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ABSTRACT

Current surgical treatments and material applications are not ideal for the treatment of orthopedic clinical injuries, such as large bone defects, cartilage defects, and vascular tendon adhesions that occur after repair. With the continuous development of tissue engineering technology, hydrogels have become important medical biomaterials. Hydrogels are three-dimensional hydrophilic network structures composed of cross-linked polymer chains. They are a new kind of polymeric material for the treatment of orthopedic diseases. Hydrogels have good biocompatibility, biodegradability, drug-carrying capacity, and controllable drug release ability and are less toxic than nanoparticle carriers. They have been widely used in wound repair, guided tissue regeneration, bacteriostasis, hemostasis, postoperative adhesion prevention, drug delivery, and 3D printing. These characteristics can be used to develop a variety of treatments for different diseases. This paper focuses on the innovative progress of hydrogels in promoting and improving bone, cartilage, tendon, and soft tissue regeneration in orthopedic clinical applications. Current and prospective applications of hydrogels in the field of orthopedics are discussed herein.

I. INTRODUCTION

Bone, cartilage, and skeletal muscle are often injured by a single injury or repeated overuse. The complex macroscopic and microscopic composition of these tissues determines the diversity of damage to these structures.[1] The ability of bone to regenerate is limited, and defects greater than a certain size cannot heal on their own. At present, autologous or allogeneic bone grafts are commonly used in the treatment of bone defects.[2–4] Autologous bone grafting is considered the gold standard of treatment.[5–8] The bone grafts are usually taken from the iliac crest of the patient and transplanted to the defective area. Autografts are biocompatible active tissues whose cells help in the healing process. Autograft remodeling occurs naturally through osteoclast-mediated bone resorption and osteoblast-mediated bone formation. However, autografts also have disadvantages, such as the limited amount of donor bone available, the need for a second operation to separate the graft, and the possibility of recurrent pain at the donor site. An alternative treatment method is the use of allograft bone that is taken from one patient and transplanted into another. Compared with autologous bone, allograft bone is avascular, non-viable tissue. Allograft bone is readily available but has an increased risk of disease transmission and the potential for immune rejection.[2,9] To solve the above problems, tissue engineering application is born. Biomolecules play a key role in engineering functional materials for biomedical applications such as tissue engineering and drug delivery systems. One of its major applications is cell and tissue engineering, where these materials are used as scaffolds for cell culture to support and regulate the biological behavior of cells to achieve the goal of creating artificial tissues/organs. Another application of biomolecular materials is the

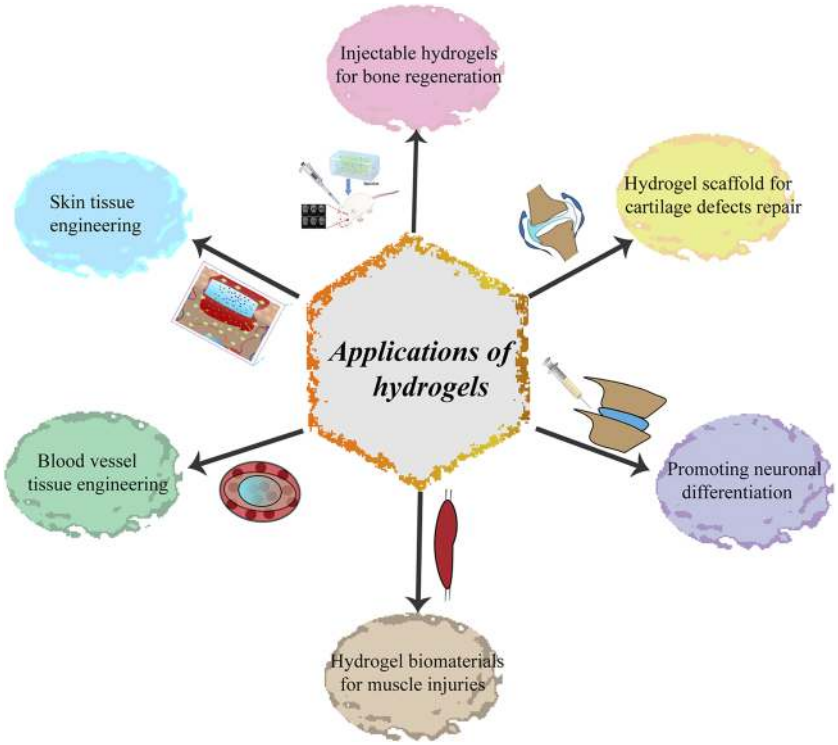

FIG. 1. Schematic diagram of applications of hydrogel materials.

drug delivery system in which materials enclosing drug molecules control the release of therapeutic drugs to improve therapeutic efficacy and reduce side effects. Biomolecules can be made into macroscopic materials, such as hydrogels, which can be implanted as drug release devices that have a locally sustained therapeutic effect.[10]

The preparation of biocompatible polymer carriers for continuous control of drug release has been extensively explored for many years. In addition, natural polymer systems outperform traditional polymers in terms of biocompatibility, biodegradability, and cost-efficiency.[11] A hydrogel is a 3D network structure composed of hydrophilic polymer chains, with a water content of 90%–99% that contributes to the efficient exchange of oxygen and materials.[12] Hydrogels are a unique scaffold material that can absorb a large volume of water without decomposition. This allows cells to adhere and differentiate onto the hydrogels. Therefore, hydrogels can simulate the natural tissue environment, provide structural support for the defective site, and enable the bone defect to be repaired through internal healing mechanisms.[13] The use of hydrogels dates back to 1960 when Wichterle and Lim introduced a cross-linked polymer (2-hydroxyethyl methacrylate) hydrophilic network as a soft contact lens material.[13,14] Tissue engineering has developed rapidly because of the mismatch between supply and demand for organ and tissue transplants.[15] The use of hydrogels is an important research field in tissue engineering that has been widely studied since it was discovered.[15,16] As scaffolds for bone tissue engineering, cells require a water environment, adequate oxygen and diffusion of nutrients, appropriate pH and osmotic pressure, and key vitamins and minerals for cell function. Some types of cells need enough space to proliferate at specific cell attachment sites and substrate properties, and hydrogels can meet these stringent requirements. Hydrogels are loose, cross-linked networks of highly hydrophilic polymers that absorb many times more water than their dry weight. The high water content makes the hydrogels highly permeable and porous, allowing oxygen and nutrients to diffuse rapidly within the scaffolds.[17] Some hydrogels are also biodegradable, providing initial support to cells and then degrading as cell populations grow and the environment changes. Hydrogels can also be created from proteins and extracellular matrix (ECM) components, including collagen and hyaluronic acid (HA), which provide environmental clues to help stem cells grow. Finally, many hydrogels can be cross-linked into solid viscoelastic structures in a cell-friendly way, which does no harm to living cells and minimizes the physiological pressure on the printed cells encapsulated in scaffolds. These properties make hydrogels close to the native microenvironment of cells, making them the best choice for tissue engineering.[18] Recent studies have demonstrated the efficacy of hydrogels in a wide range of tissue engineering applications including engineering of bone and cartilage, among others (Fig. 1).[19]

Here, we discuss the delivery pathways of hydrogels as tissue-engineered scaffolds in the treatment of orthopedic diseases and summarize the formation of different hydrogels and the methods used to promote wound healing. In addition, we analyze the success of hydrogels as drug delivery carriers and discuss prospects.

