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Strategies for constructing rotator cuff organoids: A review

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Abstract

Rotator cuff injury is a common disease of the locomotor system, causing a serious burden on the individual patient as well as society. The current treatment primarily involves surgical intervention, but it cannot completely restore the physiological integrity of the rotator cuff and carries a significant risk of postoperative re-tear. To improve the repair of rotator cuff injuries, regenerative medicine strategies have been widely explored. Organoids refer to three-dimensional (3D) tissue structures derived from stem/progenitor cells *in vitro*, which recapitulate native organ structure and function, providing an emerging platform for disease modeling, drug screening, and regenerative medicine. In this paper, we first outline the disease background of rotator cuff anatomy and current clinical treatments and subsequently summarize fabrication strategies for the rotator cuff-relevant organoids, focusing on skeletal muscle, tendon, bone, cartilage, and especially regenerative medicine approaches for the tendon-bone interface. Building upon this foundation, we describe in detail the integrative strategies for rotator cuff organoid biofabrication, encompassing cell sources, matrix materials, construction techniques, and strategies. Finally, this work also addresses the challenges in rotator cuff organoid construction and outlines possible solutions, while re-emphasizing the transformative potential of rotator cuff organoids for promoting fundamental research, accelerating drug screening, and enabling functional repair of rotator cuff diseases.

Keywords: Rotator cuff; Organoid; Regenerative medicine

1. Introduction

Rotator cuff injury (RCI) is one of the most common musculoskeletal disorders, and the main symptoms include shoulder pain, restricted mobility, and dysfunction,[1] which can cause pain and dyskinesia, thus diminishing the quality of life of the patients and placing a substantial burden on the healthcare system.[2] Based on a study conducted in Japan, the prevalence of RCI was estimated at approximately 20.7%.[3] In the United States, medical care expenditures for shoulder disorders had reached approximately US$7 billion in 2000,[4] with 65–70% of these patients experiencing rotator cuff disorders.[5] In addition, the prevalence of massive rotator cuff tears has been reported to be 10–40%, while the rate of rotator cuff re-tears is 11–68%.[6] Consequently, rotator cuff injuries represent a highly prevalent condition with profound impacts on both individual well-being and healthcare systems.

The rotator cuff consists of the supraspinatus, infraspinatus, teres minor, and subscapularis muscles. Their tendons converge to attach to the humeral head through a specialized transitional architecture, which consists of four histologically distinct zones: (1) the tendinous proper, (2) uncalcified fibrocartilage, (3) calcified fibrocartilage, and (4) mineralized bone.[7] The mechanism of RCI is complex

and is mainly thought to be caused by both injury and degeneration.[8,9] For the treatment of rotator cuff injuries, there are mainly surgical and non-surgical methods.[1,8] Non-surgical treatments—including pharmacologic interventions such as non-steroidal anti-inflammatory drugs—can alleviate the symptoms but fail to stop the progression of the disease,[9] whereas surgical treatments such as direct suture have been widely used for rotator cuff tears.[10] Liu *et al.*[11] also developed the parachute suture technique for massive rotator cuff tears and to reduce postoperative re-tear rates. However, these methods fail to alter the biological progression of the tendon. Moreover, some patients present with postoperative healing failures and the potential for re-tears.[9] Therefore, conventional therapeutic approaches for rotator cuff injuries demonstrate suboptimal clinical outcomes. In recent years, rotator cuff tissue engineering strategies have become an important direction in the treatment of rotator cuff injuries, including platelet-rich plasma (PRP),[12] growth factors,[2] stem cells,[1,13,14] and exosomes.[2] Despite the advances achieved, restoration of the injured rotator cuff remains a considerable clinical challenge.[15] As an emerging technology, organoids have significant potential to be used as a platform for studying the mechanisms of RCI and exploring novel effective therapeutic measures.

Organoids are *in vitro* cultured 3D tissue structures originating from stem or progenitor cells that can mimic the cellular composition, spatial structure, and even physiological functions of natural organs[16] (Figure 1). Organoid models are regarded as a novel and high-performing strategy, being applied to disease modeling, drug discovery, regenerative repair, and beyond. In contrast to engineered tissues, organoids are self-assembled models that utilize cells' self-organization potential to form aggregates and tissue-like arrangements, representing a bottom-up approach. Conversely, engineered tissues involve the construction of structures with relevant cell types and scaffolds, following a top-down pathway.[17,18] Since its inception, organoid technology has greatly influenced the drug screening process and the study of pathological mechanisms.[19] Following the pioneering generation of intestinal organoids by Sato *et al.* in 2009, this technology has been widely used in virtually all major tissues. Organoid protocols such as thalamus,[20] liver,[21] heart,[22] kidney,[23] and intestines[24] have been established, which have been applied in disease modeling, drug screening, regenerative medicine, and other fields. Despite their relatively recent advent, musculoskeletal system organoids have undergone rapid evolution, marked by the continuous emergence of organoids modeling bone, muscle, joint, and other tissues, which demonstrate immense potential in research and treatment of musculoskeletal system diseases.[25] Rotator cuff organoids are miniature functional models generated *in vitro* from stem cells through three-dimensional (3D) culture, induced differentiation, and self-organization to simulate key rotator cuff structures, specifically the tendon-bone interface. The application of organoid technology to RCI research can provide a robust platform that can recapitulate the disease-specific microenvironments *in vitro*, accelerate progress of RCI research, and offer hope for solving the difficulties and bottlenecks faced in the current therapeutic paradigms.

In this paper, we systematically outline the construction strategies of skeletal muscle organoids, tendon organoids, bone organoids, and cartilage organoids and discuss the application pathways of these organoids (Table 1). On this basis, we further propose construction strategies for rotator cuff organoids, analyze their applications in disease pathogenesis research and therapy development, and address prevailing technical and translational challenges.

2. Musculoskeletal system organoids

As 3D cell clusters cultured *in vitro* that can mimic the structure and function of natural tissues, organoid

	Organoids	2D cell cultures	Animal models
Biological complexity	Medium-high	Low	High
Human relevance	High	Medium	Low
Flux	Medium-high	High	Low
Disease modeling capability	High	Low	Medium
Genome editing	Y	Yes	Yes
Value of drug development	High	Medium	Medium
Ethical controversy	Low	No	High
Operating difficulty	Medium	Low	High

Figure 1. Comparison of key characteristics between organoids, 2D cell cultures, and animal models.[25-27] Created in BioRender. Shi, Q. (2025) https://BioRender.com/7k2i76x.

Table 1. Comparison of construction strategies and applications across multiple musculoskeletal system organoids

Construction and applications	Types of musculoskeletal system organoids				
	Muscle organoid	**Bone organoid**	**Tendon organoid**	**Cartilage organoid**	**Rotator cuff organoid**
Cell sources	iPSCs; hPSCs; human muscle-derived fibroblasts;	MSCs; iPSCs; ESCs; PDCs	TDSCs; BMSCs; human dermal fibroblasts	BMSCs; ADSCs; iPSCs; PDCs	BMSCs; TDSCs; ADSCs; hAMSCs
Matrix materials	Matrigel; hydrogel; Matrigel with fibrin	Matrigel; natural hydrogel; synthetic hydrogel; collagen	Matrigel; dECM; GelMA	Matrigel; hydrogel; decellularized cartilage	Matrigel; hydrogel; dECM; nanofiber
Construction techniques	CTM; 3D bioprinting	Physical stimulus; microfluidics; 3D bioprinting	3D bioprinting; microfluidics	Microfluidics; 3D bioprinting	Microfluidics; 3D bioprinting; bioreactor
Applications	Disease modeling; drug screening; therapy test	Bone modeling; regeneration simulation; repair acceleration	Drug development; studies of tenogenesis	Cartilage repair; research on pathological mechanism	Rotator cuff simulation and repair

