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Tendon organoids: Advances in bioengineering strategies and translational applications

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Abstract

As critical connective tissues transmitting from muscles to bones, tendons play a central role in movement and postural stability. However, their low cellularity, limited metabolic activity, and propensity for degeneration render them vulnerable to acute and chronic injuries. Traditional therapeutic approaches, such as autografts and allografts, are constrained by donor scarcity, immune rejection, and suboptimal functional recovery, driving the emergence of tissue engineering and organoid technologies as innovative solutions. Tendon organoids, which recapitulate the native tendon's three-dimensional (3D) structure, cellular complexity, and biomechanical niche, offer a physiologically relevant *in vitro* model for advancing our understanding of tendon development and pathology. This comprehensive review systematically examines recent advances in tendon organoid research, highlighting four key determinants in the construction of tendon organoids: (i) Selection and optimization of cell sources, particularly tendon stem/progenitor cells; (ii) regulation of biochemical cues through spatiotemporal coordination and signaling pathway modulation; (iii) design of biomimetic 3D microenvironments, including physical scaffolds and mechanical stimulation; and (iv) integration of engineering strategies, such as single-cell omics, gene editing, 3D bioprinting, and artificial intelligence (AI) for system optimization. Notably, tendon organoids demonstrate multidimensional potential in translational applications, including regenerative medicine, disease modeling, drug screening, and biomechanical research. To overcome current technical bottlenecks, future investigations should prioritize AI-driven organoid design, standardized manufacturing protocols, and solutions for clinical translation challenges. By bridging fundamental research and clinical therapeutics, this review outlines a theoretical framework and technical roadmap for the refined construction and application of tendon organoids, highlighting their transformative potential in regenerative medicine and precision healthcare.

Keywords: Tendon organoid; Tissue engineering; Regenerative medicine; Tendon stem/progenitor cells

1. Introduction

Tendons are fibrous connective tissues that link muscles to bones and facilitate the transmission of mechanical loads.[1,2] As a mechanosensitive structure of the musculoskeletal system, it serves an essential function in transmitting the force produced by muscle contraction to bones, enabling movement and maintaining posture. Tendons exhibit high tensile strength and elasticity owing to their structure that is rich in extracellular matrix (ECM), enabling them to withstand considerable mechanical loads.[3] However, tendons are susceptible to injuries, and tendon injuries are the most common disorder in the musculoskeletal system. The annual incidence in primary care settings is 4–7/1,000 people, with a prevalence of 1–3% in the general population and 7% among manual laborers. The most affected age group is 42–54 years.[4] Aging is a significant risk factor for tendinopathy, as tendons undergo degenerative changes over time, leading to an increased susceptibility to injury.[5] For example, the rotator cuff, a prevalent tendon injury, has an incidence of 9.7% in patients under 20 years old, but increases to 62% in patients aged 80 or older.[6] Tendon injuries encompass a spectrum from tendinopathy—a chronic degenerative condition typically managed non-surgically—to complete ruptures that often require surgical repair. The majority of studies and applications of tendon organoids have focused on tendinopathy, aiming to address the limited efficacy of conservative treatments. While surgical intervention is indispensable in full-thickness tendon tears, non-surgical modalities for tendinopathy still face limitations in restoring native tendon structure and function. This highlights the need for organoid-based regenerative approaches beyond *in vitro* modeling and drug screening, reinforcing their potential translational value in clinical therapy.

Current treatment options mainly focus on autografts and allografts. However, the property of donor scarcity and the potential for immune rejection for allografts limit its application. In addition, the suboptimal functional recovery of both treatments demonstrates the poor prognosis of patients.[7] Despite advancements in surgical techniques, completely restoring tendon structure and function remains elusive. Therefore, tissue engineering has emerged as a promising field for addressing these limitations. Among two-dimensional (2D) or three-dimensional (3D) tissue engineering, tendon-like organoids provide a simulation of tendons *in vitro*, structurally and functionally, contributing to regenerative medicine with high biocompatibility.[8]

Current preclinical studies on tendon pathology primarily rely on 2D cultures and animal models. Although 2D systems provide controlled environments, they fail to replicate the complex 3D architecture and ECM interactions of native tendon tissue (e.g., lack of ECM). Animal models, on the other hand, enable *in vivo* investigation of tendon injury and repair with the advantage of an *in vivo* physiological context. However, their translational relevance is limited by interspecies differences in tendon structure, gene expression (e.g., lack of *MMP1* in rodents), and biomechanics. These discrepancies hinder accurate modeling of human tendinopathy. Nevertheless, animal models remain essential for studying systemic responses and evaluating surgical or regenerative interventions. Thus, tendon organoids and animal models should be seen as complementary tools—organoids offer human-specific insights, while animal models provide a whole-organism context essential for translational research.[9,10]

In recent years, organoid technology has made remarkable progress in multifaceted fields, including neuroscience, gastroenterology, and hepatology. For example, brain organoids have been proven effective in studying neurodevelopmental disorders, while intestinal and liver organoids have become invaluable tools for investigating disease mechanisms, drug screening, and personalized medicine.[11]

Although organoid technology has been widely applied in various fields, its use in tendon research remains in the early stages. Tendon organoids offer a promising approach to addressing the biomechanical complexity of tendon tissues, potentially serving as a novel platform for studying tendon biology, modeling injuries and diseases, and testing regenerative therapies.

However, replicating the unique mechanical and biochemical environment of tendons presents significant challenges. Unlike other organs, such as the liver or intestines, tendons exhibit distinctive characteristics, including highly organized collagen fibers, low cellular density, and a complex ECM. These features make *in vitro* reconstruction of functional tendon tissue particularly difficult. Key research questions include identifying optimal cell sources, determining appropriate biochemical signals, and applying mechanical stimuli. In addition, scaffold materials and biofabrication techniques play a crucial role in mimicking tendon structure and mechanical properties.[12]

Recent advancements have addressed some of these challenges. Notable progress includes the development of high-performance scaffolds, the application of 3D bioprinting, and the use of bioreactors to simulate physiological mechanical conditions. Moreover, interdisciplinary contributions from biomechanics, materials science, and cell biology have further propelled research in this field.[13]

This review provides a comprehensive overview of recent progress in tendon organoid research, emphasizing key technical aspects and their clinical and translational potential. The discussion will cover strategies for

constructing tendon organoids, including cellular selection, biochemical and mechanical factors, and bioengineering approaches. It will also explore their applications in disease modeling and drug screening. Finally, future directions will be outlined, focusing on the integration of artificial intelligence (AI) and standardization efforts to enhance the scalability and functionality of tendon organoid technology.

2. Tendon structure and microenvironment

2.1. Tendon tissue structure

Tendons serve as dense fibrous connective tissues that anchor muscles to bones, thereby enabling the efficient transfer of mechanical forces.[1,2] Healthy tendons exhibit a bright white appearance[14] and are characterized by a low cellular content, primarily consisting of ECM (55–70%). This structural specificity sharply contrasts with parenchymal organs (e.g., liver or kidney), where cellular components dominate and ECM constitutes only a minor part.[15,16] The ECM-rich composition of tendons provides both tensile strength and elasticity, enabling their unique biomechanical function in load transmission. The composition of tendons changes with age; as tendons mature postnatally, cellular content decreases while ECM content increases.[17] The ECM is primarily constituted by proteoglycans, glycosaminoglycans, glycoproteins (with a notable presence of small leucine-rich proteoglycans), and collagen fibers (60–85% of dry weight),[18-20] which are organized into a network through the aligned arrangement of collagen fibers.[19] Collagen fibers are mainly composed of Type I collagen, accounting for 97–98% of the total collagen content in tendons, while Type III collagen constitutes 1.0–1.5%,[21] with minor amounts of collagen Types V, XI, XII, and XIV.[2] Type I collagen forms triple-helical tropocollagen molecules, which aggregate into microfibrils. These microfibrils further assemble into fibrils that exhibit a periodic "crimping" pattern under unloaded conditions.[22] Fibrils coalesce to form fibers with diameters ranging from 1 to 20 μm, which are grouped into fiber bundles (150–500 μm) enveloped and separated by the endotenon or interfascicular matrix (IFM).[21] The epitenon is a thin, dense connective tissue layer that closely envelops the tendon surface, functioning to provide lubrication and minimize friction between the tendon and surrounding tissues. The paratenon, located outside the epitenon, is composed of loose connective tissue rich in blood vessels and nerves. In tendons without a sheath, such as the Achilles tendon, the paratenon serves as the primary lubricating structure. Together, the epitenon and paratenon form the peritenon.[1,23,24] In addition to intermolecular cross-linking between collagen fibers, non-collagenous elastic components of the ECM, such as proteoglycans, glycosaminoglycans, and glycoproteins, play crucial roles in mitigating tissue deformation, enhancing viscoelasticity, and maintaining the integrity of the ECM within the fiber space.[1,21,25] These properties provide the structural foundation for tendons to transmit mechanical forces between muscles and bones and to withstand high tensile stresses (Figure 1).

