Acta Biomaterialia 101 (2020) 26–42

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actbio

Review article

# Four-dimensional bioprinting: Current developments and applications in bone tissue engineering

Zhuqing Wan, Ping Zhang, Yunsong Liu, Longwei Lv*, Yongsheng Zhou*

*Department of Prosthodontics, Peking University School and Hospital of Stomatology, National Engineering Laboratory for Digital and Material Technology of Stomatology, National Clinical Research Center for Oral Diseases, Beijing Key Laboratory of Digital Stomatology, 22 Zhongguancun Avenue South, Haidian District, Beijing 100081, PR China*

ARTICLE INFO

*Article history:*
Received 21 June 2019
Revised 20 October 2019
Accepted 25 October 2019
Available online 28 October 2019

*Keywords:*
3D bioprinting
4D bioprinting
Tissue engineering
Shape memory polymers
Shape memory hydrogels
Bone regeneration

ABSTRACT

Four-dimensional (4D) bioprinting, in which the concept of time is integrated with three-dimensional (3D) bioprinting as the fourth dimension, has currently emerged as the next-generation solution of tissue engineering as it presents the possibility of constructing complex, functional structures. 4D bioprinting can be used to fabricate dynamic 3D-patterned biological architectures that will change their shapes under various stimuli by employing stimuli-responsive materials. The functional transformation and maturation of printed cell-laden constructs over time are also regarded as 4D bioprinting, providing unprecedented potential for bone tissue engineering. The shape memory properties of printed structures cater to the need for personalized bone defect repair and the functional maturation procedures promote the osteogenic differentiation of stem cells. In this review, we introduce the application of different stimuli-responsive biomaterials in tissue engineering and a series of 4D bioprinting strategies based on functional transformation of printed structures. Furthermore, we discuss the application of 4D bioprinting in bone tissue engineering, as well as the current challenges and future perspectives.

**Statements of significance**

In this review, we have demonstrated the 4D bioprinting technologies, which integrate the concept of time within the traditional 3D bioprinting technology as the fourth dimension and facilitate the fabrications of complex, functional biological architectures. These 4D bioprinting structures could go through shape or functional transformation over time via using different stimuli-responsive biomaterials and a series of 4D bioprinting strategies. Moreover, by summarizing potential applications of 4D bioprinting in the field of bone tissue engineering, these emerging technologies could fulfill unaddressed medical requirements. The further discussions about future challenges and perspectives will give us more inspirations about widespread applications of this emerging technology for tissue engineering in biomedical field.



## Contents



* Corresponding author.
*E-mail addresses:* lvlw@bjmu.edu.cn (L. Lv), kqzhouysh@hsc.pku.edu.cn (Y. Zhou).

https://doi.org/10.1016/j.actbio.2019.10.038

## 1. Introduction

Three-dimensional (3D) printing, which was first proposed by Hull and co-workers in 1986 [1], has garnered considerable attention in the tissue engineering and biomedical fields [2–4]. Different 3D bioprinting technologies have been used to fabricate different kinds of biological structures such as blood vessels, liver tissue, bone, and heart tissue [5–7]. However, 3D bioprinting has a significant limitation that 3D bioprinting only considers the initial condition of a printed objectand assumes it to be inanimate and static. Natural tissue regeneration involves sophisticated 3D structures, microarchitectures, and extracellular matrix compositions, as well as generating tissue that possesses unique functions achieved through dynamic changes in tissue conformation. Most of these dynamic functional conformational changes are caused by built-in mechanisms that respond to intrinsic or/and external stimuli, which cannot be mimicked through 3D bioprinting [8].

In 2014, Skylar Tibbits, the director of the Self-Assembly Lab at the Massachusetts Institute of Technology (MIT), first demonstrated four-dimensional (4D) printing as a technology entailed multi-material prints with the capability to transform over time, or a customized material system that can change from one shape to another [9]. This technology has been quickly applied to the field of tissue engineering, the concept of time can be integrated within 3D bioprinting technology as the fourth dimension, leading to the development of 4D bioprinting [10]. By using stimuli-responsive materials, 4D bioprinting can be used to fabricate various 3D designed biologically active architectures capable of dynamic configuration transformations in response to different desired stimuli over time, addressing the limitations of 3D bioprinting [8]. Furthermore, Gao et al. defined the 4D bioprinting not only as the generation of cell-laden 3D-printed structures able to respond to internal cell forces or external stimuli, but also as the maturation and functionalization of cells or tissues in 3D-printed constructs with time (i.e., the configuration of the printed structures does not change) [10]. The shape and functionalities changes of printed constructs over time are the two main strategies for 4D bioprinting. These characteristics are essential for maintaining long-term homeostasis and self-renewal of biosynthetic constructs. However, the controlled degradation of materials in 3D-printed constructs that completely disappear in the dynamic process, should not be considered as 4D printing. Most 3D-printed constructs remain integrated during shape or functional transformation [11]. Thus, the configuration or function of 4D-printed constructs should be stable before and after stimulation during the 4D printing process [12].

Bone fractures and osteo-degenerative diseases lead to bone defects, necessitating bone regeneration to replace the damaged tissues [13]. Significant progress has been made in 3D bioprinting technology for bone tissue engineering over the last two decades with numerous researches demonstrating that how to combine biomaterials, cells and bioactive factors to engineer bone tissue constructs [14–18]. These technologies have promoted bone regeneration with controlled patterns and biomimetic architectures. However, there are still a series of challenges for further clinical applications of 3D bioprinting in bone tissue engineering, such as the reconstruction of large and irregular bone tissues for personalized needs, vascularization and neural regeneration in large bone defect repair, as well as the mechanical properties of 3D-printed structures [15,19,20].

The 4D-printed constructs are able to change over time under different stimulus and adapt to the native microenvironments of defect areas, providing new strategies for bone tissue engineering [21]. A series of progressive 4D strategies have been proposed to address current challenges in bone tissue engineering. For example, various stimuli-responsive shape-recovery polymers have been widely studied as suitable scaffolds and injectable hydrogels for bone tissue engineering [22–24]. The shape-transformation feature of 4D-printed bone tissue constructs could cater to the need of personalized bone regeneration, especially the irregular bone defects. In addition, the mechanical properties of 4D printed constructs could be modulated through the programmed crosslinking or reassembly of stimuli-responsive materials [25]. Meanwhile, the 4D-printed self-folding micro-tubes could be designed to engineer vascularized bone constructs. The stimuli-responsive biomaterials could make it possible to realize spatiotemporal distributions and release of bioactive cues and cells for complex heterogeneous tissue regeneration, containing both bone, vascular and nerve tissues [26]. Furthermore, the over-time functional maturation of 4D-printed bone structures could contribute to the establishment of biomimetic microenvironments, which influences the cell behaviors during the post-printing stage and enhanced the differentiation of stem cells [27–29]. These developments of 4D bioprinting in bone tissue engineering could modify the traditional 3D-printed bone constructs with enhanced shape or/and functional adaptabilities, providing additional potentials for the fabrication of elaborate printed bone constructs to fit the defect areas dynamically in the future clinical applications [30].

However, 4D bioprinting technology is still in its infancy, and its concepts and mechanisms are not yet widely understood by researchers. In this review, publications based on three databases (Pubmed, MEDLINE, Web of Science) have been overviewed by two researchers respectively. Based on these published articles, we introduce a series of stimuli-responsive materials and their cell-inherent features, as well as their shape-transformation mechanisms for 4D bioprinting, as reported in current literatures. Meanwhile, several strategies to realize the functionalization and maturation of cells or tissues in 3D-printed constructs over time have also been reviewed. Furthermore, this review focuses on the development of bone tissue 4D bioprinting and introduces its advanced applications in bone tissue regeneration, combining the current studies with potential clinical insights. Finally, we discuss the major obstacles to the development of 4D bioprinting and consider the future directions and perspectives for this revolutionary, valuable, and fascinating technology.

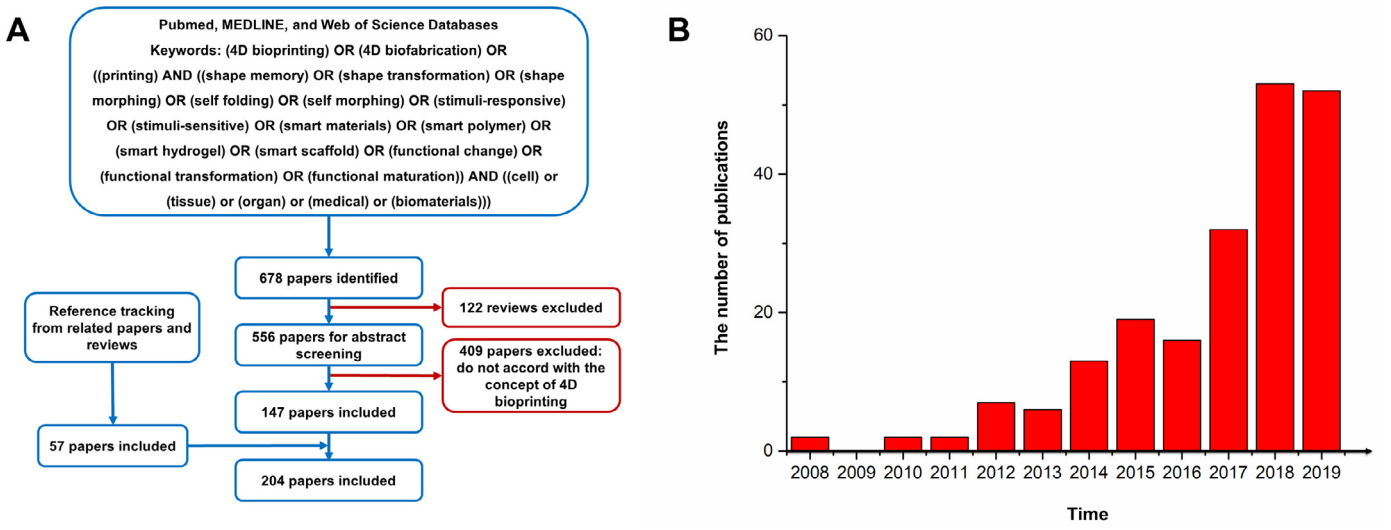


**Fig. 1.** The overview of current publications for 4D bioprinting. (A) The searching strategy for overviewing the current publications of 4D bioprinting in Pubmed, MEDLINE, and Web of Science databases (until 30 September 2019). (B) Statistics on the numbers of publications in recent years.

## 2. The overview of published articles on 4D bioprinting

Publications were reviewed by two researchers respectively based on the three databases (Pubmed, MEDLINE and Web of Science) until 30 September 2019. The following keywords were used: (4D bioprinting) OR (4D biofabrication) OR ((printing) AND ((shape memory) OR (shape transformation) OR (shape morphing) OR (self folding) OR (self morphing) OR (stimuli-responsive) OR (stimuli-sensitive) OR (smart materials) OR (smart polymer) OR (smart hydrogel) OR (smart scaffold) OR (functional change) OR (functional transformation) OR (functional maturation)) AND ((cell) or (tissue) or (organ) or (medical) or (biomaterials))). Titles and abstracts of 678 articles were reviewed and selected by two researchers independently. In addition, reference tracking of the included articles was completed to find missing articles. The final number of included articles was 204. An overview of published articles on 4D bioprinting was performed based on above included articles (Fig. 1). In this review, we introduce the 4D bioprinting technology from two fundamental mechanisms, shape-transformation and functional transformation mechanisms, based on these included research articles.