Abbreviations: ADSCs: Adipose-derived stem cells; BMSCs: Bone marrow mesenchymal stem cells; CTM: Cascade tube microfluidics; dECM: Decellularized extracellular matrix; ESCs: Embryonic stem cells; GelMA: Gelatin methacrylate; hAMSCs: Human amniotic membrane mesenchymal stem cells; hPSC: Human pluripotent stem cells; iPSCs: Induced pluripotent stem cells; MSCs: Mesenchymal stem cells; PDCs: Periosteum-derived cells; TDSCs: Tendon-derived stem cells; 3D: Three-dimensional.

cultivation methodologies share fundamental principles with traditional 2D cell culture yet exhibit critical distinctions in their capacity to recapitulate tissue-level functional complexity. The fabrication of organoids relies on suitable cell types and sources, matrix materials, biotechnology, and organoid construction strategies.[16,28] Since the rotator cuff is a complex structure composed of multiple tissues with structural and functional complexity, rotator cuff organoid cultivation requires the integration of multiple organoids, including skeletal muscle organoids, tendon organoids, bone organoids, and cartilage organoids.[2] In addition, a rigorous culture protocol is needed to ensure the successful construction of the organoids, so that the organoids can faithfully recapitulate the natural biological characteristics of rotator cuff tissues.

2.1. Skeletal muscle organoids

2.1.1. Physiological structure of skeletal muscle

Skeletal muscle accounts for approximately 40% of the total body weight and is composed mainly of water, proteins, and other substances.[29] Skeletal muscle is composed of myofibers; each myofiber is externally wrapped with an endomysium. Many myofibers are wrapped by the perimysium to form muscle bundles, and multiple muscle bundles are further encapsulated by the epimysium to form muscles, which ultimately connect to the skeleton through tendons.[30] Each myofiber consists of thousands of myofibrils, which in turn are composed of serially repeating sarcomeres-the fundamental contractile units formed by myofilaments. The sarcomere is primarily composed of thick myofilaments (myosin) and thin myofilaments (actin). The actin is regulated by tropomyosin and troponin complexes that sterically block myosin cross-bridge binding sites. When electrical excitation is transmitted to the myocyte through the transverse tubule system, the muscle completes its contractile movement through the excitation-contraction coupling.[31] As an important component of the locomotor system, skeletal muscle—along with bone, connective tissue, and nerves—executes both fine motor tasks and large movements such as grasping and walking, thus permitting the organism to accomplish daily life activities. In addition, skeletal muscle converts chemical energy into mechanical energy through contraction and at the same time, releases heat, acting as a thermoregulator. Moreover, skeletal muscle modulates the systemic glucose homeostasis through the synthesis and breakdown of glycogen.[25]

2.1.2. Construction of skeletal muscle organoids

Common cell sources for skeletal muscle organoids include induced pluripotent stem cells (iPSCs),[32] human pluripotent stem cells (hPSCs),[33] and human muscle-derived fibroblasts,[34] with hPSCs being predominantly employed in current protocols. These cells can give rise to cell lines such as myogenic progenitor cells and satellite cells through predefined cultivation strategies, which ultimately coalesce into skeletal muscle organoids. In addition to the cell sources, optimized bioengineering methodologies are equally crucial for the quality of skeletal muscle organoids. In order to construct more responsive organoids, researchers have explored the hanging drop method, dynamic cell culture in spinner flasks, and culture in low attachment wells, rotating-wall vessels, and other techniques in the construction of skeletal organoids.[35] Shin *et al.*[33] implemented a stepwise, pre-patterned protocol to

induce hPSCs into myogenic progenitor cells and myoblasts, enabling the biofabrication of human skeletal muscle organoids (hSkMOs), and observed sustainable satellite cells that can be activated for repairing damaged muscle tissue during the culture process. Starting from human hiPSC, Grass *et al.*[36] generated neuromesodermal progenitors (NMPs) through WNT and FGF signaling activation, and subsequently induced differentiation of a portion of NMPs that retain mesodermal identity to skeletal myocytes, enabling the construction of neuromuscular organoids. Bioengineering techniques have been used in organoid construction to increase the efficiency of construction and to improve the homogeneity of the organoids, thereby increasing their potential for application. For example, Li *et al.*[37] developed functional mouse skeletal muscle droplet-engineered organoids from mouse gastrocnemius muscle tissue using cascade tube microfluidics (CTM). Constructed within a shorter duration, the organoids exhibited enhanced maturation and functionality, as well as potential for scalable and reproducible production. Such an organoid production technique has high feasibility and substantial potential for both fundamental research and therapeutic applications. In addition, recapitulation of actual physiological condition, mimicry of higher-order architectural and functional complexity, and construction of multi-tissue composite organoids are being explored. Yin *et al.*[38] established neuromusculoskeletal organoids from hPSCs through a co-culture strategy (static-to-spinning culture strategy), which realized the coordinated development of three distinct tissue domains within a single organoid, demonstrating the strong self-organization ability of 3D cell culture systems *in vitro* (Table 2).

2.1.3. Application of skeletal muscle organoid

Traditional models adopted in disease research are primarily 2D cell cultures and animal models, but 2D cultures are difficult to replicate cell growth in a 3D environment *in vivo*, and animal models exhibit inherent limitations and inaccuracy in disease mechanism research and therapy development due to the interspecies divergence, while organoids can provide an ideal platform that more closely resembles the physiological environment. Shahriyari *et al.*[39] generated functional human skeletal muscle organoids (SMOs) and engineered skeletal muscle from hPSCs. Utilizing patient-derived iPSCs with a deletion of exons 48–50, they constructed an engineered skeletal muscle model with features of Duchenne muscular dystrophy (DMD), and used this model to demonstrate the therapeutic efficacy of CRISPR/Cas9 technology in DMD. Gao *et al.*[32] used iPSCs from C9orf72-ALS patients to generate neuromuscular organoids which showed ALS-associated lesion features. On this basis, they performed drug testing and demonstrated the efficacy of GSK2606414 in improving skeletal muscle contraction and decreasing the accumulation of poly (glycine-proline) dipeptide repeat protein. Organoids can also be used to study the effects of various possible pathogenic factors. For example, Jiang *et al.*[40] established hSkMOs derived from hPSC that maintained important skeletal muscle features and then exposed them to a 2 Gy dose of radiation and observed defects in organoid amplification, differentiation, and repair. In addition to this, muscle organoids can also be used for drug screening. Svobodova *et al.*[41] used hiPSC to establish a disease model of DMD *in vitro*, providing a high-throughput platform for drug screening. Furthermore, the construction of patient-specific organoids can also facilitate the development of targeted therapies (Figure 2).

2.2. Tendon organoids

2.2.1. Physiological structure of tendon

Tendon is mainly a collagen fiber bundle structure connecting muscle and bone, which is mainly composed of type I collagen, and similar to the way skeletal muscle is organized, collagen molecules form procollagen molecules, five procollagen molecules group together to form microfibrils, which subsequently aggregate into fibrils, and fibrils are combined into collagen fibers to ultimately give

Table 2. Construction of skeletal muscle organoids

Cell source	Inducing factor	Matrix material	References
iPSC	bFGF, CHIR99021, Y27632, HGF/IGF	Geltrex	32
hPSC	HGF, IGF1, FGF2	Growth factor-reduced Matrigel	33
Immortalized myoblasts	Human recombinant insulin	Hydrogel	34
hiPSC	FGF2, CHIR99021, GDF11	Matrigel	36
Primary skeletal muscle cells	IGF1	Matrigel	37
hPSC	HGF, FGF2	Collagen/Matrigel hydrogel	39
hPSC	FGF2	N/A	38
hiPSC	IGF, FGF2, HGF, CHIR99021	Matrigel	40

Abbreviations: bFGF: Basic fibroblast growth factor; FGF: Fibroblast growth factor; GDF11: Growth differentiation factor 11; HGF: Hepatocyte growth factor; hiPSC: Human-induced pluripotent stem cells; hPSC: Human pluripotent stem cells; IGF: Insulin-like growth factor; iPSC: Induced pluripotent stem cell.

