Skeletal organoids

Chen Zhang[1,2,3,#], Yingying Jing[1,2,#], Jianhua Wang[4,#], Zhidao Xia[5], Yuxiao Lai[6,], Long Bai[1,2,7,*], Jiacan Su[1,2,4,*]*

Key Words:
applications; construction strategies; organoids; skeletal system

From the Contents

ABSTRACT

The skeletal system, composed of bones, muscles, joints, ligaments, and tendons, serves as the foundation for maintaining human posture, mobility, and overall biomechanical functionality. However, with ageing, chronic overuse, and acute injuries, conditions such as osteoarthritis, intervertebral disc degeneration, muscle atrophy, and ligament or tendon tears have become increasingly prevalent and pose serious clinical challenges. These disorders not only result in pain, functional loss, and a marked reduction in patients' quality of life but also impose substantial social and economic burdens. Current treatment modalities, including surgical intervention, pharmacotherapy, and physical rehabilitation, often do not effectively restore the functionality of damaged tissues and are associated with high recurrence rates and long-term complications, highlighting significant limitations in their efficacy. Thus, there is a strong demand to develop novel and more effective therapeutic and reparative strategies. Organoid technology, as a three-dimensional micro-tissue model, can replicate the structural and functional properties of native tissues in vitro, providing a novel platform for in-depth studies of disease mechanisms, optimisation of drug screening, and promotion of tissue regeneration. In recent years, substantial advancements have been made in the research of bone, muscle, and joint organoids, demonstrating their broad application potential in personalised and regenerative medicine. Nonetheless, a comprehensive review of current research on skeletal organoids is still lacking. Therefore, this article aims to present an overview of the definition and technological foundation of organoids, systematically summarise the progress in the construction and application of skeletal organoids, and explore future opportunities and challenges in this field, offering valuable insights and references for researchers.

***Corresponding authors:**
Yuxiao Lai, yx.lai@siat.ac.cn;
Long Bai, bailong@shu.edu.cn;
Jiacan Su, drsujiacan@163.com.

#Authors equally.

http://doi.org/10.12336/biomatertransl.2024.04.005

How to cite this article:
Zhang, C.; Jing, Y.; Wang, J.; Xia, Z.; Lai, Y; Bai, L.; Su, J. Skeletal organoids. *Biomater Transl.* **2024**, *5*(4), 390-410.

Introduction

The skeletal system, composed of bones, joints, muscles, tendons, and ligaments, plays a crucial role in human movement and support, working in concert to facilitate bodily motion, maintain posture, and preserve balance. However, with ageing, sports injuries, or the progression of diseases, these components of the skeletal system are prone to degenerative changes or damage, leading to a spectrum of conditions such as bone fractures, intervertebral disc degeneration, osteoarthritis, muscle atrophy, and ligament or tendon tears (**Figure 1**). These diseases not only impair patients' mobility but also greatly impacting their quality of life and mental health, presenting significant challenges to healthcare systems. Traditional treatments often have limitations in efficacy and face high recurrence rates, underscoring the necessity for developing more advanced therapeutic approaches to safeguard human health.

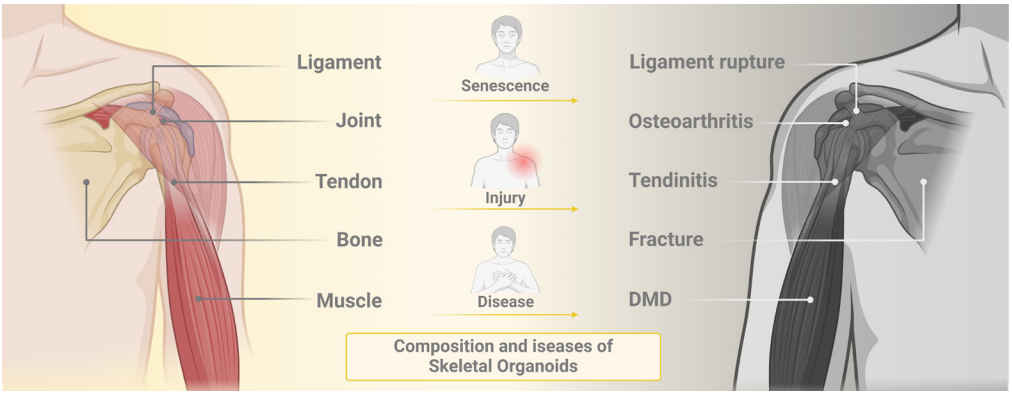


Figure 1. Skeletal system composition and diseases. Created with BioRender.com. DMD: Duchenne muscular dystrophy.

Organoids are three-dimensional (3D), *in vitro* cultured micro-organ models capable of self-assembly, reproducing the structure and function of natural tissues. In recent years, organoid technology has attracted significant attention in the field of biomedical research. As studies have progressed, organoids have provided novel methodologies for investigating tissue development, disease mechanisms, and drug screening. Research on skeletal organoids has also emerged, with successful construction of models for bones, muscles, and joints, demonstrating tremendous potential in disease research and regenerative medicine. For instance, "mini-joints" synthesised through co-culturing bone and cartilage organoids have been utilised to test drugs for arthritis treatment,[1] while gene-editing techniques have been employed to create organoid models of muscular dystrophy, aiding in the exploration of disease progression and the identification of therapeutic strategies.[2] These findings indicate that skeletal system organoids not only facilitate a more comprehensive understanding of disease onset and progression but also hold significant promise in personalised medicine and regenerative therapy.

Given the clinical importance of skeletal organoids, there remains a lack of comprehensive reviews on the subject. This review aims to address this gap by first providing an overview of the definition and technological basis of organoids, followed by a systematic summary of the developmental history and current research progress of skeletal organoids. Subsequently, this article will review various organoid models, including those of bones, muscles, joints, ligaments, and tendons, discussing in detail their construction methods, application areas, and future potential. In addition, this review will provide a thorough discussion on the future prospects, challenges, and research directions of skeletal organoids, providing valuable references for researchers and promoting further advancement in this emerging field.

Overview of Organoids and the Developmental History of Skeletal Organoid

Organoids are 3D cell clusters cultured *in vitro*. Through the induced differentiation of pluripotent stem cells or organ-derived cells, these clusters grow and self-assemble on supporting substrates, such as hydrogels, ultimately forming organ-like structures with certain physiological functions that can be maintained in long-term cultures. As a 3D culture model, organoids exhibit significant advantages in many aspects. Compared to traditional two-dimensional (2D) cultures, organoids more closely mimic natural organs regarding gene and protein expression, metabolic function, and micro-architecture. Furthermore, organoids can be maintained for extended periods, display structures more similar to human tissues, and can be employed to model disease onset, offering immense potential in drug screening and organ development research (**Figure 2**).

The study of organoids can be traced back to 1907 when Henry and colleagues successfully cultured isolated sponge tissues *in vitro*, proving the feasibility of *ex vivo* culture. This experiment also demonstrated that organoids could self-organise into multicellular structures without external intervention, which is a hallmark feature of organoids.[3] However, there was still a significant gap between *ex vivo* culture and the construction of self-organising organoids, as cells derived from organs rarely differentiate and proliferate into complete organoids. This led researchers to consider the induction and cultivation of pluripotent stem cells. However, obtaining human pluripotent stem cells raised ethical concerns, necessitating an alternative approach in organoid research.

A breakthrough came in 2006 when Takahashi and Yamanaka[4] successfully induced the formation of induced pluripotent stem cells (iPSCs) by reprogramming somatic cells into pluripotent stem cells, opening up a new direction for

1 Organoid Research Center, Institute of Translational Medicine, Shanghai University, Shanghai, China; 2 National Center for Translational Medicine (Shanghai) SHU Branch, Shanghai University, Shanghai, China; 3 School of Medicine, Shanghai University, Shanghai, China; 4 Department of Orthopaedics, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China; 5 Centre for Nanohealth, Swansea University Medical School, Swansea University, Swansea, UK; 6 Centre for Translational Medicine Research & Development, Institute of Biomedical and Health Engineering, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong Province, China; 7 Wenzhou Institute of Shanghai University, Wenzhou, Zhejiang Province, China

organoid research based on pluripotent stem cell induction. In 2009, Sato et al.[5] successfully constructed intestinal epithelial organoids, marking the beginning of formal organoid research. The following decade witnessed rapid advancements in the organoid field. In 2011, Eiraku et al.[6] constructed retinal organoids using mouse embryonic stem cells; in 2013, brain organoids were successfully created from human pluripotent stem cells;[6,7] in 2020, venom gland organoids were successfully developed;[8] in 2021, scaffold-free cartilage organoids were developed;[9] and by 2024, bone organoids for large bone repair were successfully engineered.[10] With the continuous improvement of scaffold materials and construction methods, the development of the organoid field is expected to accelerate further (**Figure 3**).

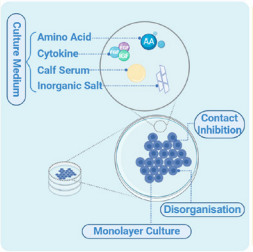
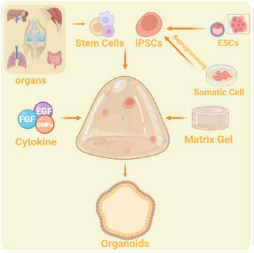
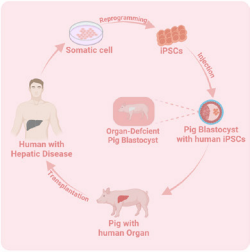
Physiologic Presentation of Different System	2D Cell Culture	Organoids	Interspecies Mammalian Chimeras
			
Disease Model	No	Yes	Yes
Vascularisation	No	No	Yes
Self-organising	No	Yes	Yes
Operating Difficulty	Easy	Medium	Hard
Culture Cycle	Days	Months	Years
Long-term Culture	No	Yes	Yes
Genome Editing	Yes	Yes	Yes

Figure 2. Comparison across multiple systems. Created with BioRender.com. 2D: two-dimensional; AA: amino acid; BMPs: bone morphogenetic proteins; EGF: epidermal growth factor; ESCs: embryonic stem cells; FGF: fibroblast growth factor; IGF: insulin-like growth factor; iPSCs: induced pluripotent stem cells.

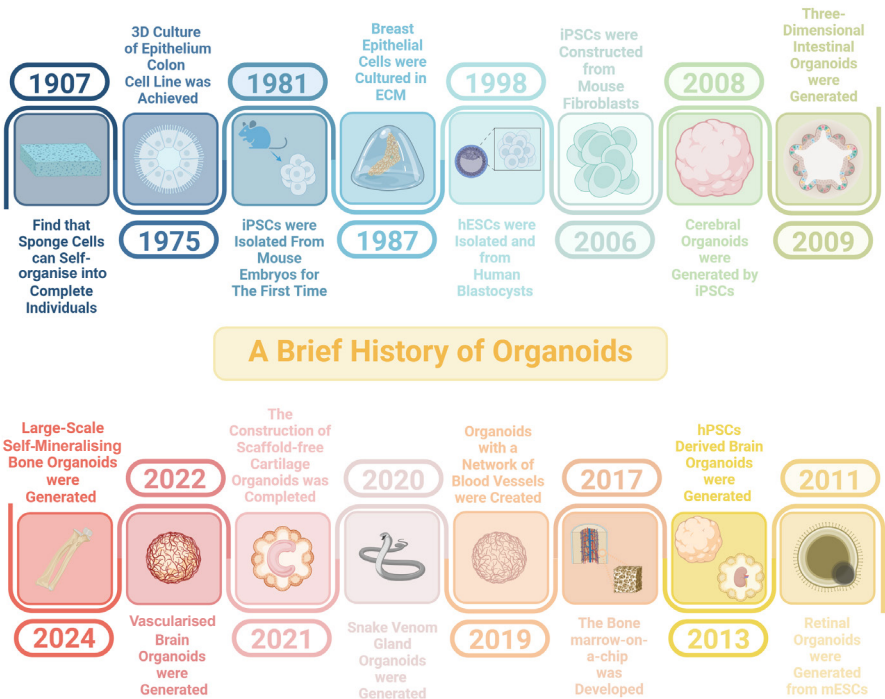


Figure 3. Development of skeletal organoids. Created with BioRender.com. ECM: extracellular matrix; hESCs: human embryonic stem cells; hPSCs: human pluripotent stem cells; iPSCs: induced pluripotent stem cells; mESCs: mouse embryonic stem cells.

Basic Strategies for Organoid Construction

As self-assembled, 3D cell clusters cultivated *in vitro*, organoids share certain similarities with traditional 2D cell culture methods, such as the need for selecting suitable cells, a supportive matrix, and inductive agents or physical cues that enhance cell growth and differentiation. The establishment of stable, long-term organoids requires the precise spatial and temporal integration of these elements.

Cell sources for organoid construction

The construction of organoids depends on an appropriate cell source, with easily culturable, highly differentiated cells serving as the foundation. Suitable cells for organoid formation are typically stem cells, such as mesenchymal stem cells, iPSCs, adipose-derived mesenchymal stem cells, embryonic stem cells, and organ-specific stem cells. Under suitable growth conditions, these stem cells can create ideal organoid models. Adipose-derived mesenchymal stem cells, which possess multi-lineage differentiation potential, are commonly found in adipose tissue, bone marrow, and umbilical cords. They have the potential to differentiate into osteocytes, chondrocytes, and adipocytes and have advantages in ease of cultivation and reduced immune rejection, making them ideal for bone organoid construction.[11]

iPSCs, produced by reprogramming adult somatic cells to regain pluripotency, resemble embryonic stem cells in their capacity to differentiate into multiple human cell types.[12] As artificially induced cells, iPSCs can be used to generate personalised cellular and tissue models for disease research and drug screening, facilitating personalised treatments and minimising immune rejection in tissue transplantation.[13] Moreover, iPSCs avoid the use of foetal tissue, thus addressing the ethical concerns associated with embryonic stem cells.[14]

Scaffold materials in organoid construction

Scaffold materials are essential in bone organoid construction, providing physical support for 3D cellular growth and mimicking the *in vivo* tissue microenvironment, which influences cell growth, differentiation, and function. Ideal scaffold materials should offer biocompatibility, mechanical strength, biodegradability, and the capacity to promote cell adhesion and proliferation.[15] Hydrogels, which create a 3D hydrated environment that supports cell adhesion and proliferation and promotes differentiation, are widely used in organoid construction due to their biomimetic properties.[16] Hydrogels can be classified as natural, synthetic, or composite, depending on their source, and they effectively simulate the natural structure of bone tissue to support cell growth and differentiation.

