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3D printing in musculoskeletal interface engineering: current progress and future directions

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The musculoskeletal system relies on critical tissue interfaces for its function; however, these interfaces are often compromised by injuries and diseases. Restoration of these interfaces is complex by nature which renders traditional treatments inadequate. An emerging solution is three-dimensional printing, which allows for precise fabrication of biomimetic scaffolds to enhance tissue regeneration. This review summarizes the utility of 3D printing in creating scaffolds for musculoskeletal interfaces, mainly focusing on advanced techniques such as multi-material printing, bioprinting, and 4D printing. We emphasize the significance of mimicking natural tissue gradients and the selection of appropriate biomaterials to ensure scaffold success. The review outlines state-of-the-art 3D printing technologies, varying from extrusion, inkjet and laser-assisted bioprinting, which are crucial for producing scaffolds with tailored mechanical and biological properties. Applications in cartilage-bone, intervertebral disc, tendon/ligament-bone, and muscle-tendon junction engineering are discussed, highlighting the potential for improved integration and functionality. Furthermore, we address challenges in material development, printing resolution, and the *in vivo* performance of scaffolds, as well as the prospects for clinical translation. The review concludes by underscoring the transformative potential of 3D printing to advance orthopedic medicine, offering a roadmap for future research at the intersection of biomaterials, drug delivery, and tissue engineering.

Keywords: 3D printing, tissue engineering, musculoskeletal interface, scaffolds, biomaterials, drug delivery, regenerative medicine.

1. Introduction

The musculoskeletal system is a complex network of tissues working together to provide structural support, protection, and movement [1]. Key interfaces within this system, such as those between bone and cartilage, intervertebral discs, tendons/ligaments and bone, and muscle-tendon junctions, exhibit unique heterogeneous structural and compositional gradients [2]. These gradients are crucial for regulating biophysical and biochemical properties, ensuring the smooth transmission of mechanical forces and biochemical signals across different tissue types [3]. Unfortunately, due to their complexity and high mechanical demands, these interfaces are particularly prone to injury and degeneration, especially in the aging population [4, 5]. This can further lead to debilitating conditions like osteoarthritis, intervertebral disc degeneration, and tendon/ligament tears. There were approximately 1.3 billion prevalent cases of musculoskeletal disorders globally, including low back pain, neck pain, osteoarthritis, rheumatoid arthritis, and gout. These disorders caused $420 billion expenditure imposing substantial economic burdens especially in US [6, 7]. Therefore, repairing and regenerating damaged tissue interfaces is crucial for maintaining joint health, preventing degenerative diseases, and enabling smooth, pain-free movement [8]. Traditional tissue engineering approaches have frequently simply scaffold design (e.g., monolithic architecture) constructed from homogeneous materials (e.g., polylactic acid) and populated with a single cell type (e.g., fibroblasts) [9-11]. Despite their prevalence, these scaffolds markedly diverge from natural tissue interfaces, which are characterized by intricate structural and compositional gradients [12, 13]. This fundamental mismatch significantly compromises the ability of the scaffolds to mimic the complex architecture and functionality of native tissues, thereby impeding both their therapeutic effectiveness and their translation into clinical applications [14, 15]. Given these limitations, there is a pressing need for innovative approaches that more accurately address the challenges inherent in the regeneration of tissue interfaces.

In recent years, 3D printing has emerged as a formidable technology for the fabrication of patient-specific scaffolds, markedly enhancing the precision with which scaffolds can be developed in terms of their structural, chemical, and biological properties[16-20]. This technology leverages a spectrum of biocompatible materials (e.g., polymers, ceramics, and hydrogels) which facilitate the production of scaffolds that closely emulate the native tissue environment [21-23]. These scaffolds

are engineered to feature graded mechanical properties and to incorporate biochemical cues essential for guiding tissue regeneration. Furthermore, the integration of growth factors, pharmaceuticals, and live cells within these scaffolds augments their regenerative potential, culminating in the creation of functional tissue interfaces [10, 24-26]. Notably, advanced 3D printing techniques such as multi-material printing, bioprinting, and 4D/5D printing have broadened the possibilities for constructing complex, biomimetic scaffolds. These innovations enable the scaffolds to more accurately mimic the intricate native tissue interfaces, enhancing tissue regeneration and integration. As a result, 3D printing holds the potential to revolutionize tissue interface engineering within the musculoskeletal system with complex tissue interfaces for enhanced integration and regeneration.

For instance, in osteochondral tissue engineering, scaffolds can be stratified to possess a biomechanical gradient from a stiffer bone-like base to a softer cartilaginous top, mirroring the natural osteochondral interface [27-29]. Similarly, in intervertebral disc regeneration, the design can feature a gradient in hydrogel properties ranging from a softer nucleus pulposus-like center to a tougher annulus fibrosus-like periphery, effectively replicating the biomechanical characteristics of native disc tissue [30, 31]. Utilizing the latest developments in 3D printing technology and biomaterials science, researchers are now able to construct complex, biomimetic scaffolds that more accurately replicate the natural interfaces of tissues to enhance tissue regeneration and integration. As research in this area progresses, it is anticipated that 3D printed scaffolds will become increasingly crucial in managing musculoskeletal disorders and in enhancing patient outcomes.

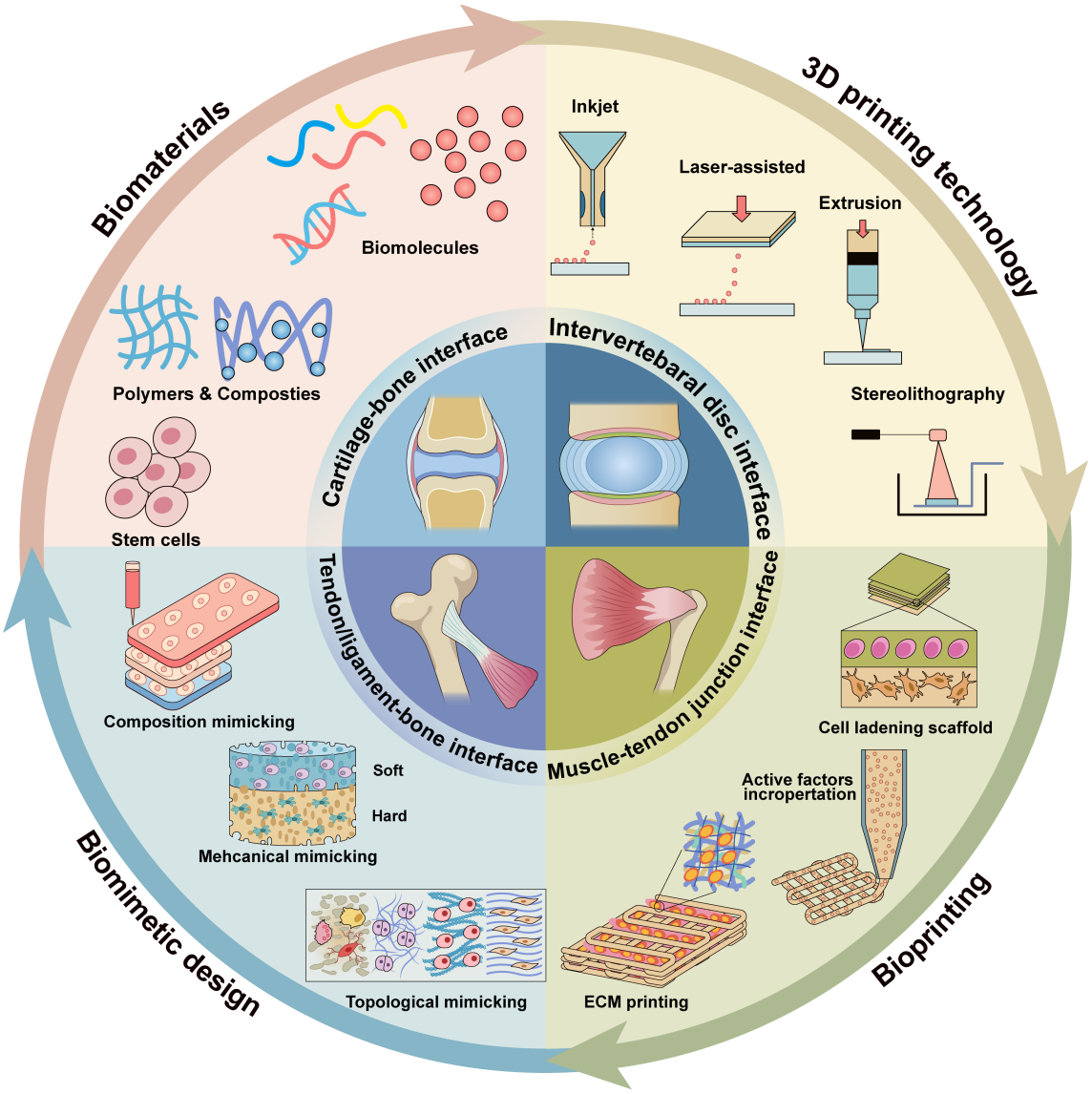

Fig.1. Overview of 3D printed scaffolds for tissue interface engineering in the musculoskeletal system. The schematic covers key elements, including biomaterials, 3D printing technologies, biomimetic design strategies and biomedical applications in regenerating tissue interface of musculoskeletal system. This comprehensive overview highlights the interdisciplinary nature of this rapidly advancing field, integrating biomaterials, biostructure, and advanced manufacturing to engineer functional tissue interface regeneration.

2. Biomaterials in musculoskeletal tissue interface engineering

Biomaterials are crucial in musculoskeletal tissue engineering, serving as substitutes that are bioactive or non-bioactive with similar, identical or even superior functions to the target tissues to

elicit or facilitate the complex and diversified physical and physiological activities of human bodies. The musculoskeletal interface contains hard-to-hard (e.g., cartilage-bone interfaces) tissues, soft-to-hard (e.g., tendon/ligament-bone interfaces) tissues, and soft-to-soft (e.g., muscle-tendon interfaces) tissues, leading to complex physiological functions that relate to body movements. Under physiological conditions, the elastic modulus of the musculoskeletal system tissues varies by several orders of magnitude and the existence of an interface helps to increase tissue contact area which minimizes the stress-shielding effect [32], as the imbalanced or unmatched mechanical properties may cause the development of different diseases (e.g. osteoarthritis) [33]. Additionally, the degradation of the biomaterial must be controlled to maintain a balance of new tissue growth while continuously sustaining the physiological loading. Furthermore, some bioactive molecules (such as stem cells [34], growth factors and drugs [35]) are also used to stimulate the host reactions of the human body and regulate the microenvironment, with a view to ultimately regenerate the body's native tissues and their functions completely. Based on these realities, multiple properties including but not limited to mechanical property, degradation and bioactivity of biomaterials need to be taken into consideration. Collectively, the design and fabrication of functional biomaterials for 3D printing musculoskeletal tissue interfaces should take adequate considerations regarding several functional requirements including but not limited to supporting and stabilizing body movements, as well as their further long-term physiological activities.