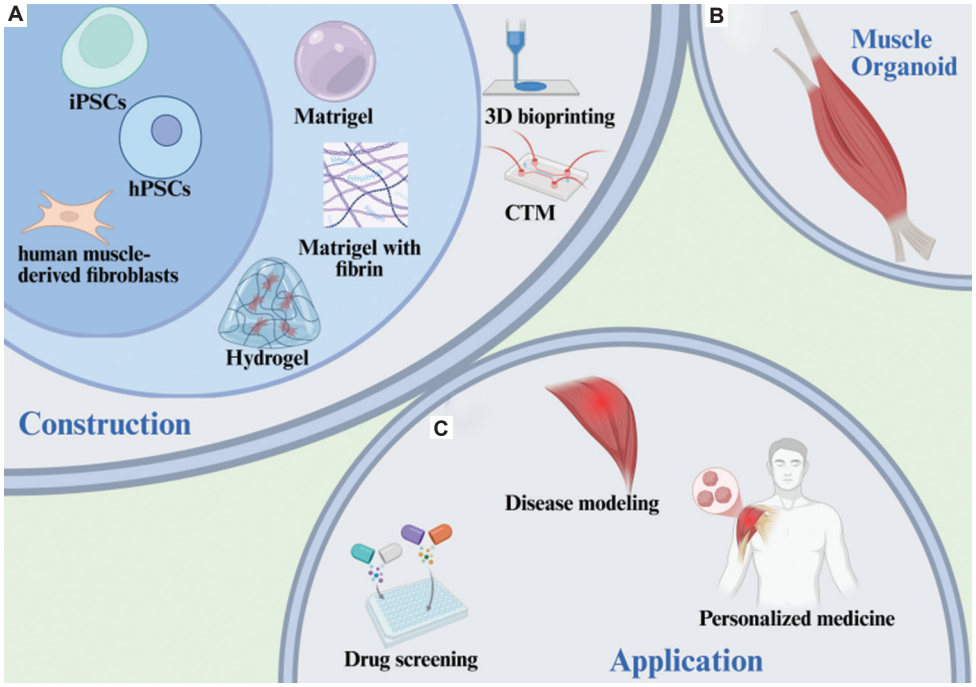

Figure 2. Construction and application of skeletal muscle organoids. (A) The construction of muscle organoids; (B) muscle organoid; (C) the applications of muscle organoids. Created in BioRender. Shi, Q. (2025) https://BioRender.com/82y9pxc.
Abbreviations: CTM: Cascade tube microfluidics; hPSC: Human pluripotent stem cells; iPSC: Induced pluripotent stem cells.

rise to the structure of tendon.[42,43] Tendons contain a small amount of cells, mainly tendon cells, which are responsible for synthesizing collagen, as well as some cell types such as endothelial cells, pericytes, and immune cells, which are distributed in different regions and have unique functions.[44] Some studies have shown that stem or progenitor cells are present in tendons and can be isolated.[45] The tendon can be divided into two parts: the midsubstance, which is mainly dense fibrous connective tissue formed by type I collagen fibrils aligned parallel to the long axis of the tendon, and the tendon-bone interface, which can be subdivided into four sequential transition areas: tendon, fibrocartilage, calcified fibrocartilage, and mineralized bone.[46] The primary function of tendons is to transmit the tension generated by muscle contraction, thereby assisting in the completion of motion.[47] Furthermore, tendons possess proprioceptive function, allowing them to play a crucial role in the adjustment of movements.[43]

2.2.2. Construction of tendon organoids

Tendon organoids are important components of rotator cuff organoids, and their cell sources include human dermal fibroblasts,[48] tendon-derived stem cells (TDSCs),[49] and bone marrow mesenchymal stem cells (BMSCs),[50] which can generate tendon organoids in suitable matrix materials that meet the needs for research and therapeutic purposes through a pre-designed cultivation strategy. Graça *et al.*[51] established 3D rod-like organoids of tendon using commercially available dermal fibroblasts through a three-step method (2D expansion, 2D stimulation, 3D maturation), and these organoids possess an extracellular matrix (ECM) and microenvironment similar to that of the native tendon structure and are suitable for tendon-related research. Kroner-Weigl *et al.*[48] similarly adopted the three-step protocol, i.e., expansion, stimulation, and maturation, to construct tendon organoid models using purchased human dermal fibroblasts as the source cells, which were used to study the effect of dexamethasone on tendon differentiation of human dermal fibroblasts. In addition, the construction of tendon organoids is not yet mature, and the optimization studies of the construction protocol are underway. For example, Yan *et al.*[49] constructed *in vitro* tendon organoids using young tendon stem cells and senescent tendon stem cells, respectively, as cell sources, which resulted in a higher failure rate, low cell density, and disorganized matrix in the model constructed from senescent stem cells. Zhao *et al.*[52] evaluated the suitability of porcine tendon-derived decellularized ECM (dECM) with different digestibility for 3D bioprinting and demonstrated that the "high-viscosity slurry" state was more suitable and enhanced targeted differentiation of stem cells, suggesting its potential as a promising biomaterial for organoid construction (Table 3).

2.2.3. Application of tendon organoids

Tendon damage is a high-impact injury with inherently limited regenerative capacity, and traditional repair modalities such as surgical treatment are difficult to fully

restore the structure and function of the tendon and are associated with many complications. Thus, organoids exhibiting a high degree of similarity emerge as potential transplantation therapies. Qiu *et al.*[53] cultured mesenchymal stem cells (MSCs) on collagen fiber scaffolds under cyclic tensile stimulation, directing them to undergo fibroblast differentiation, suggesting their significant potential for application as regenerative grafts for tendon repair. Similar to skeletal muscle organoids, another pivotal application of tendon organoids lies in their utility as a platform for drug screening and toxicity assessment. Kroner-Weigl *et al.*[48] constructed tendon organoids for testing the effect of glucocorticoids on tendon cell differentiation to validate their role in inducing tendon differentiation, although the results did not show obvious advantages. Furthermore, tendon organoids constructed from patient-derived stem cells can enable personalized therapeutic strategies, assist in exploring clinical intervention and treatment methods in a targeted manner, and promote the realization of optimal repair outcomes (Figure 3).

Table 3. Construction of tendon organoids

Cell source	Inducing factor	Matrix material	References
Human dermal fibroblasts	TGF-β3	N/A	48
TSPCs	TGF-β3	N/A	49
Human dermal fibroblasts	TGF-β3	N/A	51
MSCs	N/A	NDGA-crosslinked collagen fiber scaffolds	53

Abbreviations: MSC: Mesenchymal stem cell; NDGA: Nordihydroguaiaretic acid; TGF-β3: Transforming growth factor beta 3; TSPC: Tendon stem/progenitor cells.