Natural hydrogels in organoid engineering

Natural hydrogels consist of 3D network structures made from natural polymers with high water-retention and biocompatibility.[17] Owing to their resemblance to the *in vivo* extracellular matrix (ECM), natural hydrogels provide cellular support that promotes adhesion, proliferation, and differentiation.[16, 18] Examples include collagen, Matrigel, and fibrin:

Collagen, the predominant structural protein in human tissues, is biocompatible and biodegradable, facilitating cell attachment, proliferation, and differentiation, which makes it widely used in organoid construction.[19]

Matrigel is a complex ECM mimic derived from mouse tumour cells, composed of various proteins, glycoproteins, and growth factors. It provides structural support, promoting cell adhesion, proliferation, migration, and differentiation.[20, 21]

Fibrin, a natural protein important in tissue repair and coagulation, offers excellent biocompatibility. Fibrin scaffolds provide mechanical support to organoids, stimulate vascularisation, and enhance cell adhesion, making them ideal for tissue regeneration.

Synthetic hydrogels in organoid engineering

Synthetic hydrogels are engineered polymers with 3D network structures that absorb and retain substantial water content.[22] The adjustability of synthetic hydrogels allows for precise adjustments in structure and composition to meet different cell growth and differentiation needs. Common synthetic hydrogels include poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), and polyacrylamide. Synthetic hydrogels offer enhanced stability and tunability over natural hydrogels and avoid immunogenic responses, thus providing excellent tissue compatibility.[23] For instance, PEG is extensively utilised in tissue engineering due to its biocompatibility, low immunogenicity, tunability, and ease of processing. PEG hydrogels simulate the ECM environment, promoting cellular interactions with the scaffold.[24] Another notable synthetic hydrogel, PLGA, enables precise control over degradation rates *in vivo* by adjusting the lactic-to-glycolic acid ratio, thus facilitating customised therapies.[25]

Microenvironment construction in organoid engineering

The creation of a supportive microenvironment is vital to organoid success. Beyond scaffold materials, the microenvironment also involves chemical and biological signal transmission. Cell proliferation and differentiation are critical in bone organoid development, with growth factors and signalling pathways determining cell fate. While selecting appropriate cells and scaffold materials is the first step, guiding cells to form functional multicellular clusters is essential for successful organoid construction.

Various growth factors play crucial roles during cell growth and differentiation, including bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), insulin-like growth factors, platelet-derived growth factor, transforming growth factor-β (TGF-β), and vascular endothelial growth factor.[26] By selecting suitable growth factors in organoid culture, researchers can direct stem cell differentiation and promote tissue formation. Additionally, nanoparticles or microspheres delivering growth factors enable localised release of signalling molecules, which regulates tissue development and enhancing organoid construction.

Physical stimuli in organoid development

Physical stimuli are also critical in organoid development. Stem cells are responsive to chemical signals and physical forces such as tension, electrical stimulation, and compression. These forces are transmitted through the ECM to cell surface receptors and are further relayed to the nucleus, affecting gene expression and cellular activities. In organoid construction, physical stimuli are introduced using various methods, such as rotating bioreactors and microfluidic systems, which provide fluid shear forces that simulate blood flow. These mechanical cues promote vascularisation within the organoid and facilitate tissue maturation, thereby improving functionality and viability.[27]

Bone Organoids

Physiology and structure of bone

Biological characteristics of bone tissue

Bone is a densely mineralised connective tissue that primarily functions to support and protect vital organs in the body, as well as to participate in calcium storage, release, and haematopoiesis.[28] The basic structural unit of bone tissue is the bone matrix, which mainly comprises collagen fibres and inorganic minerals (primarily hydroxyapatite). These components confer both toughness and hardness to the bone.[29] The primary cellular components of bone include osteocytes, osteoblasts, and osteoclasts. Osteoblasts synthesise, secrete, and mineralise bone matrix, serving as the main functional cells in bone formation, while osteoclasts are involved in the resorption and degradation of the bone matrix. The coordinated actions between osteoclasts and osteoblasts facilitate bone remodelling, thereby maintaining the dynamic equilibrium of bone tissue, which is essential during bone development.[30] Additionally, osteoprogenitor cells in the periosteum can differentiate into various bone cell types, playing an essential role in bone repair and regeneration.[31] Bone tissue is highly vascularised, providing necessary nutrients and oxygen to bone cells while supporting haematopoietic activities within the bone marrow. These characteristics make bone not only the hardest organ in the body but also one with strong regenerative capacity.[32]

Role of bone tissue in the skeletal system

In the skeletal system, bone tissue serves multiple functions. First, bones form the skeletal framework of the body, supporting other soft tissues within the skeletal system and maintaining body shape. Additionally, bones, in conjunction with muscles, ligaments, and joints, form complex lever systems that facilitate various physical activities such as walking, running, and jumping. Furthermore, bone tissue acts as a buffer, absorbing and dispersing external forces exerted on the body, thereby protecting internal organs from damage.[33] Bone also serves as a major reservoir of substances like calcium and phosphorus, playing an essential role in maintaining calcium ion balance in the blood. Furthermore, bones are responsible for the renewal of the body's blood system, as the bone marrow within the medullary cavity is the primary site of haematopoiesis, producing red blood cells, platelets, and white blood cells, which are essential for oxygen supply and energy provision during physical activities.[34] Therefore, bone tissue is not only the structural core of the skeletal system but also significantly impacts the maintenance of human homeostasis through its metabolic and physiological functions. In cases of disease or injury, dysfunction in bone tissue can severely impair mobility and lead to systemic health issues, such as osteoporosis and regenerative anaemia. Thus, constructing bone organoid models that imitate the physiological functions and structures of bone is of great significance for advancing research and treatment of bone-related diseases.

Construction strategies of bone organoids

Bone organoids can be constructed using various cell types. In addition to the commonly used iPSCs and adipose-derived mesenchymal stem cells, human bone marrow-derived osteoprogenitor cells are also ideal for bone organoid construction. Similar to mesenchymal stem cells, human bone marrow-derived osteoprogenitor cells are derived from human bone marrow and can differentiate into various types of bone cells. These cells exhibit a rapid differentiation capability towards osteoblasts and demonstrate strong tissue-forming potential in bone organoid construction.[35]

Studies have shown that composite hydrogels formed by combining PEG and hyaluronic acid can be used for bone marrow organoid construction. PEG provides mechanical strength and stability, while hyaluronic acid significantly enhances cell proliferation and maintains stem cell properties. This composite hydrogel shows superior performance in bone generation and tissue regeneration, promoting human cell integration and creating a bone marrow-like microenvironment .[36]

PLGA also demonstrates excellent tissue compatibility and promotes bone regeneration, making it a key scaffold material in bone organoid research and applications. PLGA can serve as a scaffold for bone repair or as a drug delivery carrier. When combined with inorganic calcium phosphate, PLGA's osteoconductive and differentiation-inducing properties are significantly enhanced, with this composite material more effectively promoting osteoblast differentiation than single-material options.[37]

Signalling molecules play a key role in inducing cell differentiation during bone organoid construction. For instance, BMPs can induce the migration and differentiation of osteoprogenitor cells, facilitating bone tissue regeneration and repair, making BMPs key proteins in bone formation.[38] Insulin-like growth factors stimulate osteoblast synthesis and secretion of bone matrix proteins, promoting bone matrix generation, inhibiting osteoblast apoptosis, and accelerating bone injury repair by acting synergistically with other growth factors. FGF and vascular endothelial growth factor are essential for angiogenesis, supporting the formation of blood vessels, ensuring adequate blood supply, and thereby aiding bone tissue regeneration and repair.[39]

Bioink has recently emerged as a focal research area in bone organoid technology. Designed for 3D bioprinting, bioink can carry cells during the printing process and simulate biological tissue through precise 3D structures. Bioinks typically consist of biocompatible materials, cells, and bioactive

factors that support cell growth, enabling the creation of complex tissue structures such as skin, bone, and cartilage.[40] Wang and collaborators[10] developed a composite bioink composed of gelatin methacrylate, alginate methacrylate, and hydroxyapatite, which exhibits self-mineralising properties, enhances multicellular differentiation, and supports long-term culture and maturation of bone organoids. Bioink has opened new pathways for organoid culture, allowing for precise control over scaffold shape and internal structure to better imitate the complex morphology of natural bone tissue.

With continuous advancements in organoid culture techniques, strategies for constructing bone organoids are increasingly refined (**Table 1**).[41-45] The trend in bone organoid cultivation is moving toward creating larger, vascularised organoids through co-culture of multiple cell types. Technologies like 3D printing have opened new avenues for bone organoid applications, laying the foundation for more accurate modelling of complex bone structures and potentially enhancing clinical translation for bone tissue engineering.

Table 1. Methods for constructing bone organoids

Cell source	Matrix gel	Inducing factor	Application	Reference
$CD14^+$ monocytes	HA/β-TCP	Macrophage colony-stimulating factor	Simulate the bone regeneration process	41
BMSCs, human umbilical vein endothelial cells	DNA hydrogels	Apt02, tFNA	Accelerate the repair of critical-sized bone defects	42
MuSCs, BMSCs	β-TCP	BMP-2	Promote differentiation and mineralisation of cells	43
BMMs	DBP	VD3, PGE2	Local remodelling of bone tissue	44
hBMSCs, rBMSCs	GelMA	TGF-β3	Rapidly promote bone regeneration	45

Note: Apt02: Aptamer02; BMMs: bone marrow-derived macrophages; BMP-2: bone morphogenetic protein-2; BMSCs: bone mesenchymal stem cells; DBP: vitamin D binding protein; GelMA: gelatin methacryloyl; HA: hydroxyapatite; hBMSCs: human bone-derived mesenchymal stem cells; MuSCs: muscle stem cells; PEG2: prostaglandin E2; rBMSCs: rabbit bone-derived mesenchymal stem cells; tFNA: tetrahedral framework nucleic acid; TGF-β3: transforming growth factor-β3; VD3: vitamin D3; β-TCP: β-tricalcium phosphate.

The above construction method makes bone organoids have the morphology and chemical composition of bone tissue, but in order to test whether bone organoids have the mechanical properties and functional characteristics of bones, we still need to characterise their structure and function.

Morphological and functional characteristics of bone organoids

The construction of bone organoids not only involves culturing bone cells but also recreating their complex functional characteristics. The morphology of bone organoids should resemble the microstructure and macroscopic features of natural bone, such as a porous trabecular structure and a dense cortical bone layer. This morphological feature provides sufficient mechanical support, promotes the growth of blood vessels and nerves, and enhances the biological function of bone tissue.[29]

In terms of functional characteristics, bone organoids should possess osteogenic capability, vascularisation potential, and mechanical responsiveness. Osteogenic capability is demonstrated by the cells' ability to continuously secrete bone matrix and form mineralised bone tissue. Vascularisation is crucial for functional bone tissue, as an adequate blood supply is needed to maintain cell viability and facilitate metabolism. By introducing vascular endothelial cells or applying pro-angiogenic growth factors, a vascular network can be formed within the organoid, enhancing its physiological functions.[25] Additionally, bone organoids must exhibit mechanical responsiveness, with the ability to withstand external stress and perform self-repair. This feature is particularly important for applications in bone grafting and repair.

Applications of bone organoids

Application in orthopaedic disease research

As a 3D *in vitro* model, bone organoids simulate the complex microenvironment and functional characteristics of bone tissue, demonstrating significant potential in the basic research of orthopaedic diseases. Traditional 2D culture systems and animal models have contributed to the study of orthopaedic diseases; however, they are limited in replicating the structure of human bone tissue, the ECM, cell-to-cell interactions, and the mechanical environment. Bone organoids address these limitations by providing a more physiologically relevant research platform (**Figure 4**). They have proven particularly valuable in studying osteoporosis, poor bone fracture healing, bone tumours, and genetic bone disorders.[32] By simulating different pathological conditions, researchers can reproduce disease progression and pathogenesis *in vitro*. For example, bone organoids constructed with bone marrow mesenchymal stem cells and Matrigel can recreate the process of osteolytic lesions in multiple myeloma, facilitating the study of the disease's biological mechanisms and identifying potential therapeutic strategies.[46] Additionally, micron-scale bone organoids can be used to study changes in bone structure under microgravity, offering valuable insights into bone loss and remodelling processes, and providing a feasible method for osteoporosis research.[47]

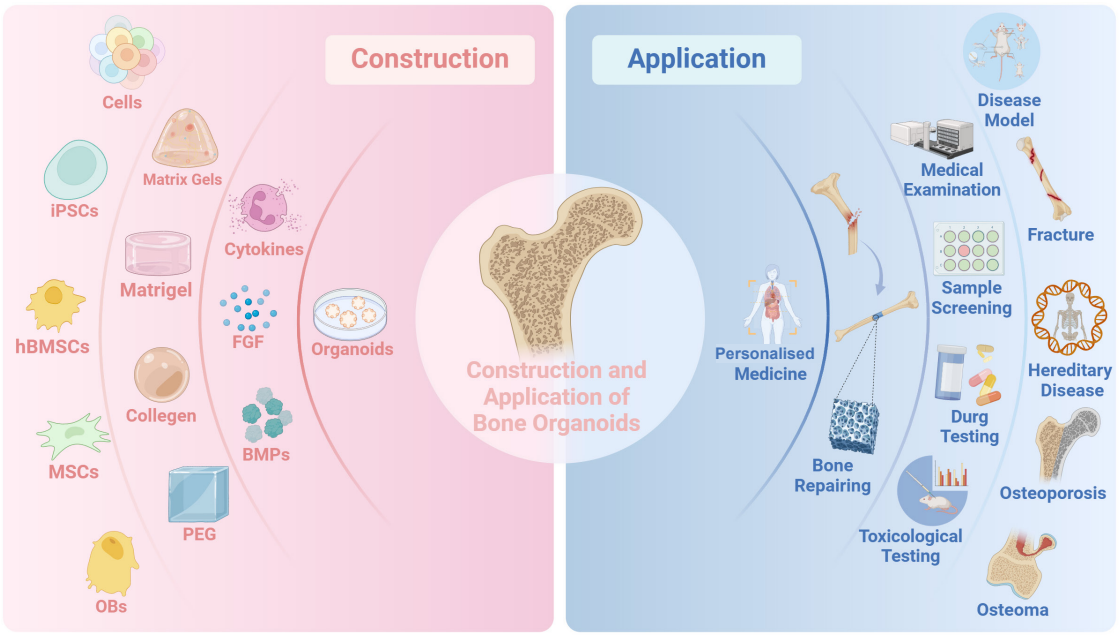


Figure 4. Construction and application of bone organoids. Created with BioRender.com. BMPs: bone morphogenetic proteins; FGF: fibroblast growth factor; hBMCs: human bone stem cells; iPSCs: induced pluripotent stem cells; MSCs: mesenchymal stem cells; OBs: osteoblasts; PEG: polyethylene glycol.