The hard-to-hard tissue interfaces typically include bone-cartilage interfaces (BCI) at joints, exhibiting a two-layered micronano structure (including a porous structure and a dense structure), representing modulus ranging from 4.15 ± 1.29 MPa to 1.87 ± 0.11 GPa [36]. In around mid-20th century, metals (e.g., titanium (Ti), magnesium (Mg)) and bioceramic materials (including bioinert ceramics and bioactive ceramics such as β-tricalcium phosphate (β-TCP) [37, 38] and bioactive glass [39] were first developed as artificial joints, which are biocompatible but lack the matching mechanical and structure-mimicking properties needed for joint replacement. Recent advances in understanding tissue architecture and host immune responses have led to significant developments in scaffold materials. These include both natural polymers (e.g., alginate [40], silk [41] and collagen [42]) and synthetic polymer (e.g., polylactic acid (PLA) [43], polycaprolactone (PCL) [44], and polyglycolic acid (PGA) [45]). When combined with bone-related cells (particularly mesenchymal stem cells (MSCs) and chondrocytes) and bioactive molecules (such as bone

morphogenetic protein (BMP-2), transforming growth factor (TGF-β), and insulin-like growth factor (IGF)) [40], these scaffolds can effectively activate chondrogenic and osteogenic pathways, creating optimal conditions for tissue regeneration. In addition, over the recent years, some clinical trials have been conducted including the BioCartilage® (a scaffold with Collagen Type II and cartilage matrix elements, NCT03696394, registered with ClinicalTrails.gov), and the Triphasic Osteochondral Scaffold (NCT05332288, registered with ClinicalTrails.gov), etc. What's more, some commercial products based on different treatment strategies have also been utilized in clinic, for example, ChondroMimetic™ (TiGenix NV, mixture of collagen, HA and β-TCP), Agili-C™ (CartiHeal, natural sources derived, containing calcium carbonate, aragonite and hyaluronic acid), Osteocel® Plus (NuVasive®, containing MSCs and osteoprogenitor cells), Carticel® (Genzyme, FDA approved, autologous cultured cells derived) [46]. These commercial products have successfully integrated into the local tissues and connect well with the surrounding articular cartilage and the subchondral bone. However, to date, we still can't fully reproduce the depth-dependent composition and tissue arrangement of the bone and chondral tissues, limiting the scaffolds' therapeutic efficacy.

The soft-to-hard tissue interfaces (the enthesis located in tendon/ligament-bone interfaces), act as the bridge between different soft and hard tissues, which plays a key role in musculoskeletal movements with a gradual mechanical transition from the tendon (tensile modulus 200 MPa) to bone (tensile modulus 20 GPa) [14], making the design and selection of biomaterials an instrumental factor for 3D printing musculoskeletal interface engineering. Among all kinds of materials, natural alginate [47], fibrin [48], silk [41], and collagen [49], and synthesized polymers PLA [35], poly(lactide-co-glycolide) (PLGA) [50], PCL [44], and polyglycolic acid (PGA) [45] have been applied as scaffolds. Additionally, enthesis-associated cells such as fibroblasts, osteoblasts, costal-cartilage-derived stem cells (CDSCs), tendon stem/progenitor cells (TSPCs), adipose-derived stem cells (ADSCs), and mesenchymal stem cells (MSCs), along with growth factors like BMPs, fibroblast growth factors (FGFs), TGF-βs, growth and differentiation factors (GDFs), platelet-derived growth factors (PDGFs), and vascular endothelial growth factors (VEGF), were used as bioactive factors for regenerating these interfaces [51]. For instance, Xie etc. developed a multiphasic scaffold that efficiently facilitated ligamentization and graft-bone

integration in a rabbit model by mimicking the native architecture of the anterior cruciate ligament and double delivery of calcium ions and some connective tissue growth factor [35].

The soft-to-soft tissue interfaces include muscle-tendon junctions (MTJ), which comprise a network of overlapping muscles (modulus ~0.012-2.8 MPa) and tendon tissues (modulus ~500-1850 MPa) [52]. Another example is the intervertebral disc (IVD), consisting of the nucleus pulposus (effective aggregate modulus of 1.0 MPa) surrounded by the annulus fibrosis (aggregate modulus of 440-750 kPa) [53]. Biocompatible polymers (including natural polymers such as silk [54] and collagen [42], and synthetic polymers such as PLA [35], and PLGA [50]) are applied as scaffolds. To structurally and compositionally mimic the native interface, various cells (e.g., MSCs, fibroblasts) [8] and growth factors (e.g., TGF-β, BMPs, IGFs, FGFs) [55] are further incorporated. For instance, Sun et al. 3D printed a bioactive fiber-reinforced hydrogel scaffold containing bone marrow derived-MSCs, providing sufficient mechanical support during force transmission, and improving the postinjury microenvironment by incorporating the immunomodulatory and antioxidant bioactivities, and eventually promoting the structural and functional regeneration of muscle-tendon interface [54]. In another study, Sun et. al. developed a 3D bioprinted intervertebral disc scaffold releasing connective tissue growth factor (CTGF) and transforming growth factor-β3 (TGF-β3). These scaffolds demonstrated zone-specific matrix regeneration and enhanced biomechanical properties, offering potential for clinical applications [56]. Regarding clinical trials, the Musculotendinous Tissue Repair Unit and Reinforcement (MTURR, an ECM scaffold, NCT01292876, registered with ClinicalTrails.gov) was carried out. What's more, the utilization of some commercial scaffolds such as GraftJacket™ (an allograft scaffold sourced from cadaver human skin), Zimmer® (a collagen scaffold derived from porcine dermis) and TissueMend (a single-layer collagen scaffold derived from fetal bovine dermis) [57], etc. for MTJ regeneration have been tried. However, there's only limited evidence about their healing efficacy. Moreover, there are only a few inventions that have been successfully commercialized for MTJ regeneration, leaving a blank gap and urging for more clinical conversion products.

In this review, we have outlined the range of biomaterials and bioactive molecules utilized in 3D-printed musculoskeletal interfacial engineering. **Table 1** summarizes some recently developed biomaterials for musculoskeletal interface engineering along with their mechanical, degradation

and biological properties, and state-of-the-art applications in musculoskeletal interfacial engineering. Furthermore, **Table 2** summarizes the bioactive molecules utilized for musculoskeletal interface regeneration including different cells, growth factors and cytokines as well as their working mechanism. This includes an array of cell types, growth factors, and cytokines, along with an elucidation of their respective functional mechanisms.

Table 1. Biomaterials for musculoskeletal tissue interface engineering.

Category	Material	Mechanical Properties	Degradation Properties	Application in Musculoskeletal Interface Engineering	Ref
Ceramic	β-TCP	Tensile strength: 154 MPa Compressive strength: 687 MPa	Tunable from years to decades	Osteochondral interface Ligament–bone interface	[37, 38, 58]
	Bioactive glass	Compressive modulus: 45-160 GPa Compressive strength: 100-1080 MPa	Tunable but overall very slow	Osteochondral interface Ligament–bone interface	[39, 59-61]
	HA	Young's modulus: 80-110 GPa Tensile strength: 0.05 GPa Compressive strength: 0.4-0.9 GPa	Slow	Osteochondral interface	[62, 63]
Natural Polymer	Alginate	Elastic modulus: <200 kPa	Fast degradation, in several weeks	Osteochondral interface Intervertebral disc interfaces Ligament–bone	[40, 47, 64, 65]

	Fibrin	Young's modulus: 5-40 kPa Elongation at break: 332%	Fast degradation, in several weeks	interface Ligament–bone interface Osteochondral interface	[48, 66-68]
	Silk	Young's modulus: 10-55 kPa. Breaking strain: 4%-38%	Tunable from days to years	Intervertebral disc interfaces Ligament–bone interface Tendon-muscle interface	[41, 54, 69-73]
	Collagen	Young's modulus: 20-600 kPa Elongation at break: 10%-86%	Rapidly degradable by matrix metalloproteinase	Osteochondral interface Intervertebral disc interfaces Tendon-bone interface. Ligament–bone interface	[42, 49, 74-77]
Synthetic Polymer	PLA	Maximum tensile strength: 48.66±1.11 MPa Young's modulus: 3107.03±26.91 MPa	Half-life: 30 weeks	Osteochondral interface Ligament–bone interface Intervertebral disc	[30, 35, 43, 78-80]

	Elongation at break: <10%		interfaces Tendon-muscle interface Ligament–bone interface	
PLGA	Young's modulus: 2-7 GPa Elongation at break: 3%-20%	Degradation time: <18 weeks	Intervertebral disc interfaces Tendon-bone interface	[54, 81-83]
PCL	Compressive modulus: 200-420 MPa	Relatively slow degradation, generally in several years	Tendon-muscle interface Osteochondral interface Intervertebral disc interfaces Tendon-bone interface	[44, 83-87]
PGA	Tensile modulus: 6-7 GPa Tensile strength: 60-99.7 MPa Elongation at break: 1.5%-20%	Fast degradation, in several months	Tendon-bone interface	[45, 88]

Table 2. Bioactive molecules for musculoskeletal tissue interface engineering.

Category	Bioactive Molecules	Mechanism	Ref
Cartilage-bone interfaces	MSCs	MSCs can differentiate into chondrocytes and osteoblasts.	[40]
	Adipose-derived Stem Cells (ASCs)	i) ASCs can differentiate into cartilage and bone cells; ii) ASCs have low immunogenicity.	[89]
	Chondrocytes	Chondrocytes are one of the components of osteochondral interfaces, which can further form cartilage.	[40]
	BMP	i) BMP-2 and BMP-7 can promote cartilage maturity; ii) BMP-2, BMP-4, BMP-6, BMP-7 can induce osteogenic differentiation.	[40]
	TGF-β	TGF-β can induce osteogenic differentiation.	[40]
	IGF	i) IGF-1 can promote cartilage maturity; ii) IGF-1 and IGF-2 can induce osteogenic differentiation.	[40]
	GDF-5	i) GDF-5 can promote IVD function cell proliferation; ii) GDF-5 can regulate ECM metabolism; iii) GDF-5 can reduce the inflammatory response.	[90]
Tendon/ligament-bone interfaces	Fibroblasts	i) Fibroblasts are one of the cell components of enthesis; ii) Fibroblasts can further transfer into fibrocytes, which is another important cell component of enthesis.	[51]
	Osteoblasts	i) Osteoblasts are one of the cell components of enthesis;	[51]

	ii) Osteoblasts	
CDSCs	i) CDSCs can differentiate into tenocytes, chondrocytes and osteocytes; ii) CDSCs can adapt to low-oxygen and low-nutrient conditions	[91]
TSPCs	i) TSPCs promote fibrogenesis during enthesis regeneration; ii) TSPCs have an anti-inflammatory effect.	[51]
ADSCs	i) ADSCs can promote enthesis regeneration by fibrogenesis and chondrogenesis; ii) ADSCs can reduce oxidative stress by inhibiting the methylation LncRNA Morf4l1.	[51]
MSCs	MSCs can differentiate and finally form cartilage and fibrocartilage.	[51]
BMPs	i) BMP-2 and BMP-7 can promote cartilage maturity; ii) BMP-2, BMP-4, BMP-6, BMP-7 can induce osteogenic differentiation.	[40]
FGFs	FGFs can promote fibrogenesis.	[51]
TGFs	i) TGF-β1 can promote chondrogenesis; ii) TGF-β3 can promote collagen generation, fibrogenesis	[51]

		and chondrogenesis.	
	GDFs	GDF-5 can promote fibrogenesis.	[51]
	PDGFs	PDGFs can induce tenogenic differentiation of ADSCs and MSCs.	[51]
	VEGF	i) VEGF can promote angiogenesis at the fibrocartilage zone; ii) VEGF can promote fibrocartilaginous regeneration;	[92]
	MSCs	MSCs can differentiate into tenocytes and myocytes;	[54]
	Fibroblasts	i) Fibroblasts are one of the cell components in myotendinous junctions; ii) Fibroblasts can further transfer into fibrocytes, which is another important cell component in myotendinous junctions.	[51]
Muscle-tendon Interfaces	TGF-β	TGF-β can promote fibrogenesis.	[51]
	BMPs	i) BMPs can induce the transformation of fibroblasts into myoblasts; ii) BMPs can promote the formation of fibrocartilage insertion in myotendinous junctions.	[93]
	IGFs	IGFs can trigger satellite cell differentiation and proliferation;	[94]