## 3. 4D bioprinting based on shape-transformation mechanism

Shape-transformation is the most common way to introduce the fourth dimension into 3D bioprinting. There are several ways to realize shape-transformation of the printed structure, which can be performed manually or by using cell traction force (CTF). It also can be performed utilizing the shape changing properties of certain biomaterials. This section reviews a range of shape-transformation mechanisms and considers their potential integration with 3D bioprinting technology to realize 4D bioprinting.

### *3.1. CTF for 4D bioprinting*

Cells are able to exert traction force originating from actin polymerization and actomyosin interactions when adhered to a substrate [31]. CTF plays an essential role in many biological processes [32], regulating cellular and tissue behaviors such as wound healing [33], angiogenesis [34], metastasis [35], inflammation [36]. Based on the mechanisms of CTF, cell origami technology, in which cells form 3D constructs by folding two-dimensional elements into pre-defined shapes, was developed [37]. This technology was utilized by Kuribayashi-Shigetomi et al. to fabricate diverse high-throughput self-folding cell-laden 3D microstructures [38]. They fabricated a parylene microplate model connected by flexible joints, and the cells were cultured across the two microplates. Thus, the cells exerted deformation at the flexible joint owing to CTF, leading to folding of the microplates. The folding angle can be managed accurately by incorporating an additional thin and flexible joint within adjacent microplates. Moreover, this cell-enabled folding technique could also be used to construct vessel-like structures by culturing human umbilical vein and arterial endothelial cells according to Kuribayashi-Shigetomi's study [38]. Multiple cell types can be combined with designed complex cell-laden structures, providing a potential strategy for 4D bioprinting.

### *3.2. Potential stimuli-responsive materials for 4D bioprinting*

The use of stimuli-responsive biomaterials has largely superseded CTF-based approach, providing a more refined strategy for effecting controlled shape-transformation of an object [39,40]. SMPs belong to smart polymers that could "remember" permanent shapes owing to physical or chemical crosslinks. This allows them to be deformed temporally, fixed by vitrification or crystallization of the polymer chains, and returned to their original shape under an external stimulus [41,42]. However, the disadvantage of the majority of solid-state SMPs in terms of 4D bioprinting is that cells can only be seeded on the surface of the materials rather than uniformly dispersed within it. Therefore, cell-laden shape memory hydrogels (SMHs), which can achieve reversible and sequential changes of their conditions and functions on demand, have been developed [43]. The typical shape memory behavior of smart hydrogels achieved through the reversible hydration of hydrophilic chain segments inside the polymer network, whereupon temporary crosslinks formed or cleaved. Owing to the strain discrepancy among their different components, 3D-printed structures can undergo self-assembly processes, which can be utilized to achieve 4D bioprinting [44].

The fabrication of printed constructs with suitable stimuli-responsive materials allows their shape-transformation and/or functional adjustment under specific external stimuli [2]. In the following section, we introduce several potential stimuli-

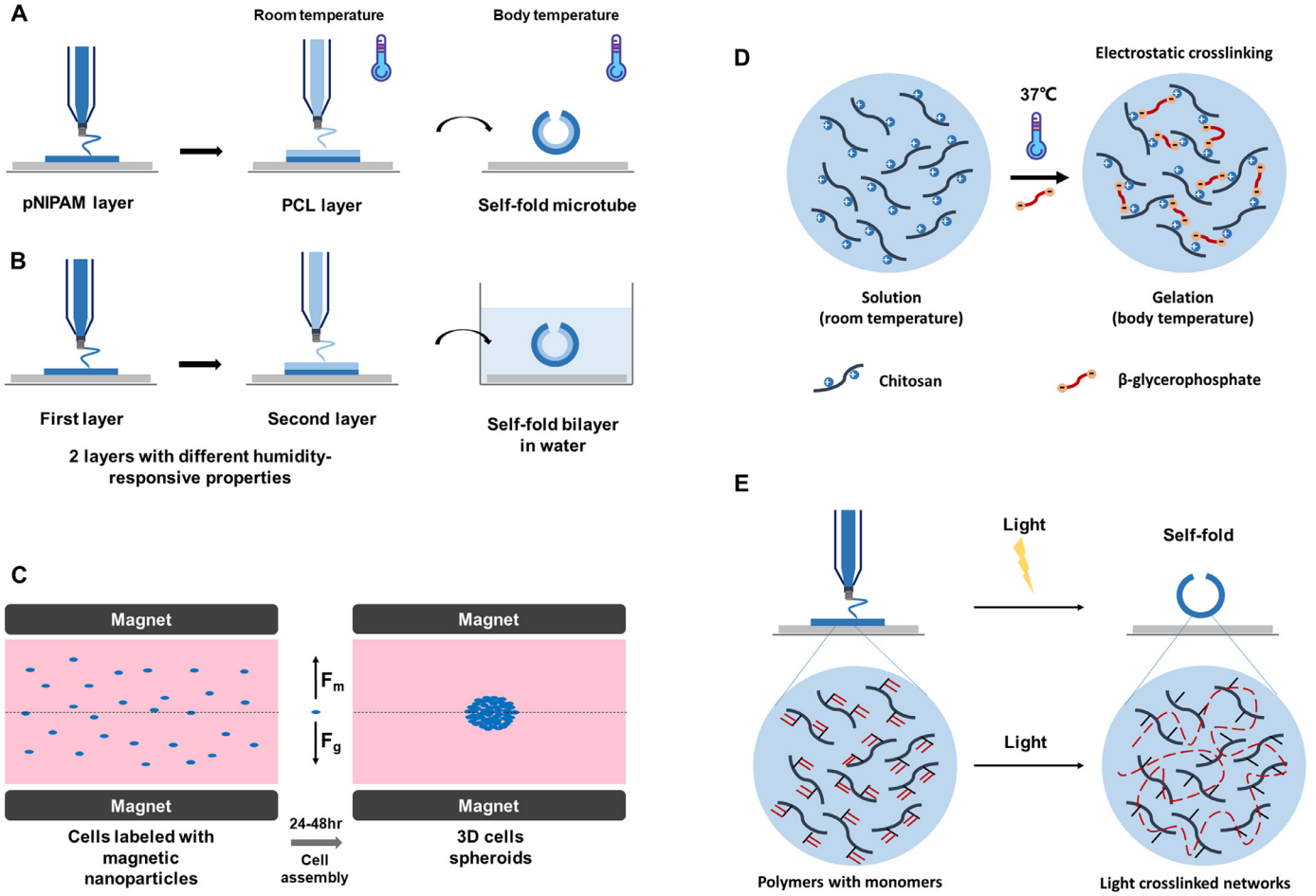


**Fig. 2.** Schematic illustrations of physical stimuli-responsive biomaterials. (A) Thermoresponsive properties of electrospun pNIPAM-PCL bilayer. No folding is observed for the bilayer at room temperature. The self-fold microtubes form at body temperature. (B) Schematic illustrations of humidity-responsive self-fold bilayer in water. The biocompatible bilayer consisting of two layers with different swelling characteristics in aqueous solutions, constructing spontaneous deformation to transform a tubular tissue architecture. (C) 3D cell culture with magnetic-based levitation. $F_m$: magnetic force. $F_g$: gravity. Magnetic force and gravity guide cells to assemble at the levitation height and form 3D cell spheriods. (D) Thermal gelation of chitosan solution in the presence of $\beta$-glycerophosphate (GP). The solution exhibits liquid state at 25 °C and solidifies to forma gel at 37 °C. Cationic chitosan chains are linked closer to the negatively charged GP molecules. The decrease in electrostatic repulsion amongst CS–CS chains, increase in electrostatic attraction between CS–GP and formation of hydrogen bonds are responsible for gelation without precipitation. (E) Schematic illustrations of the UV-assisted light-responsive polymer cross-linking process. The cross-linked composites networks invoke the shape-transformation of printed structures.

responsive materials, their self-assembly or shape memory abilities, and their potential uses in 4D bioprinting. According to the type of the stimuli to arouse 4D procedure, these smart materials can be classified into physical, chemical, and biological responsive stimuli-responsive materials Table 1.

### 3.2.1. Physical stimuli-responsive materials

*Temperature-responsive materials*: Temperature is the most frequently used physical stimulus to achieve shape-transformation in bioprinted structures [45–47]. A series of thermoresponsive materials have been developed based on the mechanism of thermal gelation. When the environmental temperature is below the low critical solution temperature, the polymeric chains adopt extension mode and transform into solution phase. However, contraction of polymeric chains turns the polymer into a gel state when temperature is above the low critical solution temperature [48–50]. This mechanism has been used to fabricate cell-laden bilayers that exhibit reversible folding/unfolding deformation under decreased or elevated temperature, respectively [51–53]. For example, Hendrikson's group fabricated a thermo-controlled 3D shape-memory polyurethane scaffold that can change shape with time during culture [54]. The transformation temperature of polyurethane is 32°C and a predetermined mechanical strain can be applied to the seed cells during the shape recovery procedure, directing the behaviours of cells seeded on the scaffolds.

A classical thermoresponsive material is poly(*N*-isopropylacrylamide) (pNIPAM) [47]. For instance, Luo et al. used 4-hydroxybutyl acrylate (4HBA) as a crosslinker to fabricate an elastic porous pNIPAM hydrogel that exhibits rapid and programmable locomotion prompted by external stimuli [55], and Apsite et al. fabricated self-folding multi-layer porous electrospun scaffolds using thermoresponsive poly(caprolactone) (PCL) and pNIPAM (Fig. 2(A) [56]. These pNIPAM-based thermoresponsive materials provide a wide range of possibilities for bioprinting, while they still have essential limitations, such as poor biocompatibility, hydrophobicity, and undegradability [57]. Moreover, pNIPAM generally requires relatively high transformation temperatures, leading to insufficient cell viability and function. Employing copolymers with pNIPAM may address these limitations. For example, cell viability can be maintained by reducing the transformation temperature to around 37°C, and cell adhesion and growth can be improved by combining with bioactive peptides, such as arginine-glycine-aspartic acid (RGD) [8].

Another transformation principle of thermoresponsive materials is based on changes in their wettability and solubility

with temperature [58,59]. For example, Inonv's group utilized the sol-gel transition of gelatin to fabricate a temperature-triggered gelatin-polycaprolactone(PCL) bilayer [60]. The self-rolled polymer bilayer folds at room temperature (22°C). The photoinitiator-modified gelatin does not undergo dissolution and is held by non-photocrosslinked gelatin, preventing the bilayer from folding at 37°C. Inonv's group also demonstrated another gelatin-PCL bilayer in which the gelatin layer swells and generates stress in aqueous environments [61]. When the PCL layer is in crystalline state at room temperature, the gelatin layer cannot bend it, while increasing temperature softens the PCL layer and the bilayer undergoes a folding process. These mechanisms, based on the reversible structure switching of PCL upon melting and crystallization, is similar to the reaction of SMPs under increasing temperature, and mechanical stress is induced by swelling of the hydrogel (gelatin) layers. Any hydrogel can be used to replace gelatin and any hydrophobic polymer with an appropriate softening point can replace the PCL. Furthermore, all the polymers used in these studies are compatible and biodegradable [57]. The cells adsorbed or encapsulated in these constructs remain alive for a considerable period of time, providing new options for 4D bioprinting.