II. HYDROGEL

In all multicellular organisms, cells are structurally supported by a complex, biologically active scaffold called the extracellular matrix (ECM).[13] The ECM is a complex mixture of proteins and

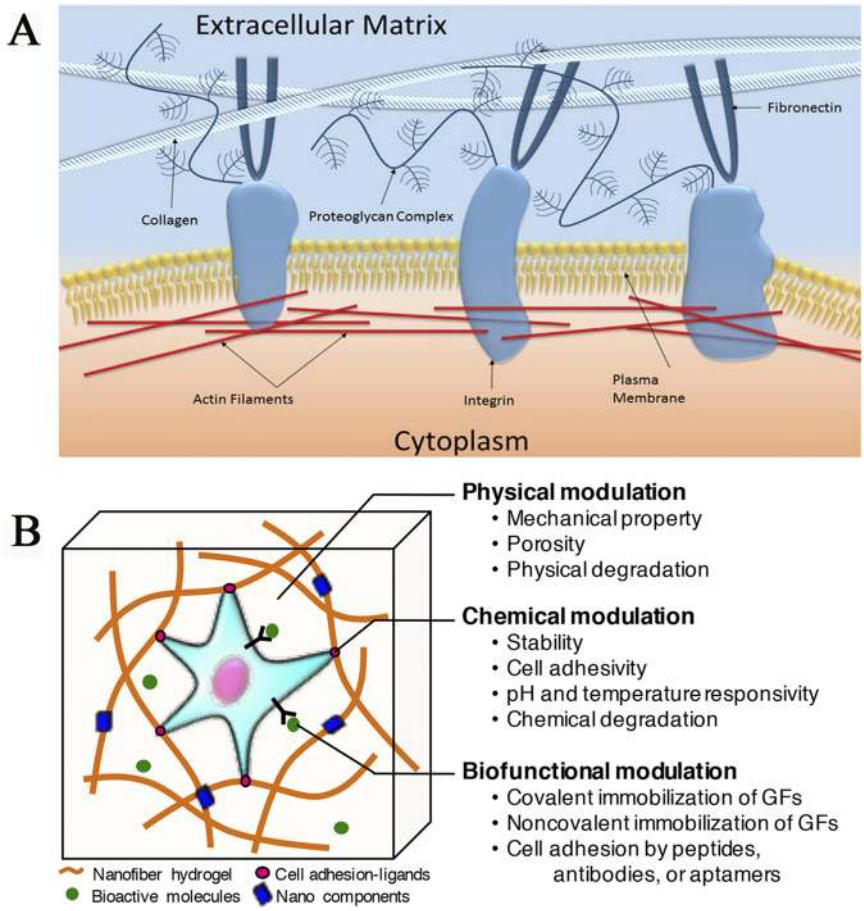

FIG. 2. (a) Schematic of the components of the extracellular matrix and the cellular receptors that interact with these components.[13] Reproduced with permission from Naahidi *et al.*, Biotechnol. Adv. **35**(5), 530–44 (2017). Copyright 2017 Elsevier. (b) Schematic diagram of physical, chemical, and biological functional regulation of 3D hydrogels in regeneration engineering.[27] Reproduced with permission from Guan *et al.*, Biotechnol. J. **12**(5), 1600394 (2017). Copyright 2017 Elsevier.

polysaccharides that are secreted by cells. It exists in specific tissues or organs and is a relatively stable substance. All the components of ECM and their arrangement outside the cell are shown in Fig. 2(a). These substances are arranged in a specific way that gives the structure its characteristics. The ECM is also involved in determining the fate of cell migration, differentiation, and apoptosis. Cells produce the macromolecular components of ECM and control their assembly. Natural ECM is an ideal biological scaffold because it contains all tissue components except living cells.[20] Hydrogels and ECM are similar in structure and composition, with an ideal hydrogel scaffold simulating the characteristics of natural ECM.[21,22] In addition, hydrogels can be used to control cell adhesion, molecular reactions, structural integrity, biodegradability, and biocompatibility and allow cells to adhere and migrate, transfer, and retain cells and biochemical factors, and ultimately exert mechanical and biological influences to change cell behavior.[22–24] Among the many materials used in tissue engineering, hydrogels have emerged as one of the most prominent and versatile.[25] Hydrogels can be designed to support cell proliferation, migration, and differentiation, allowing oxygen and nutrients to be transported in a natural, highly hydrophilic three-dimensional environment. Importantly, the properties of hydrogels are determined by the chemistry of the underlying polymer.[26]

A. Physical modulation of hydrogels

Native tissues have a wide range of physical characteristics, including rigidity and porosity.[27] As analogs of native tissues, hydrogels can provide biophysical signals to regulate cellular behavior in a controlled manner by simulating these physical features at the microscopic scale and nanoscale [Fig. 2(b)]. In tissue engineering, the mechanical properties, porosity, and degradation behavior of hydrogels provide a filling framework for regenerated tissues. In hydrogels, these properties are determined by the intrinsic and cross-linking properties of the backbone polymer (the number, type, and size of the cross-linked molecules) and environmental conditions.[15]

1. Mechanical strength

The biological properties of hydrogels are determined by the properties of polymers and crosslinking agents, gel conditions, swelling, and degradation.[28] Hydrogels can affect cell differentiation through a variety of mechanical properties, such as nuclear mechanics, actin cytoskeletal tension and integrity, integrin-mediated adhesion signal, and other mechanical pathways.[29–31] The regulation of the mechanical properties of hydrogels is of great significance in the evaluation of the applicability of hydrogels. The mechanical properties are highly dependent on the structure of the polymer,

especially the cross-linking density and degree of swelling. The mechanical properties of hydrogels can be easily regulated by changing the composition, crosslinking density, and polymerization conditions of the polymer.[28] For example, the mechanical strength of ionic cross-linked alginate brine gels increases with the addition of divalent ions with a higher affinity for alginate.[15]

2. Porosity

The porosity, permeability, and mechanical properties of scaffolds are important factors affecting cell growth, cell migration, and scaffold colonization. The porosity of the scaffold is determined by the closed and open pores of different sizes, shapes, spatial distributions, and interconnections.[32] Hydrogels have limited internal diffusion, and porosity is an important physical factor to promote nutrient and oxygen transport to maintain cell survival.[33] Pores are usually composed of interconnected pores rather than isolated uniform pore spheres, with the pore sizes of porous hydrogels varying from a few micrometers to hundreds of micrometers. A major challenge in pore analysis is how to precisely control the pore size and spatial distribution on the nanoscale.[32,34]