		IGFs can promote muscle fiber regeneration.	
	FGFs	i) FGFs can promote fibrogenesis; ii) FGFs can modulate tendon maturation.	[51, 95]
	MSCs	MSCs can differentiate into NCs or NP-like cells and support the deposition of proteoglycans and collagen.	[96, 97]
	AFs	i) Mimic the natural microenvironment of IVD tissues; ii) Promote the ECM deposition in annulus fibrosus tissue.	[98]
Intervertebral disc interfaces	NPs	i) Mimic the natural microenvironment of IVD tissues; ii) Promote the ECM deposition in nucleus pulposus tissue.	[98]
	iPSCs	iPSCs can differentiate into NCs or NP-like cells.	[99]
	CTGFs	CTGFs can promote MSCs differentiate into NP-like cells and AF-like cells;	[56]
	TGF-β	TGF-β1 can preserve disc height and to inhibit apoptosis.	[100]

3. 3D printing technologies for musculoskeletal tissue interface engineering applications

With the assistance of computer-aided design and manufacturing, 3D printing can precisely arrange materials on a spatial scale to form both bioactive and functional bionic morphologies and organism structures. The general performing principle of 3D printing is to transform a digital design into a physical object by driving the nozzles of the 3D printer to move in 3D space under the control and assistance of a computer [101]. During this process, inks (material or material-adhesive) are accumulated layer-by-layer, resulting in the creation of the final product reflecting the designed model. Correspondingly, 3D bioprinting is an emerging biomedical technology developed based on traditional 3D printing technologies, utilizing bioactive materials, cells and bioactive factors as inks during the printing process for a better biomimetic structure and improved cellular response. 3D bioprinting allows for the construction of complex biomimetic structures with precisely controlled mechanical and biological properties to satisfy the varying tissue environments.

There are several 3D printing methods including the widely used traditional fused deposition modeling (FDM) [102], inkjet [15, 103, 104], selective laser sintering (SLS) [105], and stereo lithography appearance (SLA) [106, 107]. FDM is the most widely used 3D printing method due to the low cost. This method involves extruding bioinks layer by layer using a heated needle with differentiating pressure, propelling the process. Precision is achieved through adjustments in nozzle size, pressure, and temperature, dictating print speed and clarity [108]. FDM technology is suited for highly viscous (> 30 mPa·s) materials to preserve filament integrity [15]. However, this requirement makes the nozzles susceptible to blockages, which is a key drawback of the FDM process [109]. While these techniques have reached a considerable level of precision, they remain constrained by material characteristics. To overcome these limitations, laser assisted 3D printing uses photopolymerization. In this process, laser light triggers the solidification of photosensitive materials by crosslinking them. This approach not only provides enhanced precision but also boasts quicker printing rates than the previously mentioned techniques. There are several technologies including SLA and digital light printing (DLP) [110, 111], where the common basic principle relies on the refraction of mirrors to direct a laser to the target to

form the desired shape [112]. In one study, Chen et al. successfully printed an ECM/GelMA/exosome scaffold by SLA for early osteoarthritis treatment. After the printing process, the bioactive components still can maintain their biological functions, which was proven to promote osteochondral defect generation by restoring chondrocyte mitochondrial dysfunction and enhancing chondrocyte migration [113]. Likewise, SLS is based on the high-temperature sintering of powder materials under irradiation of a laser, which is often used to manufacture wear-resistant parts [114]. Laser-based 3D printing methods (including LIFT and DLP) are further developed. Thanks to the inherent properties (directionality, brightness and coherence) of the laser, these printing methods represent high accuracy and printing speed. However, upon printing, photosensitive materials (e.g. GelMA, PEGDA, and chitosan methacrylate (CHI-MA)) [115] are required as printing inks and expensive laser systems are required as necessary hardware, resulting in the high cost. Thus, laser-assisted 3D printing is less used compared to other 3D printing technologies. In addition, the unwanted polymerization of adjacent photosensitive materials due to the high light intensity of the laser may appear, which will significantly reduce the accuracy of printing [116]. Despite these issues, it is still the quickest and most accurate 3D printing method available. The high resolution also brings the advantageous ability of ultra-fine printing to form complex structural architectures.

The emergence of 3D printing has transformed manufacturing with its precision and customization capabilities. This technology has now evolved to encompass bioprinting, which combines living cells and biomaterials to fabricate functional tissues and organs. Particularly in musculoskeletal interfacial engineering, 3D bioprinting is a pioneering technique that allows for the meticulous placement of bioinks and the creation of scaffolds resembling the natural extracellular matrix, showing great potential for tissue regeneration. 3D bioprinting methods include extrusion-based bioprinting (EBB), droplet-based bioprinting (DBB), and laser-based bioprinting (LBB). Similarly, EBB is developed based on FDM technology, thus the bioink used in EBB contains collagen, alginate, and other high-viscosity biomaterials. Inkjet, as well as DBB, is driven by either the piezoelectric effect, heat, or pneumatic pressure, spraying ink drops with a nozzle. Increasing nozzle numbers will lead to a faster printing speed. Both inkjet and DBB printing demonstrate

superior material versatility, enabling the deposition of various liquid formulations including cell-laden bioinks, polymer solutions, growth factor suspensions, and crosslinkable hydrogel precursors [117]. Extrusion related bioprinting methods are the most widely applicable method of bioprinting as elongated fibers (a common structure within musculoskeletal tissues) can be built based on the deposition of the highly viscous biomaterials [108]. For example, a bone-ligament-bone scaffold with a multiphasic biomimicking architecture was fabricated for bone and ligament regeneration, consisting of a ligament region loaded with human bone marrow stem cells (hBMSCs) and bone regions loaded with BMP-2 [118]. Cells in the ligament area aligned rapidly and bone formation was localized only in the bone areas after implantation. The *in vivo* results indicated that newborn bone and ligament tissues with similar structure and function to natural tissues formed in the corresponding areas. **Table 3** summarizes the printing properties of the above discussed printing methods, as well as their application in musculoskeletal interface engineering.

Table 3. 3D printing technologies for musculoskeletal tissue interface engineering.

Category	Technologies	Accuracy	Speed	Resolution	Cost	Material	Application	Ref
Material extrusion	FDM	Relatively low	Slow (μm/s)	100 μm	Low	High viscosity materials such as collagen, alginate and polymers such as PCL, PLA, PLGA, PEG, etc.	Osteochondral interface Intervertebral disc interfaces Ligament–bone interface Tendon-bone interface Tendon-muscle interface	[30, 54, 75, 102, 119, 120]
Injection	Inkjet	Low	Medium (mm/s)	30 – 100 μm	Low	Low viscosity materials such as collagen, alginate, PEGDA, suspension of living cells, etc.	Tendon-muscle interface Tendon-to-bone interface Ligament–bone interface	[15, 103, 104]
Laser-assisted	SLA	High	Medium (mm/s)	50 – 200 μm	High	Photocrosslinkable polymers such as	Osteochondral interface	[106, 107,

printing						GelMA, PEGDA, CHI-MA, etc.	Intervertebral disc interfaces Tendon-bone interface	113, 119]
	DLP	High	Fast (mm^3/s)	6 μm	High	Photocrosslinkable polymers such as GelMA, PEGDA, CHI-MA, etc.	Osteochondral interface	[110, 111]
Sintering	SLS	High	Slow (μm/s)	50 – 100 μm	High	Powder materials that can form interatomic connections between particles when heated by a laser such as thermoplastic polymer, wax, ceramics, etc.	Osteochondral interface	[105, 114, 119]

With the development of contemporary 3D printing methods, advanced 3D printing methods using functional hybrid materials of different properties have been successfully developed through the combination of different 3D printing techniques as well as by the invention of new techniques such as the coaxial 3D printing techniques. For instance, Gao et al. utilized a thermal-assisted three-cartridge extrusion printing technique to programmatically print a porous high-strength (0.41 MPa for tensile strength and 8.4 MPa for compressive strength) hydrogel loaded with TGF-β1 and β-TCP to facilitate hMSC adhesion, proliferation and differentiation for osteochondral regeneration [121]. However, although they took gradient compositions into consideration and formed a bilayer structure, there was a lack of attention to the gradient structure itself. The recently reported aspiration-assisted bioprinting technology, on the other hand, utilizes a bottom-up, high-precision approach which successfully achieved a compact and hierarchical tissue alignment for the first time. Additionally, the printed osteochondral interface structure remained highly biomimetic even when printed without scaffold-assisted positioning [122]. Other advanced technologies such as machine learning [123] and electrospinning [10] can also be applied to optimize printing parameters and manufacturing process and form elaborate structures even at a submicron scale.

4D and even 5D printing technologies have recently been rapidly developed for additive manufacturing technologies. 4D printing incorporates time as a regulatory element, enabling the utilization of shape-memory polymers to fabricate complex structures that can autonomously or programmatically revert to their initial shapes or functions. These transformations are triggered by diverse external stimuli, which consist of physical factors (like light and temperature), chemical factors (such as pH and catalysts), and biological factors (including sugars and enzymes) [116, 124]. In one study, Zhang and Li et al. fabricated a NIR-triggered artificial porous scaffold using shape memory polyurethane by 4D printing, to facilitate bone repair. The printed scaffold exhibited an excellent shape memory effect that fully recovered to the initial shape within 100 seconds [125], providing advantages to realize novel 4D-printed biomedical scaffolds. To date, although 4D printing technology is widely utilized to construct single structural systems, there is only little research conducted on the musculoskeletal interface system. The structures of the 4D

printed scaffolds need to be finely designed to optimize their performance based on the actual withstood force to ensure the appropriate response to the changes in tissue surface [126, 127]. 5D printing (also called 5-axis printing) utilizes five axes (adding another two axes based on 3D printing), which can produce complex curved surfaces easily with stronger mechanical properties (3-5 times stronger than 3D printed implants [128]) while using less material [124], to produce multi-dimensional objects. Thus, it is extremely beneficial to utilize 5D printing for constructing artificial musculoskeletal interface scaffolds which usually have complex multi-layer curved surfaces. As 5D printing is still in the early stage of development, very limited research has been done and mainly focuses on constructing artificial bones. Although 4D and 5D printing technologies are not yet popular in musculoskeletal interface engineering, in general, these advanced additive manufacturing technologies provide ideas for expanding new directions in biomanufacturing technology and creating disruptive innovations in orthopedics.

