Biomaterials Translational

Future perspectives: advances in bone/cartilage organoid technology and clinical potential

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Key Words:
biomaterials; bone; cartilage; organoids; stem cells; tissue engineering

From the Contents

ABSTRACT

Bone and cartilage tissues are essential for movement and structure, yet diseases like osteoarthritis affect millions. Traditional therapies have limitations, necessitating innovative approaches. Organoid technology, leveraging stem cells' regenerative potential, offers a novel platform for disease modelling and therapy. This review focuses on advancements in bone/cartilage organoid technology, highlighting the role of stem cells, biomaterials, and external factors in organoid development. We discuss the implications of these organoids for regenerative medicine, disease research, and personalised treatment strategies, presenting organoids as a promising avenue for enhancing cartilage repair and bone regeneration. Bone/cartilage organoids will play a greater role in the treatment of bone/cartilage diseases in the future, and promote the progress of biological tissue engineering.

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Introduction

Bone and cartilage tissues are integral to the human musculoskeletal system, providing essential structural integrity and function. However, bone diseases, particularly those related to cartilage such as articular cartilage injuries, are prevalent across all age groups, with a clinical incidence rate of 20–33%.[1] These injuries, often caused by degenerative diseases or sports injuries, are detected in 61–63% of arthroscopy examinations.[2] Millions suffer from varying degrees of cartilage damage annually, which can lead to severe joint diseases like osteoarthritis (OA), affecting 595 million people globally.[3] In China, the number is also significant, with 10,681,311 cases of OA in 2019, a 132.66% increase from 1990,[4] and the prevalence continues to rise with the growing rates of obesity and an ageing population.[5]

Given the importance of bone and cartilage disease models in disease research and drug development, current methods such as two-dimensional cell culture and animal experiments have limitations as they cannot fully simulate real human tissues and systems.[6] The development of more advanced *in vitro* biological models is therefore urgently needed.[7] Advances in tissue engineering and regenerative medicine, especially organoid technology, have provided new hope for cartilage repair.[8]

Cartilage injury diseases like OA are characterised by chondrocyte inflammation and extracellular matrix (ECM) destruction, and similar pathological processes occur in bone injuries. The lack of vascularity in cartilage and certain bone areas limits their self-repair capabilities. The pathogenesis of bone and cartilage diseases, such as OA, is driven by a complex interplay of genetic, mechanical, inflammatory, and enzymatic factors that lead to tissue degeneration.[3] While two-dimensional cell cultures have traditionally been used to study these diseases, they often fall short in capturing the intricate cellular interactions and ECM influences present *in vivo*. Three-dimensional (3D) organoid models, however, offer a superior system that better mimics the tissue's 3D architecture and multicellular dynamics, allowing for a more accurate investigation of disease mechanisms and therapeutic responses. This advanced model facilitates a holistic study

of cellular communication and signaling, providing a deeper understanding of pathogenesis and the discovery of new therapeutic targets.[8] Although traditional treatments such as artificial cartilage or synthetic materials have been applied in repairs, they still pose challenges in terms of durability, post-implant rejection, and tissue compatibility.[9] Fortunately, advances in tissue engineering and regenerative medicine, particularly organoid technology, offer new hope for the repair of bones and cartilage.[8]

The development of organoid technology, based on the self-renewal and self-organising capabilities of stem cells, provides a new platform for the study and treatment of bone and cartilage diseases. Bone or cartilage organoids can not only mimic the physiological microenvironment of cartilage tissue but also rapidly expand *in vitro*, offering new strategies for bone or cartilage bio-tissue engineering.[10, 11] In the construction process of organoids, stem cells are used as the basic material, which can be induced to differentiate into specific types of cells and assembled into 3D structures in appropriate scaffold materials.[12, 13] This mutually beneficial relationship makes organoid technology not only advance our understanding of human biology but also provide new strategies for disease treatment, especially in the field of regenerative medicine for tissues and organs such as bones and cartilage, showing great application potential.[14-16]

With the continuous development of bioengineering, we believe that organoid technology will become a more effective method for treating bone or cartilage injury-related diseases in the future. In this review, we discuss the role and development of bone/cartilage organoids prepared by various types of cells, biomaterials, and external factors through various assembly methods.

Stem Cells

Stem cell technology plays an essential role in organoid construction due to its distinctive diversity and regenerative capacity.[17, 18] The evolution of organoid technology, especially through the utilisation of pluripotent stem cells (PSCs) and adult stem cells, has created new opportunities for simulating and regenerating bone and cartilage tissues. Comprising both embryonic stem cells (ESCs) and induced PSCs (iPSCs), along with adult stem cells such as bone marrow mesenchymal stem cells (BMSCs), adipose-derived mesenchymal stem cells (ADSCs), and synovium-derived mesenchymal stem cells, these cells form a crucial cellular resource in organoid research.[19-21] Their abilities for self-renewal and self-organise have forged new avenues in the study and treatment of bone and cartilage diseases,[22] showing significant potential in facilitating cartilage regeneration.[23] Stem cell-derived organoids can mimic the key functions of tissues or organs, presenting extensive biomedical application prospects in the field of bone and cartilage regeneration.[24, 25]

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are highly valued in regenerative medicine for their self-renewal and multi-lineage differentiation potential.[26] Harvested from various tissues including bone marrow, adipose, and synovium, MSCs exhibit unique biological traits and robust regenerative capabilities.[18, 27]

Yang et al.[25] developed novel microcryogels to induce and induce chondrogenesis and osteogenic differentiation of MSCs, enabling the regeneration of cartilage and bone tissues to form osteochondral organoids through self-assembly *in vivo* (**Figure 1A**). The osteogenic or chondrogenic differentiation of MSCs encompasses multiple stages, including proliferation, maturation, and terminal differentiation.[28] By employing *in vitro* culture methods alongside specific growth factors and ECM molecules, it is possible to generate organoids that mimic bone or cartilage.[29] The advantage of using MSCs to develop bone or cartilage organoids lies in their accessibility, rapid expansion, and ability to differentiate into diverse mesenchymal-derived tissues.[30, 31] Notably, cells derived from bone marrow, synovium, and periosteum demonstrate the highest potential for cartilage generation.[32, 33] Xie et al.[34] have utilised digital light processing and distribution induction techniques to load BMSCs hydrogel microspheres, which, after induction, form cartilage and then transform into osteoid organoids, thus recapitulating the endochondral ossification process *in vitro*. Scotti et al.[35] have seeded BMSCs onto a collagen scaffold to serve as a developmental template and added interleukin (IL)-1β to the culture medium to promote cartilage reshaping. The resulting organoids contain haematopoietic stem cells and various types of progenitor cells, resembling natural bone in structure and function.

In the field of tissue engineering and regenerative medicine, MSCs have a wide range of applications. They can differentiate into bone or cartilage cells and also modulate the local microenvironment by secreting soluble factors and exosomes, which facilitate tissue repair and regeneration.[18] ADSCs and BMSCs are widely studied due to their similar biological characteristics.[33, 36] However, research indicates that BMSCs in osteogenic differentiation compared to ADSCs.[37] In organoid construction, the combination of MSCs with biomaterials can create 3D bone or cartilage organoids, providing a powerful tool for studying disease pathogenesis, drug screening, and therapeutic strategy assessment. Dai et al.[26] have shown that bone morphogenetic protein (BMP)-2 loaded scaffolds can promote the formation of bone organoids, offering an acellular strategy that fosters an ecological environment for the exchange between stem cells and immune cells, thus harvesting a rich source of autologous cells such as osteoblasts, fibroblasts, and haematopoietic cells (**Figure 1B**). A novel polymer-modified deoxyribonucleic acid hydrogel combined with tetrahedral

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framework nucleic acids has been shown to accelerate the osteogenic differentiation of BMSCs and the angiogenesis of human umbilical vein endothelial cells with *in vivo* rat experiments demonstrating their efficacy in promoting new bone formation[38] (**Figure 1C**). Lin et al.[39] have also proposed that embryonic joint progenitor cells expressing leucine-rich repeat-containing G-protein-coupled receptor 5-green fluorescent protein, generated through gel embedding, have the potential to form cartilage organoids (**Figure 1D**). Despite the potential of MSCs in cell-based tissue engineering and regenerative medicine, challenges remain in addressing the variability of MSC characteristics from different sources and in creating organoids that more closely resemble native bone or cartilage.[39, 40]

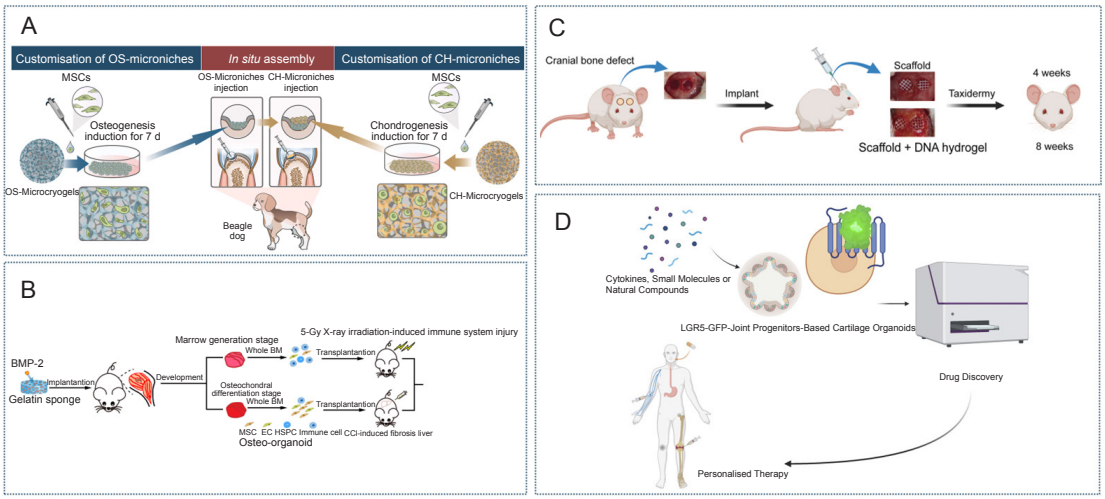

Figure 1. Organoids constructed from mesenchymal stem cells. (A) Schematic diagram of osteochondral organoid formation and repair process by inducing differentiation and regeneration of MSCs. Reprinted from Yang et al.[25] (B) The gelatin sponge loaded with BMP-2 was implanted into the inner muscle pocket near the femurs of mice to generate bone organoids. The bone organoids formed mature, exhibiting a balance between osteogenic and resorptive activity, and a stable proportion of endothelial cells and MSCs. Reprinted from Dai et al.[26] (C) *In vivo* repair of mouse skull defect with hydrogel scaffold and schematic diagram of treatment. The hydrogel recruits many BMSCs for bone formation, further promoting the growth of new bone. Reprinted from Han et al.[38] (D) LGR5-joint progenitors-based cartilage organoids for realisation of novel drug discovery, and personalised regenerative therapy of cartilage repair. Reprinted from Lin et al.[39] BM: bone marrow; BMP-2: bone morphogenetic protein-2; CCl_4: carbon tetrachloride; CH: chondrogenic differentiation; EC: endothelial cell; GFP: green fluorescent protein; HSPC: haematopoietic stem/progenitor cell; LGR5: leucine rich repeat containing g protein-coupled receptor 5; MSC: mesenchymal stem cell; OS: chondrogenic differentiation.

Pluripotent stem cells

PSCs, comprising both ESCs and iPSCs, have demonstrated immense potential in regenerative medicine and disease modelling due to their remarkable pluripotency and self-renewal capabilities.[19, 41] In recent years, iPSCs have garnered particular interest for their application in generating bone and cartilage organoids.[14, 42]

While iPSCs share similarities with ESCs, such as unlimited self-renewal, pluripotency, surface marker expression, and telomerase activity,[43-45] they offer a promising alternative cell source as they circumvent the ethical and political issues associated with ESCs by not requiring the destruction of human embryos.[44] Moreover, when compared to MSCs, iPSCs have shown greater evidence of long-term potential in bone and cartilage formation. They tend to express higher levels of types I and X collagen.[46, 47] Despite the advantages of iPSCs, challenges remain in generating functional chondrogenic organoids from iPSCs, including large-scale production and maintenance of their functionality. Additionally, the potential for dedifferentiation or tumour formation from iPSCs could impact clinical applications.[44, 48] However, iPSCs have an edge over MSCs in addressing issues such as cell quantity, fibrocartilage formation, or loss of phenotype during passaging.[47]

iPSCs have become a versatile tool in the study of chondrogenic organoid formation.[46, 49, 50] The development of chondrogenic regenerative therapies based on iPSCs is a rapidly evolving field. iPSCs have shown effectiveness in modelling monogenic cartilage diseases and have achieved positive results in simulating skeletal developmental abnormalities.[51] A significant advantage of iPSCs is their ability to reprogram mature somatic cells back to a pluripotent state akin to ESCs, thus bypassing the ethical concerns associated with the use of ESCs.[49, 52] Nilsson Hall et al.[53] proposed that periosteum-derived cells have similar characteristics to MSCs and can be used to produce chondrogenic microspheres that differentiate

into cartilage organoids, which can be used to prepare engineered bone organoids for the regenerative treatment of long bone defects. The height of new bone formation and the part connected with the old bone are comparable to natural long bones (**Figure 2A**). A recent study showed that allogeneic iPSC-derived chondrogenic organoids transplanted into the knee joints of primate models with cartilage defects integrated with the host articular cartilage and prevented further degradation of the surrounding cartilage[52] (**Figure 2B**), highlighting the clinical potential of iPSC technology in the treatment of articular cartilage defects. Tsumaki et al.[54] also raised the possibility of selecting iPSC-induced chondrocytes from the iPSC repertoire for transplantation, which demonstrated their potential for preparing cartilage organoids. O'Connor and colleagues[46] used mouse iPSCs to establish bone-cartilage organoids by exposing them to a time-dependent series of transforming growth factor-β3 and BMP-2, simulating the natural skeletal development process through endochondral ossification. The resulting organoids displayed dual tissues composed of cartilage and calcified bone regions. Furthermore, Hall et al.[50] found that the implantation of iPSC-derived chondrogenic microtissues combined with hypertrophic chondrogenic organoids in nude mice led to the formation of cartilage and bone regions. Tam et al.[49] investigated the potential of human-iPSCs (hiPSCs)-derived cartilage-like organoids in bone defect repair, showing that IL-1β could promote bone healing in human PSC osteoid organoids (**Figure 2C**). In addition, Rodríguez Ruiz et al.[55] constructed cartilage and bone organoids using human iPSCs expressing osteoprotegerin extra-long (OPG-XL) and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system, which could reduce osteoclast activity and overcome the effects of chondrogenic fibrosis and high mineralisation of bone matrix on OA. It has been reported that iPSC-derived chondrogenic organoids can also recruit osteogenic precursors for bone repair and have demonstrated the ability to repair cartilage defects in mouse models.[49] Xiahou et al.[24] developed a hydrogel with a cell response switch to induce ADSCs to spontaneously and sequentially proliferate and aggregate, producing engineered columnar cartilage microtissues with histological characteristics and biomechanical properties very similar to natural transparent cartilage. Han et al.[56] successfully made organoids by culturing synovial fluid-derived cells in a 3D culture environment, optimising the use of synovial fluid-derived cells and enhancing their application potential in cartilage repair. These findings have opened new horizons for the development of complex tissue-engineered implants, using pre-differentiated organoids as building blocks to promote region-specific functions for the establishment of articular cartilage grafts.