Tendons are characterized by relatively low vascularity, sparse cellularity, and reduced metabolic activity. A significant challenge in tendon repair is the post-injury fibrotic response mechanism, which leads to tendon sheath adhesion and excessive scar formation. This prevents the restored tendon from regaining the mechanical strength of uninjured tissue, often resulting in frequent reinjury.[26] The natural regeneration of tendons after injury involves three primary phases: Inflammation, proliferation, and remodeling.[20] During this process, fibroblast activity and the synthesis of Type III collagen significantly increase. However, healed tendons exhibit fewer cross-links and smaller collagen fibril diameters compared to healthy tendons. Fibrotic tissue can develop between the tendon and its surrounding structures, resulting in adhesions and thereby increasing the risk of reinjury.[27]

2.2. Tendon cell composition

The primary cells in tendon tissue are tenocytes (90–95%)[28] and tenoblasts (resident tendon cells).[20] Tenocytes are elongated, spindle-shaped fibroblasts with a low nucleus-to-cytoplasm ratio and low metabolic activity, distributed among collagen fibers. Their main functions include secreting ECM and releasing signals that regulate tendon formation and development.[21,29] Tenoblasts, the immature form of tenocytes, differentiate into tenocytes as the individual ages.[20] Through single-cell transcriptomic analyses, recent studies have illustrated the heterogeneity of the tendon resident cell population[27,30-33] (Figure 1). While tenocytes and their precursors form the core functional cellular component, tendon-resident cells can be broadly categorized into three major subpopulations, including functional fibroblasts that express high levels of ECM-related genes (e.g., *COL1A1*, *COL3A1*), tendon stem/progenitor cells (TSPCs), and immune-regulatory cells that express cytokines and complement factors.[3,29,34-36] The study evaluates the expression of typical tendon fibroblast markers, such as scleraxis (SCX), tenomodulin (TNMD), and Mohawk Homeobox, in each subpopulation,[1] identifying the presence of these markers in a subset of fibroblasts. The phenotypic identification of tenocytes relies on the expression of common markers in resident cell populations, including ECM proteins, such as Type 1 collagen (COLI) and Type 3 collagen (COLIII), or small leucine-rich proteoglycans (SLRPs), such as decorin, within the IFM,[1] with emerging markers, including *THBS4* and *WNT10A*, implicated in tendon development and repair.[31]

Importantly, the described cell types (tenocytes, tenoblasts, functional fibroblasts, and TSPCs) represent the

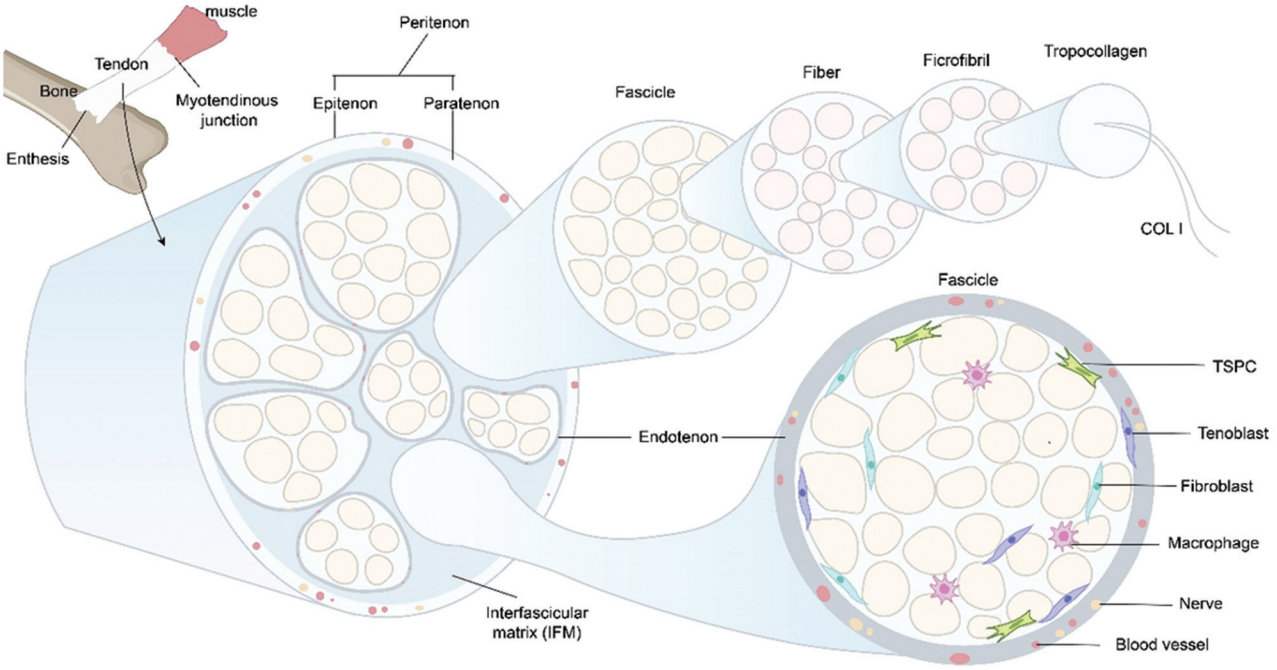

Figure 1. Structural organization of the tendon microenvironment. The tendon microenvironment is predominantly composed of ECM, constituting 55–70% of its dry weight. This ECM features a hierarchical assembly of type I collagen (97–98% of total collagen), organized from tropocollagen to microfibrils to fibril bundles, which are reinforced by proteoglycans and glycosaminoglycans to enhance tensile strength and elasticity. Tendon cells (tenocytes/tenoblasts) are sparsely distributed within the ECM. Vascular and neural networks are predominantly localized to the paratenon, while the tendon body itself is largely avascular. Created with Adobe Illustrator, Yiwen Xue (2025) https://imgur.la/images/2025/09/09/fig1.jpg.
Abbreviations: COL I: Collagen type I; ECM: Extracellular matrix; IFM: Interfascicular matrix; TSPC: Tendon stem/progenitor cells.

principal cellular populations but are not exhaustive. The tendon microenvironment, particularly within the IFM, also contains a significant complement of resident immune cells, including macrophages, mast cells, lymphocytes, neutrophils, and dendritic cells.[37,38] These immune cells are increasingly recognized for their crucial roles not only in surveillance and acute inflammatory responses but also in orchestrating tendon repair, remodeling, and modulating the local cellular environment. Nerve cells are also present and contribute to tendon function and injury responses.[39,40] A small population of TSPCs is also present in soft connective tissues,[29] capable of forming colonies *in vitro*. Nestin is a neural stem cell marker co-expressed in tendon progenitors, and a subpopulation of nestin-positive TSPCs was identified that exhibits robust tenogenic potential and enhanced regenerative capacity.[33] Several other molecular markers have been reported to identify TSPCs, including dipeptidyl peptidase-4[41] (a surface marker linked to TSPC proliferation), and *RSPO2*[42,43] (wingless-related integration site signaling enhancer promoting tenogenic differentiation). Upon tendon injury, TSPCs can activate, proliferate, and differentiate into tenocytes to promote tendon healing. Notably, TSPC subpopulations expressing functional markers, such as cathepsin K[44,45] or cytoskeleton regulator[46] exhibit distinct roles in ECM remodeling during repair. The interaction between TSPCs and the ECM is intricate; mechanical stimulation of the ECM can modulate the gene expression profiles of TSPCs, which in turn influences the composition and structure of the ECM.[21,24,47]

To construct tendon organoids, a comprehensive understanding of the structure and microenvironment of tendon tissue is essential. These factors collectively govern tendon development, functional maintenance, and post-injury repair processes. By mimicking the cellular composition, physicochemical properties of the ECM, mechanical environment, and cell-cell interactions within tendons, it becomes possible to develop functional tendon organoids that better replicate native tissue behavior.

3. Organoids

3.1. Origin and development of organoids

Organoids are 3D *in vitro* tissue analogs derived from adult stem cells or pluripotent stem cells, capable of mimicking the structure and function of organs.[48-51] They represent miniature and simplified model systems that exhibit self-organization, cellular diversity, and the ability to replicate organ functions.[49,50] The concept of organoids dates back to 1907, when Wilson[52] discovered that mechanically dissociated sponge cells could reaggregate and self-organize into functional sponge organisms. This groundbreaking

finding revealed the self-organizing and regenerative capabilities of cells, laying the foundation for organoid research. The modern era of organoid technology began in 2009, when Hans Clevers' team successfully cultured the first intestinal organoid using adult stem cells from mouse intestines,[53] marking the dawn of organoid research. In 2013, Lancaster *et al.*[54] developed brain organoids from human pluripotent stem cells, followed by the successful generation of liver,[55] kidney,[56] and pancreatic organoids.[57] These advancements have significantly contributed to the study of organ development and disease modeling, while also advancing personalized medicine and targeted therapeutic strategies.[50] In the same year, organoid technology was recognized as one of the top ten breakthroughs by *Science* magazine. The rapid advancement of organoid technology, along with its improved ability to simulate organ structure and function, has provided powerful tools for research in organogenesis, disease modeling, drug screening, and precision medicine.[50,51]

Today, organoid technology is experiencing renewed growth through interdisciplinary integration. Biomedical engineering technologies, such as hydrogels, microfluidics, and 3D printing, are addressing current limitations, including high heterogeneity, low maturity, and structural simplicity in organoids.[58-60] These innovations are enhancing the ability of organoids to replicate the complexity of real organs. Organ-on-a-chip systems, which fall within the realm of biomedical engineering technologies, facilitate the construction of higher-fidelity organoids by precisely controlling the cellular microenvironment.[61-63] For example, Hu *et al.*[62] developed a Bone/Cartilage Organoid-on-Chip device, bridging the gap between *ex vitro* cell culture, animal models, and human pathological conditions. This system allows organoids to exhibit specific pathophysiological features observed during bone and cartilage diseases. Furthermore, advancements in omics, imaging, genetics, and AI are driving the evolution of organoid technology.[12,64] AI is increasingly being applied to address challenges related to organoid assembly complexity and data analysis, including rapid screening of construction strategies, cost-effective extraction of multiscale image features, streamlined analysis of multi-omics data, and precise preclinical evaluation.[12]

3.2. Development of tendon organoids

Although the construction of fully functional tendon organoids has not yet been achieved, research in this field has progressed from simple 2D cultures to more complex 3D structures. This evolution reflects a shift from microscopic to macroscopic approaches and from single-tissue to multi-tissue collaborative construction, laying the groundwork for future tendon organoids that more closely mimic physiological environments.