4. Applications of 3D printing in musculoskeletal tissue interfaces

4.1 Bone-cartilage interfaces

4.1.1 Bone-cartilage interface structure

The bone-cartilage interface (BCI), integral to joint function, exhibits a sophisticated, multilayered architecture that plays a crucial role in stress distribution, load transfer, and joint mechanics [129]. This interface is comprised of three distinct layers: the articular cartilage, the calcified cartilage, and the subchondral bone, each characterized by unique structural and biochemical gradients that facilitate seamless movement and effective load management across the joint (**Fig. 2A**) [130]. The articular cartilage, the top layer, absorbs mechanical loads and facilitates low-friction movement through a composition of collagen types I and II as vertical parallel fibers spanning from the deeper zones to the superficial zone [131]. Below this, the calcified cartilage layer, marked by the tide mark, provides a strong mechanical link to the subchondral bone through interdigitations, with a gradient decrease in hydroxyapatite content upwards [132]. The subchondral bone, the deepest layer, bears the highest loads, characterized by its high hydroxyapatite and collagen type I content which are critical for its strength and the protection of overlying layers [133]. Together, these layers enable effective load management and mechanical stability across the joint.

Across these layers, there is a notable gradient in cell types and behaviors. The subchondral bone houses osteocytes, osteoblasts, and osteoclasts, whereas the cartilaginous regions contain chondrocytes [133]. Chondrocyte density and morphology exhibit a gradient, with cells becoming more numerous and transitioning from flat to more hypertrophic shapes as one moves from the superficial to the deep zones. This cellular arrangement is mirrored by the structural organization within the chondrons, the basic functional units in cartilage, which adapt in composition and number of enclosed chondrocytes based on depth. The mechanical properties of the BCI also demonstrate a gradient, with the elastic modulus decreasing from approximately 3.9 GPa in the subchondral bone to 0.32 GPa in the calcified cartilage, which further drops in the articular cartilage zone [134]. This gradient aids in the smooth transfer and dissipation of mechanical loads across the interface, minimizing the risk of injury and wear to the joint over time. Collectively, the intricate structural, cellular, and mechanical gradients of the bone-cartilage interface not only underscore its complexity but also highlight its essential role in joint functionality and health.

4.1.2 Regulation of materials, cells, and growth factors in 3D printed scaffolds to mimic the BCI structure

The fabrication of scaffolds through 3D printing technology allows for the simulation of complex BCI by varying the composition of materials and the incorporation of bioactive factors. The top layer of the scaffold primarily focuses on mimicking the articular cartilage by using hydrogels such as alginate and gelatin, which have soft and hydrating properties. (**Fig. 2B**) [135, 136]. The materials are able to closely mimic the characteristics of natural cartilage due to their biocompatibility and the porous nature of hydrogels which supports the growth and proliferation of chondrocytes—the cells responsible for maintaining the cartilage matrix [137, 138]. For instance, hyaluronic acid-based bioink has been observed to significantly enhance cellular functionality by both inducing an upregulation of chondrogenic gene markers and allowing for the precise deposition of matrix components, promoting the formation of functional tissue [139]. The middle layer of the scaffold serves as a transitional zone between the soft cartilage and the hard bone, which is typically

mimicked using a gradient of hydrogel-based composite material containing ceramic particles (e.g. hydroxyapatite) [140, 141]. This gradation has the characteristic of incrementally increased stiffness and compressive strength, contributing to scaffold integration as well as both migration and differentiation of mesenchymal stem cells. For example, Zhang et al. conducted a study where they developed gradient 3D scaffolds by altering the composition of nanohydroxyapatite (nHA) and sodium alginate hydrogel. Their findings revealed that a combination of 40% (w/w^{-1}) nHA and 60% (w/w^{-1}) hydrogel resulted in a successful remodeling of the calcified cartilage layer, exhibiting favorable physicochemical, mechanical, and biological properties that were well-matched [142]. The bottom layer of the scaffold is designed to mimic the subchondral bone, utilizing ceramic materials such as HA and β-TCP which are osteoconductive and boast mechanical strength similar to the bone's mineral components [143-145]. They provide a robust framework that not only supports the attachment and proliferation of osteoblasts but also facilitates the mineralization process essential for bone regeneration, restoring the structural integrity of the damaged bone. For instance, the successful integration of synthetic, degradable polymers (poly (propylene fumarate)) with osteoconductive HA nanoparticles led to the fabrication of 3D printed scaffolds that were characterized by well-defined layers and interconnected pores with enhanced bone and cartilage formation [146]. These approaches provide a tailor-made environment conducive to tissue regeneration.

The integration of bioactive growth factors into 3D printed scaffolds significantly enhances their capability to direct tissue regeneration in a site-specific manner. In the cartilage region of the scaffold, it has been proved that cartilage growth and maturation were supported by various growth factors, including TGF-β1, IGF-1, FGF-2 and BMP-2 [147]. TGF-β1 is a potent chondrogenic factor that enhances the synthesis of cartilage matrix proteins such as collagen type II and aggrecan, stimulating MSCs to adopt chondrocyte fates to form new cartilage tissue [148]. In the bone region, the incorporation of BMPs, IGF-1/2 and FGFs serves to enhance osteogenic differentiation and maturation. BMPs are well-known for their ability to induce osteoblast differentiation from progenitor cells and stimulate bone formation [149]. For instance, scaffolds enriched with BMP-2 in the lower layer have demonstrated increased mineral deposition and enhanced mechanical strength, which are

crucial for successful bone defect repair [150]. In addition, optimization is necessary to develop an adjustable and reproducible growth factor delivery system capable of triggering cartilage and bone repair mechanisms. The strategic distribution of these growth factors in a gradient across the scaffold not only promotes specific cellular activities in their respective zones but also supports the integration of newly formed tissues by mimicking the natural transition seen *in situ* [151]. Vascularization should also be taken into account during bone tissue regeneration. Therefore, Lee et al. developed 3D-printed square-pored PCL scaffolds with bioactive surfaces coated with minerals and platelet-derived growth factors. Their work showcased enhanced bone regeneration with neovessel formation, presenting significant therapeutic promise for bone tissue engineering [152]. By replicating this gradient, the scaffolds can facilitate a more natural healing process, leading to better functional recovery and tissue integration.

4.1.3 3D printing and biomimetic scaffold design

Cell-laden 3D printing, also known as bioprinting, represents a transformative approach in the field of tissue engineering and regenerative medicine [153]. This technique involves the precise deposition of biomaterials mixed with cells (bioinks) to create complex tissue constructs that mimic natural tissue structure and function. One of the most significant applications of cell-laden 3D printing is in the engineering of multi-tissue interfaces, such as the bone-cartilage interface in joints (**Fig. 2C**) [75]. The ability to precisely control the placement of different cell types within a scaffold allows for the creation of gradient structures that mimic the natural transitions between different tissue types. In bone-cartilage interface scaffolds, bioprinting can be used to deposit layers containing different concentrations of MSCs pre-differentiated towards either osteogenic or chondrogenic lineages. For instance, the top layers of the scaffold may be enriched with chondrocytes or composed of chondrogenically induced MSCs within a hydrogel matrix rich in TGF-β, promoting cartilage formation. Similarly, the bottom layers may contain osteoblasts or osteogenically induced MSCs in a ceramic-based bioink enhanced with BMPs, facilitating bone regeneration [129]. Bioprinting of anisotropic bicellular living hydrogels (ABLHs) has demonstrated efficacy in enhancing osteochondral regeneration. This is achieved by integrating articular cartilage progenitor cells and bone mesenchymal stem cells within

distinct, stratified layers. This method fosters a more cohesive BCI, which is crucial for effective tissue regeneration [154]. This approach utilizes the differential spatial regulation inherent in ABLHs to support both chondrogenic and osteogenic differentiation, offering a promising strategy for the repair of complex organ defects.

Furthermore, the precise control offered by 3D printing techniques, such as extrusion-based bioprinting and inkjet printing, is vital in tissue interface engineering. Biomimetic design, which aims to mimic the natural structures and properties of tissues, is of the utmost importance in the development of scaffolds for tissue interface engineering [155]. Through adjustments in printing parameters, researchers can fabricate scaffolds with seamless material transitions, closely replicating the natural gradients observed in the BCI (**Fig. 2D**) [156]. This biomimetic approach allows for the creation of structures that guide cell behavior and tissue development, ultimately leading to improved functional outcomes. For instance, by engineering a scaffold with a gradual increase in hydroxyapatite concentration from the cartilage to the bone layer, precise guidance of stem cell differentiation along the scaffold's gradient can be achieved [157]. This biomimetic strategy promotes the formation of distinct yet interconnected tissue types, mimicking the natural tissue interface. In addition to material gradients, biomimetic design also considers the optimization of pore architecture within the scaffold. Controlling the pore size, distribution, and interconnectivity allows for the creation of an environment conducive to nutrient diffusion and cellular infiltration which are essential for tissue regeneration [158, 159]. Complex biomimetic porous structures fabricated via 3D printing can also induce the generation of new blood vessels, thereby accelerating the repair of bone defects. Examples include Lotus seedpod-inspired designs, biomimetic Haversian bone scaffolds, and naturally occurring bone trabecular curvature structures [160-162]. Overall, the integration of biomimetic design principles into scaffold fabrication through 3D printing techniques enables the development of functional scaffolds that closely mimic natural tissue interfaces. This approach holds great promise for tissue engineering applications, as it facilitates seamless integration, promotes appropriate cell behavior, and enhances functional outcomes.

By combining these advanced material gradients with precise 3D structural configurations,

it is possible to create scaffolds that not only support the growth of differentiated tissues, but also facilitate the integration of the said tissues with existing bone and cartilage, leading to improved outcomes in joint repair and restoration. This integrated approach in scaffold design through 3D printing is paving the way for next-generation solutions in regenerative medicine, particularly in addressing complex joint injuries and degenerative diseases. The functionalization of printed structures and the incorporation of biomimetic designs are critical for enhancing interface integration and recapitulating the native tissue structure. While there are still challenges to be addressed, such as the long-term stability and integration of the scaffolds *in vivo*, the successful implementations of 3D printed scaffolds in preclinical and clinical studies highlight their potential for revolutionizing the treatment of osteochondral injuries and diseases.