2.3. Bone organoids

2.3.1. Physiological structure of bone

Bone is a mineralized connective tissue that provides structural support and protection to vital organs while participating in the movement of the body.[54] Bone serves as a reservoir of calcium and phosphorus, which regulates calcium and phosphorus homeostasis in body fluids. In addition, the bone marrow contained in the bone plays a hematopoietic role. The bone matrix is the basic non-cellular structure that constitutes bone tissue, and is composed of organic (about 35%) and inorganic (about 65%) components that provide mechanical strength and metabolic regulatory functions to bone. The cellular components of bone primarily comprise osteoblasts, osteoclasts, and osteocytes. Among them, osteocytes are the fully matured form of osteoblasts, accounting for about 90–95% of skeletal cells,[55] while osteoblasts and osteoclasts are responsible for bone formation and resorption, respectively, through tightly coupled cellular activities that drive the skeletal remodeling process.[54] Various skeletal stem/progenitor cells have also been identified, which are present in specialized compartments including periosteum and bone marrow cavity, playing a critical role in bone development.[56] Macroscopically, bones can be categorized

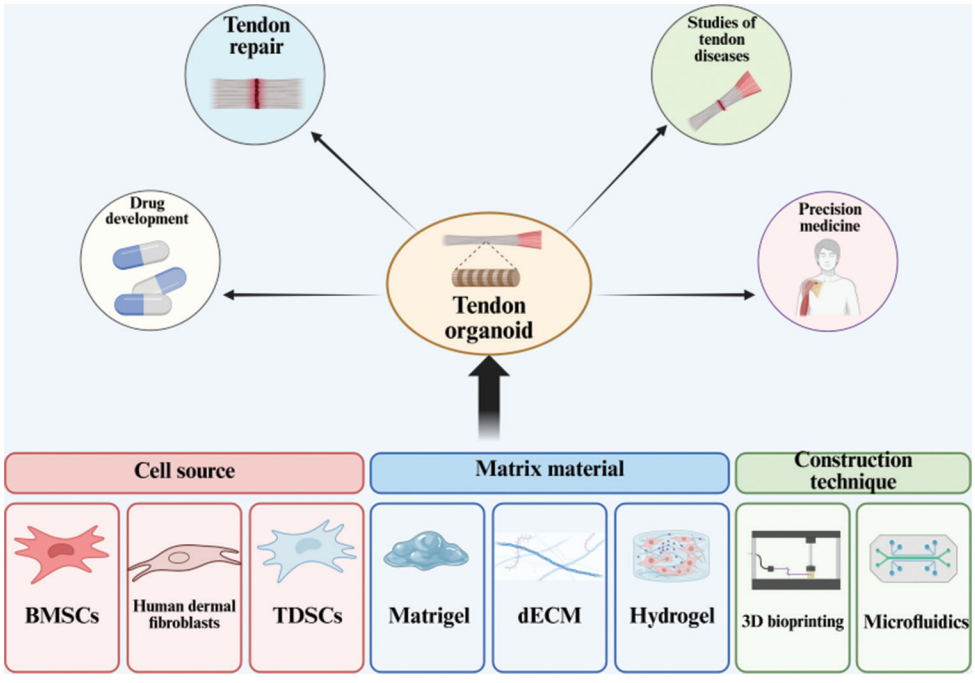

Figure 3. Construction and application of tendon organoids. Created in BioRender. Shi, Q. (2025) https://BioRender.com/6id61yz.
Abbreviations: BMSCs: Bone marrow mesenchymal stem cells; dECM: Decellularized extracellular matrix; TDSCs: Tendon-derived stem cells.

into long bones, short bones, flat bones, and irregular bones, which are distributed in different anatomical regions of the body and fulfill distinct functions. For example, the short bones distributed in the hands and feet have an irreplaceable role in the body's ability to perform complex and fine movements.[57]

2.3.2. Construction of bone organoids

Compared with other organoids of the musculoskeletal system, bone organoid fabrication has been well investigated, and the construction strategies are relatively mature. The most common cell sources for bone organoid construction are MSCs,[58] iPSCs,[59] as well as human periosteum-derived cells and embryonic stem cells. iPSCs, which are somatic cells genetically reprogrammed to have the characteristics of embryonic stem cells,[55] have been extensively applied in organoid construction. iPSCs, used as the source for mouse organoids by O'Connor *et al.*,[60] through the time-dependent sequential exposure of growth factors, successfully realized the construction of osteochondral organoid. Cardier *et al.*[61] Successfully generate osteogenic organoids using allogeneic MSCs with collagen microbeads and PRP clots as ECM and scaffolds. These organoids were subsequently used for the treatment of congenital pseudoarthrosis of the tibia. Beyond that, Fuller *et al.*[62] established cost-effective bone organoid models by inoculating the mouse preosteoblast cell lines into hydrogel extracellular matrices, which can reduce the use of animal models. Matrix materials, as the integral components of bone organoid construction, have also undergone significant advancements. Matrigels, natural biochemical hydrogels, and synthetic biochemical hydrogels have been increasingly employed and refined for the fabrication of bone organoids to increase the construction efficiency and to meet specific purposes (Table 4).[63]

2.3.3. Applications of bone organoids

Bone disease is a relatively common non-fatal disease, including fractures, osteoarthritis, osteoporosis, and bone tumors, which can impair the motor function of patients, cause economic burden, and seriously affect their quality of life. As an *in vitro* cell culture model that is highly similar to the body's bone tissue, bone organoids hold a promising application prospect in the research of skeletal diseases. Xie *et al.*[58] constructed engineered bone healing tissue organoids using BMSCs and hydrogel microspheres and used them to repair bone defects in rabbits, enabling rapid bone regeneration within 4 weeks. Park *et al.*[64] established trabecular bone organoids by co-culturing bone lining cells and bone marrow mononuclear cells on demineralized bone matrix scaffolds, thus enabling investigation of the process of local bone remodeling. Extending their utility beyond restorative therapies, bone organoids serve as versatile platforms for constructing pathomimetic disease models. Frenz-Wiessner *et al.*[65] generated human bone marrow organoids using commercially available iPSCs and leveraged them for genetic disease modeling. Iordachescu *et al.*[66] engineered trabecular bone organoids by co-culturing human osteoblasts and osteoclast precursors as well as bovine femoral trabecular fragments derived from New Zealand cattle, and then, the organoids were subsequently exposed to microgravity to simulate the process of bone loss, providing a reliable model for osteoporosis research. Furthermore, bone organoids have been utilized to model pathological conditions of diseases including bone tumors, osteomyelitis, and bone deformities (Figure 4).[67]

2.4. Cartilage organoids

2.4.1. Physiological structure of cartilage

Cartilage is a highly specialized, avascular, and aneural connective tissue that provides support, protection, shock absorption, and friction reduction in the human body. It is primarily located in synovial joints, the spine, ribs, trachea, and other regions.[68] Cartilage consists mainly of chondrocytes and ECM, with the latter determining the distinct properties of different cartilage types. Chondrocytes are responsible for synthesizing and secreting ECM components, including collagen and proteoglycans, and also secrete cytokines to regulate tissue repair and the cartilage microenvironment.[69] Within the ECM components, collagen fibrils primarily provide tensile strength and structural stability, whereas proteoglycans confer compressive resistance.[68] Based on compositional variations in the ECM, cartilage is classified into three

Table 4. Construction of bone organoids

Cell source	Inducing factor	Matrix material	References
hBMSC/rBMSC	TGF-β3, ascorbic acid, dexamethasone	GelMA	58
BMSC	N/A	CM, PRP	61
MC3T3-E1 and RAW 264.7 cell lines	BMP, β-glycerol phosphate, RANK-L, M-CSF	Matrigel	62
Bone marrow mononuclear cells	VD3, PGE2	DBP	63
Osteoblasts and osteoclasts	β-glycerol phosphate, ascorbic acid, RANK-L, M-CSF	Human blood clot-like fibrin domes	66

Abbreviations: BMP: Bone morphogenetic protein; CM: Collagen microbeads; DBP: Demineralized bone paper; GelMA: Gelatin methacrylate; hBMSC: Human bone marrow mesenchymal stem cells; M-CSF: Macrophage colony-stimulating factor; PGE2: Prostaglandin E2; PRP: Platelet-rich plasma; RANK-L: Receptor activator of nuclear factor-kappa B ligand; rBMSC: Rabbit bone marrow mesenchymal stem cells; VD3: Vitamin D3.