3.2.1. Evolution of cultivation techniques from two-dimensional to three-dimensional

The development of tendon organoids is still in its early stages, evolving from rudimentary beginnings. Initial research focused on 2D culture systems, where tendon cells or stem cells were seeded in culture dishes and exposed to specific growth factors and mechanical stimuli to study cell proliferation, differentiation, and matrix synthesis.[65] These studies provided insights into the basic biological behaviors of tendon cells *in vitro* and laid the foundation for subsequent 3D culture systems. However, 2D systems have significant limitations, as they fail to replicate the 3D structure and complex mechanical environment of native tendons, resulting in functional discrepancies between cultured cells and real tendon tissue.[66] Strategies beyond simple monolayer culture emerged to better recapitulate aspects of the tendon niche within 2D or transitional systems. For instance, indirect co-culture approaches, where target cells are exposed to the secretome of tendon tissue explants or tenocytes without direct contact, have demonstrated significant potential in programming cells toward tenogenic differentiation.[67]

Cell sheet technology has been adopted,[68] where stem cells are cultured on specific substrates, such as decellularized tendon slices,[69-71] to form cell sheets with defined thickness and structure. This approach provides a microenvironment closer to that of *in vivo* conditions, paving the way for the construction of 3D structures. Advanced 3D culture techniques now utilize biocompatible and biodegradable scaffolds (e.g., collagen hydrogels, poly(lactic-co-glycolic acid)[72]) to seed stem cells and create 3D tendon-like tissues through *in vitro* cultivation.[73] These 3D systems better replicate the mechanical and biological functions of native tendons, marking a significant step forward in tendon organoid research.

3.2.2. Breakthroughs in scale and function from micro to macro

Research on tendon organoids has expanded beyond microscopic-level cell behavior and matrix synthesis to the development of functional tissues at macroscopic scales. These larger-scale organoids not only exhibit enhanced mechanical strength but also better replicate the physiological functions of native tendons. Early studies primarily focused on the microscopic scale, such as optimizing cell differentiation and functional expression by modulating the composition and mechanical properties of the ECM. In recent years, research on centimeter-scale tendon organoids has not yet been achieved, with most efforts concentrated on millimeter-scale models. However, advancements in tissue engineering technologies are laying the groundwork for the construction of centimeter-scale organoids. For instance, the concept of "Bioprinting-

Assisted Tissue Emergence" has been proposed, utilizing 3D bioprinting to control geometric shapes and cell density, thereby facilitating the generation of centimeter-scale tissues with self-organizing characteristics.[74] The continuous evolution of tissue engineering techniques provides critical technological and theoretical support for the development of functional centimeter-scale tendon organoids.

3.2.3. From single-tissue to multi-tissue collaborative construction

Tendons do not exist in isolation within the body but interact closely with surrounding tissues, such as muscles, bones, nerves, and blood vessels. Constructing fully functional tendon organoids requires the collaborative integration of multiple tissue types. Although the successful creation of a fully functional tendon organoid has not yet been achieved, trends in multi-tissue collaborative construction have emerged in the study of other organoids.[75] For example, researchers have utilized human pluripotent stem cells and co-development strategies to successfully generate self-organizing organoid models containing neural, muscular, and skeletal tissues.[76] This multi-tissue collaborative approach offers new insights for the future development of tendon organoids, potentially enabling the integration of tendons with other relevant tissues to better replicate the physiological environment of the human body.

With ongoing advancements in stem cell technology, biomaterials, and bioengineering, tendon organoids are expected to achieve significant breakthroughs in structural complexity, functional integrity, and compatibility with human physiology. The development strategies in tendon organoid engineering are presented in Table 1.

4. Construction of tendon organoids

Although research on the construction of tendon organoids is still in its early stages, numerous studies have identified key components essential for their development. These include the selection of appropriate cell sources, the provision of a suitable physical environment and biochemical factors,[20] and the integration of engineering strategies to facilitate organoid formation.

4.1. Cell selection

As mentioned earlier, the collagen composition, directional growth, and prevention of fibrosis are critical dynamic processes in the construction of tendon organoids. The use of appropriate cell types can better control the functional realization of tendon organoids. In tendon tissue engineering, stem cells such as TSPCs, mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs) are utilized for their stemness to self-renew and differentiate into the cellular composition and collagen structure of native tendons. Most adult stem cells exhibit

Table 1. Developmental strategies in tendon organoid engineering

Development stage	Key strategies or models	Representative materials/technologies	Advantages	Limitations	References
Transitional 2D culture	• Cell sheet technology; use of decellularized tendon slices for cell sheet formation.	• Decellularized tendon slices. • Co-culture inserts.	• Closer mimicry of the *in vivo* tendon environment than monolayer.	• Still lacks full 3D spatial and mechanical properties.	68–71
Early 3D culture	• Scaffold-based 3D culture. • Hydrogel embedding of stem cells.	• Collagen hydrogels, PLGA, Matrigel.	• Supports ECM formation, tenogenic differentiation.	• Limited scalability. • Variable mechanical properties.	72,73
Advanced 3D culture	• Bioreactors; mechanical stimulation. • Self-organizing hydrogel constructs.	• Stretchable hydrogels. • Dynamic bioreactors.	• Improved structural organization. • Enhanced tenogenic phenotype.	• Still millimeter-scale. • Limited vascularization.	66,74
Macro-level	• BATE. • Large-scale, geometrically defined constructs.	• 3D bioprinting platforms. • Customized bioinks.	• Scalable to centimeter range. • Enables tissue integration studies.	• Functional maturation and long-term stability are still under study.	74
Multi-tissue organoids	• Co-development with human pluripotent stem cells. • Integration with neural, muscular, and skeletal components.	• Pluripotent stem cells. • Multi-lineage differentiation protocols.	• Potential for complex tissue integration. • Better mimics physiological conditions; supports collaborative tissue development.	• No successful, fully functional tendon model yet. • High complexity. • Technical challenges in tissue integration.	75,76

2D: Two-dimensional; 3D: Three-dimensional; BATE: Bioprinting-assisted tissue emergence; ECM: extracellular matrix; PLGA: Poly(lactic-co-glycolic acid).

tissue specificity, differentiating into a limited range of cell types within their tissue of origin.[77]

Tendon stem/progenitor cells have been identified in human and rat tendons, primarily located within the ECM containing biglycan and fibromodulin.[36] TSPCs exhibit multipotent differentiation potential and self-renewal capacity, differentiating into tenocytes, osteocytes, chondrocytes, and adipocytes after *in vitro* expansion and *in vivo* transplantation,[36] while also forming functional extracellular matrices. Compared to tenocytes, TSPCs demonstrate superior proliferative and migratory abilities,[78] supporting the formation and repair of tendon organoids. TSPCs also exhibit high stability and low immunogenicity *in vitro*. Notably, aged TSPCs show significant defects in self-renewal and clonogenic potential,[79] resulting in fragile, thin, and poorly organized tendon organoids with reduced cell density and proliferation. These organoids exhibit inferior structural and functional properties compared to those formed by young TSPCs.[80] Therefore, young and highly active TSPCs should be selected as seed cells for optimal results.

MSCs and iPSCs have been widely used in animal studies and clinical tendon repair.[81-83] MSCs are multipotent stem cells capable of self-renewal and differentiation into tissue-specific cells *in vitro*. In addition, their immunomodulatory functions and anti-inflammatory properties contribute to tendon remodeling.[37] MSCs secrete bioactive molecules and exosomes,[84] such as cytokines, growth factors, and chemokines, which facilitate tissue construction. Studies have shown that human umbilical cord MSCs[85] and adipose-derived stem cells,[86-88] when combined with appropriate biochemical factors and biomaterials, can be utilized in tendon tissue engineering and serve as materials for constructing tendon organoids. Differentiating iPSCs into MSCs and further inducing their differentiation into tenocytes can generate functional tendon-like tissues. When iPSC-derived MSCs are seeded onto well-aligned ultrafine fibers, they differentiate into tenocyte-like cells through the activation of mechanosensitive signaling pathways. This process is characterized by increased expression of SCS and COL1, and the production of mature collagen.[89] This approach not only addresses limitations in cell sourcing but also provides a potential pathway for personalized therapies.

Complementing these sources, amniotic-derived cells—encompassing both amniotic epithelial cells (AECs) and amniotic mesenchymal stromal/stem cells (AMSCs)—represent another promising cell type for tendon organoid construction.[90] Derived from the placenta, these cells possess inherent low immunogenicity, anti-inflammatory properties, and immunomodulatory capacities,[90,91] akin to MSCs. Both AECs and AMSCs exhibit a well-documented potential for tenogenic differentiation.[67,92-95] Studies have demonstrated that AECs can be effectively programmed toward the tendon lineage, expressing key tenogenic markers, such as SCX and TNMD,[67,92,95,96] particularly when exposed to appropriate tendon-specific cues or microenvironments. Similarly, AMSCs have shown the ability to differentiate into tenocytes and contribute to tendon repair in preclinical models.[93] Their accessibility, minimal ethical concerns, and dual functionality (tenogenic potential and immunomodulation) make amniotic-derived cells a valuable addition to the toolbox for developing immunocompatible and functionally robust tendon organoids.[97]

Future research should focus on optimizing cell selection strategies, exploring alternative cell sources, and integrating gene editing and biomaterial technologies to develop organoid models that more closely mimic native tendons. The characteristics of the different cell sources are presented in Table 2.