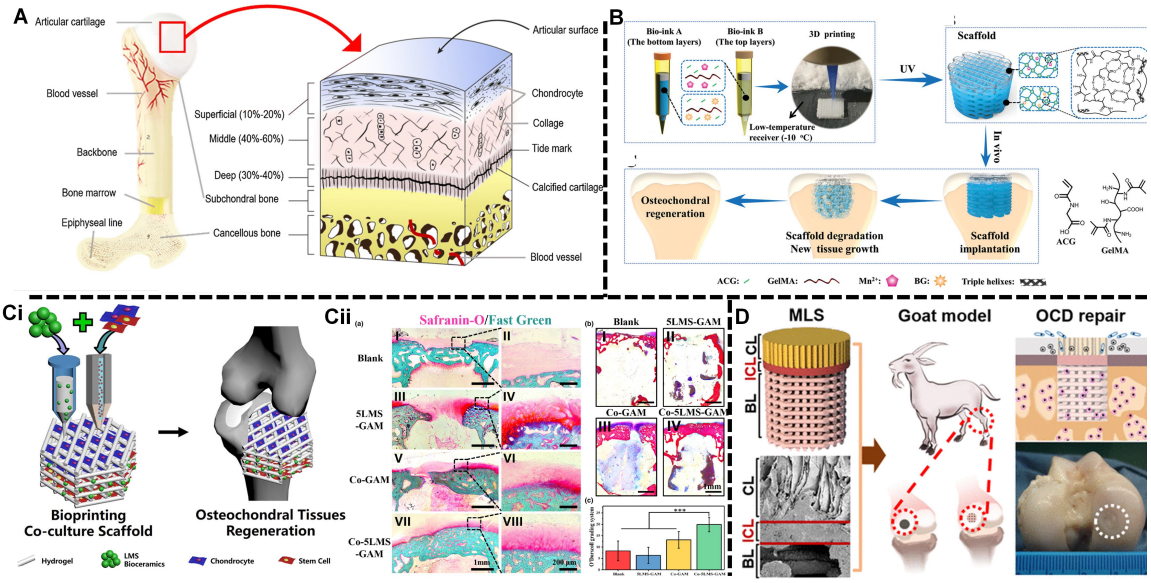

Fig. 2. (A) Structure of the BCI, illustrating the superficial zone with fewer chondrocytes and collagen fibers, the intermediate zone with larger collagen fibers, the deep zone with tightly packed fibers, active cells, and lower water content, and the calcified zone where the transition to stiff subchondral bone occurs. Reprinted with permission from Ref. [163] Copyright Springer Nature. (B) 3D printing of biohybrid gradient scaffolds using GelMA and PAGE hydrogels for osteochondral defect repair. Ref [164] Copyright Wiley. (Ci) Schematic of bioprinted bilayer scaffolds composed of Li-Mg-Si bioceramics and MSCs for osteochondral tissue regeneration. Ref [165] Copyright Elsevier. (Cii) Safranin-O/Fast

Green histological staining of 3D bioprinted scaffolds, demonstrating a synergistic effect on the regeneration of cartilage and subchondral bone tissues. Ref [165] Copyright Elsevier. (D) Multilayered scaffold with hierarchical organization and heterogeneous composition to mimic the stratified structure and complex components of natural osteochondral tissues. Ref [166] Copyright American Chemical Society.

4.2 The tendon/ligament-bone interfaces

4.2.1 The structure and function of the tendon/ligament-bone interfaces

The tendon/ligament-bone interface, a transitional region between soft and hard tissues [8], is a typical example of a composite hierarchical structure that both determines functions such as structural support, load transmission and contributes to human movement [167, 168]. The tendon/ligament-bone junction is comprised of four distinct but continuous sections: tendon/ligament, non-mineralized fibrocartilage, mineralized fibrocartilage, and bone (**Fig. 3A**). From the tendon/ligament section to the bone section, the aligned and parallel fibrous tissue gradually bend and cross in a disorganized way, which leads to smooth stress distribution and enhances the bond strength [169]. Meanwhile, an increase in mineral content and a decrease in collagenous tissue can be found when closer to the bone [168, 170]. This hierarchical structure allows for soft and hard tissue to coexist at one interface, where the tendon is tough and extensible with a stiffness of about 200 MPa, while the bone tissue is hard and brittle with a stiffness of about 20 GPa, endowing the tendon/ligament region to effectively sustain force, and the bone is able to bear compressive loads [171]. Due to the special functions and unique biological properties of this interface, many common orthopedic injuries (such as anterior cruciate ligament (ACL) rupture and tendon rotator cuff injury) often necessitate the repair of the ruptured tendon/ligament's bony attachments [172-174]. Therefore, the development of integrated fixated scaffolds that reestablish the critical connection between bone and tendon/ligament and support functional scaffold-osteointegration is imperative.

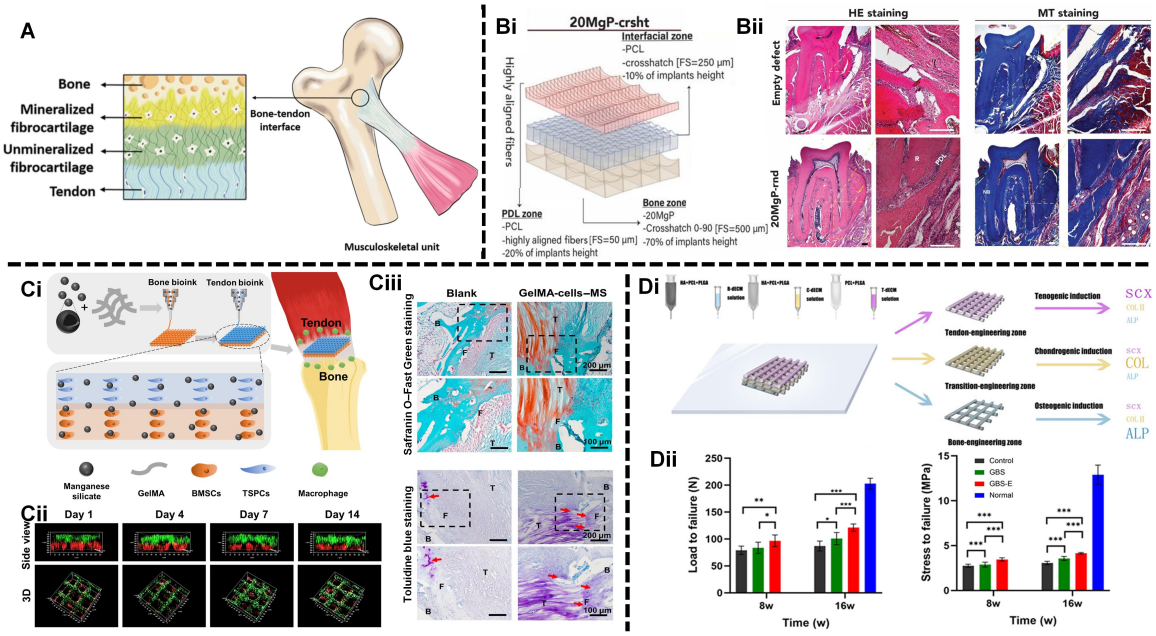

Fig. 3. (A) Structure of the tendon/ligament-bone interface, consisting of tendon/ligament (blue area), non-mineralized and mineralized fibrocartilage (green and yellow area) and bone (orange area). Reprinted with permission from Ref. [175] Copyright Elsevier. (Bi) Illustration of structurally graded three-zone scaffold. (Bii) Hematoxylin and eosin (HE) staining and Masson's trichrome (MT) staining of periodontal defects, with PDL referring to the periodontal ligament-to-bone interface and NB referring to new bone formation. Reprinted with permission from Ref. [176] Copyright American Chemical Society. (Ci) Schematic diagram of immunomodulatory multicellular scaffolds. (Cii) Confocal laser scanning microscopy (CLSM) images show a bilayered structure with spatial distribution of BMSCs (red) and TSPCs (green). (Ciii) Representative Safranin O-Fast Green (top) and toluidine blue (bottom) staining images of the tendon-to-bone interface. Reprinted with permission from Ref. [177] Copyright Science. (Di) Fabrication and illustration of mechanics-graded scaffold. (Dii) Biomechanical analysis results after implantation for 8 and 16 weeks. Reprinted with permission from Ref. [178] Copyright American Chemical Society.

4.2.2 Regulation of materials, cells, and growth factors in 3D printed scaffolds to mimic the tendon/ligament-bone structure

As a promising technology in tissue engineering, 3D printing is capable of fabricating tendon/ligament-bone tissues with heterogeneous architecture and good mechanical property as well as creating a healthy microenvironment by integrating biomolecular cues.

As tissues with stratified and changing structural gradients, tendon/ligament-bone tissues should be repaired with a multilayered scaffold. In a study by Duan et al., a single-layer PLGA scaffold and two types of multilayer scaffolds were prepared by 3D printing to validate the importance of multilayer structures [179]. The first type of multilayer scaffold was a layer-by-layer printed PLGA scaffold with a collagen fibrin hydrogel inserted in the middle, while the second was a multilayer scaffold composed of parallel-printed PLGA and Pluronic F127 with a collagen hydrogel injection on the outside. The experimental results showed that compared to the single-layer scaffold, both multilayer scaffolds exhibited improved mechanical properties and supported tenogenic differentiation. Furthermore, Malda et al. used melt electro writing 3D printing technology to develop a structurally graded three-zone scaffold for interface regeneration [176]. The scaffold consisted of a PCL layer and a magnesium phosphate (MgP)-PCL mixture layer, with a 0/90° angle cross-hatch pattern printed into the interface area, simulating the connection between ligaments (PCL layer) and bone (MgP layer) (**Fig. 3Bi**). The *in vivo* results revealed that the interface region promoted the coordinated regeneration of several tissues (**Fig. 3Bii**).

In addition to the structural remodeling strategy, scientists are also working on reshaping the interfacial microenvironment by reproducing its biochemical composition. For example, Wu et al. fabricated several immunomodulatory multicellular scaffolds [177], utilizing a bioink composed of manganese silicate nanoparticles and GelMA. Tendon stem progenitor cells (TSPC) and BMSCs were added in a layered manner within the scaffold to simulate the tendon-bone interface (**Fig. 3Ci and Cii**). The manganese ions were observed to have the ability to regulate the immune responses to promote cell differentiation for tendon-bone integration regeneration (**Fig. 3Ciii**). In addition, considering the biochemical cues of tissue composition, Lee et al. designed a scaffold enriched with growth factors by encapsulating different functional factors within PLGA microspheres and incorporating them into a PCL scaffold [180]. To summarize the construction of the scaffolds briefly, connective tissue

growth factor (CTGF) microspheres were printed in the upper layer of the scaffold for tendon formation, then a mixture of CTGF and transforming TGF-β3 microspheres were printed in the middle layer for fibrocartilage formation, while BMP2 microspheres were dispersed in the bottom later for bone formation. This approach enabled the targeted delivery of multiple factors for precise repair. Furthermore, to better mimic the innate tissue microenvironment, scientists used tissue-specific dECMs as bioink to provide multiple environmental cues such as tissue-specific proteins, glycans as well as other biological factors [172]. For example, Cho et al. combined structural cues with dECMs to create a scaffold [181]. They used PU/PCL to print a scaffold with lattice (bone and fibrocartilage area) and alignment (tendon area) patterns, and then selectively deposited tendon-, tendon/bone hybrid- and bone-dECMs into each corresponding phase. This scaffold provided environmentally instructive support for the regeneration of the complex interface.

