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Three-dimensional bioprinting for musculoskeletal regeneration and disease modeling

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Abstract

Musculoskeletal disease and injury are highly prevalent disorders that impose tremendous medical and socioeconomic burdens. Tissue engineering has attracted increasing attention as a promising technique of regenerative medicine to restore degenerative or damaged tissues and is used to produce functional disease models. As a revolutionary technology, three-dimensional (3D) bioprinting has demonstrated a considerable potential in enhancing the versatility of tissue engineering. 3D bioprinting allows for the rapid and accurate spatial patterning of cells, growth factors, and biomaterials to generate biomimetic tissue constructs. Meanwhile, 3D-bioprinted *in vitro* models also offer a viable option to enable precise pharmacological interventions in various diseases. This review provides an overview of 3D bioprinting methods and bioinks for therapeutic applications and describes their potential for musculoskeletal tissue regeneration. We also highlight the fabrication of 3D-bioprinted models for drug development targeting musculoskeletal disease. Finally, the existing challenges and future perspectives of 3D bioprinting for musculoskeletal regeneration and disease modeling are discussed.

Keywords: 3D bioprinting; Bioink; Musculoskeletal tissue; Regeneration; Disease modeling

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1. Introduction

The musculoskeletal system is made of bone, skeletal muscle, cartilage, tendon, meniscus, and intervertebral disc (IVD), which are responsible for motion as well as provide structural support for the human body and protect internal organs. In daily activities, musculoskeletal tissues are susceptible to small tears or other injuries due to various mechanical loads. Unlike cartilage, tendon, meniscus, and IVD, bone and skeletal muscle have high regenerative capacity after slight injuries. However, severe damage beyond the self-repair ability can lead to a range of musculoskeletal disorders (MSDs).

MSDs affect 1.7 billion people and have become the leading cause of morbidity worldwide according to the Global Burden of Disease Study.[1,2] Moreover, the incidence of MSDs shows an increasing trend with the aging of the population. In the United States, for example, there are at least 70 million clinic visits and 130 million clinical contacts for MSDs each year, resulting in more than $150 billion in the national healthcare system costs.[3] Mild MSDs can be addressed with physical therapy or drug intervention. Severe MSDs, on the other hand, require surgical reconstruction. Autograft represents the gold standard for the treatment of severe MSDs, but is limited by donor site scarcity, morbidity, and pain.[4] Allografts and xenografts are feasible alternatives, although concerns regarding immunological incompatibility, rejection risk, and infectious agent transmission remain.[5] Therefore, novel approaches to regenerating damaged musculoskeletal tissues are urgently needed.

Tissue engineering enables the creation of viable scaffolds for the regeneration of damaged tissues. Since the beginning, tissue engineering has the prospect of generating tissues for a variety of purposes, ranging from *in vitro* disease modeling to *in vivo* tissue regeneration. Tissue-engineered scaffolds provide a hospitable microenvironment for cell adhesion, spreading, proliferation, migration, and differentiation. Moreover, the addition of bioactive molecules, such as drugs or growth factors, can further enhance the ability of scaffolds to promote cell differentiation and induce the formation of target tissues. However, generating tissues that precisely mimic the structural and functional features of native tissues remain unattainable in musculoskeletal tissue engineering, despite the promising translational potential of tissue engineering approaches. This is primarily due to the fact that conventional manufacturing technologies lack the ability to accurately regulate the spatial arrangement of construction elements.[6] Furthermore, while spontaneous cellular organization processes can build certain types of fundamental biostructures, they are extremely difficult to regulate and manage. Few technologies have so far been able to reconstruct the complex tissue architecture and cell spatial heterogeneity, which are required to mimic the physiologic function.

Recently, three-dimensional (3D) bioprinting is applied in a variety of biomedical scenes, such as tissue engineering, disease modeling, and drug screening.[7,8] Compared with traditional tissue engineering approaches, 3D bioprinting has several advantages, such as determining tissue form prior to printing, and acts as a bridge to clinical application. The advancement of 3D bioprinting has substantially expanded the field of musculoskeletal tissue engineering by allowing the development of scaffolds that can effectively replicate desired mechanical characteristics and structures. 3D bioprinting allows for the precise and controlled spatial arrangement of cells in 3D scaffold materials. The development of increasingly sophisticated and biomimetic tissue-engineered analogues holds the promise for producing patient-derived functional grafts as well as clinically predictive drug testing tools. Therefore, it is an emerging strategy of constructing tissues for musculoskeletal regeneration, disease modeling, and drug development by 3D bioprinting.

In this review, we provide a concise review of 3D bioprinting, including several common 3D bioprinting techniques and bioinks. The application of these techniques in musculoskeletal tissue regeneration is highlighted. Following that, recent advances of 3D bioprinting for musculoskeletal disease modeling and drug screening are summarized. Finally, we discuss the existing challenges and future perspectives of 3D bioprinting for musculoskeletal regeneration and disease modeling.

2. Brief overview of 3D bioprinting

3D bioprinting is the process of patterning and assembling bioactive materials, such as growth factors, cells, and biomaterials based on predefined 3D designs, leading to the creation of a functional tissue construct.[9] 3D bioprinting technology is a subclass of 3D printing technology that is primarily used in the biomedical field. Traditional 3D printing often uses plastic or alloy materials for printing, whereas the materials used in 3D bioprinting are called bioinks, which consist of living cells alone or together with supporting biomaterials such as hydrogels.[10] The major advantage of 3D bioprinting over other approaches, such as microengineering and cell sheet engineering, is its ability to create spatially complex and heterogeneous tissue constructs consisting of cells and/or various biomaterials.[11] Through 3D bioprinting, diverse cells and biomaterials can be localized to replicate the structural complexity of tissues. The 3D bioprinting process can be achieved through different technologies and each technique is based on its own principles and has distinct requirements for the materials to be used. Therefore, bioinks and bioprinting techniques need to be attuned to each other. The following is a brief introduction to several common 3D bioprinting technologies and bioinks.

2.1. Bioprinting technologies

3D bioprinting technologies create functional tissue constructs based on the principles of layer-by-layer stacking and consistent self-assembly.[12] According to the adopted bioprinting techniques, these layers can be integrated by different means, such as heat, light radiation, and chemical crosslinking. The current mainstream 3D

bioprinting methods are inkjet bioprinting, extrusion-based bioprinting, and light-based bioprinting. Briefly, inkjet bioprinting typically involves spraying low-viscosity bioinks onto a substrate in discrete droplets, while extrusion-based bioprinting extrudes viscous bioink into continuous filaments. The bioinks for light-based bioprinting are composed of photoresponsive materials, which are solidified by light irradiation.

2.1.1. Inkjet bioprinting

Inkjet bioprinting is the earliest developed 3D bioprinting technology and its concept is the same as that of traditional 2D inkjet printing.[13] The technology, also known as drop-on-demand bioprinting, uses various energy sources to allow for pattern deposition of discrete droplets onto a substrate layer.[14] The system achieves the deposition of droplets by applying pressure pulses to overcome the surface tension of the materials. By adjusting the energy parameters, the density, shape, and size of the droplets can be controlled. These droplets can be ejected to predetermined positions to create a 3D construct with different concentration gradients. Inkjet printers with a reservoir connected to multiple nozzles enable simultaneous printing of different cells and biological components. Moreover, this technology has relatively fast printing speed and is ideal for printing structures for soft tissue regeneration.[8] However, it is limited by several disadvantages. Due to the low driving force of inkjet printers, bioinks with a higher viscosity are not suitable for inkjet printing, narrowing the selection range of printable materials.[15] The use of lower-viscosity bioinks results in poor mechanical strength of scaffolds, which fail to meet the requirements of *in vitro* culture and transplantation. In addition, it is difficult to print constructs with physiologic cell density because of the nozzle clogging caused by high cell density bioinks.

2.1.2. Extrusion-based bioprinting

Currently, extrusion-based bioprinting has become one of the most popular technologies of 3D bioprinting due to its versatility and affordability.[16] This method usually fabricates a 3D construct by utilizing mechanical forces driven by air pressure or a motor to extrude viscous cell-laden bioinks through a nozzle in a controlled and filamentous manner. The precision of the printed construct can be adjusted by controlling the printing speed, extrusion speed, printing temperature, nozzle size, and other parameters. Extrusion-based bioprinting allows successful fabrication of constructs with high cell density (>10^8 cells per mL).[17] Another major advantage for extrusion-based bioprinting is that any materials with sufficient viscosity can be used as candidates for bioinks.[18] Higher-viscosity materials provide structural support for the printed structure and lower-viscosity materials are beneficial for maintaining cell survival and function. The trade-off between printability and cell viability needs to be considered in the selection of bioinks. Bioinks with different ranges of viscosity (30 to over 6×10^7 mPa·s) for use in extrusion-based bioprinting have been reported.[19] Extrusion-based bioprinting is the most common printing method for musculoskeletal tissue engineering, mostly because of its advantages, including a wide selection of available bioinks, ease of operation, fast printing, and ability to create large and complex constructs.

2.1.3. Light-based bioprinting

Light-based bioprinting is an additive manufacturing technology with very high resolution and accuracy. The technology uses a tuned light source to solidify or deposit bioinks. The printed structure supports higher cell survival (85%–95%) due to the absence of high temperature and extrusion shear force damage.[20] Stereolithography (SLA) and digital light processing (DLP) are typical light-based bioprinting technologies that could crosslink polymer solutions based on the light pattern on each layer to fabricate desired constructs. The samples printed by these methods usually present high precision and smooth surfaces. Another common light-based bioprinting method is laser-assisted bioprinting (LAB), which does not depend on printheads, and the structures printed by this method can support high cell viability (>95%).[21] For LAB, laser pulses are manipulated to induce the bioink droplets to transfer from the donor layer to the collecting substrate and form 3D structures. Volumetric bioprinting has recently become a potent tool because of its ability to quickly fabricate tissue constructs.[22,23] The bioinks polymerize and form expected structure when exposed to a specific light source. The process can be completed in seconds without the need for support and sacrificial materials, significantly improving the suitability of biomaterials.[24] Compared with the traditional extrusion-based and laser-assisted bioprinting technologies, volumetric bioprinting has obvious advantages in accuracy, resolution, and cell viability, opening new possibilities for musculoskeletal regeneration and disease modeling.[25] Overall, the major advantage of light-based bioprinting technologies is their capacity to fabricate complex designs with high resolution and instantly print structures without supporting materials. Despite these advantages, there are also some limitations, such as high cost and limited choice of photopolymerizable bioinks.

2.2. Bioinks

In 3D bioprinting, living cells encapsulated in bioinks are used and printing parameters are adjusted in the fabrication process of living tissues. The printability of bioinks is defined as the capacity to generate 3D structures with good fidelity

and integrity.[26] Relative to the printing method used, the printability of bioinks mainly depends on their rheological characteristics and gelation kinetics.[27] The printability of bioinks and the regulation of their physicochemical properties on cell behaviors are the key to the regeneration of tissues and organs. In general, bioinks need to possess some essential characteristics that meet the basic requirements of 3D bioprinting. Bioinks must have good biocompatibility, which requires that the chosen materials and their degradation products must be nontoxic. Moreover, bioinks must provide cell adhesion sites that allow cells to survive, adhere, and proliferate. When used for printing different musculoskeletal tissues, bioinks must meet the tissue-specific requirements. For bone tissue, bioinks need to have angiogenic and osteogenic bioactivity as well as strong mechanical properties. For skeletal muscle tissue, bioinks must be able to promote cell alignment and myogenic differentiation and maturation to simulate muscle-oriented fibrous structures. For cartilage, meniscus and IVD tissue, region-specific extracellular matrix (ECM) deposition is a concern when designing bioinks. Bioink materials commonly used for 3D bioprinting of musculoskeletal tissues include natural materials and synthetic materials. They provide suitable environment for cell growth and are used together with cells for bioprinting of target tissues or organs.

2.2.1. Natural materials

Natural materials such as collagen, gelatin, alginate, fibrinogen, hyaluronic acid, and decellularized extracellular matrix (dECM) are common components in the bioink formulation. As a key structural component of ECM, collagen has the advantages of low immunogenicity, good biocompatibility, and biodegradation. The concentration of collagen affects the printing accuracy and the structural fidelity of the target constructs. The constructs printed using bioinks composed of low-concentration collagen are fragile, which is not conducive to the long-term maintenance of structural stability. To that end, Beketov *et al*. developed a bioink based on high-concentration collagen for the bioprinting of chondrocytes.[28] Cartilaginous tissue formation was observed 5–6 weeks after subcutaneous implantation. Gelatin is the product of partial hydrolysis of collagen, and its structure is similar to that of ECM. Compared with collagen, gelatin has a higher water solubility. Gelatin remains a gel at low temperature (<20°C) and dissolves into a liquid at high temperature (37°C). This temperature-sensitive property makes gelatin one of the most common bioink components. However, gelatin-based bioinks alone cannot form a stable network structure for subsequent cell culture. To address this, a common strategy is used to modify gelatin with methacrylate groups to obtain a photocrosslinkable hydrogel, namely gelatin methacrylate (GelMA).[29]

Another strategy is to combine gelatin with other polymers, such as alginate or fibrinogen, to form a hybrid bioink.[30,31] Alginate, a polysaccharide derived from natural algae, is considered nontoxic and biologically inert to mammalian cells. A major advantage of alginate is that it can be rapidly crosslinked into a gel in the presence of divalent cations.[32] Due to the lack of biological cues, alginate is often combined with other components such as gelatin or collagen to form a bioink with biological activity.[33] Fibrinogen, a glycoprotein found in the blood, can be converted to insoluble fibrin under the catalysis of thrombin, forming a stable network structure to promote tissue repair. Fibrin has good biocompatibility and biodegradability, and there are some amino acid sequences, such as RGD (Arg-Gly-Asp), in its structure which can promote cell binding.[34] Despite these advantages, mechanically stable constructs cannot be bioprinted with pristine fibrinogen solutions because of their low viscosity. Other components, such as alginate and GelMA, are often incorporated to fibrinogen solutions to improve their printing feasibility.[35,36] Hyaluronic acid (HA) is one of the main constituents of ECM and has been extensively employed in tissue engineering because of its anti-inflammatory and angiogenic properties. Due to its versatility in structure modification, it has proved to be an excellent bioink successfully applied to 3D bioprinting in recent years. The addition of HA can improve the dispersion uniformity of the bioinks.[37] Like gelatin, HA has been mainly used in bioinks in combination with other polymers. Recently, dECM-based bioinks have gained popularity in 3D bioprinting applications. As a novel bioink derived from native tissue, a dECM-based bioink retains native ECM components and necessary biological cues, which can enhance cell viability and tissue-specific functionality.[38,39] Lee *et al*. employed bone-derived dECM to incorporate human adipose-derived stem cells and printed 3D bone construct.[40] It was found that bioinks composed of bone dECM and alginate promoted cell viability and osteogenic differentiation compared with pristine alginate-based bioinks.

2.2.2. Synthetic materials

Synthetic polymers provide greater design flexibility and structural complexity than natural polymers, which is advantageous for bioprinting. With the incorporation of ECM elements and extra crosslinking, synthetic polymers can exhibit improved mechanical and biological performance. Pluronic is a nontoxic FDA-approved block copolymer that is often used in 3D printing.[41] Depending on their molecular weight and the ratio of poly (ethylene oxide) (PEO) to poly (propylene oxide) (PPO) in the Pluronic chain, several grades of Pluronics are available in different states, such as liquid, paste, and solid. Among them,

Pluronic F127 is most commonly used in 3D bioprinting. Pluronic F127 solution can flow at low temperature (<10°C), which is conducive to cell encapsulation and dispersion.[42] As the temperature rises, the solution gradually transitions to a gel state by self-assembly. Due to its inverse thermogelling properties, Pluronic F127 gained much attention in the field of 3D bioprinting. Mozetic *et al.* developed a thermosensitive bioink based on Pluronic/alginate blends and investigated its effect on the behaviors of C2C12 cells.[43] This system enables printing of cell-laden structures with good shape retention under physiological conditions. Shearing forces generated during the printing process induced cellular alignment along the deposition direction. The resulting constructs demonstrated high cell viability and enhanced myogenic gene expression. Polyethylene glycol (PEG) is another common synthetic material used in 3D bioprinting. Polyethylene glycol diacrylate (PEGDA), a derivative of PEG, has reactive acrylate groups at both ends and can be used to prepare hydrogels by photocuring. A study has demonstrated that the mechanical performance of bioprinted constructs can be flexibly adjusted by altering the concentration of PEGDA in bioinks.[44] As a synthetic polyether, PEO is broadly used in the field of 3D bioprinting owing to its biocompatibility, inertness, and ease of molecular modification. Several studies have demonstrated that the addition of PEO can enhance the strength of hydrogen bonding between gelatin chains, leading to phase separation of gelatin/PEO aqueous solution. Therefore, PEO often functions as a porogen in the bioink system for the generation of micropores in the printed construct.[45,46] Based on this principle, Ying *et al.* developed a novel bioink consisting of GelMA and PEO and induced the formation of uniformly dispersed PEO droplets in the continuous GelMA phase.[45] The printed construct with highly interconnected pores was generated by removing the PEO phase from the photocrosslinked GelMA hydrogel.

3. 3D bioprinting for musculoskeletal regeneration

Tissue defects caused by trauma, tumor removal, or congenital malformations require reconstruction of anatomy and restoration of function through the introduction of custom-made constructs to fill the defects. Various tissue constructs fabricated by 3D bioprinting have shown great application potential in the field of musculoskeletal tissue engineering. In this section, we discuss the recent advances in 3D bioprinting for musculoskeletal tissue regeneration.

3.1. Bone

Bone tissue is a hard connective tissue consisting of cancellous and cortical bone. It not only offers structural support and protection but also sustains various metabolic activities including mineral transfer, hematopoiesis, and hormone modulation. The cell types of bone tissue include bone progenitor cells, osteoblasts, osteocytes, and osteoclasts, which are responsible for regulating the process of bone formation and resorption. Despite the remarkable regenerative capacity of bone tissue, significant challenges remain when it comes to repairing large segmental bone defects caused by various factors, such as tumor resection, infections, or trauma.[47,48] Clinicians often have to resort to surgical intervention in cases where significant bone defects need to be repaired, with autografts, allografts, xenografts, and inorganic grafts being the most commonly used approaches for repairing bone defects.[49,50] However, existing clinical treatments for bone repair suffer from several shortcomings, such as donor-site morbidity, anatomical mismatch, inadequate bone volume, graft absorption, and rejection.[51] To address these limitations, the demand for tissue-engineered bone substitutes has been on the rise, leading to the development of new, converging technologies that offer hope for more effective and sustainable bone repair solutions. As a cutting-edge technology, 3D bioprinting has been widely used in the field of bone regeneration due to its significant potential to create functional bone grafts (Table 1). For example, recent advances in 3D bioprinting have enabled the development of multicell co-culture models that hold promise for simulating the intricate cellular interactions present in native bone tissue. By constructing a sophisticated microenvironment, these models provide the necessary conditions to investigate and understand the delicate cell–cell interactions that underpin the function of bone tissue. Tang *et al.* used GelMA to bioprint a bone construct in which Hertwig's epithelial root sheath cells and dental papilla cells were recombined to mimic the microenvironment of cell–cell interaction *in vivo*.[52] The formation of the mineralization texture and improved bone regeneration were observed after implantation of the construct in an alveolar bone defect model, which may be attributed to cell–cell interactions (Figure 1).