Drug screening research

Bone organoids offer significant advantages in drug screening and toxicological studies, particularly in developing and evaluating drugs related to bone tissue. Compared to traditional 2D cell culture models, bone organoids provide a more realistic cellular environment and biomechanical stress, which are crucial for assessing drug efficacy, cytotoxicity, and biometabolism.[48] In drug screening, bone organoids can be used for high-throughput screening, especially for drug development targeting diseases such as osteoporosis, bone tumours, and osteoarthritis. By using trabecular bone organoids constructed with demineralised bone paper made from bone biomaterials, *in vitro* mineralisation can be achieved, significantly enhancing clinical predictive power and reducing the time required for osteoporosis drug screening.[44] Furthermore, the 3D structure of bone organoids offers more targets for identifying potential drug molecules and their mechanisms of action.

Potential in regenerative medicine

Bone organoids exhibit tremendous potential in regenerative medicine, particularly in bone tissue repair and reconstruction. Traditional bone grafting and repair techniques face challenges, including limited donor availability, immune rejection, and long-term complications. Utilising bone organoids for personalised treatment may help overcome these limitations. Bone organoids constructed from a patient's own cells can be stimulated to differentiate into bone tissue for transplantation or repair. This personalised bone grafting strategy not only reduces immune rejection but also better replicates the specific bone structure and function of the patient. Additionally, bone organoids can be combined with 3D printing technology to create bone tissue of specific shapes using scaffold materials, facilitating the repair of large or complex bone defects.[45]

In bone regeneration and skeletal tissue engineering, bone organoids can also serve as an ideal platform for evaluating new scaffold materials, growth factors, and cell therapy strategies. Researchers can use bone organoids to test the biocompatibility and the mechanical properties of various materials *in vitro* and assess their effects on promoting bone tissue regeneration. Leveraging this advantage, future developments may lead to safer and more effective bone regeneration techniques for clinical bone defect repair and other orthopaedic applications in regenerative medicine.

Muscle Organoids

Physiology and structure of muscle

Structure and function of skeletal muscle

Skeletal muscle is a highly specialised striated muscle attached to bones, responsible for body movement and posture control. By contracting, it converts chemical energy into mechanical energy, generating the force needed to drive skeletal motion. Comprising approximately 40% of body weight, skeletal muscle consists of multinucleated muscle fibres that exhibit a distinct striped appearance, with lengths reaching up to 1 cm and diameters of about 100 μm. Skeletal muscle is wrapped in three layers of connective tissue: the epimysium surrounding the entire muscle, the perimysium enclosing muscle bundles, and the endomysium covering individual muscle fibres.[49] Within each muscle fibre are myofilaments, which can be categorised into thick and thin filaments based on their protein

composition. Thick filaments are mainly composed of myosin, while thin filaments are composed of actin, both forming the fundamental contractile unit known as the sarcomere.[50] Muscle contraction is achieved through the sliding filament theory, where neural signals trigger membrane depolarisation, resulting in the release of calcium ions that bind to myosin, causing conformational changes. Through the hydrolysis of ATP, actin and myosin filaments slide past each other, resulting in sarcomere shortening and inducing muscle contraction. Additionally, the ECM surrounding muscle fibres serves a vital role in providing structural support, integrating biochemical signals, and maintaining tissue integrity.[51]

Role of muscle tissue in the skeletal system

Skeletal muscle plays a key crucial in the skeletal system, as it connects to bones through tendons and acts as the main executor of movement. By converting chemical energy into mechanical work, skeletal muscle produces force through contraction, enabling complex bodily movements ranging from fine motor skills to large-scale coordinated activities. The interaction between skeletal muscles and bones is crucial for maintaining body balance and supporting vital functions. Beyond its mechanical function, skeletal muscle also serves as an essential glucose reservoir, helping to maintain glucose homeostasis in the body through glycogen synthesis and breakdown.[52] Mechanical stimulation of muscle fibres leads to hypertrophy, enabling muscles to adapt to increased mechanical loads, enhancing strength and endurance—explaining why physical training increases muscular strength.[53] This process also underscores the importance of mechanical stimulation in promoting muscle tissue growth, a factor integral to organoid cultivation.

Construction of muscle organoids

There are multiple cell options available for muscle organoid construction, among which human pluripotent stem cells are most commonly used. These cells can be induced to differentiate into muscle-related cell lineages such as myogenic progenitor cells or satellite cells through specific differentiation strategies.[54] Scaffold materials are one of the core components in muscle organoid construction, with biocompatible materials such as collagen, gelatin, and fibrin commonly selected to provide a microenvironment conducive to cell adhesion, growth, and differentiation.[55] Alternatively, collagen can be combined with Matrigel, where the cells are mixed with the scaffold material and gelled in a mould to create a 3D muscle organoid structure.[56]

A 3D culture system offers conditions closer to the *in vivo* environment for muscle organoid construction. By mimicking mechanical tension found in muscle tissue, researchers can promote the alignment, fusion, and formation of muscle fibre-like structures *in vitro*.[57] Additionally, some studies employ bioreactors to dynamically regulate the culture environment, providing mechanical stretching stimuli that enhance the functionality of muscle organoids, such as contractile force and muscle fibre alignment.[58]

The cultivation of muscle organoids also requires the activation and inhibition of relevant signalling pathways to simulate the muscle development process. During differentiation, key molecules such as Wnt signalling pathway, BMP, and FGF are used to induce mesodermal progenitor cells to further differentiate into myogenic cells. The Wnt protein family plays a critical regulatory role in adult skeletal muscle regeneration, with Wnt signalling regulating satellite cell proliferation and differentiation through β-catenin. When Wnt signalling is successfully activated, β-catenin translocates from the cytoplasm to the nucleus, activating satellite cell proliferation.[55]

BMP has a specific role in skeletal muscle regeneration. At certain concentrations, BMP induces mesodermal cells to differentiate towards bone tissue while inhibiting muscle differentiation.[59, 60] On the other hand, BMP signalling promotes growth within muscle tissue through the Smad1/5/8 pathway, supporting muscle hypertrophy and preventing muscle atrophy.[60] The FGF family, composed of 22 conserved members, is extensively involved in embryonic development, tissue homeostasis, and injury repair. Among them, FGF2 and FGF6 play significant roles in skeletal muscle regeneration, where FGF signalling maintains satellite cells in a proliferative state by inhibiting myogenic differentiation, thus supporting muscle regeneration.[61] In summary, selecting appropriate induction and differentiation strategies is crucial in muscle organoid culture (**Table 2**). [62-66]

Table 2. Methods for constructing muscle organoids

Cell source	Matrix gel	Inducing factor	Application	Reference
hiPSCs	Matrigel	HGF	Model for studying muscle development and related diseaseS	62
	Matrigel with fibrin	Tamoxifen	Study of muscle pathology, test of gene and cell therapy approaches	63
Primary muscle cells	Matrigel with fibrin	–	Building disease model	64
Myoblasts	Fibrin hydrogels	CDFDA, NanoLuc	Model for simulating drug metabolism	65
Mouse myoblasts	Matrigel with collagen	IGF-1	Evaluation of drug effects on muscle disorders	66

Note: CDFDA: 5 (and 6)-carboxy-2′,7′-dichloroflourescein diacetate; HGF: hepatocyte growth factor; hiPSCs: human induced pluripotent stem cells; IGF-1: insulin-like growth factor 1.

Functional characterisation of muscle organoids

Functional characterisation of muscle organoids is crucial for evaluating their biological properties and functional integrity. Firstly, morphological analysis is the most fundamental characterisation method, involving the observation of muscle tissue structures, such as muscle fibre alignment, nuclear positioning, and ECM distribution. Using immunofluorescence staining and microscopy, researchers can detect specific muscle markers, such as α-actinin and myosin, to assess the maturation level of muscle organoids.[49]

Secondly, functional assessment of muscle organoids, including measurements of their contractile ability, is a key indicator to determine if they possess the functions of normal muscle tissue. By applying electrical stimulation to muscle organoids in a 3D culture environment, researchers can induce contraction responses and subsequently measure the magnitude and persistence of contraction forces.[58]

Finally, gene expression analysis is another important method for characterising the developmental and functional status of muscle organoids. Techniques such as real-time polymerase chain reaction and RNA sequencing enable researchers to examine the expression of muscle-specific genes, such as Pax genes 7 and myogenin, to evaluate the differentiation and maturation status of muscle organoids.[57]

Overall, the construction of muscle organoids relies on various cell sources and differentiation strategies, combined with advanced tissue engineering technologies and 3D culture systems, to emulate the structure and function of *in vivo* muscle tissue. Functional characterisation methods, including morphological analysis, functional assessment, and gene expression analysis, provide a reliable model system for advancing studies on muscle development, regeneration, and disease mechanisms.

Applications of muscle organoids

Research on muscle degenerative diseases

Muscle organoids play a crucial role in modelling muscle degenerative diseases, particularly those that cannot be adequately replicated using traditional animal models. By differentiating patient-derived iPSCs into muscle organoids, researchers can recreate the microenvironment of diseased muscle tissue *in vitro*, providing an ideal platform for studying the pathogenesis of muscle degenerative diseases (**Figure 5**). For instance, Duchenne muscular dystrophy, caused by mutations leading to the absence of dystrophin on the muscle cell membrane, cannot be accurately simulated using conventional cell culture models.[67] By utilising clustered regularly interspaced palindromic repeats (CRISPR) CRISPR-associated (Cas) nucleases 9 technology to perform gene editing on iPSCs, researchers can construct muscle organoid models with Duchenne muscular dystrophy gene mutations, offering new pathways for understanding the pathogenesis of the disease and identify potential therapeutic strategies.[2]

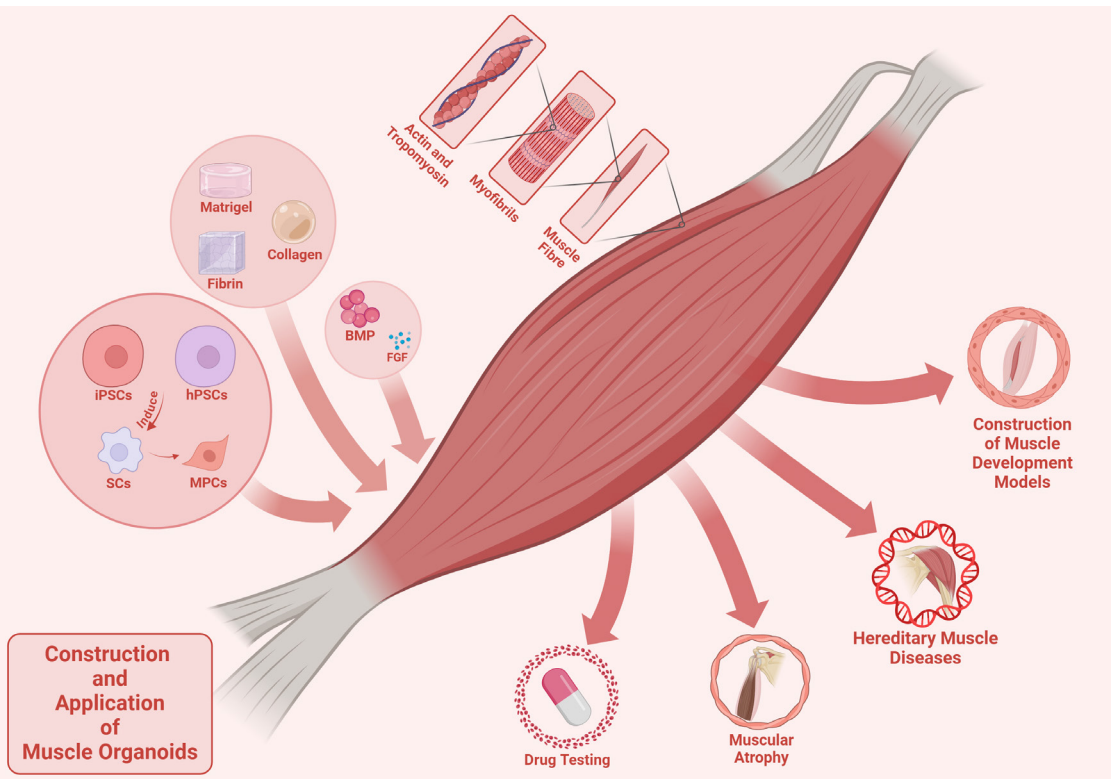


Figure 5. Construction and application of muscle organoids. Created with BioRender.com. BMP: bone morphogenetic proteins; FGF: fibroblast growth factor; hPSCs: human pluripotent stem cells; iPSCs: induced pluripotent stem cells; MPCs: muscle progenitor cells; SCs: satellite cells.