Another characteristic of such integrated soft-hard tissue interface is mechanical variation, so gradient changes in mechanical properties are also important in scaffold design. In a study by Reinwald et al., they prepared collagen-agarose scaffolds with the addition of different concentrations of hydroxyapatite to create a stiffness gradient tissue (i.e., 0%, 0.2%, and 40% (v/v) for muscle, tendon and bone, respectively) [182]. The Young's modulus of the three-phase scaffold was 20, 140 and 240 kPa for muscle, tendon and bone-mimic parts, respectively. The scaffolds were also designed with different surface morphologies (pores in the bone region, and ridges and channels in the tendon and muscle areas) to promote cell alignment. In another study, Zhao et al. fabricated a three-layer scaffold made of tendon, cartilage, and bone-derived ECM and bioink in different proportions [178]. They used PCL/PLGA/HA to prepare the tendon (material ratio: 1:1:0) area, the transition (1:1:2) area, and the bone (1:1:4) area (**Fig. 3Di**). After printing, different dECMs were coated on their corresponding scaffold areas, forming the graded biomimetic scaffold. The results revealed that as the mineral content increased, the corresponding Young's modulus gradually increased from the tendon to the bone engineering areas. The *in vivo* experiment showed that this scaffold had good biomechanical properties and was able to enhance tendon-bone repair (**Fig. 3Dii**).

4.2.3 3D printing and biomimetic scaffold design

The design of tendon/ligament-bone scaffolds requires reference to multilayered and hierarchical structures, thus further exploration is needed to achieve a biomimetic design and to enhance tissue integration. The structure of the tendon/ligament-bone region possesses strong biomechanical properties, requiring sufficient strength to withstand wounds and muscle contraction stresses during both tissue repair and movement [170]. Hybrid bioprinting of biomaterials offers a promising approach, as it enables the creation of composite scaffolds with a wide range of mechanical properties by combining elastic and rigid biomaterials [52]. In addition, gradient changes in mechanical properties such as stiffness provide physical clues for cell differentiation, as cells will be highly sensitive to the physical properties of the matrix [183]. Moreover, an ideal scaffold should also have an internal bionic structure for cell adhesion and blood vessel growth, which requires the inclusion of suitable and interconnected pores within, facilitating inward cell growth and nutrient transportation. On the other hand, pore size is a critical factor to consider, as researchers have observed variations in cell behavior based on pore sizes. Studies show that osteoblasts and chondrocytes are more abundant in regions with pore sizes between 380 - 405 μm, while fibroblasts are more concentrated in regions with pore sizes between 186 - 200 μm, suggesting that different pore diameters have a significant impact on cell behaviors [184].

In terms of composition, it is crucial to consider the varying biomechanical and biochemical properties of different biomaterials [185], specifically the *in vivo* behaviors, since even if the scaffold meets the mechanical requirements *in vitro*, its performance will change due to degradation caused by prolonged implantation [186]. To overcome the drawbacks of using one type of biomaterial, a combination of multiple materials can be employed to capitalize on their respective strengths to confer superior performance to the composite scaffolds. For example, mixing biodegradable and nondegradable materials to create artificial ligament-bone can both promote new bone ingrowth while preserving biomechanical properties upon degradation [187]. On the other hand, from a bionic perspective, the application of dECM scaffolds result in better mimicry of natural tissues,

as they can provide the necessary physical support and vital biochemical and mechanical elements for tissue morphogenesis and homeostasis [188].

Moreover, the surface topology of the scaffold provides geometric cues that can influence cell orientation and morphology through “contact guidance” [189]. For example, regional differences in the orientation and shape of collagen fibers can lead to distinct arrangements and phenotypes of adherent cells; aligned patterns promote tendon differentiation, while randomly arranged fibers induce increased bone formation [190]. Additionally, to enhance the interface strength, triangular and reverse trapezoidal patterns can be prepared to achieve an interlocking design [191]. Adhesives can also be employed to regulate the tension, roughness or chemical properties of the interface, thereby improving joint durability and integration [192, 193]. Furthermore, surface functionalization can be explored to optimize cell/tissue-biomaterial interactions for better integration, such as surface modification with poly(L-lysine) and hyaluronic acid or the application of polydopamine coatings [194, 195], which not only facilitate the encapsulation or grafting of therapeutic molecules, but also improve cell adhesion and spreading.

4.3 Muscle–tendon junction

4.3.1 The structure and function of the muscle–tendon junction

The muscle–tendon junction (MTJ) is a highly specific tissue interface where the muscle’s fascia intersects with the extracellular matrix of the tendon [196]. Moreover, the junction plays a pivotal role in transmitting forces through the tendon to the bone, generated by contracting skeletal muscles. Macroscopically, the transition from muscle to tendon is not a precise, flat boundary (**Fig. 4A**). At the interface, muscle fibers and tendon tissues intermingle, forming an intricate network that increases the adhesive surface area between the two types of tissue, thus facilitating a stronger bond [197]. On a microscopic scale, the MTJ establishes a crucial connection between myogenic cells originating from somites, contributing to the formation of both limb muscles and tendons derived from the lateral plate [198]. The MTJ is comprised of four distinct ultrastructural domains, facilitating the connection between the actin filaments of the terminal sarcomere and the collagen fibers of the tendon [199]. These domains are comprised of distinct components: an intracellular

domain known as the internal lamina, a connecting domain that links the internal lamina to the lamina densa of the external lamina, the lamina densa itself, and a domain responsible for attaching the lamina densa to the collagen fibers, referred to as the matrix [200]. This precise structural and molecular organization allows forces generated by muscle contraction to be efficiently transmitted to the tendon and skeleton. The significant disparity in stiffness and strength between tendon and muscle tissues results in discontinuous mechanical performance at the interface. Consequently, muscle injuries often occur at the interface itself, impairing muscle function [201]. The regenerative capacity of tendons after injury is limited. Postoperative scar tissue often does not match the biomechanical properties or architecture of muscle-tendon junction tissue. This has led to a growing interest in tissue engineering. Researchers are exploring ways to mitigate MTJ injuries and restore tissue integrity.

4.3.2 Regulation of materials, cells, and growth factors in 3D printed scaffolds

Engineering the muscle-tendon interface faces a myriad of challenges owing to the intricate interplay of heterogeneous components with distinct mechanical properties, notably the actin filaments of muscle and the collagen fibers of tendon. Reconstruction of a functional MTJ hinges significantly upon the alignment of muscle fiber bundles on the muscle side and extensive collagen fiber deposition on the tendon side [202]. While conventional fabrication methods struggle to replicate the graded composition and nanoscale interdigitation of native MTJ architecture, recent strides in multi-material 3D bioprinting offer promising avenues for engineering biomimetic MTJ interfaces. In the pursuit of artificial muscle development, facilitating the transmission of contractile forces to tendons and subsequently to bones is essential for maintaining skeletal mobility [203]. To tackle the challenge of replicating the complex muscle-tendon junction, Merceron et al. used 3D bioprinting technology to replicate the muscle and tendon matrices using thermoplastic polyurethane (PU) and PCL respectively, creating a sophisticated muscle tendon interface [203]. The engineered construct featured a gradient structure, with each side displaying strain-stress curves that are indicative of different material properties: the elastomeric fabricated by PU for the muscle-like region, the plastic fabricated by PCL for the tendon-like area, and a blend of elastomeric and plastic fabricated by generating a 10% overlap of

PU and PCL patterns for the transition zone, effectively emulating the natural muscle/tendon interface (**Fig. 4B**). The printed composite scaffolds exhibited regionally changed mechanical properties: the muscle cells loaded PU elastomeric muscle region (Young's modulus of 0.39 ± 0.05 MPa), the tendon cells loaded PCL stiff tendon side (Young's modulus of 46.67 ± 2.67 MPa), and the both cells loaded PU-PCL overlapped interface region (Young's modulus of 1.03 ± 0.14 MPa). Notably, GDF-5 has been found to promote the proliferation and differentiation of tenocytes, a process critical for improving tendon repair and recovery of function. Furthermore, the bioprinted construct demonstrated promising outcomes in terms of high cell viability and cellular differentiation (**Fig. 4C**). Sun et al. utilized 3D printing technology to develop a biologically active fibrous-enhanced hydrogel for structural and functional regeneration of the MTJ [204]. Additionally, a study by Zhang et al. demonstrated that a self-assembling dipeptide hydrogel synthesized from P11-4 and P11-8 polypeptides has been shown to bind with GDF-5, forming a novel functional hydrogel [205]. Through precise 3D printing, a scaffold made from PLGA fibers was created and loaded with MSCs and transmembrane glycoprotein Klotho combined in a silk fibroin methacryloyl (SilMA) hydrogel to form a fiber-reinforced multifunctional hydrogel. MSCs were incorporated into the hydrogel to improve cell regeneration, and recombinant transmembrane glycoprotein Klotho was added to serve as an antioxidant and immunomodulatory biomolecule. The implementation of Klotho is an important factor to consider, as the study indicates that the presence of Klotho regulates the inflammatory response, guiding macrophage polarization from the M1 (inflammatory) to M2 (anti-inflammatory) phenotype. This effect downregulates proinflammatory cytokines (e.g. IL-1β, tumor necrosis factor-α) which inhibits the viability of MSCs, while upregulating growth factors (e.g. TGF-β, IL-10) and promoting MSC survivability and growth. The synergy of Klotho with the implanted MSCs, along with the mechanical support of the PLGA, promotes the MTJ repair. These advancements in 3D printing and tissue engineering offer promising strategies for the development of biomimetic muscle-tendon junctions. However, achieving seamless integration between 3D printed muscle-tendon junction scaffolds and surrounding tissues remains to be a challenge.

4.3.3 Bioprinting and biomimetic scaffold design

The use of bioprinting in the development and integration of scaffolds for the muscle-tendon junction has been a major focus in facilitating the regeneration of related tissues [206]. The goals of these methods are to endow the scaffolds with unique properties important for both biocompatibility and mechanical compatibility, and to generate a degradation profile which matches the tissue regeneration process. One major focus in bioprinting is biomaterials, specifically bioinks: the substances the scaffolds are composed of which can greatly influence cell behaviors. For example, Laternser et al. utilized bioprinting techniques within a porous scaffold to create muscle-tendon-like structures for high-throughput drug screening at the muscle-tendon interface [207]. Circular dumbbell-shaped pillars were fabricated and inserted into a polystyrene-coated porous plate. Alternating layers of GelMA-polyethylene glycol diacrylate bioink and tendon cells were extruded and inkjet-printed around the pillars to mimic tendon-like tissue (**Fig. 4D**). GelMA and muscle cells were then bioprinted in an alternating fashion between the pillars, resulting in the formation of muscle-like tissue. This approach facilitated the organized arrangement of muscle cells which induced rapid differentiation of muscle fibers and tendon formation due to the biocompatibility of the GelMA bioink. Furthermore, electrical stimulation was employed to promote muscle fiber contraction and support muscle functionality. In addition, growth factors such as TGF-β1 and IGF-1, when immobilized in a spatially regulated fashion using surface chemistry approaches, may also facilitate site-directed tissue regeneration [196]. Nanofibers have also been shown to confer mechanical properties well-suited for tissue-engineered tendons. Specifically, aligned electrospun nanofibers may be uniquely applicable in tendon tissue engineering by virtue of their ability to provide topographical signals [208]. It has been proposed that nanoyarn nanofibers generate favorable microenvironments conducive to cell adhesion, alignment, and infiltration, which are properties considered advantageous for tendon regeneration, through the development of a highly porous 3D microarchitecture [209]. When regenerating the muscle-tendon interface, functionalizing scaffold degradation kinetics to coincide with tissue ingrowth is of critical importance. 3D printing scaffolds using materials permitting adjustable degradation properties and incorporating protease-sensitive domains enable optimization of degradation dynamics which supports replacement by neo-tissue.