3. Physical degradation

When used as biomaterial scaffolds, the physical degradation of hydrogels can provide space for cell migration and vascular infiltration to make tissue regeneration successful.[35] The rate of hydrogel degradation should match the rate of new tissue formation so that the treated tissue area can maintain the integrity of the new tissue and mechanical stability.[27] The degradability of hydrogels depends on various physical factors such as the properties of the materials constituting the hydrogels and the microenvironmental conditions. For example, alginate is widely used as an ion-crosslinked hydrogel because of its high biocompatibility and easy gelation. Partial oxidation of the alginate chain makes alginate degradable under physiological conditions. Micro-oxidized alginate, which is biodegradable in aqueous media, is a biomaterial with proven potential as a vehicle for drug and cell delivery.[27,36,37]

B. Chemical modulation of hydrogels

In tissue engineering, biomaterial scaffolds used to form gels in the body must promote both the application (e.g., adhesion, proliferation, and differentiation) and the cellular functions required for tissue development without causing severe chronic inflammation.[15] Micro- and nanotechnologies have been used to regulate the chemical properties of hydrogels, such as stability, pH and temperature sensitivity, and chemical degradability based on the dynamic physicochemical properties of hydrogels. However, the combination of chemical groups and the long-term cross-linking process may affect the activity of the cells encapsulated in the hydrogel.[27] Therefore, we discuss the chemical modulation of highly biocompatible hydrogels as scaffolds for biomaterials.

1. Stability

The stability of hydrogels is an important factor in maintaining the original structure and function *in vivo*, allowing them enough time to induce successful tissue regeneration.[38] Hydrogels have good thermal stability and good surface coverage and swelling degree at high temperature.[39] In particular, the shrinkage rate is one of the important factors affecting the dimensional stability of hydrogels. Materials with a high shrinkage rate will lead to a mismatch between implant and tissue.[40]

2. pH and temperature

Some hydrogels are sensitive to external stimuli (such as temperature and pH) that facilitate tissue regeneration, effectively delivering biomolecules to the target region. For example, pH-sensitive hydrogels are polymer gels whose volume varies with the pH of the external environment.[41,42] The gel contains a large number of bases, such as acids, carboxyl groups, and amino groups, which are easy to hydrolyze or protonize, and the dissociation of these groups is affected by the external pH value.[43,44] When the external pH value changes, the dissociation of these groups will destroy the corresponding hydrogen in the gel. The destruction of this bond reduces the crosslink points of the gel network, resulting in a change in the gel structure.[45–47] These characteristics enable hydrogels to have a good biocompatibility. Thermosensitive hydrogels are the most common stimulus-responsive hydrogels in recent years. Above the low critical solution temperature (LCST), the gel is in a gel state, otherwise it exists in a solution state. Therefore, LCST hydrogels at room temperature and body temperature are ideal for *in situ* gelation after injection.[48]

3. Chemical degradation

It is very important to control the degradation rate of hydrogels. The chemical degradation rate of hydrogels can be regulated by controlling the network crosslinked density and enzymatic hydrolysis by cell-mediated proteases.[49]

III. APPLICATION OF HYDROGEL IN BONE SCIENCE

The rapidly developing field of bone tissue regeneration is in urgent need of biomaterials to provide temporary stents for new bone formation. Scaffolds are an important part of bone tissue engineering. Scaffolds used for bone tissue engineering should have good biocompatibility, biodegradability, mechanical properties, and pore structure with high interconnectedness of porosity to ensure the infiltration of cells and the full diffusion of nutrients to cells.[50–55] For example, electrospinning has also made great progress in the treatment of bone diseases.[56] However, *in situ* hydrogels, which are similar to natural ECM and can be implanted easily using the needle implantation technique, have been widely used in bone tissue engineering (Table I). Gel scaffolds have similar chemical and structural properties to natural bone, can improve the osteogenic behavior of stem cells, and can provide a good scaffold to promote the differentiation of neural stem cells into neurons to restore spinal cord regeneration.[57,58] Hydrogel materials have unique advantages in the application of bone tissue engineering, and the development of various preparation techniques and materials provides more targets for the repair of the different bone defects. It is difficult for cartilage to repair itself since it lacks blood vessels, nerves, lymphatic networks, and primitive cells, and once damaged, it usually needs to be surgically replaced.[59] The purpose of cartilage tissue engineering is to prepare functional and scar-free tissues. Hydrogels have been widely studied as potential scaffold materials suitable for tissue engineering.[60]

TABLE I. Application of hydrogel in bone science.

Category	Conditions	Hydrogel	Loaded content	Advantages	References
Bone	Long bone defect	Injection *in situ* hydrogel	nHAp	Bone defects can be repaired with injectable *in situ* hydrogel	63–65, 73, 75, and 83
		Collagen + DCN gels	DCN	The vehicle carrying the BMP-2	
		GelMA-C-OPG hydrogel	OGP	Good mechanical properties	
	Fracture healing	Dissolved staphylococcal hydrogel	Lysostaphylbcoccus	Deliver therapeutic agents and cells	
		Poly (ethylene glycol) (PEG)-based hydrogels	siRNA/NPs	Speed up bone formation and improve biomechanical strength	
		PEM hydrogel	Type I collagen hydrogel	Osteogenesis differentiation	
Cartilage	Cartilage defect	Porous hydrogel	B–S–H	Using technologies such as 3D printing, spinning, and biodegradable pore or microsphere doped hydrogels	87, 89, 93, 94, and 100
		The CCH hydrogel	Three-phase collagen, chondroitin sulfate, hyaluronic acid	It shows the characteristic hyaline cartilage, which is stiffer than collagen	
	Osteoarthritis	Chitosan hydrogel	Chitosan	Hydrogel fibers and porous hydrogels were manufactured to simulate cartilage tissue, innate immune regulation	
		Hyaluronic acid hydrogel	HA, human umbilical cord-derived MSCs	It can be used for regeneration therapy of full-thickness cartilage degradation	
Skeletal muscle	Rotator cuff injury	Chitosan thermal gel	Chitosan	It is used to prevent adhesion at the surgical site	107–109, 114, and 117
		Tendon hydrogel (tHG)	Type I collagen	The addition of tendon hydrogels alone can fully improve the biomechanical control of RTC	

TABLE I. (*Continued.*)

Category	Conditions	Hydrogel	Loaded content	Advantages	References
	Tendon injury	Solution hydrogels	Gelatin	The highly viscous hydrogel as a scaffold around the tendon	
		Thermosensitive hydrogels	Butane diisocyanate, collagen hydrogel	Repair cells in minimally invasive treatment of partial tendinopathy	
		PEG hydrogel	PEG	The layered scaffold contains bony and tendinous tissue compartments	