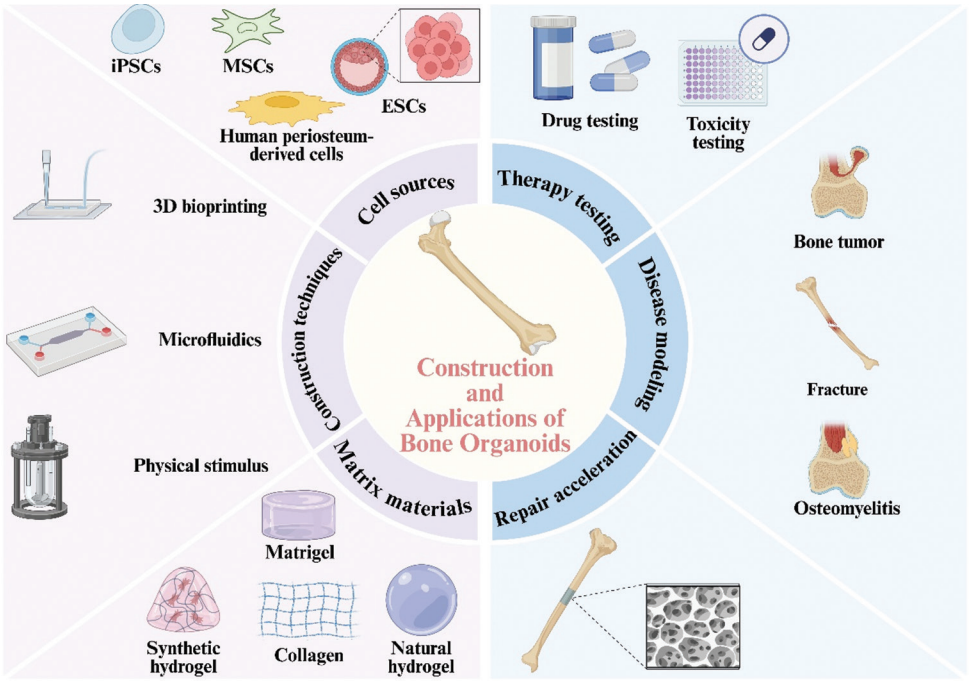

Figure 4. Construction and applications of bone organoids. Created in BioRender. Shi, Q. (2025) https://BioRender.com/bt6t6ga.
Abbreviations: ESCs: Embryonic stem cells; iPSCs: Induced pluripotent stem cells; MSCs: Mesenchymal stem cells.

types: hyaline cartilage, fibrocartilage, and elastic cartilage. Hyaline cartilage, the most prevalent type in humans, is found on articular surfaces of synovial joints, the nose, trachea, and other areas. It is composed mainly of type II collagen, proteoglycans, water, and a sparse population of chondrocytes.[70] Fibrocartilage, present in the intervertebral discs, menisci, and tendon-bone interfaces, consists largely of type I collagen and exhibits high tensile strength. The primary cartilage type at the rotator cuff tendon-bone interface is fibrocartilage.

2.4.2. Construction of cartilage organoids

Due to the lack of neural and vascular supply, cartilage has limited self-repair capacity once injured, as exemplified by osteoarthritis resulting from progressive wear of articular cartilage. Furthermore, cartilage-related pathologies—including developmental disorders, cartilage tumors, and inflammatory arthropathies—pose significant clinical challenges and create a demand for innovative, applicable disease models and therapeutic strategies. Consequently, cartilage organoids have attracted widespread research interest. Shen *et al.*[71] developed a novel hydrogel microsphere using the microfluidic system and seeded BMSCs onto these microspheres to form cartilage organoid precursors, which markedly enhanced cartilage repair. Wu *et al.* constructed organoids that mimic the native cartilage matrix microenvironment by controlling the orientation of fibers and chondroitin sulfate concentration gradients within collagen hydrogels, offering a promising strategy for functional cartilage regeneration.[72] Zhang *et al.*[73] established cartilage organoids from BMSCs and subsequently induced an inflammatory cartilage organoid model using interleukin (IL)-1β, providing a robust platform for investigating the impact of inflammation on cartilage structure. In addition, various strategies have been proposed to enhance the applicability of cartilage organoids. Studies have shown that incorporating PRP can improve cell viability, proliferation, and differentiation within cartilage organoids, thereby advancing their utility in pathological mechanism research and therapeutic development (Table 5).[74]

2.4.3. Applications of cartilage organoids

Cartilage-related disorders, particularly osteoarthritis, impose significant burdens on patients and markedly diminish their quality of life. As methods for generating cartilage organoids have advanced, these models are increasingly being applied to the investigation of disease mechanisms and cartilage repair. Abe *et al.*[75] generated allogeneic iPSC-derived cartilage organoids and transplanted them into the knee joint cartilage defect model in cynomolgus macaques, demonstrating successful cartilage repair and highlighting the potential of organoid-based approaches for clinical application. Lin *et al.*[76] showed that LGR5-expressing joint progenitor cells can be used to generate cartilage organoids suitable for disease modeling and drug screening. Rothbauer *et al.*[77] established a chip-based co-cultivation system of synovial and cartilage organoids to study reciprocal cross talk in arthritis. Strategies incorporating vascular microenvironments

have also been explored: Chen *et al.*[78] leveraged cartilage organoids by precisely engineering pro-/anti-angiogenic microenvironments to achieve osteochondral defect repair (Figure 5).

3. Construction of rotator cuff organoids

Similar to most diseases, existing *in vitro* models for rotator cuff disorders—primarily conventional cell cultures as well as animal models—exhibit some limitations which make it difficult to meet the needs of comprehensive disease research. In addition, since rotator cuff tears predominantly occur at the tendon-bone interface and have a high rate of postoperative re-tear, there is an urgent need for an innovative and effective strategy to achieve better restoration of rotator cuff injuries. Based on this, multiple rotator cuff regeneration strategies are under constant exploration, including stem cell-based therapies (MSCs, ADSCs), scaffold materials (collagen matrices, nano/micro-fibrous matrices), PRP, and physical stimulation modalities.[79,80] Most of the strategies focus on developing structural materials that recapitulate the properties of natural tissues to improve the healing,[81] while substantial evidence has demonstrated that these regeneration approaches have achieved some improvements, but some significant challenges persist in this domain. Given the structural complexity of the rotator cuff, the creation of multi-tissue units represents a promising approach to achieving good rotator cuff repair.

Table 5. Construction of cartilage organoids

Cell source	Inducing factor	Matrix material	References
BMSCs	TGF-β3, dexamethasone, ascorbic Acid	RGD-SF-DNA hydrogel microsphere	71
Chondrocytes	TGF-β1, IGF-1,	Collagen hydrogel	72
BMSCs	CTCC-Y002 medium	N/A	73
iPSCs	TGF-β1, Ascorbic Acid, BMP2, GDF5	Matrigel	75
Chondrocytes	411D-250 medium	Hydrogel	76

Notes: CTCC-Y002 is a commercial chondrogenic differentiation medium, whereas 411D-250 is a commercial chondrogenic differentiation medium.
Abbreviations: BMP2: Bone morphogenetic protein 2; BMSCs: Bone marrow mesenchymal stem cells; GDF5: Growth differentiation factor 5; IGF-1: Insulin-like growth factor 1; iPSCs: Induced pluripotent stem cells; RGD: Arginine-glycine-aspartic acid; SF: Silk fibroin; TGF-β3: Transforming growth factor beta 3.