Collectively, such functionalization approaches may generate biomimetic microenvironments with enhanced capacity to emulate the native tissue-tissue interface.

Incorporating biomimetic design principles into 3D printed scaffolds for musculoskeletal tissue interfaces is essential for effectively regenerating these complex tissues. The native muscle-tendon junction boasts intricate hierarchical structures and precise biochemical compositions that have evolved over time to optimally transmit forces while facilitating cellular processes. To regenerate this specialized interface using tissue engineering approaches, it is imperative that the scaffold mimics key aspects of the native microenvironment. Biomimetic design aims to endow scaffolds with properties analogous to the natural extracellular matrix by employing various strategies. For instance, the use of gradient structures and materials can replicate the mechanical property transitions observed across different tissue types [210]. Incorporating biochemical signals through growth factor immobilization or decellularized matrices may also provide instructive cues for cellular behavior. A study by Kim et al. used a novel bioprinting technique to mimic various MTJ components through the use of different bioinks resembling key components, such as muscle-specific, tendon-specific and collagen bioinks [211]. The bioinks were created using two key components, dECMs (derived from porcine muscle and tendon) and type I collagen. Muscle and tendon dECMs were used accordingly to create muscle-specific and tendon-specific bioinks, which were both seeded with human adipose-derived stem cells (hADSCs). It was found that by using these bioinks alongside the collagen I bioink to create a biphasic gradient with a specialized 3D printing nozzle that the scaffold could mimic the function of the MTJ, outperforming the collagen-only control, indicated by the assessment of gene expression and differentiation capability. Additionally, topographical features such as aligned nanofibers aim to emulate natural structural cues [212]. Adopting a biomimetic design paradigm that incorporates multi-level structural, compositional, and functional mimicry holds promise for generating scaffolds capable of seamlessly integrating with host tissues to regenerate the muscle-tendon interface. However, the current scene of 3D printing technology regarding the MTJ has not been fully explored. Continued efforts in refining biomimetic design will be crucial for advancing the field of musculoskeletal interface tissue engineering.

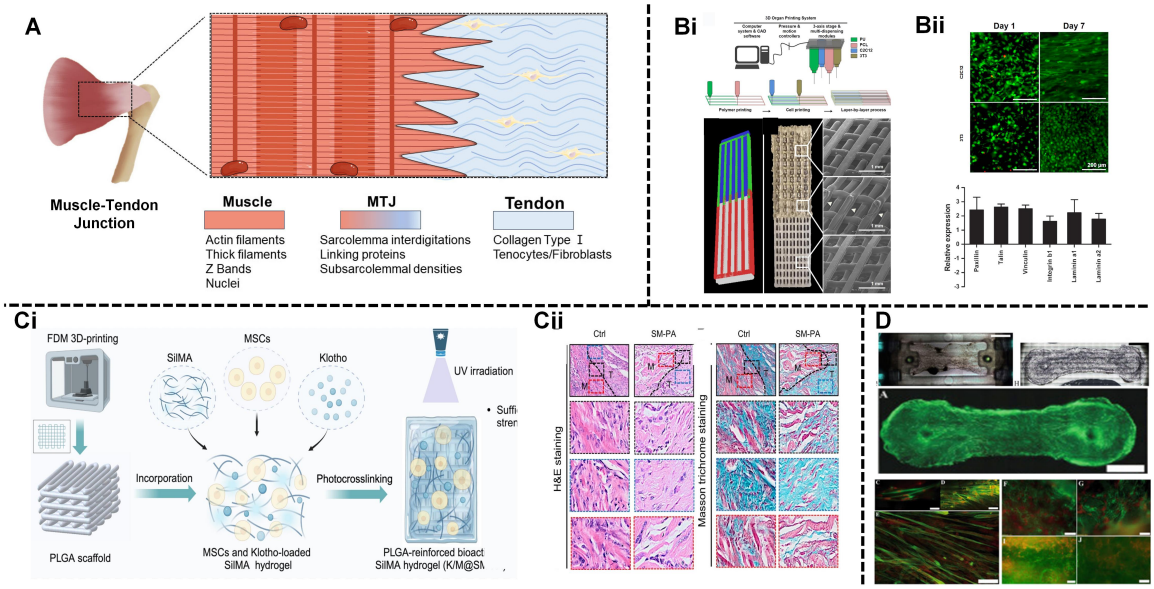

Fig. 4. (A) Structure of the muscle-tendon junction (MTJ), consisting of tendon (blue area), muscle (red area) and the MTJ (overlap region). Reprinted with permission from Ref. [196]. Copyright MDPI. (Bi) 3D printing method schematic (top) and MTJ scaffold structure with SEM images (bottom). (Bii) Cell viability (top) and gene expression (bottom) associated data. Reprinted with permission from Ref. [203] Copyright IOPScience. (Ci) Schematic of bioactive fiber-enforced hydrogel scaffold fabrication. (Cii) Hematoxylin and eosin (HE) staining (left) and Masson's trichome staining (right) of fiber-enforced hydrogel scaffold in rat MTJ defects. Reprinted with permission from Ref. [204]. Copyright Science. (D) 3D bioprinting of myoblasts and tenocytes for evaluating monoculture tissue model differentiation**.** Reprinted with permission from Ref. [207]. Copyright SAGE Publications.

4.4 Intervertebral disc interfaces

4.4.1 Intervertebral disc interface structure

IVD is an intricately designed composite structure essential for the biomechanical functionality of the spinal column, facilitating both flexibility and load distribution across vertebral bodies [213]. Composed of three primary regions, each region contributes uniquely to the IVD's function and structural integrity. The nucleus pulposus (NP), a centrally located gelatinous core, provides the disc with the ability to absorb and distribute loads due to its high water content and proteoglycan composition **(Fig. 5A)** [214]. Surrounding the NP is the annulus fibrosus (AF), a robust, multilaminar fibrous ring that

confers tensile strength and maintains the structural integrity of the disc under mechanical stress [215]. The cartilaginous endplates (CEPs) cap the NP and AF at both ends, serving as interfaces with the adjacent vertebral bodies and playing a crucial role in nutrient transportation to the disc [216]. Despite its critical functions, the IVD is susceptible to degeneration, characterized by biochemical and structural changes within these components, leading to diminished disc function, chronic back pain, and potential disability. This degeneration is a significant concern given its impact on quality of life and the economic implications associated with treatment and loss of productivity.

4.4.2 Regulation of materials, cells, and growth factors in 3D printed scaffolds to mimic the IVD structure

3D printing technology has revolutionized the field of tissue engineering, particularly in the fabrication of scaffolds that replicate the intricate structure and functional gradients of the IVD [217]. A key development in tissue-engineered IVD scaffolds is the strategic use of diverse materials in combination with cells and bioactive factors. Recent studies have demonstrated significant advancements in 3D printing techniques for IVD tissue engineering which offers promising solutions for the treatment of IVD disc diseases.

Hydrogels are frequently employed in tissue engineering to mimic the soft and hydrated properties of the NP within the IVD (**Fig. 5B)** [218]. Several types of hydrogels, including chitosan, alginate, hyaluronan, gelatin, and agarose, have been commonly utilized for this purpose. These hydrogels create a supportive environment that replicates the gelatinous nature of the NP [219, 220]. However, hydrogel alone often lacks sufficient mechanical properties for IVD tissue engineering. To overcome this limitation, recent studies have explored techniques such as double network crosslinking or the addition of nanofibers to enhance the mechanical strength of hydrogels [97, 221]. Conversely, tougher and fibrous materials like PCL and PLGA are employed to replicate the structure of the AF [222, 223]. The creation of a functional gradient between these materials is crucial to mimic the native NP-AF transition. Multi-material 3D printing techniques allow for the modulation of material properties along the scaffold, ensuring a seamless mechanical transition that closely mirrors the natural disc [224]. By adjusting parameters such as the ratio of hydrogel

to polymer, scaffolds can be crafted with varying stiffness and biochemical characteristics, facilitating the desired gradient formation. The strategic placement of NP and AF cells within the scaffold further enhances its regenerative capabilities, enabling the development of structurally and functionally accurate IVD tissue.

4.5.3 3D printing and biomimetic scaffold design

The development of tissue-engineered IVDs using 3D printing techniques offers the potential to fabricate scaffolds that closely mimic the complex, multi-layered structure of the native disc [225]. By utilizing specific materials and/or cells in the 3D printing process, it is possible to replicate the unique mechanical and biological properties of each component of the IVD. On the one hand, the NPs, which are softer and more elastic tissues at the core of the IVD, can be emulated using hydrogel-based bioinks. These bioinks, often containing cells such as chondrocytes or NP cells, are enriched with growth factors like TGF-β to promote cell differentiation and to maintain a high-water content, resembling the gelatinous nature of native NPs (**Fig. 5C)**. To enhance the biofunctionality of the scaffolds, bioactive factors like TGF-β3 and insulin-like FGF are often incorporated into the bioinks [56, 226]. These factors play a pivotal role in guiding the differentiation of embedded mesenchymal stem cells into NP and AF phenotypes, promoting region-specific cellular activities and tissue regeneration. On the other hand, the AF, which is tougher and the load-bearing portion of the IVD, can be replicated using fibrous bioinks. These bioinks consist of aligned fibers and can be loaded with fibroblast-like cells or AF cells [223]. Additionally, the incorporation of connective tissue growth factors can further enhance the formation of fibrous tissue within the printed construct, replicating the anisotropic mechanical properties of the AF [56]. A notable example of a novel bioink for IVDs that was presented involved gellan gum-poly (ethylene glycol) diacrylate (GG-PEGDA) and PLA which were combined into a tunable, 3D printed cartilage system, with PLA forming the stiffer AF regions and GG-PEGDA hydrogels seeded with mesenchymal stem cells occupying the softer NP regions [227]. This approach demonstrated excellent mechanical properties, cell viability, and degradation characteristics, making it a highly biomimetic IVD replacement candidate. By combining biomaterials, polymers, growth factors, cells, and nanoparticles, researchers have created highly biomimetic IVD replacements capable of inducing the

differentiation of cells into AF and NP phenotypes. These approaches utilize specific patterning and controlled release of growth factors to guide the differentiation process, resulting in the formation of tissue that closely resembles the native IVD structure. While there is still progress to be made in achieving fully biomimetic IVD replacements, these cutting-edge advancements in bioprinting techniques and bioink development hold great promise. The ability to create complex structures with spatial control and induce site-specific cell differentiation offers exciting possibilities for the future of IVD tissue engineering and regeneration.