A. Hydrogel promotes bone regeneration

1. Treatment of long bone defects

Bone is one of the most common grafts. While most bone defects can heal themselves or be repaired with autologous bone grafts, long bone defects caused by severe trauma or malignancy remain a major challenge. Large bone defects are a common and debilitating clinical symptom.[61,62] Obtaining large amounts of bone from other parts of the body to treat large bone defects inevitably leads to serious complications at the donor site.[8] Hydrogels have superior sensitivity, injectability, and minimally invasive properties. An article on injectable *in situ* hydrogel repair of bone defects reported that unlike prefabricated stents that require surgical implantation, hydrogels can be injected into the defective site through minimally invasive surgery to combat any geometric deformation. Hydrogels are thus ideal for repairing bone defects in non-load-bearing sites that do not require high mechanical strength.[63–65] Injectable hydrogels can be classified into physical or chemical hydrogels based on the crosslinking mechanism of hydrogel gelation.[65–67] In addition, injectable hydrogels can be used as carriers to deliver biomolecular therapeutic agents and cells to the defective site to enhance the repair of damaged bone tissue. Studies have shown that polymer additions, bioceramic inclusion bodies, drugs, and cellular binding to injectable hydrogels improve their physicochemical and biological properties thus enhancing osteogenesis.[65] Thermosensitive hydrogels or thermal gels undergo a reversible sol–gel transition with the temperature change.[68] At room temperature (25 °C), thermosensitive hydrogels are usually in the sol state, and as the temperature increases to body temperature (37 °C), the hydrogels change to the gel state.[69] It is reported that injectable *in situ* hydrogels have been widely used in orthopedic clinics.[65,70,71] Based on the visible light curing, glycol injectable hydrogel of chitosan (CS), after binding to bone morphogenetic proteins (BMP-2) and (or) transforming growth factor (TGF-β), can be sustained for the release of growth factors within 30 days. Studies have shown *in vivo* and *in vitro* that a gel can promote collagen type alkaline phosphatase, collagen type I, and osteocalcin (OCN) mRNA expression; can increase the tibia defect, bone mass, and bone mineral density; and can prompt hydrogel application values in the treatment of bone defect.[72,73]

Hydrogels have also been found to be osteoconductive, promoting the differentiation of mouse mesenchymal stem cells (mMSCs) into osteoblasts by upregulating the expression of Runx2. Dhivya *et al.* evaluated the bone healing properties of hydrogels in rats with tibial defect and the role of nanohydroxyapatite (nHAp) in the hydrogels. The study showed that the addition of nHAp to heat-sensitive chitosan (CS)-based hydrogels improved their physical and biological properties. The addition of nHAp increased protein adsorption, inhibited swelling, and reduced lysozyme degradation, which to some extent proved that hydrogels could promote long bone healing.[74] A recent novel osteogenic polypeptide hydrogel (GelMA-C-OGP) was created from a photo-cross-linked common cross-linking template gelatin (GelMA) and a photo-cross-linked osteogenic growth peptide (OGP) using ultraviolet radiation. Gels make it possible to form hydrogels. Photo-cross-linked OGP has good mechanical properties to promote bone regeneration. GelMA-C-OPG hydrogels can significantly enhance the expression of osteoblast-related genes BMP-2, OCN, and Osteopontin (OPN), increase the calcium salt precipitation in osteoblasts, and thus accelerate the bone formation process of pre-osteoblasts. GelMA-C-OGP hydrogels also promote bone regeneration *in vivo* (Fig. 3).[75]

2. Treatment of delayed fracture healing

The healing of fractures is a multistage repair process.[76] Some factors contribute to the failure of normal fracture healing, including fracture location, degree of soft tissue damage, degree of osteoporosis, and infection, as well as instability due to improper treatment, patient age, and use of medication.[77,78] Infection is a serious complication of orthopedic surgery during fracture repair.[79] Estimates of periprosthetic joint infection (PJI) rates after primary knee or hip replacement range from 0.3% to 1.9% and may exceed 10% in patients with specific risk factors.[79–81] After internal fixation with intramedullary nail, steel plate, and screw, the infection rate of closed fracture is 1%–2% and that of open fracture is more than 30%.[79] Johnson *et al.* designed injectable hydrogels to treat *Staphylococcus aureus* orthopedic implant infections to support fracture repair. They studied the activity, stability, release, and antibacterial properties of hydrogel encapsulated staphylococcin. The *in vivo* conductivity of hydrogels was studied in a mouse model of the infection of

