues in terms of elasticity, strength, porosity, biocompatibility and biochemistry. These scaffolds are widely used to repair AC defects in preclinical studies, to study the pathogenesis of OA and to screen candidate drugs (Lozito *et al.*, 2013; Rai *et al.*, 2017).

The 3D culture model shows high reproducibility and controllability. Additionally, it is better for investigating cell-cell or material-cell interaction compared with the 2D culture model. Nevertheless, it still cannot reflect the native tissue microenvironment with regard to physiological complexity and gradients of oxygen and nutrients in the AC (Schwab *et al.*, 2017).

Animal model

Several animal species and methods have been used as *in vivo* models to simulate the onset and progression of OA (McCoy, 2015). As for classification, they include chemically or surgically induced models as well as naturally occurring or genetically modified spontaneous models (Kuyinu *et al.*, 2016). Although small animal models established using mice and rats are relatively inexpensive, rapidly generated and can be genetically modified, AC thickness and layers in small animals are different from humans, leading to difficulties in tissue extraction and clinical translation (McCoy, 2015; Seok *et al.*, 2013). With large animals, such as sheep, goats and dogs, the process of establishing OA is time-consuming, expensive and

challenging to control (McCoy, 2015). Animal studies also raise ethical considerations against 3R principles.

OCE model

To overcome ethical issues and maximally simulate the natural joint environment, *ex vivo* models established using AC or OCEs have been developed. The tissue explant model is inexpensive and easy to produce and control (Johnson *et al.*, 2016). Research on cell-cell, cell-ECM and inter-tissue crosstalk is possible within native tissue surroundings (Cope *et al.*, 2019). AC is avascular and aneural, facilitating its *ex vivo* culture. Schwab *et al.* (2017) demonstrated that OCEs could maintain tissue viability for up to 56 d in a system that separates bone and AC into two compartments and provides a tissue-specific medium. By comparing OCEs with cartilage-only explants, other studies have found that soluble factors released by subchondral bone preserve chondrocytes' survival and expression of anabolic genes (Amin *et al.*, 2009; de Vries-van Melle *et al.*, 2012). Furthermore, subchondral bone plays a key role in inducing chondrogenesis of mesenchymal stem cells in an osteochondral environment (de Vries-van Melle *et al.*, 2014). It is easier to obtain intact AC for OCEs than AC explants without causing damage to the deep layer of AC during explant harvest. Studies have shown that with OCEs from animals, the onset and development of early OA can be modelled (Byron and Trahan, 2017). With OCE plugs from human OA joints, potential therapeutics can be evaluated, without species disparity (Geurts *et al.*, 2018). Vainieri *et al.* (2018) established a bovine OCE defect model to evaluate biomaterials for AC repair. As a preclinical tool, OCEs from native joints of large animals or humans outperform cell culture models and small animal models with regard to the clinical translation of findings.

OCE sources and harvest

An OCE unit (Fig. 1) is composed of intact AC, subchondral bone, part of cancellous bone and bone marrow, closely representing the AC milieu in a native joint. As the sole cell type in AC, chondrocytes are regularly distributed within three characteristic zones, namely the superficial, middle and deep zone (Hunter and Bierma-Zeinstra, 2019). Calcified cartilage is a transition zone between the tide mark and subchondral bone, which includes various cells such as osteocytes, osteoblasts and osteoclasts. Vessels and sensory neurons could invade subchondral bone, contributing to joint pain and interaction with AC during OA progression (Hu *et al.*, 2020). Since OCEs are usually extracted from joints of adult donors, the bone marrow cavity in the cancellous bone is filled with yellow bone marrow, which mostly contains adipocytes (fat) and mesenchymal stem cells, rather than haematopoietic stem cells and blood cells. Lipid droplets are clearly seen floating at the surface of the medium during both bovine and human OCE culture, suggesting that bone marrow components may drain during *ex vivo* culture without the protection of a closed cavity.

OCE sources

The main sources of OCEs are joints from animals and humans (Table 2**a**,**b**). As for animals, healthy fetlock or stifle joints from large animals (such as bovine,

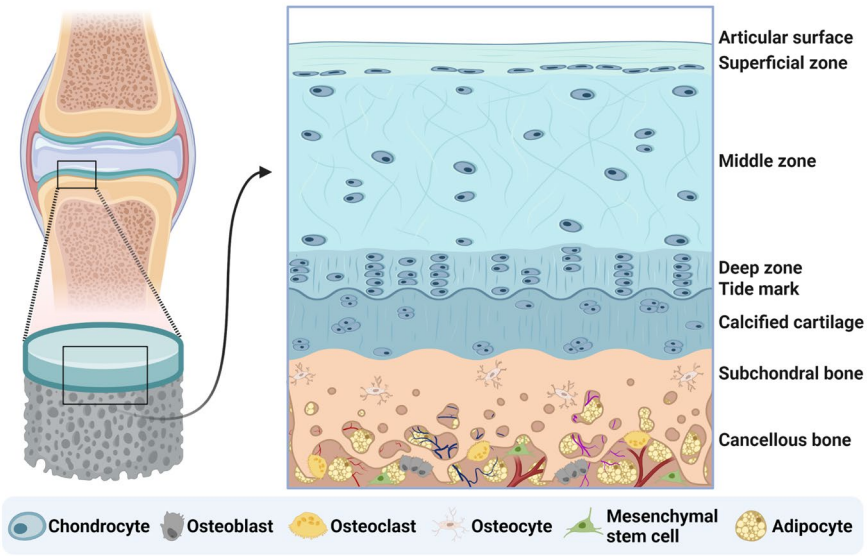

Fig. 1. OCE microstructure. The left part shows the OCE column isolated from the diarthrodial joint comprising the full-thickness AC and underlying bone. The right part illustrates the microstructure of the whole OCE unit. The AC is longitudinally divided into a superficial zone, middle zone, deep zone, tide mark and calcified cartilage. The corresponding subchondral and cancellous bone consists of various cells, such as osteocytes, osteoclasts, osteoblasts, mesenchymal stem cells and adipocytes. Figure made using Biorender.