Abbreviations: DFC, dental follicle cell; DPC, dental papilla cell; GelMA: gelatin methacrylate; HERS, Hertwig's epithelial root sheath; LAP, lithium phenyl-2, 4, 6-trimethylbenzoylphosphinate; UV, ultraviolet.

Angiogenesis and osteogenesis are considered tightly coupled during bone development and regeneration.[71] Vascularization is one of the key factors affecting the effectiveness of bioprinted scaffolds for bone regeneration in bone tissue engineering.[72] The constructs bioprinted using stem cells and endothelial cells demonstrated higher osteogenic potential than the stem cell constructs.[73] Nulty *et al.* used fibrin-based bioinks to prepare a prevascularized construct with customized shapes and sizes.[53] The construct can significantly promote the formation and development

Table 1. Advances in 3D bioprinting for bone regeneration

Bioprinting technology	Materials	Cell type	Cell density (cells/mL)	Key outcomes	Ref.
Extrusion	HA, fibrinogen, gelatin, and glycerol	HUVECs and BMSCs	1×10^7	Supported robust vascular development and higher levels of new bone formation	[53]
	GelMA	BMSCs	5×10^6	Promoted new bone formation in vivo	[54]
	Collagen, chitosan, and β-GP	BMSCs	5×10^7	Facilitated osteogenic differentiation and bone regeneration in vivo	[55]
	Bone ECM	ADSCs	1.2×10^7	Promoted new bone formation and more competent vascular development	[56]
	HAMA and GelMA	C3H10T1/2	1×10^7	Promoted osteoblast differentiation and induced ectopic bone formation	[57]
	GelMA, PEG, gelatin, and MSN	BMSCs	1×10^7	Promoted osteogenic differentiation and accelerated diabetic bone repair	[58]
	ACuMBGNs, oxidized alginate, and gelatin	BMSCs	1×10^6	Promoted osteogenic differentiation and angiogenesis	[59]
	HAMA, GelMA, alginate, and graphene oxide	BMSCs and macrophages	2×10^6	Promoted the M2-type polarization of macrophages and promoted bone repair	[60]
	HA, gelatin, PCL, fibrinogen, PF-127, glycerol, and thrombin	BMSCs and EPCs	1.5×10^7	Promoted the new blood vessels and new bone formation	[61]
	GelMA,	HERS cells and DPCs	1×10^6	Generated mineralization texture and promoted alveolar bone regeneration	[52]
	Fibrinogen, gelatin, glycerol, HA, and PCL	BMSCs	5×10^6	Supported bone formation and vascularization	[62]
	GelMA, gum methacrylate	HUVECs, BMSCs	2×10^6	Promoted bone regeneration and angiogenesis	[63]
	Graphene oxide, alginate, and gelatin	BMSCs	5×10^7	Promoted osteogenic differentiation	[66]
	Bone ECM	HUVECs, MSCs	1×10^7	Led to the formation of interconnected vascular networks	[65]
Robotic in situ extrusion	PEGDA, GelMA, and alginate	MC3T3-E1 cells	-	Promoted the repair of long segmental defects	[66]
VBP	GelMA	HUVECs, BMSCs	3×10^6	Promoted osteogenic differentiation	[67]
LAB	BioRoot RCS® and collagen	Stromal cells	7×10^7	Promoted osteogenic differentiation and bone formation	[68]
DLP	GelMA and dextran	BMSCs	-	Promoted bone regeneration in vivo	[69]
	SilMA	MC3T3-E1 cells	2×10^6	Drove osteogenesis	[70]

Abbreviations: VBP: volumetric bioprinting, LAB: laser-assisted bioprinting, DLP: digital light processing, HA: hyaluronic acid, GelMA: gelatin methacrylate, ECM: extracellular matrix, HAMA: hyaluronic acid methacrylate, MSN: mesoporous silica nanoparticle, PCL: polycaprolactone, PEGDA, SilMA: silk fibroin methacrylate, β-GP: β-glycerophosphate, PF-127: Pluronic F-127, HUVECs: human umbilical vein endothelial cells, BMSCs: bone marrow stem cells, ADSCs: adipose-derived stem cells, EPCs: endothelial progenitor cells, HERS: Hertwig's epithelial root sheath, DPCs: dental papilla cells, ACuMBGNs: amine-functionalized copper (Cu)-doped mesoporous bioactive glass nanoparticles

of vascular networks, which facilitate the repair of critical bone defects. Shen *et al.* developed a bioprinting strategy to fabricate bone tissue-engineered scaffolds in which endothelial cells were able to form *in situ* networks of blood vessels.[54] The *in vivo* bioprinted *in situ* vascularized scaffolds have shown excellent performance in new bone formation in a rat model with cranial critical-sized defects.[54] Another study used intraoperative bioprinting to prepare a scaffold that enabled simultaneous delivery of pPDGF-B and pBMP-2 for the repair of critical-sized bone defects. Platelet-derived growth factor (PDGF) has been reported to exhibit angiogenic effects by promoting the

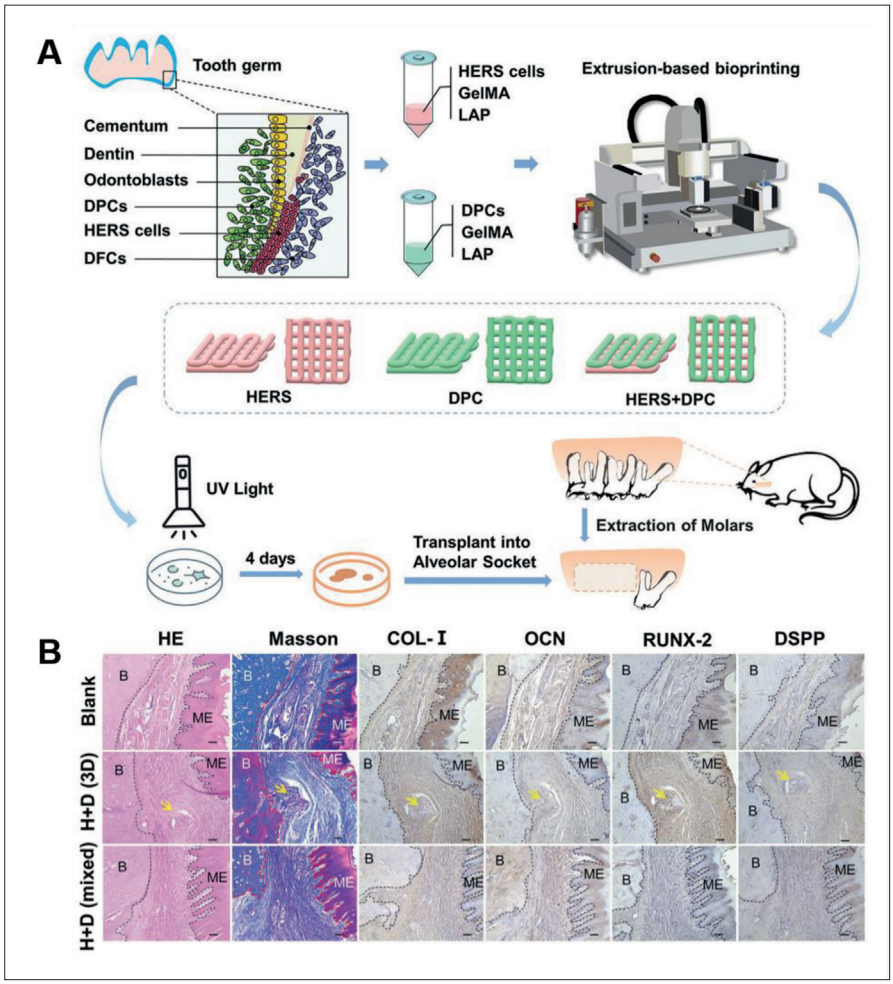

Figure 1. 3D bioprinting for bone regeneration. (A) Schematic diagram of 3D bioprinting and transplantation of bone constructs. (B) Hematoxylin and eosin staining, Masson's trichrome staining and immunohistochemical evaluations of implantation in alveolar bone after 8 weeks. Adapted from Tang *et al.*[54].

expression of vascular endothelial growth factor, which is conducive to osteoblast proliferation and cell migration.[55] Kim *et al.* used 3D bioprinting to prepare a construct loaded with endothelial cell spheroids and human adipose stem cells.[56] The spheroid-laden construct demonstrated higher angiogenesis and osteogenic ability compared with traditional multiple-cell construct. Moreover, *in vivo* experimental results showed that spheroid-laden multicell construct can induce new bone formation and neovascularization more effectively, which further confirmed its potential for bone regeneration. A study evaluated the effect of 3D-bioprinted scaffold structures on angiogenesis.[61] It was found that the increase in the number of hierarchical microchannels in bone biomimetic scaffolds, especially the transverse Volkmann canals, accelerated the formation of new blood vessels. This is probably because microchannels promote the exchange of nutrients and thus improve angiogenesis.

The pathophysiological microenvironment is critical for tissue regeneration after injury, which can significantly affect cell growth, differentiation, apoptosis, and other cell functions.[74] For patients with primary diseases such as diabetes, the inflammatory microenvironment in the injured bone can lead to vascular occlusion and decreased neovascularization. A bioactive scaffold containing bone morphogenetic protein (BMP)-4-loaded mesoporous silica nanoparticle (MSNs), bone marrow stem cells (BMSCs), and RAW264.7 cells was bioprinted for use in diabetic bone repair. BMP-4 in the scaffold facilitated the polarization of RAW264.7 toward M2-type macrophages, secreting more anti-inflammatory mediators to improve the local microenvironment. Furthermore, BMP-4 and BMP-2 released by M2-type macrophages worked together to enhance the osteogenic differentiation of BMSCs. With the implantation of the scaffolds, the process of bone repair was significantly accelerated.[58] Infection is a potential

complication following bone defect repair, and the risk of infection is heightened in the presence of open wounds or orthopedic implants.

When infectious bone defects occur, bacteria adhere to aggregate and proliferate on the scaffold surface to form biofilms that impair the function of osteoblasts, leading to delayed union or nonunion.[75,76] It has been reported that doxycycline can be released from a 3D-bioprinted scaffold, which is capable of inhibiting bacteria to reduce the risk of infection, to promote the expression of BMP-2 for stimulating new bone formation.[57]

3.2. Cartilage

Cartilage is an important tissue responsible for a variety of critical functions, including cushioning stress, reducing friction between adjacent bones, and composing organs. Cartilage consists mainly of proteoglycans, water, type II collagen, and a few chondrocytes. The articular cartilage has a specific zonal orientation (superficial, middle, deep, and calcified zones), and its structure and composition vary in a depth-dependent manner.[77] Trauma, aging, disease, and other factors can increase the risk of damage to cartilage, especially articular cartilage, resulting in joint dysfunction. According to the depth of the lesion, articular cartilage defects can be divided into partial cartilage defects, full-thickness cartilage defects, and osteochondral defects. Due to the inherent characteristics such as low cell density and absence of blood vessels and nerves, the self-healing ability of articular cartilage is significantly limited.[78] Without timely and potent intervention, chondral lesions often progress to secondary osteoarthritis, leading to severe pain and even disability.[79] Eventually, patients with end-stage diseases have to undergo total joint replacement. Therefore, the repair and regeneration of cartilage tissue has attracted much attention. The common clinical treatment strategies for cartilage defects include debridement, bone marrow stimulation, and osteochondral transplantation.[80,81] Among them, debridement and bone marrow stimulation are classified as palliative treatments, which cannot achieve the curative effect.[82] The application of transplant technology is constrained by several shortcomings, such as the need for reoperation, insufficient donor tissue, and increased risk of immune rejection and disease transmission.[83] The current available treatments, which are not widely available, often result in the development of fibrotic tissue, which is unfavorable to the native articular cartilage and increases the tendency to degeneration.[84] Thus, it is imperative to develop innovative techniques capable of effectively enhancing the regeneration of cartilage tissue. The emergence of bioprinting technology represents a significant advancement in the field of cartilage regeneration. Bioprinting is a potential method for producing functional grafts that more closely resemble native tissue architectures and is therefore a promising approach to cartilage tissue repair. Recent 3D bioprinting studies for cartilage regeneration are listed in Table 2.

Numerous studies have attempted to evaluate the effects of formulations or physical properties of bioinks (such as matrix stiffness) on the maintenance of chondrocyte phenotype and subsequent influence on cartilage-specific ECM production. Conventional bioprinted hydrogels usually have poor mechanical strength, so it is a challenge to engineer mechanically robust cartilage constructs that can withstand high load-bearing environments. A feasible strategy for improving the mechanical strength of tissue constructs is to incorporate stiffer polymer components into the bioink to strengthen its network.[106,107] Inspired by this strategy, an alginate hydrogel reinforced with short submicron polylactide was designed as a bioink for the bioprinting of cartilaginous construct. Round chondrocytes with high cell viability were observed in the bioprinted constructs which had an elastic modulus three times higher than that of the pristine alginate constructs.[108] A similar approach was used in another study to develop fiber-reinforced cartilage ECM-based bioinks for cartilage regeneration. The incorporation of ECM promoted the growth and chondrogenic differentiation of stem cells in the bioink. Furthermore, the bioprinted constructs augmented with polycaprolactone (PCL) fibers displayed a compression modulus comparable to that of native articular cartilage.[86] In addition to the mechanical performance required by motion forces, the engineering of biomimetic cartilage tissues should focus on their chondrogenic function.[35] To address this issue, de Melo *et al.* developed a new tissue design option for cartilage regeneration.[35] Based on spatially organized bioprinting, this strategy enables human mesenchymal stem cell (hMSC) spheroids to maintain the chondrogenic behavior without detriment to the macro mechanical properties of engineered tissues.[35] Pei *et al.* used extrusion printing to construct a cartilage repair scaffold in which mesenchymal stem cells (MSCs) were transfected with microRNA-410.[109] The up-regulation of microRNA-410 enhanced the migration, proliferation, and chondrogenic differentiation of loaded cells. Compared with the nontransfected group, the transfected group showed better cartilage regeneration in the rabbit cartilage defect model (Figure 2). Another important issue with the bioprinted grafts is their integration with native host tissue, which is deemed vital for successful cartilage regeneration.[110] In response to this concern, a visible-light-responsive bioink was designed for chondral repair. The bioink material consists mainly of a dual-functionalized tyramine and GelMA and tris (2,2′-bipyridyl) ruthenium (II) chloride and sodium persulfate (Ru/SPS) that acts as

Table 2. Advances in 3D bioprinting for cartilage regeneration

Bioprinting technology	Materials	Cell type	Cell density (cells/mL)	Key outcomes	Ref.
Extrusion	GelMA-Tyr and Ru/SPS	ACPCs	2×10^7	Promoted neo-cartilage formation	[85]
	Alginate, cartilage ECM	BMSCs	2×10^7	Promoted chondrogenesis	[86]
	Gelatin, PCL, fibrinogen, HA glycerol, and PLGA	BMSCs	1×10^7	Enhanced anisotropic cartilage regeneration	[87]
	β-CD and PNIPAm	ADSCs	1×10^6	Formed cartilage-like tissue in vitro	[88]
	Gellan gum and lignin	MSCs	3.5×10^6	Improved chondrogenesis	[89]
	Alginate and GelMA	MSCs	2×10^7	Promoted cartilage-specific ECM deposition	[90]
	HA-PBA and PVA	ADSCs	3.5×10^6	Promoted ECM deposition	[91]
	PRP and SF	Chondrocytes	2.5×10^6	Favored ECM deposition	[92]
	Methacrylated kappa-carrageenan	ATDC5 cells	2×10^7	Enhanced the viability, proliferation, and GAGs deposition	[93]
	Alginate, HA, and PLA	Chondrocytes	1×10^6	Promoted ECM deposition	[94]
	PCL, gelatin, HA, glycerol, and fibrinogen	BMSCs	1×10^7	Promoted cartilage repair in vivo	[95]
	Alginate, GelMA, and β-tricalcium phosphate	BMSCs	1×10^7	Enhanced the formation of calcified cartilage tissue	[96]
	Norbornene-modified HA	MSCs	2×10^7	Promoted ECM deposition	[97]
	GelMA and HAMA	ADSCs	1×10^7	Led to hyaline-like cartilage formation	[98]
DLP	Methylacryloyl naringin and GelMA	Chondrocytes	1×10^7	Improved cartilage defect repair	[99]
	γ-PGA-GMA	Chondrocytes	1×10^6	Promoted ECM deposition	[100]
Robotic-assisted DLP	Alginate and PEGDA	-	-	Promoted focal cartilage defect restoration	[101]
	4-Armed PEG-ACLT and HAMA	-	-	Promoted in vivo cartilage regeneration	[102]
SLA	GelMA and PEGDA	BMSCs	2×10^6	Improved chondrogenic differentiation	[103]
Inkjet	PEGDMA	Chondrocytes	5×10^6	Promoted ECM deposition	[104]
	-	BMSCs	-	Promoted GAGs deposition and collagen network organization	[105]

Abbreviations: DLP: digital light processing, SLA: stereolithography, GelMA: gelatin methacrylate, ECM: extracellular matrix, HA: hyaluronic acid, PCL: polycaprolactone, PLGA: poly(lactic-co-glycolic acid), β-CD: β-cyclodextrin, PVA: polyvinyl alcohol, HA-PBA: phenylboronic acid grafted hyaluronic acid, SF: silk fibroin, PRP: platelet-rich plasma, PLA: polylactic acid, γ-PGA-GMA: γ-poly(glutamic) acid-glycidyl methacrylate, PEGDA: polyethylene glycol diacrylate, HAMA: hyaluronic acid methacrylate, PEGDMA: polyethylene glycol dimethacrylate, ACPCs: articular chondroprogenitor cells, BMSCs: bone marrow stem cells, ADSCs: adipose-derived stem cells, MSCs: mesenchymal stem cells, GAGs: glycosaminoglycans

initiators. After one-step photoactivation, the adhesive strength of bioink, which acts as a cartilage-binding glue, had increased 15-fold, by forming covalent bonds with tyrosine residues in natural cartilage tissue compared with GelMA alone.[85]