*Humidity-responsive materials*: Humidity-responsiveness widely exists in natural systems, where plants such as pinecones and wheat utilize humidity shifts to trigger structural transformations in order to disperse their seeds under beneficial conditions [62,63]. These phenomena have inspired the development of humidity-responsive materials that change their shapes and sizes during swelling and shrinking processes upon variations in humidity (Fig. 2(B)) [64,65].

For instance, Jamal et al. have reported the spontaneous deformation of a poly(ethylene glycol) biocompatible bilayer consisting of two cell-laden layers with different swelling characteristics in aqueous solutions, constructing a series of anatomical microscale geometrical structures [66]. Zhang et al. combined a poly(ethylene glycol)-conjugated azobenzene derivative (PCAD) with agarose (AG) (PCAD@AG) to fabricate a new biomaterial that was capable of shape reconfiguration upon humidity change [67]. After combining pure agarose with PCAD, its rate of absorption decreased while the rate of desorption enhanced. This property is critical to the fast locomotion of PCAD@AG.

Cellulose stearoly esters (CSEs) also can be used to fabricate moisture-responsive, self-standing films [68]. As a result of the abor desorption of water molecules, CSE films with the low degree of stearoyl substitution of 0.3%, labeled CSE0.3, could fold or unfold to exhibit rhythmical bending motions, while CSE3 (stearoyl substitution of 3%) is hydrophobic and exhibits thermoresponsive properties. Humidity-responsive bilayer films with hydrophobic surfaces were obtained by spraying CSE3 nanoparticles onto the surface of CSE0.3 films. This kind of bilayer can perform fast reversible bending motions and continuous shape-transformation in solution.

These technologies represent the earliest versions of 4D printing, where the concept of multilayer structures was compatible with the future design of 4D bioprinting using biocompatible humidity-responsive materials. However, the culture environment of cells should be maintained under constantly high humidity and specific osmotic pressure. Therefore, the degree of shape-transformation may be limited due to humidity/osmotic pressure limitations. These challenges could be addressed by adjusting the sensitivity of humidity-responsive materials to within the range of cell endurance [8].

*Electro-responsive materials*: Most electro-responsive materials are polyelectrolyic polymers, which can swell, shrink, or fold under an external electric field. The properties of these materials can be regulated by the direction or strength of the electric field [69]. These developed electrically conductive biomaterials could provide new insights into biomedical applications and drug delivery [70–73]. Moreover, some hydrogels containing electrically conductive polymers, such as poly(pyrrole)s, poly(aniline)s, and poly(thiophene)s, can exhibit favorable biocompatibility and printability, giving potential for 4D bioprinting [74,75]. For example, a conductive electroactive hydrogel was fabricated by combining 3D printing with polypyrrole interfacial polymerization has been reported [76]. The printed constructs could be applied for developing new bioelectronics interfaces and neuroprosthetic devices.

The electro-responsive carbon-based nano-biomaterials, such as graphene and carbon nanotubes (CNTs), have also attracted extensive attention in recent years as tools to investigate and control the biology and fate of stem cells due to their unique mechanical properties, adjustable surface chemistry, and favorable electrical conductivity, which have more advantages for nerve tissue engineering [77,78]. Nanoparticles of these materials have the potential to be used as bioinks for 4D bioprinting [79–81]. For instance, Servant et al. designed a graphene-based macroporous hydrogel matrix that is able to control the release of small molecules under particular electrical stimulation via reversible deswelling of the hybrid gel [82]. Moreover, CNTs also show desired mechanical, electrical, and cytocompatible properties for 4D bioprinting. For instance, Shin's group has dispersed CNTs into gelatin methacryloyl and hyaluronic acid bioinks to facilitate the manufacture of elaborate foldable biosensors and functionalized tissue-engineering constructs [81]. The *in vitro* and *in vivo* studies showed that printed scaffolds coated with graphene and carbon nanotubes nanocomposites could accelerate the osteogenic differentiation of seeded stem cells obviously [83–85]. In conclusion, these biocompatible and electrically conductive carbon-based nano-biomaterials could be used to fabricate stimuli-responsive 4D architectures, providing more possibilities for neural and bone tissue regeneration [86].

*Magnetic responsive materials*: Magnetic responsive materials consist of ferromagnetic or paramagnetic magnetic nanoparticles (MNPs) that can respond to magnetic fields [87]. These materials have been widely used in biomedical applications, such as controlled drug release system and tissue engineering [88–92]. For example, a magnetic responsive drug delivery system can be constructed by combining $Fe_3O_4$ nanoparticles with polyethylene glycol/agar hydrogel networks, representing a promising alternative for soft tissue injury treatment [93]. In addition, the $Fe_3O_4$ nanoparticles can also be encapsulated into the organogel to prepare a magnetic gel with remarkable magnetic responsive and self-healing properties, which shows favorable rheological properties for bioprinting [94].

Magnetic 3D bioprinting technology, which controls the orientation and assembly of anisotropic micro-biomaterials as building blocks during the printing process, has been proposed as a biofabrication strategy for generating innervated secretory epithelial organoids [2,95]. By magnetizing with magnetic nanoparticles, cells in monolayers arranged spatially with magnet dots to produce 3D spheroids. These magnetic 3D bioprinting spheroids exhibit higher cell viability and steadier intracellular activity compared with magnetic-nanoparticle-free spheroids. This magnetic 3D bioprinting technology also was used to pattern human myometrium cells into rings that then were monitored for contractility and function over time [96].

Recently, the magnetic assembly of cells or tissue spheroids, or living materials has been studied, providing great potential for 4D bioprinting (Fig. 2(C)) [97]. The paramagnetic properties of MNPs could be used to realize magnetic levitational assembly of cells or micro-tissues. For example, Souza et al. utilized the magnetic levitation of cells to establish a 3D tissue culture system in which cells or a matrix are labeled with gold, iron oxide nanoparticles, or filamentous phages. The geometry of the cell spheroids is controlled via the spatial control of the magnetic field, and co-cultures of different types of cells could be realized [98]. Furthermore, Sun

**Table 1**
Stimuli-responsive shape-memory materials and methods.

| Stimulus | Materials/methods | Mechanisms | Application | Pros | Cons | References |
|---|---|---|---|---|---|---|
| Temperature | poly(*N*-isopropylacrylamide) (pNIPAM) | Sol-gel transition | Cell-laden bilayers for soft tissue | Easy availability; High sensitivity to temperature | Not well biocompatibility; Hydrophobicity; Low biodegradability; Low control precision. | Wei et al. [48] Bakarich et al. [47] Breger et al. [49] Pei et al. [50] Zakharchenko et al. [51] Luo et al. [55] Apsite et al. [56] |
| | Polyurethane | Shape transformation | Cell-laden scaffolds for bone, muscle, cardiovascular tissue repair | Suitable shape transition temperature | Complex mechanical stimuli control | Hendrikson et al. [54] |
| | Gelatin-Polycaprolactone(PCL) | Sol-gel transition; Wettability and solubility differences between two materials | Bilayers, Cell-laden bioscaffolds for tissue engineering | Compatible; Biodegradable | Unsuitable for complex shape transformation | Stroganov et al. [60,61] Ionov L et al. [57] |
| Humidity | Poly-ethylene glycol (PEG) | Swelling properties | Cell-laden bilayers | Biocompatibility | Humidity/osmotic limitation | Jamal et al. [66] Zhang et al. [67] |
| | Agarose | Water sorption/water desorption | Hybrid films | Native; Strong hygroscopic | Humidity/osmotic limitation | Zhang et al. [67] |
| | Cellulose stearoly esters (CSEs) | Water sorption/water desorption | Bio-sensors or bio-actuators | Biocompatible | Humidity/osmotic limitation | Zhang et al. [68] |
| Electric field | Polypyrrole(PPy); Polyaniline; Polythiophene | Electrically conductive properties | Drug delivery; Biomimetic or bioinspired systems | Improved conductive characteristics | Undesirable biocompatibility | Green et al. [74] Song et al. [75] Fantino et al. [76] |
| | Carbon-based nanobiomaterials (graphene, carbon nanotubes) | Electro-responsive properties | Drug delivery; Biosensors; Cell-laden scaffolds for bone and nerve tissue engineering | Suitable mechanical properties; Adjustable surface chemistry; Excellent electrical conductivity; Cytocompatibility. | Restricted electrical stimulation | Ahadian et al. [77] Ramon et al. [78] Shin et al. [81] Servant et al. [82] Miao et al. [184] |
| Magnetic field | Magnetic nanoparticles (MNPs) | Magnetic-responsive properties; Self-assembly | Drug delivery; Cell/tissue spheroids (epithelial organoids; myomethrium rings; 3D embryoid body; microvascular-like structures) | Remote control; High control precision; Harmlessness to cells | Complex control system; Challenging sufficient magnetic field gradient achievement | Zhang et al. [90] Lalitha et al. [94] Souza et al. [96,98] Du et al. [97] Sun et al. [99] Adine et al. [95] |
| | Gadolinium ($Gd^{3+}$) | Magnetic levitation | Cell/Tissue spheroids; Drug delivery; Cancer therapy | Low toxicity; Noninvasiveness; Easy-to-use | Undesirable cytotoxic effect at high concentration | Tocchio et al. [100] Parfenov et al. [102] Fattah et al. [103] Turker et al. [104] |
| light | Photoinitiators (e.g.: benzophenone, hydrazone bonds, methylene blue) | Photocrosslink; Dynamic covalent chemistry | Soft robotics; Cell delivery; in-situ photo-curing structures | High shape fidelity; Stability relaxation; Cytocompatibility | Low light tissue penetration | Wei et al. [113] Kuang et al. [114] |
| | Photodegradable moieties(e.g.: coumarin, o-nitrobenzyl ether groups) | Programmable photodegradation | Cell delivery; Cell-laden structures; 3D vascular networks | Precise multistaged light program | Low tissue penetration; Phototoxicity. | Griffin et al. [116] Arakawa et al. [117] |
| Acoustic | Surface acoustic waves (SAWs) | Acoustic force assembly | 3D cellular patterns and constructs | Non-invasive; Cytocompatible; Biocompatible | Limited line patterns; Micro dimensions only | Nasser et al. [119] |

(*continued on next page*)