Table 2a. Representative OCE sources and characteristics of reported studies on diarthrodial joint diseases.
[1] Sizes presented with diameter followed by height or depth. NA, not available.

Application	Study	Species; age; joint or region	OCE size[1]	Defect size[1] or other intervention	Culture duration	Major readout; aim or finding
AC defect	Vainieri *et al.*, 2018	Bovine; 3-5 months; femoral condyles	7.6 mm; 6.1 mm	4 mm; 3 and 4 mm defect; mechanical load	10 d	Tissue viability, histology, gene expression; establishment of a mechanically loaded OCE model for evaluation of biomaterials in AC repair
AC defect	Schwab *et al.*, 2017	Pig; 5-7 months; medial femoral condyles	8 mm; 5 mm	1, 2 and 4 mm; full thickness defect	28 d	Tissue viability, histology, biochemical analysis; critical size AC defect in the OCEs as an *ex vivo* platform for assessment of AC repair strategies
AC defect	Mouser *et al.*, 2018	Equine; 3 and 5 years; metacarpophalangeal joints	8.5 mm; 4 mm	4 mm; full thickness defect	57 d	Histology, GAG, DNA; OCE model unravelling cell distribution in hydrogels influencing AC repair
AC defect	de Vries-van Melle *et al.*, 2012	Bovine; 3-8 months; metacarpophalangeal joints	8 mm; 5 mm	6 mm; chondral, subchondral and osteochondral defect	28 d	Histology, qPCR, tissue viability; OCE model to study AC repair treatments and mechanisms
AC defect	Theodoropoulos *et al.*, 2011	Bovine; 6-9 months; metacarpophalangeal joints	15 mm; NA	4.5 mm; 10 mm	8 weeks	Histology, DNA, collagen content, TEM; OCE model to evaluate integration of tissue engineered cartilage with host AC
AC defect	Yeung *et al.*, 2018	Human; 67-82 years; knee joints	3 mm; 5-6 mm	1 mm; NA	8 weeks	Histology, GAG, ELISA; AC defect established using OCEs from human OA joints – feasible for evaluation of emerging AC therapies
OA	Haltmayer *et al.*, 2019	Horse; 11, 17 and 18 years; medial femoral condyles	6 mm; NA	10 ng/mL IL-1β, TNF-α and 2 mm partial defect	21 d	Histology, nitric oxide, urea, ELISA; co-culture of synovium and OCEs treated with inflammatory cytokines in combination with partial thickness AC defect as an *ex vivo* OA model
OA	Houtman *et al.*, 2021	Human; NA; knee joints of OA patients	8 mm; NA	10 ng/mL IL-1β, 10 nmol/L triiodothyronine, 65 % strain	13 d	GAG, qPCR, histology, mechanical test; OCEs treated by three different triggers reflecting reliable biomimetic models for OA investigation and treatment
OA	Kleuskens *et al.*, 2021	Human; 54-84 years; smooth and fibrillated tibia plateaux	10 mm; 4 mm	No treatment	4 weeks	Histology, tissue viability, biochemical analysis; establishment of an *ex vivo* model with human OCEs at different OA stages
OA	Byron and Trahan, 2017	Horse; NA; femoro-patellar joints	7-9 mm; 6 mm	10 ng/mL IL-1β; synovium coculture	4 d	PGE_2, TNF-α, MMP13, DMMB, LDH assays; different effects of IL-1β on the co-culture system of OCE and synovium compared with AC explant
OA	Geurts *et al.*, 2018	Human; 72 ± 5.7 years, knee tibial plateaus; 74 ± 5.9 years, facet joints of spine	NA	1 μg/mL lipopolysaccharides	7 d	MTT staining, ELISA; human OCE model of knee and spine OA joints enabling assessment of responses to drug treatment
OA	Li *et al.*, 2021	Human; 59 - 86 years; femoral heads	8 mm; 5 mm	1, 5, 10 ng/mL IL-1β and TNF-α	7 d	Tissue viability, histology, qPCR, ELISA, GAG; establishment of an inflammatory OA model using human OCEs
AC injury	van Haaften *et al.*, 2017	Porcine; 5-7 months; femoral condyles	8 mm; 4 mm	Indentation loading, 25 N, 5 cycles at 120 mm/min in 20 s	13 d	Tissue viability, proteoglycan and collagen content; OCEs to investigate initial AC damage by indentation injury

Table 2b. Representative OCE sources and characteristics of reported studies on diarthrodial joint diseases. [1] Sizes presented with diameter followed by height or depth. NA, not available.