4.2. Biochemical factors

The construction of tendon organoids relies on a precisely regulated biochemical microenvironment, which directly influences cell fate determination, matrix synthesis, and tissue function. Biochemical factors primarily include growth factors, cytokines, and small chemical molecules. These bioactive molecules regulate cell proliferation, differentiation, migration, and matrix remodeling during organoid development by activating specific cell fate and developmental signaling pathways.[77,98] Cell signaling pathways and intercellular interactions provide essential support for the development and functional maintenance of tendon organoids. Integrating bioactive molecules into scaffolds represents a synergistic tissue engineering strategy.[20]

Among these biochemical cues, growth factors play a critical role in the construction of tendon organoids. Insulin-like growth factor 1 (IGF-1) has been identified as a key regulator of collagen synthesis and cell proliferation in tenocytes.[99] Research has demonstrated that IGF-1 signaling promotes cell proliferation and protein synthesis by activating the phosphoinositide 3-kinase/protein kinase B and extracellular signal-regulated kinase pathways, thereby supporting normal tendon growth.[99] In addition, transforming growth factor-beta (TGF-β) and bone morphogenetic proteins are crucial for tendon development and repair,[100,101] and have been applied to enhance tendon graft healing.[83] TGF-β1[102,103] and TGF-β3[88,104] are pivotal regulators of tendon development and repair, promoting the proliferation and tenogenic differentiation of TSPCs while stimulating the synthesis of collagen and other ECM components.[102] Members of the fibroblast growth factor (FGF) family, such as FGF2, enhance tenocyte proliferation and migration and upregulate the expression of tendon-specific markers, such as SCX and TNDM.[105,106] FGF7 has been shown to enhance tenogenesis in human TSPCs through

Table 2. Cell sources in tendon organoid engineering

Cell type	Key characteristics	Advantages	Limitations	References
TSPCs	Reside in ECM rich in BGN and FMOD. • Multipotent (differentiates into tenocytes, osteocytes, chondrocytes, adipocytes). • High self-renewal proliferation/ migration ability.	• Superior proliferative/ migratory ability vs. tenocytes. • High stability and low immunogenicity *in vitro*. • Form functional ECM.	• Aged TSPCs show reduced self-renewal, clonogenicity, and produce fragile organoids with poor organization.	36,78–80
MSCs	• Multipotent; self-renew and differentiate into tissue-specific cells. • Secrete bioactive molecules (cytokines, exosomes). • Immunomodulatory and anti-inflammatory properties.	• Enhance tendon remodeling. • hUCMSCs and ADSCs are compatible with biomaterials for organoid construction.	• Variability in tenogenic differentiation efficiency depending on source and culture conditions.	37,81–88
iPSCs	• Differentiated into iPSC-MSCs, then tenocyte-like cells. • Mechanosensitive differentiation on aligned ultrafine fibers.	• Addresses donor scarcity. • Enables personalized therapies. • Express tenogenic markers (e.g., SCX, COL1).	• Complex differentiation protocols. • Risk of incomplete tenogenic commitment.	89
Amniotic-Derived Cells	• Include AECs and AMSCs. • Low immunogenicity and anti-inflammatory properties.	• High tenogenic potential (expresses SCX, TNMD). • Minimal ethical concerns. • Dual functionality (tenogenesis and immunomodulation).	• Limited long-term stability data in organoid systems.	67,90–97

Abbreviations: ADSCs: Adipose-derived stem cells; AECs: amniotic epithelial cells; AMSCs: amniotic mesenchymal stromal cells; BGN: Biglycan; COL1: Type 1 collagen; ECM: extracellular matrix; FMOD: Fibromodulin; hUCMSCs: Human umbilical cord mesenchymal stem cells; iPSCs: Induced Pluripotent Stem Cells; MSCs: Mesenchymal Stem Cells; SCX: Scleraxis; TNMD: Tenomodulin; TSPCs: Tendon Stem/Progenitor Cells.

intercellular interactions.[66] Platelet-derived growth factor (PDGF) promotes tenocyte proliferation and migration[46,107] and stimulates angiogenesis without increasing fibrosis.[46] Beyond growth factors, cytokines represent another key regulator in tendon organoid development by mediating intercellular communication and immune responses. Incorporating specific regulatory cytokines into 3D scaffolds has emerged as a promising strategy for organoid cultivation. For example, hydrogels capable of sustained release of the corticosteroid triamcinolone acetonide have been used to modulate chemokines, reduce inflammation, and recruit TSPCs.[108] Together, these biochemical cues are essential for the functional maintenance and injury repair of tendon organoids.

By strategically selecting and combining different biochemical factors, it is possible to control cell signaling pathways and precisely regulate cell fate and behavior, thereby constructing tendon organoids with specific structures and functions. The combined use of multiple biochemical factors is a widely recognized approach.[73] However, current studies predominantly focus on single signaling pathways, although native tendon development and repair rely on spatiotemporally coordinated crosstalk between multiple pathways.[105] For instance, synergistic activation of TGF-β3 and mechanical loading has been shown to enhance tenogenic differentiation more effectively than TGF-β3 alone,[109] underscoring the necessity of combinatorial signal modulation. Future research will further explore the synergistic mechanisms of these biochemical elements, providing more comprehensive theoretical support for the construction of tendon organoids. Another critical limitation lies in the lack of temporal control over biochemical cues. Current organoid systems often apply static biochemical factors, failing to recapitulate this dynamic sequence. Emerging strategies, such as microfluidic gradient generators, offer potential solutions but have yet to be systematically applied to tendon organoids. Such approaches will bridge the gap between reductionist single-factor studies and the complexity of native microenvironments, ultimately enabling the construction of mature, functional tendon organoids.

4.3. Physical factors

The self-assembly of stem cells into organoids requires additional stimuli and the establishment of specific cellular niches to exhibit biological properties similar to those of actual organs. Physical factors play a crucial role in simulating the dynamic environment within native tissues.[110,111] These factors primarily include the selection and physical properties of scaffold materials, as well as the application of biomechanical stimuli. Together, they provide essential support for the development and functional maintenance of tendon organoids.

4.3.1. Scaffolds

Homeostatic collagen degradation is a crucial mechanism for maintaining the dynamic equilibrium of tendon tissue. Under physiological conditions, controlled collagen breakdown creates spatial microenvironments that facilitate the migration and differentiation of stem cells. However, abnormal collagen degradation disrupts the topological architecture of the ECM, initiating a vicious cycle: Disorganized mechanical signaling impairs the directional differentiation of tendon stem cells, driving them to secrete pro-fibrotic factors that exacerbate abnormal collagen cross-linking.[66,112] Our prior work demonstrates that targeting critical nodes in this cycle can halt degenerative progression.[112,113] Consequently, effective scaffold design for tendon organoids must transcend mere structural support and actively address this biological complexity. It must achieve dual objectives—mimicking the phased degradation of healthy collagen to support dynamic cellular demands while simultaneously blocking pathological degradation pathways that compromise ECM and cellular integrity. This necessitates a multifaceted design approach.

Scaffolds provide structural support for stem cells and facilitate the formation of 3D structures that mimic the cellular niches found in native tendon tissue. As mentioned earlier, the ECM is a major component of tendons, with collagen proteins conferring unique mechanical properties and biocompatibility. Studies have shown that optimizing the material composition and structural design of scaffolds can better replicate the physiological environment of tendons, promoting the formation of tendon organoids. Beyond material selection, the design of scaffolds involves deliberate engineering of biomimetic architecture, dynamic degradation profile, mechanical properties, and bioactivity. Tendon regeneration is sensitive to the topology of the substitute,[114] so replicating the highly organized, aligned fibrous structure of native tendons is paramount for guiding cell orientation, force transmission, and ultimately, functional tissue formation.[115] Adequate porosity and interconnectivity are crucial for uniform cell distribution, efficient nutrient/waste exchange, and potential vascularization in larger constructs. The scaffold's degradation kinetics also should be tuned to mirror physiological ECM turnover, creating space for new matrix deposition and cell activity without triggering instability or pathological cascades.[71] Critically, the concept of temporally matched biological constraints has been proposed, where scaffolds are engineered to provide spatially and temporally evolving mechanical cues that actively guide the sequential phases of tendon repair and maturation, as exemplified by studies employing micro-nano hierarchical designs to deliver stage-specific mechanical stimuli.[116] The mechanical properties of stem cell culture substrates, such as stiffness and adhesiveness, significantly influence key cellular behavior, including adhesion, survival, migration, proliferation, and differentiation *in vitro*, so the scaffold should possess mechanical properties (e.g., stiffness, elasticity, strength) appropriate for tendon development.[117]

To translate these design principles into practice, the choice of scaffold material is crucial, as it must support cellular functions while providing appropriate mechanical and biochemical cues. Ideal scaffolds should support cell migration, proliferation, and matrix deposition.[118] They must also exhibit excellent biocompatibility, biodegradability, and mechanical performance. In organoid research, Matrigel—a reconstituted basement membrane matrix rich in laminin, collagen IV, and growth factors—has been extensively used as a default scaffold for diverse organoids due to its ability to support 3D self-organization.[119-121] Its composition, rich in basement membrane proteins, enables it to mimic the ECM, while also containing biochemical factors that enhance cell-matrix interactions and promote tissue development. However, due to the complex composition of Matrigel, proteomic analyses have revealed considerable variability.[122] In addition, batch-to-batch differences in mechanical properties, such as elastic modulus, can lead to inconsistent local mechanical performance in 3D culture systems, significantly impacting organoid cultivation.[122,123] These limitations underscore the need for more defined and tunable scaffold systems specifically engineered for tendon organoids.

Hydrogels, which are highly hydrated polymer networks, have been widely used in the *in vitro* construction of organoids.[18] Their high-water content mimics the native ECM environment, and their physicochemical properties can be extensively customized through design. Hydrogel scaffold materials can be derived from natural, synthetic, or composite sources. Natural materials, such as collagen,[124] gelatin,[125] silk fibroin,[115] hyaluronic acid,[126] and decellularized tendon slices, are commonly used in tendon tissue engineering due to their biocompatibility and biodegradability. However, natural materials often lack sufficient mechanical strength and have degradation rates that are difficult to control precisely. To address these limitations, they are frequently combined with other materials to modify their properties. Synthetic materials, including polylactic acid, polyglycolic acid, and polyethylene glycol (PEG), can be chemically modified to introduce functional groups and enhance bioactivity, of which the biocompatibility is often inferior to that of natural hydrogels. Consequently, composite materials have gained popularity.[72,88,127] This composite approach enables the independent tuning of mechanical properties, degradation rate, and bioactivity. For example, one study utilized rigid aramid nanofibers and flexible polyvinyl alcohol to create a highly aligned network that mimics the

microstructural interactions between collagen fibers and soft proteoglycans.[127] The resulting anisotropic composite hydrogel exhibited high mechanical performance comparable to native tendons while maintaining a water content of approximately 60%, similar to actual tendon tissue. Composite hydrogels combine biocompatibility with ideal mechanical properties, and their performance can be precisely tuned by adjusting the proportions of their components. The strategy of constructing organoids using hydrogels has been successfully applied to the development of various organoids, including cartilage organoids.[121] Notably, recent work by Zhang *et al.*[66] further underscores the potential of 3D hydrogel systems for constructing tendon organoids. By encapsulating TSPCs in a collagen-hyaluronic acid composite hydrogel, their study revealed that the 3D microenvironment dynamically regulates TSPC subpopulations via FGF7-mediated mechanosignaling, demonstrating the potential of hydrogel in constructing tendon organoids.