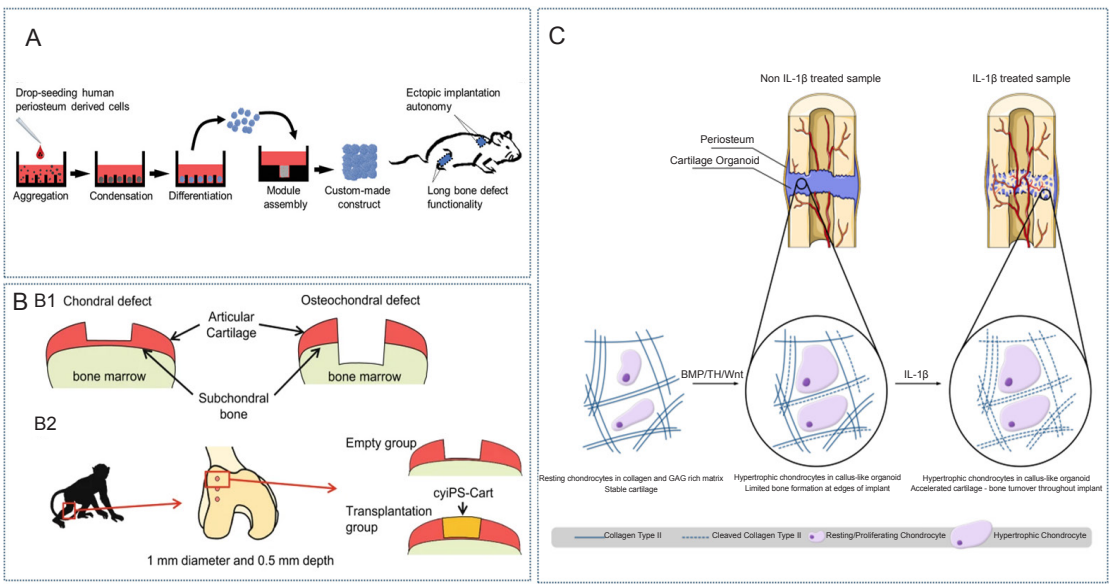

Figure 2. Organoids constructed from pluripotent stem cells. (A) Bioengineering process starting with iPSCs cellular aggregation, condensation, and differentiation followed by callus organoid assembly and implantation in ectopic and orthotopic environments. Reprinted from Nilsson Hall et al.[53] (B) Transplantation of cyiPS-Cart into primate knee joints. (B1) Two types of articular cartilage injuries: The left panel shows chondral lesions that reach but do not penetrate the subchondral bone. The right panel displays osteochondral lesions that extend into the subchondral bone. (B2) Experimental setup for cyiPS-Cart transplantation in primates. In cynomolgus monkeys, chondral lesions were induced on the femoral trochlear ridge of the right knee. The experimental group received cyiPS-Cart transplants, while the control group was left untreated. Reprinted from Abe et al.[52] (C) IL-1β promotes bone regeneration in callus-like organoids derived from human PSCs. In summary, our research suggests that IL-1β enhances bone healing, possibly by boosting the breakdown of cartilage matrix via matrix metalloproteinase 13). Reprinted from Tam et al.[49] BMP: bone morphogenetic protein; cyiPS-Cart: cynomolgus monkey iPSC-derived cartilage organoid; GAG: glycosaminoglycan; IL-1β: interleukin-1β; iPSC: induced PSC; PSC: pluripotent stem cell; TH: tyrosine hydroxylase.

Other cells

In addition to MSCs and iPSCs, other cell types such as immortalised cells and adult cells have also shown potential in constructing organoids for bone and cartilage repair.

Osteoblasts, derived from MSCs, are the cells responsible for matrix deposition and migrate to areas of bone remodelling via the local soft tissue or peripheral vascular system. Osteoblasts are pivotal for bone remodelling and offer targeted advantages for bone organoid construction, such as their direct role in bone matrix deposition and their ability to form mature bone cells. This makes them ideal for creating organoids that accurately represent bone tissue. However, their limited plasticity and reliance on specific maturation factors can be more restrictive compared to the broader differentiation potential of MSCs and the extensive self-renewal capabilities of iPSCs.[57] Additionally, osteoblasts' lack of self-renewal and susceptibility to senescence may impact the long-term vitality of organoids. These osteoprogenitor cells subsequently differentiate into mature osteoblasts under a multitude of influences, including BMPs and phosphorus-rich compounds that act as substrates for alkaline phosphatase--a key enzyme expressed by both developing and mature osteoblasts.[57-59] Fuller et al.[60] established a 3D bone organoid model using mouse osteoblasts in hydrogels, providing an economical and efficient platform for bone research (**Figure 3A**). Iordachescu's team[61] constructed an organoid model composed of osteoblasts and osteoclasts seeded on the trabecular surface of the femoral head. This model effectively mimics the process of bone loss and overcomes the limitations of traditional laboratory simulations of the bone microenvironment, offering an *ex vivo* platform (**Figure 3B**). Similarly, Park et al.[62] found that using bone biomaterial to guide the structural mineralisation of osteoblasts and constructing bone organoids through repeated bone remodelling cycles can locally regulate the trabecular bone to maintain physiological bone homeostasis. Knowles et al.[63] successfully created a mature 3D human osteocyte organoid by culturing primary human osteoblasts in fibrinogen/thrombin gels, providing a valuable tool for drug screening and disease mechanism research (**Figure 3C**).

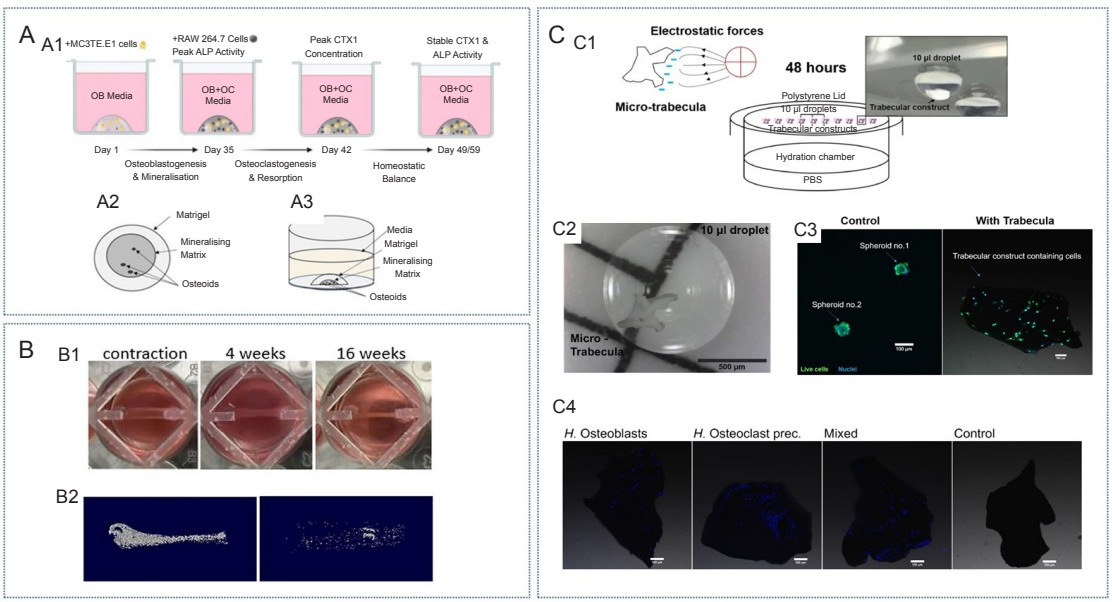

Figure 3. Organoids constructed from other cells. (A) A comprehensive illustration of the 3D mcBOM matrix creation process is outlined as (A1) A comprehensive view of the *in vitro* model is presented, encapsulating the key developmental points and interventions. (A2) A dorsal cartoon diagram is provided, depicting the 3D bone culture setup. (A3) A lateral cartoon diagram is included, offering another perspective on the 3D bone culture. Reprinted from Fuller et al.[60] (B) Human osteocyte constructs demonstrate mineralisation and maintain high viability over 20 weeks. (B1) When human osteoblasts are seeded into fibrin/thrombin gels, they cause the gel to contract between the posts, resulting in a noticeable thickening over time. (B2) Representative micro-CT reconstructions from two different donors are shown after a 20-week differentiation period in osteogenic media. Reprinted from Knowles et al.[63] (C) The micro-trabeculae, which are naturally highly electrostatic (left), were utilised for their ability to be integrated into liquid cell suspension droplets (right), thereby creating miniaturised bone avatars. (C1, 2) A hanging-drop culture system was employed to suspend the trabeculae along with primary female bone effector cells, guiding their attachment to the trabecular surface via gravitational sedimentation. (C3) The cultures were maintained for 48 hours to optimize cell surface colonisation and self-organisation. In scenarios devoid of bone scaffolds, osteoblasts interact directly to form spheroids (left). However, when a trabecula is present, these cells effectively colonize the surface and exhibit osteogenic characteristics (right). (C4) Furthermore, primary female osteoclast precursors can individually adhere and when co-cultured with osteoblasts, they form a comprehensive remodelling unit. Their presence on the trabeculae can be discerned, as the nuclei of osteoblasts are larger compared to those of osteoclasts. Reprinted from Iordachescu et al.[61] 3D: three-dimensional; ALP: alkaline phosphatase; CT: computed tomography; CTX1: type 1 collagen cross-linked C telopeptide; H. Osteoclast prec.: human osteoclast precursors; mcBOM: murine-cell-derived bone organoid model; OB: osteoblast; OC: osteoclast; PBS: phosphate-buffered saline.

Bone/cartilage organoids are constructed from immortalised cells such as chondrocytes obtained from osteoblast culture, which makes organoid construction easier and more economical. Kleuskens et al.[64] successfully made cartilage organoids from human chondrocytes derived from OA and non-degenerative sources by using a 3D culture environment with matrices from porcine embryonic axial cells, showing good potential for repair. More notably, Crispim and Ito[65] successfully produced cartilage organoids from bovine chondrocytes through a new suspension expansion protocol. These organoids not only showed significant cell proliferation and vitality but also formed structures similar to transparent cartilage through self-assembly, with matrix content and structure very similar to natural cartilage, which is expected to completely change the research methods in the field of cartilage research and related fields.

In summary, stem cells play a fundamental role in the development of bone or cartilage organoids. They not only provide the necessary cellular sources for the formation of bone or cartilage organoids but also promote the self-organisation and functional simulation of tissues.

Biomaterials

Biomaterials play a pivotal role in advancing stem cell research and organoid technology. They are crucial for replicating the mechanical properties and 3D architecture of bones in organoid construction. Beyond selecting the appropriate cells, scaffolds and signalling factors—encompassing biochemical, chemophysical, and physical cues—are vital in the fabrication of bone or cartilage organoids. The resulting organoids closely mimic natural tissues in terms of chemistry, physics, and functionality, which is essential for successful research outcomes.[66] Biomaterials, with or without growth factors, offer a promising platform for achieving spatial complexity within the microenvironment to fully harness cellular capabilities.[67]

Generally, ideal natural or synthetic scaffolds demand biocompatibility, bioactivity, protective mechanical strength, adhesive morphological ability, the capacity to support cell proliferation and/or differentiation, the ability to mimic the natural ECM, biointegration, and biodegradability.[68, 69] Additionally, scaffolds designed for bone or cartilage organoids require osteoinductivity, osteoconductivity, and specific mechanical properties.[70, 71]

The construction of bone or cartilage organoids employs a variety of biocompatible materials that provide an optimal environment for stem cell differentiation.[65, 72] These materials include natural materials such as alginate,[73, 74] chitosan,[75] collagen,[64, 76] and gelatin,[77] as well as synthetic materials like poly(lactic-co-glycolic acid),[78] polycaprolactone,[79] and polyethylene glycol (PEG).[80] These biomaterials are significant because they not only possess excellent biocompatibility and adjustable physicochemical properties that support cell growth and tissue regeneration but also effectively simulate the ECM, guide cell differentiation, and construct complex tissue engineering scaffolds. They enable scientists to simulate the microenvironment of human tissues and organs more accurately, providing powerful tools for disease modelling, drug screening, and the development of regenerative medical strategies.

Natural materials

Through the application of these natural biomaterials, the potential of organoid technology in regenerative medicine has been further demonstrated. They not only provide researchers with a platform for simulating complex bone/cartilage biological microenvironments and pathological states but also provide new strategies and directions for future clinical treatment. Especially in cartilage and bone regeneration, by precisely controlling the structure and function of organoids, a better understanding of tissue repair mechanisms can be achieved, opening new avenues for bone/cartilage repair.