Application	Study	Species; age; joint or region	OCE size[1]	Defect size[1] or other intervention	Culture duration	Major readout; aim or finding
AC injury	Martin *et al.*, 2009	Bovine; NA; tibial plateaux	25 mm; 4-8 mm	Impact loading, 7, 14 J/cm^2	14 d	Histology, tissue viability, GAG; early treatment with N-acetylcysteine after impact injury to OCEs reducing cell death and stabilising AC
Osteochondral grafts	Kang *et al.*, 2010	Bovine; 6-8 months; stifle trochleae	8 mm; 10 mm	Impact loading, 37.5 N 74 hits, 75 N 37 hits, 150 N 21 hits, 300 N 11 hits	8 d	Tissue viability, histology, nitric oxide and GAG release; impact loading parameters influencing tissue viability of OCEs as grafts
Diagnosis	Williams *et al.*, 2010	Human, cadaveric samples; 18, 76, 77 years; tibial plateaux	8.5 mm; NA	No treatment	No culture	MRI; human OCEs to assess AC degeneration with ultra-short echo time UTE T_2^* mapping
Diagnosis	Stewart *et al.*, 2013	Bovine; NA; femoral condyles	7 mm; NA	No treatment	No culture	CT; OCE model to test a high-affinity cationic contrast agent CA^{4+} to GAG for high-quality imaging *ex vivo*
Diagnosis	Bear *et al.*, 2010	Human; NA; knee joints	8.5 mm; NA	No treatment	No culture	OCT, MRI; human OCE models suggesting the sensitivity of MRI T2 mapping and OCT to changes of collagen structure in AC
Diagnosis	Griebel *et al.*, 2013	Human; 68.1 ± 9.6 years; different regions in knee joints	6.0 mm; 3.5 mm	No treatment	No culture	MRI, dualMRI under loading, histology; assessment on OCEs showing the potential of dualMRI as a novel diagnostic method for early OA

equine, ovine and porcine) are widely used. Using OCEs from healthy animal joints, either mechanically or chemically induced OA models can be established to mimic the initiation and progression of early OA (Byron and Trahan, 2017). AC properties of large animals are more similar to humans, while they are quite different in small animals (McCoy, 2015). In mice, AC thickness is around 30 μm, lacking distinct layers from the superficial surface to the deep zone (Glasson *et al.*, 2010). In addition, the calcified cartilage layer in mice is equal to or even thicker than the noncalcified cartilage layer (Glasson *et al.*, 2010). In small animals, their small size restricts the amount of AC tissue that can be obtained for biochemical tests. AC thickness in horses ranges from 1.5 mm to 2.0 mm, which is close to the 2.2-2.5 mm in humans (Frisbie *et al.*, 2006; Malda *et al.*, 2012). Additionally, other properties of horse AC, including anatomical structure, biochemical composition and biomechanical characteristics, more closely resemble those of human AC (Frisbie *et al.*, 2006; Malda *et al.*, 2012). Furthermore, samples from large animals are easy to get from abattoirs without requirement of an ethical approval for animal experimentation. Thus, large animals are commonly used for generation of OCEs for AC research.

OCEs can also be harvested from knee joints or femoral heads of patients undergoing joint replacement, mainly due to late-stage OA (Li *et al.*, 2021b). In most experiments, OCEs are extracted from regions with macroscopically intact AC. However, depending on the research question, different levels of degenerated AC should be included, for example to investigate the function of a certain gene in OA progression. The AC lesion of the femoral head, a sphere-like structure, is often limited to the top loaded region. Thus, several OCEs can be isolated from other regions, which are comparable regarding anatomical and biomechanical properties. However, it is not easy to obtain several comparable OCEs from a knee joint. Usually, meniscus-uncovered AC severely degenerates. AC and subchondral bone in the meniscus-covered region have a different thickness, biomechanical property and quantity compared with exposed AC (Thambyah *et al.*, 2006). In knee replacement surgery, the remaining subchondral bone is often too thin to extract OCEs. Nevertheless, OCE samples from humans have important advantages over those from animals. There is no species discrepancy with OCEs from native human joints, which facilitates the clinical translation of findings. Furthermore, the variability among donors exactly reflects the variability in the clinical setting. However, limited sources hamper the research using human-originated OCEs.

OA prevalence differs widely in different joint sites, with structural OA reaching around 60 % in the hands, 33 % in the knee joints and 5 % in the hip in adults over 65 years in North America and Europe (van Saase *et al.*, 1989). OCEs extracted from different joint sites might also respond differently to *ex vivo* stimulation. Geurts *et al.* (2018) found that OCEs from human spine facet and knee joints display different basal protein secretion levels of pro-COL I, IL-6 and

MCP-1. Upon inhibition of TGF-β receptor type I signalling under inflammatory conditions, MCP-1 was remarkably upregulated in knee OCEs but not in facet OCEs (Geurts *et al.*, 2018). This variation in cartilage might arise from different native-joint anatomical structures and different risk factors for development of OA in different joint sites (Martel-Pelletier *et al.*, 2016). Since few studies have focused on this variation, more research is expected to use OCEs from different joint sites to unravel joint-specific OA mechanisms.

Osteochondral properties vary even though samples are harvested from the same joint. Using MRI, Li *et al.* (2005) revealed the diversity of cartilage thickness distribution in the human tibiofemoral joint. The regions of AC-to-AC contact on the medial femoral condyle, lateral femoral condyle and medial and lateral tibial plateau are 40 %, 20 %, 40 % and 50 % thicker than the regions without AC-to-AC contact, respectively (Li *et al.*, 2005). Thambyah *et al.* (2006) observed that the thickness of the calcified layer and subchondral bone quantity in exposed AC in the human tibia is significantly reduced in the regions covered by the meniscus. Further *ex vivo* studies showed different responses to mechanical loading among AC from different regions. Compression loading on cylindrical AC disks extracted from seven different regions of canine hind joints indicated the mechanical property of the femoral AC is stiffer than that of the tibial AC, with the femoral groove being the stiffest (Li *et al.*, 2021a). After being exposed to impact compression, porcine OCEs from meniscus-covered AC present an inferior resistance with evidence for lower peak impact stress and smaller AC volume from day 7 to day 14 (Yeow *et al.*, 2009). A similar OCE study showed that in comparison with the uncovered AC, the AC covered by menisci has a larger water content as well as a lower density of superficial collagen layer and subchondral bone, which makes it more susceptive to direct mechanical loading after meniscectomy and prone to develop OA (Iijima *et al.*, 2014). Although these studies revealed different mechanical, histological and structural properties among AC from different regions of the same joint, no differences regrading cell response or molecular mechanisms have been identified. On one side, further studies using OCEs from different regions should focus on potential differences in cellular phenotypes, which might be helpful to unravel the mechanisms of post-traumatic OA and optimise chondrocyte source selection for tissue engineering. One the other side, extracting OCEs from the same AC region may minimise variations in preclinical experiments.