Native tendons possess a highly organized, aligned fibrous structure, making the architectural design of scaffolds critical for mimicking the spatial organization of the actual cellular microenvironment. To replicate this complex structure, a variety of advanced fabrication techniques have been developed and applied in tendon tissue engineering and the construction of organoids.

Electrospinning techniques utilize a high-voltage electric field to draw polymer solutions or melts into ultrafine fibers collected on a grounded target.[128] This technique enables the fabrication of scaffolds composed of aligned microfibers specifically designed to replicate the diameter range (typically 1–20 μm) and oriented arrangement of collagen fibers found in natural tendons.[129,130] This dimensional and architectural mimicry is crucial, as it promotes directional cell growth, enhances functional expression (e.g., tenogenic marker production and ECM alignment), and has been employed in numerous studies of tendon tissue engineering. Nanofiber scaffolds fabricated using electrospinning techniques can replicate the aligned fiber arrangement of natural tendons,[130,131] promoting directional cell growth and functional expression. Adjusting the porosity (60–90%) of scaffolds facilitates uniform cell distribution, nutrient diffusion, and vascularization, addressing the mass transport design requirement. In addition, nanoscale roughness or microgroove structures can guide cell alignment and differentiation, enhancing the mechanical properties of tendon organoids. While powerful for creating 2D mats or thin 3D structures, achieving thick, truly 3D constructs with deep cellular infiltration remains a challenge with standard electrospinning.[132]

Wet-spinning involves extruding a polymer solution through a spinneret into a coagulation bath, causing the polymer to precipitate or solidify, forming continuous filaments or fibers.[128] Wet-spinning is highly effective for producing fibers with larger diameters (tens to hundreds of micrometers) and high mechanical strength, making it suitable for generating bundles or yarns that better mimic the fascicular structure of tendons.[133] By controlling the coagulation kinetics and applying post-stretching, significant alignment and enhanced mechanical properties can be imparted to the fibers. Aligned wet-spun fiber bundles have been used as core scaffolds to guide tenocyte alignment and promote organized ECM deposition.[133,134] Wet spinning facilitates the fabrication of highly biocompatible fibers by avoiding the use of toxic reagents during the spinning process; however, compared with those in electrospinning scaffolds, it may be challenging to attain desirable mechanical properties for the as-prepared fibers.[132] Electrohydrodynamic Jetting is an advanced form of electrospinning that employs precise control over the jetting process using a smaller nozzle and lower flow rates. This level of spatial control surpasses traditional random electrospinning and allows for the fabrication of scaffolds with complex, predefined architectures,[135] that can meticulously replicate specific tendon tissue geometries or create tailored mechanical microenvironments.[136] It offers exceptional precision for patterning bioactive molecules or multiple cell types within the scaffold. However, the process can be slower than bulk electrospinning and requires sophisticated control systems.

The selection of the optimal fabrication technique depends on the specific design goals for the tendon organoid, balancing factors such as the required fiber diameter/alignment, mechanical strength, structural complexity (2D vs. 3D), resolution, biomimicry level, porosity, and integration with cells/biomolecules. Combining techniques is also a promising strategy to leverage their respective strengths.[116,136,137]

4.3.2. Mechanical stimulation

The physiological function of tendons is highly dependent on their mechanical properties. Studies have shown that the response of tendon cells to mechanical stress significantly influences their phenotype and function. Cyclic mechanical stretching promotes collagen synthesis and tenogenic differentiation in tendon cells,[131,138] enhances the alignment of the ECM, and improves the mechanical properties of tendon organoids. Research investigating the effects of mechanical deprivation on tendons has demonstrated that mechanical stimulation plays a critical role in the formation of fibril bipolar structures and collagen remodeling.[139] Furthermore, mechanical stretching on 3D simulated scaffolds has been shown to promote the expression of tenocyte phenotypes.[138] In addition to bioreactors for applying mechanical stimuli, microfluidic systems can simulate *in vivo* hydrodynamic environments,[62] providing precise mechanical stimulation

for tendon organoids. Appropriate mechanical stimuli can induce cells to differentiate into tenocyte phenotypes, which is crucial for the maturation and functionalization of tendon organoids.

Future organoid platforms should aim to model both fibrotic and healthy tendon microenvironments, enabling interventions to disrupt degenerative cycles while promoting physiological collagen remodeling. This approach allows stage-specific customization of therapies tailored to the progression of tendon degeneration. To fibrosis-specific organoids, although resembling native fibril morphology, their disordered collagen cross-linking results in compromised mechanical properties. Such models can evaluate fibrosis-associated molecular events or test therapeutic strategies. By comparing responses between healthy and fibrotic organoids—such as collagen reorganization efficiency under mechanical loading—critical thresholds in pathological progression can be identified, offering precise targets for clinically reversing fibrosis. By optimizing the mechanical environment, the physical properties of the ECM, the selection of scaffold materials, and the application of biomechanical stimuli, it is possible to replicate the physiological and pathological processes of tendons, promoting the formation and functionalization of tendon organoids. However, the widespread implementation of these approaches faces significant challenges. A major limitation lies in the lack of standardized engineering platforms tailored for tendon organoid culture, which require seamless integration of scaffold customization and dynamic loading of biomechanics.[140] Current systems often depend on bulky bioreactors or 2D stretching devices that fail to recapitulate the 3D multiaxial mechanical cues (e.g., tension, shear, and compression) experienced by tendons *in vivo*.[141-143] Moreover, scalable and user-friendly technologies for applying spatially resolved mechanical stimuli within high-throughput organoid arrays are still underdeveloped, hindering systematic exploration of mechanobiological responses.[144] Future research should further explore the synergistic mechanisms of these physical factors while addressing these engineering challenges, providing more comprehensive theoretical and technical support for the construction of tendon organoids.

4.4. Engineering strategies

As outlined above, the construction of tendon organoids involves the multidimensional synergy of cells, materials, mechanical environments, and biochemical signals. Advanced engineering technologies enable precise design and optimization from the molecular level to the macroscopic scale. Through interdisciplinary collaboration and technological innovation, engineering strategies provide the technical foundation for constructing tendon organoids and drive the field toward greater refinement (Figure 2).

4.4.1. Optimizing materials for omics and gene editing technologies

The optimization of cell sources, scaffolds, and biochemical factors is critical for enhancing the functionality and biomimicry of organoids. Single-cell RNA sequencing (RNA-seq) technology can resolve the heterogeneity of stem cell populations,[32,33] identifying the most suitable stem cell subpopulations for tendon differentiation as seed cells for tendon organoid construction. Single-cell analysis can characterize the gene expression profiles of individual cells isolated from tendon tissue, revealing subpopulations of nestin$^+$ TSPCs with strong tenogenic potential,[33] which can be utilized for tendon organoid development. Studies using RNA-seq have demonstrated that changes in the microenvironment during 3D culture of human TSPCs affect the proportions of different cell subpopulations, thereby regulating stem cell function.[66] Analyzing epigenetic modifications in stem cells, such as DNA methylation and histone modifications, can reveal key epigenetic mechanisms governing tendon differentiation, optimizing the directed differentiation of stem cells. By examining gene expression profiles under different biochemical treatments, the most effective combinations of small molecules for promoting tendon differentiation can be identified. Similarly, analyzing protein expression profiles on scaffold surfaces can help select materials that enhance cell adhesion, proliferation, and differentiation.[145,146] For example, proteomic studies have demonstrated that lower material stiffness promotes tenogenic differentiation in stem cells, providing evidence for optimizing the design of bioactive scaffolds in tendon tissue engineering.[146] The application of novel genome editing technologies and high-throughput screening methods further supports the optimization of stem cell culture conditions.

Organ-on-a-chip technology is a biomimetic platform based on microfluidics that can simulate the microenvironment of human tissues,[61,62] offering a novel tool for the construction and investigation of tendon organoids. By integrating cells, scaffold materials, and mechanical stimuli into a microchip, this technology precisely regulates the microenvironment of tendon organoids, enabling dynamic research on their development, function, and pathological processes. Leveraging microfluidic technology, this platform facilitates accurate drug delivery and mimics *in vivo* fluid transport, while also applying dynamic stretching or shear forces to replicate the mechanical environment of tendons within the body. This approach accurately simulates the biochemical and physical conditions of actual organs, supporting high-throughput screening and drug testing.[147-149] In addition, organ-on-a-chip technology can