**Table 1** (*continued*)

| Stimulus | Materials/methods | Mechanisms | Application | Pros | Cons | References |
|---|---|---|---|---|---|---|
| | Near field standing waves | Acoustic radiation forces | 3D brain-like constructs | Easy to use; Cytocompatible | Limited cell populations; Homogenous tissues only | Bouyer et al. [122] |
| | Ultrasound | Ultrasound-disrupted ionically crosslink | Drug release; Chemotherapy | Non-invasive; Biocompatible | Need further test mimicking | Huebsch et al. [121] |
| pH | Amino acids (e.g.: polypeptide, I-arginine grafted alginate) | Noncovalent crosslink; pH determined swelling properties | $\beta$-sheet structure; Protein delivery | Tunable morphology and mechanical properties | Specific pH environments; Unsuitability for complex shape-transformation | Clarke et al. [128] Eldin et al. [129] |
| | Chitosan-based polymers (N-succinyl chitosan grafted polyacrylamide, chitosan-based tripolyphosphate) | pH determined swelling properties; Electrostatic crosslink | Drug delivery; Bone regenerative therapies | Better controlled release of drugs; Tunable mechanical properties | Limited pH change range | Mukhopadhyay et al. [131] Xu et al. [132] |
| Ion | $Zn^{2+}$/$Ca^{2+}$-responsive hydrogels | Reversible chemical crosslink | Cell-laden shape memory structures; Hollow self-folding tubes (blood vessels) | Suitable strength; Tunable mechanical properties; Biocompatibility | Difficult to control in vivo | Liu et al. [135,136] Lonov et al. [140] |
| Biological | Polypeptides; Polynucleotides | Hydrogen bond crosslinks or ionic interactions | Shape memory hydrogels for drug delivery, programmed cell adhesion matrices | Biodegradable and biocompatible | Low mechanical strength | Skrzeszewska et al. [142] Hao et al. [143] Guo et al. [144] Todhunter et al. [145] |
| | Enzymes (e.g.: matrix metalloproteinase, thrombin, Sortase A, horseradish peroxidase) | Enzymatic mediated crosslink | Drug delivery; Bioinspired multi-activities object for blood vessels /bone /cartilage tissue regeneration | Biological substance; Cyto-compatibility; Suitable degradation; Tunable morphology | Complex regulation; Unidentified immune response | Kim et al. [146] Broguiere et al. [147] Costa et al. [148] Yan et al. [149] |

et al. presented an innovative strategy based on magnetic alginate microfibers as scaffolding elements to fabricate microvascular-like structures, allowing direct cell-to-cell interaction, which is essential for the formation of vessel-like structures [99].

Another universal system for levitating and assembling cells using a gadolinium ($Gd^{3+}$)-based nonionic paramagnetic agent has been reported for fabricating scaffold-free living architectures [100,101]. Parfenov et al. developed a prototype device equipped with magnetic levitation capabilities using gadolinium in culture media [102], Similarly, Fattah et al. demonstrated a new technology for fabricating 3D cellular structures, co-culturing breast cancer MCF-7 cells and human umbilical vein endothelial cells utilizing a magnet array to manipulate diamagnetic cells in a paramagnetic gadopentatic acid medium [103]. Three different gadolinium chelates were utilized to magnetize the cell culture environment, thus realizing scaffold-free levitation and assembly of cells [104].

Moreover, Tasoglu's group manipulated and assembled a cell-encapsulating poly(ethylene glycol) hydrogel in magnetic fields using permanent magnets by exploiting the paramagnetic characteristic of free radicals without using magnetic nanoparticles [105]. They also fabricated tunable and magnetic self-assembly microgels using a cell-laden gelatin methacryloyl hydrogel that is paramagnetized upon submerging in a stable free-radical solution [106]. In conclusion, these innovative magnetic levitational assembly systems show enormous promise for complex cellular assemblies and tissue engineering, providing a new paradigm for the application of 4D bioprinting.

*Photoresponsive materials*: Photoresponsive materials could capture externally applied optical signals and convert them into mechanical responses. Photoresponsive biomaterials can be activated by light in a relatively wide wavelength range, including near-infrared (NIR), infrared (IR), and ultraviolet (UV) regions, which have been broadly applied in biomedical applications such as controlled drug release and tissue engineering [107–112].

Photoisomerization and photodegradation of polymer chains are the most common response mechanisms for light-responsive materials, which have been widely applied to fabricate active 4D shape-changing structures. For instance, Wei et al. printed a tubular shape-memory poly(lactic acid) (PLA) structure by introducing a UV crosslinking agent (Fig. 2(E)) [113], while Kuang et al. reported a UV-light-assisted printing bioink containing urethane diacrylate and a linear semi-crystalline polymer [114]. The bioink showed capability of high strain shape memory as well as self-healing feature, paving the way for the development of 4D bioprinting.

In addition, photodegradation of biomaterials provides real-time temporal and spatial control during hydrogel degradation. Photodegradation of biomaterials can be induced by exploiting their photoresponsive features, producing dynamic hydrogel environments [115] For instance, Griffin et al. added photodegradable moieties, such as o-nitrobenzyl ether groups and coumarin, into hydrogels to tune the rate of its biodegradation [116]. Furthermore, Arakawa et al. exploited a programmable biomaterial photodegradation strategy to construct 3D multicellular endothelial vascular networks within cell-laden hydrogels [117]. Networks of microchannels with similar sizes and scales to those of the native human vascular systems could be easily generated through programmable 4D control using multiphoton lithography technology. Therefore, these photoresponsive 4D bioinks show the potential to

mimic the dynamic characteristics of natural extracellular matrix degradation.

However, the strong attenuation of light by biological tissues is a challenge. This challenge may be addressed by exploring the use of ultra-IR light, which has lower living tissue absorbency and phototoxicity as well more effective tissue penetration than UV light. Meanwhile, multiple kinds of photo-initiators have limitations in applications of tissue engineering due to their undesired cytotoxicity. New photo-polymerized systems with significant mitigated adverse effects on cellular metabolic activities and proliferative capacities have been developed, giving potential for applications including cell encapsulation and biofabrication of injectable hydrogels [118].

*Acoustic responsive materials*: Acoustic responsive materials have been used in drug release systems and tissue engineering. Acoustic force patterning is a potential technology that can be used to construct location-controlled cell platforms in a contactless, rapid, and accurate manner [119,120]. Huebsch et al. developed an alginate-based, self-healing, ultrasound-responsive hydrogel for near-digital spatiotemporal control of drug delivery and used ultrasound stimulation to disrupt the ionic crosslinks within the hydrogels [121]. Furthermore, Naseer et al. reported a biological acoustic-force-mediated micro-patterning technique to achieve rapid arrangement of cells within gelatin methacryloyl hydrogels by using surface acoustic waves [119]. A multilayered 3D brain-like construct was also fabricated via levitating neuroprogenitors through acoustic radiation forces based on near field standing waves, indicating that acoustic responsive hydrogels have the potential to be applied to 4D bioprinting in tissue engineering [122]. These bioacoustic levitational assembly technologies are non-invasive and biocompatible, while limited to line patterns and homogenous cell populations. More sophisticated methods are needed to fabricate complex heterogeneous tissue cellular architectures.

### 3.2.2. Chemical stimuli-responsive materials

*pH-responsive materials*: pH-responsive materials containing chemical groups (carboxyl, pyridine, sulfonic, phosphate, etc.) that can release or accept protons with changing pH have been applied to the fabrication of self-assembled structures [123]. These pH-responsive materials exhibit globule-to-coil transition at a critical pH value. When the functional group of the polymer is neutralized, the polymer chains are converted into globule structures from their coil forms in electrostatically repulsion states [124]. Nadgorny et al. described a 3D-printed pH-responsive construct by using poly(2-vinylpyridine) [125]. These 3D-printed structures exhibit reversible and dynamic pH-dependent swelling characteristics. The hydrogel can be used as a flow-regulating valve to regulate flow rate by controlling the changes of pH. Few synthetic pH-responsive polymers have been reported in the field of bioprinting, while the applications of natural proteins have gained great attention [124,126,127]. For instance, three different charge pentapeptide sequences were used to fabricate a robust pH-responsive hydrogel [128]. The mechanical properties of the hydrogel could be regulated significantly by tuning the charge distribution and concentration of the pentapeptide sequences. Moreover, a pH-responsive hydrogel using *L*-arginine-grafted alginate (Arg-g-Alg) hydrogel beads has been synthesized as a new type of carrier for protein delivery at specific pH environments [129]. The swelling characteristics of the hydrogel beads were determined by pH changes. These pH-responsive hydrogels with tunable stiffness and adjustable morphology are promising for applications in injectable drug delivery and tissue engineering.

Meanwhile, a series of pH-responsive chitosan hydrogels also have been widely used in drug delivery and bioprinting (Fig. 3(A)) [130,131]. Xu's group reported a chitosan-based tripolyphosphate scaffold with regulated primary amine content, which influenced the pH-responsive resorption and made the scaffold enable to absorb or release water under the changes of pH [132]. These pH-responsive materials modified scaffolds with tunable morphology and mechanical properties possess the possibility for biomedical applications, providing new possibilities for 4D bioprinting.

*Ion-sensitive hydrogels*: Many recent studies have reported strategies for the fabrication of sufficiently strong scaffolds to support clinical-scale cell-laden structures [133]. Crosslinking with multivalent ions such as $Ca^{2+}$ and $Zn^{2+}$ has been exploited in bioprinting to obtain scaffolds with suitable strength and tunable mechanical properties (Fig. 3(B)) [134,135]. For example, Liu et al. fabricated a reversible shape-memory ultrahigh strength cell-laden hydrogel utilizing the dipole-dipole interactions of poly(acrylonitrile) chains that are reversible in response to $Zn^{2+}$ [136]. They also utilized imidazole-zinc ion coordination to synthesize another shape memory hydrogel [137]. The permanent shapes of these printed constructs can be restored by extracting zinc ions using chelating agents, and this memory process is reversible. The flat cell-laden hydrogel sheet can be fold into a temporary tubular construct and fixed in culture medium containing zinc ions.

In addition, a crosslinking mechanism involving sodium-calcium ion exchange between alginate and calcium chloride has been widely applied in bioprinting [138,139]. Hydrogels based on a hydrogen bonding/calcium ion crosslinking mechanism exhibited shape memory abilities in response to reversible $Ca^{2+}$ crosslinking. Such hydrogels with weaker hydrogen bonding interactions exhibit sharp volume changes triggered by calcium ions [135]. Furthermore, Lonov et al. have reported an advanced 4D bioprinting approach to cell-laden, stimuli-triggered, shape-changing alginate and hyaluronic acid hydrogels, which were modified with methacrylate groups to endow them with photo-crosslinkable properties [140]. The photo-crosslinking hydrogel exhibited strong $Ca^{2+}$-ion-concentration dependent rheological properties. Cells can tolerate calcium ion changes in the extracellular environment, allowing $Ca^{2+}$ ions to act as biocompatible shape-transformation stimuli. This bioprinting strategy based on $Ca^{2+}$ stimuli-triggered polymers could be used to print hollow tubular structures with diameters equivalent to those of the narrowest blood vessels. In a word, these ion-sensitive crosslinking hydrogels pave the new ways for presentation of tunable cell-laden shape-morphing structures for tissue engineering and 4D bioprinting applications.