OCE harvest methods and readouts

The equipment and techniques for harvesting OCEs vary greatly among different laboratories. Generally, a trephine or drill is first used to get an osteochondral cylinder with the desired diameter. Then, the requested height of an OCE is obtained using a circular saw (Vainieri *et al.*, 2018). Moreover, a punch with a smaller diameter than the OCE can be used (Vainieri *et al.*, 2018) for establishing an AC defect with a different depth. To date, no consensus has been reached concerning the optimal size of OCEs. However, the diameter of an OCE is around 8 mm and the height ranges from 4 to 6 mm in most studies (Table 2**a**,**b**). Due to the poor self-healing ability of AC in large animals and humans, an AC defect with a diameter of 1 mm is also regarded as the critical AC defect in OCE models (Schwab *et al.*, 2017). Depending on the study aim, either a partial-thickness, full thickness or osteochondral defect should be generated.

To overcome the scarcity of human samples, Spinnen *et al.* (2021) proposed the idea of sliced OCEs with a thickness of only 500 to 800 μm, which could yield up to 100 replicates from one single human sample by a simple process. Over a 21 d culture, the slices behaved similarly to conventional punched OCEs and showed promising applicability for large-scale preclinical assessment (Spinnen *et al.*, 2021).

According to previous OCE studies (Table 2**a**,**b**), usually, both culture medium and OCE samples are collected for further analysis after stimulation and/or treatment. The medium is used to analyse the mediators/metabolites secreted by AC, while AC tissue is excised for gene expression analysis, protein extraction, GAG/DNA assay and histological evaluation. To assess OA progression and promotion of AC regeneration, readouts closely related to anabolic/catabolic metabolism and inflammation are generally applied. The most frequently used anabolic markers are collagen type II, aggrecan, COMP and PRG4 or lubricin. Catabolic markers, namely proteolytic enzymes, widely tested are MMPs and ADAMTS. Inflammatory markers such as IL-1β, IL-6, IL-8, TNF-α, nitric oxide and PGE_2 are often assessed. In addition, chondrogenic markers SOX9 and TGF-β, dedifferentiation marker collagen type I, hypertrophy marker collagen type X and trans-differentiation marker RUNX2 can be taken into account based on the experimental purposes. The above-mentioned markers can be evaluated at the transcriptional level by qPCR, at the protein level by western blot, immunostaining or ELISA, or through functional assays.

OCE culture

After extraction, OCEs can be equilibrated in the medium for 24 h to rule out samples with potential contamination or low metabolic activity. Media with different compositions have been reported for culturing OCEs and there is no consensus on which composition is superior. To make the study feasible, the medium should keep the OCEs viable and allow for minimal GAG release during the culture. As a basic medium, DMEM with high glucose concentration (25 mmol/L) has widely been

used in OCE culture. The studies choosing foetal bovine serum as a supplement are suggested to be comparable to those using ITS + Premix, although no research has specifically compared their effects on OCEs. Ascorbic acid has often been added because it is reported to help stimulate the synthesis of both collagen and aggrecan in AC explants (Clark *et al.*, 2002). Dexamethasone could enhance the biochemical content and mechanical properties of juvenile bovine AC explants after a 2-week culture (Bian *et al.*, 2010). Sodium pyruvate and non-essential amino acids can also be beneficial for the anabolism of the explants. As a buffering agent, HEPES has been applied in some studies. Depending on the purpose of the research, OCEs can be cultured under different conditions and with different stimuli (Fig. 2). The culture period and readouts of OCEs should depend on the research purposes. For evaluation of AC repair strategies, a long-term culture duration of around 8 weeks is recommended, whereby assessment of tissue viability should be a priority. Moreover, biochemical, histological and transcriptional analysis of OCEs and culture medium are possible.

OCE culture in separated compartments

OCEs cultured in separate compartments with the tissue-specific medium can maintain cell viability and tissue ECM integrity for at least 56 d (Schwab *et al.*, 2017). The AC medium in the upper compartment consists of DMEM high glucose, 1 % ITS + Premix, 50 μg/mL L-ascorbic acid-2-phosphate, 100 nmol/L dexamethasone, 40 μg/mL L-proline and antibiotics, while the bone medium in the lower compartment comprises DMEM high glucose, 10 % foetal bovine serum, 50 μg/mL L-ascorbic acid-2-phosphate, 100 nmol/L dexamethasone, 10 mmol/L β-glycerophosphate and antibiotics (Schwab *et al.*, 2017). Moreover, the separated system is suitable for investigating the tissue crosstalk at the bone-AC interface because no interaction with other tissues exists (Kleuskens *et al.*, 2021).