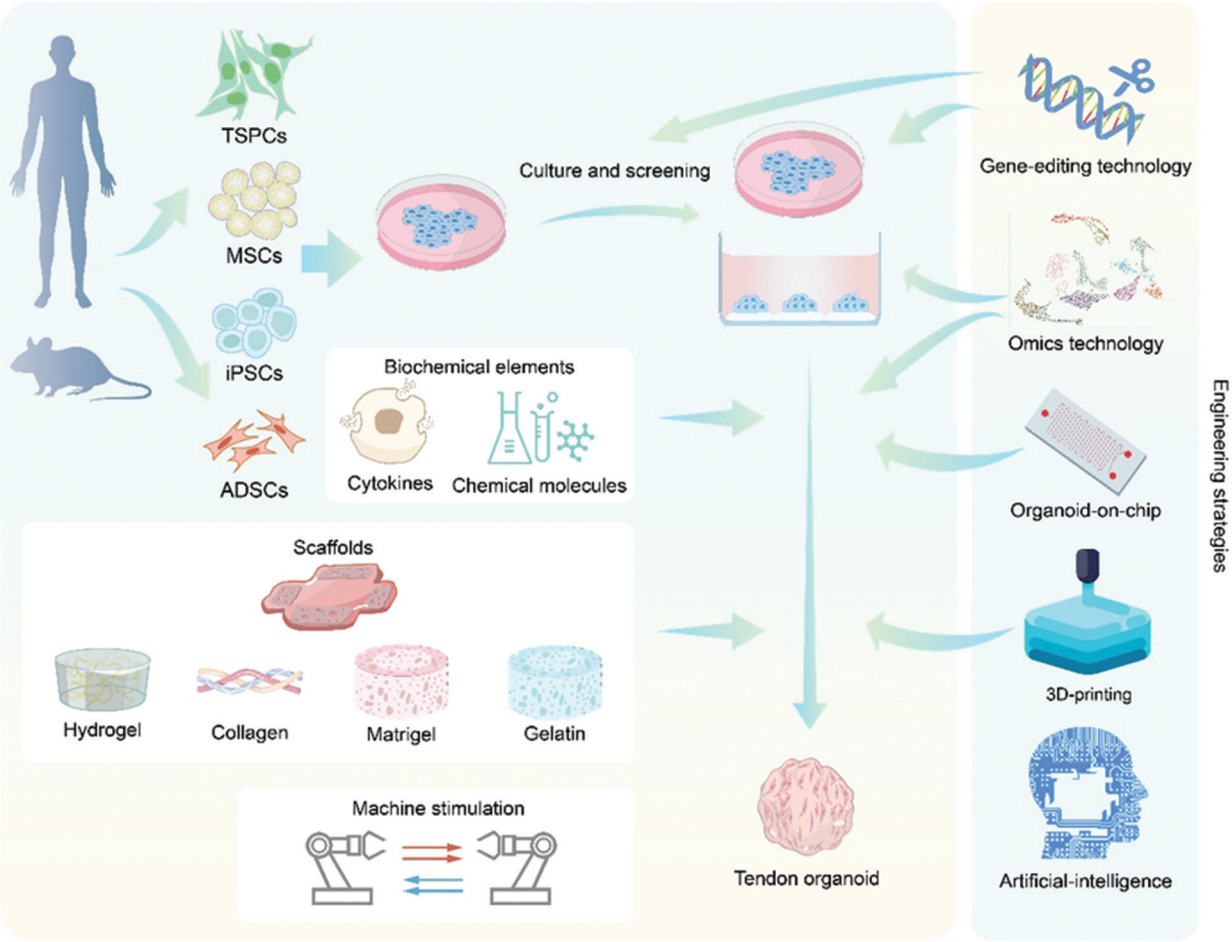

Figure 2. Construction workflow and multidimensional synergistic strategies for tendon organoids. This schematic integrates four core modules—cell selection, biochemical regulation, physical microenvironment design, and engineering strategies—to systematically outline the construction of tendon organoids. This integrated workflow emphasizes the multidimensional synergy of cellular, biochemical, biomechanical, and engineering strategies, establishing a biomimetic platform for constructing functional tendon organoids. These organoids advance applications in regenerative medicine and pathological mechanism research by replicating native tendon physiology and enabling high-precision therapeutic development. Created with Adobe Illustrator, Yiwen Xue (2025) https://imgur.la/images/2025/09/09/fig2.jpg.
Abbreviations: 3D: Three-dimensional; ADSCs: Adipose-derived stem cells; iPSCs: Induced pluripotent stem cells; MSCs: Mesenchymal stem cells; TSPCs: Tendon stem/progenitor cells.

integrate tendon cells with other relevant tissues, such as muscle, bone, and blood vessels, on the same chip, enabling the study of multi-tissue collaborative construction.[147,150]

4.4.2. Optimizing assembly for three-dimensional printing

Three-dimensional bioprinting technology offers capabilities in high-precision cell and material deposition, enabling the rapid fabrication of complex 3D structures. By selecting suitable bioink materials, including natural polymers, synthetic polymers, or composites,[151] stable and tunable tissue engineering scaffolds can be constructed.[152] Bioinks used in 3D bioprinting form 3D structures through cross-linking, achieving the strength and stability required to maintain print fidelity and resolution. The choice of bioink materials determines the mechanical and biological properties of the scaffold, while the printing design dictates the spatial arrangement of seeded cells. Decellularized ECM[87,153] and biocompatible hydrogels[151] are commonly used as materials for 3D bioprinting. For instance, one study adjusted the viscosity of decellularized tendon ECM as a bioink,[153] enhancing 7-day cell viability and enabling the fabrication of complex 3D organoid structures. The application of novel bioink materials with improved biocompatibility and biodegradability,[151,154] such as a combination of gelatin methacrylate/alginate methacrylate/hydroxyapatite, enables the construction of highly complex ECM analogs.[154] These advancements have progressively improved stem cell viability and better replicated the native organ niche.

Recent advancements in digital light processing-based lithography printing have made it easier to produce high-

resolution, freeform lattice and patterned structures compared to other 3D printing methods. David Collins' team developed a 3D printing technique called "Dynamic Interface Printing",[155] which rapidly generates centimeter-scale, single-cell-resolution 3D structures in seconds. This method successfully printed objects measuring 3 cm in diameter and 7 cm in length, with a resolution of 15 μm. The progress in 3D printing technology facilitates the simulation of complex tissue architectures and optimizes the spatial configuration of tendon organoid construction.

4.4.3. Prediction, evaluation, and optimized design for data analysis based on AI

The application of data analysis technologies provides powerful tools for optimizing design. By integrating multi-source data, including omics, mechanical properties, and imaging data, and combining advanced algorithms and models, researchers can extract critical information from vast datasets[156] to optimize the design parameters of tendon organoids,[157] thereby enhancing their biomimicry and functionality.

Organoid research primarily encompasses three aspects: Construction strategies, data analysis, and efficacy verification.[12] In particular, AI and deep learning, with their robust capabilities in big data processing, algorithmic computation, and self-learning, can significantly accelerate the development of tendon organoid research.[158,159] AI can process and integrate large volumes of data involved in tendon organoid construction, enabling predictive modeling and design optimization. Moreover, automation facilitated by AI also simplifies experiments, improves reproducibility, and reduces experimental bias.[158] Trained AI algorithms can analyze and extract features from extensive imaging data,[160,161] reconstruct 3D structures of tendon organoids from 2D imaging data, and monitor dynamic construction processes in real time. In addition, AI can screen ideal small molecule combinations and suitable cell subpopulations from massive omics datasets.[162-164] By integrating multi-dimensional data, AI can simulate biological processes and use algorithms to analyze and predict tendon organoid construction at both microscopic (cellular) and macroscopic (tissue) levels. Based on these predictions, AI can optimize materials, structures, and culture conditions.[165] For example, in a study on AI-assisted 3D printing of biomimetic tendon interfaces, researchers used AI-trained algorithms to define printing parameters. By adjusting exposure time and light intensity during the 3D printing process, Kiratitanaporn *et al.*[166] tailored the local mechanical properties of the scaffold to match those of different regions in native tendon tissue. Deep computing and AI-assisted design offer powerful tools for constructing tendon organoids by simulating complex biological and mechanical processes, optimizing design parameters, and accelerating experimental iterations, thereby significantly improving design efficiency and functionality. Future research will further explore the synergistic application of deep computing and AI-assisted design with other engineering strategies, such as omics analysis and 3D printing, to provide comprehensive support for tendon organoid construction.

5. Applications

Although still in the early stages of development, tendon organoids offer significant advantages over traditional 2D tissue cultures and animal models, as they more accurately replicate key aspects of tendon biology *in vitro*. These organoids provide structured and reproducible cellular constructs that mimic the 3D architecture and cellular composition of human tendons. As Figure 3 demonstrates, tendon organoids are valuable tools for applications in regenerative medicine, disease modeling, drug screening, and biomechanics.

5.1. Regenerative medicine

Tendinopathy, rotator cuff tears, and Achilles tendon ruptures are common tendon injuries that often result in chronic pain, restricted mobility, and reduced quality of life.[167] Current treatments, such as autografts and allografts, face significant limitations, including donor tissue shortages, immune rejection, and incomplete tendon strength restoration.[168]

Tendon organoids offer a promising regenerative solution by enabling the engineering of functional grafts that replicate the biological and biomechanical properties of damaged tendons. Typically, the process begins with patient-derived TSPCs, which are cultured under controlled conditions to differentiate into tendon-like cells, or tenocytes. Compared to direct cell therapy, organoids more closely resemble native tendon tissue, demonstrating higher survival rates and improved integration potential.

Integration is further enhanced by vascularization strategies, such as PDGF-induced angiogenesis, which promotes blood vessel formation without excessive fibrosis.[169] As a result, tendon organoids exhibit prolonged therapeutic effects while requiring fewer transplanted cells.[170] They can also incorporate features like gradient stiffness to mimic the transition zones between tendons and bones or muscles.[171]

Unlike traditional grafts, they can be patient-specific, reducing the risk of immune rejection and enhancing tissue integration. Moreover, by leveraging biochemical cues and growth factors, they facilitate the production of essential ECM components, such as collagen, to support tendon regeneration.[172,173]

By combining these elements, tendon organoids bridge the gap between biological complexity and clinical