Rotator cuff organoids, integrating skeletal muscle, tendon, cartilage, and bone structures, are one of the types of bone-tendon-muscle multitissue structures that may meet the needs of rotator cuff repair. However, given its complexity, strategies for the development of rotator cuff organoids are not yet mature. In the following, we will introduce the construction methodology for rotator cuff organoids from three critical dimensions: cell source,

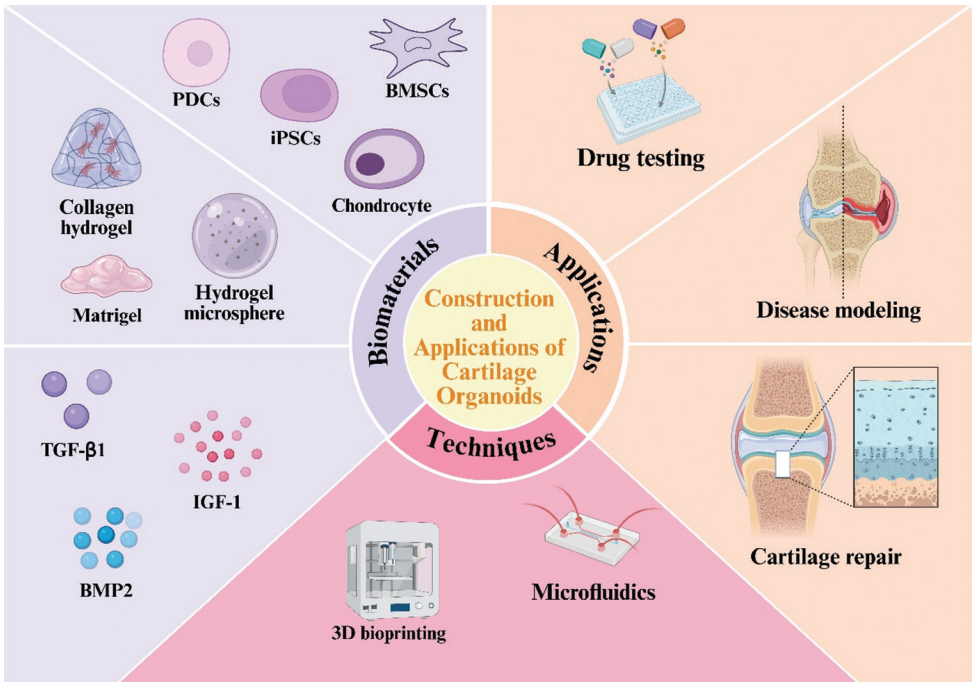

Figure 5. Construction and applications of cartilage organoids. Created in BioRender. Shi, Q. (2025) https://BioRender.com/1c8ds9y.
Abbreviations: BMP2: Bone morphogenetic protein 2; BMSCs: Bone marrow mesenchymal stem cells; IGF-1: Insulin-like growth factor 1; iPSCs: Induced pluripotent stem cells; PDCs: Periosteum-derived cells; TGF-β1: Transforming growth factor beta 1.

matrix material, and construction technique and strategy (Figure 6).

3.1. Cell sources

Cells are the basis of organoid construction. To successfully construct a mature rotator cuff organoid that meets the requirements, it is necessary to select suitable and personalized cell sources, and then, with the assistance of specific matrix materials, induce directional differentiation of cells through cytokines combined with physical stimulation, to recapitulate the structure and function as well as the microenvironment of the natural rotator cuff. Stem cells serve as a cornerstone in organoid construction due to their robust dual competencies in self-renewal and multipotent differentiation.

MSCs are critical cellular building blocks for constructing rotator cuff organoids. As a type of adult multipotent stem cell, MSCs, which exhibit strong self-renewal ability, multipotent differentiation potential, and immunomodulatory properties, are ubiquitously found in bone marrow, adipose, mucous membrane, amniotic membranes, and other tissues, and can be induced to differentiate to produce bone, cartilage, tendon, and muscle tissues.[82] Beyond this, they can also secrete matrix components such as osteocalcin and type II collagen.[83] Owing to these properties, they are utilized in the construction of musculoskeletal system organoids. In the fabrication of rotator cuff organoids, BMSCs and adipose-derived stem cells (ADSCs) are predominantly utilized.[14]

3.1.1. BMSCs

As the first MSC population type discovered, BMSCs are mainly found in the bone marrow stroma, accounting for about 0.001–0.01% of bone marrow mononuclear cells.[84,85] Owing to their technical accessibility and robust multilineage differentiation ability *in vitro*, BMSCs have been widely validated for diverse applications such as tissue repair and regeneration,[86] treatment of immune-related diseases, and drug delivery as well as gene therapy.[87] In rotator cuff organoid constructions, BMSCs are usually loaded into matrix materials along with cytokines to achieve the formation of tendon and bone and can be implanted along with endothelial cells to improve vascularization.[88] Liu *et al.*[89] developed a tendon-fibrocartilage-bone composite bridging patch loaded with BMSCs cell sheets, and the complex was subsequently placed in a bioreactor for dynamic culture, which is expected to improve rotator cuff repair. Du *et al.*[90] used BMSC-loaded polydopamine-modified 3D-printed polycaprolactone (PCL) scaffolds in combination with growth factor-loaded liposomes for the repair of infraspinatus tendon tears, showing therapeutic efficacy superior to that of direct suture. In addition, with the aim of improving the rotator cuff repair capacity of organoids, some researchers have used gene editing techniques to modify stem cells. For example, Liu *et al.*[91] genetically modified BMSCs to overexpress Msx1, which was subsequently embedded in the hydrogel scaffolds, and demonstrated formation of bone, cartilage, and collagen following implantation of the scaffolds into the tendon-bone interface.

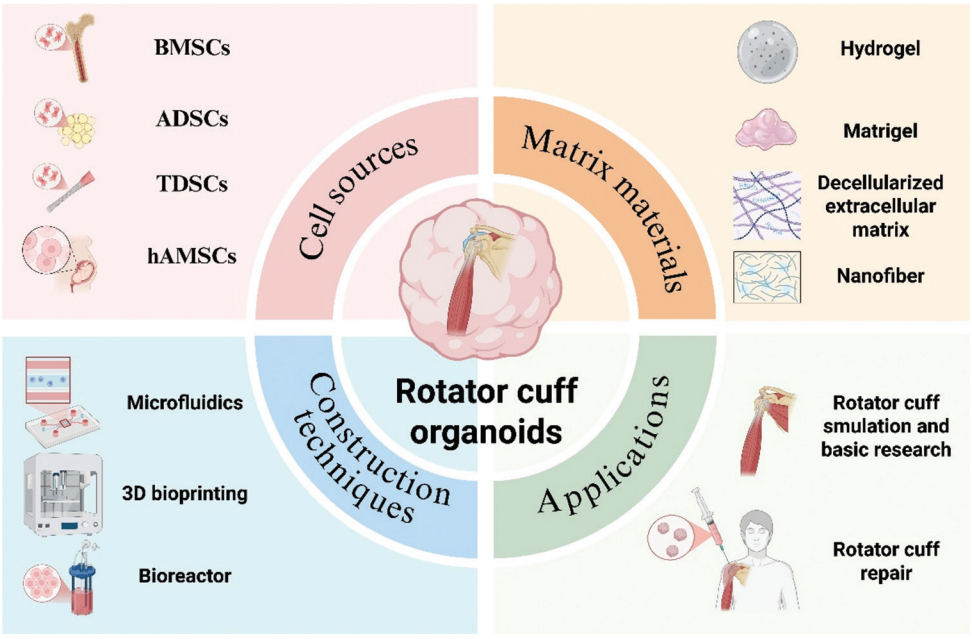

Figure 6. Construction strategies and application prospects for rotator cuff organoids. This includes descriptions of cell sources, matrix materials, core methodologies, and application prospects, such as anatomical structure simulation and regenerative repair. Created in BioRender. Shi, Q. (2025) https://BioRender.com/s7ghtnr.