Mechanical loading on OCEs

Mechanical stimuli play a protective role in AC development and maintenance while overloading acts as a detrimental risk factor for AC degeneration. Thus, the mechanical component should not be neglected in AC studies. Bioreactors are devices able to recapitulate joint kinematics, including compression and shear stress (Peroglio *et al.*, 2018; Wimmer *et al.*, 2004). 1 h of mechanical compression (0.1 Hz, 5 % strain) superimposed with shear stress (0.6 Hz, ± 60° oscillation) applied by a ceramic ball twice daily during 3 consecutive days upregulates the expression of important ECM proteins, such as COMP, in AC explants (Wimmer *et al.*, 2004). Similarly, Vainieri *et al.* (2018) showed that 1 h of mechanical loading (0.5 Hz, 10-26 % strain, ± 25° oscillation) performed twice per day for 5 consecutive days elevates anabolic gene expression in chondrocyte-seeded scaffolds implanted into AC defects in bovine OCEs, which could be used as a promising *ex vivo* model to screen new biomaterials. Van Haaften *et al.* (2017) showed that mechanically induced damage in porcine OCEs is not followed by an initial healing response due to low cell viability after overloading, implying irreversible

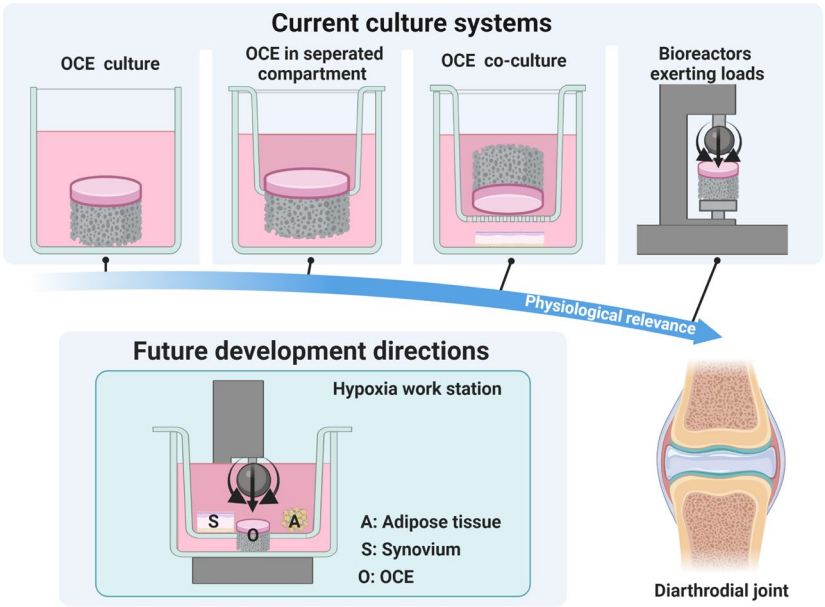

Fig. 2. Current culture systems for OCEs and future development directions. Several OCE culture systems have been developed sequentially according to their physiological relevance: OCE monoculture; OCE monoculture with overlying AC and subchondral bone in separated compartments incubated with different culture media; OCE coculture with synovial tissue; OCE culture system with applied mechanical loading. In the future, a more comprehensive OCE culture system is preferred, which takes into account synovium, adipose tissue, mechanical loading and separated culture compartments in a hypoxia workstation. Figure made using Biorender.

AC degeneration following injury. Using bovine OCEs, it has been demonstrated that physiological strains (0.25 MPa compression, 0.5 Hz) for 3 h/d on 7 consecutive days contributes to mitochondrial ROS production, ATP and matrix synthesis in the AC, while repeated overloading (1.0 MPa compression, 0.5 Hz) results in mitochondrial dysfunction, a pathophysiological mechanism in OA (Coleman *et al.*, 2016; Wolff *et al.*, 2013). Mechanically loaded tissue culture provides a setting closer to the *in vivo* joint, facilitating the preclinical relevance and clinical translation (Peroglio *et al.*, 2018).

OCE co-culture systems

OA is a diarthrodial articulation disease affecting the tissues of the entire joint, including AC, synovium, subchondral bone, meniscus and IPFP (Coleman *et al.*, 2016; Hunter and Bierma-Zeinstra, 2019). Intra-articular tissue interaction could accelerate OA development (Estell *et al.*, 2019; Findlay and Kuliwaba, 2016; Sellam and Berenbaum, 2010; Zeng *et al.*, 2020). The OCE itself is a co-culture model of AC and subchondral bone, in which the latter could help to preserve AC viability (Amin *et al.*, 2009; de Vries-van Melle *et al.*, 2012). Co-culturing of OCEs with other intra-articular tissues makes this *ex vivo* system more similar to the native joint milieu.

Haltmayer *et al.* (2019) found that OCEs co-culturing with synovial explants better mimics the OA condition than OCE monoculture with macrophages, as indicated by the presence of pro-inflammatory phenotype (M1) macrophages in the former system. In another inflammatory OA model established using AC explants or OCEs in combination with synovium, the AC response in the groups significantly differed, especially in terms of MMP-13 expression (Byron and Trahan, 2017). In human osteoarthritic AC culture systems with or without synovial tissue, it was observed that synovium inhibited proteoglycan production, which could be relieved by triamcinolone treatment (Beekhuizen *et al.*, 2011). Another study showed that bovine synovium and fibrous joint capsule could increase aggrecanase and MMP activity of the co-cultured bovine AC explants (Sward *et al.*, 2017).

Nishimuta *et al.* (2017) observed higher GAG release in bovine AC and IPFP co-culture than in the isolated AC culture, although the GAG content in AC explants showed no remarkable alterations. In a pig adipose tissue and AC explant co-culture system, adipose tissue could reduce AC tissue viability and inhibit increased PGE_2 release from AC explants stimulated by LPS (Pearson *et al.*, 2020).