### 3.2.3. Biological stimuli-responsive materials

Besides gelation through ion-crosslinking processes, shape-memory hydrogels exhibit self-assembly abilities via reversible hydrogen bond crosslinks or ionic interactions with polypeptides or polynucleotides [141]. For example, a thermally induced shape-memory hydrogel was formed by utilizing biocompatible and biodegradable recombinant telechelic polypeptides that combine with random coil-like middle blocks and collagen-like end blocks, forming of triple helices and allowing the fixation of temporary shapes upon cooling [142]. The programmed shape of hydrogels containing lysine residues was achieved through chemical crosslinking of random coils. The triple helix led to permanent shape recovery upon opening of the crosslinks during heating and melting. Similarly, by utilizing DNA as a programmable and sequence-specific glue, shape-controlled hydrogel units can be self-assembled into diverse prescribed structures in aqueous or interfacial agitation systems [143]. In addition, the cytosine-rich nucleotide sequences generated an i-motif construct through self-assembly at low pH and dissociated into random coil conformations at pH 8, leading to a reversible shape-transformation between a "quasi-liquid" state and a predesigned shape structure [144]. Furthermore, Todhunter's group performed multicellular tissue organizations utilizing a DNA-programmed cell-assembly strategy [145]. This technology exploits dissociated cells functionalized

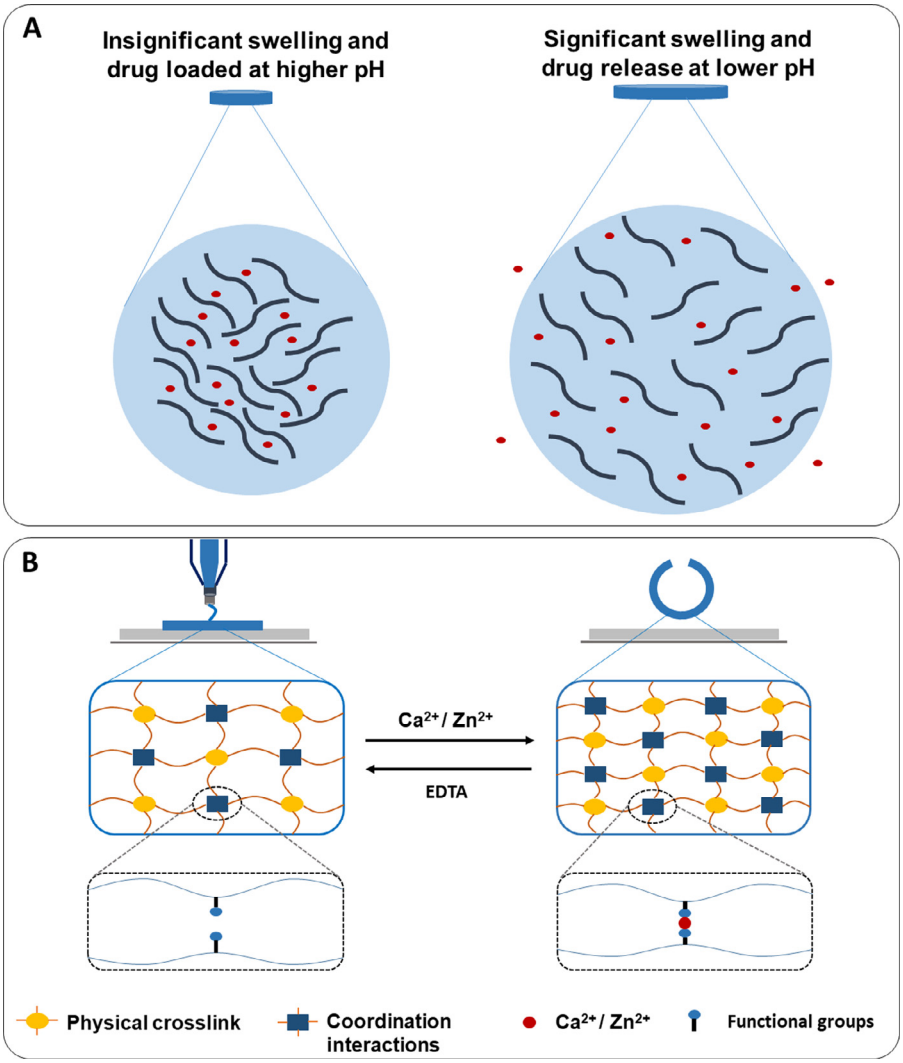


**Fig. 3.** Schematic illustrations of chemical stimuli-responsive biomaterials. (A) Schematic presentation of swelling and drug release pattern of the pH-responsive hydrogel. (B) A schematic diagram depicting the mechanism underlying a small number of calcium/zinc ions triggered reversible shape memory behavior. The functional groups coordinate with $Ca^{2+}$ /$Zn^{2+}$, while the $Ca^{2+}$ /$Zn^{2+}$ functional group linkages are dissociated in EDTA solution.

chemically with degradable oligonucleotides that can adhere to other cell surfaces labeled with complementary DNA sequences. The rapid, reversible, and specific cell adhesion induces layer-by-layer DNA-programmed assembly, fabricating 3D tissue constructs with programmed shapes, sizes, and spatial heterogeneities above a template, providing a potential approach for 4D bioprinting.

Moreover, active biological molecules, such as antibodies and enzymes, can be entrapped in 3D objects to obtain 4D structures (Fig. 4). Enzymes play important roles in processes of many biochemical reactions. The human body has multiple kinds of enzymes that can be utilized as triggering factors for shape memory properties of hydrogels. The substrates of enzymes should serve as functional side groups or crosslinkers of hydrogels [73]. For example, matrix metalloproteinase is an important protease related to the degradation of extracellular matrix components. Matrix metalloproteinase sensitive hyaluronic acid-based hydrogels showed tunable swelling and degradation capacities with dramatic cell attachment [146]. In addition, the bacterial ligase sortase A (SA) and its mutated variants have been used as crosslinking enzymes for hydrogel-based tissue engineering [147]. Modifying hyaluronan (HA) with SA-substrate peptides achieved near-instantaneous gel formation of HA. Similarly, Costa et al. demonstrated a fast-gelling silk fibroin bioink capable of enzymatic crosslinking for 3D bioprinting [148]. The hydrogels can transform from random coil configurations into $\beta$-sheet conformations over time, and can be used to fabricate different fine-tuned structures, such as shape-memory patient-specific implants, with good reliability, resolution, and reproducibility [149]. These enzyme-sensitive hydrogels with fast-crosslinking, suitable degradation and tunable morphology characteristics could be applied for tissue defect regeneration and complex tissue engineering.

## 4. 4D bioprinting based on functional transformation mechanism

Advanced biology studies have extended the original definition of 4D bioprinting, which was limited to the geometric change of 3D-printed objects, to include the transformation of shape, properties, and physical, chemical, or biological compositions of 3D constructs [150]. The functional transformation and maturation of 3D-printed cell/tissue constructs over time have also been regarded as constituting 4D bioprinting [10,151,152]. Natural tissues and organs are structurally anisotropic and highly organized architectures [3,153]. The establishment of biomimetic constructs that mimic the native extracellular matrix could guide and support the growth and differentiation of stem cells during the

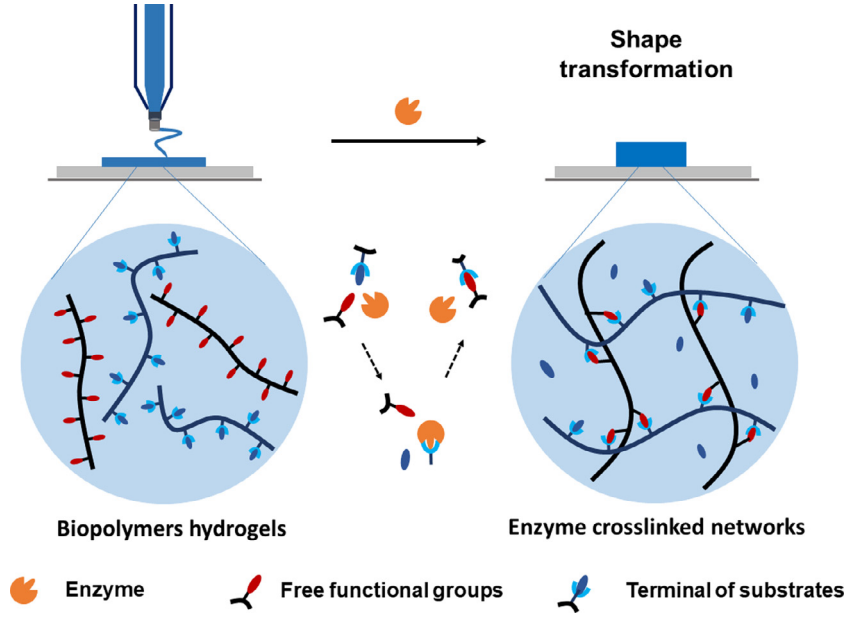


**Fig. 4.** Schematic illustrations of enzymatic-crosslinked process. The enzyme removes the terminal of substrates and then attacks the free functional groups to form crosslinks of hydrogels.

post-bioprinting stage, making it possible to fabricate on-demand implantable and functional multilayered tissues such as cartilage, skin, and skeletal muscle [154–156].

The 4D-printed constructs with aligned micro-patterns may exhibit functional differentiation and maturation during the cultivation process. For example, Betsch et al. presented an advanced bioprinting strategy utilizing a straightforward magnetic-based technology in agarose/type I collagen hydrogels to align collagen fibers [157]. The strategy realized real-time matrix remodeling of structural microarchitectures by magnetism. The unidirectional anisotropic scaffold imitates the natural tissue fiber morphology, guiding the proliferation and differentiation behaviors of cells and maturation of printed tissue in a highly desired way.

Moreover, Miao's group created biomimetic hierarchical 4D micro-patterns with smart soybean oil epoxidized acrylate (SOEA) bioinks by using a special photolithographic-stereolithographic-tandem strategy [158]. The topographical surfaces of these architectures regulate the cardio-myogenic behaviors of human MSCs effectively. These printed scaffolds could be applied to osteochondral and neural tissue engineering because of their ability to differentiate and proliferate human MSCs. This scaffold fabricated not only shows dynamic 4D shape change upon external stimulation but also provides subtle surface micro-patterns to regulate the multilineage differentiation of stem cells. Similarly, they also fabricated a 4D anisotropic skeletal muscle tissue using staircase effect strategy and investigated the effects of topographical cues on the skeletal muscle differentiation of human bone marrow mesenchymal stem cells [159]. The expression of myogenic genes was enhanced, confirming that this biomimetic strategy can reconstruct highly organized functionalized skeletal muscle tissues. In conclusion, by fabricating aligned and hierarchical microstructures, more potential bioprinting strategies have emerged to mimick the microenvironments of cell cultures, regulate the differentiation of seeded cells, and accelerate the maturation of complex constructs, providing new directions for 4D bioprinting.