The treatment of severe cartilage injury, especially osteochondral defects, poses a huge challenge for clinicians due to the complexity of the biphasic layered structure of osteochondral units. The ideal scaffolds for the repair of osteochondral defects should mimic the heterogeneous structure of native cartilage, characterized by compartmentalized zonal microstructure and composition. Cartilage with heterogeneity and anisotropy is typically studied as a layered structure of "zones" with mechanical performance dependent on the constituents and architecture of each zone.[111] Inspired by this, Idaszek *et al.* developed an extrusion printing system with a microfluidic print head to bioprint tissue constructs with cell and biomaterial gradients.[112] The bioprinted constructs simulate the layered cartilage structure consisting of hyaline and calcified cartilage. *In vivo* results in rat models confirmed that the constructs can promote full-thickness cartilage regeneration.[112] Another study offered a novel

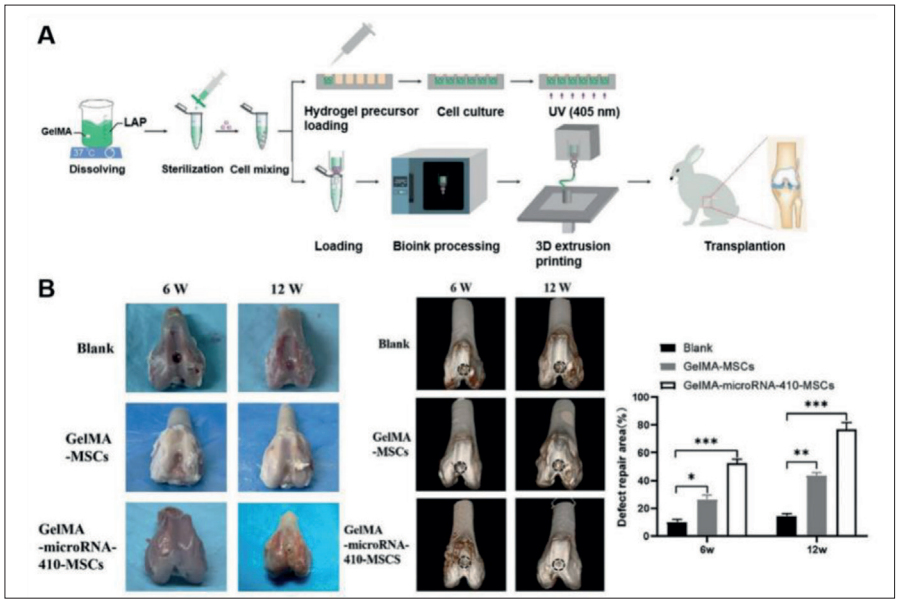

Figure 2. 3D bioprinting for cartilage regeneration. (A) Schematic illustration of 3D bioprinting of cell-laden GelMA hydrogel for repairing cartilage defects. (B) Gross view and imaging evaluation of 3D-bioprinted scaffolds for repairing cartilage defects. Adapted from Pei *et al.*[111]

hybrid bioprinting strategy to fabricate zonally stratified articular cartilage to simulate the anatomical structure of native cartilage utilizing cartilage tissue strands consisting of densely packed cells and matrix. Tissue strands show excellent printability and mechanical stability and can rapidly fuse into large-scale tissues. Predifferentiated cartilage tissue strands showed higher mechanical strength and expression of cartilage-specific genes compared with differentiated group. Moreover, the printed construct exhibits a compression modulus comparable to that of human articular cartilage (approximately 1.1 MPa)[113]. In order to more accurately guide cells in each layer to achieve region-specific differentiation and extracellular matrix deposition, Sun *et al.* developed a dual-factor release construct with gradient structure via bioprinting. The bioprinted construct was incorporated with growth factor-mediated biochemical cues and biomechanical cues mediated by small pore size, which demonstrated a strong potential to promote the whole-layer regeneration of anisotropic cartilage.[87] Recently, Dai *et al.* described a novel host–guest modulated dynamic hydrogel bioink for osteochondral regeneration.[114] The dynamic network formed by the interaction of host and guest is conducive to the achievement of improved cell adaptability, enhanced cell adhesion, bolstered mechanical strength, and adjustable stiffness of the construct. Employing the cavity of β-cyclodextrin, a tissue-specific microenvironment can be provided by releasing kartogenin and melatonin in the upper zone with lower stiffness and the lower zone with higher stiffness, respectively, to facilitate the fabrication of the heterogeneous construct.[114]

3.3. Skeletal muscle

Skeletal muscle makes up 45% of the body mass and enables a variety of vital functions including support, movement, stability and metabolic regulation.[25,115,116] Skeletal muscle is composed of myofibers, blood vessels, nerves, and connective tissue. The functional unit of skeletal muscle is myofiber, which consists of a number of aligned myofibrils wrapped by the sarcolemma.[117] The activation and contraction of skeletal muscles are achieved by connecting with a network of neurons. The movement is then accomplished by the connection between tendons and bones. The vascular network connecting the muscles is responsible for the transport of nutrients and metabolic wastes. Skeletal muscle has high regenerative ability, and small injuries below a certain threshold can be self-repaired in a highly orchestrated manner.[118] However, extensive injuries involving volumetric muscle loss (VML) overwhelm the inherent repair capacity of the remaining muscles, resulting in severe dysfunction of the locomotion system.[119] Frequent causes for VML include combat injuries, high-energy traffic accidents, tumor resection, and degenerative diseases. The regeneration phase after VML injuries involves abnormal inflammatory responses and excessive collagen deposition. Necrosis of myofibers stimulates the infiltration of immune cells, mainly neutrophils and macrophages, which participate in the clearance of necrotic myofibers and secrete specific cytokines and growth factors that regulate the activation and differentiation of satellite cells and direct the surrounding cells to partake in the ECM remodeling and angiogenesis. Currently, the treatment options

for VML are limited. The most common procedure is muscle flap transplantation, which involves the transfer of autologous tissue with blood and nerve supply from the donor site to the injured site in the patient. Despite some beneficial outcomes, this treatment suffers from the common drawbacks of autologous tissue transplantation, such as donor tissue deficiency, donor site morbidity, and potential graft failure.[120,121] Another treatment option is physical therapy, which compensates for the functional deficits associated with VML defects by hypertrophy of the remaining muscles.[122] However, this treatment is not suitable for large-scale VML defects, and VML patients are often unable to perform physical exercise, limiting its use in clinic. These concerns have led to the investigation of novel regenerative medicine treatments.

A variety of 3D bioprinting techniques have been investigated in order to create skeletal muscle grafts with regenerative potential for VML repair (Table 3). Choi *et al.* developed a granule-based printing reservoir to fabricate volumetric muscle constructs based on cell-laden dECM bioinks.[123] The resultant constructs supported high cell viability and enhanced muscle formation to promote muscle regeneration. Behre *et al.* prepared patient-specific scaffolds for VML repair using ECM-based bioinks.[124] This fabrication process was implemented with the freeform reversible embedding of suspended hydrogels (FRESH) 3D bioprinting technology, which allows the ECM hydrogel to match the tissue defects and manage the characteristics of the construct microstructure. The creation of anisotropic muscle tissues remains a challenge for traditional 3D extrusion bioprinting. In combination with the ice-templating method, Luo *et al.* developed an innovative bioprinting technology, namely vertical 3D extrusion cryo-bioprinting.[125] With precise temperature control, GelMA-based bioinks can be bioprinted into freestanding filamentous constructs with interconnected, anisotropic, and gradient microchannels. Using this technology, the printed muscle-tendon units showed high cell survival and desired cell arrangement. Without using the toxic materials, Mostafavi *et al.* developed GelMA-based foam bioinks for the preparation of tissue engineering scaffolds.[126] Homogeneous and interconnected pores were generated by mechanical stirring of the precursor gel solution at a high rate, which facilitated cell infiltration and spreading in the hydrogels. The porous bioinks were compatible with both conventional and handheld bioprinters (Figure 3A). Moreover, the constructs bioprinted based on the bioinks presented significant regenerative potential as evidenced by a mouse VML model. Successful biofabrication of skeletal muscle constructs for VML repair requires precisely replicating the structural and functional features of natural skeletal muscle. Kim *et al.* fabricated human skeletal muscle constructs that were integrated with neural cells via bioprinting and evaluated the effects of neural input on the bioprinted constructs.[127] The results showed that the neural-skeletal muscle constructs achieved rapid integration with the host neural network and enhanced the recovery of muscle function. 3D-bioprinted constructs have mechanical properties that are similar to native tissue, which is especially important for musculoskeletal tissue regeneration. A new bioprinting strategy, assembled cell-decorated collagen (AC-DC) bioprinting, was invented to fabricate musculoskeletal tissue implants for the reconstruction of damaged tissues.[128] The mechanical properties of resultant implants consisting of robust glyoxal crosslinked collagen microfibers and human-related cells were comparable to or better than those of native tissue, and they could facilitate function restoration.

Muscle fiber bundles fuse to form skeletal muscle with a highly parallel-aligned structure that is essential for effective force transfer and anisotropic locomotion.[140-142] Therefore, the fabrication of biomimetic muscle constructs to simulate the aligned structure, which can stimulate 3D cell alignment, is crucial for skeletal muscle tissue regeneration. Numerous attempts have been made in muscle cell alignment by improving the bioprinting strategies.[28,128,136] Li *et al.* developed bioinks based on viscoelastic hydrogels, which enhanced the arrangement of the cell microenvironment.[34] Combined with the gel-in-gel strategy, the bioprinted biomimetic scaffold with aligned structure was prepared for VML repair. The scaffold demonstrated the capacity to induce the alignment and elongation of 3D myoblasts. Distler *et al.* demonstrated that the microstructure of the hydrogel could be oriented by adjusting printing conditions, such as nozzle diameter and extrusion pressure, thus guiding the orientation of cell growth.[113] During the 3D printing process, the orientation of C2C12 cells in the printing direction increased with the rise of the shear force in the printing head. Kim *et al.* described an innovative bioprinting strategy for the guidance of the muscle cells.[132] To induce the alignment of laden myoblasts, they designed collagen-based bioinks mixed with gold nanowires, which provided aligned topological clues to the cells in response to the external electric field (Figure 3B and C). The bioink supported high cell viability, and the printed structures demonstrated excellent myoblast alignment and efficient myotube formation. Yeo *et al.* described a novel bioprinting method in combination with the electrohydrodynamic-direct-writing (EHD-DW) procedure, which enabled the biofabrication of high-resolution microscale structures.[133] Alginate/fibrin bioinks loaded with myoblasts or endothelial cells can be printed into spatially patterned

Table 3. Advances in 3D bioprinting for skeletal muscle regeneration

Bioprinting technology	Materials	Cell type	Cell density (cells/mL)	Key outcomes	Ref.
Extrusion	GelMA	ASCs	1×10^7	Accelerated muscle regeneration	[29]
	PEDOT and GelMA	C2C12 cells	2×10^6	Enhanced the formation of muscle fibers	[129]
	GelMA and fibrinogen	C2C12 cells	2×10^5	Recruited native muscle cells and promoted revascularization in situ	[36]
	GelMA	C2C12 cells	-	Achieved significant functional recovery and higher muscle forces	[126]
	HA, gelatin, fibrinogen, glycerol, and PCL	hMPCs and hNSCs	3×10^7	Facilitated rapid innervation and maturation into organized muscle tissue	[127]
	Gelatin and fibrinogen	C2C12 cells	1×10^7	Promoted myotube formation	[31]
	Oxidized alginate-gelatin	C2C12 cells	8×10^6	Enhanced cell differentiation into ordered myotube clusters	[115]
	Fibrinogen, gelatin, HA, and glycerol	hMPCs	1×10^7	Showed a highly organized multi-layered muscle bundle and significant functional recovery	[130]
Electric field-assisted extrusion	GelMA	C2C12 cells	1.5×10^7	Promoted myotube formation and maturation	[131]
	Collagen and Au nanowires	C2C12 cells	1×10^7	Enhanced myoblast alignment and efficient myotube formation	[132]
Extrusion cryo(bio) printing	GelMA, DMSO, and D-(+)-melezitose hydrate	C2C12 cells	1×10^6	Enhanced cell viability, spreading, and alignment	[125]
AC-DC bioprinting	HA	hMSCs	$1\text{-}5 \times 10^6$	Increased total muscle fiber count, median muscle fiber size, and cellularization	[128]
Inkjet	Alginate, fibrin, and PEO	C2C12 cells	5×10^6	Presented fully aligned myotube formation and greater myogenic differentiation	[133]
DNP-based 3D printing	GelMA and UCNP@LAP nanoinitiators	ADSCs	1×10^7	Obtained a muscle tissue repair-able cell-laden conformal scaffold without surgery implantation	[134]
	HCC-PEG and gelatin	Muscle-derived stem cells	$2\text{-}4 \times 10^6$	Lead to the de novo formation of myofibers	[135]

Abbreviations: AC-DC: assembled cell-decorated collagen, DNP: digital near-infrared photopolymerization, GelMA: gelatin methacrylate, PEDOT: poly-3,4-ethylene dioxythiophene, HA: hyaluronic acid, PCL: polycaprolactone, SAPs: self-assembling peptides, DMSO: dimethyl sulfoxide, PEO: poly (ethylene oxide), HCC-PEG: 7-hydroxycoumarin-3-carboxylate–polyethylene glycol, hMPCs: human muscle progenitor cells, hNSCs: human neural stem cells, hMSCs: human mesenchymal stem cells, ADSCs: adipose-derived stem cells

constructs by adjusting a series of printing parameters, such as the electric field, the distance from the nozzle to the loading platform, and the nozzle moving speed. The constructs bioprinted with myoblasts and endothelial cells demonstrated completely aligned myotube formation and higher myogenic differentiation potential than those bioprinted with myoblasts alone, which may be attributed to angiogenic cytokines secreted by endothelial cells. Yang *et al.* described a novel one-step printing system in which an electric field was applied simultaneously to induce the orientation and differentiation of C2C12 cells while the bioinks were being extruded.[131] The rate of myotube formation and maturation was significantly faster in the printed structures stimulated by an electric field than in the

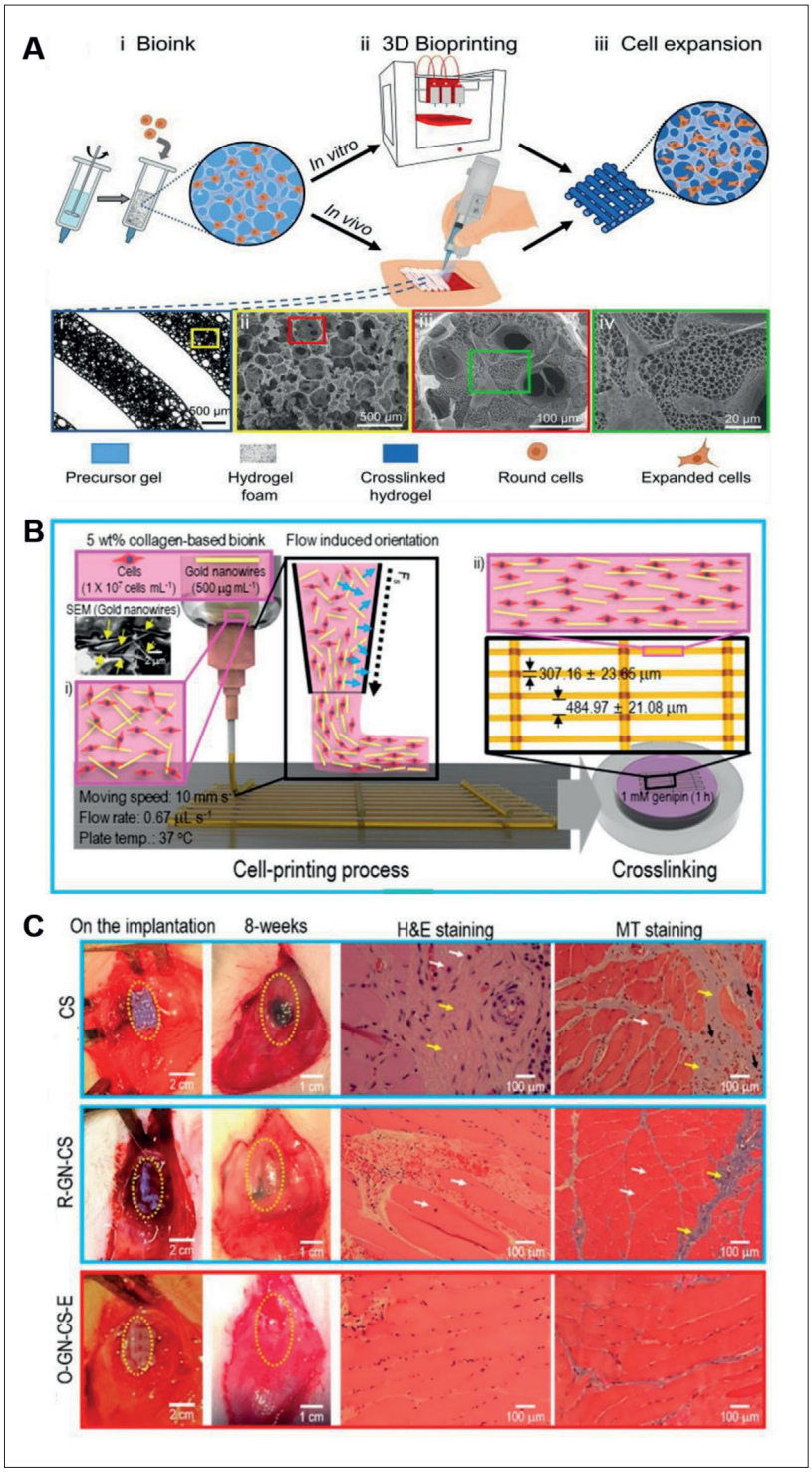

Figure 3. 3D bioprinting for skeletal muscle regeneration. (A) Schematic illustration of 3D bioprinting of multiscale porous structures using the adhesive foam-based bioink. Adapted from Mostafavi *et al.*,[126] with permission from Elsevier. (B) A collagen bioink with fully aligned Au nanowires inducing myoblast alignment under an electric field in the printing process. Figures 3B and 3C are adapted from Kim *et al.*, with permission from American Chemical Society[132] (C) Gross view and histological evaluation of 3D-printed scaffolds for repairing muscle defects at 8 weeks after surgery. Adapted from Kim *et al.*,[132] with permission from American Chemical Society.

control group. Utilizing the swelling properties of gelatin, 4D-conceptualized gelatins films with grooves were further fabricated to bundle the cell-laden GelMA microfibers, thereby simulating the structure of the native perimysium. Despite these advances, the dynamic changes of aligned muscle cells during myotube formation and maturation in large-scale bioprinted construct remain elusive. To this end, Fan *et al*. constructed skeletal muscle fiber bundles with different widths by 3D bioprinting and evaluated the effect of different spatial constraints on the alignment and differentiation of muscle cells.[31] The results showed that the degree of myotube differentiation was negatively correlated with the thickness of the printed muscle bundle. Moreover, the alignment and maturation of muscle fibers may be affected by the structure width and the forces exerted. It is suggested that physical factors play an indispensable role in the generation of skeletal muscle tissue.