Moreover, muscle organoid models can also be employed to study other muscle degenerative diseases, such as amyotrophic lateral sclerosis and mitochondrial myopathies. Amyotrophic lateral sclerosis is an idiopathic, fatal neurodegenerative disease that affects the human motor system, often leading to respiratory failure in patients.[68] By inducing specific pathological states within organoids, researchers can observe disease progression at the cellular level and explore abnormalities in related signalling pathways. More importantly, these organoid models can be used for high-throughput screening of potential drugs, providing new hope for treating muscle degenerative diseases.[69]

Mechanical performance testing and drug development

Muscle organoids serve as a reliable *in vitro* model for mechanical performance testing, which is of great significance in studying muscle function and drug development. By applying electrical stimulation to muscle organoids to induce contraction responses, researchers can measure their mechanical properties, such as contractile force, endurance, and dynamic responsiveness. These indicators reflect the maturity and functional state of the muscle organoids and can be used to assess the effects of exogenous compounds (e.g., growth factors or drugs) on muscle function.[70]

In drug development, muscle organoids provide an efficient and cost-effective platform for assessing the efficacy and safety of new drugs on muscle tissue. For example, researchers can observe the effects of drugs on muscle fibre proliferation, differentiation, and contraction by culturing muscle organoids in a medium containing the drug, as well as analyse changes in related signalling pathways. Furthermore, muscle organoids can also be used to evaluate the effectiveness of gene therapy strategies. By introducing gene delivery vectors into muscle organoids, researchers can assess the effects of restoring or knocking down specific gene expressions on muscle function, providing reliable data support for the gene therapy of muscle diseases.[56]

Applications in muscle regeneration and rehabilitation research

Muscle regeneration is a key research area in clinical and regenerative medicine, where muscle organoids hold unique advantages in studying muscle regeneration and rehabilitation. Satellite cells play a critical role in muscle regeneration during muscle injury and repair. Muscle organoid models can simulate the activation, proliferation, and differentiation of satellite cells in damaged muscle, enabling the study of muscle regeneration mechanisms.[55]

Additionally, muscle organoids have promising applications in rehabilitation research. They can simulate the effects of different rehabilitation strategies (e.g., mechanical stretching, cyclic electrical stimulation) on muscle tissue to explore the mechanisms at the cellular and molecular levels.[71] Furthermore, muscle organoids can be used to evaluate the effectiveness of tissue-engineered muscle transplantation. Researchers can combine various scaffold materials, cell types, and growth factors within muscle organoids to assess their impact on muscle tissue formation and functional recovery. By observing muscle organoids' integration, vascularisation, and functional recovery post-transplantation, valuable scientific evidence can be provided for clinical muscle regeneration therapies.[72]

In conclusion, muscle organoids have extensive applications in muscle degenerative disease research, mechanical performance testing, drug development, muscle regeneration, and rehabilitation research. They not only provide an advanced platform for basic research in muscle biology but also offer new perspectives and methods for optimising clinical treatments and rehabilitation strategies for muscle-related disease.

Joint Organoids

Physiology and structure of joints

Anatomy and biomechanical properties of joints

Joints are vital components of the human skeletal system, playing a critical role in biomechanics. The structure of a joint typically includes the joint capsule, articular cartilage, joint cavity, and synovial fluid. Articular cartilage, primarily composed of hyaline cartilage, has low-friction characteristics that support the free movement and weight-bearing capacity of joints. The primary cells in cartilage are chondrocytes, which are embedded within an abundant ECM.[73] The ECM contains a high concentration of type II collagen and proteoglycans that work together to ensure the elasticity and wear resistance of cartilage.[74] Moreover, the biomechanical properties of joints are also influenced by the joint capsule and synovial fluid. The joint capsule, made of fibrous tissue, provides protection and support during movement. Synovial fluid, acting as a lubricant, fills the joint cavity, reducing friction between joint surfaces and enhancing joint stability and smoothness of movement. This structural design allows joints to maintain a high degree of flexibility and stability while bearing external forces, helping prevent joint wear and damage.[75]

Role of joints in the skeletal system

The primary function of joints within the skeletal system is to facilitate relative movement between bones, thus enabling the execution of various motions. Through their biomechanical properties, joints can not only move freely in different directions but also effectively absorb external impacts, protecting other fragile bone structures. Articular cartilage, as an essential component of the skeletal system, has unique viscoelastic properties, allowing it to remain stable under cyclic loading. Its primary function is to provide smooth, low-friction joint movement and to resist compressive and tensile forces, thereby protecting bones from injury.[76]

The joint capsule, ligaments, and tendons surrounding the joint play key roles in joint function. The joint capsule provides passive stability by limiting unwanted motion and promotes active stability through its proprioceptive nerve endings. Additionally, specialised structures within the joint capsule are involved in the formation of joint surfaces and can adapt to age-related and pathological changes.[77] From a regenerative medicine perspective, understanding the structure and function of joints is crucial for repairing or replacing damaged joints.

Construction of joint organoids

Selection of cells and matrix materials

Joint organoids, comprising cells such as osteocytes, chondrocytes, and synoviocytes, represent a multicellular structured tissue. Consequently, constructing joint organoids requires cells with high pluripotency and differentiation potential. Besides iPSCs and mesenchymal stem cells, chondroprogenitor cells are also considered ideal due to their diversity in developmental differentiation.[78] Additionally, leucine-rich repeat-containing G protein-coupled receptor 5 positive embryonic joint progenitor cells have been identified as potential cell sources, capable of forming cartilage-functional organoids under suitable conditions.[79]

The choice of scaffold material is crucial for cell proliferation, differentiation, and functional maintenance in joint organoid construction (**Table 3**).[80-84] In recent years, decellularised ECM has gained popularity in joint organoid construction due to its excellent biocompatibility and biomechanical properties.[73, 85] Decellularised ECM, derived by removing cells from natural tissues while retaining the ECM, preserves structural proteins and bioactive factors found in natural joint tissues. This material provides essential biological signals that support the regeneration of cartilage and bone tissue.[85] Other natural materials, such as alginate, gelatin, and collagen-based hydrogels, are also commonly used as matrix materials for joint organoids to create a supportive 3D environment for cell growth and differentiation. To better mimic the microenvironment of cartilage tissue, researchers have explored biomaterials modified with specific signalling molecules, such as peptide hydrogels incorporating Wnt signalling molecules to guide chondrocyte differentiation.[86]

Table 3. Methods for constructing joint organoids

Cell source	Matrix gel	Inducing factor	Application	Reference
SMSCs	Agarose microwells	miR-138	Construction of osteoarthritis model	80
	Collagen, fibrin	miR-24	Cartilage repair and tissue regeneration in osteoarthritis	81
Chondrocytes	Matrigel, TISSEEL fibrin gel	TNF-α, TGF-β3	Construction of cartilage inflammation model	82
	NCM	Cytokines from NCM	Cartilage regeneration and repair of articular cartilage damage	83
iPSCs	Agarose microwells	BMP-2, TGF-β1, BMP-6, FGF-2	Repairment of osteochondral defects	84

Note: bone morphogenetic protein; FGF: fibroblast growth factor; iPSCs: induced pluripotent stem cells; NCM: neo-cartilage formation; SMSCs: synovial mesenchymal stromal cells; TGF: transforming growth factor; TNF-α: tumour necrosis factor-α.

Cytokine and signalling pathway regulation in joint organoids

The cultivation of joint organoids involves regulation by cytokines and signalling pathways. Similar to bone organoid construction, joint organoids undergo differentiation and proliferation regulated by the TGF-β family, BMP, FGF family, and the Wnt/β-catenin signalling pathway, among others. By adding TGF-β3 and BMP-2, iPSCs can be successfully induced to differentiate into organoids with cartilage and bone characteristics, replicating signalling pathway changes observed in osteochondral development.[78]

3D microenvironment and mechanical simulation in joint organoid cultivation

Simulating a 3D microenvironment is essential in joint organoid construction. The ECM of articular cartilage comprises collagen, proteoglycans, and other components that provide mechanical support and facilitate signal transmission. Hydrogels are commonly used as 3D scaffolds in research, along with cell embedding techniques and bioprinting technology, to construct organoids with structures similar to natural cartilage tissue. To more accurately simulate the joint environment, microfluidic chip technology (joint-on-a-chip) has been developed, introducing fluid shear stress and pressure during organoid cultivation to simulate mechanical stresses experienced in joint movement. This approach enables the study of the effects of mechanical stimuli on organoid development and function.[87]

Functional validation of joint organoids

To verify whether joint organoids possess the desired structure and function, a series of biological and biomechanical tests are conducted. Histological and immunohistochemical staining are common methods used to evaluate the distribution and morphology of chondrocytes and the composition of the matrix within the organoids. For example, haematoxylin-eosin staining and immunofluorescence staining can be employed to observe the distribution of type II collagen and proteoglycans in the organoids, determining if the formation of the cartilage matrix resembles that of natural cartilage tissue.[86]

To validate the biomechanical properties of joint organoids, mechanical tests such as compression and shear stress assessments are performed to evaluate the organoids' response and resilience under mechanical stimuli. Additionally, some studies transplant organoids into animal models to observe their integration with host tissues and regenerative effects, thereby assessing the clinical application potential of the organoids.[74] Through these validation methods, the structure, function, and biomechanical characteristics of joint organoids can be evaluated, providing a solid foundation for their application in drug screening, disease modelling, and regenerative medicine.

Applications of joint organoids

Research on osteoarthritis and intervertebral disc degeneration

Osteoarthritis is a common degenerative joint disease

characterised by cartilage degeneration and joint dysfunction. It frequently occurs in the elderly population, causing pain and mobility issues, thereby posing an economic burden on society. The aetiology of osteoarthritis is complex, involving multiple factors such as obesity, ageing, and physical trauma.[88] Current treatment methods mainly involve medication, including oral non-steroidal anti-inflammatory drugs and intra-articular steroid injections to alleviate pain, as well as hyaluronic acid injections for treating joint inflammation.[89] However, these treatments primarily address symptoms without altering the disease's progression and may have adverse side effects.

Organoid technology enables the dynamic simulation of cartilage degeneration and repair processes *in vitro*, enabling researchers to investigate the molecular mechanisms leading to cartilage matrix destruction and inflammation. For instance, joint organoids can be exposed to senescence and inflammation-related factors to study their impact on cartilage tissue. By inducing senescent joint organoids to express pro-inflammatory cytokines (e.g., interleukin-1β, interleukin-6) and tumour necrosis factor-α, the cartilage degradation observed in osteoarthritis patients can be replicated, thereby modelling the disease progression.[79]

Furthermore, joint organoids can simulate the process of intervertebral disc degeneration, where changes in chondrocytes and the ECM are crucial features. During disc degeneration, chondrocytes undergo various changes, including senescence, altered secretion, and functional impairment. The ECM shows a marked decrease in collagen and proteoglycan content, leading to reduced elasticity and cushioning capacity of the disc.[87] By constructing degeneration models, researchers can study the molecular mechanisms of chondrocyte and matrix changes within the disc, providing new perspectives for treatment strategies of intervertebral disc degeneration (**Figure 6**).

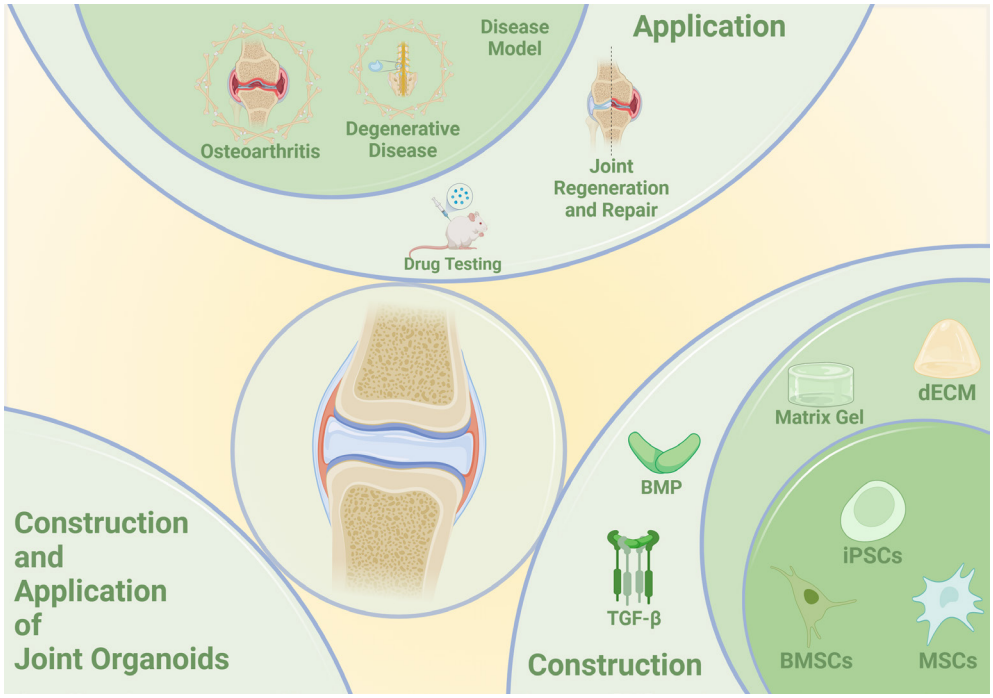


Figure 6. Construction and application of joint organoids. Created with BioRender.com. BMP: bone morphogenetic protein; BMSCs: bone mesenchymal stem cells; dECM: decellularised extracellular matrix; iPSCs: induced pluripotent stem cells; MSCs: mesenchymal stem cells; TGF-β: transforming growth factor-β.