Another technology, synthetic printed droplet networks, which contain thousands of programmed patterned communicating aqueous droplets, can be used as substrates in tissue engineering and may be modified to mimic living tissues [160]. For instance, Booth et al. used this technology to create a 3D synthetic tissue consisting of hundreds of synthetic cells [161]. A kind of light-activated DNA (LA-DNA) promoter was used to turn on gene expression within the synthetic cells, giving the synthetic tissues the ability to synthesize protein through the translation of encapsulated DNA under UV light control. By using light activation, protein pores were expressed in 3D-printed synthetic tissues, incorporating into certain bilayer interfaces and mediating electrical communication between neighboring cell subsets. This functional simulation of neuronal transmission can be controlled precisely, providing new strategies for 4D bioprinting.

## 5. Applications of 4D printing in bone tissue engineering

4D printing technology has revolutionized the 3D-printed constructs with shape and functional modification over time. Innovative 4D printing strategies present the potential for the fabrication of complex multilayer tissue constructs, providing many advantages for tissue engineering and applications [162–165]. Here, we focused on the 4D bone tissue engineering and introduce various existing applications of 4D printing in bone tissue engineering (Fig. 5). These applications exhibit overwhelming superiority in personalized bone defect repair, especially the irregular or minor size bone defects. The self-remodeling and functional maturation features of 4D-printed structures would help to fabricate biological complex hierarchical constructions similar to the native bone tissue. In addition, multiple 4D-printed strategies could be used to develop microvascular system and nervous networks within bone constructs, which is essential for large bone-graft-substitute.

### *5.1. 4D printing of bone tissue based on injectable stimuli-responsive hydrogels*

A series of injectable thermoresponsive polysaccharide hydrogels, which could play the role as a carrier for different cells, growth factors or inorganic composites (such as hydroxyapatite, calcium phosphate cements, and bioactive glass), have been developed for bone tissue engineering [24]. These modified biomaterials, such as hydroxypropyl methylcellulose, hydroxybutyl chitosan and hydroxypropyl guar-graft-poly(N-vinylcaprolactam), exhibit suitable lower critical solution temperature between

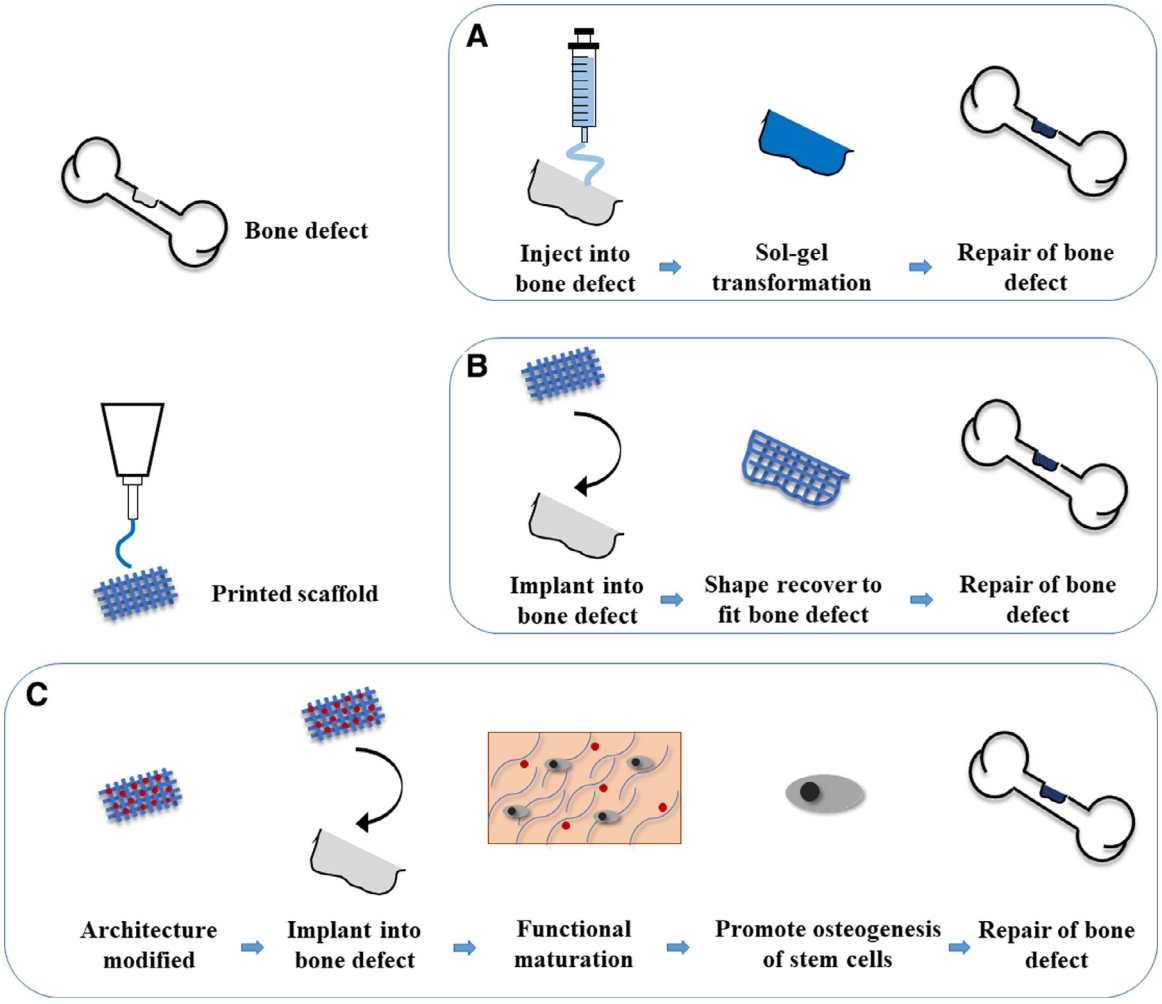


**Fig. 5.** Applications of 4D printing in bone tissue engineering. (A) Injectable thermosensitive hydrogels for 4D bone tissue regeneration: the hydrogel could be injected into the irregular defect area and transform to gel state under body temperature. (B) 4D printing of bone tissue based on shape-transformation mechanism: a shape memory scaffold changes its size to occupy the void space, realizing personalized bone defect repair. (C) 4D printing of bone tissue based on the establishment of biomimetic microenvironment: the 4D printed biomimetic scaffold with modified architectures can induce the functional maturation of neo-bone tissue and promote the osteogenesis of stem cells, enhancing the formation of new bone tissue.

physiological temperature and manipulative room temperature and could translate into gel state under body temperature [166–169]. The classical thermoresponsive material, pNIPAM, has been incorporated with hyaluronic acid and chitosan to fabricate an injectable hydrogel for bone tissue regeneration [170–173]. Similarly, chitosan could form a thermoresponsive hydrogel by combining with $\beta$-glycerophosphate salt, which modulates the hydrogen bonding and electrostatic, hydrophobic crosslinkings during the gel formation [22,174]. This kind of chitosan hydrogel exhibited desirable injectability and rapid gelation at body temperature (Fig. 2(D)). Another kind of available thermosensitive material is a copolymer of poly(lactic acid), poly(ethylene glycol) and poly(glycolic acid), which converts to a gel state under physiological conditions [175].

The mechanical strength and load-bearing capacity of these hydrogels were improved by including different mineral components, such as tricalcium phosphate, nano-hydroxyapatite and bioactive glass [176–179]. These injectable hybrid hydrogels with desirable rheological feature and *in vivo* self-setting ability served as desirable carriers for osteoblast cells with improved alkaline phosphatase activity and calcium deposition, making them enable to fill small, irregular-shaped defects and form gel at body temperature and providing a significant potential in mini-invasive repair of bone defects. Bioactive cues, such as osteogenic and angiogenetic growth factors, have been involved into the hydrogel systems to enhance the differentiations of MSCs, providing new strategies for bone defect repair [180–182]. Therefore, the composite material can be used as an injectable osteogenic material for orthopedic applications, providing new insights for clinical translation.

### *5.2. 4D printing of bone tissue based on shape memory scaffolds*

4D printing has been used to print hard-tissue constructs. For example, shape-recovery polylactide and hydroxyapatite porous scaffolds were obtained by fused filament fabrication [23], in which direct heating stimulated the shape-memory effect. The polylactide/hydroxyapatite hybrid porous scaffolds with high levels of shape recovery ability could be used as self-fitting implants to repair small bone defects. Thus, the shape-transformation features of 4D printing constructs may realize personalized bone defect repair. Such biomaterials could be used to repair bone defects in which the scaffold shape changes to occupy the void space after implantation [21].

Moreover, Miao et al. utilized PCL and crosslinkers with predetermined amounts of castor oil to synthesize smart renewable bio-scaffolds, which exhibit favorable shape-memory effects and shaperecovery at physiological temperature [183]. The surface morphology, shape memory, mechanical properties, biocompatibility and biodegradability of the synthesized smart polymers were

demonstrated to be satisfactory [184]. Similarly, the authors also fabricated a biocompatible temperature-responsive shape-memory scaffold comprising epoxidized acrylate materials based on renewable soybean oil using a 3D laser printing technique [185]. The porous scaffolds are biocompatible and exhibit comparable attachment and proliferation abilities to those of multipotent human bone marrow mesenchymal stem cells. Thus, these studies proposed renewable biomedical scaffolds that could potentially contribute to the development of 4D constructs in bone engineering.

### *5.3. 4D printing of bone tissue based on functional transformation mechanism*

The biomimetic bony microenvironment can improve the biological functionality of 3D-printed scaffolds and drive osteogenesis of stem cells during the post-bioprinting stage. It inspires us whether the establishment of this biomimetic microenvironment, which enhanced the functional maturation of 3D-printed constructs, would be considered as 4D printing in tissue engineering. Pati et al. [186] reformed polymeric 3D-printed scaffolds by ornamenting them with a cell-laden mineralized extracellular matrix to mimic bony microenvironments. The printed bone structures became mature after culturing in a rotary flask bioreactor over time. The results showed that the extracellular-matrix-ornamented scaffolds exhibit better osteoinductive and osteoconductive properties than those of bare 3D-printed scaffolds.

Moreover, the complex hierarchical structures of bone tissue possess anisotropic mechanical and electromechanical properties [187]. The smart biomaterial with piezoelectric effect, such as barium titanate, could stimulate the physiological electrical microenvironment in response to applied stress and promote the growth of osteoblasts, showing favorable biocompatibility and bone-inducing abilities [188–190]. This promising development of piezoelectric materials could be used to enhance the functional maturation of printed constructs during the post-printing stage, presenting new strategies for 4D bone tissue bioprinting.

### *5.4. 4D-printed bone constructs with blood vessels and nervous networks*

The major challenge in large bone-graft-substitute engineering is the regeneration of microvasculature and nervous networks in the substitute [191–193]. A series of 4D strategies have been proposed to fabricate microvasculature constructs. For example, hollow self-folding tubes with diameters comparable to those of the smallest blood vessels have been fabricated by combining mouse MSCs with methacrylate alginate and hyaluronic acid hybrid hydrogels [140]. In addition, localized and pre-programmed calcification and direct fibrin biofilm formation could be triggered through the entrapment of enzymes within the 4D hydrogel during the bioprinting processes. The bioinspired 3D constructs in this study were composed of bone-like structures surrounding a blood-vessel-like structure, making it possible to fabricate vascularized alveolar bone constructs [194]. These enzymes could be used alone or co-immobilized to create bioinspired constructs with multi-activity. This technology was the first to demonstrate the fabrication of such 4D-printed constructs with multi-activity, providing a potential approach for complex bone tissue engineering.