The ability to control the spatial distribution of structural and biochemical elements within a scaffold is a critical advantage of 3D printing technology. This precision is essential in recreating the complex architecture of the IVD, particularly at the interfaces between the NP, AF, and CEPs. Advanced 3D printing methods, such as extrusion and inkjet printing, are employed to fabricate scaffolds with defined, gradient structures that ensure a gradual transition in material properties. For instance, one approach involves the fabrication of scaffolds with a tailored distribution of hydroxyapatite, which mimics the mineral gradient from the CEPs to the vertebral bodies. This gradient facilitates the mimicry of load distribution and the nutrient transport functions of the endplates. Researchers have successfully utilized 3D printing to create such scaffolds with precise hydroxyapatite distribution, enabling the replication of the native IVD structure. In another study, researchers aimed to replicate the angle-ply architecture of the annulus AF, which is crucial for IVD regeneration [222]. They employed a newly developed electrohydrodynamic 3D printing technique to fabricate high-resolution PCL scaffolds (**Fig. 5D)** [96]. Finite element analysis (FEA) was used to verify the structural advantages of these scaffolds. The PCL scaffolds were then further assembled into AF constructs, accurately replicating the angle-ply architecture of the AF. FEA and mechanical testing were instrumental in identifying the most effective assembly methods for annulus fibrosus (AF) scaffolds. These methods ensured that the 3D printed AF scaffolds, constructed using a micro-extrusion-based 3D printing approach with silk-carrageenan filaments, were not only anatomically accurate but also exhibited superior mechanical properties. The *in vitro* studies confirmed the excellent biocompatibility of these 3D printed scaffolds, which promoted the adhesion and

proliferation of AF cells. The fiber alignment in the 3D-printed constructs was such that it mirrored the native tissue when rotated 90° across the periphery, a key feature for mimicking the natural angle-ply structure of the AF. The mechanical strength of these constructs reached approximately 78 kPa, surpassing that of native tissue, while also supporting cell viability, proliferation, and the deposition of AF-specific extracellular matrices. These findings suggest the potential utility of these 3D-printed AF constructs for disc replacement therapy [228].

These examples highlight how 3D printing technology can be utilized to closely mimic the structural characteristics of the intervertebral disc. By precisely controlling the spatial distribution of materials and incorporating biomimetic features, researchers can create scaffolds that replicate the complex architecture and biological functions of the IVD. These advancements hold great potential for IVD tissue engineering and the development of effective treatments for degenerative disc diseases.

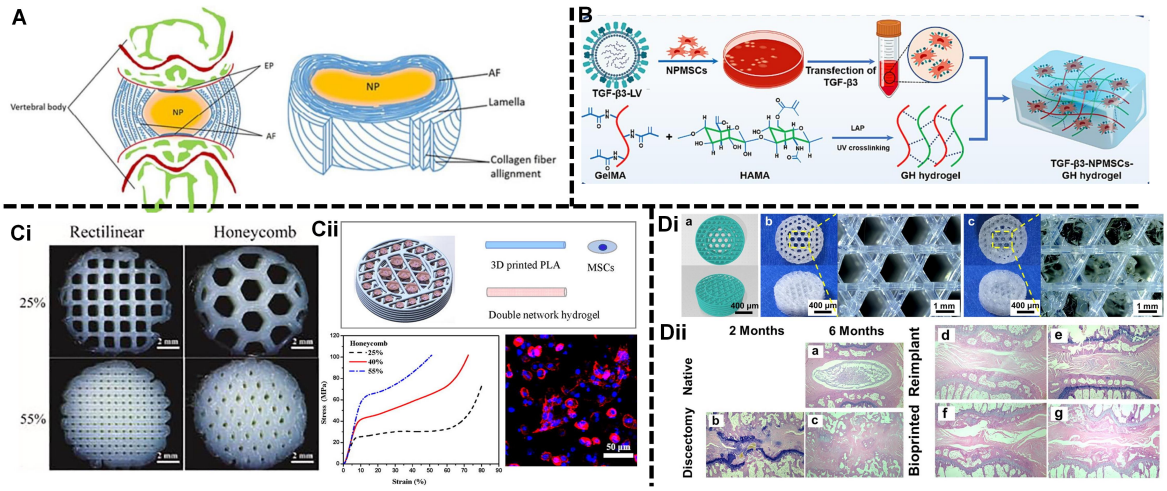

Fig. 5. (A) Schematic representation of the IVD showing the NP as a gelatinous core surrounded by the AF, with CEPs at both ends. Reprinted with permission from Ref. [220]. Copyright Frontiers. (B) Schematic illustrating the construction of genetically engineered TGF-β3-NPMSCs within GelMA and HAMA bicomponent hydrogels. Reprinted with permission from Ref. [218]. Copyright Elsevier. (Ci) Optical images of rectilinear and honeycomb IVD scaffolds. Reprinted with permission from Ref. [225]. Copyright Elsevier. (Cii) Assessment of mechanical properties and cell biocompatibility of cell-laden

honeycomb 3D IVD scaffolds. Reprinted with permission from Ref. [225]. Copyright Elsevier. (Di) In silico 3D IVD models and photographs of 3D printed IVD scaffolds. Reprinted with permission from Ref. [96] Copyright Elsevier. (Dii) H&E staining demonstrates therapeutic efficacy in maintaining disc space and producing new extracellular matrix. Reprinted with permission from Ref. [96] Copyright Elsevier.

5. Challenges and limitations

Despite the remarkable progress in 3D printing for musculoskeletal tissue engineering, several challenges must be surmounted to transition these technologies into clinical practice. A primary issue is the scarcity of biocompatible, printable materials that can replicate the intricate mechanical and biological attributes of native tissues. Although polymers, ceramics, and hydrogels have been utilized, many lack the requisite strength, elasticity, or degradation rates necessary for sustained tissue regeneration. Furthermore, the printability of these materials often necessitates harsh solvents, high temperatures, or crosslinking agents that may impair cell viability and function. There is an urgent need for the development of new bioinks and printing strategies that can harmonize printability, biocompatibility, and scaffold functionality. Another challenge pertains to the limited resolution and speed of current 3D printing technologies. While high-resolution techniques like stereolithography and two-photon polymerization can produce micro-scale features, they are constrained by slow printing speeds and high costs. Conversely, high-speed extrusion-based printing often results in low resolution and inadequate control over cell and bioactive factor distribution. There is a clear demand for advanced printing technologies that can achieve high resolution, speed, and precision to fabricate complex tissue constructs with defined architectures and functions.

The long-term stability and *in vivo* integration of 3D printed scaffolds present significant hurdles. Scaffolds, though initially designed to mimic native tissue structure and composition, often fail to maintain their integrity and function over time due to material degradation, mechanical failure, or immune rejection. Additionally, poor integration with surrounding tissues can lead to fibrous capsule formation or voids, which can hinder the regenerative process. Strategies to enhance scaffold stability and integration are needed,

such as incorporating anti-inflammatory agents, modulating immune responses, or preconditioning constructs in bioreactors prior to implantation. The scalability and cost-effectiveness of 3D printing technologies must also be addressed for widespread clinical adoption. While desktop 3D printers can fabricate small-scale scaffolds, large-scale, patient-specific implants require industrial-grade printers with significant capital and operational costs. Furthermore, regulatory and quality control aspects for 3D printed implants are still evolving, with limited guidelines and standards for their design, manufacture, and testing. There is a need for cost-effective, scalable printing platforms and the establishment of clear regulatory pathways and quality assurance protocols to facilitate clinical translation.

Finally, the biological complexity and variability of musculoskeletal tissues pose substantial challenges for regeneration using 3D printed scaffolds. Scaffolds provide initial structural and biochemical cues for tissue formation but cannot fully replicate the dynamic and heterogeneous microenvironment of native tissues, which are regulated by complex interactions of cells, growth factors, and mechanical stimuli. The regenerative potential of scaffolds may also vary based on patient-specific factors and the nature and severity of the tissue defect. Personalized and adaptive printing strategies are required to tailor scaffolds to individual patient needs and defects, along with advanced biomanufacturing approaches such as induced pluripotent stem cells or gene editing to enhance the biological functionality and specificity of the constructs. In summary, to harness the full potential of 3D printing in musculoskeletal tissue engineering, challenges such as the development of novel bioinks and printing technologies, enhancement of scaffold stability and integration, scalability and cost-effectiveness of manufacturing, and personalization of constructs must be addressed. Multidisciplinary research and collaboration are essential to transform 3D printing from a niche technology into a mainstream solution for musculoskeletal tissue regeneration.

6. Conclusion and future perspectives

The field of 3D printing for musculoskeletal tissue engineering is rapidly advancing with new technologies, materials, and applications emerging at an unprecedented pace.

Integration with other advanced manufacturing technologies, such as robotic assembly [154], machine learning [229], and artificial intelligence [230], holds great promise. These technologies can automate and optimize the design, fabrication, and quality of complex tissue constructs based on patient-specific data and feedback. Robotic systems can assemble multiple 3D printed components into larger functional constructs, while machine learning algorithms can predict optimal printing parameters and scaffold designs. Additionally, real-time monitoring and adjustment using artificial intelligence can ensure consistent and high-quality constructs [231]. Another exciting direction is the development of 4D printing, enabling dynamic and responsive scaffolds that can change their shape, composition, or function over time in response to external stimuli or biological cues [123]. These scaffolds can degrade or remodel *in vivo*, release bioactive factors or cells in a controlled manner, and respond to mechanical loads or environmental changes, enhancing tissue regeneration.

The convergence of 3D printing with nanomedicine, gene editing, and synthetic biology can revolutionize musculoskeletal tissue regeneration. The integration of nanoparticles or nanovesicles into bioinks enables the targeted delivery of drugs, growth factors, or genetic material [232, 233]. Gene editing tools like CRISPR-Cas9 can precisely modify cell genomes, allowing for the correction of genetic defects or the introduction of therapeutic genes. CRISPR-Cas9 has been successfully applied in various scenarios, such as gene knock-in for treating single-gene disorders like sickle cell anemia and gene knock-out for studying gene functions in disease models [234]. Synthetic biology approaches enable the design of artificial extracellular matrices and programmed cell-cell communication, which can improve tissue constructs. For example, synthetic biology tools have been used to engineer cells with specific adhesive properties, influencing the self-assembly of 3D structures in tissue engineering

Standardization and translation of 3D printed musculoskeletal tissues require robust protocols, reliable predictive models, and engagement of multidisciplinary teams. Establishing good manufacturing practices (GMP) for bioprinting, validating *in vitro* and *in vivo* models, and conducting well-designed clinical trials are crucial [235]. Regulatory

frameworks must evolve to provide clear guidance for approval and commercialization. Collaboration among engineers, biologists, clinicians, and regulation experts is vital for responsible and effective translation.

Moreover, the incorporation of bioinformatics into this interdisciplinary field can further enhance the precision and efficiency of tissue regeneration strategies [236, 237]. Bioinformatics can analyze vast amount of data to identify potential therapeutic targets and optimize the design of 3D-printed scaffolds. For example, by analyzing gene expression data and protein interaction network, bioinformatics can pinpoint key genes and pathways involved in tissue repair and regeneration, guiding the selection of appropriate growth factors and the design of bioinks [238]. Additionally, bioinformatics can assist in the development of predictive models for tissue behavior and the optimization of scaffold architecture to better mimic the native tissue environment.

The future of 3D printing for musculoskeletal tissue engineering is filled with opportunities. Advanced manufacturing, 4D printing, nanomedicine, gene editing, synthetic biology and bioinformatics can lead to personalized and functional tissue constructs. Overcoming challenges and seizing these opportunities will revolutionize the field, benefiting millions of patients worldwide.

Declaration of Competing Interest

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

Data availability

No data was used for the research described in the article.

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