Role of in vitro models in drug screening

Traditional drug screening methods are often based on 2D cell culture models, which lack the complex signalling and tissue specificity of the *in vivo* joint microenvironment. This discrepancy can result in significant differences between the drug's actual efficacy in the body and the outcomes observed *in vitro*. Joint organoids provide a highly physiological 3D environment, better simulating the pathological state of *in vivo* joint tissues and the interactions between cells.

By constructing joint organoids, researchers can conduct high-throughput screening of candidate drugs for their efficacy and toxicity. For example, in osteoarthritis models, simulating the inflammation and matrix degradation process of joint tissue allows for evaluating the efficacy of anti-inflammatory drugs and matrix-regenerating agents, providing reliable data for the development of osteoarthritis treatments.[80] Additionally, utilising microRNA/organoid composite hydrogels enables the screening and assessment of anti-ageing drugs' effects in improving chondrocyte homeostasis and inhibiting joint degeneration.[81] The diversity and controllability of joint organoids make personalised drug screening possible, allowing drug testing based on specific pathological features of different

patients, thereby enhancing the efficiency and precision of drug development.[90]

Application in repair and regeneration strategies

Joint organoids have broad application prospects in tissue engineering and regenerative medicine, especially in cartilage and bone tissue repair and regeneration. Since cartilage tissue has limited self-repair capabilities, treating osteoarthritis and osteochondral defects has been a clinical challenge. Organoid technology offers a new strategy for cartilage regeneration by simulating the development of joint tissues and generating 3D structures similar to natural joint tissues.

For instance, researchers use mesenchymal stem cells and iPSCs to generate joint organoids that mimic the formation of cartilage and bone tissue, exploring the mechanisms of cartilage and bone regeneration.[78] These organoids can further be combined with biomaterials to construct grafts for joint repair. By optimising culture conditions and growth factor combinations during *in vitro* culture, researchers can induce chondrocyte differentiation and matrix deposition, producing functional and stable organoids. The combination of 3D bioprinting technology with organoids also provides new possibilities for constructing joint tissues with complex structures and functions, enabling precise design of the organoid's shape, cell arrangement, and mechanical properties to better simulate natural joints.[74]

Moreover, studies have found that organoids can regulate chondrocyte homeostasis, thereby promoting cartilage regeneration. For example, introducing microRNAs (e.g., miR-24) and specific signalling pathways can inhibit chondrocyte senescence, enhance their proliferation and differentiation capabilities, and ultimately improve cartilage repair outcomes.[81] This offers a novel approach for developing organoid-based precision therapies to repair damaged joints.

Ligament and Tendon Organoids

Physiology and structure of ligaments and tendons

Tissue structure and biomechanical function of ligaments and tendons

Ligaments and tendons are connective tissues characterised by their complex structures and biomechanical properties. Both are primarily composed of collagen fibres (mainly type I collagen) and elastin embedded in a proteoglycan-water matrix. Tendons primarily function to transmit the force generated by muscles to bones, facilitating joint movement. Tendons are flexible but inelastic, capable of resisting tension and compression forces. For instance, the Achilles tendon, the largest tendon in the body, can withstand loads up to 17 times the body's weight.[91, 92] At the microscopic level, the collagen fibres in tendons and ligaments are not only aligned longitudinally but also arranged transversely and horizontally, forming a helical cross-linked structure. This unique 3D organisation grants tendons and ligaments high mechanical strength and elasticity, allowing them to resist forces from multiple directions during movement.[92]

Ligaments connect bones to bones, providing stability and support to the joints. While their structure is similar to that of tendons, they differ slightly in cell types, cellular activity, collagen content, and fibre arrangement. Ligaments contain a higher proportion of type III collagen, proteoglycans, and water, and their collagen fibres exhibit a more irregular arrangement. Ligaments are primarily composed of ligamentocytes, which play a critical role in maintaining tissue homeostasis and facilitating healing after injury.[91, 93] Moreover, ligaments and tendons exhibit different morphological and biochemical characteristics depending on their physiological states and developmental stages. For example, ligaments are more metabolically active, containing more DNA, a higher level of reducible cross-links, more type III collagen, and more proteoglycans.[93]

Role and importance in the skeletal system

Ligaments and tendons are essential components of the skeletal system. Tendons connect muscles to bones, transmitting the force generated by muscles to bones to facilitate joint movement. For example, the quadriceps and triceps have short and broad tendons to withstand greater forces, while finger flexors possess long and thin tendons to enable fine motor control.[92] During force transmission, tendon fibres can absorb and release energy, effectively buffering external forces and preventing joint damage.

Ligaments, on the other hand, connect bones to bones, providing joint stability and guiding the range of motion during activity. When exposed to external forces, ligaments stabilise the joint, preventing abnormal bone displacement and protecting it from injury. In the knee joint, for instance, the anterior cruciate ligament and posterior cruciate ligament work together to maintain joint stability. Due to the critical roles of ligaments and tendons, their injuries often lead to severe mobility impairments, affecting the quality of daily life.[91, 94]

Research indicates that the tensile strength of tendons and ligaments largely depends on collagen fibre cross-linking.[95] Damage to ligaments and tendons can result in serious complications, such as joint instability, osteoarthritis, pain, and limited function. Traditional repair methods, such as surgical suturing and tissue grafting, often fail to restore the original structure and function completely. For certain tendons and ligaments, the re-rupture rates after surgical repair may be high.[96] Consequently, tissue engineering and regenerative medicine for ligaments and tendons are becoming promising therapeutic strategies, aiming to promote the repair and regeneration of damaged tissues by combining cells, scaffold materials, and bioactive molecules.[94]

Construction of ligament and tendon organoids

In tissue engineering, multiple cell sources are often utilised to simulate the functions of ligaments and tendons (**Table 4**).[97-99] Commonly used cell sources include bone marrow-derived mesenchymal stem cells, adipose-derived stem cells, and stem cells derived from tendons or ligaments. These cells can be cultured on suitable biomaterial scaffolds and induced to differentiate into cells that exhibit ligament or tendon tissue characteristics, forming organoid structures.[100]

Table 4. Methods for constructing ligament/tendon organoids

Cell source	Matrix gel	Inducing factor	Application	Reference
SkMDCs, tenocytes	GelMA and PEGDMA	–	Drug development and screening	97
Periodontal ligament cells	BME	FGF-10, EGF	Construction of ligament organoid model	98
Normal adult human dermal fibroblasts	–	TGF-β3	*In vitro* studies of tenogenesis	99

Note: BME: basement membrane-like matrix; EGF: epidermal growth factor; FGF-10: fibroblast growth factor-10; GelMA: gelatin methacryloyl; PEGDMA: poly(ethylene glycol) dimethacrylate; skMDCs: skeletal muscle-derived cells; TGF-β3: transforming growth factor-β3.

The choice of matrix material is crucial in constructing ligament and tendon organoids, as its properties must align with the mechanical and biological characteristics of these tissues. Commonly used matrices include natural materials such as collagen, hyaluronic acid, gelatin, and alginate, as well as synthetic materials like polylactic acid and polycaprolactone. Collagen and elastin, the main components of the ECM in tendon and ligament tissues, are ideal choices for constructing organoids.[101] Petrigliano attempted to use polycaprolactone nanofibres to reconstruct rodent ligaments, but the resulting organoids lacked sufficient strength and exhibited low biocompatibility.[102] Matrix materials not only provide a scaffold for cell growth and differentiation but also influence cell behaviour and tissue formation by modulating material stiffness, porosity, and degradation rates.[94] For example, multilayered nanofibre scaffolds produced via electrospinning, coated with tendon or ligament-derived ECM, can promote cell differentiation towards tendon/ligament lineages, thereby enhancing the mechanical properties and biological function of the organoids.[103]

Creating ideal matrix materials that mimic ECM is essential to establish a microenvironment similar to the natural biochemical composition of tendons and ligaments, which can initiate specific cellular responses to promote faster tissue regeneration.[104] Cell-scaffold composites combine cells with biomaterial scaffolds to form functional tissues. By introducing ligament- or tendon-specific cells into the scaffold, along with growth factors (such as TGF-β, BMP-12, and early growth response factor-1 (EGR-1)), cells can be induced to differentiate toward ligament or tendon lineages, forming organoids.[105] For instance, EGR-1 plays a role in early tendon differentiation; in tendon cells, EGR-1 expression correlates with increased collagen expression during embryonic tendon cell differentiation.[106] Additionally, to more exactly simulate the natural structure and function of ligaments and tendons, researchers apply mechanical stress (e.g., stretching or compression) using bioreactors during organoid construction to promote cellular characteristics that mirror *in vivo* tissue properties.[100, 107]

In cell self-assembly strategies, cells can autonomously form organoids with ligament or tendon characteristics under appropriate culture conditions. Alternatively, 3D bioprinting technology uses bioinks to layer cells and biomaterials in a designed structure, enabling the construction of complex 3D tissue structures.[108] These methods, which combine different cell sources, growth factors, and scaffold materials, allow precision control over the morphology and function of organoids, simulating the microenvironment of natural ligament and tendon tissue and providing ideal tissue replacements for clinical repair.

Mechanical properties and functional evaluation of organoids

The mechanical properties and functional evaluation of organoids are crucial indicators for assessing their quality and potential applications. Ideal ligament and tendon organoids should possess mechanical strength, elastic modulus, and fracture elongation similar to natural tissues, ensuring their capability to support and transmit forces in the body. Studies have shown that regulating the fibre alignment and density of electrospun scaffolds can significantly enhance the tensile strength and elasticity of organoids, mimicking the biomechanical properties of natural tendons.[103]

To evaluate the functionality of organoids, it is essential to assess their biological characteristics both *in vitro* and *in vivo*, including cell viability, collagen synthesis capacity, ECM deposition, and adaptability to mechanical stimuli. The performance of organoids after *in vivo* transplantation is also an important functional evaluation criterion, which includes their role in tissue repair, the degree of integration with surrounding tissues, and long-term biological stability. Among these evaluations, the mechanical performance of the organoids is the most critical parameter, as it directly impacts the organoids' efficacy and safety in clinical applications.

Applications of ligament and tendon organoids

Research on sports injury repair

Ligament and tendon organoids show great potential in the research of sports injury repair (**Figure 7**). As the intensity of physical activity increases among athletes and the general population, ligament and tendon injuries, especially anterior cruciate ligament and Achilles tendon injuries, have become more prevalent. Traditional treatment methods such as surgical repair and grafting often fail to fully reestablish the structure and functionality of damaged tissue and carry a high risk of complications and long-term functional impairment.[94] Organoid technology offers a highly biomimetic 3D structure that can promote the regeneration of damaged ligament and tendon tissues. In experimental studies, tissue-engineered ligament organoids are used as an alternative to traditional grafting techniques. By combining biomaterials and stem cells, and through the regulation of growth factors (e.g., EGR-1, FGF) and the application of mechanical loading, it is possible to better facilitate cellular repair and functional recovery.[109]

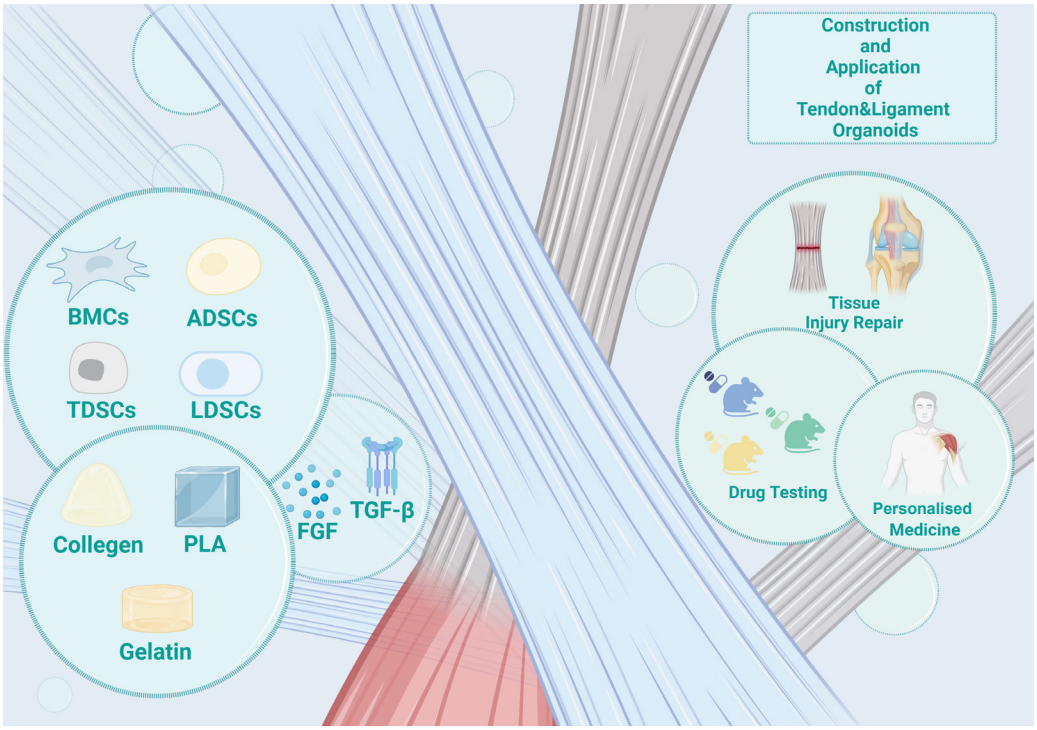


Figure 7. Construction and application of ligament and tendon organoids. Created with BioRender.com. ADSCs: adipose-derived stem cells; BMCs: bone marrow cells; FGF: fibroblast growth factor; LDSCs: lipoma-derived stem cells; PLA: polylactic acid; TDSCs: tendon-derived stem cells; TGF-β: transforming growth factor-β.