Furthermore, the 4D bioprinting of the electro-responsive biomaterials have shown great potential for nerve tissue regeneration. Miao's group demonstrated a multi-responsive graphene hybrid 4D-printed architecture providing multiple nerve regeneration characteristics, such as physical guidance, chemical cues, and seamless integration [195]. This stimuli-responsive 4D technique could be combined with the bone tissue fabrications, paving the way for repairing bone defects with nerve damage.

## 6. Future perspectives and current challenges

4D bioprinting, incorporating "time" as the fourth dimension within 3D bioprinting, is expected to allow the creation of complicated structures with on-demand dynamically controllable shapes and functions, considering to be the next generation of tissue engineering technology [8]. In the last few years, with emerging development of stimuli-responsive biomaterials and better understanding of tissue regeneration, 4D bioprinting technology has gained lots of attention in biomedical area and clinical applications [53,196–201]. For example, 4D bioprinting technology provide enormous application prospects in the field of personalized tissue regeneration. The 4D-printed implantation with programmed shape and size would fit the defect sites with precise geometry [202–205]. The functional transformation of implantation during the post-printing stage would show bio-mimicking features, facilitating the tissue remodeling and maturation. At the same time, the recent progress of computational model system has provided new opportunities to program neo-tissue growth in personalized tissue engineering [73,206].

Meanwhile, the transformation features of 4D bioprinting could also benefit the treatment of adolescent patients through fabricating self-growing constructs. For example, Morrison et al. demonstrated the successful application of personalized 4D-printed medical device for the treatment of pediatric tracheobronchomalacia [164]. The 4D-printed PCL airway splints exhibited optimal adjustment to tissue growth with designed mechanical and degradation behaviors over time, which indicates a self-growing feature. The self-transformation and self-maturation abilities of 4D-printed constructs would give a new perspective to produce specific implants with time-dependent growth behavior, which shows great advantages in the treatment of adolescent patients with congenital malformation [207].

In addition, stimuli-responsive cell-assembly and tissue remodeling technologies could be used in the clinical applications of drug delivery and cell therapy [208–210]. An effective magnetic force driven stem cells delivery system has been applied in spinal cord injury repair [211]. Similarly, these stimuli-responsive cell carriers could exhibit directional migration and cell homing features *in vivo*, serving as a clinically applicable vehicle for the injury repair at special site [212].

To summarize, the shape and function transformation features of 4D bioprinting could be utilized to design and control the printed constructs with special shapes, sizes, functional time and working sites over time, which will meet the requirements of tissue engineering and clinical applications. These 4D bioprinting technologies could provide great potential for personalized treatment and precision medicine, which have been regarded as the paramount trend in the field of tissue engineering [213,214]. Although a series of stimuli-responsive biomaterials and multiple innovative strategies have been developed, 4D bioprinting is still in its infancy and multiple challenges are needed to be addressed.

Firstly, it is still challenging to make the existing stimuli-responsive biomaterials printable and transforming them to optimized bioinks [8]. Although several stimuli-responsive biomaterials have been thoroughly investigated for biofabrication approaches, and the cytocompatibility and *in vivo* suitability of SMP materials have been verified [215–219], their direct application into bioinks may not be simple. In addition, more researches are required to address the multiple challenges in 4D bioprinting, such as the negative effects of the printing procedures on cell-laden bio-scaffolds and the possibilities for scaling-up and high-throughput productions.

Secondly, the existing shape-transformation procedures of 4D-printed structures are still simple deformations, such as folding or assembling, which can not meet complex needs in clinical ap-

plications. More efforts should be made to improve the accurate spatiotemporal control of the shape-transformation and printing resolution for a wide spectrum of tissue engineering applications. The precise control of the generation or release of internal stress is also required during the use of stimuli-responsive materials when shape-transformation occurs. The stimuli-responsive abilities should be maintained during the long-term application process without losing their unique properties. Raviv et al. reported that repeated folding/unfolding led to an obvious decrease of the mechanical properties of printed structures. These scaffolds could only be fully recovered to the original shape in limited situations [220]. In addition, the mechanical strength of printed structures are usually insufficient to withstand a high pressure [57]. Thus, it is necessary to develop 4D-bioprinted structures with robust shape-transformation properties, especially when repeated responsiveness is required.

Moreover, some limitations are expected to be overcome before further applications in clinical practice. There are still substantial restrictions in terms of incitation mechanisms that can be utilized to trigger deformation procedures. For instance, dramatic changes in UV level and pH may be unsuitable because of their possible negative effects on cell viability [221], while temperature (between 4 and 40 °C) and $Ca^{2+}$ concentration can be altered without detrimental effects to living cells [57]. Relatively moderate incitation mechanisms or stimuli for 4D transformation are expected to be proposed to make 4D-pined technology more friendly to the host environments. Meanwhile, it is also important to investigate the interactions between stimuli-responsive materials and the immune system, facilitating the integration between printed structures and the microenvironment of recipient sites. Thus, the interactions between printed structures and microenvironments of the hosts are expected to be harmonized in a smarter and more controllable manner.

Furthermore, the real-life physiological activities of human tissues are much more complex, and cellular activities can be influenced by a variety of stimuli, such as neuro-regulation, humoral regulation, and self-regulation [10,20]. Printed biological constructs usually go through multiple transforming processes before achieving their full functionality. Thus, it is still challenging to fabricate printed objects that can undergo complex shape-transformation processes and functional transitions under multiple stimuli simultaneously.

To advance the scaling-up production and realize the elaborate programmed control of 4D bioprinting in tissue engineering, it is important to introduce computer design technologies and sophisticated multiple stimuli-responsive procedures to realize the fabrication of complex self-transformation objects. The Project Cyborg software designed by MIT is a platform that offers the abilities to simulate self-assembly and programmable materials, as well as the optimization of design constructs [222]. Biosensors and bioactuators have been used for monitoring physical activities in the body [223]. While, further work is required to provide precise control of the stimuli applied to bioprinted structures in a minimally invasive manner.

However, the cost of these stimuli-responsive and programmable biomaterials could be expensive and the manufacture of such materials with sophisticated computer design systems are usually costly. Meanwhile, the scaling-up production should be affordable and manageable. Thus, there is still a trade-off between the feasibility of manufacture and the superiority of 4D bioprinted constructs.

## 7. Conclusions

In conclusion, 4D bioprinting offers the ability to fabricate shape-programmed and functional structures in a controlled way, emerging as a promising approach to construct an active, multiple-layered and functional bone tissue with complex structures. In addition, the advancement of 4D bioprinting will fulfill the upgraded medical requirements and explore its widespread application in the biomedical field. At the same time, more studies are expected to solve the existing challenges before 4D printing serves as a powerful method to establish dynamic and hierarchical architectures of natural tissues or organs.

## Declaration of Competing Interest

The authors declare no conflict of interest.

## Acknowledgment

This work was supported by the National Key Research and Development Program of China (grant number 2018YFB1106900), the National Natural Science Foundation of China (grant number 31600787), the Beijing Natural Science Foundation (7192228), the Project for Culturing Leading Talents in Scientific and Technological Innovation of Beijing (grant number Z171100001117169), the Peking University School of Stomatology for Talented Young Investigators (grant number PKUSS20150107), and Joint Grant from PKUHSC-KCL Joint Institute for Medical Research.

## References

[1] C.W. Hull, Apparatus for production of threedimensional objects by stereolithograph, US Patent US4575330 A (1986).
[2] D. Kokkinis, M. Schaffner, A.R. Studart, Multimaterial magnetically assisted 3D printing of composite materials, Nat. Commun. 6 (2015) 8643.
[3] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, Nat. Biotechnol. 32 (8) (2014) 773–785.
[4] Z. Qin, B.G. Compton, J.A. Lewis, M.J. Buehler, Structural optimization of 3D-printed synthetic spider webs for high strength, Nat. Commun. 6 (2015) 7038.
[5] A. Arslan-Yildiz, R. El Assal, P. Chen, S. Guven, F. Inci, U. Demirci, Towards artificial tissue models: past, present, and future of 3D bioprinting, Biofabrication 8 (1) (2016) 014103.
[6] C. Yu, X. Ma, W. Zhu, P. Wang, K.L. Miller, J. Stupin, A. Koroleva-Maharajh, A. Hairabedian, S. Chen, Scanningless and continuous 3D bioprinting of human tissues with decellularized extracellular matrix, Biomaterials 194 (2019) 1–13.
[7] H. Cui, M. Nowicki, J.P. Fisher, L.G. Zhang, 3D Bioprinting for organ regeneration, Adv. Healthc. Mater. 6 (1) (2016) 1601118.
[8] Y.C. Li, Y.S. Zhang, A. Akpek, S.R. Shin, A. Khademhosseini, 4D bioprinting: the next-generation technology for biofabrication enabled by stimuli-responsive materials, Biofabrication 9 (1) (2016) 012001.
[9] S. Tibbits, 4D Printing: multi-Material shape change, Archit. Des. 84 (1) (2014) 116–121.
[10] B. Gao, Q. Yang, X. Zhao, G. Jin, Y. Ma, F. Xu, 4D Bioprinting for biomedical applications, Trends. Biotechnol. 158 (9) (2016) 746–756.
[11] S. Miao, N. Castro, M. Nowicki, L. Xia, H. Cui, X. Zhou, W. Zhu, S.J. Lee, K. Sarkar, G. Vozzi, Y. Tabata, J. Fisher, L.G. Zhang, 4D printing of polymeric materials for tissue and organ regeneration, Mater. Today 20 (2017) 577–591.
[12] Y. Zhou, W.M. Huang, S.F. Kang, X.L. Wu, H.B. Lu, J. Fu, H.P. Cui, From 3D to 4D printing: approaches and typical applications, J. Mech. Sci. Technol. 29 (10) (2015) 4281–4288.
[13] L.S. Baptista, G.S. Kronemberger, K.R. Silva, J.M. Granjeiro, Spheroids of stem cells as endochondral templates for improved bone engineering, Front. Biosci. (Landmark Ed.) 23 (2018) 1969–1986.
[14] N. Jones, Science in three dimensions: the print revolution, Nature 487 (7405) (2012) 22–23.
[15] A.C. Daly, F.E. Freeman, T. Gonzalez-Fernandez, S.E. Critchley, J. Nulty, D.J. Kelly, 3D Bioprinting for cartilage and osteochondral tissue engineering, Adv. Healthc. Mater. 6 (22) (2017), doi:10.1002/adhm.201700298.
[16] J. Yang, Y.S. Zhang, K. Yue, A. Khademhosseini, Cell-laden hydrogels for osteochondral and cartilage tissue engineering, Acta Biomater. 57 (2017) 1–25.
[17] A.R. Amini, C.T. Laurencin, S.P. Nukavarapu, Bone tissue engineering: recent advances and challenges, Crit. Rev. Biomed. Eng. 40 (5) (2012) 363–408.
[18] S. Bose, M. Roy, A. Bandyopadhyay, Recent advances in bone tissue engineering scaffolds, Trends Biotechnol. 30 (10) (2012) 546–554.
[19] N. Ashammakhi, A. Hasan, O. Kaarela, B. Byambaa, A. Sheikhi, A.K. Gaharwar, A. Khademhosseini, Advancing frontiers in bone bioprinting, Adv. Healthc. Mater. 8 (7) (2019) e1801048.
[20] M. Qasim, D.S. Chae, N.Y. Lee, Advancements and frontiers in nano-based 3D and 4D scaffolds for bone and cartilage tissue engineering, Int. J. Nanomed. 14 (2019) 4333–4351.