3.4. Meniscus

The meniscus is a semilunar wedge-shaped fibrocartilage tissue, which acts a pivotal part in knee locomotion. Its primary functions include the distribution and transfer of mechanical load, shock absorption, joint lubrication, and stability.[140,141] The meniscus has a distinctive zonal organization and structure. The outer region (the red-red zone) is more ligament-like and contains elongated fibroblast-like cells. This region has predominantly type I collagen and is equipped with self-healing ability due to the presence of blood supply. The inner region, also known as the white–white zone, is dominated by round chondrocyte-like cells that are embedded within an ECM rich in type II collagen and glycosaminoglycans (GAGs). This region demonstrates limited regenerative capacity owing to its deficient vascularization. The red–white zone, a transitional zone with features of both red–red zone and white–white zone, separates the two zones. Meniscus lesion is a prevalent orthopedic sports injury that affects knee balance and causes pain and joint dysfunction. Suturing of defects and partial meniscus replacement are often used to repair smaller meniscal tears, which restore the function of the meniscus to some extent. For irreparable meniscal tears, surgical interventions including meniscectomy or meniscus allograft transplantation are required. The removal of unstable, damaged meniscus tissues through partial meniscectomy is still the gold-standard surgical intervention of meniscal tears, accounting for half of arthroscopic knee surgeries in the United States.[142] Nevertheless, meniscectomy disrupts the biomechanics of the joint, leading to a dramatically increased risk of development of knee osteoarthritis in the long term.[143] Meniscus allograft transplantation also has limitations, such as unfavorable compatibility, inappropriate graft sizing, risk of immunogenicity, and limited tissue availability.[144-146] Currently, effective treatment options are lacking due to some challenges, including poor blood supply, complex 3D structure with personalized size parameters, deformability, and unique resistance to tension and compression of the meniscus.[140,147] Therefore, advanced strategies, including 3D bioprinting for the engineering of fibrocartilage tissues, are urgently needed. Table 4 presents the recent 3D bioprinting studies on meniscus regeneration.

Meniscus regeneration is severely hampered by a poor match between the implanted scaffolds and the host, because even the slight adjustments in implant position can influence contact stress and joint biomechanics.[157] To address this issue, an anatomically shaped and patient-specific construct was developed via inkjet bioprinting for meniscus regeneration (Figure 4). First, MRI data from a healthy volunteer's medial meniscus were obtained to design a STL model. The 3D model was then imported into the printer system to guide the subsequent printing process. The bioprinted construct showed good biocompatibility while satisfying shape adaptation.[155] Likewise, Stocco *et al*. employed an extrusion 3D bioprinter to fabricate a meniscus biomimetic scaffold with compatible anatomical shape using type I collagen and aligned electrospun nanofibrous mats.[149] The bioprinting was implemented using a virtual meniscus model created from patient MRI images. The structural integrity, shape fidelity, and mechanical strength of the scaffolds were enhanced by the addition of aligned nanofibers sheets.[149] In general, hydrogel-based bioinks alone are too mechanically weak to form self-supporting stable constructs. Biocompatible synthetic polymers are often used to help maintain the construct's shape and improve its mechanical strength. Chae *et al*. developed a biocompatible and functional meniscus construct using polyurethane_poly(ε-caprolactone) (PU_PCL) and a dECM-derived bioink.[141] The ECM components in the bioink provided the embedded cells with a friendly microenvironment for proliferation and differentiation while PU_PCL imparted robust mechanical properties and structural stability to the construct.[141] Jian *et al*. used a dual-nozzle printing system and a mixture of PCL and cell-laden GelMA/MECM bioink to create a biomimetic meniscal scaffold.[148] The scaffold resembled the native meniscus in terms of morphology and composition and promoted the formation of meniscal tissues in a nude mouse model.[148] The organization of cellular and matrix components is essential for musculoskeletal tissues to perform their functions.[158,159] For the meniscus, the circumferential organization of collagen fibers and cellular components in the outer region enables them to withstand hoop stresses.[160] Thus, in addition to replicating

Table 4. Advances in 3D bioprinting for meniscus regeneration

Bioprinting technology	Materials	Cell type	Cell density (cells/mL)	Key outcomes	Ref.
Extrusion	MECM, PCL, and PU	BMSCs	5×10^6	Promoted neofibrocartilage formation	[141]
	GelMA, PCL, and MECM	MFCs	1×10^6	Assisted in the formation of meniscal structures	[148]
	Collagen, PCL, and CNT	BMSCs	6×10^6	Improved the mechanical properties of the bioprinted construct without affecting cell viability	[149]
	Fibrinogen, gelatin, and cartilage ECM	MPCs	2×10^6	Enabled the spatial control of capillary formation in the bioprinted construct	[150]
	Gelatin, CMC, and alginate	MG63-osteosarcoma cells	1×10^5	Promoted collagen secretion and cell proliferation	[151]
	Gelatin, fibrinogen, HA, and glycerol	MSCs	1×10^7	Generated regional differential cell and ECM depositions	[152]
	Oxidized cellulose, alginate and collagen	MFCs	1×10^7	Promoted collagen deposition	[153]
	GelMA, HAMA, MECM, and PCL	MSCs	5×10^5	Promoted neomeniscal regeneration in vivo	[154]
	Gellan gum, fibrinogen, and SilMA	Meniscus cells	1.5×10^7	Led to the formation of fibrocartilaginous tissue in vivo	[147]
	Collagen	BMSCs	3.8×10^7	Provided an anatomically shaped, patient-specific construct with viable cells	[155]
	Alginate	ADSCs	1×10^6	Preferentially organized cellular arrays within constructs	[156]

Abbreviations: MECM: meniscal extracellular matrix, GelMA: gelatin methacrylate, PCL: polycaprolactone, PU: polyurethane, ECM: extracellular matrix, CMC: carboxymethyl cellulose, CNT: carbon nanotubes, HA: hyaluronic acid, HAMA: hyaluronic acid methacrylate, SilMA: silk fibroin methacrylate, BMSCs: bone marrow stem cells, MPCs: meniscus progenitor cell, MFCs: meniscal fibrocartilage chondrocytes, MSCs: mesenchymal stem cells, ADSCs: adipose-derived stem cells

patient-specific macroscopic dimensions, it is of equal importance to reproduce tissue-specific microscopic spatial organization of cells for meniscus regeneration. Chansoria *et al*. developed an ultrasound-assisted 3D bioprinting strategy for meniscus regeneration.[156] The cells suspended in the bioink were aligned at multiple length scales under the force of the superimposed ultrasonic bulk acoustic waves. By adjusting acoustic parameters, the cells can be manipulated into a controlled spatial aligned pattern to simulate the circumferential organization of the meniscus.[156]

Forming the anisotropic architecture of the meniscus is one of the difficulties in engineering biomimetic meniscus constructs. Hao *et al*. employed 3D printing technology to prepare a composite scaffold that enabled the co-delivering of platelet-derived growth factor-BB (PDGF-BB) and kartogenin (KGN).[154] These two bioactive factors can be controlled-release to promote stem cell migration and differentiation toward cartilage. The new tissue formation of the meniscus was observed half a year after implantation of the dual drug-loaded scaffolds. The study provides a promising strategy for the generation of meniscal constructs with biomimetic anisotropic microarchitecture. In addition to restoring the anisotropic properties of the menisci, engineered meniscus tissue requires growth of peripheral blood vessels (PBV) for nutrition supply, which is necessary for long-term stress tolerance and prevention of osteoarthritis progression.[162] Sun *et al*. reported a bioprinted anisotropic meniscus scaffold.[152] This scaffold demonstrated PBV infiltration, regional differential cells, and matrix deposition. The implantation of the functional scaffold is beneficial to the maintenance of joint function and the prevention of joint degeneration.[152] In partially vascularized tissues, such as menisci, the spatial distribution of microvessels is precisely confined.[150] Typically observed in degenerative tissues such as menisci, intervertebral discs, and cartilage, vascular growth into nonvascularized region can result in changes of tissue characteristics.[163-166] Therefore, the recapitulation of the spatial microvascular distribution is imperative for the successful fabrication of biomimetic meniscal constructs. To that end, Terpstra *et al*. have developed bioinks with pro- or antiangiogenic properties, which enabled spatial regulation of blood capillary formation in the bioprinted meniscal constructs.[150]

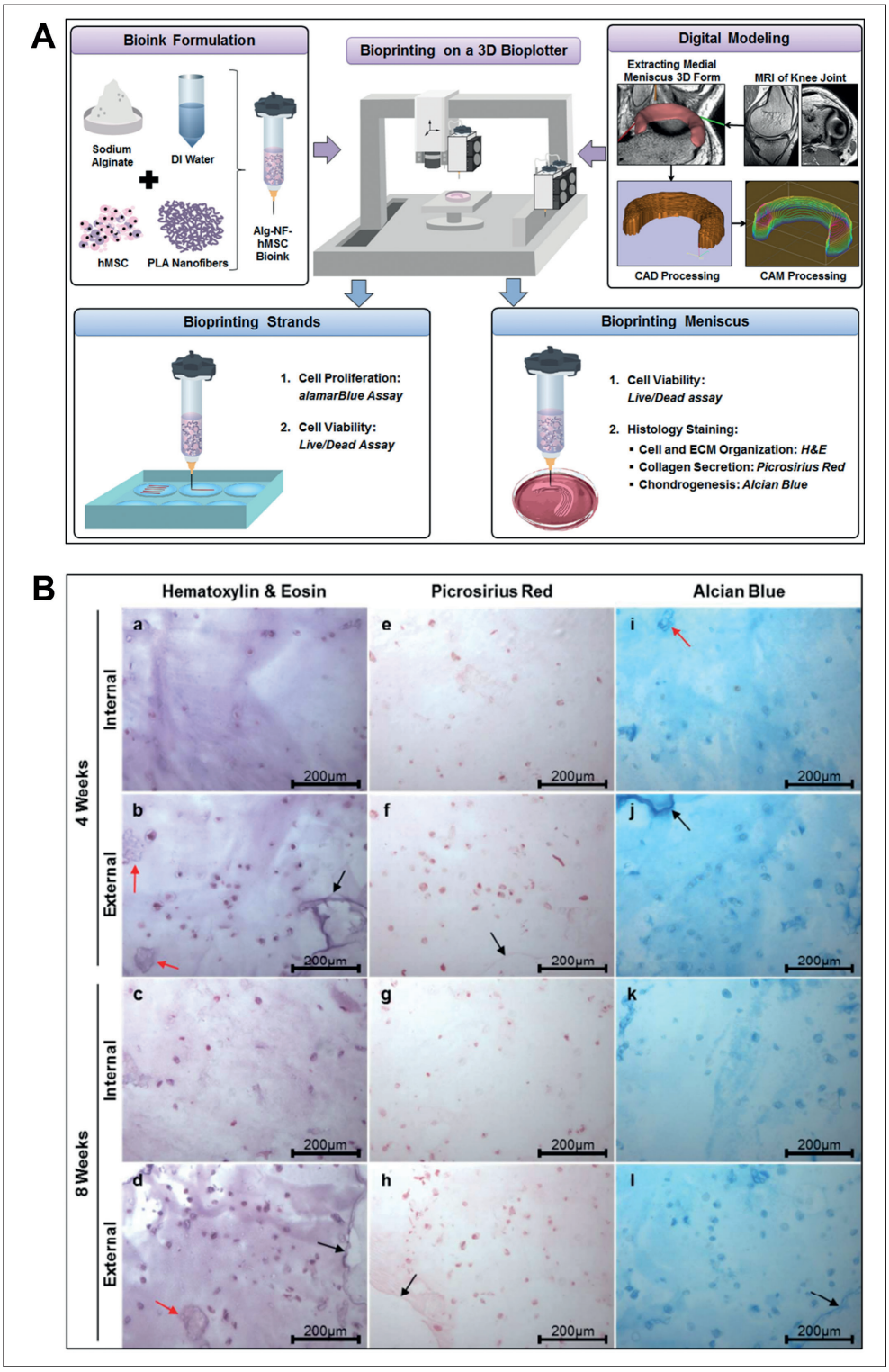

Figure 4. 3D bioprinting for meniscus regeneration. (A) Schematic representation of the bioprinting process of a meniscus construct. (B) Representative microscope images of stained meniscus construct sections after 4 (a, b, e, f, i, j) and 8 (c, d, g, h, k, l) weeks in culture. Adapted from Narayanan *et al.*,[161] with permission from American Chemical Society.

3.5. IVD

Located between adjacent vertebrae, IVD consists of three elements: nucleus pulposus (NP), annulus fibrosus (AF), and cartilaginous endplate. It is a complex fibrocartilaginous structure that absorbs and transfers mechanical load from various directions and allows flexible movement of the spine.[167] IVD is prone to degradation and has poor self-healing ability due to its avascularity.[168] IVD degeneration (IVDD) is a pathological process characterized by disorder of ECM structure, loss of proteoglycan, herniation of NP, and loss of disc height. The etiology of IVDD is complex and involves many pathogenic factors, such as trauma, aging, spinal deformities, and genetic factors.[169] As the most common cause of low back pain, IVDD results in a large number of patients with disability. Every year, more than 500 million people worldwide suffer from low back pain, imposing tremendous socioeconomic burden on humans[170]. The current treatment for IVDD includes conservative treatments and surgical treatments. The former includes steroid injections, nonsteroidal anti-inflammatory drugs, and physiotherapy, and the latter includes spinal fusion, total IVD replacement, and discectomy. These interventions can relieve symptoms; however, none of them has been successful in reversing IVDD progression and restoring disc function. Moreover, some treatments, such as spinal fusion, can alter the biomechanics of the spine, leading to an increased risk of degeneration of adjacent discs.[171] Hence, novel intervention measures that can effectively slow down the degeneration process and regenerate degenerated IVD are urgently needed.

Several studies have attempted to use tissue engineering for IVD regeneration but have encountered many challenges.[172-174] Among them, the preparation of engineered scaffolds is a tricky problem because of the complex microstructure of IVD, especially the AF. Accurate simulation of biomimetic AF anatomical structure is the key to the function restoration of IVD. This relies on advanced scaffold preparation methods. Electrospinning is a widely used technique for the preparation of fibers, which can be several microns or even nanometers in diameter. Electrospun nanofibers are considered excellent engineered materials due to their good biocompatibility, controllable mechanical properties, and similar characteristics to natural ECM. To mimic the hydrophilic environment and hierarchical structure of native AF, Yang *et al*. used electrospinning technology to prepare a scaffold consisting of PCL, poly(lactic-co-glycolic acid) (PLGA) and type I collagen.[175] *In vivo* experiments showed that the scaffold achieved good integration with the surrounding host tissues and promoted the recovery of disc function. In recent years, 3D printing technology has risen in popularity and has been used in IVD tissue engineering. In contrast to electrospinning, 3D printing allows customization of the scaffold without additional assembly steps. Bhunia *et al*. fabricated an engineered AF scaffold based on silk fibroin (SF) and carrageenan by 3D printing technology.[176] The scaffold simulated the multilamellar structure of the native AF and showed good mechanical properties. In addition, the scaffold supported cell growth and promoted the production of AF-specific ECM. The accuracy of printing is an important factor affecting the structure and function of 3D-printed scaffolds. Liu *et al*. used the electrohydrodynamic 3D printing technique to prepare an AF scaffold with high resolution for IVD regeneration (Figure 5).[177] After finite element analysis, the design of the structure was optimized before printing. The implanted scaffold maintained the height of the disc and promoted the partial recovery of the biomechanical function of IVD. Hu *et al*. developed a bioink composed of gellan gum and PEGDA for the bioprinting of IVD in combination with poly(lactic acid) (PLA).[178] The bioprinted construct exhibited excellent mechanical properties and supported high cell viability. Although bioprinted tissue constructs have shown promising results in musculoskeletal tissue engineering, 3D bioprinting of IVD is still rudimentary.

4. 3D bioprinting for disease modeling

For a long time, preclinical drug screening mainly relies on the use of animal models.[8] As an alternative to human disease research, animal models offer a controlled experimental system, which maintains the overall complexity of cells, tissues, and other factors within organ systems. However, biomedical results of animal models often do not fully represent the actual status of human diseases due to the vast genetic, phenotypic, and physiological differences between animals and humans.[179] Even by means of genetically engineered animal models, it is difficult to simulate the critical biological characteristics of diseased cells and their microenvironment, diseased tissues or organs, or their physiology in patients. Moreover, ethical concerns must be taken into account when carrying out animal experiments.[8] These limitations impede the translation of results from animal experiments into human treatments.[180] Another approach for drug validation is 2D culture of human cells that provides valuable insights into pathological mechanisms in a more controlled manner. This approach has the advantages of ease of use, low cost, and potentially high throughput, thus enabling the testing of multiple conditions and treatments in a short time. Despite these advantages, 2D cultured cells are obviously deficient in complex 3D structures and interactions found *in vivo*, which are essential for maintaining proper functional phenotypes in the musculoskeletal system. Shortcomings in existing drug screening strategies have led to a growing

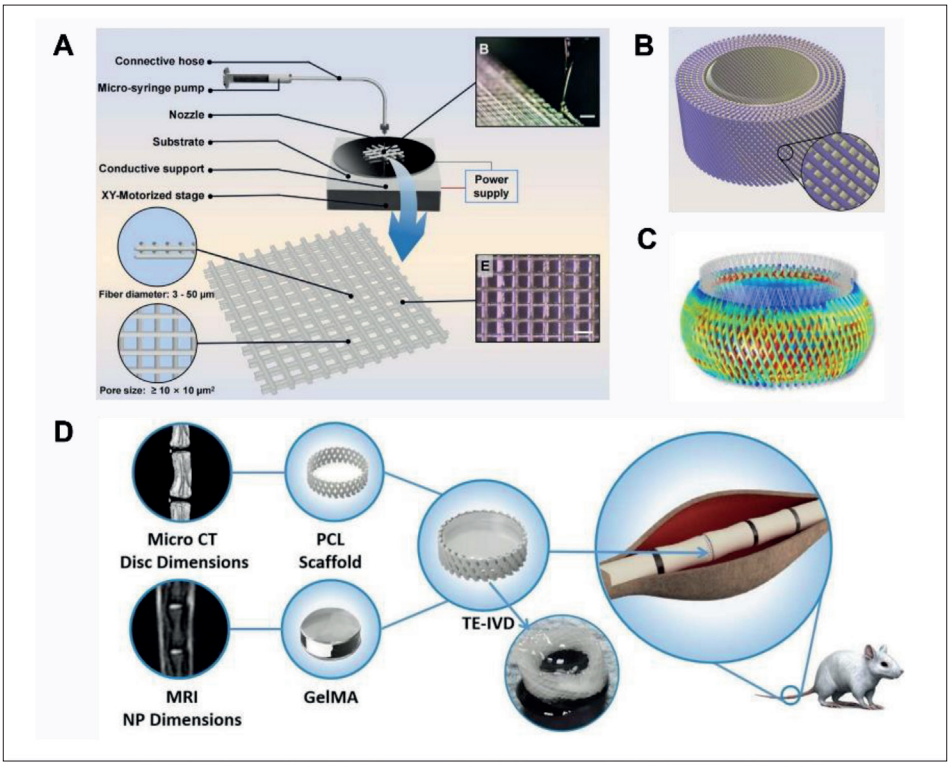

Figure 5. 3D bioprinting for IVD regeneration. (A) Schematic diagram of 3D electrohydrodynamic printing technology. (B) Scaffold structure design based on natural AF. (C) The simulation of the printed AF scaffold based on finite element analysis. (D) *In vivo* evaluation of the assembled construct. Adapted from Liu *et al*., with permission from the authors.[182]

need for better *in vitro* models. These tissue-engineered 3D disease models enable simulation of *in vivo* complex 3D structures and interactions by incorporating human cells in a genetically and environmentally controlled experimental system, which overcomes the shortcomings of 2D culture methods and has the potential to complement or even replace the use of animal models.[7] Research into disease mechanisms and drug development will increasingly benefit from sophisticated engineered tissues such as *in vitro* models of human disease.[179] As an advanced manufacturing technique to manipulate cells and biomaterials, 3D bioprinting can recapitulate the sophisticated architecture and function of human tissues and has great potential in the construction of disease models.