Alginate

Alginate-based hydrogels have become invaluable in the field of regenerative medicine due to their biocompatibility and ability to encapsulate cells, providing a supportive environment for tissue regeneration. They have shown significant efficacy in promoting cartilage and bone regeneration and have been widely applied in the engineering of complex tissue constructs.[81, 82]

In the realm of cartilage regeneration, alginate constructs high-interconnectivity scaffolds that foster an environment conducive to chondrocyte proliferation and matrix production, ensuring good shape retention and cellular activity. Kleuskens et al.[64] combined alginate gel with a matrix from porcine notochord cells to create cartilage organoids composed of human chondrocytes. The use of alginate gel not only promotes the proliferation and matrix deposition of organoids but also fuses organoids into a homogeneous tissue similar to natural cartilage. Shehzad et al.[77] proposed the combination of alginate with other biocompatible materials such as gelatin to prepare hydrogel scaffolds that not only promote cell spreading and survival but also provide structural support for the fabrication of bone organoids (**Figure 4A**). The design of these scaffolds balances shape fidelity with cellular viability, especially their large pore size, which is crucial for cell migration and tissue regeneration.

Chitosan

As a natural biomaterial, chitosan plays a key role in regenerative medicine, particularly in the construction of organoid models and the regeneration of cartilage and bone tissues.[83] It provides a 3D scaffold environment for cells and promotes the proliferation and differentiation of specific cell types by mimicking the characteristics of the ECM, showing great potential in organoid development. Functionalised chitosan further enhances its anti-inflammatory and metabolic regulatory roles in organoid models by combining with specific bioactive molecules, such as the A-type endothelin receptor antagonist (BQ-123-CHI) and the B-type bradykinin receptor antagonist (R-954-HA).[75] In a horse joint cartilage inflammation model induced by IL-1β, this functionalised chitosan showed significant therapeutic effects, effectively reducing markers of inflammation and metabolic degradation, thereby demonstrating its potential in organoid research and

clinical applications. Moreover, the degradation products of sulfated chitosan can specifically increase the number of therapeutic haematopoietic stem/progenitor cells in bone organoids[84] (**Figure 4B**), providing a potential new cell source for bone marrow transplantation. These findings not only enrich our understanding of the application of chitosan in regenerative medicine but also provide new directions and opportunities for future therapeutic strategies.

Hyaluronic acid

Hyaluronic acid (HA), with its excellent biocompatibility and ability to regulate cell behaviour, plays a key role in organoid construction.[85] New biocompatible materials, especially hydrogels, provide a favourable microenvironment for stem cell differentiation due to their high water content and adjustable physicochemical properties that mimic the ECM.[12] Biomimetic hydrogels, such as composites of HA and hydroxyapatite (HYP), have been proven to be highly effective in replicating tissue microenvironments.[13, 86] Yang et al.[25] successfully developed a customised gelatin microgel based on HA and HYP. This innovative material not only enhances the ability of MSCs to spontaneously assemble into bone-cartilage organoids through bone-cartilage units *in vivo* but also demonstrates the broad application prospects of organoid technology in the field of bone-cartilage repair by promoting the regeneration of cartilage and bone tissues (**Figure 4C**).

Collagen

Collagen, as a major component of the ECM, plays an essential scaffold role in tissue engineering to support cell growth and simulate a natural environment for tissue regeneration.[68] Cardier et al.[76] used allogeneic BMSCs from healthy donors, collagen microbeads (CM), and platelet-rich plasma clots to successfully develop an innovative composite material. As an osteogenic organoid, this material was applied to non-healing tibial segments and promoted osteogenesis in patients with bone formation failure (such as congenital pseudoarthrosis of the tibia). The application of collagen in cartilage and bone tissue engineering is also remarkable. In hydrogel studies containing non-degenerate organoids, Kleuskens et al.[64] observed the distribution of type I collagen among organoids, which formed a continuous tissue composed of cells, proteoglycans, and type II collagen around the organoids, highlighting the key role of collagen in simulating the natural cartilage environment.

Gelatin

Gelatin, a partially hydrolysed form of collagen, has been widely used in the field of tissue engineering due to its excellent biocompatibility and scaffold-forming ability. Studies have confirmed that gelatin-based hydrogels perform exceptionally well in the field of tissue engineering, especially in bone tissue engineering and the manufacture of bone organoids. The large pore size and highly interconnected scaffold structure of these hydrogels significantly promote cell diffusion and activity.[77] Deng et al.[87] also used a gelatin sponge scaffold loaded with recombinant human BMP-2 to create osteoid organoids, successfully constructing mature osteoid organoids with a bone marrow-like structure. These organoids, through the separation of MSCs from bone organs in the body to replace autologous MSCs, show superior proliferation ability and tri-lineage differentiation potential, becoming a new source of obtaining stem cells with excellent performance. Wang et al.[88] innovatively invented a new type of bio-ink, composed of gelatin methacrylate (GelMA), alginate methacrylate, and HYP, to print highly complex bone ECM analogues. This scaffold helps to culture and mature bone organoids and promotes multicellular differentiation, and its self-mineralisation characteristics are similar to natural bone, effectively enhancing bone repair ability and providing new possibilities for clinical applications (**Figure 4D**). Not only for bone repair ability, but organoids also have great potential in the treatment of OA. Han et al.[56] used transglutaminase-crosslinked gelatin to make osteoid organoids and successfully constructed tissue models with good structural stability. Transglutaminase-crosslinked gelatin has excellent mechanical properties and biocompatibility, which enables it to support cell growth and tissue function maintenance. These osteoid organoids show good application potential in simulating joint cartilage degeneration and related arthritis, especially in the research and treatment of OA. Coincidentally, Sun et al.[89] designed a type of organoid hydrogel (miR-24μS/SMSC organoid hydrogel [MSOH]) for ageing synovial MSCs. This hydrogel, by regulating the agieng-related miR-24/tau kinase 1 signalling pathway, slows down the ageing of chondrocytes and promotes cartilage regeneration. In a rat animal experiment, MSOH showed excellent cartilage repair ability, indicating its potential to become a new therapy for treating arthritis cartilage defects (**Figure 4E**, and **F**). Shehzad successfully made bone tissue organoids using gelatin-based hydrogels. These hydrogels combine gelatin, GelMA, and alginate, a combination that optimises the printing accuracy, stability, and biocompatibility of hydrogels, making them suitable for 3D bioprinting. Experimental results show that the developed hydrogels have good cell survival rates and biodegradability and can effectively support the mineralisation and differentiation of bone tissue. This method provides an efficient and economical solution for bone tissue engineering and *in vitro* models.[77] Zhang et al.[90] developed a new type of bio-ink, using granular hydrogels to simplify the production and 3D printing process of stem cell spheroids. It can spontaneously form stem cell spheroids at temperature changes to adsorb chondrocytes and form cartilage-like tissue.

Artificial materials

Synthetic materials play a vital role in the field of biomaterials, offering superior biocompatibility and broad applications in bone and cartilage repair.

Polylactic acid

Polylactic acid (PLA), as a biodegradable material, serves not only as a scaffold for stem cell growth and differentiation but also demonstrates immense potential in bone remodelling. A hydrogel developed by Xiahou et al.[24] combines PLA with poly(L-glutamic acid)/fatty acid diazide, utilising dual crosslinking mechanisms—photo-induced and ionic bonding—to effectively support the spontaneous proliferation

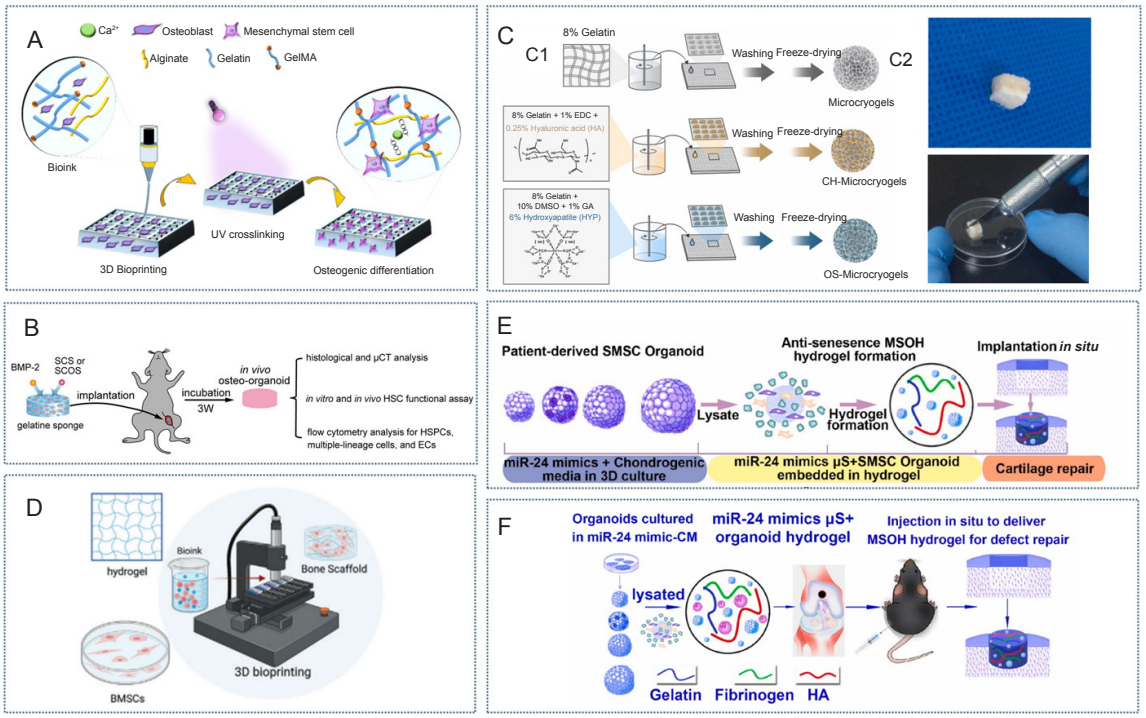

Figure 4. Organoids constructed from natural materials. (A) Fabrication of 3D printed Alg/Gel/GelMA-based cell-laden scaffolds. Reprinted from Shehzad et al.[77] (B) A schematic overview of the process for collecting and evaluating HSPCs from osteo-organoids in mice, induced by implanting freeze-dried gelatin scaffolds loaded with BMP-2 alone or in combination with SCS or SCOS, involves implanting these scaffolds into the muscle of the lower limbs, allowing them to incubate for three weeks, and then explanted the resulting osteo-organoids for further analysis. Reprinted from Dai et al.[84] (C) Microscopic observation and cytocompatibility analysis of control microcryogels, CH-microcryogels, and OS-microcryogels. (C1) Schematic of fabricating customised microcryogels. (C2) Gross observation of self-assembled osteochondral organoids. The self-assembled osteochondral organoid was incised in the axial position to allow separate analysis of the chondrogenic and osteogenic components. Reprinted from Yang et al.[25] (D) Biocompatibility of bioprinted BMSCs-laden GelMA, GelMA/AlgMA bioprinted scaffolds, and GelMA/AlgMA/HYP scaffolds. Reprinted from Wang et al.[88] Copyright 2024 Wiley - VCH GmbH. (E) Generating miR-24 transfected SMSC organoids for anti-senescence and pro-chondrogenesis. Fabrication of senescence-targeted miR-24 μS/SMSC organoid hydrogel for potential applications in chondrogenesis and further cartilage repair treatment. (F) Schematic illustration of the study design with 3D cultured SMSC organoids for cartilage damage treatment by intra-articular injection in rats. Reprinted from Sun et al.[89] 3D: three-dimensional; Alg: alginate; AlgMA: Alg methacrylate; BMP-2: bone morphogenetic protein-2; BMSC: bone marrow mesenchymal stem cell; CH: chondrogenic differentiation; CM: collagen microbead; DMSO: dimethyl sulfoxide; EC: endothelial cell; EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; GA: glutaraldehyde; Gel gelatin; GelMA: Gel methacrylate; HA: hyaluronic acid; HSC: haematopoietic stem cell; HSPC: haematopoietic stem/progenitor cell; HYP: hydroxyapatite; MSOH: miR-24 PLGA μS/SMSC organoid hydrogel; OS: chondrogenic differentiation; PLGA: poly (lactic-co-glycolic acid); SCOS: sulfonated chito-oligosaccharide; SCS: sulfonated chitosan; SMSC: synovial mesenchymal stem cell; UV: ultraviolet; μCT: micro-computed tomography; μS: microspheres.

and aggregation of ADSCs. This hydrogel can automatically regulate cell adhesion, detachment, and aggregation through a cell response switch mechanism, leading to the formation of native-like cartilage microtissues. It exhibits excellent histological and biomechanical properties in cartilage tissue engineering (**Figure 5A**). Toni's team[91] constructed a 3D bone organoid model using poly-L-lactic acid and rat MSCs, replicating the micro-anatomical structure and vascular network of flat and short bones. By combining the decellularised adult male rat scapula matrix with poly-L-lactic acid-reconstructed vasculature and functionalising it with rat tail collagen, they successfully simulated the cortical and trabecular structures. This organoid can guide the osteogenic process of MSCs and support the attachment and survival of MSC-like cells on the reconstructed vascular walls, offering the potential for studying bone remodelling mechanisms in specific skeletal regions. Moreover, Ma et al.[92] showed that poly(lactic-co-glycolic acid) scaffolds support osteogenic differentiation and mineralisation, making them promising candidates for bone tissue engineering applications.

Polycaprolactone and polyethylene glycol

PCL is widely used to construct bone tissue engineering scaffolds due to its excellent biocompatibility and biodegradability. PCL scaffolds can mimic the natural bone matrix, promoting vascularisation and nerve innervation, thereby reducing inflammation and promoting osteointegration[79] (**Figure 5B**). This makes PCL an ideal material choice for promoting bone healing and regeneration.

The development of PEG-based hydrogels provides an alternative to traditional Matrigel models for organoid culture. PEG hydrogels are biocompatible and have adjustable mechanical properties, making them suitable for organoid tissue engineering models.[80, 93] These studies reveal the potential of PEG-based hydrogels in providing physiologically relevant models and promoting cell differentiation. Shen et al.[94] developed arginine-glycine-aspartic acid (RGD)-silk fibroin-DNA hydrogel microspheres (RSD-MS) for cartilage organoid fabrication. These microspheres combine the excellent mechanical properties of silk fibroin-DNA double network hydrogels with the cell adhesion capability of RGD peptides. RSD-MS can load BMSCs, promoting their chondrogenic differentiation and forming cartilage organoid precursors. Both *in vitro* and *in vivo* experiments show that RSD-MS significantly promotes cartilage regeneration and repair. This research provides new materials and strategies for cartilage regeneration and tissue engineering.