Abbreviations: ADSCs: Adipose-derived stem cells; BMSCs: Bone marrow mesenchymal stem cells; hAMSCs: Human amniotic membrane mesenchymal stem cells; TDSCs: Tendon-derived stem cells.

3.1.2. ADSCs

Although BMSCs remain the primary cell source, the utilization of ADSCs has gradually broadened due to their ease of accessibility, simple isolation process, and high proliferation efficiency.[92] For example, Shi *et al.*[93] loaded ADSCs together with human amniotic membrane MSCs (hAMSCs) onto decellularized activated living hyaline cartilage grafts (LHCG) and implanted them into rat models, which achieved the repair of tendon-bone interface and promoted the healing of RCI. Meanwhile, this study also provided preliminary evidence of the feasibility of hAMSCs for rotator cuff organoid construction. In addition, interestingly, Song *et al.*[94] concluded that the frozen ADSC slices are off-the-shelf scaffold that can efficiently repair rabbit supraspinatus tendon tears. Similarly, Shin *et al.*[95] effectively repaired rotator cuff tears by employing cell slices prepared using ADSCs and transplanting them to the tear site. In addition, Fu *et al.*[96] demonstrated that hydrogel scaffolds containing ADSC-derived exosomes could regulate the growth and differentiation of TDSCs, thereby promoting the healing of rotator cuff injuries.

3.1.3. TDSCs

Compared to other stem cells, TDSCs exhibit a more mature tendon phenotype, while demonstrating powerful proliferation and differentiation capacities, which make this cell type an ideal cell type for tendon regeneration.[97] Central to RCI repair is the repair of the tendon-bone interface, and TDSCs also serve as potential cell sources for rotator cuff organoid construction. For example, He *et al.*[98] found that TDSC-derived exosomes could modulate inflammation and improve the structure and function of the tendon-bone interface. Then, they loaded the exosomes onto type I collagen and polydopamine-modified scaffolds, and subsequently implanted these scaffolds into the torn supraspinatus muscle of rats, which led to more enhanced reparative effects and lower inflammatory response compared with control group and rats receiving only scaffold implantation treatment. Zhang *et al.*[99] achieved rotator cuff repair in rabbit rotator cuff tear models by employing PCL-based 3D-bioprinted scaffolds loaded with TDSC-derived exosomes together with type I collagen, and the composite scaffolds could promote the migration, proliferation, and differentiation of BMSCs.

3.2. Matrix materials

Matrix materials play a pivotal supporting role in the construction of rotator cuff organoids by mimicking the natural tissue microenvironment and inducing cell growth, proliferation, migration, and differentiation,[16] thus optimizing the structural function of rotator cuff organoids. Good matrix materials also contribute to the superior mechanical properties and biocompatibility of the resulting organoids. Matrigel is the basic matrix material for organoid construction, but due to the complexity of its composition and poor mechanical properties, a variety of new and personalized matrix materials are gradually being developed, including natural hydrogels, synthetic hydrogels, and dECM. In the following, we will introduce matrix materials that can be used for rotator cuff organoid construction.

3.2.1. Hydrogel

Hydrogels are matrix materials with biomimetic properties that have controlled mechanical properties, porosity, and viscoelasticity compared with natural extracellular matrices, which can promote cell adhesion, migration, and proliferation differentiation.[100] Due to its excellent properties, hydrogel plays an important role in organoid construction and tissue repair, and has also been used for rotator cuff repair. Ni *et al.*[101] developed a PCL scaffold with the help of 3D printing technology and loaded the scaffold with BMSCs and basic fibroblast growth factor, which was then implanted into rat supraspinatus tendon tear sites to enhance the interfacial healing of rotator cuff injuries. Hydrogels offer exceptional utility in stem cell delivery due to their adjustable physical and chemical properties. Dai *et al.*[102] used gelatin methacrylate (GelMA) to deliver ADSCs with porous Se@SiO_2 nanoparticles. The composite construct was subsequently implanted into the rat tendon-bone interface, not only to minimize the loss of stem cells, but also to promote the repair of RCI. Similarly, Yuan *et al.*[103] employed porous hyaluronic acid methacrylate hydrogel to encapsulate ADSCs and BMP2 and used it for RCI repair in rats, achieving fibrocartilage reconstruction and thus promoting repair. Given the transitional, layered structure of the tendon–bone interface, multiphase scaffolds combined with hydrogels have emerged as a more complex yet more faithful strategy for mimicking the natural tissue hierarchy. Cao *et al.*[104] generated multiphase scaffolds to recapitulate the transitional interfacial structure of the rotator cuff through 3D printing, and encapsulated fibroblasts, BMSCs, and osteoblasts hierarchically in GelMA, thus constructing a cell/GelMA-multiphase scaffold composite, which has substantial potential in rotator cuff repair.

3.2.2. Decellularized ECM

Within natural tissues, the ECM fulfills critical functions such as offering mechanical support, mediating signaling cascades, and providing adhesion. As a result, animal-derived ECM scaffolds have been approved for use in valve replacement, orthopedic implants, hernia repair, and other areas.[105] Decellularized ECM derived from human or animal sources has been successfully used in the generation of various organoid models. Chin *et al.*[106] treated fascial ECMs with high-molecular-weight tyramine, instead of hyaluronic acid, and implanted them into rat models,

which reduced inflammation and improved tissue remodeling. In addition, Shi *et al.*[93] manufactured LHCG and decellularized them to produce dLHCG, which were loaded with MSCs and used for rotator cuff repair. The biological effects of the stem cell secretomes have been emphasized, and thus, decellularized stem cell medium can also be considered a broadly dECM. Chen *et al.*[107] collected hBMSC-derived mediums and demonstrated that they could promote tendon-bone healing in the rotator cuff by modulating macrophages.

3.3. Construction techniques and strategies

Given the complexity of rotator cuff organoids, it is necessary to synthesize a variety of biotechnologies and methods to better integrate muscle, tendon, and bone organoids to realize the successful construction of rotator cuff organoids and unleash the application potential of organoids. 3D bioprinting and microfluidics technologies represent established bioengineering approaches for fabricating architecturally complex organoids, while bioreactor systems constitute an emerging strategy with significant potential.[108]

3D bioprinting technology enables precise control of spatial structure and can be employed to fabricate complex and highly ordered scaffold structures. Compared with traditional techniques, 3D printing technology can better mimic the ECM environment, produce layered tissue structures, and improve organoid performance. Jiang *et al.*[109] generated layer-by-layer scaffolds and three-layer scaffolds by using polylactic-co-glycolic acid inks through 3D printing technology, and combined these scaffolds with stem cells together with hydrogels, which have the potential for realizing rotator cuff tendon repair. To further improve organoid performance, the use of cell-loaded dECM bioinks is feasible. Chae *et al.*[110] designed a gradient multi-tissue model by bioprinting using dECM bioinks containing hBMSCs, which was therapeutically useful for rat supraspinatus tendon tears. In addition to this, gene transfection of stem cells by recombinant adenovirus can be synergistically used with 3D printing technology for achieving regenerative repair of the rotator cuff tears.[111]