Above-mentioned studies revealed the crosstalk between AC and synovium or adipose tissues. The latter influenced AC degeneration by changing either anabolic/catabolic metabolism or inflammation of AC. Tissue interactions found in the *ex vivo* systems are consistent with previous discoveries about OA (Chang *et al.*, 2018; Sanchez-Lopez *et al.*, 2022), suggesting OCE co-culture systems could be used as a promising tool to explore OA mechanisms and possible interventions. However, no studies have evaluated the tissue viability of synovium and adipose tissue. As both tissues have vascular and neural support in native joints, it is important to first evaluate their viability after long-term *ex vivo* culture. Additionally, no consensus has been achieved on the weight ratio of AC and co-cultured tissue, which may also influence their interaction and the experimental results.

OCE applications and main findings

As an *ex vivo* model, OCEs are mainly applied in OA research and AC repair (Fig. 3). In chemically or mechanically induced OA-like models, inflammatory or post-traumatic OA phenotypes can be reproduced using OCEs. With this model, the underlying mechanisms of onset and development of OA can be investigated in addition to potential therapeutics. OCEs with different types of AC defect can be utilised as a tool to explore the applicability of biomaterials with or without embedded cells for AC repair. Furthermore, OCEs have also been used in other fields related to diarthrodial joint diseases (Fig. 3).

Investigating OA mechanisms

Numerous previous studies have uncovered the transcriptional, biochemical and histological characteristics of human OA joints. Due to the high heterogeneity and complexity of OA, to date, no model has been able to completely simulate authentic native OA onset and progression. OA has been classified into several phenotypes, mainly according to its heterogeneity (Van Spil *et al.*, 2019). Researchers have used OCEs to simulate a certain phenotype of OA according to its pathogenic mechanisms. Houtman *et al.* (2021) successfully established three different biomimetic OA models, including an inflammatory, mechanically induced and hypertrophic phenotype using human OCEs. Such *ex vivo* models reflecting different OA phenotypes could help to evaluate tailored treatments. Using rat OCEs as well as chondrocytes, researchers have demonstrated that TGF-α stimulates AC degradation by activating Rho/ROCK, MEK/ERK signalling pathway and endothelin receptor A, suggesting novel therapeutic targets in OA (Appleton *et al.*, 2010; Usmani *et al.*, 2012). In a bovine OCE injury model caused by impact loading, agents promoting cytoskeletal dissolution could mitigate oxidant release from mitochondria by disrupting mitochondrial-cytoskeletal linkage and reduce chondrocyte mortality, suggesting new approaches to prevent post-traumatic OA (Sauter *et al.*, 2012).

Exploring biomarkers and radiographic techniques for early OA diagnosis

Identifying novel early OA biomarkers following clinical studies is very challenging because patients

with early-stage OA are usually asymptomatic. Clutterbuck *et al.* (2011) identified many secreted proteins involved in ECM functions in an inflammatory culture system of equine OCEs by high throughput techniques, which may serve as a reference for discovering candidate biomarkers in human early-stage OA. In a canine OCE impact injury model, PGE_2 was found to be correlated with impact severity; hence, this study may justify the clinical use of PGE_2 as a biomarker to monitor early post-traumatic OA after joint trauma (Waters *et al.*, 2015). Using human OCEs, correlations were revealed among proinflammatory and catabolic biomarkers, histological scoring, ECM content and tissue viability (Werner *et al.*, 2021). Further analysis of the data may provide individual diagnostic and therapeutic targets for OA (Werner *et al.*, 2021). The secreted form of clusterin, possessing AC protective function, was attenuated by inflammatory cytokine stimulation in equine OCEs, so its downregulation in synovial fluid can potentially be a novel candidate biomarker for OA (Matta *et al.*, 2021). Overall, OCE models could be invaluable preclinical tools to explore OA biomarkers. Moreover, OCEs are helpful to test new diagnostic techniques for early OA. In bovine and rabbit OCEs, a cationic iodinated agent was demonstrated to be highly sensitive to the negatively charged AC; thus, its application in contrast-enhanced CT may be helpful to monitor AC degeneration (Stewart *et al.*, 2013). OCEs from the human tibial plateau were widely used to evaluate non-invasive imaging techniques such as MRI and OCT to optimise mapping methods for the clinical detection of early OA (Bear *et al.*, 2010; Griebel *et al.*, 2013; Williams *et al.*, 2010).

Screening potential disease-modifying OA drugs and assessing clinically applied drugs

Numerous disease-modifying OA drugs have been discovered in cell culture or small-animal OA models, but none exist in clinics due to translational obstacles. OCE models are suitable preclinical tools to screen and assess potential OA drugs before their clinical translation. Geurts *et al.* (2018) successfully created an inflammatory OA model using OCEs from human joints and further demonstrated that inhibitor of TGF-β signalling could mitigate inflammation; hence, this culture system could be considered as a preclinical model to aid in the validation of new drugs (Geurts *et al.*, 2018). In an AC injury model of bovine OCEs subjected to a single impact, immediate exposure to drugs such as N-acetylcysteine and rotenone proved to reduce chondrocyte death and protect AC integrity (Goodwin *et al.*, 2010; Martin *et al.*, 2009). The AC of bovine OCEs pre-treated with an IPN was shown to improve the tribological function of AC during the following friction test (Cooper *et al.*, 2017). In a canine OCE model, bupivacaine, a local anaesthetic, showed cytotoxic effects on chondrocytes and should, thus, not be recommended for clinical use (Hennig *et al.*, 2010). On the other