Hydroxyapatite

HYP) has good osteoconductive properties, promoting direct cell-matrix interactions, and is a key material for bone regeneration, widely used in bone tissue engineering.[95] Li et al.[96] used MSCs embedded in autocrine ECM and, with the help of osteogenic HYP nanoparticles, directly differentiated MSCs into osteogenic precursor cells and induced mineralisation within the cell-ECM structure. Concurrently, they also used iPSCs to generate multipotent mesenchymal precursor cells and further constructed organoids mimicking joint tissue. Wang et al.[88] used a mixed bioink of GelMA/alginate methacrylate/HYP to successfully fabricate bone organoids. This bioink simulates the complex ECM of bone tissue, with the ability for self-mineralisation and cell differentiation. Through 3D bioprinting technology, the constructed scaffolds can be long-term cultured and promote the maturation of bone organoids, with mechanical properties close to natural bone. This research is of great significance in the field of bone tissue engineering, especially in bone regeneration and repair, with broad application potential (**Figure 5C**). Li et al.[97] embedded HYP nanoparticles into MSC spheroids to create bone microtissues, which further self-organised into bone organoids. This method can enhance the osteogenic differentiation ability of MSCs, promote the formation and mineralisation of bone matrix, and generate bone organoids with a trabecular structure. HYP nanoparticles play a key role in promoting the development of bone microtissues, including improving cell survival rates and osteogenic capabilities. The method also shows good application prospects in the treatment of bone defects. He et al.[98] constructed an organoid model using β-tricalcium phosphate/HYP), encapsulating it in rat muscle tissue or implanting it in a muscle pouch for culture, resulting in a system that supports tissue survival, angiogenesis, and osteogenic gene expression, potentially generating bone tissue suitable for clinical transplantation. This provides new ideas for future bone tissue transplantation engineering applications.

Graphene oxide

Graphene oxide (GO), derived from graphite oxidation, is a promising nanomaterial for tissue engineering and regenerative medicine due to its atomically thin sheet, large surface area, and hydrophilic functional groups that facilitate chemical modifications and absorption of various molecules and cations.[99] Studies have shown that GO enhances human MSC (hMSC) adhesion, proliferation,[100] cell growth,[101, 102] and osteogenic differentiation.[103] In 3D studies, GO has been integrated into hydrogel scaffolds such as alginate,[104, 105] collagen,[106] and GelMA,[107] creating 3D GO hybrid scaffolds. Shin et al.[107] demonstrated that GO-GelMA hybrid hydrogels support fibroblast spreading and alignment with improved viability and proliferation. Choe et al.[105] found that adding GO to alginate enhanced the bioink's structural stability and reduced swelling. However, higher alginate concentrations in 3D bio-printed scaffolds led to increased mechanical properties, inhibiting cell spreading and osteogenic differentiation of hMSCs.[108] Incorporating GO at an optimal concentration into low-density alginate/gelatin-based bioink could improve scaffold fidelity, enhance osteogenic differentiation of hMSCs, and increase ECM mineralisation for bone tissue engineering. Furthermore, GO/alginate/gelatin composite bioink materials not only enhance scaffold mechanical strength and stability but also promote cell adhesion and proliferation, thereby improving osteogenic differentiation and mineralisation efficiency.[109] Zhang et al.[110] successfully created functional bone organoids by applying mechanical loading to 3D bioprinted hMSCs with GO composite scaffolds, which increased mineral density, stiffness, and osteoblast differentiation, forming bone cell-like structures (**Figure 5D**). These organoids offer an *in vitro* human model for studying skeletal pathophysiology and drug screening, potentially replacing animal experiments.

Organoid External Factors

The advancement of organoid technology provides a unique model for simulating the complex biology of cartilage and bone. The formation of these organoids relies on the precise regulation of signalling factors that activate at specific time points, guiding cells to self-organise along particular developmental pathways.[111] Although various protocols promoting bone or cartilage formation have been developed, each involves different biofactors, intermediate steps, and culture durations, and no consensus on the most effective method has been reached.[112] Therefore, systematically comparing the factors and preparation methods that play a role is crucial for optimising the formation of bone or cartilage organoids.

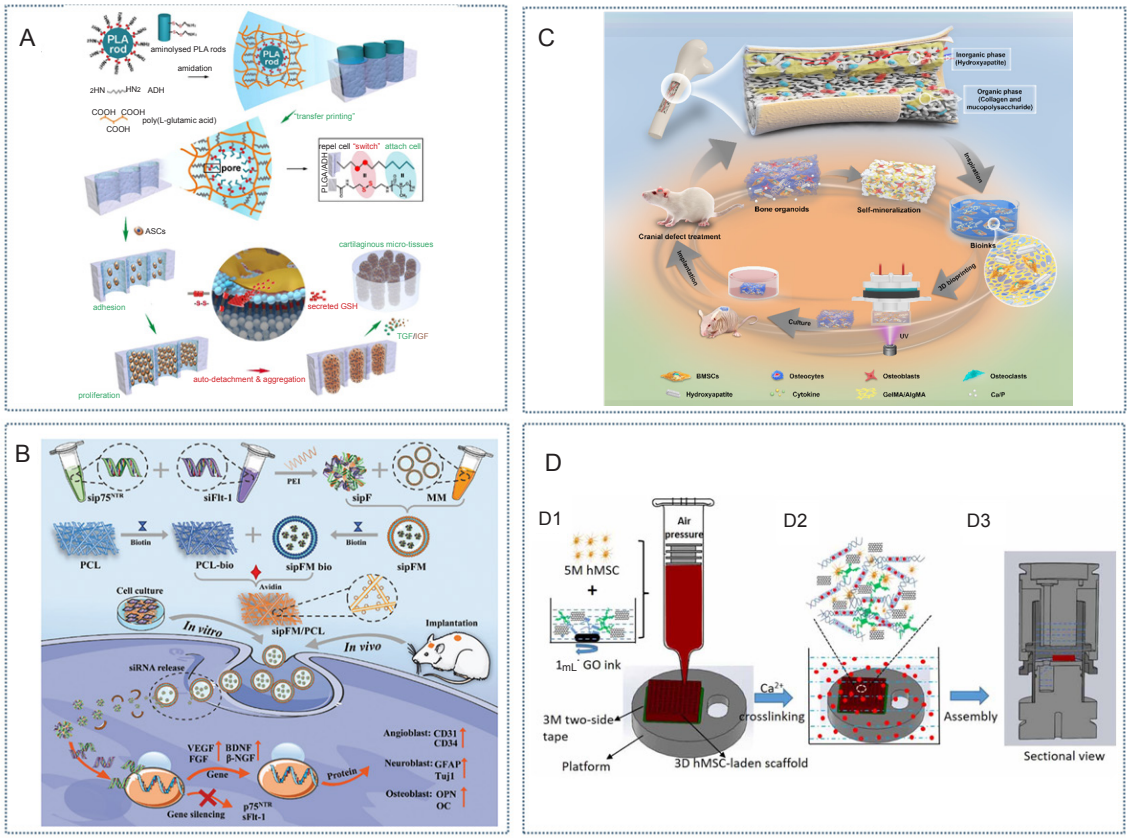

Figure 5. Organoids constructed by artificial materials. (A) Fabrication and function of hydrogel with tubular pores. Reprinted from Xiahou et al.[24] Copyright 2020 American Chemical Society. (B) A schematic depiction of the dual siRNA-loaded cell membrane-coated scaffolds showcases their role in enhancing bone regeneration through the concurrent stimulation of angiogenesis and neurogenesis. These gene-regulating matrices can modulate the expression of genes associated with blood vessels and nerves, and they enhance the paracrine activity of both vascular and nerve growth factors *in vitro*. By leveraging the synergistic effects of angiogenesis and neurogenesis, the bone repair scaffold not only ameliorates the microenvironment of bone defects but also facilitates the restoration and integration of bone tissue. Reprinted from Qiao et al.[79] Copyright 2023 Wiley - VCH GmbH(C) Fabrication of a highly intricate bone ECM analog using a novel bioink composed of GelMA/AlgMA/HYP. Reprinted from Wang et al.[88] Copyright 2024 Wiley-VCH GmbH. (D) (D1) 3D bioprinting processes. (D2) Ca^{2+} crosslinking post-bioprinting. (D) Section view to show the position of the 3D cell-laden construct in the compression bioreactor. Adapted from Zhang et al.[110] 3D: three-dimensional; AlgMA: alginate methacrylate; ASC: adult stem cell; BDNF: brain-derived neurotrophic factor; ECM: extracellular matrix; FGF: fibroblast growth factor: GelMA: gelatin methacrylate; GFAP: glial fibrillary acidic protein; GO: graphene oxide; hMSC: human mesenchymal stem cell; HYP: hydroxyapatite; MM: membrane; OC: osteoclast; OPN: osteopontin; $p75^{NTR}$: p75 neurotrophic factor receptor; PCL: polycaprolactone; PLA: polylactic acid; sFlt-1: soluble fms-like tyrosine kinase-1; siRNA: small interfering RNA; TGF: transforming growth factor; Tuj1: neuron-specific class III beta-tubulin; UV: ultraviolet; VEGF: vascular endothelial growth factor; β-NGF: β-nerve growth factor.

Biological factors and chemical signals

The differentiation of bone or cartilage formation is a complex process regulated by multiple signalling pathways that control the aggregation, differentiation, and ultimate formation of mesenchymal progenitor cells into bone or cartilage.[112] Key signalling molecules such as BMP, growth factor, transcription termination factor B, Wnt, and cell adhesion molecules (neural cell adhesion molecule, N-cadherin, β-catenin) play an essential role in this process.[113-115] Sox9, a cartilage-specific transcription factor, is crucial for maintaining the chondrocyte phenotype and promoting bone or cartilage formation differentiation.[112] Moreover, BMP-2, as an osteogenic growth factor, has been widely used to promote the differentiation of stem cells into osteoblasts.[116] Osteomodulin can actively coordinate the osteogenic process through the BMP-2/suppressor of mother against decapentaplegic signalling pathway.[117] In a scaffold-free micro mass environment, finely tuning the concentration of growth factors such as transforming growth factor-β can effectively guide iPSCs to differentiate into chondrocytes.[118] These studies highlight the importance of biomolecules in mimicking the *in vivo* microenvironment and promoting organoid formation. Using specific chemical small molecule inhibitors can regulate the Wnt and melanocyte inducing transcription factor signalling pathways, thereby improving the efficiency and uniformity of hiPSC chondrogenic formation.[119]

Physical stimulation

Physical stimulation such as mechanical forces, electrical

stimulation, and hypoxia are crucial for the formation and function of cartilage organoids. For instance, cartilage is often subject to compression and shear forces, which can promote the synthesis of ECM and tissue formation.[120] *In vitro* studies that mimic these physical stimuli can better simulate the *in vivo* environment, thereby enhancing the maturity and functionality of organoids. Mechanical forces can promote organoid maturation by affecting cell morphology and the synthesis of ECM.[121] Electrical stimulation is another physical stimulus that affects the regeneration of bone and cartilage tissues. A study has shown that Electrical stimulation can regulate cell behaviour, including proliferation, differentiation, and the synthesis of ECM.[91] The use of electroactive biomaterials can mimic endogenous bioelectric phenomena, thereby promoting the regeneration process of bone and cartilage.[98] Hypoxia is an essential factor in cartilage tissue engineering. Chondrocytes can better maintain their phenotype and function in a hypoxic environment.[122] *In vitro* culture that simulates a hypoxic environment can improve the formation efficiency and quality of cartilage organoids. The stable expression of hypoxia-inducible factor-1α under hypoxic conditions is significant for maintaining the homeostasis and function of chondrocytes.[123-125]

Organoid Assembly

Self-assembly technology

Self-assembly techniques offer a scaffold-free method for cellular self-organisation, utilising specific biomaterials and culture conditions to induce stem cell differentiation and form organoids. For instance, Yang et al.[25] employed gelatin-based microcryogels customised with HA and HYP to induce cartilage and bone regeneration, respectively, assembling into osteochondral organoids *in vivo*. Nilsson Hall's team[53] allowed human periosteum-derived cells to self-assemble into microspheroids within specially designed microwell plates made from 3% agarose. These microspheroids then differentiated into mini-tissues with chondrogenic properties *in vitro* and further fused to form larger tissue structures, eventually generating bone organoids *in vivo* and healing critical-sized long bone defects in rats *ex vivo*. Tam's group[49] successfully cultivated chondrogenic organoids using induced hiPSCs and self-assembly techniques, demonstrating stable chondrogenic characteristics upon *in vitro* culture and *in vivo* implantation. Stimulation with factors such as BMP-4, BIO, and 3,3,5-triiodo-L-thyronine induced the maturation and mineralisation of these organoids, particularly after the addition of IL-1β, which effectively bridged critical-sized long bone defects *in vivo* models, confirming the feasibility of using hiPSCs and self-assembly strategies to promote bone repair. This approach not only allows for precise control over the size and characteristics of bone or cartilage organoids but also enables accurate simulation of the cartilage intermediates in bone tissue engineering by mimicking the natural fracture healing process.

3D Printing

The application of 3D printing technology in organoid construction enables researchers to precisely control the shape and structure of organoids. Wang et al.[88] used bioinks such as GelMA/ alginate methacrylate /HYP to print scaffolds that mimic the microstructure of bone tissue, on which bone organoids were cultivated. Xie et al.[34] utilised digital light processing 3D printing technology to load BMSCs into hydrogel microspheres, printing and inducing the formation of osteo-callus organoids, effectively simulating the endochondral ossification process and significantly accelerating bone defect repair. Bolander's team[126] combined human bone membrane-derived cells pre-treated *ex vivo* with thiol-ene alginate hydrogel to successfully fabricate callus organoids that mimic fracture-healing tissue. These not only verified cell viability and the ability to maintain chondrogenic and osteogenic differentiation *in vitro* but also showed that these biomaterials provided the necessary 3D cues for cells to organize tissue formation without additional stimulatory molecules *in vivo*. Li et al.[127] also used 3D printing technology with digital light processing printing methods to create a mini joint system simulating the knee joint using human BMSCs. This system integrates an engineered osteochondral complex, synovial fibro-like tissue, and adipose tissue, capable of simulating the interactions and inflammatory responses between joint tissues, providing an effective *in vitro* model for joint disease research and drug testing. This method offers new possibilities for the precise repair of bone defects.