Microfluidics is another powerful biomedical engineering technology for organoid construction, which enables the fabrication of materials with complex adjustable size, shape, and composition. The cell culture environment can be accurately controlled through the control of flow rate, viscosity, and other parameters to assist in the high-throughput and highly consistent production of organoid models.[112] Li *et al.*[37] employed CTM to dramatically shorten the construction time of skeletal muscle organoids and achieve large-scale production of functional skeletal muscle organoids. By using microfluidics and 3D printing technology, Jiang *et al.*[113] built an organoid platform to generate homogeneous organoid models for achieving high-throughput screening. Owing to its ability of fine-tuning, multitissue organoids were successfully constructed. For example, Nguyen *et al.*[114] established kidney and liver multiorgan microarrays through microfluidics and employed them to investigate the therapeutic potential of extracellular vesicles in diseases. Furthermore, microfluidics provides important support for the regenerative repair process of the rotator cuff. Ding *et al.*[115] developed hydrogel microrobots and loaded them with Mg^{2+} and Zn^{2+} through a microfluidic platform to promote the healing of the tendon-bone interface in rotator cuff tears. The microfluidics technology can also be used to establish a concentration gradient of cytokines to promote gradient differentiation of stem cells and better recapitulate the structure of rotator cuff.[116]

Mechanical stimulation plays a critical role in rotator cuff healing. Studies have demonstrated that applying uniaxial strain can induce cellular alignment along the direction of mechanical loading and enhance ECM deposition.[117] Furthermore, mechanical loading promotes MSCs differentiation, proliferation, and ECM synthesis.[118] Consequently, applying appropriate mechanical stimulation during cultivation can facilitate the maturation and functionalization of rotator cuff organoids. For instance, Liu *et al.*[89] fabricated TFBCs subjected to mechanical stimulation for up to 7 days, which demonstrated superior performance including enhanced cell migration and more uniform cell distribution. Mechanical stimulation also serves a distinct function in the establishment of pathological models. For example, building on their previous work with trabecular bone organoids, Iordachescu *et al.*[66] successfully established an osteoporosis model through the application of mechanical stimulation. In addition, mechanical stimulation may promote organoid vascularization by upregulating proangiogenic factors.

Co-culture serves as a modular assembly strategy. Skardal *et al.*[119] established an integrated three-tissue organ-on-a-chip system through the co-culture of liver, heart, and lung organoids and applied it to drug screening. Furthermore, innovative techniques such as magnetic-assisted assembly and acoustic-based assembly have been explored. Chen *et al.*[120] demonstrated scaffold-free assembly of organoids using the acoustic node assembly technique.

4. Challenges and outlook

Rotator cuff organoids hold promising translational potential due to the excellent capabilities of organoid technology and the high prevalence of rotator cuff injuries. However, there are still some challenges and obstacles in the development of rotator cuff organoids.

Most of the current organoid constructions only recapitulate partial aspects of the organ's structure and lack

the complete and complex spatial composition, making it difficult to accurately emulate the functional characteristics of the adult organs. The development of musculoskeletal system organoids began relatively late, and the inherent complexity of musculoskeletal tissues—with their multiple functions such as support, protection, and hematopoiesis—makes the creation of organoids capable of faithfully replicating these physiological structures particularly challenging.[55] The rotator cuff is a representative component of the musculoskeletal system, characterized by a gradient transition zone from bone to calcified fibrocartilage, fibrocartilage, tendon, and muscle. This region exhibits gradual changes in cell types and ECM composition, presenting a major challenge for developing rotator cuff organoids that accurately reconstruct this multi-tissue architecture. Several recent studies have predicted that this challenge can be surmounted through the design of scaffold materials by means of 3D printing and microfluidics. In addition, microfluidic organ chips have been reported as a technology that enables multitissue crosstalk, and the concept of musculoskeletal system organoid chips has also been proposed,[121,122] showing significant advantages in solving the problem of complex organoid construction.

Inadequate vascularization constitutes a critical barrier compromising the release of the application potential of rotator cuff organoid techniques. Poor healing of rotator cuff injuries is usually associated with poor local blood supply. Therefore, the application of organoids for rotator cuff repair requires the construction of rotator cuff organoids with a functional vascular network to support the survival of large volumes of tissues and to improve repair outcomes. This is actually a common problem for most organoids. To overcome this challenge, some researchers have adopted co-culture with endothelial cells, pericytes, MSCs, *etc.*, to achieve vascularization of the organoids.[123] In addition, Garreta *et al.*[124] reported a method for vascularization of renal organoids, through which they utilized renal dECM hydrogels and assembled hPSC-derived endothelial organoids with renal organoids to produce organoid models possessing vascular-like structures. The vascularization problem inherent to rotator cuff organoids can also be solved by the construction and assembly of vascular organoids. Mechanical stimulation, electrical stimulation, shear stress, and other methods that may improve the vascularization of musculoskeletal system organoids have also been explored.[87] Beyond the vascular system, factors released by the nervous system play crucial roles in bone metabolism and regeneration processes.[125] However, most of the current organoid models lack robust neural innervation, limiting their ability to recapitulate complex physiological microenvironments. With advancing technologies such as 3D bioprinting, creating sophisticated organoid systems with functional neural integration is becoming increasingly feasible. For example, Grass *et al.*[36] generated neuromuscular organoids containing spinal motor neurons through a defined induction protocol. Current organoid models often lack organ-specific cell types, which may play critical roles in disease pathogenesis and treatment—such as macrophages and other immune cells.[126] Consequently, recapitulating the crosstalk between the immune and musculoskeletal systems represents a pressing challenge that must be addressed to advance the application of rotator cuff organoids in regenerative repair.

Development of the musculoskeletal system organoids is still at an early stage, and poor reproducibility represents a prominent issue. Poor reproducibility of organoids, stemming from variability in cell types, matrix materials, cytokines, culture protocols, and other factors, affects the comparability of results and the difficulty of clinical translation. The generation of organoids relies heavily on the robust self-organization capacity of cells, a process that is inherently difficult to control, leading to considerable batch-to-batch heterogeneity and insufficient standardization.[28] Furthermore, the absence of generally recognized international criteria specifying the essential attributes of qualified organoids constitutes another major impediment to standardization. Standardization of originating cells, optimization of culture systems and microfluidic organ-on-a-chip, and adoption of strict quality control standards are essential for achieving standardized construction of organoids.[127] It is hoped that in the future, challenges related to organoid reproducibility will be resolved, barriers to clinical translation will be overcome,[128] and the full application potential of organoid technology will be realized. For instance, Lawlor *et al.*[129] demonstrated that extrusion-based 3D cellular bioprinting technology enhances organoid reproducibility and enables high-throughput production. Separately, Brandenberg *et al.*[130] achieved standardized organoid generation through microcavity array platforms, significantly reducing batch-to-batch heterogeneity and facilitating applications in drug discovery pipelines.

Furthermore, the high maintenance costs of organoid culture, along with inherent dimensional constraints and scalability challenges, hinder their broader applications in the biomedical field.[131]

5. Conclusion

Research on musculoskeletal system organoids remains in its early stage, yet it has already demonstrated considerable potential, with applications emerging in regenerative medicine, drug screening, and disease modeling. Despite existing limitations, the potential of organoids is expected to be further unlocked through technological advancements and the establishment of standardized cultivation protocols, thereby accelerating their transition

into clinical applications. As an emerging model that mimics the complex structures and functions of the human rotator cuff, rotator cuff organoids accurately integrate the biomimetic structures of the bone-tendon-muscle multitissue interface, providing a 3D interferable platform for analyzing the developmental process, homeostatic maintenance, and regenerative repair of the rotator cuff. As research on rotator cuff organoids continues to advance, the rotator cuff organoids provide valuable platforms for fundamental studies and the development of therapeutic strategies for rotator cuff diseases.

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Conflict of interest

The authors declare that they have no competing interests.

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Writing–review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Availability of data

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