The development of 3D disease models depends on the availability of cell types that precisely mimic disease phenotypes.[181] In general, cell sources that are commonly used to build *in vitro* disease models include primary cells, cell lines, pluripotent stem cells (PSCs), or adult stem cells (ASCs). Primary cells isolated from animal tissues and organs have obvious advantages in reproducing specific tissue functions. However, the isolation of primary cells involves complex procedures, and the resulting mixed cell population usually requires further extraction of cells of interest. In addition, there are problems such as limited proliferative capacity and loss of phenotype during *in vitro* expansion when using primary cells. Cell lines are low-cost and more readily available, and usually follow standard culture and expansion procedures. They have uniform genotypic and phenotypic characteristics, allowing repeated *in vitro* culture. However, most of these cells are modified, so their structural and functional properties may differ from those of the target cells. Stem cells are able to overcome these limitations.[187] Since ASCs can only be derived from organs with a certain regenerative capacity, *in vitro* models derived from ASCs exist only in a limited number of organs. PSCs have unlimited self-renewal capacity and plasticity and can differentiate into almost any cell type *in vitro*. Over the past decade, there has been a remarkable progress in the development of PSC differentiation methods, which are able to generate 3D tissue-like structures such as organoid models *in vitro*.[183] These organoid models demonstrate similar morphology, cell composition, and function of the parts of developing organs *in vivo*. However, it is important to note that almost all specialized cell types derived from PSCs still exhibit immature phenotypes. These immature cells may be relevant to the study of early-onset disease processes, but whether their biological response can be extrapolated to

the types of mature and functional cells that are typically present in adult organs remains unknown. Therefore, it is necessary to develop powerful methods to differentiate PSCs and promote their maturation. The process of stem cell differentiation in the body is highly sophisticated and it is difficult to recapitulate all the cues *in vitro*. Besides, the combination of physicochemical factors required to induce differentiation of human PSCs into specific lineages remains unknown.[184] Thus, the interaction between cells and ECM is another important factor affecting the construction of *in vitro* models. ECM biomimetic materials such as Matrigel are popular options for building *in vitro* models. Matrigel is purified extract derived from ECM-producing tumors that provide both structural support and growth factors necessary for cell growth and differentiation. Despite practical properties such as cell adhesion and biodegradability, animal-derived materials are limited by poor mechanical properties and batch differences. In addition to the material itself, material design is also an important part of *in vitro* model system construction.[185] For example, in order to accurately guide stem cell differentiation, a series of biocompatible materials such as multifunctional hydrogels were designed to simulate the mechanical strength and 3D biological structure of bone.[186] These hydrogels are promising candidates for bioinks due to their unique high water content structure and adjustable physicochemical properties.

3D bioprinting offers a powerful tool for the creation of a variety of *in vitro* disease models due to its high precision, resolution, reproducibility, and capability to scale up scaffold production.[187] Kim *et al.* used 3D bioprinting to engineer a 3D diseased skin tissue with pathophysiological characteristics of type II diabetes *in vitro* and validated its feasibility as a drug screening tool.[188] Bin *et al.* developed bioinks composed of scar dECM and alginate–gelatin (Alg–Gel) hydrogels with desired mechanical properties to mimic the native architecture and microenvironmental factors of human hypertrophic scar (HHS).[189] The bioprinted HHS model demonstrated hallmarks of early-stage HHS and suitability for rapid drug testing. Since solid tumors possess complex and heterogeneous structures based on various cell types and ECM, 3D-bioprinted tumor models are potential tools for advancing our understanding of cancer biology and mechanism of therapeutics.[190,191] Han *et al.* bioprinted *in vitro* breast cancer models, which can accurately recapitulate the pathological micromorphology of heterogeneous cancer tissues and trigger drug responses similar to those of human cancers.[192] Neufeld *et al.* developed fibrin glioblastoma bioinks for the bioprinting of a glioblastoma model.[193] The bioprinted glioblastoma model contains complex blood vessels through which blood cells and drugs can be administered, achieving a faithful simulation of the tumor. Hakobyan *et al.* described the fabrication of exocrine pancreas spheroid models using laser-assisted bioprinting approach, which closely resembled the initial stages and progression of pancreatic ductal adenocarcinoma.[194] These bioprinted tumor models offer an opportunity to produce high-throughput drug testing platforms and mimic patient-specific drug reaction for individualized anticancer therapies.[195]

Recently, 3D-bioprinted constructs have been increasingly investigated as *in vitro* disease models for

Table 5. Advances in musculoskeletal disease models

Bioprinting technology	Materials	Cell type	Cell density (cells/mL)	Disease model	Characteristic	Drugs	Content evaluated	Ref.
Extrusion	Silk, PVP, and nano-HA	ADSCs	1×10^7	Osteoarthritis	Three layers; each layer for cartilage, bone, and interfacial phase, respectively	Celecoxib and Rhein	Anti-inflammatory effect	[195]
	Alginate	Chondrocytes	-	Joint infection	-	Antibiotic	Chondrotoxicity	[198]
Inkjet	Matrigel	hSkMDC	2×10^7	Muscle wasting disease	Contractile and aligned myofibers	Caffeine and Tirasemtiv	EPS-induced contractile force	[197]
Microneedle-based spheroid assembling	-	BMSCs	-	Metabolic bone disease	ECM abundance comparable to natural tissues	PD98059, U0126, Icariin, and purmorphamine	Osteogenic differentiation	[192]

Abbreviations: PVP: polyvinylpyrrolidone, nano-HA: nano-hydroxyapatites, ADSCs: adipose-derived stem cells, hSkMDC: human skeletal muscle-derived cells, BMSCs: bone marrow stem cells, ECM: extracellular matrix, EPS: electrical pulse stimulation

exploring molecular mechanisms and screening drug candidates for MSDs (Table 5). Metabolic bone disease (MBD) encompasses a broad spectrum of conditions characterized by abnormalities in bone mineral or bone matrix, affecting over 500 million people worldwide.[196] Among them, osteoporosis is the most common and associated with high risk of fractures.[197] To better understand the bone metabolic pathologies and to develop therapeutic drugs, *in vitro* models of bone tissue are urgently needed. Breathwaite *et al.* developed an *in vitro* bone model system using scaffold-free 3D bioprinting.[198,199] The morphological features of the bioprinted constructs including an abundance of ECM around the lacunar were comparable to natural tissues isolated from human donors. In this system, the effects of four drugs on osteogenic differentiation of BMSCs were analyzed. It was found that the differences of alkaline phosphatase (ALP) activity and the expression of osteogenic genes relative to untreated group were greater in 3D-bioprinted constructs compared with 2D culture group. The results indicate that the 3D-bioprinted model provides a more sensitive and biologically relevant opportunity to screen novel drugs against MBD. Osteoarthritis is a serious chronic and degenerative disease that is increasingly prevalent in aging and obese populations.[200] Little is known in the case of the molecular mechanisms of the onset and progression of osteoarthritis, and thus, most current treatments for osteoarthritis alleviate symptoms without repairing the cartilage tissues. Understanding the interaction between osteochondral tissues, symptoms, and related signaling pathways will provide better options for the treatment of osteoarthritis. Toward this, Singh *et al.* bioprinted human osteochondral units using silk-based materials and predifferentiated stem cells (Figure 6).[201] The osteochondral units, consisting of three layers, with each layer for cartilage, bone, and an interfacial phase, respectively, had macroscopic grid structures with open spaces and interconnected pores permitting cell–cell interactions. These osteochondral units were then subjected to the stimulation with pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-1 beta to model the early stage of osteoarthritis for efficient evaluation of anti-inflammatory drugs. Muscle wasting disease is a commonly encountered disorder, which arises from a variety of causes such as tumors, aging,

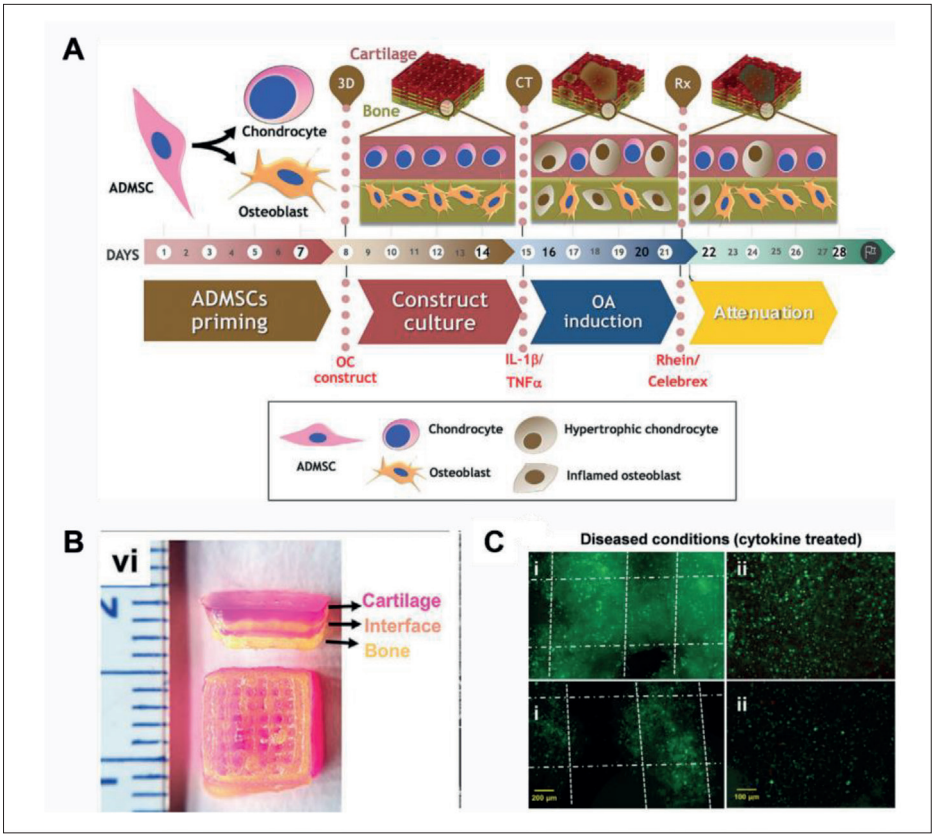

Figure 6. 3D bioprinting for disease modeling. (A) Schematic illustration of the preparation process of osteochondral models. (B) Representative images of the bioprinted osteochondral models. (C) Cell viability assessment with Live/Dead staining after bioprinting. Adapted from Singh *et al.*, with permission from John Wiley and Sons.[201]

prolonged bed rest, heart failure, and chronic obstructive pulmonary disease.[202] Loss of mobility by reduction of muscle mass and function result in poor quality of life and huge health care costs. There are not many medications available for treating skeletal muscle disorders, and drug interventions for muscle wasting diseases remain scarce. To this end, Reyes-Furrer *et al.* developed a 3D microphysiological system (MPS) based on human skeletal muscle models made of human skeletal muscle precursor cells and Matrigel using drop-on-demand bioprinting.[203] The bioprinted muscle models demonstrated contractile and aligned myofibers after a week of culture. In addition, contractile force of the models induced by electrical pulse stimulation was significantly promoted upon the intervention of known muscle stimulants, such as caffeine and Tirasemtiv, validating the huge potential of these models in the screening and development of drugs against muscle wasting diseases. Infection has always been a huge challenge for orthopedic surgeons, and the rise of antibiotic-resistant strains has further worsened the problem. The development of safe and effective antibiotics is urgently needed, and cytotoxicity is one of the main concerns for the screening of antibiotics. Bioprinted musculoskeletal constructs allow low-cost and efficient determination of the toxicity of drugs on cells. Datta *et al.* described a novel approach to manufacturing scalable tissue strands, which serve as the basic structural unit for bioprinting *in vitro* tissue models.[204] As a novel scalable bioink, tissue strands allow scaffold-free bioprinting for rapid generation of biomimetically mature tissues. The diameter of tissue strands remains stable, and they can maintain their original shape during culture to ensure the repeatability of the bioprinting process, enabling rapid fabrication of scale-up tissues. These bioprinted scaffold-free cartilage models closely mimic the physiology of articular cartilage and show great potential for drug screening.

5. Current challenges and future perspectives

Tissue engineering has made great strides over the past decade, with recent advances in bio-manufacturing technology, especially 3D bioprinting, being the main driving force. 3D bioprinting technologies have demonstrated great promise in musculoskeletal tissue engineering and drug development. However, there are some challenges that should be taken into account for future applications (Figure 7).

Bioinks possess properties required for 3D-bioprinting complex tissues and offer particular biological cues that facilitate tissue maturation *in vitro* and *in vivo*.[34] To generate biologically functional 3D constructs, bioinks must be compatible with corresponding bioprinting technology, which fulfills some critical characteristics, including rheology, physicochemical properties, and biological function. With advances in bioprinting technology, especially extrusion-based bioprinting, hydrogel-based bioinks have become one of the most common options. For extrusion-based bioprinting, hydrogel-based bioinks serve as a cell carrier to protect cells from shear forces while providing mechanical support and biological cues to guide cell growth and function. Maintaining the balance between physicochemical properties and biological functions poses a continuous challenge for 3D bioprinting. More precisely, 3D bioprinting is generally anticipated to produce a mechanically robust construct, but the encapsulated cells in bioinks typically need mild handling procedures and a fairly soft substrate environment.[205] Strong hydrogels are

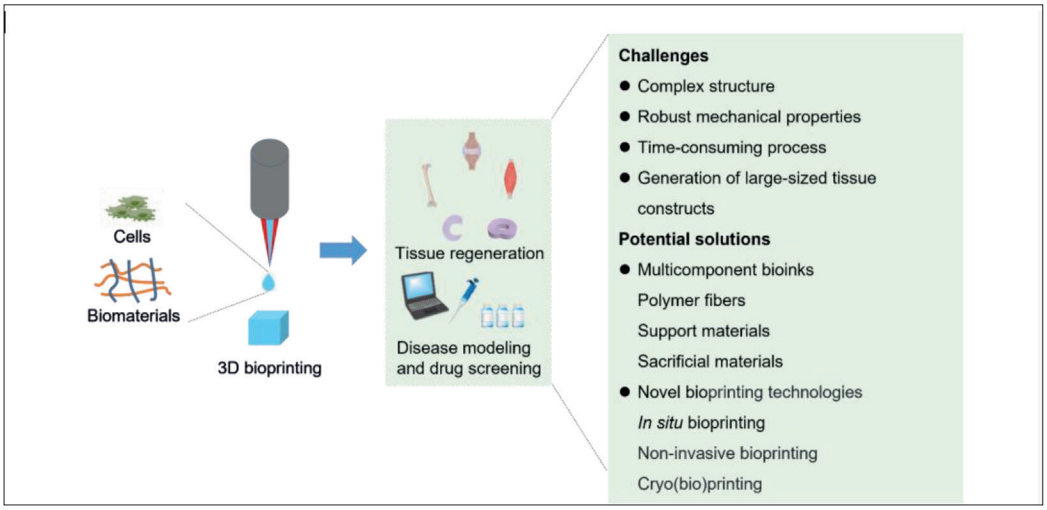

Figure 7. Current challenges and potential solutions of 3D bioprinting for musculoskeletal regeneration and disease modeling.

thought to provide more stable structural support for the growth of viable cells after printing than soft hydrogels. In order to maintain high shape fidelity during cell culture, a minimum stiffness of 10 kPa is required in bioprinted constructs.[206] In addition to meeting the requirements of cell culture, the bioprinted constructs also need to withstand the complex mechanical environment faced by musculoskeletal tissues upon implantation. Such strict requirements have led to a shortage of bioinks available for musculoskeletal tissue regeneration. The development of new formulation of bioinks is a research focus in this field. The simultaneous possession of all the required properties by a single component bioink is a challenging task for 3D bioprinting of functional tissues. Researchers are focusing their attention on multicomponent bioinks, which not only contribute to the expansion of biofabrication windows, but also enhance the functionality and complexity of bioprinted constructs. For example, a nanoengineered ionic covalent entanglement (NICE) bioink was described for the bioprinting of complex and large-scale tissue constructs.[207] Because of the unique rheological properties and biological clues of the bioink, the encapsulated cells can proliferate stably and maintain a high survival rate in the bioink. Moreover, the printed constructs demonstrated good shape fidelity and mechanical strength through the synergistic action of multiple crosslinking mechanisms. The incorporation of polymer fibers into bioinks can also increase the mechanical properties of printed constructs. For example, the combination of porous PCL fiber meshes and GelMA hydrogels loaded with amorphous magnesium phosphate significantly improved the mechanical properties of the printed structure and delayed its degradation, providing mechanical support for the recruitment and differentiation of progenitor cells to promote bone tissue regeneration.[208] In addition to improving the bioink formulation, the strategy of combining 3D bioprinting with 3D-printed scaffold as a support material can significantly improve the mechanical properties of the entire structure. For example, MSCs-laden fibroin-based bioinks were bioprinted into 3D-printed PCL frameworks to create constructs with enhanced mechanical properties. The mechanically reinforced constructs supported robust vascularization and graft mineralization when implanted *in vivo*.[62]

The creation and functionalization of large-sized tissue constructs remains a great challenge in 3D bioprinting. The vascular system within the tissue/organ provides the necessary nutrients and allows for metabolic exchange. The construction of the nutrient network is necessary when the size of the printed tissue construct is greater than 200 μm, which exceeds the diffusion limit of nutrients and oxygen. A common solution is to bioprint the cell-laden porous construct, but its structure is prone to collapse due to the poor mechanical properties of hydrogels. Especially for the centimeter-scale construct, the internal porous structure is difficult to maintain effectively. Another solution is a synchronous bioprinting strategy that incorporates sacrificial materials.[209] The synergistic interaction between cells and sacrificial biomaterials enhances the printing performance of each component, making it easier to manufacture complex constructs.