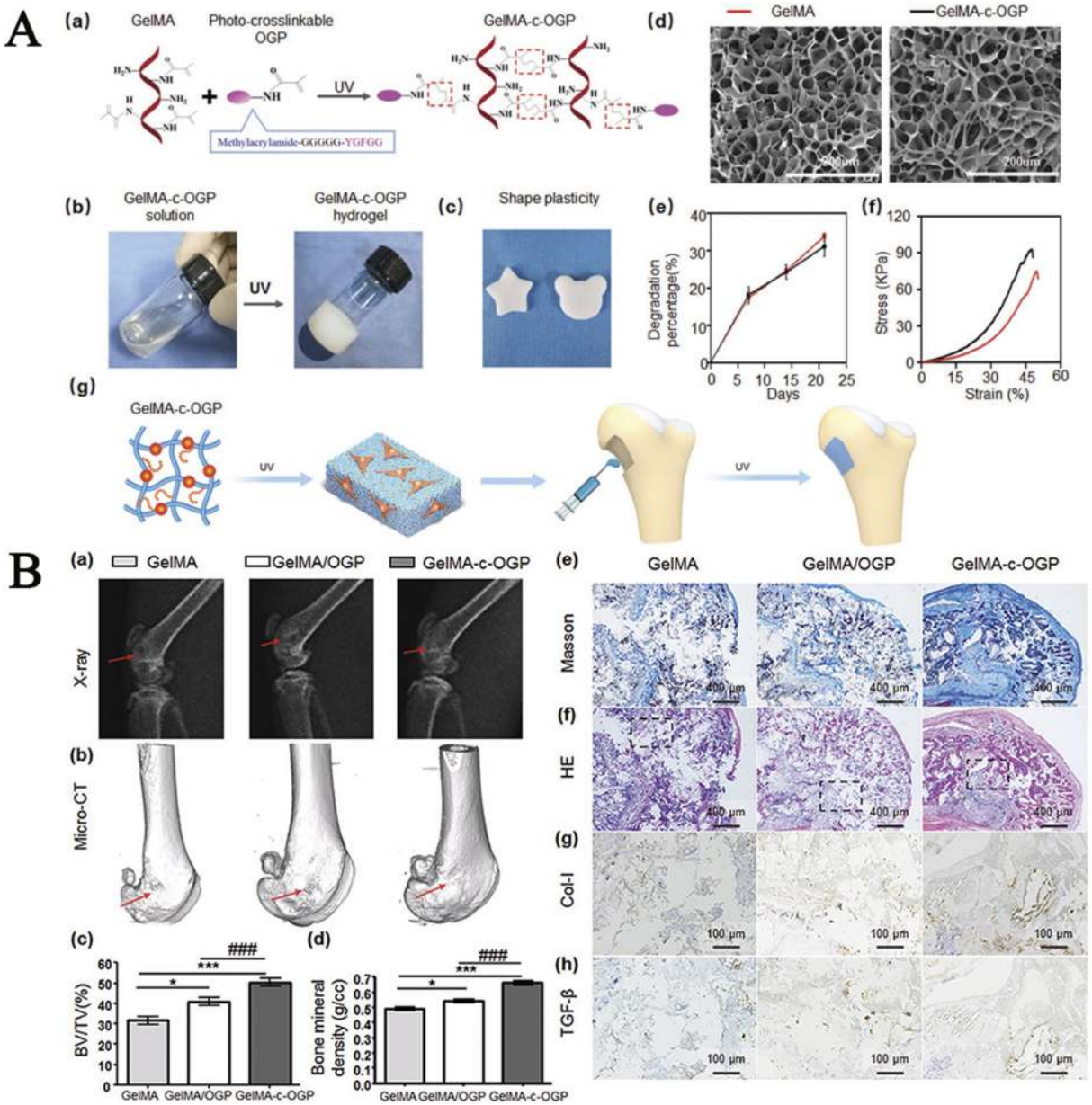

FIG. 3. A) Flow chart of the gel-C-OGP hydrogel structure and its mechanical properties. (a) Esterification of OGP polypeptide and gelatin (GelMA) and the chemical molecular structure of GelMA-C-OGP under UV light. (b) Photographs of hydrogel formation after UV exposure. (c) Fixed form of gel after crosslinking. (d) SEM images of GelMA and GelMA-C-OGP hydrogels. [(e) and (f)] Degradation rate and stress of GelMA and GelMA-C-OGP hydrogels. (g) Schematic diagram of GelMA-C-OGP for bone regeneration. (B) Osteogenic properties of the gel-C-OGP hydrogel *in vivo*. (a) X rays of the distal femur defect after 8 weeks of treatment. (b) 3D reconstruction of the defective area of the distal femur after μ-CT scan. (c) Bone mineral density (BMD) at the site of distal femoral defect. (d) BV/TV at the site of distal femoral defect was detected by CT. [(e)–(h)] Histological examination of the bone defect area after different treatment methods.[75] Reproduced with permission from Qiao *et al.*, Adv. Healthcare Mater. **9**(1), e1901239 (2020). Copyright 2020 Wiley.

femoral fractures to evaluate the therapeutic potential of lysostaphy-lococcin hydrogel therapy.[82] It was shown that hydrogels can overcome the delay in fracture healing caused by infection. Hydrogels can be used as vehicles to deliver growth factors directly added to the gelatin to promote the healing of the fracture. The degradation of water gel ratio and the release of growth factors can be controlled by the change of water content; it can be to a certain control of the release of growth factors, even in the case of quick release. Growth factors added to the hydrogel have also been shown to promote bone formation.[83] Qiu *et al.* made a periosteal extracellular matrix (PEM)

of injectable hydrogels, which dynamically integrates a variety of biological functions and can be in the different stages of the fracture healing process to work; the PEM hydrogels with type I collagen have been fully analyzed, and the *in vitro* PEM water gel effect on different stages of healing has been studied. PEM hydrogels can induce macrophage recruitment and M2 polarization, promote the differentiation of mesenchymal stem cells into endothelium-like cells, promote the osteogenic differentiation of primary cranial osteoblasts and mesenchymal stem cells, and induce mineralization in simulated body fluids. Hydrogel evaluation was performed *in vivo* using the dynamic and multiphase effects of a critical large-size skull defect model in rats (Fig. 4).[84]

B. Hydrogel promotes cartilage regeneration

1. Treatment of cartilage defect

Osteochondral tissue consists mainly of articular cartilage and subchondral bone.[55,85] The ideal treatment strategy for osteochondral defects is to regenerate both articular cartilage and subchondral bone.[55] At present, cartilage tissue engineering is considered one of the most promising treatment methods for cartilage defects.[86] A large number of hydrogels have been developed to repair articular cartilage defects due to their advantages of biodegradability, biocompatibility, and control of cell–matrix interactions.[87] In recent years, great progress has been made in cartilage tissue engineering,