Induced cell differentiation

Adding specific growth factors to 3D culture systems such as hydrogels can effectively induce stem cells to differentiate into osteoblasts or chondrocytes. For example, Fuller et al.[60] used a hydrogel ECM to culture a pre-osteoblastic cell line, controlling its differentiation into functional osteoblasts, thus forming bone organoids. Iordachescu et al.[61] developed a trabecular bone organ model to study the regulatory mechanisms of local bone remodelling. This model assembles trabecular bone organoids by inducing cell differentiation simulating the bone formation process in bone tissue engineering. These constructs serve as multicellular, organotypic units, forming large bone cell protrusions and tubular structures that develop on a millimeter scale and initiate new matrix formation away from the original structure. This strategy not only aids in studying the differentiation mechanisms of bone cells but also provides a new cellular source for bone tissue engineering.

Demineralised bone matrix technology

Demineralised bone matrix (DBM) technology provides a natural scaffold for cells by removing cellular components while retaining the original structure and bioactive molecules of the ECM.[61] With excellent biocompatibility in constructing bone and cartilage organoids, DBM aids in cell adhesion, proliferation, and differentiation.[49] Park et al.[62] found that inducing cell differentiation within DBM using demineralised bone paper biomaterial simulates unmineralised bone matrix, guiding osteoblasts to deposit mineralised bone tissue and activating quiescent osteocytes and inducing osteoclastogenesis under chemical stimulation such as vitamin D3 and prostaglandin E2. The use of the DBM matrix offers an effective strategy for tissue engineering, especially in the repair of bone and cartilage defects.

Microfluidic technology

Microfluidic technology provides a highly controllable environment for organoid culture by precisely controlling fluid flow. Shen et al.[94] developed a novel RSD-MS using a microfluidic system combining photopolymerisation and self-assembly techniques, promoting the proliferation, adhesion, and chondrogenic differentiation of BMSCs, offering innovative strategies and material choices for cartilage regeneration and tissue engineering. Paggi's team[128] created a "cartilage-on-chip" system using microfluidic technology, which can simulate the microenvironment of chondrocytes *in vitro*. This system allows researchers to apply precisely defined compression and multi-directional mechanical stimuli to a 3D agarose hydrogel filled with chondrocytes. This microenvironment simulation method enables researchers to explore how different types of mechanical stimuli affect the phenotype of chondrocytes and the production of ECM. Mechanical stimulation increases the expression of specific chondrocyte markers and promotes the production of glycosaminoglycans, a major component of the native cartilage ECM (**Figure 6**). Additionally, Alamán-Díez et al.[129] embedded pre-differentiated chondrogenic stem cells in a microfluidic chip and provided suitable culture conditions to simulate the cartilage tissue microenvironment, promoting cell maturation and matrix formation, thus providing an efficient *in vitro* model for cartilage tissue engineering. This technology is particularly useful in cartilage tissue engineering as it can simulate the microenvironment of cartilage, facilitating the formation of chondrogenic organoids. Bahmaee et al.[130] designed a microfluidic chip with a 3D microenvironment, combining fluid shear stress to simulate the growth conditions of cartilage tissue, promoting the differentiation and matrix formation of chondrogenic cells, thus providing a controllable and efficient *in vitro* model for cartilage tissue engineering.

Applications

Organoid technology has revolutionised the field of biomedical research, offering unprecedented opportunities for studying complex biological systems and advancing clinical applications. Particularly, bone and cartilage organoids have emerged as invaluable tools due to their ability to recapitulate the intricate microenvironments of human tissues.[15] The rapid advancement of organoid technology has established it as a vital platform for studying the self-renewal and self-organising capabilities of stem cells, including ESCs, iPSCs, and adult stem cells.[22] The *in vitro* models of organoids, with their ability to simulate complex natural tissue structures, have become crucial tools in both fundamental biological research and clinical applications. Particularly in the realm of bone and cartilage organoids, these models have successfully emulated the physiological characteristics of human cartilage at a microscopic level and have filled the gap for high-fidelity models of cartilage diseases.[14-16]

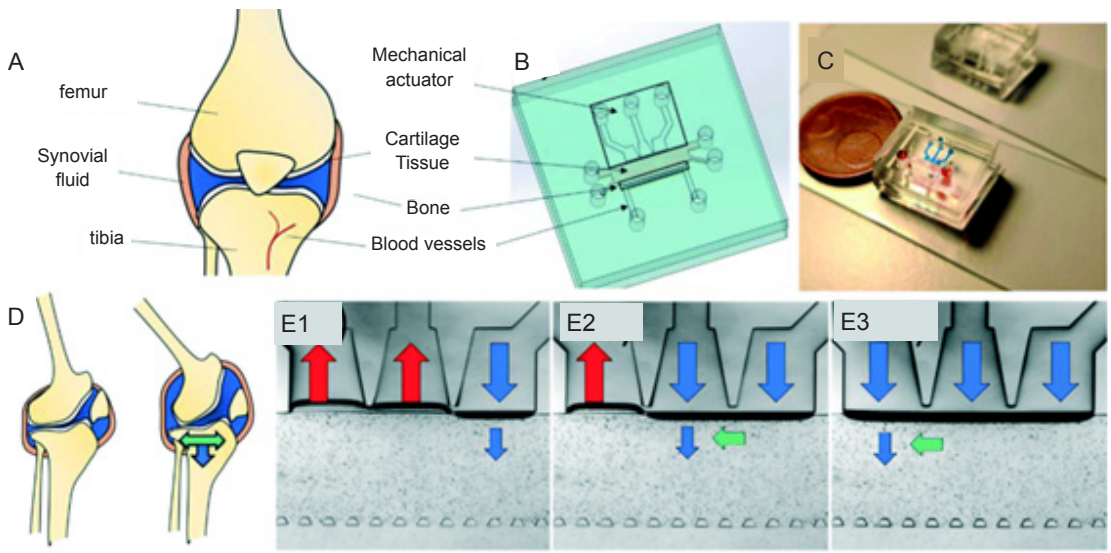

Figure 6. Cartilage-on chip design, operation, and characterisation. (A) A diagrammatic representation of the knee joint is provided. (B) The design of the cartilage-on-chip device is outlined, emphasising its key components. (C) A top-down view of the cartilage-on-chip device, utilising food dyes for clarity – the actuation unit is coloured blue, the cell-hydrogel chamber is red, and the perfusion channel is yellow. (D) A side view of the joint in motion, illustrating the creation of multi directional mechanical stimulation (shear strain indicated by green arrows and compression by blue arrows). (E1–3) A top-down view of the cartilage-on-chip device filled with human chondrocytes in agarose and subjected to a sequence that induces multi-directional mechanical stimulation. Red Arrows indicate the deposition of a thin extracellular matrix shell (1–5 μm) and intercellular matrix around three-dimensional-cultured chondrocytes under multi-directional mechanical stimulation. Blue arrows represent compression, while green arrows indicate shear strain. Adapted from Paggi et al.[128]

Bone regeneration

MSCs have made significant advances in the field of tissue engineering, particularly in bone regeneration.[23] By integrating MSCs with a gelatin sponge scaffold loaded with human BMP-2, Deng et al.[87] successfully constructed bone organoids *in vivo* to promote bone regeneration. This innovative approach not only addresses the limitation of autologous cell supply but also minimises the associated risks of using allogeneic cells in stem cell transplantation. Furthermore, Zhang et al.[131] discovered that cells derived from bone organoids can rapidly and effectively rebuild the compromised peripheral and solid immune organs of irradiated mice. This finding presents a potential alternative source of haematopoietic stem/progenitor cells for transplantation, potentially benefiting a broader range of patients with bone regeneration disorders. Qiao et al.'s[79] neuro-vascularised bone organoid, constructed from a double small interfering RNA loaded cell membrane coated scaffold, utilised the synergistic effects of angiogenesis and neurogenesis to not only improve the microenvironment of bone defects, but also promote bone tissue repair and integration.

Regeneration of transparent cartilage

The regeneration of transparent cartilage has posed a significant challenge in the field of tissue engineering. However, innovative organoid cultivation strategies have led to groundbreaking progress. These strategies harness the self-organising capabilities of dissociated cells to successfully simulate the natural regeneration process of cartilage, effectively recapturing classic models of cartilage regeneration.[132, 133] Notably, by utilising fibroblasts derived from terminal phalangeal elements as a source of chondrogenic progenitor cells, researchers have successfully facilitated the regeneration of transparent cartilage.[132, 134] This breakthrough not only presents a fresh viewpoint on cartilage regeneration but also highlights the pivotal role of cellular self-organisation. The crux of this innovative method is its reliance on the cells' intrinsic potential to catalyze tissue formation and healing, thus minimising the need for external growth factors or invasive procedures. This paradigm shift in cartilage regeneration heralds a new era of regenerative medicine, offering hope for the treatment of cartilage-related diseases and injuries.

Drug screening

The rapid development of organoid technology has revolutionised the field of drug screening, demonstrating immense potential, particularly in the study of bone disease treatments.[16, 135] Li et al.[136] indicates that organoid-on-a-chip technology provides an innovative method for constructing *in vitro* models that simulate the physiological environment of specific organs, such as cartilage. This approach successfully overcomes the limitations of traditional two-dimensional cell culture models and animal models in the drug screening process. By constructing bone organoids, researchers can assess the efficacy of drugs in a more physiologically relevant environment. This not only reduces the time and cost associated with drug screening but also decreases the toxicity of drug testing. Moreover, organoid technology enhances intercellular interactions, allowing for a more accurate simulation of human physiological and pathological conditions. This highly mimetic method significantly improves the efficiency and accuracy of drug development and provides a new model system for a deeper understanding of drug mechanisms of action.

Furthermore, organoid technology has shown unique advantages in simulating disease histological characteristics and heterogeneity. It offers important tools for precision medicine and personalised therapy, assisting researchers in tailoring treatment plans based on the specific pathological features of patients.

Precision medicine

Precision medicine, as an emerging trend in disease treatment, has shown particular importance in the field of bone disease therapy.[16] By utilising patient-derived organoids, researchers can delve into the genetic heterogeneity among individuals, offering a novel strategy for personalised healthcare. This approach not only aids in predicting patients' responses to specific treatments but also simulates the differences between tumours and individual patients, providing a robust supportive tool for clinical decision-making. As research progresses and develops, patient-specific bone organoids are expected to play an increasingly critical role in the realm of precision medicine. Human OA cartilage organoids have become vital tools for uncovering new molecular mechanisms of cartilage degeneration and provide a new platform for assessing targeted treatment pathways.[137] In the exploration of novel treatment strategies, iPSC-derived organoids have also been employed to identify pathogenic mutations in families with early-onset OA and to conduct functional studies on exon-edited hiPSCs, offering key insights into the gene expression of chondrocytes.[138] Furthermore, organoids and organoid extracellular vesicles have demonstrated tremendous potential as nanocarriers due to their acellular nature, stable drug-loading capacity, nanoscale size, and excellent biocompatibility. Particularly in the repair of osteoporotic fractures, the application of organoid extracellular vesicles has already shown its significance.[139]

These *in vitro* models, capable of intricately simulating natural tissue structures, have become indispensable tools in basic biological research and clinical applications. Especially in the study of bone and cartilage organoids, these models not only mimic the micro-physiology of human cartilage to a certain extent but also fill the gap for high-fidelity cartilage disease models, which is crucial for understanding the pathogenesis of cartilage diseases and the development of new drugs.[14-16]

Future Perspectives

The potential of organoid technology in advancing the treatment of bone/cartilage injuries and diseases, particularly OA, is immense. This regenerative medicine approach has opened new avenues for disease modelling and therapeutic development. However, as we look to the future, it is essential to recognise the challenges and limitations that organoid research may encounter. One of the primary challenges is the discrepancy between organoids and human tissues, which may impact the accuracy of drug screening and disease studies. Maintaining the long-term stability and functionality of

organoids for extended use in disease research and therapy is another significant hurdle. Achieving consistency and replicability in organoid production for large-scale applications is a technical challenge that needs to be addressed. Economic considerations are also crucial, as the high costs associated with organoid generation and maintenance could limit their broad application. Potential clinical risks, such as immune rejection and safety concerns, must be thoroughly investigated before organoid technology can be translated into clinical practice. Furthermore, despite the availability of various natural and synthetic materials, identifying an ideal biomaterial for organoid construction remains a challenge. In vitro organoid models have limitations in mimicking the complexity of *in vivo* physiological and pathological processes, which affects their predictive power for clinical outcomes.

Despite these challenges, the future of organoid technology holds great promise. The integration of organoid technology with advanced bioengineering techniques, such as 3D printing, microfluidics, and gene editing, presents a promising frontier. These interdisciplinary advancements could lead to more sophisticated disease models and tailored treatment strategies, enhancing both the efficiency and accuracy of medical interventions. In the realm of bone/cartilage regeneration, organoids offer a unique advantage by providing a controlled microenvironment for stem cell differentiation and tissue development. This controlled system allows researchers to study the intricate processes of tissue regeneration and to test the efficacy of potential therapeutics in a physiologically relevant context.

The implications of organoid technology for precision medicine are particularly noteworthy. By utilising patient-derived organoids, researchers can explore the genetic heterogeneity among individuals, leading to a more personalised approach to treatment. This strategy has the potential to predict patient responses to specific treatments and to model the variability between tumours and individual patients, thus informing clinical decision-making. As we look to the future, the role of organoid technology in addressing the challenges faced in translational medicine is clear. The development of more complex tissue-engineered implants, using pre-differentiated organoids as building blocks, could revolutionise the way we approach cartilage repair and bone regeneration.