Printing vascularized constructs holds the promise of overcoming size limitations. Printing individual blood vessels is relatively easy to achieve. However, the construction of the entire blood vessel network (from large-scale to small-scale vessels) is an important issue to be solved in the field of 3D bioprinting. Brassard *et al.* developed a novel organoid printing technology, BATE, which successfully constructed highly biomimetic centimeter-scale tissues, including branch vascular system, opening up new ways for bioprinting and vascularization of large-sized constructs.[210] The functionalization of printed constructs is highly dependent on the maturity of the tissue. By altering the physicochemical signals in the printed construct, cell behaviors can be regulated to promote tissue maturation. The culture conditions after printing also affect the process of tissue maturation.

For clinical use, 3D-bioprinted tissue constructs are either surgically implanted in the body after *in vitro* incubation for maturation or directly generated in tissue defects by *in situ* bioprinting. The former strategy requires a long time to complete the entire process, which is not conducive to clinical translational application. By means of a robotic manipulator, *in situ* bioprinting allows for the direct construction of functional tissue constructs at target locations based on imaging information.[68] To obtain target structures, traditional bioprinting methods require direct access to the printing location and allow the printing head to move freely along the *x*, *y*, and *z* axes. Thus, the current application of *in situ* 3D bioprinting is limited to externally exposed damaged areas or sites requiring surgical exposure. Developing new 3D printing technologies to expand the application scope would be a promising solution. Minimally invasive or noninvasive approach is one of the major trends in clinical treatments. In this context, the concept of noninvasive *in vivo* 3D bioprinting attracts increasing attention. Based on that, Chen *et al.* explored near-infrared (NIR) light-responsive 3D printing technology to fabricate tissue constructs *in vivo* in a non-invasive manner.[138] By modulation and irradiation of NIR, the injected bioinks can be bioprinted into tissue constructs with customized patterns. With this approach, living

tissue constructs, including ears and muscles, were successfully formed without surgical exposure. Taking a similar principle, Urciuolo *et al*. developed a new bioprinting technique, which enables direct fabrication of functional tissues in living animals, and named it intravital 3D bioprinting.[139] The technique allows *in situ* bioprinting of a variety of complex tissue constructs such as dermis, skeletal muscle and brain.

When it comes to disease modeling, 3D bioprinting is a powerful tool and has been employed to create complex and dynamic models of various types of tumors.[196] The emergence of bioprinted models has enhanced our understanding of the onset and progression of disease. They also provide a valuable platform for the screening and development of therapeutic drug for MSDs. However, the development of 3D bioprinting for creating musculoskeletal disease models is still in its infancy, and the number of relevant studies available for review is limited. Most existing studies utilized simplified bioprinted models to screen high-throughput drugs or answer simple research questions. The future of bioprinting models for personalized therapy of MSDs may lie in the creation of more biomimetic *in vitro* disease models.

6. Conclusion

Due to its powerful ability to instantly and accurately transform digital images into 3D entities with biological function, 3D bioprinting offers an advanced method for the construction of complex tissue constructs as well as drug development. Bioprinted tissue constructs have shown promising performance in the studies concerning the regeneration of musculoskeletal tissues, including bone, cartilage, skeletal muscle, and meniscus. Aided by the development of newly synthesized materials and novel bioprinting technologies, the application of 3D bioprinting for musculoskeletal tissue engineering will be significantly expanded in the future.

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Conflict of interest

The authors declare no conflicts of interest.

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Consent for publication

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Availability of data

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References

1. Khodabukus A, Guyer T, Moore AC, Stevens MM, Guldberg RE, Bursac N. Translating musculoskeletal bioengineering into tissue regeneration therapies. *Sci Transl Med*. 2022;14:eabn9074.
doi: 10.1126/scitranslmed.abn9074

2. Cieza A, Causey K, Kamenov K, Hanson SW, Chatterji S, Vos T. Global estimates of the need for rehabilitation based on the Global Burden of Disease study 2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2021;396:2006-2017.
doi: 10.1016/s0140-6736(20)32340-0

3. Zhang S, Xing M, Li B. Recent advances in musculoskeletal local drug delivery. *Acta Biomater*. 2019;93:135-151.
doi: 10.1016/j.actbio.2019.01.043

4. Agarwal R, Williams K, Umscheid CA, Welch WC. Osteoinductive bone graft substitutes for lumbar fusion: A systematic review. *J Neurosurg Spine*. 2009;11:729-740.
doi: 10.3171/2009.6.Spine08669

5. Charalambides C, Beer M, Cobb AG. Poor results after augmenting autograft with xenograft (Surgibone) in hip revision surgery: A report of 27 cases. *Acta Orthop*. 2005;76:544-549.
doi: 10.1080/17453670510041547

6. Shapira A, Dvir T. 3D tissue and organ printing—Hope and reality. *Adv Sci*. 2021;8:2003751.
doi: 10.1002/advs.202003751

7. Vanderburgh J, Sterling JA, Guelcher SA. 3D printing of tissue engineered constructs for in vitro modeling of

disease progression and drug screening. *Ann Biomed Eng.* 2017;45:164-179.
doi: 10.1007/s10439-016-1640-4

8. Cui H, Miao S, Esworthy T, et al. 3D bioprinting for cardiovascular regeneration and pharmacology. *Adv Drug Deliv Rev.* 2018;132:252-269.
doi: 10.1016/j.addr.2018.07.014

9. Groll J, Boland T, Blunk T, et al. Biofabrication: Reappraising the definition of an evolving field. *Biofabrication.* 2016;8:013001.
doi: 10.1088/1758-5090/8/1/013001

10. Abaci A, Guvendiren M. Designing decellularized extracellular matrix-based bioinks for 3D bioprinting. *Adv Healthc Mater.* 2020;9:e2000734.
doi: 10.1002/adhm.202000734

11. Zhang Y, Haghiashtiani G, Hübscher T, et al. 3D extrusion bioprinting. *Nat Rev Methods Primers.* 2021;1:75.
doi: 10.1038/s43586-021-00073-8

12. Zhang T, Zhao W, Xiahou Z, Wang X, Zhang K, Yin J. Bioink design for extrusion-based bioprinting. *Appl Mater Today.* 2021;25:101227.
doi: 10.1016/j.apmt.2021.101227

13. Mandrycky C, Wang Z, Kim K, Kim D-H. 3D bioprinting for engineering complex tissues. *Biotechnol Adv.* 2016;34: 422-434.
doi: 10.1016/j.biotechadv.2015.12.011

14. Cui H, Nowicki M, Fisher JP, Zhang LG. 3D bioprinting for organ regeneration. *Adv Healthc Mater.* 2017;6:1601118.
doi: 10.1002/adhm.201601118

15. Li X, Liu B, Pei B, et al. Inkjet bioprinting of biomaterials. *Chem Rev.* 2020;120:10793-10833.
doi: 10.1021/acs.chemrev.0c00008

16. Cui X, Li J, Hartanto Y, et al. Advances in extrusion 3D bioprinting: A focus on multicomponent hydrogel-based bioinks. *Adv Healthc Mater.* 2020;9:1901648.
doi: 10.1002/adhm.201901648

17. Hölzl K, Lin S, Tytgat L, Vlierberghe SV, Gu L, Ovsianikov A. Bioink properties before, during and after 3D bioprinting. *Biofabrication*. 2016;8:032002.
doi: 10.1088/1758-5090/8/3/032002

18. Derakhshanfar S, Mbeleck R, Xu K, Zhang X, Zhong W, Xing M. 3D bioprinting for biomedical devices and tissue engineering: A review of recent trends and advances. *Bioact Mater.* 2018;3:144-156.
doi: 10.1016/j.bioactmat.2017.11.008

19. Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol.* 2014;32:773-785.
doi: 10.1038/nbt.2958

20. Xu K, Han Y, Huang Y, Wei P, Yin J, Jiang J.The application of 3D bioprinting in urological diseases. *Mater Today Bio*. 2022;16:100388.
doi: 10.1016/j.mtbio.2022.100388

21. Li M, Sun D, Zhang J, Wang Y, Wei Q, Wang Y. Application and development of 3D bioprinting in cartilage tissue engineering. *Biomater Sci*. 2022;10:5430-5458.
doi: 10.1039/D2BM00709F

22. Bernal PN, Delrot P, Loterie D, et al. Volumetric bioprinting of complex living-tissue constructs within seconds. *Adv Mater.* 2019;31:e1904209.
doi: 10.1002/adma.201904209

23. Bernal PN, Bouwmeester M, Madrid-Wolff J, et al. Volumetric bioprinting of organoids and optically tuned hydrogels to build liver-like metabolic biofactories. *Adv Mater.* 2022;34:e2110054.
doi: 10.1002/adma.202110054

24. Rizzo R, Ruetsche D, Liu H, Zenobi-Wong M. Optimized photoclick (bio)resins for fast volumetric bioprinting. *Adv Mater.* 2021;33:e2102900.
doi: 10.1002/adma.202102900

25. Gehlen J, Qiu W, Schädli GN, Müller R, Qin X-H. Tomographic volumetric bioprinting of heterocellular bone-like tissues in seconds. *Acta Biomater*. 2023;156:49-60.
doi: 10.1016/j.actbio.2022.06.020

26. Ouyang L, Yao R, Zhao Y, Sun W. Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication.* 2016;8:035020.
doi: 10.1088/1758-5090/8/3/035020

27. Samandari M, Quint J, Rodríguez-delaRosa A, Pourquié O, Tamayol A. Bioinks and bioprinting strategies for skeletal muscle tissue engineering. *Adv Mater.* 2022;34:e2105883.
doi: 10.1002/adma.202105883

28. Beketov EE, Isaeva EV, Yakovleva ND, et al. Bioprinting of cartilage with bioink based on high-concentration collagen and chondrocytes. *Int J Mol Sci.* 2021;22:11351.
doi: 10.3390/ijms222111351

29. Hwangbo H, Lee H, Jin EJ, et al. Bio-printing of aligned GelMa-based cell-laden structure for muscle tissue regeneration. *Bioact Mater.* 2022;8:57-70.
doi: 10.1016/j.bioactmat.2021.06.031

30. Sonaye SY, Ertugral EG, Kothapalli CR, Sikder P. Extrusion 3D (bio)printing of alginate-gelatin-based composite scaffolds for skeletal muscle tissue engineering. *Materials*. 2022;15:7945.
doi: 10.3390/ma15227945

31. Fan T, Wang S, Jiang Z, et al. Controllable assembly of skeletal muscle-like bundles through 3D bioprinting. *Biofabrication*. 2021;14:015009.
doi: 10.1088/1758-5090/ac3aca

32. De Santis MM, Alsafadi HN, Tas S, et al. Extracellular-matrix-reinforced bioinks for 3D bioprinting human tissue. *Adv Mater.* 2021;33:e2005476.
doi: 10.1002/adma.202005476

33. Yang X, Lu Z, Wu H, Li W, Zheng L, Zhao J. Collagen-alginate as bioink for three-dimensional (3D) cell printing based

cartilage tissue engineering. *Mater Sci Eng C Mater Biol Appl.* 2018;83:195-201.
doi: 10.1016/j.msec.2017.09.002

34. de Melo BAG, Jodat YA, Cruz EM, Benincasa JC, Shin SR, Porcionatto MA. Strategies to use fibrinogen as bioink for 3D bioprinting fibrin-based soft and hard tissues. *Acta Biomater.* 2020;117:60-76.
doi: 10.1016/j.actbio.2020.09.024

35. de Melo BAG, Jodat YA, Mehrotra S, et al. 3D printed cartilage-like tissue constructs with spatially controlled mechanical properties. *Adv Funct Mater.* 2019;29: 1906330.
doi: 10.1002/adfm.201906330

36. Li T, Hou J, Wang L, et al. Bioprinted anisotropic scaffolds with fast stress relaxation bioink for engineering 3D skeletal muscle and repairing volumetric muscle loss. *Acta Biomater.* 2022;156:21-36.
doi: 10.1016/j.actbio.2022.08.037

37. Visscher DO, Lee H, van Zuijlen PPM, et al. A photo-crosslinkable cartilage-derived extracellular matrix bioink for auricular cartilage tissue engineering. *Acta Biomater.* 2021;121:193-203.
doi: 10.1016/j.actbio.2020.11.029

38. Sahranavard M, Sarkari S, Safavi S, Ghorbani F. Three-dimensional bio-printing of decellularized extracellular matrix-based bio-inks for cartilage regeneration: A systematic review. *Biomater Transl.* 2022;3:105-115.
doi: 10.12336/biomatertransl.2022.02.004

39. Kim BS, Das S, Jang J, Cho D-W. Decellularized extracellular matrix-based bioinks for engineering tissue- and organ-specific microenvironments. *Chem Rev.* 2020;120:10608-10661.
doi: 10.1021/acs.chemrev.9b00808

40. Lee J, Hong J, Kim W, Kim GH. Bone-derived dECM/alginate bioink for fabricating a 3D cell-laden mesh structure for bone tissue engineering. *Carbohydr Polym.* 2020;250:116914.
doi: 10.1016/j.carbpol.2020.116914

41. Yoncheva K, Calleja P, Agüeros M, et al. Stabilized micelles as delivery vehicles for paclitaxel. *Int J Pharm.* 2012;436:258-264.
doi: 10.1016/j.ijpharm.2012.06.030

42. Shamma RN, Sayed RH, Madry H, El Sayed NS, Cucchiarini M. Triblock copolymer bioinks in hydrogel three-dimensional printing for regenerative medicine: A focus on pluronic f127. *Tissue Eng Part B Rev.* 2022;28:451-463.
doi: 10.1089/ten.TEB.2021.0026

43. Mozetic P, Giannitelli SM, Gori M, Trombetta M, Rainer A. Engineering muscle cell alignment through 3D bioprinting. *J Biomed Mater Res A.* 2017;105:2582-2588.
doi: 10.1002/jbm.a.36117

44. Shin YJ, Shafranek RT, Tsui JH, Walcott J, Nelson A, Kim D-H. 3D bioprinting of mechanically tuned bioinks derived from cardiac decellularized extracellular matrix. *Acta Biomater.* 2021;119:75-88.
doi: 10.1016/j.actbio.2020.11.006

45. Ying GL, Jiang N, Maharjan S, et al. Aqueous two-phase emulsion bioink-enabled 3D bioprinting of porous hydrogels. *Adv Mater.* 2018;30:e1805460.
doi: 10.1002/adma.201805460

46. Jia L, Hua Y, Zeng J, et al. Bioprinting and regeneration of auricular cartilage using a bioactive bioink based on microporous photocrosslinkable acellular cartilage matrix. *Bioact Mater.* 2022;16:66-81.
doi: 10.1016/j.bioactmat.2022.02.032

47. Zhang W, Wang N, Yang M, et al. Periosteum and development of the tissue-engineered periosteum for guided bone regeneration. *J Orthop Translat.* 2022;33: 41-54.
doi: 10.1016/j.jot.2022.01.002

48. Li Y, Pan Q, Xu J, et al. Overview of methods for enhancing bone regeneration in distraction osteogenesis: Potential roles of biometals. *J Orthop Translat.* 2021;27:110-118.
doi: 10.1016/j.jot.2020.11.008

49. Agarwal R, García AJ. Biomaterial strategies for engineering implants for enhanced osseointegration and bone repair. *Adv Drug Deliv Rev.* 2015;94:53-62.
doi: 10.1016/j.addr.2015.03.013

50. Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C. Bone regeneration strategies: Engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. *Biomaterials.* 2018;180:143-162.
doi: 10.1016/j.biomaterials.2018.07.017

51. Mauffrey C, Barlow BT, Smith W. Management of segmental bone defects. *J Am Acad Orthop Surg.* 2015;23:143-153.
doi: 10.5435/jaaos-d-14-00018

52. Tang H, Bi F, Chen G, et al. 3D-bioprinted recombination structure of Hertwig's epithelial root sheath cells and dental papilla cells for alveolar bone regeneration. *Int J Bioprint.* 2022;8:512.
doi: 10.18063/ijb.v8i3.512

53. Nulty J, Freeman FE, Browe DC, et al. 3D bioprinting of prevascularised implants for the repair of critically-sized bone defects. *Acta Biomater.* 2021;126:154-169.
doi: 10.1016/j.actbio.2021.03.003

54. Shen M, Wang L, Gao Y, et al. 3D bioprinting of in situ vascularized tissue engineered bone for repairing large segmental bone defects. *Mater Today Bio.* 2022;16: 100382.
doi: 10.1016/j.mtbio.2022.100382

55. Moncal KK, Tigli Aydın RS, Godzik KP, et al. Controlled Co-delivery of pPDGF-B and pBMP-2 from intraoperatively bioprinted bone constructs improves the repair of calvarial defects in rats. *Biomaterials.* 2022;281:121333.
doi: 10.1016/j.biomaterials.2021.121333

56. Kim W, Jang CH, Kim G. Bone tissue engineering supported by bioprinted cell constructs with endothelial cell spheroids. *Theranostics.* 2022;12:5404-5417. doi: 10.7150/thno.74852

57. Wang M, Li H, Yang Y, et al. A 3D-bioprinted scaffold with doxycycline-controlled BMP2-expressing cells for inducing bone regeneration and inhibiting bacterial infection. *Bioact Mater.* 2021;6:1318-1329. doi: 10.1016/j.bioactmat.2020.10.022

58. Sun X, Ma Z, Zhao X, et al. Three-dimensional bioprinting of multicell-laden scaffolds containing bone morphogenic protein-4 for promoting M2 macrophage polarization and accelerating bone defect repair in diabetes mellitus. *Bioact Mater.* 2021;6:757-769. doi: 10.1016/j.bioactmat.2020.08.030

59. Zhu H, Monavari M, Zheng K, et al. 3D bioprinting of multifunctional dynamic nanocomposite bioinks incorporating Cu-doped mesoporous bioactive glass nanoparticles for bone tissue engineering. *Small.* 2022;18:e2104996. doi: 10.1002/smll.202104996

60. Yu K, Huangfu H, Qin Q, et al. Application of bone marrow-derived macrophages combined with bone mesenchymal stem cells in dual-channel three-dimensional bioprinting scaffolds for early immune regulation and osteogenic induction in rat calvarial defects. *ACS Appl Mater Interfaces.* 2022;14:47052-47065. doi: 10.1021/acsami.2c13557