Drug testing and regenerative medicine applications

Ligament and tendon organoids provide an innovative platform for drug testing. Compared to traditional 2D cell cultures, organoids can better simulate the *in vivo* 3D environment, providing a more realistic tissue response. By testing anti-inflammatory drugs, tissue repair promoters, or growth factors on organoids, researchers can assess the efficacy and safety of these drugs in a more realistic setting, thereby reducing the reliance on animal experiments.

Moreover, ligament and tendon organoids have extensive applications in regenerative medicine. They can be used to repair damaged skeletal tissues and assess their effectiveness in accelerating tissue regeneration and reducing scar tissue formation. Additionally, organoid models constructed from aged tendon stem cells allow researchers to study the effects of ageing on tendon structure. Comparative studies have shown that aged cells exhibit significant defects in forming 3D tendon tissue organoids, with marked degeneration in cell adhesion, migration, and self-renewal capabilities.[110]

Applications in preclinical models and personalised medicine

Ligament and tendon organoids play a crucial role in preclinical models, particularly demonstrating significant potential in personalised medicine. By utilising a patient's autologous stem cells or iPSCs to construct specific organoids, researchers can create tissue models that reflect the patient's unique pathological characteristics or evaluate the tissue response following surgery.

In personalised medicine, organoid construction can be tailored to the patient's injury severity, genetic background, and biomechanical needs. This approach is particularly suitable for complex ligament and tendon injury repair since the organoids can be designed based on the patient's specific condition, using customised combinations of biomaterials and cells to ensure optimal tissue regeneration outcomes.

In summary, ligament and tendon organoids, as advanced tissue engineering tools, are transforming existing methods of sports injury repair, drug testing, and personalised medicine. With the ongoing advancement of technology, the potential for these organoids in clinical applications will be further expanded and validated.

Integrated Application of Skeletal Organoids and Future Prospects

Integration and interaction of multiple tissue organoids

Combination construction and research of multiple skeletal organoids

The skeletal system is composed of various tissues, including bones, muscles, cartilage, ligaments, and tendons, whose coordinated functioning is essential for human activity. Focusing solely on a single tissue organoid model may not comprehensively reflect the overall function and interaction within the skeletal system. To replicate the complex functions of these tissues *in vitro*, researchers have begun constructing and integrating multiple organoids into a unified system. Through 3D cell culture and microfluidic technologies, researchers

have developed multi-organoid models, including those for the intestine and heart.[111] Additionally, bone microfluidic chips have been successfully constructed, showing potential in clinical diagnostics.[112] By integrating multiple tissue organoids, researchers can more realistically simulate the skeletal system's complex biological environment more realistically, providing more accurate disease models and platforms for therapeutic evaluation.

Research on interactions among different organoids

Understanding the interactions among different organoids is crucial in multi-tissue organoid research. In the skeletal system, various tissues interact in complex ways under both physiological and pathological conditions. For instance, muscle force transmission influences bone growth, and joint inflammation can affect surrounding ligaments and tendons. Organoid technology enables researchers to systematically study these interactions in a controlled environment. Advancing research on multi-tissue organoids will further reveal the complexities of skeletal system diseases and aid in developing more targeted treatment strategies.

The potential of organoids in personalised medicine

Development of precision medicine and personalised therapies

Precision medicine and personalised therapies have become crucial in modern medicine, focusing on tailoring treatments according to individual differences in genetics, environment, and lifestyle. Organoid technology provides an essential tool for precision medicine, as organoids can be generated from a patient's own cells, creating 3D tissue models that closely match the patient's physiological state. These models can be used in the laboratory for drug screening, toxicity testing, and disease research, thereby facilitating the formulation of personalised treatment strategies. Particularly for skeletal diseases, personalised organoid models can accurately predict a patient's response to specific treatments, avoiding ineffective or harmful interventions and improving treatment success rates.[113]

Application prospects of skeletal organoids in personalised medicine

In personalised medicine applications of skeletal organoids, bone organoids have shown potential in predicting osteoporosis patients' responses to medications. By generating bone organoids from patient-derived cells, researchers can evaluate the impact of different drugs on bone density and structure, thereby determining the most appropriate drug therapy for each patient.[47] Similarly, personalised muscle organoid models can be employed to study rare muscle diseases, such as Duchenne muscular dystrophy, and to test the effectiveness of gene therapies or drug treatments.[2] The construction of joint organoids offers new diagnostic and therapeutic approaches for arthritis patients, enabling the use of patient-derived chondrocytes to assess drug efficacy.[83] Additionally, patient-specific cancer organoids have shown promising results in personalised drug screening, facilitating tailored treatment approaches with potentially improved therapeutic outcomes.[114, 115] These applications not only improve treatment outcomes but also reduce unnecessary clinical trials, offering new pathways for the advancement of personalised medicine (**Figure 8**).

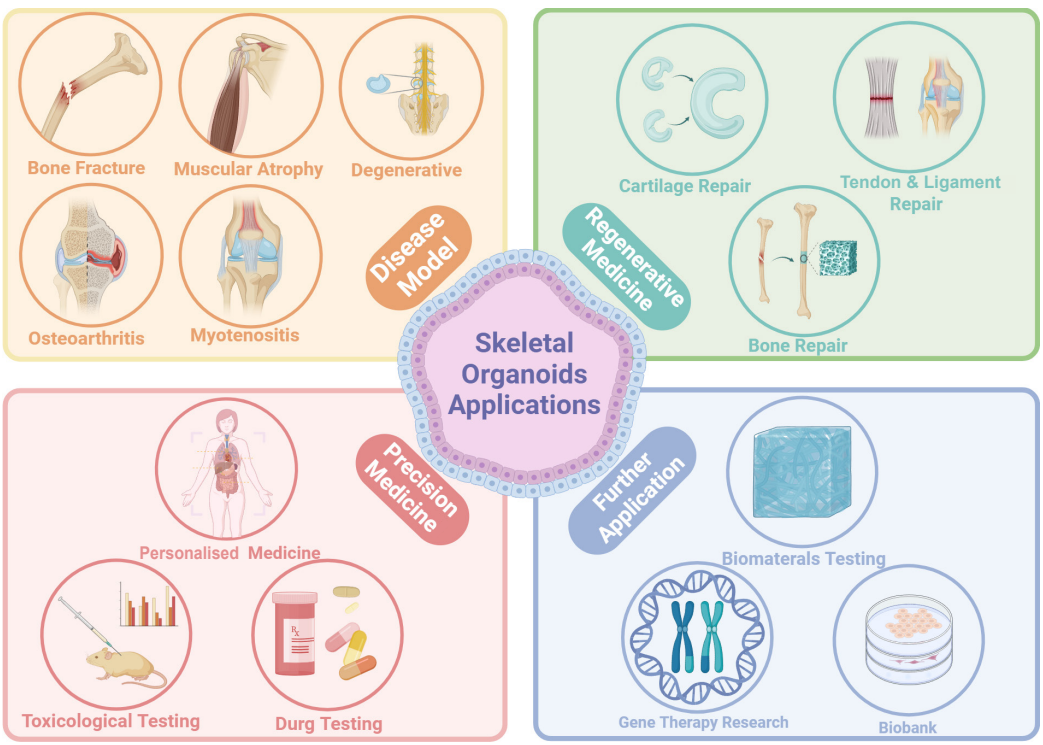


Figure 8. Applications of skeletal organoids. Created with BioRender.com.

Future research directions and challenges

Technical challenges and opportunities in skeletal organoids

Although organoid technology has shown immense potential in skeletal research, technical challenges remain. One primary issue is the standardisation of organoid construction, as the current processes are complex and yield variable results. Achieving higher reproducibility and standardisation is crucial. Additionally, simulating the organoid microenvironment needs further refinement to more accurately reflect the physical and biochemical conditions *in vivo*. Another challenge is the large-scale production and long-term functional maintenance of organoids. However, these challenges present opportunities for innovation. For instance, constructing anti-ageing organoid scaffolds through 3D bioprinting technology can effectively mitigate cellular senescence and alleviate symptoms of osteoarthritis.[116] Alternatively, combining microfluidic technology with DNA hydrogels to construct cartilage organoids can enhance tissue regeneration.[117] Furthermore, multi-omics analysis of organoids can reveal the gene regulatory mechanisms underlying diseases, enabling more precise, personalised therapeutic approaches.[115]

Development trends in new organoid construction technologies

The future of skeletal organoid construction is expected to move towards greater complexity and precision. Co-culturing multiple cell types, utilising dynamic culture systems, and integrating biomechanical signals will further enhance the physiological relevance of organoids. The integration of tissue engineering and regenerative medicine will also drive the application of organoids in damaged tissue repair. Furthermore, the introduction of artificial intelligence and machine learning into the organoid generation process may optimise construction parameters and predict the developmental processes of organoids. With technological advancements, the functionality of organoids will increasingly resemble that of natural tissues, and their application range will continue to expand.

Ethical and regulatory issues in skeletal organoid research

The rapid development of organoid technology raises ethical and regulatory challenges. Particularly in research involving patient-derived cells, ensuring patient privacy and data security is of utmost importance. Furthermore, the application of organoids may extend the ethical boundaries of human experimentation, necessitating a clear legal and ethical framework. Regulatory agencies need to keep pace with technological advancements, formulating appropriate standards and guidelines to ensure the safety and efficacy of organoid use in research and clinical applications. Additionally, public acceptance and understanding of organoid technology need to be enhanced. Through science education and transparent communication, concerns can be alleviated, promoting the widespread application of organoid technology.

Conclusion

In this review, we systematically summarised the progress, construction strategies, and applications of skeletal organoids in disease research and medical fields. While we discussed the construction of skeletal organoids, a standardised strategy for organoid construction has not yet been established due to the variability in construction approaches chosen by researchers based on specific application goals. Future research may allow for quantifying the influence of different factors within organoid construction strategies, thereby standardising organoid construction practices.

While we summarised the components of skeletal organoids, examples of combining these components in practical applications are lacking. Complex organoid construction involving multiple organoid types may present a promising research direction. Although we provided various examples of organoid construction, most of these remain small-scale, limited to laboratory environments, and large-scale industrial production of organoids is a challenge yet to be addressed. Establishing standardised organoid construction protocols may propel advancements in this area.

In summary, skeletal organoids offer significant practical value and developmental potential. Skeletal organoids provide a 3D model of tissues like bone, muscle, joints, ligaments, and tendons, creating new platforms for studying skeletal system disease mechanisms, drug screening, and tissue regeneration. However, challenges remain in the field, including standardisation, the complexity of multi-organoid integration, and barriers to clinical application. Therefore, further optimisation of organoid construction techniques is necessary to enhance organoid stability, facilitating their translation into personalised medicine, complex disease modelling, and clinical applications.

Author contributions

YJ and JW conceptualised the review; ZC drafted the manuscript; CZ, LB, YJ, JS, LY, and ZX revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Financial support

This work was financially supported by National Natural Science Foundation of China (Nos. 32471396, 82230071, 82172098), Integrated Project of Major Research Plan of National Natural Science Foundation of China (No. 92249303), Shanghai Committee of Science and Technology (No. 23141900600, Laboratory Animal Research Project), Shanghai Clinical Research Plan (No. SHDC2023CRT01), Shanghai Municipal Demonstration Project for Innovative Medical Device Applications (No. 23SHS05700), and Young Elite Scientist Sponsorship Program by China Association for Science and Technology (No. YESS20230049).

Acknowledgement

None.