Limitations

Exploring the clinical potential of bone/cartilage organoids in disease modelling reveals several key limitations. These include discrepancies between organoids and human tissues that may impact drug screening and disease studies, as well as challenges in maintaining the long-term stability and functionality of organoids for extended use in disease research and therapy. Additionally, the technical challenge of achieving consistency and replicability in organoid production for large-scale applications, the high costs associated with organoid generation and maintenance, and potential clinical risks such as immune rejection and safety concerns must be addressed. Furthermore, despite the availability of various natural and synthetic materials, identifying an ideal biomaterial for organoid construction remains a challenge. Lastly, in vitro organoid models have limitations in mimicking the complexity of *in vivo* physiological and pathological processes, affecting their predictive power for clinical outcomes. Addressing these challenges is essential for enhancing the physiological relevance of organoid models and unlocking their full potential in regenerative medicine and precision healthcare.

Conclusions

In conclusion, the advancement of organoid technology holds great promise for the future of medicine, particularly in the fields of bone and cartilage regeneration. As research continues to push the boundaries of this technology, we can anticipate a future where organoids play a critical role in medical research, drug development, and clinical treatment, offering patients a wider range of therapeutic options and improved outcomes. The ongoing evolution of organoid technology is a testament to the power of interdisciplinary collaboration in driving biomedical innovation.

Author contributions

Concept and design: JH, CL, XZ, JL; acquisition, analysis, or interpretation of data: JH, AL, RL, JL; critical revision of the manuscript for important intellectual content: JH, XW, CL, SJ, JC; administrative, technical, or material support: JL, XZ, CL; supervision: JL, XZ, JH. All authors drafted of the manuscript and read and approved the final version of the manuscript.

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Conflicts of interest statement

All authors declared no conflicts of interest.

1. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* **2018,** *392,* 1789-1858.
2. Li, M.; Yin, H.; Yan, Z.; Li, H.; Wu, J.; Wang, Y.; Wei, F.; Tian, G.; Ning, C.; Li, H.; Gao, C.; Fu, L.; Jiang, S.; Chen, M.; Sui, X.; Liu, S.; Chen, Z.; Guo, Q. The immune microenvironment in cartilage injury and repair. *Acta Biomater.* **2022,** *140,* 23-42.
3. GBD 2021 Osteoarthritis Collaborators. Global, regional, and national burden of osteoarthritis, 1990-2020 and projections to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *The Lancet Rheumatology.* **2023,** *5,* e508-e522.
4. Li, H.; Kong, W.; Liang, Y.; Sun, H. Burden of osteoarthritis in China, 1990-2019: findings from the Global Burden of Disease Study 2019. *Clin Rheumatol.* **2024,** *43,* 1189-1197.
5. von Mentzer, U.; Corciulo, C.; Stubelius, A. Biomaterial integration

in the joint: pathological considerations, immunomodulation, and the extracellular matrix. *Macromol Biosci.* **2022,** *22*, e2200037.
6. Lin, A.; Sved Skottvoll, F.; Rayner, S.; Pedersen-Bjergaard, S.; Sullivan, G.; Krauss, S.; Ray Wilson, S.; Harrison, S. 3D cell culture models and organ-on-a-chip: meet separation science and mass spectrometry. *Electrophoresis.* **2020,** *41*, 56-64.
7. Ballard, D. H.; Boyer, C. J.; Alexander, J. S. Organoids - preclinical models of human disease. *N Engl J Med.* **2019,** *380*, 1981-1982.
8. Zhang, Y.; Li, G.; Wang, J.; Zhou, F.; Ren, X.; Su, J. Small joint organoids 3D bioprinting: construction strategy and application. *Small.* **2024,** *20*, e2302506.
9. Wu, M.; Zheng, K.; Li, W.; He, W.; Qian, C.; Lin, Z.; Xiao, H.; Yang, H.; Xu, Y.; Wei, M.; Bai, J.; Geng, D. Nature-inspired strategies for the treatment of osteoarthritis. *Adv Funct Mater.* **2024,** *34*, 2305603.
10. Abraham, D. M.; Herman, C.; Witek, L.; Cronstein, B. N.; Flores, R. L.; Coelho, P. G. Self-assembling human skeletal organoids for disease modeling and drug testing. *J Biomed Mater Res B Appl Biomater.* **2022,** *110*, 871-884.
11. Sánchez-Porras, D.; Durand-Herrera, D.; Paes, A. B.; Chato-Astrain, J.; Verplancke, R.; Vanfleteren, J.; Sánchez-López, J. D.; García-García Ó, D.; Campos, F.; Carriel, V. Ex vivo generation and characterization of human hyaline and elastic cartilaginous microtissues for tissue engineering applications. *Biomedicines.* **2021,** *9*, 292.
12. Manjula-Basavanna, A.; Duraj-Thatte, A. M.; Joshi, N. S. Robust self-regeneratable stiff living materials fabricated from microbial cells. *Adv Funct Mater.* **2021,** *31*, 2010784.
13. Kim, I. L.; Mauck, R. L.; Burdick, J. A. Hydrogel design for cartilage tissue engineering: a case study with hyaluronic acid. *Biomaterials.* **2011,** *32*, 8771-8782.
14. Wang, X. H.; Liu, N.; Zhang, H.; Yin, Z. S.; Zha, Z. G. From cells to organs: progress and potential in cartilaginous organoids research. *J Transl Med.* **2023,** *21*, 926.
15. Zhao, D.; Saiding, Q.; Li, Y.; Tang, Y.; Cui, W. Bone organoids: recent advances and future challenges. *Adv Healthc Mater.* **2024,** *13*, e2302088.
16. Huang, J.; Zhang, L.; Lu, A.; Liang, C. Organoids as innovative models for bone and joint diseases. *Cells.* **2023,** *12*, 1590.
17. Lin, J.; Huang, J.; Jiao, Z.; Nian, M.; Li, C.; Dai, Y.; Jia, S.; Zhang, X. Mesenchymal stem cells for osteoarthritis: recent advances in related cell therapy. *Bioeng Transl Med.* **2024**, e10701.
18. Mendes-Pinheiro, B.; Campos, J.; Marote, A.; Soares-Cunha, C.; Nickels, S. L.; Monzel, A. S.; Cibrão, J. R.; Loureiro-Campos, E.; Serra, S. C.; Barata-Antunes, S.; Duarte-Silva, S.; Pinto, L.; Schwamborn, J. C.; Salgado, A. J. Treating Parkinson's disease with human bone marrow mesenchymal stem cell secretome: a translational investigation using human brain organoids and different routes of in vivo administration. *Cells.* **2023,** *12*, 2565.
19. Brassard, J. A.; Lutolf, M. P. Engineering stem cell self-organization to build better organoids. *Cell Stem Cell.* **2019,** *24*, 860-876.
20. Aurora, M.; Spence, J. R. hPSC-derived lung and intestinal organoids as models of human fetal tissue. *Dev Biol.* **2016,** *420*, 230-238.
21. Yang, J.; Zhang, Y. S.; Yue, K.; Khademhosseini, A. Cell-laden hydrogels for osteochondral and cartilage tissue engineering. *Acta Biomater.* **2017,** *57*, 1-25.
22. Fatehullah, A.; Tan, S. H.; Barker, N. Organoids as an in vitro model of human development and disease. *Nat Cell Biol.* **2016,** *18*, 246-254.
23. Kleuskens, M. W. A.; Crispim, J. F.; van Donkelaar, C. C.; Janssen, R. P. A.; Ito, K. Evaluating initial integration of cell-based chondrogenic constructs in human osteochondral explants. *Tissue Eng Part C Methods.* **2022,** *28*, 34-44.
24. Xiahou, Z.; She, Y.; Zhang, J.; Qin, Y.; Li, G.; Zhang, L.; Fang, H.; Zhang, K.; Chen, C.; Yin, J. Designer hydrogel with intelligently switchable stem-cell contact for incubating cartilaginous microtissues. *ACS Appl Mater Interfaces.* **2020,** *12*, 40163-40175.
25. Yang, Z.; Wang, B.; Liu, W.; Li, X.; Liang, K.; Fan, Z.; Li, J. J.; Niu, Y.; He, Z.; Li, H.; Wang, D.; Lin, J.; Du, Y.; Lin, J.; Xing, D. In situ self-assembled organoid for osteochondral tissue regeneration with dual functional units. *Bioact Mater.* **2023,** *27*, 200-215.
26. Dai, K.; Zhang, Q.; Deng, S.; Yu, Y.; Zhu, F.; Zhang, S.; Pan, Y.; Long, D.; Wang, J.; Liu, C. A BMP-2-triggered in vivo osteo-organoid for cell therapy. *Sci Adv.* **2023,** *9*, eadd1541.
27. Li, Q.; Yu, H.; Sun, M.; Yang, P.; Hu, X.; Ao, Y.; Cheng, J. The tissue origin effect of extracellular vesicles on cartilage and bone regeneration. *Acta Biomater.* **2021,** *125*, 253-266.
28. Shimizu, H.; Yokoyama, S.; Asahara, H. Growth and differentiation of the developing limb bud from the perspective of chondrogenesis. *Dev Growth Differ.* **2007,** *49*, 449-454.
29. Gao, L.; Orth, P.; Cucchiarini, M.; Madry, H. Effects of solid acellular type-I/III collagen biomaterials on in vitro and in vivo chondrogenesis of mesenchymal stem cells. *Expert Rev Med Devices.* **2017,** *14*, 717-732.
30. Chamberlain, G.; Fox, J.; Ashton, B.; Middleton, J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells.* **2007,** *25*, 2739-2749.
31. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.; Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* **2006,** *8*, 315-317.
32. Sakaguchi, Y.; Sekiya, I.; Yagishita, K.; Muneta, T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum.* **2005,** *52*, 2521-2529.
33. Yoshimura, H.; Muneta, T.; Nimura, A.; Yokoyama, A.; Koga, H.; Sekiya, I. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. *Cell Tissue Res.* **2007,** *327*, 449-462.
34. Xie, C.; Liang, R.; Ye, J.; Peng, Z.; Sun, H.; Zhu, Q.; Shen, X.; Hong, Y.; Wu, H.; Sun, W.; Yao, X.; Li, J.; Zhang, S.; Zhang, X.; Ouyang, H. High-efficient engineering of osteo-callus organoids for rapid bone regeneration within one month. *Biomaterials.* **2022,** *288*, 121741.
35. Scotti, C.; Piccinini, E.; Takizawa, H.; Todorov, A.; Bourgine, P.; Papadimitropoulos, A.; Barbero, A.; Manz, M. G.; Martin, I. Engineering of a functional bone organ through endochondral ossification. *Proc Natl Acad Sci U S A.* **2013,** *110*, 3997-4002.
36. Hamid, A. A.; Idrus, R. B.; Saim, A. B.; Sathappan, S.; Chua, K. H. Characterization of human adipose-derived stem cells and expression of chondrogenic genes during induction of cartilage differentiation. *Clinics (Sao Paulo).* **2012,** *67*, 99-106.
37. Baksh, D.; Yao, R.; Tuan, R. S. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. *Stem Cells.* **2007,** *25*, 1384-1392.
38. Han, Y.; Wu, Y.; Wang, F.; Li, G.; Wang, J.; Wu, X.; Deng, A.; Ren, X.; Wang, X.; Gao, J.; Shi, Z.; Bai, L.; Su, J. Heterogeneous DNA hydrogel loaded with Apt02 modified tetrahedral framework nucleic acid accelerated critical-size bone defect repair. *Bioact Mater.* **2024,** *35*, 1-16.