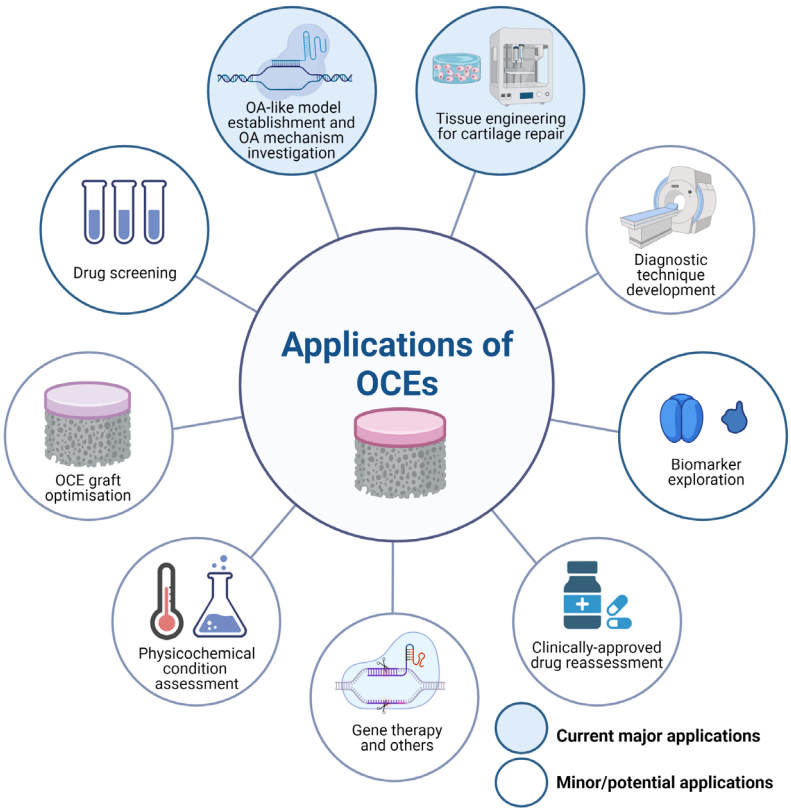

Fig. 3. Applications of OCE culture systems. The main applications of OCEs are in the fields of OA research and tissue engineering for AC regeneration. Other applications include diagnostic technique development, biomarker exploration, drug screening and reassessment, graft optimisation, physicochemical condition assessment, gene therapy and others. Figure made using Biorender.

hand, clinical doses of tranexamic acid, an effective topical haemostatic used during a surgical operation, showed no cytotoxicity to AC, supporting its use in joint surgery (Ambra *et al.*, 2019).

Validating tissue engineering strategies for AC repair

AC tissue engineering is a common and promising direction for AC repair. OCEs with an authentic AC microenvironment are valuable models to validate the efficacy of these strategies before clinical translation. Yeung *et al.* (2018) demonstrated that a 1 mm diameter AC defect established in human knee OCEs remained stable for a long time and engineered AC grafts implanted in the defect presented a hyaline cartilage phenotype over an 8-week culture period. Moreover, environmental factors and drugs like serum supplementation, oxygen tension, mechanical compression and a MMP inhibitor could alter the phenotype of the engineeredAC (Yeung *et al.*, 2018). This *ex vivo* model with OCEs from human OA joints shows its feasibility to evaluate emerging AC regeneration treatments. To closely mimic clinical AC defects with various depths, de Vries-van Melle *et al.* (2012) explored bovine OCE models with different AC defect depths; the defect type was shown to influence new AC formation and could be used as a model to evaluate tissue engineering treatments. Mouser *et al.* (2018) established a full thickness AC defect model in equine OCEs and assessed the effects of spatial chondrocyte distribution in hydrogels on AC-like tissue formation. Vainieri *et al.* (2020) placed HA-based hydrogels with or without chemotactic factors into defects of OCEs and then implanted them into the subcutaneous tissue of athymic mice. HA-based constructs could enhance endogenous cell recruitment and further promote AC repair (Vainieri *et al.*, 2020). An HA-enriched fibrin hydrogel could promote AC repair in chondral defects in porcine OCEs by using a hADSC-based tissue engineering approach (Wu *et al.*, 2018). Abbas (2017) demonstrated that the combination of BM-MSCs and homogenised AC enhances AC regeneration in a human OCE defect model.

Assessing physicochemical conditions to minimise AC damage during surgery

Arthroscopic surgery has become a mainstay of sports medicine and continuous irrigation is indispensable during the operation. Physicochemical conditions of the irrigation solution such as osmolarity, temperature and constituents may show different effects on AC. Using a porcine OCE model, Kocaoglu *et al.* (2011) found that colder solutions at room temperature show more detrimental effects on AC than warmer irrigation solutions close to body temperature. Hyperosmolar saline solution at 37 °C decreases chondrocyte death rate in scalpel-induced AC injury in bovine OCEs; thus, increasing the solution osmolarity (480 mOsm) could be chondroprotective during joint surgery (Amin *et al.*, 2008; Eltawil *et al.*, 2018). During the drilling process of bovine OCEs, irrigation solution, especially with raised osmolarity and reduced calcium ion content, remarkably mitigates chondrocyte death, which may be important for orthopaedic surgery requiring AC drilling (Farhan-Alanie and Hall, 2014).