Conflicts of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. 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.
2. Shahriyari, M.; Islam, M. R.; Sakib, S. M.; Rinn, M.; Rika, A.; Krüger, D.; Kaurani, L.; Gisa, V.; Winterhoff, M.; Anandakumar, H.; Shomroni, O.; Schmidt, M.; Salinas, G.; Unger, A.; Linke, W. A.; Zschüntzsch, J.; Schmidt, J.; Bassel-Duby, R.; Olson, E. N.; Fischer, A.; Zimmermann, W. H.; Tiburcy, M. Engineered skeletal muscle recapitulates human muscle development, regeneration and dystrophy. *J Cachexia Sarcopenia Muscle.* **2022,** *13*, 3106-3121.
3. Davies, J. A. Chapter 1 - Organoids and mini-organs: Introduction, history, and potential. In *Organoids and Mini-Organs*, Davies, J. A.; Lawrence, M. L., eds.; Academic Press: 2018; pp 3-23.
4. Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* **2006,** *126*, 663-676.
5. Sato, T.; Vries, R. G.; Snippert, H. J.; van de Wetering, M.; Barker, N.; Stange, D. E.; van Es, J. H.; Abo, A.; Kujala, P.; Peters, P. J.; Clevers, H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature.* **2009,** *459*, 262-265.
6. 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.
7. Lancaster, M. A.; Renner, M.; Martin, C. A.; Wenzel, D.; Bicknell, L. S.; Hurles, M. E.; Homfray, T.; Penninger, J. M.; Jackson, A. P.; Knoblich, J. A. Cerebral organoids model human brain development and microcephaly. *Nature.* **2013,** *501*, 373-379.
8. Post, Y.; Puschhof, J.; Beumer, J.; Kerkkamp, H. M.; de Bakker, M. A. G.; Slagboom, J.; de Barbanson, B.; Wevers, N. R.; Spijkers, X. M.; Olivier, T.; Kazandjian, T. D.; Ainsworth, S.; Iglesias, C. L.; van de Wetering, W. J.; Heinz, M. C.; van Ineveld, R. L.; van Kleef, R.; Begthel, H.; Korving, J.; Bar-Ephraim, Y. E.; Getreuer, W.; Rios, A. C.; Westerink, R. H. S.; Snippert, H. J. G.; van Oudenaarden, A.; Peters, P. J.; Vonk, F. J.; Kool, J.; Richardson, M. K.; Casewell, N. R.; Clevers, H. Snake venom gland organoids. *Cell.* **2020,** *180*, 233-247.e21.
9. 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.
10. 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.
11. Cable, J.; Fuchs, E.; Weissman, I.; Jasper, H.; Glass, D.; Rando, T. A.; Blau, H.; Debnath, S.; Oliva, A.; Park, S.; Passegué, E.; Kim, C.; Krasnow, M. A. Adult stem cells and regenerative medicine-a symposium report. *Ann N Y Acad Sci.* **2020,** *1462*, 27-36.
12. Doss, M. X.; Sachinidis, A. Current challenges of iPSC-based disease modeling and therapeutic implications. *Cells.* **2019,** *8*, 403.
13. Omole, A. E.; Fakoya, A. O. J. Ten years of progress and promise of induced pluripotent stem cells: historical origins, characteristics, mechanisms, limitations, and potential applications. *PeerJ.* **2018,** *6*, e4370.
14. Qian, S.; Mao, J.; Liu, Z.; Zhao, B.; Zhao, Q.; Lu, B.; Zhang, L.; Mao, X.; Cheng, L.; Cui, W.; Zhang, Y.; Sun, X. Stem cells for organoids. *Smart Med.* **2022,** *1*, e20220007.
15. Wu, S.; Wu, X.; Wang, X.; Su, J. Hydrogels for bone organoid construction: From a materiobiological perspective. *J Mater Sci Technol.* **2023,** *136*, 21-31.
16. Ma, P.; Chen, Y.; Lai, X.; Zheng, J.; Ye, E.; Loh, X. J.; Zhao, Y.; Parikh, B. H.; Su, X.; You, M.; Wu, Y. L.; Li, Z. The translational application of hydrogel for organoid technology: challenges and future perspectives. *Macromol Biosci.* **2021,** *21*, e2100191.
17. Catoira, M. C.; Fusaro, L.; Di Francesco, D.; Ramella, M.; Boccafoschi, F. Overview of natural hydrogels for regenerative medicine applications. *J Mater Sci Mater Med.* **2019,** *30*, 115.
18. Kozlowski, M. T.; Crook, C. J.; Ku, H. T. Towards organoid culture without Matrigel. *Commun Biol.* **2021,** *4*, 1387.
19. Jee, J. H.; Lee, D. H.; Ko, J.; Hahn, S.; Jeong, S. Y.; Kim, H. K.; Park, E.; Choi, S. Y.; Jeong, S.; Lee, J. W.; Cho, H. J.; Kwon, M. S.; Yoo, J. Development of collagen-based 3D matrix for gastrointestinal tract-derived organoid culture. *Stem Cells Int.* **2019,** *2019*, 8472712.
20. Ye, W.; Luo, C.; Li, C.; Huang, J.; Liu, F. Organoids to study immune functions, immunological diseases and immunotherapy. *Cancer Lett.* **2020,** *477*, 31-40.
21. Li, C.; Zhang, Y.; Du, Y.; Hou, Z.; Zhang, Y.; Cui, W.; Chen, W. A review of advanced biomaterials and cells for the production of bone organoid. *Small Sci.* **2023,** *3*, 2370015.
22. Madduma-Bandarage, U. S. K.; Madihally, S. V. Synthetic hydrogels: synthesis, novel trends, and applications. *J Appl Polym Sci.* **2021,** *138*, 50376.
23. Aisenbrey, E. A.; Murphy, W. L. Synthetic alternatives to Matrigel. *Nat Rev Mater.* **2020,** *5*, 539-551.
24. Wilson, R. L.; Swaminathan, G.; Ettayebi, K.; Bomidi, C.; Zeng, X. L.; Blutt, S. E.; Estes, M. K.; Grande-Allen, K. J. Protein-functionalized poly(ethylene glycol) hydrogels as scaffolds for monolayer organoid culture. *Tissue Eng Part C Methods.* **2021,** *27*, 12-23.
25. Jin, S.; Xia, X.; Huang, J.; Yuan, C.; Zuo, Y.; Li, Y.; Li, J. Recent advances in PLGA-based biomaterials for bone tissue regeneration. *Acta Biomater.* **2021,** *127*, 56-79.
26. Wozney, J. M.; Rosen, V.; Byrne, M.; Celeste, A. J.; Moutsatsos, I.; Wang, E. A. Growth factors influencing bone development. *J Cell Sci Suppl.* **1990,** *13*, 149-156.
27. Tortorella, I.; Argentati, C.; Emiliani, C.; Martino, S.; Morena, F. The role of physical cues in the development of stem cell-derived organoids. *Eur Biophys J.* **2022,** *51*, 105-117.
28. Farokhi, M.; Mottaghitalab, F.; Shokrgozar, M. A.; Ou, K. L.; Mao, C.; Hosseinkhani, H. Importance of dual delivery systems for bone tissue engineering. *J Control Release.* **2016,** *225*, 152-169.
29. Olszta, M. J.; Cheng, X.; Jee, S. S.; Kumar, R.; Kim, Y. Y.; Kaufman, M. J.; Douglas, E. P.; Gower, L. B. Bone structure and formation: A new perspective. *Mater Sci Eng R Rep.* **2007,** *58*, 77-116.
30. Buckwalter, J. A.; Glimcher, M. J.; Cooper, R. R.; Recker, R. Bone biology. *JBJS.* **1995,** *77*, 1276-1289.
31. Buck, D. W., 2nd; Dumanian, G. A. Bone biology and physiology: Part I. The fundamentals. *Plast Reconstr Surg.* **2012,** *129*, 1314-1320.
32. Huang, J.; Zhang, L.; Lu, A.; Liang, C. Organoids as innovative models for bone and joint diseases. *Cells.* **2023,** *12*, 1590.
33. Grabowski, P. Physiology of bone. In *Calcium and bone disorders in children and adolescents.* Allgrove, J.; Shaw, N., eds. Karger Publishers: 2009; Vol. 16, pp 32-48.
34. Porter, J. R.; Ruckh, T. T.; Popat, K. C. Bone tissue engineering: a review in bone biomimetics and drug delivery strategies. *Biotechnol Prog.* **2009,** *25*, 1539-1560.
35. Li, B.; Shi, Y.; Liu, M.; Wu, F.; Hu, X.; Yu, F.; Wang, C.; Ye, L. Attenuates of NAD(+) impair BMSC osteogenesis and fracture repair through OXPHOS. *Stem Cell Res Ther.* **2022,** *13*, 77.

36. Vallmajo-Martin, Q.; Broguiere, N.; Millan, C.; Zenobi-Wong, M.; Ehrbar, M. PEG/HA hybrid hydrogels for biologically and mechanically tailorable bone marrow organoids. *Adv Funct Mater.* **2020,** *30,* 1910282.
37. Li, M.; Liu, W.; Sun, J.; Xianyu, Y.; Wang, J.; Zhang, W.; Zheng, W.; Huang, D.; Di, S.; Long, Y. Z.; Jiang, X. Culturing primary human osteoblasts on electrospun poly(lactic-co-glycolic acid) and poly(lactic-co-glycolic acid)/nanohydroxyapatite scaffolds for bone tissue engineering. *ACS Appl Mater Interfaces.* **2013,** *5,* 5921-5926.
38. Schmidt-Bleek, K.; Willie, B. M.; Schwabe, P.; Seemann, P.; Duda, G. N. BMPs in bone regeneration: less is more effective, a paradigm-shift. *Cytokine Growth Factor Rev.* **2016,** *27,* 141-148.
39. Akiva, A.; Melke, J.; Ansari, S.; Liv, N.; van der Meijden, R.; van Erp, M.; Zhao, F.; Stout, M.; Nijhuis, W. H.; de Heus, C.; Muñiz Ortera, C.; Fermie, J.; Klumperman, J.; Ito, K.; Sommerdijk, N.; Hofmann, S. An organoid for woven bone. *Adv Funct Mater.* **2021,** *31,* 2010524.
40. Hospodiuk, M.; Dey, M.; Sosnoski, D.; Ozbolat, I. T. The bioink: a comprehensive review on bioprintable materials. *Biotechnol Adv.* **2017,** *35,* 217-239.
41. Papadimitropoulos, A.; Scherberich, A.; Güven, S.; Theilgaard, N.; Crooijmans, H. J.; Santini, F.; Scheffler, K.; Zallone, A.; Martin, I. A 3D in vitro bone organ model using human progenitor cells. *Eur Cell Mater.* **2011,** *21,* 445-458; discussion 458.
42. 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.
43. Yuasa, M.; Yamada, T.; Taniyama, T.; Masaoka, T.; Xuetao, W.; Yoshii, T.; Horie, M.; Yasuda, H.; Uemura, T.; Okawa, A.; Sotome, S. Dexamethasone enhances osteogenic differentiation of bone marrow- and muscle-derived stromal cells and augments ectopic bone formation induced by bone morphogenetic protein-2. *PLoS One.* **2015,** *10,* e0116462.
44. 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.
45. 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.
46. Visconti, R. J.; Kolaja, K.; Cottrell, J. A. A functional three-dimensional microphysiological human model of myeloma bone disease. *J Bone Miner Res.* **2021,** *36,* 1914-1930.
47. 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.
48. Gupta, N.; Liu, J. R.; Patel, B.; Solomon, D. E.; Vaidya, B.; Gupta, V. Microfluidics-based 3D cell culture models: Utility in novel drug discovery and delivery research. *Bioeng Transl Med.* **2016,** *1,* 63-81.
49. Frontera, W. R.; Ochala, J. Skeletal muscle: a brief review of structure and function. *Calcif Tissue Int.* **2015,** *96,* 183-195.
50. Au, Y. The muscle ultrastructure: a structural perspective of the sarcomere. *Cell Mol Life Sci.* **2004,** *61,* 3016-3033.
51. Vandenburgh, H.; Shansky, J.; Del Tatto, M.; Chromiak, J. Organogenesis of skeletal muscle in tissue culture. *Methods Mol Med.* **1999,** *18,* 217-225.
52. Sinacore, D. R.; Gulve, E. A. The role of skeletal muscle in glucose transport, glucose homeostasis, and insulin resistance: implications for physical therapy. *Phys Ther.* **1993,** *73,* 878-891.
53. Powell, C. A.; Smiley, B. L.; Mills, J.; Vandenburgh, H. H. Mechanical stimulation improves tissue-engineered human skeletal muscle. *Am J Physiol Cell Physiol.* **2002,** *283,* C1557-1565.
54. Kindler, U.; Zaehres, H.; Mavrommatis, L. Generation of skeletal muscle organoids from human pluripotent stem cells. *Bio Protoc.* **2024,** *14,* e4984.
55. Otto, A.; Schmidt, C.; Luke, G.; Allen, S.; Valasek, P.; Muntoni, F.; Lawrence-Watt, D.; Patel, K. Canonical Wnt signalling induces satellite-cell proliferation during adult skeletal muscle regeneration. *J Cell Sci.* **2008,** *121,* 2939-2950.
56. Vandenburgh, H.; Del Tatto, M.; Shansky, J.; Lemaire, J.; Chang, A.; Payumo, F.; Lee, P.; Goodyear, A.; Raven, L. Tissue-engineered skeletal muscle organoids for reversible gene therapy. *Hum Gene Ther.* **1996,** *7,* 2195-2200.
57. Shin, M. K.; Bang, J. S.; Lee, J. E.; Tran, H. D.; Park, G.; Lee, D. R.; Jo, J. Generation of skeletal muscle organoids from human pluripotent stem cells to model myogenesis and muscle regeneration. *Int J Mol Sci.* **2022,** *23,* 5108.
58. Shahriyari, M.; Rinn, M.; Hofemeier, A. D.; Babych, A.; Zimmermann, W. H.; Tiburcy, M. Protocol to develop force-generating human skeletal muscle organoids. *STAR Protoc.* **2024,** *5,* 102794.
59. Borok, M. J.; Mademtzoglou, D.; Relaix, F. Bu-M-P-ing iron: how BMP signaling regulates muscle growth and regeneration. *J Dev Biol.* **2020,** *8,* 4.
60. Sartori, R.; Schirwis, E.; Blaauw, B.; Bortolanza, S.; Zhao, J.; Enzo, E.; Stantzou, A.; Mouisel, E.; Toniolo, L.; Ferry, A.; Stricker, S.; Goldberg, A. L.; Dupont, S.; Piccolo, S.; Amthor, H.; Sandri, M. BMP signaling controls muscle mass. *Nat Genet.* **2013,** *45,* 1309-1318.
61. Pawlikowski, B.; Vogler, T. O.; Gadek, K.; Olwin, B. B. Regulation of skeletal muscle stem cells by fibroblast growth factors. *Dev Dyn.* **2017,** *246,* 359-367.
62. Mavrommatis, L.; Jeong, H. W.; Kindler, U.; Gomez-Giro, G.; Kienitz, M. C.; Stehling, M.; Psathaki, O. E.; Zeuschner, D.; Bixel, M. G.; Han, D.; Morosan-Puopolo, G.; Gerovska, D.; Yang, J. H.; Kim, J. B.; Arauzo-Bravo, M. J.; Schwamborn, J. C.; Hahn, S. A.; Adams, R. H.; Schöler, H. R.; Vorgerd, M.; Brand-Saberi, B.; Zaehres, H. Human skeletal muscle organoids model fetal myogenesis and sustain uncommitted PAX7 myogenic progenitors. *Elife.* **2023,** *12,* RP87081.
63. Pinton, L.; Khedr, M.; Lionello, V. M.; Sarcar, S.; Maffioletti, S. M.; Dastidar, S.; Negroni, E.; Choi, S.; Khokhar, N.; Bigot, A.; Counsell, J. R.; Bernardo, A. S.; Zammit, P. S.; Tedesco, F. S. 3D human induced pluripotent stem cell-derived bioengineered skeletal muscles for tissue, disease and therapy modeling. *Nat Protoc.* **2023,** *18,* 1337-1376.
64. Wang, J.; Zhou, C. J.; Khodabukus, A.; Tran, S.; Han, S. O.; Carlson, A. L.; Madden, L.; Kishnani, P. S.; Koeberl, D. D.; Bursac, N. Three-dimensional tissue-engineered human skeletal muscle model of Pompe disease. *Commun Biol.* **2021,** *4,* 524.
65. Gholobova, D.; Gerard, M.; Decroix, L.; Desender, L.; Callewaert, N.; Annaert, P.; Thorrez, L. Human tissue-engineered skeletal muscle: a novel 3D in vitro model for drug disposition and toxicity after intramuscular injection. *Sci Rep.* **2018,** *8,* 12206.
66. Vandenburgh, H.; Shansky, J.; Benesch-Lee, F.; Barbata, V.; Reid, J.; Thorrez, L.; Valentini, R.; Crawford, G. Drug-screening platform based on the contractility of tissue-engineered muscle. *Muscle Nerve.* **2008,** *37,* 438-447.
67. Duan, D.; Goemans, N.; Takeda, S.; Mercuri, E.; Aartsma-Rus, A. Duchenne muscular dystrophy. *Nat Rev Dis Primers.* **2021,** *7,* 13.