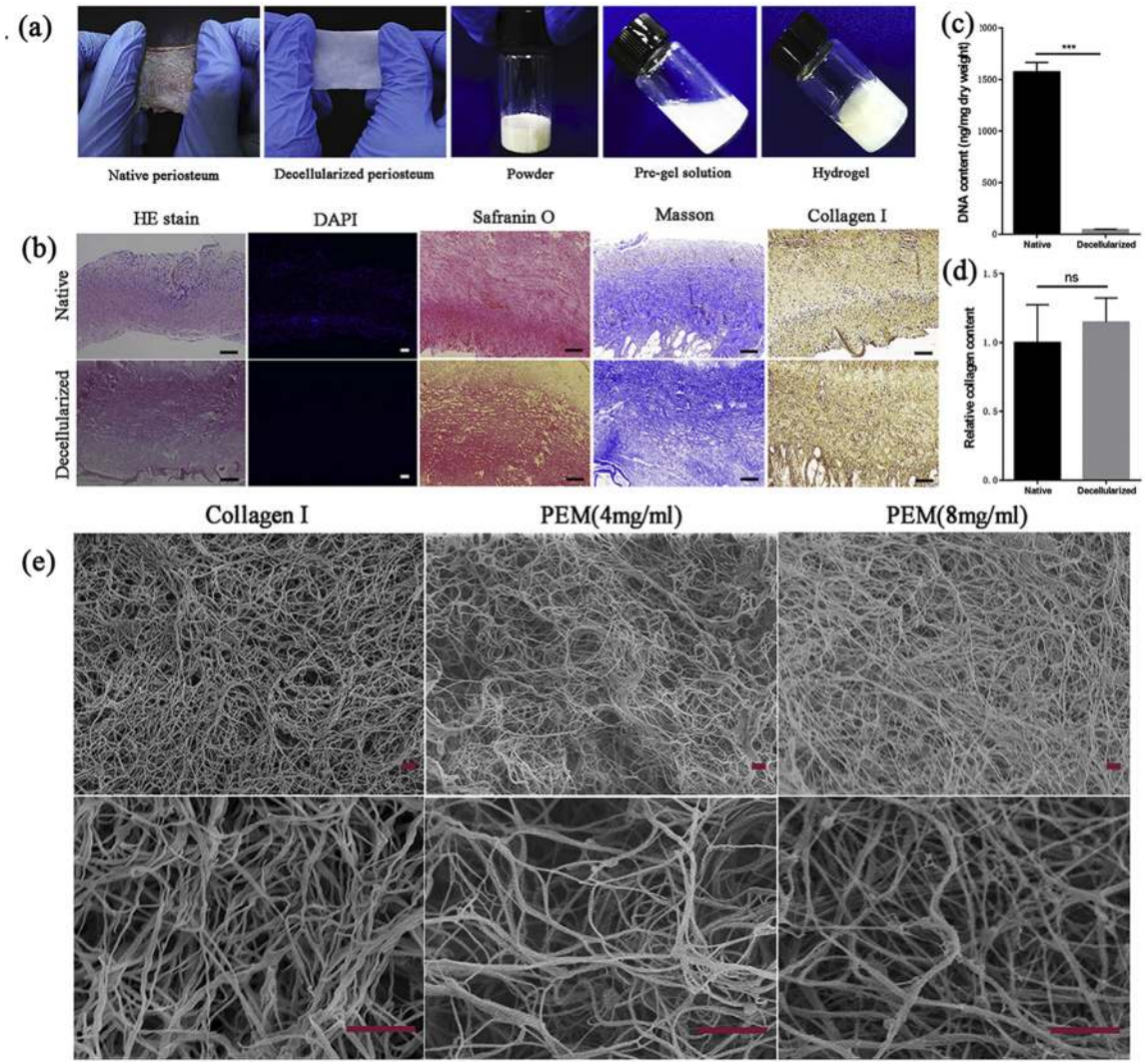

FIG. 4. Preparation and characterization of acellular periosteum hydrogels. (a) Acellular periosteum and hydrogel preparation images. (b) H & E and DAPI staining, Safranin O staining, and Mason's trichromatic staining. (c) Quantitative detection of DNA removal. (d) Collagen retention. (e) SEM analysis of the structure of PEM and type I collagen hydrogel nanofibers under different amplification conditions. Bone regeneration in a skull defect model.[84] Reproduced with permission from Qiu *et al.*, Biomaterials **227**, 119552 (2020). Copyright 2020 Elsevier.

such as the use of 3D printing, spinning and hydrogels doped with porous or biodegradable microspheres to help maintain cell survival and cartilage structure induction. These technologies used in the manufacture of hydrogel induction and consolidation of the surrounding tissue can promote cartilage formation in the body at the same time. New processing techniques are also used to make hydrogel fibers and porous hydrogels to improve cartilage formation and to create layers of hydrogels that mimic the banded structure of natural cartilage. In scaffolds with closed pores, the distribution of cells inside the scaffolds may be restricted, resulting in the generation of non-uniform ECM and poor mechanical properties. Therefore, the porosity of scaffolds also plays an important role in cartilage tissue engineering.[88]

As cell-free implants, hydrogels exhibit mechanical, swelling, and lubricating behavior similar to that of articular cartilage in structure and mechanics. In addition, they allow for payload transfer and promote chondrogenesis by encapsulating cells.[87,89,90] Hydrogels used to replace articular cartilage are available in two forms, one as a cellular carrier material to promote tissue cell regeneration and the other as a permanent implant to replace damaged cartilage.[87] Polyethylene glycol diacrylate (PEGDA)-crosslinked hydrogels, consisting of thiolated hyaluronic acid and thiolated gelatin, have a variety of applications in tendon, cartilage, and other soft tissue repair and wound healing. Polyethylene glycol (PEG), a hydrophilic polymer, has been widely used in biological applications. For example, PEG-modified drugs can improve the sustainability and biocompatibility of drugs. PEG material surfaces have been used to control cell adhesion, in particular, photocrosslinked PEG hydrogels (such as PEG bisacrylate [PEGDA]) to support *in vitro* cell growth and *in vivo* tissue regeneration scaffolds. Zhu *et al.* combined 3D-printed acellular chondrocytes, Extracellular Matrix (ECM), and polyethylene glycol diacrylate (PEGDA) integrated scaffold (PEGDA/ECM) with natural compound Honokiol (Hon) to determine the effect of the combination on the regeneration of osteochondral defects (Fig. 5).[91] Yuan *et al.* prepared a double network of bovine serum albumin/sodium alginate cartilage containing hydroxyapatite nanowire composite material (B–S–H) hydrogel scaffold.[92] Application of hydrogel fibers and porous hydrogels to simulate cartilage tissue for repair of cartilage defects.[93]

2. Treatment of osteoarthritis

Osteoarthritis (OA) is a debilitating degenerative disease that affects synovial joints and causes cartilage to deteriorate.[94] OA is characterized by damage or destruction of articular cartilage and subchondral bone, especially in the hands, hips, and knees.[95] The