Optimising osteochondral graft transplantation in clinical practice

OCE transplantation is a common surgical strategy for patients with full-thickness AC lesions. Exploring advanced surgical techniques in *ex vivo* OCE models may improve the success of operations and post-operative prognosis. By applying impact loads on *ex vivo* bovine OCEs, Kang *et al.* (2010) revealed that more hits of loading with low magnitudes could maintain higher cell viability in the bovine OCE grafts and, thus, ensure better graft integration into the osteochondral defect site. In a similar *ex vivo* model, tissue-engineered biphasic AC bone implants showed better integration in comparison with autologous OCEs as indicated by chondrocyte migration into the host cartilage (Theodoropoulos *et al.*, 2011).

Overall, the OCE model is a promising preclinical tool suitable for applications in broad fields related to diarthrodial joint diseases. Basically, different types of OA models could be emulated using OCEs to study the underlying mechanisms of OA. Another application is the assessment of the toxicity, effectiveness and penetration of promising DMOADs. With the creation of defects, OCEs could be used to investigate the mechanisms of chondrogenic repair, screen biomaterials with or without cells, investigate the chondrogenic ability of stem cells and test physiological biomimetic conditions related to AC repair. Moreover, OCEs are also suitable for exploring diagnostic biomarkers and radiographic techniques for OA, optimising surgical details in sports medicine and assessing gene therapy for OA treatment (Madry and Cucchiarini, 2016; Madry *et al.*, 2003).

Future development of OCEs

As an emerging model to study diarthrodial joint diseases, OCEs have the potential to closely simulate the complex native joint microenvironment. However, current OCE culture systems still face several challenges and future studies should focus on new strategies to optimise the OCE model.

OCEs from porcine femoral medial condyles could remain alive *ex vivo* for up to 56 d (Schwab *et al.*, 2017). For AC repair research, especially in large animals, longer culture periods of OCEs may be necessary. Shortening the time interval between tissue harvesting from human joints or the abattoir and OCE isolation may maximise cell survival. Additionally, the use of irrigation solutions during OCE extraction can lower the drilling temperature and help to maintain cell viability (Farhan-Alanie and Hall, 2014). No studies have explored whether

OCE culture under physiological oxygen conditions could keep the tissue viable for a longer period.

To date, no consensus has been achieved on a standardised source, size and culture system for OCEs. However, OCEs from large animals, such as horses, that have closer anatomical and biochemical properties to human joints should be preferred. While access to samples from healthy human joints is strongly limited, OCEs from patients undergoing joint replacement should not be wasted. Due to the thickness and biomechanical differences of AC even within the same joint (Li *et al.*, 2005; Li *et al.*, 2021a), OCEs should be extracted from the same region to minimise specimen variations. In most *ex vivo* studies, AC and subchondral bone of OCEs are cultured in the same compartment, although they are separated under *in vivo* conditions, with AC in an articular cavity and subchondral bone in a bone marrow cavity. To better mimic the native joint circumstances, a culture system with separated compartments and different culture media should be applied in future studies (Schwab *et al.*, 2017).

Currently, most OCE studies use cytokines to induce an inflammatory OA-like model or one strike loading to mimic post-traumatic OA. Although using these models it is possible to observe transcriptional, histological or biochemical changes to AC similar to a certain phenotype of OA, the natural process of OA is largely dependent on long-term injurious loading, especially the changes to the subchondral bone (Burr and Gallant, 2012; Chang *et al.*, 2019; Visser *et al.*, 2015). Therefore, it is worth establishing an *ex vivo* OA model with OCEs by applying long-term detrimental biomechanical loading (Fig. 2). Moreover, investigators could incorporate synovium or adipose tissues into this model to explore and demonstrate the crosstalk between intraarticular tissues. In general, more attention should be paid to the analysis of subchondral bone. Taking hypoxia and separated culture compartments into account could be an ideal *ex vivo* model maximally mimicking the native OA onset and progression. Furthermore, the dense and thick ECM in the AC tissue may cause the translational failure of novel therapeutics from cell culture or small animal models to clinical practice. OCEs are appropriate to evaluate the uptake, penetration and distribution of novel drugs or controlled release of drug delivery systems before clinical translation (Colella *et al.*, 2020).

Conclusions

One obvious reason that hinders the development of therapeutics for joint diseases such as OA and AC defects is that there are no all-encompassing models reflecting the onset and progression of human joint diseases. OCEs from large animals and humans have been proved to remain alive during long-term culture and could advance towards a versatile preclinical system. Studies have applied OCEs to establish OA-like and AC defect models *ex vivo*, which are useful to unravel disease mechanisms, screen disease-modifying drugs and evaluate AC repair strategies. To make OCE models more authentic, native joint conditions such as mechanical loading, tissue co-culture and physioxia must be fulfilled. Such a biomimetic preclinical OCE system can advance the research on OA, AC degeneration and other joint disorders, facilitating the translation into clinical practice.

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Discussion with Reviewer

Jereon Geurts: If the authors were to propose guidelines for the use of OCEs in OA/AC research, what standardised readouts would they recommend to enable comparison between studies conducted by different research groups?

Authors: To make the studies using OCEs more comparable, we recommend assays to evaluate at least the following three aspects. Transcriptional analysis should include anabolic, catabolic and inflammatory gene expression in chondrocytes. Biochemically, the amount of GAG and inflammatory markers released into the medium should be assessed. Histological staining and semi-quantitative analysis reflecting proteoglycan content in AC are recommended. Depending on experimental purposes, assessment of chondrogenesis, hypertrophy, dedifferentiation, trans-differentiation, apoptosis, senescence and biomechanics might be needed.

Editor's note: The Scientific Editor responsible for this paper was Stephen Ferguson.