61. Sun X, Jiao X, Yang X, et al. 3D bioprinting of osteon-mimetic scaffolds with hierarchical microchannels for vascularized bone tissue regeneration. *Biofabrication.* 2022;14:035008. doi: 10.1088/1758-5090/ac6700

62. Pitacco P, Sadowska JM, O'Brien FJ, Kelly DJ. 3D bioprinting of cartilaginous templates for large bone defect healing. *Acta Biomater.* 2023;156:61-74. doi: 10.1016/j.actbio.2022.07.037

63. Li Z, Li S, Yang J, et al. 3D bioprinted gelatin/gellan gum-based scaffold with double-crosslinking network for vascularized bone regeneration. *Carbohydr Polym.* 2022;290:119469. doi: 10.1016/j.carbpol.2022.119469

64. Zhang J, Eyisoylu H, Qin XH, Rubert M, Müller R. 3D bioprinting of graphene oxide-incorporated cell-laden bone mimicking scaffolds for promoting scaffold fidelity, osteogenic differentiation and mineralization. *Acta Biomater.* 2021;121:637-652. doi: 10.1016/j.actbio.2020.12.026

65. Parthiban SP, Athirasala A, Tahayeri A, Abdelmoniem R, George A, Bertassoni LE. BoneMA-synthesis and characterization of a methacrylated bone-derived hydrogel for bioprinting of in-vitro vascularized tissue constructs. *Biofabrication.* 2021;13:035031. doi: 10.1088/1758-5090/abb11f

66. Li L, Shi J, Ma K, et al. Robotic in situ 3D bio-printing technology for repairing large segmental bone defects. *J Adv Res.* 2021;30:75-84. doi: 10.1016/j.jare.2020.11.011

67. Gehlen J, Qiu W, Schädli GN, Müller R, Qin X-H. Tomographic volumetric bioprinting of heterocellular bone-like tissues in seconds. *Acta Biomater.* 2022;156:49-60. doi: 10.1016/j.actbio.2022.06.020

68. Touya N, Devun M, Handschin C, et al. *In vitro* and *in vivo* characterization of a novel tricalcium silicate-based ink for bone regeneration using laser-assisted bioprinting. *Biofabrication.* 2022;14:024104. doi: 10.1088/1758-5090/ac584b

69. Tao J, Zhu S, Liao X, et al. DLP-based bioprinting of void-forming hydrogels for enhanced stem-cell-mediated bone regeneration. *Mater Today Bio.* 2022;17:100487. doi: 10.1016/j.mtbio.2022.100487

70. Rajput M, Mondal P, Yadav P, Chatterjee K. Light-based 3D bioprinting of bone tissue scaffolds with tunable mechanical properties and architecture from photocurable silk fibroin. *Int J Biol Macromol.* 2022;202:644-656. doi: 10.1016/j.ijbiomac.2022.01.081

71. Ryan EJ, Ryan AJ, González-Vázquez A, et al. Collagen scaffolds functionalised with copper-eluting bioactive glass reduce infection and enhance osteogenesis and angiogenesis both in vitro and in vivo. *Biomaterials.* 2019;197:405-416. doi: 10.1016/j.biomaterials.2019.01.031

72. Wang J, Wang H, Wang Y, et al. Endothelialized microvessels fabricated by microfluidics facilitate osteogenic differentiation and promote bone repair. *Acta Biomater.* 2022;142:85-98. doi: 10.1016/j.actbio.2022.01.055

73. Amler AK, Thomas A, Tüzüner S, et al. 3D bioprinting of tissue-specific osteoblasts and endothelial cells to model the human jawbone. *Sci Rep.* 2021;11:4876. doi: 10.1038/s41598-021-84483-4

74. Li J, Han F, Ma J, et al. Targeting endogenous hydrogen peroxide at bone defects promotes bone repair. *Adv Funct Mater.* 2022;32:2111208. doi: 10.1002/adfm.202111208

75. Arciola CR, Campoccia D, Montanaro L. Implant infections: Adhesion, biofilm formation and immune evasion. *Nat Rev Microbiol.* 2018;16:397-409. doi: 10.1038/s41579-018-0019-y

76. Josse J, Valour F, Maali Y, et al. Interaction between staphylococcal biofilm and bone: How does the presence of biofilm promote prosthesis loosening? *Front Microbiol.* 2019;10:1602. doi: 10.3389/fmicb.2019.01602

77. Schon BS, Hooper GJ, Woodfield TBF. Modular tissue assembly strategies for biofabrication of engineered cartilage. *Ann Biomed Eng.* 2017;45:100-114. doi: 10.1007/s10439-016-1609-3

78. Wei W, Dai H. Articular cartilage and osteochondral tissue engineering techniques: Recent advances and challenges. *Bioact Mater.* 2021;6:4830-4855. doi: 10.1016/j.bioactmat.2021.05.011

79. Borrelli J, Jr., Olson SA, Godbout C, Schemitsch EH, Stannard JP, Giannoudis PV. Understanding articular cartilage injury and potential treatments. *J Orthop Trauma.* 2019;33(Suppl 6):S6-S12. doi: 10.1097/bot.0000000000001472

80. Daly AC, Freeman FE, Gonzalez-Fernandez T, Critchley SE, Nulty J, Kelly DJ. 3D bioprinting for cartilage and osteochondral tissue engineering. *Adv Healthc Mater.* 2017;6:1700298. doi: 10.1002/adhm.201700298

81. Liu Y, Shah KM, Luo J. Strategies for articular cartilage repair and regeneration. *Front Bioeng Biotechnol.* 2021;9:770655. doi: 10.3389/fbioe.2021.770655

82. Zhu S, Chen P, Chen Y, et al. 3D-printed extracellular matrix/polyethylene glycol diacrylate hydrogel incorporating the anti-inflammatory phytomolecule honokiol for regeneration of osteochondral defects. *Am J Sports Med.* 2020;48:2808-2818. doi: 10.1177/0363546520941842

83. Hotham WE, Malviya A. A systematic review of surgical methods to restore articular cartilage in the hip. *Bone Joint Res.* 2018;7:336-342. doi: 10.1302/2046-3758.75.Bjr-2017-0331

84. Hunziker EB. Articular cartilage repair: Basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis Cartilage.* 2002;10:432-463. doi: 10.1053/joca.2002.0801

85. Lim KS, Abinzano F, Bernal PN, et al. One-step photoactivation of a dual-functionalized bioink as cell carrier and cartilage-binding glue for chondral regeneration. *Adv Healthc Mater.* 2020;9:e1901792. doi: 10.1002/adhm.201901792

86. Rathan S, Dejob L, Schipani R, Haffner B, Möbius ME, Kelly DJ. Fiber reinforced cartilage ECM functionalized bioinks for functional cartilage tissue engineering. *Adv Healthc Mater.* 2019;8:e1801501. doi: 10.1002/adhm.201801501

87. Sun Y, You Y, Jiang W, Wang B, Wu Q, Dai K. 3D bioprinting dual-factor releasing and gradient-structured constructs ready to implant for anisotropic cartilage regeneration. *Sci Adv.* 2020;6:eaay1422. doi: 10.1126/sciadv.aay1422

88. Zhang L, Tang H, Xiahou Z, et al. Solid multifunctional granular bioink for constructing chondroid basing on stem cell spheroids and chondrocytes. *Biofabrication.* 2022;14:035003. doi: 10.1088/1758-5090/ac63ee

89. Bonifacio MA, Cometa S, Cochis A, et al. A bioprintable gellan gum/lignin hydrogel: A smart and sustainable route for cartilage regeneration. *Int J Biol Macromol.* 2022;216:336-346. doi: 10.1016/j.ijbiomac.2022.07.002

90. Wang B, Diaz-Payno PJ, Browe DC, et al. Affinity-bound growth factor within sulfated interpenetrating network bioinks for bioprinting cartilaginous tissues. *Acta Biomater.* 2021;128:130-142. doi: 10.1016/j.actbio.2021.04.016

91. Shi W, Fang F, Kong Y, et al. Dynamic hyaluronic acid hydrogel with covalent linked gelatin as an anti-oxidative bioink for cartilage tissue engineering. *Biofabrication.* 2021;14:014107. doi: 10.1088/1758-5090/ac42de

92. Li Z, Zhang X, Yuan T, et al. Addition of platelet-rich plasma to silk fibroin hydrogel bioprinting for cartilage regeneration. *Tissue Eng Part A.* 2020;26:886-895. doi: 10.1089/ten.TEA.2019.0304

93. İlhan GT, Irmak G, Gümüşderelioğlu M. Microwave assisted methacrylation of Kappa carrageenan: A bioink for cartilage tissue engineering. *Int J Biol Macromol.* 2020;164:3523-3534. doi: 10.1016/j.ijbiomac.2020.08.241

94. Antich C, de Vicente J, Jiménez G, et al. Bio-inspired hydrogel composed of hyaluronic acid and alginate as a potential bioink for 3D bioprinting of articular cartilage engineering constructs. *Acta Biomater.* 2020;106:114-123. doi: 10.1016/j.actbio.2020.01.046

95. Sun Y, You Y, Jiang W, Zhai Z, Dai K. 3D-bioprinting a genetically inspired cartilage scaffold with GDF5-conjugated BMSC-laden hydrogel and polymer for cartilage repair. *Theranostics.* 2019;9:6949-6961. doi: 10.7150/thno.38061

96. Kosik-Kozioł A, Costantini M, Mróz A, et al. 3D bioprinted hydrogel model incorporating β-tricalcium phosphate for calcified cartilage tissue engineering. *Biofabrication.* 2019;11:035016. doi: 10.1088/1758-5090/ab15cb

97. Galarraga JH, Kwon MY, Burdick JA. 3D bioprinting via an in situ crosslinking technique towards engineering cartilage tissue. *Sci Rep.* 2019;9:19987. doi: 10.1038/s41598-019-56117-3

98. Onofrillo C, Duchi S, O'Connell CD, et al. Biofabrication of human articular cartilage: A path towards the development of a clinical treatment. *Biofabrication.* 2018;10:045006. doi: 10.1088/1758-5090/aad8d9

99. Huang Y, Meng X, Zhou Z, et al. A naringin-derived bioink enhances the shape fidelity of 3D bioprinting and efficiency of cartilage defect repair. *J Mater Chem B.* 2022;10:7030-7044. doi: 10.1039/d2tb01247b

100. Lee AK, Lin YH, Tsai CH, Chang W-T, Lin T-L, Shie M-Y. Digital light processing bioprinted human chondrocyte-laden poly (γ-glutamic acid)/hyaluronic acid bio-ink towards cartilage tissue engineering. *Biomedicines.* 2021;9:714. doi: 10.3390/biomedicines9070714

101. Lipskas J, Deep K, Yao W. Robotic-assisted 3D bio-printing for repairing bone and cartilage defects through a minimally invasive approach. *Sci Rep.* 2019;9:3746. doi: 10.1038/s41598-019-38972-2

102. Ma K, Zhao T, Yang L, et al. Application of robotic-assisted in situ 3D printing in cartilage regeneration with HAMA hydrogel: An in vivo study. *J Adv Res*. 2020;23:123-132. doi: 10.1016/j.jare.2020.01.010

103. Zhu W, Cui H, Boualam B, et al. 3D bioprinting mesenchymal stem cell-laden construct with core-shell nanospheres for cartilage tissue engineering. *Nanotechnology.* 2018;29:185101. doi: 10.1088/1361-6528/aaafa1

104. Cui X, Breitenkamp K, Finn MG, Martin Lotz, D'Lima DD. Direct human cartilage repair using three-dimensional bioprinting technology. *Tissue Eng Part A*. 2012;18:1304-1312. doi: 10.1089/ten.TEA.2011.0543

105. Burdis R, Chariyev-Prinz F, Kelly DJ. Bioprinting of biomimetic self-organised cartilage with a supporting joint fixation device. *Biofabrication.* 2021;14:015008. doi: 10.1088/1758-5090/ac36be

106. Daly AC, Cunniffe GM, Sathy BN, Jeon O, Alsberg E, Kelly DJ. 3D bioprinting of developmentally inspired templates for whole bone organ engineering. *Adv Healthc Mater.* 2016;5: 2352-2352. doi: 10.1002/adhm.201670100

107. Critchley SE, Kelly DJ. Bioinks for bioprinting functional meniscus and articular cartilage. *J 3D Print Med*. 2017;1: 269-290. doi: 10.2217/3dp-2017-0012

108. Kosik-Kozioł A, Costantini M, Bolek T, et al. PLA short sub-micron fiber reinforcement of 3D bioprinted alginate constructs for cartilage regeneration. *Biofabrication.* 2017;9:044105. doi: 10.1088/1758-5090/aa90d7

109. Pei Z, Gao M, Xing J, et al. Experimental study on repair of cartilage defects in the rabbits with GelMA-MSCs scaffold prepared by three-dimensional bioprinting. *Int J Bioprint*. 2023;9. doi: 10.18063/ijb.v9i2.662

110. Mancini IAD, Vindas Bolaños RA, Brommer H, et al. Fixation of hydrogel constructs for cartilage repair in the equine model: A challenging issue. *Tissue Eng Part C Methods.* 2017;23:804-814. doi: 10.1089/ten.TEC.2017.0200

111. Ofek G, Athanasiou K. Micromechanical properties of chondrocytes and chondrons: Relevance to articular cartilage tissue engineering. *J Mech Mater Struct*. 2007;2:1059-1086. doi: 10.2140/jomms.2007.2.1059

112. Idaszek J, Costantini M, Karlsen TA, et al. 3D bioprinting of hydrogel constructs with cell and material gradients for the regeneration of full-thickness chondral defect using a microfluidic printing head. *Biofabrication.* 2019;11:044101. doi: 10.1088/1758-5090/ab2622

113. Wu Y, Ayan B, Moncal KK, et al. Hybrid bioprinting of zonally stratified human articular cartilage using scaffold-free tissue strands as building blocks. *Adv Healthc Mater.* 2020;9:e2001657. doi: 10.1002/adhm.202001657

114. Dai W, Zhang L, Yu Y, et al. 3D bioprinting of heterogeneous constructs providing tissue-specific microenvironment based on host–guest modulated dynamic hydrogel bioink for osteochondral regeneration. *Adv Funct Mater.* 2022;32:2200710. doi: 10.1002/adfm.202200710

115. Distler T, Solisito AA, Schneidereit D, Friedrich O, Detsch R, Boccaccini AR. 3D printed oxidized alginate-gelatin bioink provides guidance for C2C12 muscle precursor cell orientation and differentiation via shear stress during bioprinting. *Biofabrication*. 2020;12:045005. doi: 10.1088/1758-5090/ab98e4

116. Liu J, Saul D, Böker KO, Ernst J, Lehman W, Schilling AF. Current methods for skeletal muscle tissue repair and regeneration. *Biomed Res Int.* 2018;2018:1984879. doi: 10.1155/2018/1984879

117. Ostrovidov S, Salehi S, Costantini M, et al. 3D bioprinting in skeletal muscle tissue engineering. *Small.* 2019;15:e1805530. doi: 10.1002/smll.201805530

118. Tidball JG. Mechanisms of muscle injury, repair, and regeneration. *Compr Physiol.* 2011;1:2029-2062. doi: 10.1002/cphy.c100092

119. Corona BT, Rivera JC, Owens JG, Wenke JC, Rathbone CR. Volumetric muscle loss leads to permanent disability following extremity trauma. *J Rehabil Res Dev.* 2015;52:785-792. doi: 10.1682/JRRD.2014.07.0165

120. Langridge B, Griffin M, Butler PE. Regenerative medicine for skeletal muscle loss: A review of current tissue engineering approaches. *J Mater Sci Mater Med.* 2021;32:15. doi: 10.1007/s10856-020-06476-5

121. Lin CH, Lin YT, Yeh JT, Chen C-T. Free functioning muscle transfer for lower extremity posttraumatic composite structure and functional defect. *Plast Reconstr Surg.* 2007;119:2118-2126. doi: 10.1097/01.prs.0000260595.85557.41

122. Aguilar CA, Greising SM, Watts A, et al. Multiscale analysis of a regenerative therapy for treatment of volumetric muscle loss injury. *Cell Death Discov.* 2018;4:33. doi: 10.1038/s41420-018-0027-8

123. Choi YJ, Jun YJ, Kim DY, et al. A 3D cell printed muscle construct with tissue-derived bioink for the treatment of volumetric muscle loss. *Biomaterials.* 2019;206:160-169. doi: 10.1016/j.biomaterials.2019.03.036

124. Behre A, Tashman JW, Dikyol C, et al. 3D bioprinted patient-specific extracellular matrix scaffolds for soft tissue defects. *Adv Healthc Mater.* 2022;11:e2200866. doi: 10.1002/adhm.202200866

125. Luo Z, Tang G, Ravanbakhsh H, et al. Vertical extrusion cryo(bio)printing for anisotropic tissue manufacturing. *Adv Mater.* 2022;34:e2108931. doi: 10.1002/adma.202108931

126. Mostafavi A, Samandari M, Karvar M, et al. Colloidal multiscale porous adhesive (bio)inks facilitate scaffold integration. *Appl Phys Rev.* 2021;8:041415. doi: 10.1063/5.0062823

127. Kim JH, Kim I, Seol YJ, et al. Neural cell integration into 3D bioprinted skeletal muscle constructs accelerates restoration of muscle function. *Nat Commun.* 2020;11:1025. doi: 10.1038/s41467-020-14930-9

128. Christensen KW, Turner J, Coughenour K, et al. Assembled cell-decorated collagen (AC-DC) fiber bioprinted implants with musculoskeletal tissue properties promote functional recovery in volumetric muscle loss. *Adv Healthc Mater.* 2022;11:e2101357. doi: 10.1002/adhm.202101357

129. Wang Y, Wang Q, Luo S, et al. 3D bioprinting of conductive hydrogel for enhanced myogenic differentiation. *Regen Biomater.* 2021;8:rbab035. doi: 10.1093/rb/rbab035

130. Kim JH, Seol YJ, Ko IK, et al. 3D bioprinted human skeletal muscle constructs for muscle function restoration. *Sci Rep.* 2018;8:12307. doi: 10.1038/s41598-018-29968-5

131. Yang GH, Kim W, Kim J, Kim GH. A skeleton muscle model using GelMA-based cell-aligned bioink processed with an electric-field assisted 3D/4D bioprinting. *Theranostics.* 2021;11:48-63. doi: 10.7150/thno.50794

132. Kim W, Jang CH, Kim GH. A myoblast-laden collagen bioink with fully aligned Au nanowires for muscle-tissue regeneration. *Nano Lett.* 2019;19:8612-8620. doi: 10.1021/acs.nanolett.9b03182

133. Yeo M, Kim G. Electrohydrodynamic-direct-printed cell-laden microfibrous structure using alginate-based bioink for effective myotube formation. *Carbohydr Polym.* 2021;272:118444. doi: 10.1016/j.carbpol.2021.118444