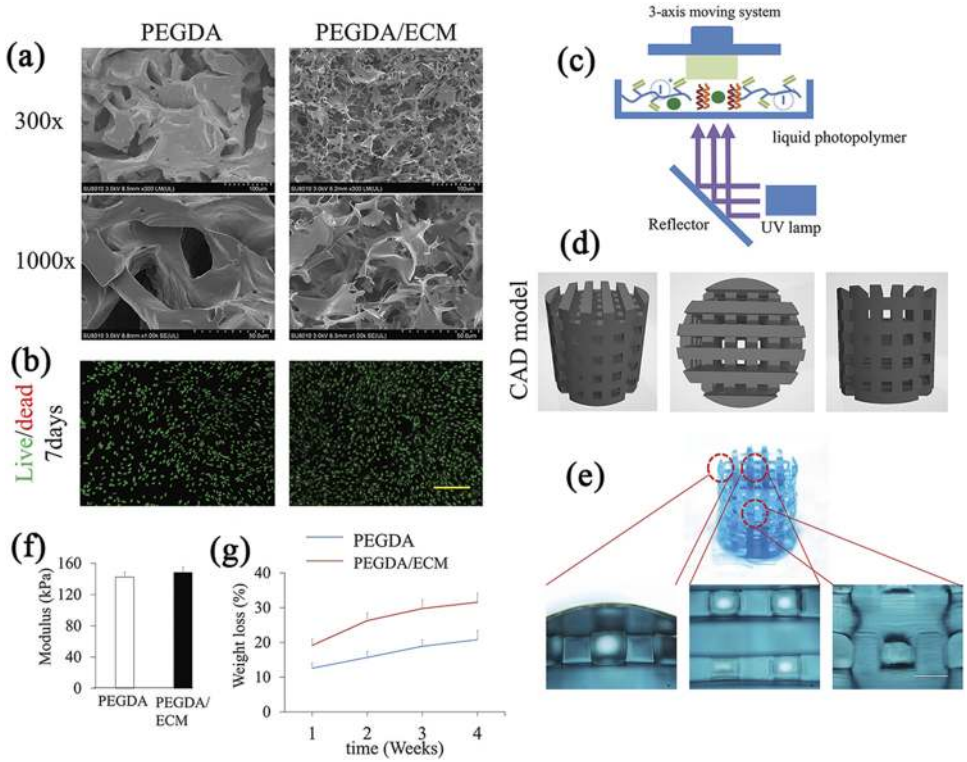

FIG. 5. Characterization of polyethylene glycol diacrylate (PEGDA)/Extracellular Matrix (ECM) hydrogels and fabrication of PEGDA/ECM bio-inks with dynamic projection stereoscopic lithography. (a) Scanning electron microscopy of PEGDA and PEGDA/ECM hydrogels. (b) Live/dead cell analysis of PEGDA and PEGDA/ECM hydrogel cultured cells. (c) Schematic of PEGDA/ECM bio-ink prepared using dynamic projection stereoscopic lithography. (d) Computer-aided design (CAD) pre-designed scaffold models. (e) Naked eye image of PEGDA/ECM scaffold. (f) Compression modulus of PEGDA and PEGDA/ECM brackets. (g) Degradation tests of PEGDA and PEGDA/ECM scaffolds *in vitro*.[91] Reproduced with permission from Zhu *et al.*, Am. J. Sports Med. **48**(11), 2808–18 (2020). Copyright 2020 SAGE Publications.

application of hydrogels in the treatment of OA can regulate the polarization and activity of immune cells, go beyond the transmission of anti-inflammatory components, and have the inherent function of immune regulation.[94] For example, natural scaffolds such as chitosan hydrogel do not produce toxic substances in the treatment of cartilage damage due to their good biodegradability and biocompatibility.[96–98] The structure of chitosan hydrogel is similar to that of sulfated gel, which can provide an appropriate

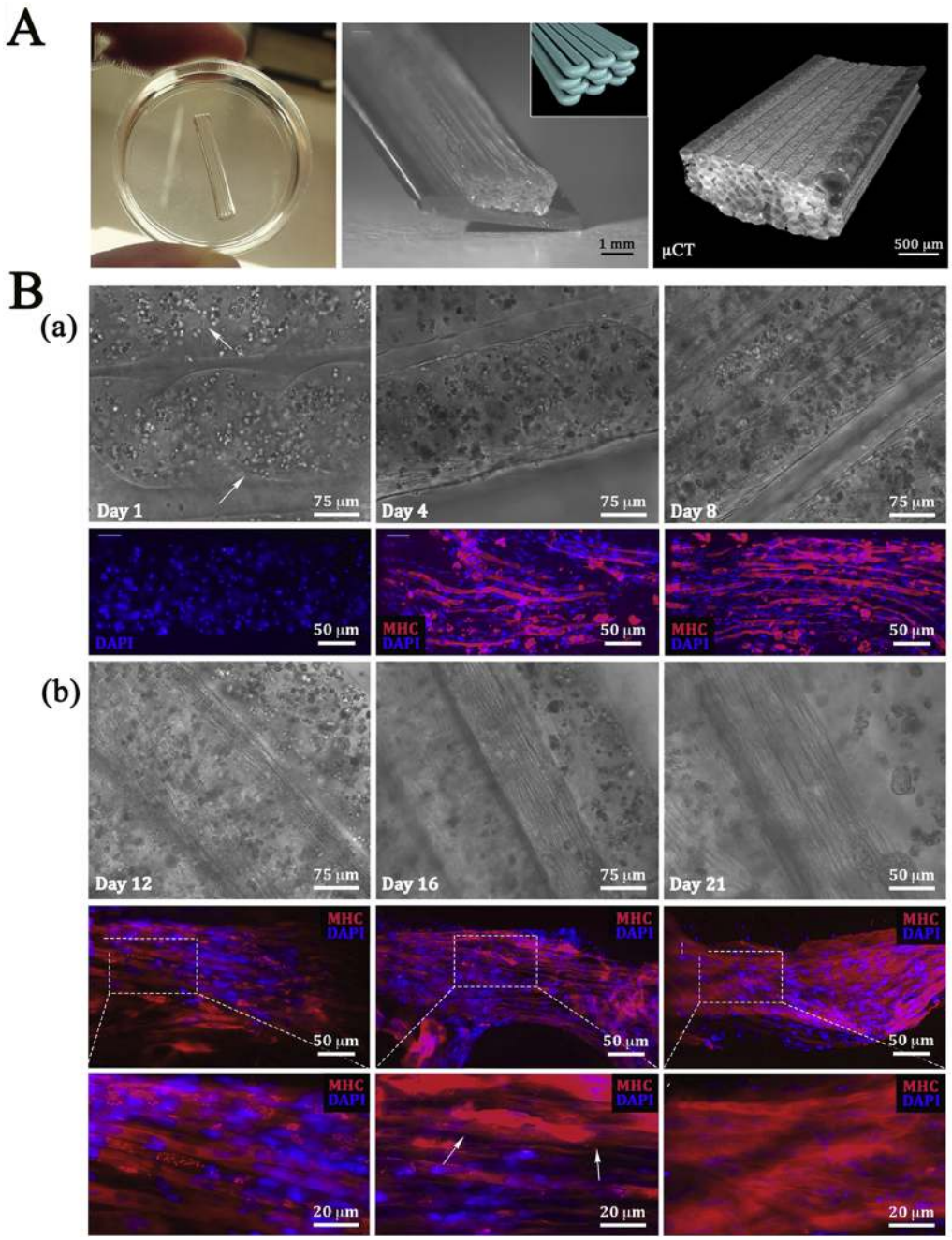

FIG. 6. (A) 3D bioprinted structure composed of unidirectional aligned PF hydrogel fibers. (B) Bright field and MHC immunofluorescence photomicrographs of C2C12 myogenic cells at different time points in 3D bio-printing. (a) MHC-negative C2C12 cells were round on day 1. Myogenic differentiation was observed on day 4 besides MHC expression. Day 8 showed an increase in the formation of myotubules expressing MHC. (b) On day 12, the muscular tubes are neatly arranged and compact; well-organized muscular structures were shown on day 16; and day 21 showed progressive myofibrogenesis and prominent sarcomere tissue. The core is counterstained with DAPI.[118] Reproduced with permission from Costantini *et al.*, Biomaterials **131**, 98–110 (2017). Copyright 2020 Elsevier.

microenvironment for chondrocyte proliferation and ECM production, maintain the correct phenotype, and promote chondrogenesis.[97–99] The therapeutic potential of transplantation of the HA hydrogel and human umbilical cord mesenchymal stem cells (MSCs) into the porcine OA model was studied. Studies have shown that using a mixture of HUCMSCs and HA to regenerate cartilage in large animal models may be an effective treatment for OA.[100] Dimitrios *et al.* also evaluated the potential of a hybrid hydrogel crosslinked from biodegradable and thermosensitive triblock copolymers. They injected the drug into a mouse model of OA, and the hydrogel was shown to restore bone mineralization, proteoglycan production, and Sox-9 and Runx-2 levels to some extent.[95]