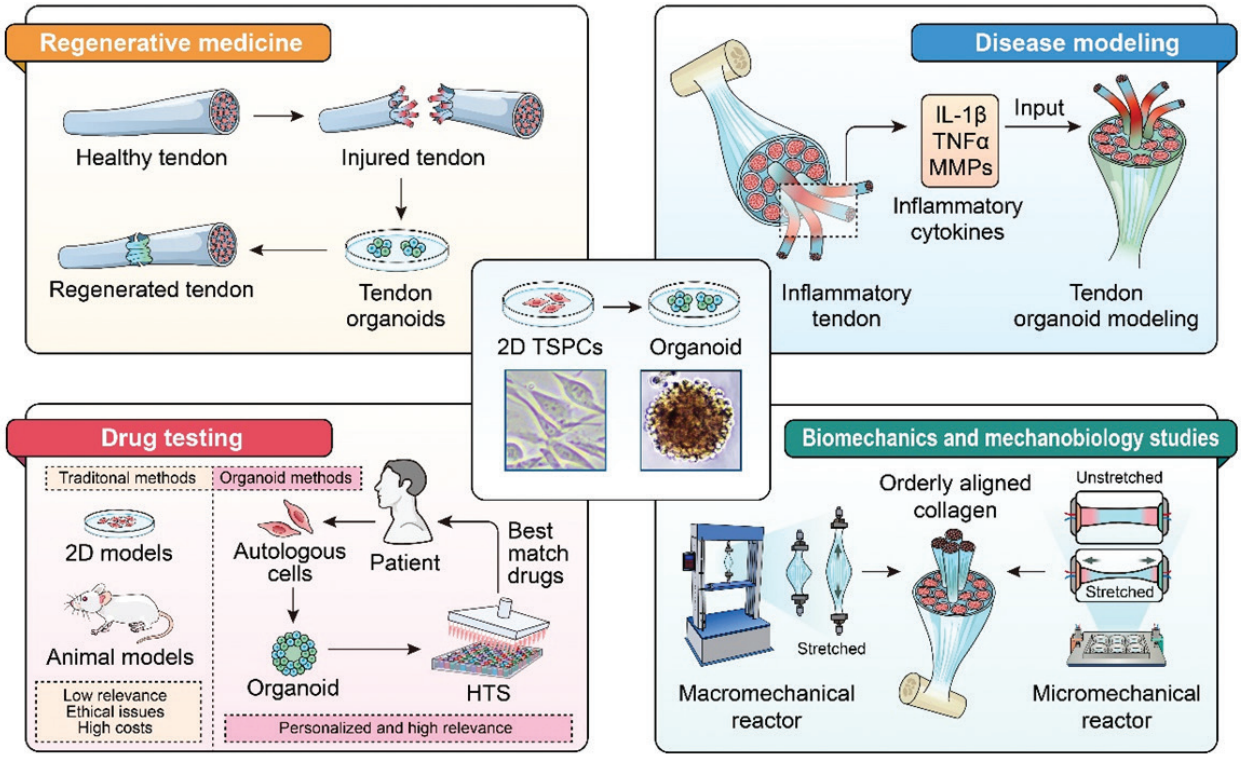

Figure 3. The diverse applications of tendon organoids. At the center, a schematic representation and electron microscopy images of tendon organoids provide an overview of their structure. The top left panel illustrates regenerative medicine, where tendon organoids are transplanted into injured tendons to promote healing and tissue regeneration. The top right panel is about disease modeling, where inflammatory tendons exhibit elevated levels of specific inflammatory factors, TNF-α, IL-1β, and MMPs. By introducing these factors into tendon organoid cultures, researchers can simulate pathological conditions, enabling *in vitro* exploration of inflammation mechanisms and signaling pathways. The bottom left panel shows a comparison between traditional methods and organoid-based approaches in terms of drug testing. Conventional models, including 2D cultures and animal studies, often suffer from low relevance to human biology, ethical concerns, and high costs. In contrast, organoid-based methods involve isolating patient-derived tendon cells, inducing them into organoids, and utilizing them for high-throughput drug screening. This strategy enhances relevance, eliminates ethical issues, and enables personalized medicine by identifying the most effective treatment for each patient. The bottom right panel demonstrates biomechanics research, where tendon organoids are subjected to tensile force using a stretch bioreactor to investigate optimal biomechanical conditions for growth and repair. The findings inform rehabilitation strategies, ensuring that controlled mechanical stimulation—such as appropriate exercise—enhances tendon recovery. Created with Adobe Photoshop Yixi Wu, Zi Yin (2025) https://imgur.la/images/2025/09/09/figure3.jpg.
Abbreviations: 2D: Two-dimensional; HTS: High-throughput screening; IL-1β: Interleukin-1 beta; MMPs: Matrix metalloproteinases; TNF-α: Tumor necrosis factor-alpha; TSPCs: Tendon stem/progenitor cells.

application. They provide a versatile platform for refining regenerative therapies while offering a more effective and personalized solution for tendon repair.

5.2. Disease modeling

Tendon organoids serve as a powerful *in vitro* platform for studying various tendon disorders, including tendinopathy, rotator cuff injuries, Achilles tendon ruptures, and chronic tendon degeneration. These conditions often result from overuse, aging, or systemic diseases, such as diabetes, which compromise tendon structure and function.[167] Tendinopathy, for example, is characterized by chronic inflammation, matrix degradation, and collagen disorganization, leading to pain and reduced mobility.[174] Similarly, chronic rotator cuff tears involve progressive collagen breakdown and cellular senescence, further complicating repair. By providing a controlled research environment, tendon organoids enable the investigation of these pathological processes and their underlying mechanisms.[175]

Emerging evidence suggests that tendinopathy comprises distinct subtypes, each with unique pathological characteristics. This distinction underscores the need for subtype-specific models to enhance understanding of disease mechanisms and treatment responses. Tendon organoids offer a versatile alternative to *in vivo* models, enabling the precise replication of different disease states.[176] Pathological conditions in tendon organoids can be recreated by modulating biochemical, mechanical, and cellular factors. Inflammatory and catabolic environments are simulated by introducing key cytokines and enzymes, such as tumor necrosis factor-alpha (TNF-α),

interleukin-1β (IL-1β), and matrix metalloproteinases (MMPs).[177] Mechanical stress—such as cyclic stretching, overloading, or shear force—is applied using bioreactors to mimic repetitive strain and mechanical overuse, which contribute to tendon injuries.[170]

Aging, a major factor in tendon degeneration, can be modeled using senescent cells or oxidative stress inducers, such as hydrogen peroxide, allowing researchers to examine age-related changes in tenocyte function, including reduced matrix production and increased susceptibility to damage.[169] Similarly, genetic predispositions are investigated by introducing specific mutations or using patient-derived cells with known genetic risk factors, providing insights into the genetic and epigenetic influences on tendon maintenance and degeneration.[178]

Tendon organoids facilitate the study of critical processes, such as inflammation, collagen disorganization, and impaired matrix remodeling. Furthermore, they serve as a testing platform for novel therapeutic strategies, including inhibitors of catabolic enzymes, matrix-repair agents, and anti-inflammatory treatments. By bridging the gap between basic research and clinical applications, tendon organoids represent a valuable tool for understanding disease progression and developing targeted therapies for tendon disorders.

5.3. Drug testing

Tendon organoids are valuable tools for evaluating the safety and efficacy of drugs targeting tendon injuries and degenerative diseases. Current clinical treatments for tendon pathologies include non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and naproxen, corticosteroid injections, and platelet-rich plasma (PRP) therapy. While NSAIDs and corticosteroids alleviate pain and inflammation, they do not repair structural tendon damage and may even hinder healing with prolonged use. PRP therapy, which delivers growth factors, such as PDGF and TGF-β, to promote tendon repair, has shown inconsistent outcomes.[171] These variations may stem from differences in disease stages or subtype heterogeneity, suggesting that classification-based screening using tendon organoids could improve treatment evaluation.

The limitations of existing therapies underscore the need for next-generation drugs targeting key molecular pathways involved in tendon repair. Potential approaches include enhancing collagen synthesis, preventing matrix degradation, and modulating inflammation with greater specificity. The Connectivity Map (CMap) database, a large-scale computational drug discovery tool, compares disease-associated gene expression profiles with drug-induced expression patterns to identify therapeutic candidates. While CMap has successfully facilitated drug repurposing, primarily in oncology research.[179] However, its application to tendon diseases remains limited due to the absence of tendon-specific cellular and drug response datasets. Establishing specialized databases to capture interactions between therapeutic agents and tendon-specific cells could significantly improve targeted drug development.[180]

Drug discovery using tendon organoids begins with constructing models that replicate the native properties of tendons. Researchers culture organoids with aligned collagen fibers and apply mechanical stimuli to simulate physiological forces. Pathological conditions are recreated by introducing inflammatory cytokines, including IL-1β and TNF-α, or proteolytic enzymes like matrix MMP-13, enabling controlled testing of drug candidates.[181]

Current drug candidates for tendon repair include small-molecule inhibitors of MMPs to reduce collagen degradation and growth factors, such as connective tissue growth factor, to enhance ECM production and accelerate healing. In addition, biologics that regulate inflammation—such as IL-10 mimetics or inhibitors of proinflammatory cytokines—offer potential solutions for controlling inflammation without impairing tendon regeneration.[182]

Tendon organoids also hold promise for personalized medicine. Patient-derived TSPCs or tenocytes can be used to generate customized organoids, allowing researchers to assess drug responses based on individual genetic and epigenetic factors. For example, organoids derived from patients predisposed to tendinopathy or chronic inflammation can identify the most effective treatments for specific conditions. This personalized approach moves beyond the one-size-fits-all model, optimizing therapeutic outcomes.[183]

Tendon organoids streamline drug discovery by providing a human-relevant, reproducible, and cost-effective model. They reduce reliance on animal studies, which often fail to fully replicate human tendon biology, and enable high-throughput screening of therapeutic candidates. By simulating the tendon microenvironment, organoids facilitate rigorous preclinical evaluation of new therapies for safety and efficacy before progression to clinical trials.

5.4. Biomechanics and mechanobiology studies

Tendon organoids serve as a powerful model for investigating the interaction between cells and mechanical forces, a fundamental aspect of tendon biology. By applying cyclic stretching or other mechanical stimuli, researchers can replicate the mechanical loading conditions that tendons experience *in vivo*. This enables the study of how mechanical forces regulate matrix organization, collagen fiber alignment, and tenocyte differentiation.[143]

Mechanical loading plays a crucial role in maintaining the structure and function of tendons. When subjected

to controlled mechanical stress, tendon organoids exhibit aligned collagen fibers, closely resembling the hierarchical organization of native tendons. This structural adaptation enhances tissue strength and improves its capacity to transmit mechanical forces. Conversely, insufficient mechanical stimulation results in disorganized collagen networks and weaker matrix composition, mirroring pathological conditions, such as tendon injuries or immobilization.[182] These studies provide valuable insights into how tendons respond to mechanical forces under both physiological and pathological conditions.

Excessive mechanical stress contributes to overuse injuries, such as tendinopathy, whereas inadequate loading during recovery can impede healing.[143] Tendon organoids enable researchers to explore these biomechanical dynamics in a controlled setting, identifying optimal mechanical loading thresholds for tendon repair and injury prevention.

Research utilizing tendon organoids to model mechanical loading has significant implications for rehabilitation and sports medicine. Understanding the effects of varying mechanical stimuli on tendon cells and tissue can inform the development of effective therapeutic strategies. These findings could enhance physical therapy protocols, optimize athletic training regimens, and advance the engineering of tendon constructs for clinical applications.