134. Chen Y, Zhang J, Liu X, et al. Noninvasive in vivo 3D bioprinting. *Sci Adv.* 2020;6:eaba7406. doi: 10.1126/sciadv.aba7406

135. Urciuolo A, Poli I, Brandolino L, et al. Intravital three-dimensional bioprinting. *Nat Biomed Eng.* 2020;4:901-915. doi: 10.1038/s41551-020-0568-z

136. Jana S, Levengood SK, Zhang M. Anisotropic materials for skeletal-muscle-tissue engineering. *Adv Mater.* 2016;28:10588-10612. doi: 10.1002/adma.201600240

137. Zhang Y, Zhang Z, Wang Y, Su Y, Chen M. 3D myotube guidance on hierarchically organized anisotropic and conductive fibers for skeletal muscle tissue engineering. *Mater Sci Eng C Mater Biol Appl.* 2020;116:111070. doi: 10.1016/j.msec.2020.111070

138. Ostrovidov S, Hosseini V, Ahadian S, et al. Skeletal muscle tissue engineering: Methods to form skeletal myotubes and their applications. *Tissue Eng Part B Rev.* 2014;20:403-436. doi: 10.1089/ten.TEB.2013.0534

139. Yeo M, Kim G. Three-dimensional microfibrous bundle structure fabricated using an electric field-assisted/cell printing process for muscle tissue regeneration. *ACS Biomater Sci Eng.* 2018;4:728-738. doi: 10.1021/acsbiomaterials.7b00983

140. Bilgen B, Jayasuriya CT, Owens BD. Current concepts in meniscus tissue engineering and repair. *Adv Healthc Mater.* 2018;7:1701407. doi: 10.1002/adhm.201701407

141. Chae S, Lee SS, Choi YJ, et al. 3D cell-printing of biocompatible and functional meniscus constructs using meniscus-derived bioink. *Biomaterials.* 2021;267:120466. doi: 10.1016/j.biomaterials.2020.120466

142. Kwon H, Brown WE, Lee CA, et al. Surgical and tissue engineering strategies for articular cartilage and meniscus repair. *Nat Rev Rheumatol.* 2019;15:550-570. doi: 10.1038/s41584-019-0255-1

143. Roemer FW, Kwoh CK, Hannon MJ, et al. Partial meniscectomy is associated with increased risk of incident radiographic osteoarthritis and worsening cartilage damage in the following year. *Eur Radiol.* 2017;27:404-413. doi: 10.1007/s00330-016-4361-z

144. Noyes FR, Barber-Westin SD. Long-term survivorship and function of meniscus transplantation. *Am J Sports Med.* 2016; 44:2330-2338. doi: 10.1177/0363546516646375

145. Rosso F, Bisicchia S, Bonasia DE, Amendola A. Meniscal allograft transplantation: A systematic review. *Am J Sports Med.* 2015;43:998-1007. doi: 10.1177/0363546514536021

146. Jiang D, Ao YF, Gong X, Wang Y-J, Zheng Z-Z, Yu J-K. Comparative study on immediate versus delayed meniscus allograft transplantation: 4- to 6-year follow-up. *Am J Sports Med.* 2014;42:2329-2337. doi: 10.1177/0363546514541653

147. Costa JB, Park J, Jorgensen AM, et al. 3D bioprinted highly elastic hybrid constructs for advanced fibrocartilaginous tissue regeneration. *Chem Mater.* 2020;32: 8733-8746. doi: 10.1021/acs.chemmater.0c03556

148. Jian Z, Zhuang T, Qinyu T, et al. 3D bioprinting of a biomimetic meniscal scaffold for application in tissue engineering. *Bioact Mater.* 2021;6:1711-1726. doi: 10.1016/j.bioactmat.2020.11.027

149. Stocco TD, Moreira Silva MC, Corat MAF, AO L. Towards bioinspired meniscus-regenerative scaffolds: Engineering a novel 3D bioprinted patient-specific construct reinforced by biomimetically aligned nanofibers. *Int J Nanomed.* 2022;17:1111-1124.
doi: 10.2147/ijn.S353937

150. Terpstra ML, Li J, Mensinga A, et al. Bioink with cartilage-derived extracellular matrix microfibers enables spatial control of vascular capillary formation in bioprinted constructs. *Biofabrication.* 2022;14:034104.
doi: 10.1088/1758-5090/ac6282

151. Sathish PB, Gayathri S, Priyanka J, et al. Tricomposite gelatin-carboxymethylcellulose-alginate bioink for direct and indirect 3D printing of human knee meniscal scaffold. *Int J Biol Macromol.* 2022;195:179-189.
doi: 10.1016/j.ijbiomac.2021.11.184

152. Sun Y, Zhang Y, Wu Q, et al. 3D-bioprinting ready-to-implant anisotropic menisci recapitulate healthy meniscus phenotype and prevent secondary joint degeneration. *Theranostics*. 2021;11:5160-5173.
doi: 10.7150/thno.54864

153. Lan X, Ma Z, Szojka ARA, et al. TEMPO-oxidized cellulose nanofiber-alginate hydrogel as a bioink for human meniscus tissue engineering. *Front Bioeng Biotechnol.* 2021;9: 766399.
doi: 10.3389/fbioe.2021.766399

154. Hao L, Tianyuan Z, Zhen Y, et al. Biofabrication of cell-free dual drug-releasing biomimetic scaffolds for meniscal regeneration. *Biofabrication.* 2021;14:015001.
doi: 10.1088/1758-5090/ac2cd7

155. Filardo G, Petretta M, Cavallo C, et al. Patient-specific meniscus prototype based on 3D bioprinting of human cell-laden scaffold. *Bone Joint Res.* 2019;8:101-106.
doi: 10.1302/2046-3758.82.Bjr-2018-0134.R1

156. Chansoria P, Narayanan LK, Schuchard K, Shirwaiker R. Ultrasound-assisted biofabrication and bioprinting of preferentially aligned three-dimensional cellular constructs. *Biofabrication*. 2019;11:035015.
doi: 10.1088/1758-5090/ab15cf

157. Ali AM, Newman SDS, Hooper PA, Davies CM, Cobb JP. The effect of implant position on bone strain following lateral unicompartmental knee arthroplasty: A biomechanical model using digital image correlation. *Bone Joint Res*. 2017;6: 522-529.
doi: 10.1302/2046-3758.68.Bjr-2017-0067.R1

158. Badylak SF. The extracellular matrix as a biologic scaffold material. *Biomaterials*. 2007;28:3587-3593.
doi: 10.1016/j.biomaterials.2007.04.043

159. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. *J Cell Sci.* 2010;123:4195-4200.
doi: 10.1242/jcs.023820

160. Makris EA, Hadidi P, Athanasiou KA. The knee meniscus: Structure-function, pathophysiology, current repair techniques, and prospects for regeneration. *Biomaterials.* 2011;32:7411-7431.
doi: 10.1016/j.biomaterials.2011.06.037

161. Narayanan LK, Huebner P, Fisher MB, Spang JT, Starly B, Shirwaiker RA. 3D-bioprinting of polylactic acid (PLA) nanofiber-alginate hydrogel bioink containing human adipose-derived stem cells. *ACS Biomater Sci Eng.* 2016;2:1732-1742.
doi: 10.1021/acsbiomaterials.6b00196

162. MacBarb RF, Chen AL, Hu JC, Athanasiou KA. Engineering functional anisotropy in fibrocartilage neotissues. *Biomaterials.* 2013;34:9980-9989.
doi: 10.1016/j.biomaterials.2013.09.026

163. Bonnet CS, Walsh DA. Osteoarthritis, angiogenesis and inflammation. *Rheumatology.* 2005;44:7-16.
doi: 10.1093/rheumatology/keh344

164. Mapp PI, Walsh DA. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. *Nat Rev Rheumatol.* 2012;8:390-398.
doi: 10.1038/nrrheum.2012.80

165. Ashraf S, Wibberley H, Mapp PI, Hill R, Wilson D, Walsh DA. Increased vascular penetration and nerve growth in the meniscus: A potential source of pain in osteoarthritis. *Ann Rheum Dis.* 2011;70:523-529.
doi: 10.1136/ard.2010.137844

166. Freemont AJ, Watkins A, Le Maitre C, et al. Nerve growth factor expression and innervation of the painful intervertebral disc. *J Pathol.* 2002;197:286-292.
doi: 10.1002/path.1108

167. Ohnishi T, Iwasaki N, Sudo H. Causes of and molecular targets for the treatment of intervertebral disc degeneration: A review. *Cells.* 2022;11:394.
doi: 10.3390/cells11030394

168. Peng Y, Qing X, Shu H, et al. Proper animal experimental designs for preclinical research of biomaterials for intervertebral disc regeneration. *Biomater Transl.* 2021;2:91-142.
doi: 10.12336/biomatertransl.2021.02.003

169. Feng Y, Egan B, Wang J. Genetic factors in intervertebral disc degeneration. *Genes Dis.* 2016;3:178-185.
doi: 10.1016/j.gendis.2016.04.005

170. Wu D, Li G, Zhou X, Zhang W. Repair strategies and bioactive functional materials for intervertebral disc. *Adv Funct Mater.* 2022;32:2209471.
doi: 10.1002/adfm.202209471

171. van Uden S, Silva-Correia J, Oliveira JM, Reis RL. Current strategies for treatment of intervertebral disc degeneration: Substitution and regeneration possibilities. *Biomater Res.* 2017;21:22.
doi: 10.1186/s40824-017-0106-6

172. JT M, AH M, JA C, et al. Translation of an engineered nanofibrous disc-like angle-ply structure for intervertebral disc replacement in a small animal model. *Acta Biomater.* 2014;10:2473-2481.
doi: 10.1016/j.actbio.2014.02.024

173. Yang J, Wang L, Zhang W, et al. Reverse reconstruction and bioprinting of bacterial cellulose-based functional total intervertebral disc for therapeutic implantation. *Small.* 2018;14:1702582.
doi: 10.1002/smll.201702582

174. Gullbrand SE, Kim DH, Bonnevie E, et al. Towards the scale up of tissue engineered intervertebral discs for clinical application. *Acta Biomater.* 2018;70:154-164.
doi: 10.1016/j.actbio.2018.01.050

175. Yang J, Yang X, Wang L, et al. Biomimetic nanofibers can construct effective tissue-engineered intervertebral discs for therapeutic implantation. *Nanoscale.* 2017;9:13095.
doi: 10.1039/c7nr03944a

176. Bhunia BK, Dey S, Bandyopadhyay A, Mandal BB. 3D printing of annulus fibrosus anatomical equivalents recapitulating angle-ply architecture for intervertebral disc replacement. *Appl Mater Today.* 2021;23:101031.
doi: 10.1016/j.apmt.2021.101031

177. Liu Z, Wang H, Yuan Z, et al. High-resolution 3D printing of angle-ply annulus fibrosus scaffolds for intervertebral disc regeneration. *Biofabrication.* 2022;15:015015.
doi: 10.1088/1758-5090/aca71f

178. Hu D, Wu D, Huang L, et al. 3D bioprinting of cell-laden scaffolds for intervertebral disc regeneration. *Mater Lett.* 2018;223:219-222.
doi: 10.1016/j.matlet.2018.03.204

179. Parker KK, Healy K, Bursac N. Tissue-engineered disease models. *Nat Biomed Eng.* 2018;2:879-880.
doi: 10.1038/s41551-018-0339-2

180. Moran CJ, Ramesh A, Brama PA, O'Byrne JM, O'Brien FJ, Levingstone TJ. The benefits and limitations of animal models for translational research in cartilage repair. *J Exp Orthop.* 2016;3:1.
doi: 10.1186/s40634-015-0037-x

181. Benam KH, Dauth S, Hassell B, et al. Engineered in vitro disease models. *Annu Rev Pathol.* 2015;10:195-262.
doi: 10.1146/annurev-pathol-012414-040418

182. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006;126:663-676.
doi: 10.1016/j.cell.2006.07.024

183. Vandana JJ, Manrique C, Lacko LA, Chen S. Human pluripotent-stem-cell-derived organoids for drug discovery and evaluation. *Cell Stem Cell.* 2023;30:571-591.
doi: 10.1016/j.stem.2023.04.011

184. Corsini NS, Knoblich JA. Human organoids: New strategies and methods for analyzing human development and disease. *Cell.* 2022;185:2756-2769.
doi: 10.1016/j.cell.2022.06.051

185. Mota C, Camarero-Espinosa S, Baker MB, Wieringa P, Moroni L. Bioprinting: From tissue and organ development to in vitro models. *Chem Rev.* 2020;120:10547-10607.
doi: 10.1021/acs.chemrev.9b00789

186. Kurian AG, Singh RK, Patel KD, Lee J-H, Kim H-W. Multifunctional GelMA platforms with nanomaterials for advanced tissue therapeutics. *Bioact Mater.* 2022;8:267-295.
doi: 10.1016/j.bioactmat.2021.06.027

187. Kang HW, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol.* 2016;34:312-319.
doi: 10.1038/nbt.3413

188. Kim BS, Ahn M, Cho WW, Gao G, Jang J, Cho D-W. Engineering of diseased human skin equivalent using 3D cell printing for representing pathophysiological hallmarks of type 2 diabetes in vitro. *Biomaterials.* 2021;272:120776.
doi: 10.1016/j.biomaterials.2021.120776

189. Bin Y, Dongzhen Z, Xiaoli C, et al. Modeling human hypertrophic scars with 3D preformed cellular aggregates bioprinting. *Bioact Mater.* 2022;10:247-254.
doi: 10.1016/j.bioactmat.2021.09.004

190. Neufeld L, Yeini E, Pozzi S, Satchi-Fainaro R. 3D bioprinted cancer models: From basic biology to drug development. *Nat Rev Cancer.* 2022;22:679-692.
doi: 10.1038/s41568-022-00514-w

191. Jain P, Kathuria H, Dubey N. Advances in 3D bioprinting of tissues/organs for regenerative medicine and in-vitro models. *Biomaterials*. 2022;287:121639.
doi: 10.1016/j.biomaterials.2022.121639

192. Han J, Jeon S, Kim MK, Jeong W, Yoo JJ, Kang H-W. In vitro breast cancer model with patient-specific morphological features for personalized medicine. *Biofabrication.* 2022;14:034102.
doi: 10.1088/1758-5090/ac6127

193. Neufeld L, Yeini E, Reisman N, et al. Microengineered perfusable 3D-bioprinted glioblastoma model for in vivo mimicry of tumor microenvironment. *Sci Adv.* 2021;7:eabi9119.
doi: 10.1126/sciadv.abi9119

194. Hakobyan D, Médina C, Dusserre N, et al. Laser-assisted 3D bioprinting of exocrine pancreas spheroid models for cancer initiation study. *Biofabrication.* 2020;12:035001.
doi: 10.1088/1758-5090/ab7cb8

195. Tang M, Rich JN, Chen S. Biomaterials and 3D bioprinting strategies to model glioblastoma and the blood-brain barrier. *Adv Mater.* 2021;33:e2004776.
doi: 10.1002/adma.202004776

196. Gao Y, Fu Z, Guan J, Liu X, Zhang Q. The role of Notch signaling pathway in metabolic bone diseases. *Biochem Pharmacol.* 2023;207:115377.
doi: 10.1016/j.bcp.2022.115377

197. Greenblatt MB, Tsai JN, Wein MN. Bone turnover markers in the diagnosis and monitoring of metabolic bone disease. *Clin Chem.* 2017;63:464-474. doi: 10.1373/clinchem.2016.259085

198. Breathwaite E, Weaver J, Odanga J, dela Pena-Ponce M, Lee JB. 3D bioprinted osteogenic tissue models for in vitro drug screening. *Molecules.* 2020;25:3442. doi: 10.3390/molecules25153442

199. Breathwaite EK, Weaver JR, Murchison AC, Treadwell ML, Odanga JJ, Lee JB. Scaffold-free bioprinted osteogenic and chondrogenic systems to model osteochondral physiology. *Biomed Mater.* 2019;14:065010. doi: 10.1088/1748-605X/ab4243

200. Martel-Pelletier J, Barr AJ, Cicuttini FM, et al. Osteoarthritis. *Nat Rev Dis Primers.* 2016;2:16072. doi: 10.1038/nrdp.2016.72

201. Singh YP, Moses JC, Bandyopadhyay A, Mandal BB. 3D bioprinted silk-based in vitro osteochondral model for osteoarthritis therapeutics. *Adv Healthc Mater.* 2022;11:2200209. doi: 10.1002/adhm.202200209

202. Furrer R, Handschin C. Muscle wasting diseases: Novel targets and treatments. *Annu Rev Pharmacol Toxicol.* 2019;59:315-339. doi: 10.1146/annurev-pharmtox-010818-021041

203. Alave Reyes-Furrer A, De Andrade S, Bachmann D, et al. Matrigel 3D bioprinting of contractile human skeletal muscle models recapitulating exercise and pharmacological responses. *Commun Biol.* 2021;4:1183. doi: 10.1038/s42003-021-02691-0

204. Datta P, Wu Y, Yu Y, Moncal KK, Ozbolat IT. A scaffold free 3D bioprinted cartilage model for in vitro toxicology. *Methods Mol Biol.* 2021;2147:175-183. doi: 10.1007/978-1-0716-0611-7_15

205. Ouyang L. Pushing the rheological and mechanical boundaries of extrusion-based 3D bioprinting. *Trends Biotechnol.* 2022;40:891-902. doi: 10.1016/j.tibtech.2022.01.001

206. Ouyang L, Armstrong JPK, Lin Y, et al. Expanding and optimizing 3D bioprinting capabilities using complementary network bioinks. *Sci Adv.* 2020;6:eabc5529. doi: 10.1126/sciadv.abc5529

207. Chimene D, Peak CW, Gentry JL, et al. Nanoengineered ionic–covalent entanglement (nice) bioinks for 3D bioprinting. *ACS Appl Mater Interfaces.* 2018;10:9957-9968. doi: 10.1021/acsami.7b19808

208. Dubey N, Ferreira JA, Daghrery A, et al. Highly tunable bioactive fiber-reinforced hydrogel for guided bone regeneration. *Acta Biomater.* 2020;113:164-176. doi: 10.1016/j.actbio.2020.06.011

209. Shao L, Gao Q, Xie C, Fu J, Xiang M, He Y. Synchronous 3D bioprinting of large-scale cell-laden constructs with nutrient networks. *Adv Healthc Mater.* 2020;9:e1901142. doi: 10.1002/adhm.201901142

210. Brassard JA, Nikolaev M, Hübscher T, Hofer M, Lutolf MP. Recapitulating macro-scale tissue self-organization through organoid bioprinting. *Nat Mater.* 2021;20:22-29. doi: 10.1038/s41563-020-00803-5