C. Hydrogel for skeletal muscle injury

1. Treatment of rotator cuff injury

Skeletal muscle has a strong ability to repair itself and can fully recover when slightly damaged, but when major muscle loss occurs, the repair process of skeletal muscle is hindered.[101] Skeletal muscle regeneration includes three stages: inflammation and degeneration, regeneration and repair, and remodeling.[102,103] Therefore, any repair material should have high biocompatibility, biodegradability, high affinity to the biological surface, and proper elasticity to allow contraction of skeletal muscle.[104,105] The use of cell carriers is particularly necessary to facilitate the colonization of implanted cells in the defective areas of skeletal muscle and to prevent cell loss. Hydrogels have been widely used in skeletal muscle tissue engineering as biomaterials.[101,106,107] One of the most common postoperative complications in the repair of rotator cuff injury is adhesion, which leads to joint stiffness. Even when the rotator cuff heals well after repair, postoperative patients and doctors are usually not satisfied with the degree of stiffness.[108] Therefore, to reduce postoperative stiffness, a variety of materials have been developed, including polylactic acid membrane, medical chitosan, medical sodium hyaluronate gel, collagen, and gelatin, to prevent adhesion at the surgical site.[109] A solution gel is the most widely used rotator cuff repair material.[110,111]

2. Treatment of tendon injury

When tendons are injured, the tendons transfer the force generated by the muscle to the bone and act as a barrier to limit the muscle injury by absorbing external forces. Tendons have high tensile strength, good flexibility, and the best level of elasticity.[112] Tendon injuries are caused by internal or external trauma alone or in combination and can be classified as acute or chronic. The limited ability of tendons to self-repair and the lack of overall efficiency in the current treatment schemes have given the necessary motivation for engineering tissue for tendon repair.[113] As a prefabricated scaffold, the hydrogel can be used to guide the formation of muscle tissue *in vitro* or coordinate the *in situ* regeneration of muscle tissue *in vivo*.[114] Postoperative adhesion is associated with the proliferation of fibroblasts, where excessive proliferation of fibroblasts may lead to adhesion, and the fibroblasts are transformed into scar tissue.[115,116] However, fibroblast proliferation is an important process in tendon repair. Therefore, fibroblast proliferation and hemostasis are important factors in the reduction of postoperative adhesion. The tendon healing process progresses slowly after the application of anticoagulants. Chitosan and gelatin can act as anti-adhesion agents that promote the proliferation of epithelial and endothelial cells but inhibit the proliferation of fibroblasts. High viscosity of hydrogels may also delay tendon healing due to the inhibition of fibroblast proliferation during the first few weeks when the anti-adhesion agent is retained *in vivo*. When the highly viscous hydrogel acts as a scaffold around the tendon, it prevents the growth of fibers in the surrounding tissue. It has been reported that the application of chitosan thermal gels successfully reduces the thickness of fibrosis between the supraspinatus and the deltoid. Since excessive fibrosis can lead to adhesions, these reports suggest that the chitosan thermal gel can also act as an anti-adhesion agent.[108] The tendons are inserted into the bone through the fibrous cartilaginous interfaces, thereby reducing mechanical strain and tissue failure. Despite this toughening mechanism, tendon tears due to acute injury (overload) or degeneration (aging) can still occur. Fixing a torn tendon to the bone causes a layer of scar tissue with less biomechanical properties. The process of tendon fibrocartilage regeneration requires the use of selected biomaterials to protect cells from interfacial strain.[117] To address this problem, professor Ali Khademhosseini's team from the University of California has proposed a new strategy based on innovative 3D bio-printing methods to create functional skeletal muscle tissues. Hydrogel fibers containing muscle precursor cells (C2C12) were used for high-resolution 3D bio-printing. To promote myogenic differentiation, a custom bioink was developed using a photocurable semi-synthetic biopolymer (PEG-fibrinogen) to encapsulate cells in a three-dimensional structure composed of oriented hydrogel fibers [Fig. 6(a)]. *In vitro*, myoblasts encapsulated at the initial stage of culture began to migrate and fuse, forming multinucleated myotubes within the 3D bio-printed fibers [Fig. 6(b)]. The obtained muscle tubes show a high arrangement along the direction of hydrogel fiber deposition. After subcutaneous implantation in the back of immunodeficient mice, the bio-print was built into the body to create tissue artificial muscle tissue. The results showed enhanced myogenic differentiation with the parallel formation of long-range myotubules. This research method has far-reaching prospects for expanding skeletal muscle tissue engineering for human clinical applications.[118]

IV. CONCLUSION AND PROSPECTS

Hydrogel is one of the most suitable biopolymer materials for tissue engineering because of its wide application prospects and unique properties. A wide range of natural and synthetic hydrogels have been shown to promote healing in bone, cartilage, and skeletal muscle. In particular, hydrogels with heterogeneous structures and multiple cellular components are expected to promote the regeneration of whole organs in the near future.[119,120] Our study has some limitations. First, the application of hydrogels has its limitations, such as hydrophilic expansibility and the lack of consensus in determining optimal pore size for precisely controlling the cell movement at the nanoscale. Second, the fabrication of hydrogel scaffolds to match the high strength mechanical properties of natural musculoskeletal tissues remains a major challenge in the field of weight-bearing. Specific organizations also need to customize the features of the scaffolding. Specifically, in bone, cartilage, and disk tissue engineering, producing structures with controllable pore size and degradation rate, and good mechanical properties is critical. In this Review, the preparation of various types of hydrogel scaffolds

was described, and it was shown that such biomaterials could play an effective role in the repair of various orthopedic diseases and injuries. For example, a new biomimetic design was developed in which the hydrogel interface was added between different collagen scaffolds. This effectively disperses the local strain between different biomaterial environments and reduces the occurrence of interface failure during tendon repair. The change of the properties of hydrogels, especially the time required for the transition of the hydrogel network from viscosity to elasticity and the change of the final elastic properties, provides a powerful condition for improving the mechanical properties of three-phase biomaterials. This robust composite biomaterial model can provide different insights into the biological enhancement of layered composite materials and the design of a series of rigid tissue scaffolds for orthopedic insertion injuries. These materials offer real advantages to the organizational engineering community and are likely to attract increasing attention in the coming years. It is hoped that in the future, researchers will explore the functions of various hydrogel derivatives in biomaterial formulations, which will be a great opportunity to develop new materials with new properties. Different types of hydrogel materials can be specially modified and functionalized to achieve better performance. In addition, many newly developed materials perform *in vitro* tests well but face challenges to overcome *in vivo*. From *in vitro* experiments to *in vivo*, we need a more systematic approach to develop strategies for advanced biomaterials. With continued innovation, tissue engineering will become increasingly influential, driving the design and clinical transformation of new hydrogels and delivering tangible benefits to patients in the years ahead.

AUTHORS' CONTRIBUTIONS

Y.Z. and Z.L. contributed equally to this work.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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