39. Lin, W.; Wang, M.; Xu, L.; Tortorella, M.; Li, G. Cartilage organoids for cartilage development and cartilage-associated disease modeling. *Front Cell Dev Biol.* **2023,** *11*, 1125405.
40. Vail, D. J.; Somoza, R. A.; Caplan, A. I. MicroRNA regulation of bone marrow mesenchymal stem cell chondrogenesis: toward articular cartilage. *Tissue Eng Part A.* **2022,** *28*, 254-269.
41. Lemarié, L.; Dargar, T.; Grosjean, I.; Gache, V.; Courtial, E. J.; Sohier, J. Human induced pluripotent spheroids' growth is driven by viscoelastic properties and macrostructure of 3D hydrogel environment. *Bioengineering (Basel).* **2023,** *10*, 1418.
42. Adkar, S. S.; Brunger, J. M.; Willard, V. P.; Wu, C. L.; Gersbach, C. A.; Guilak, F. Genome engineering for personalized arthritis therapeutics. *Trends Mol Med.* **2017,** *23*, 917-931.
43. Narsinh, K. H.; Plews, J.; Wu, J. C. Comparison of human induced pluripotent and embryonic stem cells: fraternal or identical twins? *Mol Ther.* **2011,** *19*, 635-638.
44. Hirschi, K. K.; Li, S.; Roy, K. Induced pluripotent stem cells for regenerative medicine. *Annu Rev Biomed Eng.* **2014,** *16*, 277-294.
45. Zhao, J.; Jiang, W. J.; Sun, C.; Hou, C. Z.; Yang, X. M.; Gao, J. G. Induced pluripotent stem cells: origins, applications, and future perspectives. *J Zhejiang Univ Sci B.* **2013,** *14*, 1059-1069.
46. O'Connor, S. K.; Katz, D. B.; Oswald, S. J.; Groneck, L.; Guilak, F. Formation of osteochondral organoids from murine induced pluripotent stem cells. *Tissue Eng Part A.* **2021,** *27*, 1099-1109.
47. Larson, B. L.; Yu, S. N.; Park, H.; Estes, B. T.; Moutos, F. T.; Bloomquist, C. J.; Wu, P. B.; Welter, J. F.; Langer, R.; Guilak, F.; Freed, L. E. Chondrogenic, hypertrophic, and osteochondral differentiation of human mesenchymal stem cells on three-dimensionally woven scaffolds. *J Tissue Eng Regen Med.* **2019,** *13*, 1453-1465.
48. Liu, Z.; Tang, Y.; Lü, S.; Zhou, J.; Du, Z.; Duan, C.; Li, Z.; Wang, C. The tumourigenicity of iPS cells and their differentiated derivates. *J Cell Mol Med.* **2013,** *17*, 782-791.
49. Tam, W. L.; Freitas Mendes, L.; Chen, X.; Lesage, R.; Van Hoven, I.; Leysen, E.; Kerckhofs, G.; Bosmans, K.; Chai, Y. C.; Yamashita, A.; Tsumaki, N.; Geris, L.; Roberts, S. J.; Luyten, F. P. Human pluripotent stem cell-derived cartilaginous organoids promote scaffold-free healing of critical size long bone defects. *Stem Cell Res Ther.* **2021,** *12*, 513.
50. Hall, G. N.; Tam, W. L.; Andrikopoulos, K. S.; Casas-Fraile, L.; Voyiatzis, G. A.; Geris, L.; Luyten, F. P.; Papantoniou, I. Patterned, organoid-based cartilaginous implants exhibit zone specific functionality forming osteochondral-like tissues in vivo. *Biomaterials.* **2021,** *273*, 120820.
51. Liu, H.; Yang, L.; Yu, F. F.; Wang, S.; Wu, C.; Qu, C.; Lammi, M. J.; Guo, X. The potential of induced pluripotent stem cells as a tool to study skeletal dysplasias and cartilage-related pathologic conditions. *Osteoarthritis Cartilage.* **2017,** *25*, 616-624.
52. Abe, K.; Yamashita, A.; Morioka, M.; Horike, N.; Takei, Y.; Koyamatsu, S.; Okita, K.; Matsuda, S.; Tsumaki, N. Engraftment of allogeneic iPS cell-derived cartilage organoid in a primate model of articular cartilage defect. *Nat Commun.* **2023,** *14*, 804.
53. Nilsson Hall, G.; Mendes, L. F.; Gklava, C.; Geris, L.; Luyten, F. P.; Papantoniou, I. Developmentally engineered callus organoid bioassemblies exhibit predictive in vivo long bone healing. *Adv Sci (Weinh).* **2020,** *7*, 1902295.
54. Tsumaki, N.; Okada, M.; Yamashita, A. iPS cell technologies and cartilage regeneration. *Bone.* **2015,** *70*, 48-54.
55. Rodríguez Ruiz, A.; van Hoolwerff, M.; Sprangers, S.; Suchiman, E.; Schoenmaker, T.; Dibbets-Schneider, P.; Bloem, J. L.; Nelissen, R.; Freund, C.; Mummery, C.; Everts, V.; de Vries, T. J.; Ramos, Y. F. M.; Meulenbelt, I. Mutation in the CCAL1 locus accounts for bidirectional process of human subchondral bone turnover and cartilage mineralization. *Rheumatology (Oxford).* **2022,** *62*, 360-372.
56. Han, B.; Fang, W.; Yang, Z.; Wang, Y.; Zhao, S.; Hoang, B. X.; Vangsness, C. T., Jr. Enhancement of chondrogenic markers by exosomes derived from cultured human synovial fluid-derived cells: a comparative analysis of 2D and 3D conditions. *Biomedicines.* **2023,** *11*, 3145.
57. Amarasekara, D. S.; Kim, S.; Rho, J. Regulation of osteoblast differentiation by cytokine networks. *Int J Mol Sci.* **2021,** *22*, 2851.
58. Breslin, S.; O'Driscoll, L. Three-dimensional cell culture: the missing link in drug discovery. *Drug Discov Today.* **2013,** *18*, 240-249.
59. Fang, Y.; Eglen, R. M. Three-dimensional cell cultures in drug discovery and development. *SLAS Discov.* **2017,** *22*, 456-472.
60. Fuller, J.; Lefferts, K. S.; Shah, P.; Cottrell, J. A. Methodology and characterization of a 3D bone organoid model derived from murine cells. *Int J Mol Sci.* **2024,** *25*, 4225.
61. Iordachescu, A.; Hughes, E. A. B.; Joseph, S.; Hill, E. J.; Grover, L. M.; Metcalfe, A. D. Trabecular bone organoids: a micron-scale 'humanised' prototype designed to study the effects of microgravity and degeneration. *NPJ Microgravity.* **2021,** *7*, 17.
62. Park, Y.; Cheong, E.; Kwak, J. G.; Carpenter, R.; Shim, J. H.; Lee, J. Trabecular bone organoid model for studying the regulation of localized bone remodeling. *Sci Adv.* **2021,** *7*, eabd6495.
63. Knowles, H. J.; Chanalaris, A.; Koutsikouni, A.; Cribbs, A. P.; Grover, L. M.; Hulley, P. A. Mature primary human osteocytes in mini organotypic cultures secrete FGF23 and PTH1-34-regulated sclerostin. *Front Endocrinol (Lausanne).* **2023,** *14*, 1167734.
64. Kleuskens, M. W. A.; Crispim, J. F.; van Doeselaar, M.; van Donkelaar, C. C.; Janssen, R. P. A.; Ito, K. Neo-cartilage formation using human nondegenerate versus osteoarthritic chondrocyte-derived cartilage organoids in a viscoelastic hydrogel. *J Orthop Res.* **2023,** *41*, 1902-1915.
65. Crispim, J. F.; Ito, K. De novo neo-hyaline-cartilage from bovine organoids in viscoelastic hydrogels. *Acta Biomater.* **2021,** *128*, 236-249.
66. Wei, W.; Dai, H. Articular cartilage and osteochondral tissue engineering techniques: recent advances and challenges. *Bioact Mater.* **2021,** *6*, 4830-4855.
67. D'Costa, K.; Kosic, M.; Lam, A.; Moradipour, A.; Zhao, Y.; Radisic, M. Biomaterials and culture systems for development of organoid and organ-on-a-chip models. *Ann Biomed Eng.* **2020,** *48*, 2002-2027.
68. Haghwerdi, F.; Khozaei Ravari, M.; Taghiyar, L.; Shamekhi, M. A.; Jahangir, S.; Haririan, I.; Baghaban Eslaminejad, M. Application of bone and cartilage extracellular matrices in articular cartilage regeneration. *Biomed Mater.* **2021,** *16*, 042014.
69. Chen, L.; Ren, W. Three-dimensional (3D) and drug-eluting nanofiber coating for prosthetic implants. In *Racing for the surface: antimicrobial and interface tissue engineering*, Li, B.; Moriarty, T. F.; Webster, T.; Xing, M., eds.; Springer International Publishing: Cham, 2020; pp 91-114.
70. Wang, Z.; Wang, Y.; Yan, J.; Zhang, K.; Lin, F.; Xiang, L.; Deng, L.; Guan, Z.; Cui, W.; Zhang, H. Pharmaceutical electrospinning and 3D printing scaffold design for bone regeneration. *Adv Drug Deliv Rev.* **2021,** *174*, 504-534.
71. Zhou, J.; Xiong, S.; Liu, M.; Yang, H.; Wei, P.; Yi, F.; Ouyang, M.; Xi, H.; Long, Z.; Liu, Y.; Li, J.; Ding, L.; Xiong, L. Study on the influence of scaffold morphology and structure on osteogenic performance. *Front Bioeng Biotechnol.* **2023,** *11*, 1127162.
72. Chen, S.; Chen, X.; Geng, Z.; Su, J. The horizon of bone organoid: a

perspective on construction and application. *Bioact Mater.* **2022,** *18*, 15-25.
73. Chae, S.; Lee, H.; Ryu, D.; Kim, G. Macroscale pseudo-spheroids fabricated using methacrylated collagen-coated cells. *Theranostics.* **2024,** *14*, 924-939.
74. Nerger, B. A.; Sinha, S.; Lee, N. N.; Cheriyan, M.; Bertsch, P.; Johnson, C. P.; Mahadevan, L.; Bonventre, J. V.; Mooney, D. J. 3D hydrogel encapsulation regulates nephrogenesis in kidney organoids. *Adv Mater.* **2024,** *36*, e2308325.
75. Cullier, A.; Cassé, F.; Manivong, S.; Contentin, R.; Legendre, F.; Garcia Ac, A.; Sirois, P.; Roullin, G.; Banquy, X.; Moldovan, F.; Bertoni, L.; Audigié, F.; Galéra, P.; Demoor, M. Functionalized nanogels with endothelin-1 and bradykinin receptor antagonist peptides decrease inflammatory and cartilage degradation markers of osteoarthritis in a horse organoid model of cartilage. *Int J Mol Sci.* **2022,** *23*, 8949.
76. Cardier, J. E.; Diaz-Solano, D.; Wittig, O.; Sierra, G.; Pulido, J.; Moreno, R.; Fuentes, S.; Leal, F. Osteogenic organoid for bone regeneration: Healing of bone defect in congenital pseudoarthrosis of the tibia. *Int J Artif Organs.* **2024,** *47*, 107-114.
77. Shehzad, A.; Mukasheva, F.; Moazzam, M.; Sultanova, D.; Abdikhan, B.; Trifonov, A.; Akilbekova, D. Dual-crosslinking of gelatin-based hydrogels: promising compositions for a 3D printed organotypic bone model. *Bioengineering (Basel).* **2023,** *10*, 704.
78. Luo, Z.; Xian, B.; Li, K.; Li, K.; Yang, R.; Chen, M.; Xu, C.; Tang, M.; Rong, H.; Hu, D.; Ye, M.; Yang, S.; Lu, S.; Zhang, H.; Ge, J. Biodegradable scaffolds facilitate epiretinal transplantation of hiPSC-Derived retinal neurons in nonhuman primates. *Acta Biomater.* **2021,** *134*, 289-301.
79. Qiao, F.; Zou, Y.; Bie, B.; Lv, Y. Dual siRNA-loaded cell membrane functionalized matrix facilitates bone regeneration with angiogenesis and neurogenesis. *Small.* **2024,** *20*, e2307062.
80. Majumder, J.; Torr, E. E.; Aisenbrey, E. A.; Lebakken, C. S.; Favreau, P. F.; Richards, W. D.; Yin, Y.; Chang, Q.; Murphy, W. L. Human induced pluripotent stem cell-derived planar neural organoids assembled on synthetic hydrogels. *J Tissue Eng.* **2024,** *15*, 20417314241230633.
81. Zhang, F. X.; Liu, P.; Ding, W.; Meng, Q. B.; Su, D. H.; Zhang, Q. C.; Lian, R. X.; Yu, B. Q.; Zhao, M. D.; Dong, J.; Li, Y. L.; Jiang, L. B. Injectable mussel-inspired highly adhesive hydrogel with exosomes for endogenous cell recruitment and cartilage defect regeneration. *Biomaterials.* **2021,** *278*, 121169.
82. Eiraku, M.; Takata, N.; Ishibashi, H.; Kawada, M.; Sakakura, E.; Okuda, S.; Sekiguchi, K.; Adachi, T.; Sasai, Y. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature.* **2011,** *472*, 51-56.
83. Banihashemian, S. A.; Zamanlui Benisi, S.; Hosseinzadeh, S.; Shojaei, S.; Abbaszadeh, H. A. Chitosan/hyaluronan and alginate-nanohydroxyapatite biphasic scaffold as a promising matrix for osteoarthritis disorders. *Adv Pharm Bull.* **2024,** *14*, 176-191.
84. Dai, K.; Zhang, W.; Deng, S.; Wang, J.; Liu, C. Sulfated polysaccharide regulates the homing of HSPCs in a BMP-2-triggered in vivo osteo-organoid. *Adv Sci (Weinh).* **2023,** *10*, e2301592.
85. Vasvani, S.; Kulkarni, P.; Rawtani, D. Hyaluronic acid: A review on its biology, aspects of drug delivery, route of administrations and a special emphasis on its approved marketed products and recent clinical studies. *Int J Biol Macromol.* **2020,** *151*, 1012-1029.
86. Paggi, C. A.; Teixeira, L. M.; Le Gac, S.; Karperien, M. Joint-on-chip platforms: entering a new era of in vitro models for arthritis. *Nat Rev Rheumatol.* **2022,** *18*, 217-231.
87. Deng, S.; Zhu, F.; Dai, K.; Wang, J.; Liu, C. Harvest of functional mesenchymal stem cells derived from in vivo osteo-organoids. *Biomater Transl.* **2023,** *4*, 270-279.
88. Wang, J.; Wu, Y.; Li, G.; Zhou, F.; Wu, X.; Wang, M.; Liu, X.; Tang, H.; Bai, L.; Geng, Z.; Song, P.; Shi, Z.; Ren, X.; Su, J. Engineering large-scale self-mineralizing bone organoids with bone matrix-inspired hydroxyapatite hybrid bioinks. *Adv Mater.* **2024,** *36*, e2309875.
89. Sun, Y.; You, Y.; Wu, Q.; Hu, R.; Dai, K. Senescence-targeted MicroRNA/Organoid composite hydrogel repair cartilage defect and prevention joint degeneration via improved chondrocyte homeostasis. *Bioact Mater.* **2024,** *39*, 427-442.
90. Zhang, L.; Tang, H.; Xiahou, Z.; Zhang, J.; She, Y.; Zhang, K.; Hu, X.; Yin, J.; Chen, C. Solid multifunctional granular bioink for constructing chondroid basing on stem cell spheroids and chondrocytes. *Biofabrication.* **2022,** *14*, 035003.
91. Toni, R.; Barbaro, F.; Di Conza, G.; Zini, N.; Remaggi, G.; Elviri, L.; Spaletta, G.; Quarantini, E.; Quarantini, M.; Mosca, S.; Caravelli, S.; Mosca, M.; Ravanetti, F.; Sprio, S.; Tampieri, A. A bioartificial and vasculomorphic bone matrix-based organoid mimicking microanatomy of flat and short bones. *J Biomed Mater Res B Appl Biomater.* **2024,** *112*, e35329.
92. Ma, C.; Peng, Y.; Li, H.; Chen, W. Organ-on-a-chip: a new paradigm for drug development. *Trends Pharmacol Sci.* **2021,** *42*, 119-133.
93. Klotz, B. J.; Oosterhoff, L. A.; Utomo, L.; Lim, K. S.; Vallmajo-Martin, Q.; Clevers, H.; Woodfield, T. B. F.; Rosenberg, A.; Malda, J.; Ehrbar, M.; Spee, B.; Gawlitta, D. A versatile biosynthetic hydrogel platform for engineering of tissue analogues. *Adv Healthc Mater.* **2019,** *8*, e1900979.
94. Shen, C.; Wang, J.; Li, G.; Hao, S.; Wu, Y.; Song, P.; Han, Y.; Li, M.; Wang, G.; Xu, K.; Zhang, H.; Ren, X.; Jing, Y.; Yang, R.; Geng, Z.; Su, J. Boosting cartilage repair with silk fibroin-DNA hydrogel-based cartilage organoid precursor. *Bioact Mater.* **2024,** *35*, 429-444.
95. Pina, S.; Rebelo, R.; Correlo, V. M.; Oliveira, J. M.; Reis, R. L. Bioceramics for osteochondral tissue engineering and regeneration. *Adv Exp Med Biol.* **2018,** *1058*, 53-75.
96. Li, Z. A.; Shang, J.; Xiang, S.; Li, E. N.; Yagi, H.; Riewruja, K.; Lin, H.; Tuan, R. S. Articular tissue-mimicking organoids derived from mesenchymal stem cells and induced pluripotent stem cells. *Organoids.* **2022,** *1*, 135-148.
97. Li, L.; Li, H.; Wang, Q.; Xue, Y.; Dai, Y.; Dong, Y.; Shao, M.; Lyu, F. Hydroxyapatite nanoparticles promote the development of bone microtissues for accelerated bone regeneration by activating the FAK/Akt pathway. *ACS Biomater Sci Eng.* **2024,** *10*, 4463-4479.
98. He, T.; Hausdorf, J.; Chevalier, Y.; Klar, R. M. Trauma induced tissue survival in vitro with a muscle-biomaterial based osteogenic organoid system: a proof of concept study. *BMC Biotechnol.* **2020,** *20*, 8.
99. Chung, C.; Kim, Y. K.; Shin, D.; Ryoo, S. R.; Hong, B. H.; Min, D. H. Biomedical applications of graphene and graphene oxide. *Acc Chem Res.* **2013,** *46*, 2211-2224.
100. Park, S. Y.; Park, J.; Sim, S. H.; Sung, M. G.; Kim, K. S.; Hong, B. H.; Hong, S. Enhanced differentiation of human neural stem cells into neurons on graphene. *Adv Mater.* **2011,** *23*, H263-267.
101. Chen, H.; Müller, M. B.; Gilmore, K. J.; Wallace, G. G.; Li, D. Mechanically strong, electrically conductive, and biocompatible graphene paper. *Adv Mater.* **2008,** *20*, 3557-3561.
102. Lee, W. C.; Lim, C. H.; Shi, H.; Tang, L. A.; Wang, Y.; Lim, C. T.; Loh, K. P. Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide. *ACS Nano.* **2011,** *5*, 7334-7341.
103. Nayak, T. R.; Andersen, H.; Makam, V. S.; Khaw, C.; Bae, S.; Xu, X.; Ee, P. L.; Ahn, J. H.; Hong, B. H.; Pastorin, G.; Özyilmaz, B. Graphene