68. Kiernan, M. C.; Vucic, S.; Cheah, B. C.; Turner, M. R.; Eisen, A.; Hardiman, O.; Burrell, J. R.; Zoing, M. C. Amyotrophic lateral sclerosis. *Lancet.* **2011,** *377,* 942-955.
69. Bombieri, C.; Corsi, A.; Trabetti, E.; Ruggiero, A.; Marchetto, G.; Vattemi, G.; Valenti, M. T.; Zipeto, D.; Romanelli, M. G. Advanced cellular models for rare disease study: exploring neural, muscle and skeletal organoids. *Int J Mol Sci.* **2024,** *25,* 1014.
70. Osaki, T.; Uzel, S. G. M.; Kamm, R. D. Microphysiological 3D model of amyotrophic lateral sclerosis (ALS) from human iPS-derived muscle cells and optogenetic motor neurons. *Sci Adv.* **2018,** *4,* eaat5847.
71. Chen, Z.; Li, B.; Zhan, R. Z.; Rao, L.; Bursac, N. Exercise mimetics and JAK inhibition attenuate IFN-γ-induced wasting in engineered human skeletal muscle. *Sci Adv.* **2021,** *7,* eabd9502.
72. Makris, E. A.; Gomoll, A. H.; Malizos, K. N.; Hu, J. C.; Athanasiou, K. A. Repair and tissue engineering techniques for articular cartilage. *Nat Rev Rheumatol.* **2015,** *11,* 21-34.
73. Zhu, J.; Lun, W.; Feng, Q.; Cao, X.; Li, Q. Mesenchymal stromal cells modulate YAP by verteporfin to mimic cartilage development and construct cartilage organoids based on decellularized matrix scaffolds. *J Mater Chem B.* **2023,** *11,* 7442-7453.
74. 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.
75. Ralphs, J. R.; Benjamin, M. The joint capsule: structure, composition, ageing and disease. *J Anat.* **1994,** *184 (Pt 3),* 503-509.
76. Sophia Fox, A. J.; Bedi, A.; Rodeo, S. A. The basic science of articular cartilage: structure, composition, and function. *Sports Health.* **2009,** *1,* 461-468.
77. Forrester, D. M. Diagnosis of Bone and Joint Disorders. Donald Resnick, MD and Gen Niwayama, MD. 3 Volumes. Philadelphia, W. B. Saunders, 1981. 3277 pages, 2670 illustrations, $295. *Arthritis Rheum.* **1982,** *25,* 480.
78. 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.
79. 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.
80. Sun, Y.; Wu, Q.; Dai, K.; You, Y.; Jiang, W. Generating 3D-cultured organoids for pre-clinical modeling and treatment of degenerative joint disease. *Signal Transduct Target Ther.* **2021,** *6,* 380.
81. 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.
82. Rothbauer, M.; Byrne, R. A.; Schobesberger, S.; Olmos Calvo, I.; Fischer, A.; Reihs, E. I.; Spitz, S.; Bachmann, B.; Sevelda, F.; Holinka, J.; Holnthoner, W.; Redl, H.; Toegel, S.; Windhager, R.; Kiener, H. P.; Ertl, P. Establishment of a human three-dimensional chip-based chondro-synovial coculture joint model for reciprocal cross talk studies in arthritis research. *Lab Chip.* **2021,** *21,* 4128-4143.
83. 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.
84. 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.
85. Giobbe, G. G.; Crowley, C.; Luni, C.; Campinoti, S.; Khedr, M.; Kretzschmar, K.; De Santis, M. M.; Zambaiti, E.; Michielin, F.; Meran, L.; Hu, Q.; van Son, G.; Urbani, L.; Manfredi, A.; Giomo, M.; Eaton, S.; Cacchiarelli, D.; Li, V. S. W.; Clevers, H.; Bonfanti, P.; Elvassore, N.; De Coppi, P. Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture. *Nat Commun.* **2019,** *10,* 5658.
86. Deng, Y.; Lei, G.; Lin, Z.; Yang, Y.; Lin, H.; Tuan, R. S. Engineering hyaline cartilage from mesenchymal stem cells with low hypertrophy potential via modulation of culture conditions and Wnt/β-catenin pathway. *Biomaterials.* **2019,** *192,* 569-578.
87. Li, Z. A.; Sant, S.; Cho, S. K.; Goodman, S. B.; Bunnell, B. A.; Tuan, R. S.; Gold, M. S.; Lin, H. Synovial joint-on-a-chip for modeling arthritis: progress, pitfalls, and potential. *Trends Biotechnol.* **2023,** *41,* 511-527.
88. Wieland, H. A.; Michaelis, M.; Kirschbaum, B. J.; Rudolphi, K. A. Osteoarthritis - an untreatable disease? *Nat Rev Drug Discov.* **2005,** *4,* 331-344.
89. Richard, M. J.; Driban, J. B.; McAlindon, T. E. Pharmaceutical treatment of osteoarthritis. *Osteoarthritis Cartilage.* **2023,** *31,* 458-466.
90. 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.
91. Lim, W. L.; Liau, L. L.; Ng, M. H.; Chowdhury, S. R.; Law, J. X. Current progress in tendon and ligament tissue engineering. *Tissue Eng Regen Med.* **2019,** *16,* 549-571.
92. Kannus, P. Structure of the tendon connective tissue. *Scand J Med Sci Sports.* **2000,** *10,* 312-320.
93. Amiel, D.; Frank, C.; Harwood, F.; Fronek, J.; Akeson, W. Tendons and ligaments: a morphological and biochemical comparison. *J Orthop Res.* **1984,** *1,* 257-265.
94. Koski, J. A.; Ibarra, C.; Rodeo, S. A. Tissue-engineered ligament: cells, matrix, and growth factors. *Orthop Clin North Am.* **2000,** *31,* 437-452.
95. Xu, B.; Chow, M. J.; Zhang, Y. Experimental and modeling study of collagen scaffolds with the effects of crosslinking and fiber alignment. *Int J Biomater.* **2011,** *2011,* 172389.
96. Randelli, P.; Spennacchio, P.; Ragone, V.; Arrigoni, P.; Casella, A.; Cabitza, P. Complications associated with arthroscopic rotator cuff repair: a literature review. *Musculoskelet Surg.* **2012,** *96,* 9-16.
97. Laternser, S.; Keller, H.; Leupin, O.; Rausch, M.; Graf-Hausner, U.; Rimann, M. A novel microplate 3D bioprinting platform for the engineering of muscle and tendon tissues. *SLAS Technol.* **2018,** *23,* 599-613.
98. Hsieh, M. S.; Chen, M. Y.; Chang, Y. S.; Huang, C. S.; Hsu, T. N.; Huang, M. S.; Yeh, C. T.; Tzeng, Y. M. Targeting the Neuropilin-1 receptor with Ovatodiolide and progress in using periodontal ligament organoids for COVID-19 research and therapy. *Life Sci.* **2024,** *351,* 122764.
99. Graça, A. L.; Kroner-Weigl, N.; Reyes Alcaraz, V.; Müller-Deubert, S.; Rudert, M.; Docheva, D. Demonstration of self-assembled cell sheet culture and manual generation of a 3d tendon/ligament-like organoid by using human dermal fibroblasts. *J Vis Exp.* **2024**, e66047.
100. Donderwinkel, I.; Tuan, R. S.; Cameron, N. R.; Frith, J. E. Tendon tissue engineering: Current progress towards an optimized tenogenic differentiation protocol for human stem cells. *Acta Biomater.* **2022,** *145,* 25-42.

101. Kirkendall, D. T.; Garrett, W. E. Function and biomechanics of tendons. *Scand J Med Sci Sports.* **1997,** *7*, 62-66.
102. Petrigliano, F. A.; Arom, G. A.; Nazemi, A. N.; Yeranosian, M. G.; Wu, B. M.; McAllister, D. R. In vivo evaluation of electrospun polycaprolactone graft for anterior cruciate ligament engineering. *Tissue Eng Part A.* **2015,** *21*, 1228-1236.
103. Chainani, A.; Hippensteel, K. J.; Kishan, A.; Garrigues, N. W.; Ruch, D. S.; Guilak, F.; Little, D. Multilayered electrospun scaffolds for tendon tissue engineering. *Tissue Eng Part A.* **2013,** *19*, 2594-2604.
104. Yang, G.; Rothrauff, B. B.; Lin, H.; Yu, S.; Tuan, R. S. Tendon-derived extracellular matrix enhances transforming growth factor-β3-induced tenogenic differentiation of human adipose-derived stem cells. *Tissue Eng Part A.* **2017,** *23*, 166-176.
105. Ker, D. F. E.; Tan, C.; Cartmell, S. Editorial: Tendons and ligaments: development, pathogenesis, tissue engineering, and regenerative medicine. *Front Bioeng Biotechnol.* **2024,** *12*, 1489256.
106. Lejard, V.; Blais, F.; Guerquin, M. J.; Bonnet, A.; Bonnin, M. A.; Havis, E.; Malbouyres, M.; Bidaud, C. B.; Maro, G.; Gilardi-Hebenstreit, P.; Rossert, J.; Ruggiero, F.; Duprez, D. EGR1 and EGR2 involvement in vertebrate tendon differentiation. *J Biol Chem.* **2011,** *286*, 5855-5867.
107. Bourdón-Santoyo, M.; Quiñones-Uriostegui, I.; Martínez-López, V.; Sánchez-Arévalo, F.; Alessi-Montero, A.; Velasquillo, C.; Ibarra-Ponce de León, C. Preliminary study of an in vitro development of new tissue applying mechanical stimulation with a bioreactor as an alternative for ligament reconstruction. *Rev Invest Clin.* **2014,** *66 Suppl 1*, S100-110.
108. Zhang, Y.; Lei, T.; Tang, C.; Chen, Y.; Liao, Y.; Ju, W.; Zhang, H.; Zhou, B.; Liang, R.; Zhang, T.; Fan, C.; Chen, X.; Zhao, Y.; Xie, Y.; Ye, J.; Heng, B. C.; Chen, X.; Hong, Y.; Shen, W.; Yin, Z. 3D printing of chemical-empowered tendon stem/progenitor cells for functional tissue repair. *Biomaterials.* **2021,** *271*, 120722.
109. Qiu, Y.; Lei, J.; Koob, T. J.; Temenoff, J. S. Cyclic tension promotes fibroblastic differentiation of human MSCs cultured on collagen-fibre scaffolds. *J Tissue Eng Regen Med.* **2016,** *10*, 989-999.
110. Yan, Z.; Yin, H.; Brochhausen, C.; Pfeifer, C. G.; Alt, V.; Docheva, D. Aged tendon stem/progenitor cells are less competent to form 3D tendon organoids due to cell autonomous and matrix production deficits. *Front Bioeng Biotechnol.* **2020,** *8*, 406.
111. Fang, G.; Chen, Y. C.; Lu, H.; Jin, D. Advances in spheroids and organoids on a chip. *Adv Funct Mater.* **2023,** *33*, 2215043.
112. Mansoorifar, A.; Gordon, R.; Bergan, R.; Bertassoni, L. E. Bone-on-a-chip: microfluidic technologies and microphysiologic models of bone tissue. *Adv Funct Mater.* **2021,** *31*, 2006796.
113. Perkhofer, L.; Frappart, P. O.; Müller, M.; Kleger, A. Importance of organoids for personalized medicine. *Per Med.* **2018,** *15*, 461-465.
114. Driehuis, E.; van Hoeck, A.; Moore, K.; Kolders, S.; Francies, H. E.; Gulersonmez, M. C.; Stigter, E. C. A.; Burgering, B.; Geurts, V.; Gracanin, A.; Bounova, G.; Morsink, F. H.; Vries, R.; Boj, S.; van Es, J.; Offerhaus, G. J. A.; Kranenburg, O.; Garnett, M. J.; Wessels, L.; Cuppen, E.; Brosens, L. A. A.; Clevers, H. Pancreatic cancer organoids recapitulate disease and allow personalized drug screening. *Proc Natl Acad Sci U S A.* **2019,** *116*, 26580-26590.
115. Zhu, Y.; Tang, S.; Yuan, Q.; Fu, J.; He, J.; Liu, Z.; Zhao, X.; Li, Y.; Zhao, Y.; Zhang, Y.; Zhang, X.; Zhang, Y.; Zhu, Y.; Wang, W.; Zheng, B.; Wu, R.; Wu, T.; Yang, S.; Qiu, X.; Shen, S.; Hu, J.; Chen, L.; Wang, Y.; Wang, H.; Gao, D.; Chen, L. Integrated characterization of hepatobiliary tumor organoids provides a potential landscape of pharmacogenomic interactions. *Cell Rep Med.* **2024,** *5*, 101375.
116. Wang, Y.; You, Y.; Chen, H.; Liu, J.; Wu, Q.; Dai, K.; Sun, Y. 3D-bioprinted anti-senescence organoid scaffolds repair cartilage defect and prevent joint degeneration via miR-23b/ELOVL5-mediated metabolic rewiring. *Chem Eng J.* **2024,** *491*, 152049.
117. 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.

Received: October 7, 2024
Revised: November 1, 2024
Accepted: November 3, 2024
Available online: November 15, 2024