6. Future directions

As tendon organoid research advances from foundational discoveries to translational applications, three interconnected themes—AI, scalable manufacturing standardization, and clinical translation—are expected to drive its future development. This section examines emerging trends, technological synergies, and remaining challenges that will shape the next generation of tendon organoid systems (Figure 4).

6.1. AI-powered revolution in tendon organoid design and analysis

The incorporation of AI into tendon organoid research has revolutionized traditional trial-and-error methodologies, replacing them with data-driven, predictive frameworks. Conventional approaches for optimizing culture conditions, scaffold properties, and mechanical stimulation protocols rely on iterative experimentation, a process that is both time-consuming and resource-intensive.[183] Machine learning (ML) now enables the rapid analysis of multidimensional datasets—spanning transcriptomics, proteomics, and biomechanical metrics—to predict optimal conditions for tenogenic differentiation and collagen fibril alignment, both essential for functional tendon development.[12] For example, deep neural networks trained on high-content imaging data can detect subtle morphological patterns in tendon organoids, such as early markers of collagen organization or cell-matrix interactions, which may escape human observation.[54] These models also predict synergistic combinations of growth factors (e.g., TGF-β3 and FGF2) and mechanical loading regimens, expediting the maturation of tendon-like tissues *in vitro*.[184]

A breakthrough in AI-driven tendon research lies in the *in silico* design of biomaterials. Generative adversarial networks simulate the performance of synthetic or decellularized ECM scaffolds under varying stiffness and topographical conditions, significantly reducing reliance on physical prototyping.[185] This approach aligns with advances in hydrogel design, where AI-driven platforms predict polymer compositions that mimic native tendon ECM, optimizing elasticity, porosity, and degradability.[186] For example, Bai *et al.*[12] demonstrated that ML-guided optimization of synthetic hydrogels, such as PEG-based matrices, enhances organoid scalability while preserving biomechanical fidelity. These AI-driven methodologies accelerate discovery while also reducing the need for animal experimentation, aligning with ethical research priorities.[187]

Despite these advancements, challenges remain in data standardization and model interpretability. Variability in data formats across laboratories and the "black box" nature of deep learning models hinder reproducibility and translational potential.[188] Emerging solutions, such as federated learning systems, enable decentralized model training without compromising data privacy, addressing intellectual property concerns while fostering global research collaboration. Initiatives like the Organoid Cell Atlas provide standardized lineage-specific markers and differentiation protocols, ensuring cross-institutional validation of AI-generated insights.[189]

6.2. Scalable and standardized manufacturing

The clinical translation of tendon organoids depends on overcoming scalability and reproducibility challenges inherent in current protocols. Most laboratory-scale systems rely on manual handling, resulting in batch-to-batch variability in cellular composition and ECM organization. To mitigate these inconsistencies, the field is shifting toward automated bioreactor platforms equipped with closed-loop feedback systems. Microfluidic devices that precisely regulate biochemical gradients, coupled with robotic handling systems, offer a scalable approach to standardizing organoid maturation across thousands of replicates.[190]

A key component of this transition is the establishment of universally accepted quality control (QC) metrics. While metrics, such as collagen type I/III ratios and tensile modulus, provide functional benchmarks, they often fail to capture the complexity of native tendon hierarchy. Advanced QC

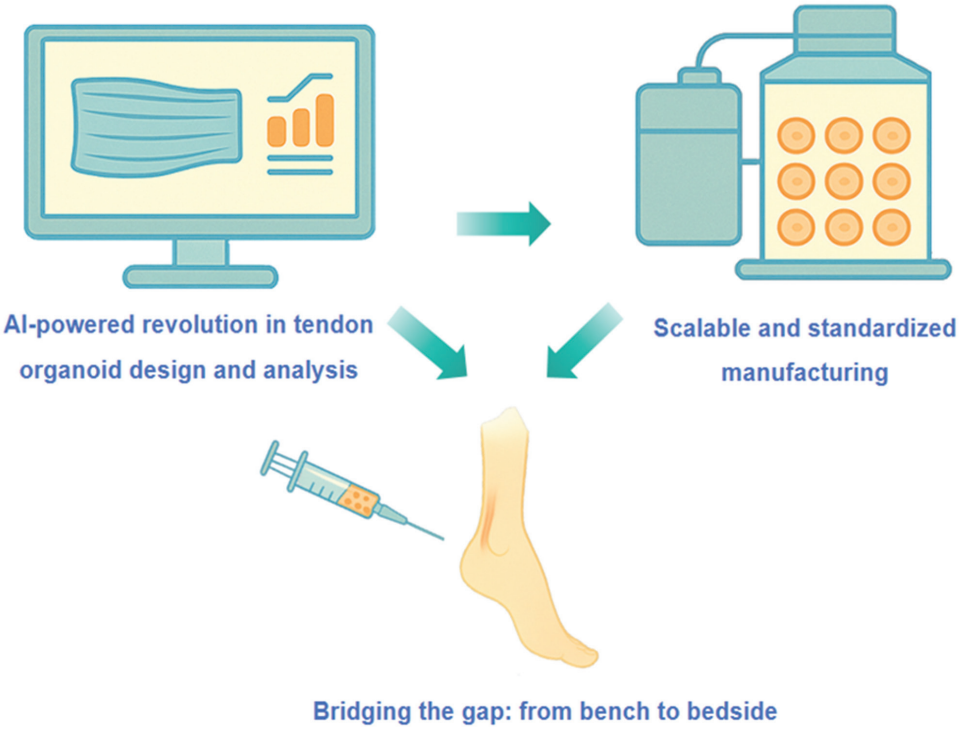

Figure 4. Integrated pipeline for tendon organoid development and clinical translation. A conceptual workflow illustrating the progression from *in silico* design to therapeutic application. Left, AI-powered platforms integrate multi-modal datasets to guide the design of biomimetic tendon organoids. Bottom, engineered organoids are implanted into injury sites—such as the Achilles tendon—highlighting the translational path from bench to bedside. Right, scalable and standardized biomanufacturing ensures reproducibility and throughput. Image created by the authors. Created with Microsoft PowerPoint Yixi Wu (2025) https://imgur.la/images/2025/09/16/7D58E7DD-B2D3-4FAB-87C4-34F462FDF01E.png.
Abbreviation: AI: Artificial intelligence.

pipelines incorporating multiphoton microscopy (for 3D ECM visualization) and Raman spectroscopy (for molecular fingerprinting) are being explored. The recent advent of organoid-on-a-chip systems with embedded sensors allows non-destructive monitoring of oxygen tension, pH, and mechanical forces during culture—a prerequisite for Good Manufacturing Practice compliance.[191]

Standardization efforts must also address biological variability. Donor-specific differences in progenitor cells (e.g., tendon-derived stem cells vs. iPSCs) significantly impact organoid phenotypes. Clustered regularly interspaced short palindromic repeats-based synthetic biology tools offer a solution by introducing genetic "kill switches" or homogenizing pathways, such as yes-associated protein/transcriptional co-activator with PDZ-binding motif signaling, to reduce clonal heterogeneity. International consortia, akin to the Organoid Cell Atlas initiative, are needed to define lineage-specific markers and differentiation protocols validated across institutions.[192]

6.3. Bridging the gap from bench to bedside

The ultimate test for tendon organoids lies in their ability to address unmet clinical needs, particularly in treating chronic tendinopathies and large-scale tendon ruptures. However, translating these advances into clinical therapies faces multiple hurdles across biological, engineering, and regulatory domains. Biologically, most organoids lack the vascular and neural networks essential for integration into host tissues. Emerging strategies include co-culturing with endothelial progenitor cells to prevascularize constructs. For instance, co-culturing human umbilical vein endothelial cells with stem cells has led to the formation of vascular systems within organoids.[193] In addition, incorporating piezoelectric materials that generate electrical stimuli mimicking native mechanotransduction is being explored.

In parallel with efforts to overcome these fundamental challenges, researchers are advancing the translational potential of tendon organoids through personalized and reconstructive approaches. Personalized medicine represents a key frontier. Patient-derived organoids could serve as avatars for drug screening, predicting individual responses to therapies like PRP or sclerosing agents. In reconstructive applications, 3D bioprinting of organoid-laden scaffolds tailored to a patient's anatomical defect (e.g., rotator cuff geometry) is being explored. Early-stage trials have demonstrated feasibility in rodent models, where bioengineered tendon organoids seeded on aligned nanofibrous meshes restored ~70% of native tensile strength post-implantation.[191]

As tendon organoids move closer to clinical application, regulatory and economic considerations become increasingly critical. Regulatory pathways, however, remain nebulous. Regulatory agencies like the Food and Drug Administration (FDA) currently lack specific guidelines for organoid-based products. In December 2022, the United States enacted the FDA Modernization Act 2.0, amending the Food, Drug, and Cosmetic Act to remove the compulsory requirement for animal experimentation in drug development. This legislative change permits the use of alternative methods, including organoid models, for evaluating drug safety and efficacy before human clinical trials. More specific official regulations for replacing animals with organoids in experiments are forthcoming, necessitating collaborations between researchers and policymakers.[194] Adaptive licensing frameworks, similar to those proposed for gene therapies, may accelerate translation while ensuring safety. Concurrently, cost-effectiveness analyses must address whether organoid-based therapies offer advantages over existing options, such as autografts, particularly in resource-limited settings.

7. Conclusion

The maturation of tendon organoid technology will depend on synergistic advances across AI, manufacturing, and translational science. Cross-disciplinary collaborations—material scientists working with ML experts, clinicians partnering with regulatory specialists—will be indispensable. As these systems evolve from simplified models to functionally graded tissues recapitulating the enthesis (tendon-to-bone interface), they may unlock therapies for historically intractable conditions, such as degenerative rotator cuff disease. Ultimately, the journey from petri dish to patient embodies the promise of regenerative medicine: Not Merely to repair, but to rebuild life's structural masterpieces with cellular precision.

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Conflict of interest

The authors declared that they have no competing interests.

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Availability of data

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