for controlled and accelerated osteogenic differentiation of human mesenchymal stem cells. *ACS Nano.* **2011,** *5*, 4670-4678.
104. Marrella, A.; Lagazzo, A.; Barberis, F.; Catelani, T.; Quarto, R.; Scaglione, S. Enhanced mechanical performances and bioactivity of cell laden-graphene oxide/alginate hydrogels open new scenario for articular tissue engineering applications. *Carbon.* **2017,** *115*, 608-616.
105. Choe, G.; Oh, S.; Seok, J. M.; Park, S. A.; Lee, J. Y. Graphene oxide/alginate composites as novel bioinks for three-dimensional mesenchymal stem cell printing and bone regeneration applications. *Nanoscale.* **2019,** *11*, 23275-23285.
106. Kang, S.; Park, J. B.; Lee, T.-J.; Ryu, S.; Bhang, S. H.; La, W.-G.; Noh, M.-K.; Hong, B. H.; Kim, B.-S. Covalent conjugation of mechanically stiff graphene oxide flakes to three-dimensional collagen scaffolds for osteogenic differentiation of human mesenchymal stem cells. *Carbon.* **2015,** *83*, 162-172.
107. Shin, S. R.; Aghaei-Ghareh-Bolagh, B.; Dang, T. T.; Topkaya, S. N.; Gao, X.; Yang, S. Y.; Jung, S. M.; Oh, J. H.; Dokmeci, M. R.; Tang, X. S.; Khademhosseini, A. Cell-laden microengineered and mechanically tunable hybrid hydrogels of gelatin and graphene oxide. *Adv Mater.* **2013,** *25*, 6385-6391.
108. Zhang, J.; Wehrle, E.; Adamek, P.; Paul, G. R.; Qin, X. H.; Rubert, M.; Müller, R. Optimization of mechanical stiffness and cell density of 3D bioprinted cell-laden scaffolds improves extracellular matrix mineralization and cellular organization for bone tissue engineering. *Acta Biomater.* **2020,** *114*, 307-322.
109. Zhang, J.; Eyisoylu, H.; Qin, X. H.; Rubert, M.; Müller, R. 3D bioprinting of graphene oxide-incorporated cell-laden bone mimicking scaffolds for promoting scaffold fidelity, osteogenic differentiation and mineralization. *Acta Biomater.* **2021,** *121*, 637-652.
110. Zhang, J.; Griesbach, J.; Ganeyev, M.; Zehnder, A. K.; Zeng, P.; Schädli, G. N.; Leeuw, A.; Lai, Y.; Rubert, M.; Müller, R. Long-term mechanical loading is required for the formation of 3D bioprinted functional osteocyte bone organoids. *Biofabrication.* **2022,** *14*, 035018.
111. Rossi, G.; Manfrin, A.; Lutolf, M. P. Progress and potential in organoid research. *Nat Rev Genet.* **2018,** *19*, 671-687.
112. Li, J.; Dong, S. The signaling pathways involved in chondrocyte differentiation and hypertrophic differentiation. *Stem Cells Int.* **2016,** *2016*, 2470351.
113. Mendes, L. F.; Tam, W. L.; Chai, Y. C.; Geris, L.; Luyten, F. P.; Roberts, S. J. Combinatorial analysis of growth factors reveals the contribution of bone morphogenetic proteins to chondrogenic differentiation of human periosteal cells. *Tissue Eng Part C Methods.* **2016,** *22*, 473-486.
114. Murphy, M. K.; Huey, D. J.; Hu, J. C.; Athanasiou, K. A. TGF-β1, GDF-5, and BMP-2 stimulation induces chondrogenesis in expanded human articular chondrocytes and marrow-derived stromal cells. *Stem Cells.* **2015,** *33*, 762-773.
115. Matta, C.; Mobasheri, A. Regulation of chondrogenesis by protein kinase C: Emerging new roles in calcium signalling. *Cell Signal.* **2014,** *26*, 979-1000.
116. Min, S. K.; Kim, M.; Park, J. B. Bone morphogenetic protein 2-enhanced osteogenic differentiation of stem cell spheres by regulation of Runx2 expression. *Exp Ther Med.* **2020,** *20*, 79.
117. Lin, W.; Zhu, X.; Gao, L.; Mao, M.; Gao, D.; Huang, Z. Osteomodulin positively regulates osteogenesis through interaction with BMP2. *Cell Death Dis.* **2021,** *12*, 147.
118. Limraksasin, P.; Kondo, T.; Zhang, M.; Okawa, H.; Osathanon, T.; Pavasant, P.; Egusa, H. In vitro fabrication of hybrid bone/cartilage complex using mouse induced pluripotent stem cells. *Int J Mol Sci.* **2020,** *21*, 581.
119. Dicks, A. R.; Steward, N.; Guilak, F.; Wu, C. L. Chondrogenic differentiation of human-induced pluripotent stem cells. *Methods Mol Biol.* **2023,** *2598*, 87-114.
120. Belluzzi, E.; Todros, S.; Pozzuoli, A.; Ruggieri, P.; Carniel, E. L.; Berardo, A. Human cartilage biomechanics: experimental and theoretical approaches towards the identification of mechanical properties in healthy and osteoarthritic conditions. *Processes.* **2023,** *11*, 1014.
121. Ansari, S.; Khorshidi, S.; Karkhaneh, A. Engineering of gradient osteochondral tissue: From nature to lab. *Acta Biomater.* **2019,** *87*, 41-54.
122. Bilic, J.; Izpisua Belmonte, J. C. Concise review: Induced pluripotent stem cells versus embryonic stem cells: close enough or yet too far apart? *Stem Cells.* **2012,** *30*, 33-41.
123. Juhász, K. Z.; Hajdú, T.; Kovács, P.; Vágó, J.; Matta, C.; Takács, R. Hypoxic conditions modulate chondrogenesis through the circadian clock: the role of hypoxia-inducible factor-1α. *Cells.* **2024,** *13*, 512.
124. Arai, Y.; Cha, R.; Nakagawa, S.; Inoue, A.; Nakamura, K.; Takahashi, K. Cartilage homeostasis under physioxia. *Int J Mol Sci.* **2024,** *25*, 9398.
125. Yu, Y.; Jiang, Y.; Ge, H.; Fan, X.; Gao, H.; Zhou, Z. HIF-1α in cartilage homeostasis, apoptosis, and glycolysis in mice with steroid-induced osteonecrosis of the femoral head. *J Cell Physiol.* **2024,** *239*, e31224.
126. Bolander, J.; Mota, C.; Ooi, H. W.; Agten, H.; Baker, M. B.; Moroni, L.; Luyten, F. P. Bioinspired development of an in vitro engineered fracture callus for the treatment of critical long bone defects. *Adv Funct Mater.* **2021,** *31*, 2104159.
127. Li, Z.; Lin, Z.; Liu, S.; Yagi, H.; Zhang, X.; Yocum, L.; Romero-Lopez, M.; Rhee, C.; Makarcyzk, M. J.; Yu, I.; Li, E. N.; Fritch, M. R.; Gao, Q.; Goh, K. B.; O'Donnell, B.; Hao, T.; Alexander, P. G.; Mahadik, B.; Fisher, J. P.; Goodman, S. B.; Bunnell, B. A.; Tuan, R. S.; Lin, H. Human mesenchymal stem cell-derived miniature joint system for disease modeling and drug testing. *Adv Sci (Weinh).* **2022,** *9*, e2105909.
128. Paggi, C. A.; Hendriks, J.; Karperien, M.; Le Gac, S. Emulating the chondrocyte microenvironment using multi-directional mechanical stimulation in a cartilage-on-chip. *Lab Chip.* **2022,** *22*, 1815-1828.
129. Alamán-Díez, P.; García-Gareta, E.; Arruebo, M.; Pérez, M. A bone-on-a-chip collagen hydrogel-based model using pre-differentiated adipose-derived stem cells for personalized bone tissue engineering. *J Biomed Mater Res A.* **2023,** *111*, 88-105.
130. Bahmaee, H.; Owen, R.; Boyle, L.; Perrault, C. M.; Garcia-Granada, A. A.; Reilly, G. C.; Claeyssens, F. Design and evaluation of an osteogenesis-on-a-chip microfluidic device incorporating 3D cell culture. *Front Bioeng Biotechnol.* **2020,** *8*, 557111.
131. Zhang, W.; Wei, X.; Wang, Q.; Dai, K.; Wang, J.; Liu, C. In vivo osteo-organoid approach for harvesting therapeutic hematopoietic stem/progenitor cells. *J Vis Exp.* **2024**, e66026.
132. Yu, L.; Lin, Y. L.; Yan, M.; Li, T.; Wu, E. Y.; Zimmel, K.; Qureshi, O.; Falck, A.; Sherman, K. M.; Huggins, S. S.; Hurtado, D. O.; Suva, L. J.; Gaddy, D.; Cai, J.; Brunauer, R.; Dawson, L. A.; Muneoka, K. Hyaline cartilage differentiation of fibroblasts in regeneration and regenerative medicine. *Development.* **2022,** *149*, dev200249.
133. Wilson, H. V. A new method by which sponges may be artificially reared. *Science.* **1907,** *25*, 912-915.
134. Wu, Y.; Wang, K.; Karapetyan, A.; Fernando, W. A.; Simkin, J.; Han, M.; Rugg, E. L.; Muneoka, K. Connective tissue fibroblast properties are position-dependent during mouse digit tip regeneration. *PLoS One.* **2013,** *8*, e54764.
135. Yan, H. H. N.; Siu, H. C.; Law, S.; Ho, S. L.; Yue, S. S. K.; Tsui, W. Y.;

Chan, D.; Chan, A. S.; Ma, S.; Lam, K. O.; Bartfeld, S.; Man, A. H. Y.; Lee, B. C. H.; Chan, A. S. Y.; Wong, J. W. H.; Cheng, P. S. W.; Chan, A. K. W.; Zhang, J.; Shi, J.; Fan, X.; Kwong, D. L. W.; Mak, T. W.; Yuen, S. T.; Clevers, H.; Leung, S. Y. A comprehensive human gastric cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening. *Cell Stem Cell*. **2018,** *23*, 882-897.e11.

136. Li, Z.; Yu, D.; Zhou, C.; Wang, F.; Lu, K.; Liu, Y.; Xu, J.; Xuan, L.; Wang, X. Engineering vascularised organoid-on-a-chip: strategies, advances and future perspectives. *Biomater Transl*. **2024,** *5*, 21-32.
137. Dönges, L.; Damle, A.; Mainardi, A.; Bock, T.; Schönenberger, M.; Martin, I.; Barbero, A. Engineered human osteoarthritic cartilage organoids. *Biomaterials*. **2024,** *308*, 122549.
138. van Hoolwerff, M.; Rodríguez Ruiz, A.; Bouma, M.; Suchiman, H. E. D.; Koning, R. I.; Jost, C. R.; Mulder, A. A.; Freund, C.; Guilak, F.; Ramos, Y. F. M.; Meulenbelt, I. High-impact FN1 mutation decreases chondrogenic potential and affects cartilage deposition via decreased binding to collagen type II. *Sci Adv*. **2021,** *7*, eabg8583.
139. Liu, H.; Su, J. Organoid and organoid extracellular vesicles for osteoporotic fractures therapy: current status and future perspectives. *Interdiscip Med*. **2023,** *1*, e20230011.

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