[21] L. Zhang, G. Yang, B.N. Johnson, X. Jia, Three-dimensional (3D) printed scaffold and material selection for bone repair, Acta Biomater. 84 (2019) 16–33.
[22] S. Saravanan, S. Vimalraj, P. Thanikaivelan, S. Banudevi, G. Manivasagam, A review on injectable chitosan/beta glycerophosphate hydrogels for bone tissue regeneration, Int. J. Biol. Macromol. 121 (2019) 38–54.
[23] F.S. Senatov, K.V. Niaza, M.Y. Zadorozhnyy, A.V. Maksimkin, S.D. Kaloshkin, Y.Z. Estrin, Mechanical properties and shape memory effect of 3D-printed PLA-based porous scaffolds, J. Mech. Behav. Biomed. Mater. 57 (2016) 139–148.
[24] S. Graham, P.F. Marina, A. Blencowe, Thermoresponsive polysaccharides and their thermoreversible physical hydrogel networks, Carbohydr. Polym. 207 (2019) 143–159.
[25] H. Suo, D. Zhang, J. Yin, J. Qian, Z.L. Wu, J. Fu, Interpenetrating polymer network hydrogels composed of chitosan and photocrosslinkable gelatin with enhanced mechanical properties for tissue engineering, Mater. Sci. Eng. C Mater. Biol. Appl. 92 (2018) 612–620.
[26] C. Li, J.P. Armstrong, I.J. Pence, W. Kit-Anan, J.L. Puetzer, S. Correia Carreira, A.C. Moore, M.M. Stevens, Glycosylated superparamagnetic nanoparticle gradients for osteochondral tissue engineering, Biomaterials 176 (2018) 24–33.
[27] H.D. Kim, S. Amirthalingam, S.L. Kim, S.S. Lee, J. Rangasamy, N.S. Hwang, Biomimetic materials and fabrication approaches for bone tissue engineering, Adv. Healthc. Mater. 6 (23) (2017), doi:10.1002/adhm.201700612.
[28] J. Vaithilingam, P. Sanjuan-Alberte, S. Campora, G.A. Rance, L. Jiang, J. Thorpe, L. Burroughs, C.J. Tuck, C. Denning, R.D. Wildman, R.J.M. Hague, M.R. Alexander, F.J. Rawson, Multifunctional bioinstructive 3D architectures to modulate cellular behavior, Adv. Funct. Mater. 29 (38) (2019), doi:10.1002/adfm.201902016.
[29] Z. Luo, S. Zhang, J. Pan, R. Shi, H. Liu, Y. Lyu, X. Han, Y. Li, Y. Yang, Z. Xu, Y. Sui, E. Luo, Y. Zhang, S. Wei, Time-responsive osteogenic niche of stem cells: a sequentially triggered, dual-peptide loaded, alginate hybrid system for promoting cell activity and osteo-differentiation, Biomaterials 163 (2018) 25–42.
[30] Q. Ge, A.H. Sakhaei, H. Lee, C.K. Dunn, N.X. Fang, M.L. Dunn, Multimaterial 4D printing with tailorable shape memory polymers, Sci. Rep. 6 (2016) 31110.
[31] J.L. Tan, J. Tien, D.M. Pirone, D.S. Gray, K. Bhadriraju, C.S. Chen, Cells lying on a bed of microneedles: an approach to isolate mechanical force, Proc. Natl. Acad. Sci. USA 100 (4) (2003) 1484–1489.
[32] K. Franze, The mechanical control of nervous system development, Development 140 (15) (2013) 3069–3077.
[33] A. Brugués, E. Anon, V. Conte, J.H. Veldhuis, M. Gupta, J. Colombelli, J.J. Muñoz, G.W. Brodland, B. Ladoux, X. Trepat, Forces driving epithelial wound healing, Nat. Phys. 10 (9) (2014) 683–690.
[34] C.J. Underwood, L.T. Edgar, J.B. Hoying, J.A. Weiss, Cell-generated traction forces and the resulting matrix deformation modulate microvascular alignment and growth during angiogenesis, Am. J. Physiol. Heart Circ. Physiol. 307 (2) (2015) H152–H164.
[35] C.M. Kraningrush, J.P. Califano, C.A. Reinhartking, Cellular traction stresses increase with increasing metastatic potential, PLoS ONE 7 (2) (2012) e32572.
[36] R.T. Tranquillo, J.D. Murray, Continuum model of fibroblast-driven wound contraction: inflammation-Mediation, J. Theor. Biol. 158 (2) (1992) 135–172.
[37] N. Ashammakhi, S. Ahadian, F. Zengjie, K. Suthiwanich, F. Lorestani, G. Orive, S. Ostrovidov, A. Khademhosseini, Advances and future perspectives in 4D bioprinting, Biotechnol. J. 13 (12) (2018) 1800148.
[38] K. Kuribayashi-Shigetomi, H. Onoe, S. Takeuchi, Cell origami: self-Folding of three-dimensional cell-laden microstructures driven by cell traction force, PLoS ONE 7 (12) (2012) e51085.
[39] A.C. Schone, B. Schulz, A. Lendlein, Stimuli responsive and multifunctional polymers: progress in materials and applications, Macromol. Rapid Commun. 37 (23) (2016) 1856–1859.
[40] A.S. Gladman, E.A. Matsumoto, R.G. Nuzzo, L. Mahadevan, J.A. Lewis, Biomimetic 4D printing, Nat. Mater. 15 (4) (2016) 413–418.
[41] J.W. Su, X. Tao, H. Deng, C. Zhang, S. Jiang, Y. Lin, J. Lin, 4D printing of a self-morphing polymer driven by a swellable guest medium, Soft Matter 14 (5) (2018) 765–772.
[42] C. Liu, H. Qin, P.T. Mather, Review of progress in shape-memory polymers, J. Mater. Chem. 17 (16) (2007) 1543–1558.
[43] C. LãWenberg, M. Balk, C. Wischke, M. Behl, A. Lendlein, Shape-Memory hydrogels: evolution of structural principles to enable shape switching of hydrophilic polymer networks, Acc. Chem. Res. 50 (4) (2017) 723–732.
[44] S. Naficy, R. Gately, R. Gorkin, H. Xin, G.M. Spinks, 4D Printing of reversible shape morphing hydrogel structures, Macromol. Mater. Eng. 302 (2017) 1600212.
[45] L. Klouda, A.G. Mikos, Thermoresponsive hydrogels in biomedical applications, Eur. J. Pharm. Biopharm. 68 (1) (2008) 34–45.
[46] X. Wang, Y. Sun, C. Peng, H. Luo, R. Wang, D. Zhang, Transitional suspensions containing thermosensitive dispersant for three-dimensional printing, ACS Appl. Mater. Interfaces 7 (47) (2015) 26131–26136.
[47] S.E. Bakarich, R. Gorkin 3rd., M. in het Panhuis, G. Spinks, 4D Printing with mechanically robust, thermally actuating hydrogels, Macromol. Rapid Commun. 36 (12) (2015) 1211–1217.
[48] H. Wei, S.X. Cheng, X.Z. Zhang, R.X. Zhuo, Thermo-sensitive polymeric micelles based on poly(N -isopropylacrylamide) as drug carriers, Prog. Polym. Sci. 34 (9) (2009) 893–910.
[49] J.C. Breger, C. Yoon, R. Xiao, H.R. Kwag, M.O. Wang, J.P. Fisher, T.D. Nguyen, D.H. Gracias, Self-folding thermo-magnetically responsive soft microgrippers, ACS Appl. Mater. Interfaces 7 (5) (2016) 3398–3405.
[50] Y. Pei, J. Chen, L. Yang, L. Shi, Q. Tao, B. Hui, J. Li, The effect of pH on the LCST of poly(N-isopropylacrylamide) and poly(N-isopropylacrylamide-co-acrylic acid), J. Biomater. Sci. Polym. Ed. 15 (5) (2004) 585–594.
[51] S. Zakharchenko, N. Puretskiy, G. Stoychev, M. Stamm, L. Ionov, Temperature controlled encapsulation and release using partially biodegradable thermo–magneto-sensitive self-rolling tubes, Soft Mater. 6 (12) (2010) 2633–2636.
[52] Y. Gao, M. Wei, X. Li, W. Xu, A. Ahiabu, J. Perdiz, Z. Liu, M.J. Serpe, Stimuli-responsive polymers: fundamental considerations and applications, Macromol. Res. 6 (25) (2017) 513–527.
[53] S.H. Hsiao, S.H. Hsu, Synthesis and characterization of dual stimuli-sensitive biodegradable polyurethane soft hydrogels for 3D cell-laden bioprinting, ACS Appl. Mater. Interfaces 10 (35) (2018) 29273–29287.
[54] W.J. Hendrikson, J. Rouwkema, F. Clementi, C.A. van Blitterswijk, S. Farè, L. Moroni, Towards 4D printed scaffolds for tissue engineering: exploiting 3D shape memory polymers to deliver time-controlled stimulus on cultured cells, Biofabrication 9 (3) (2017) 031001.
[55] R. Luo, J. Wu, N.D. Dinh, C.H. Chen, Hydrogels: gradient porous elastic hydrogels with shape-memory property and anisotropic responses for programmable locomotion, Adv. Funct. Mater. 25 (47) (2016) 7272–7279.
[56] I. Apsite, G. Stoychev, W. Zhang, D. Jehnichen, J. Xie, L. Ionov, Porous stimuli-responsive self-folding electrospun mats for 4D biofabrication, Biomacromolecules 18 (10) (2017) 3178–3184.
[57] L. Ionov, 4D Biofabrication: materials, methods, and applications, Adv. Healthc. Mater. 7 (17) (2018) e1800412.
[58] N.A. Peppas, P. Bures, W. Leobandung, H. Ichikawa, Hydrogels in pharmaceutical formulations, Eur. J. Pharm. Biopharm. 50 (1) (2000) 27–46.
[59] L. Li, H. Shan, C.Y. Yue, Y.C. Lam, K.C. Tam, X. Hu, Thermally induced association and dissociation of methylcellulose in aqueous solutions, Langmuir 18 (20) (2002) 7291–7298.
[60] V. Stroganov, S. Zakharchenko, E. Sperling, A.K. Meyer, O.G. Schmidt, L. Ionov, Biodegradable self-folding polymer films with controlled thermo-triggered folding, Adv. Funct. Mater. 24 (27) (2014) 4357–4363.
[61] V. Stroganov, M. Alhussein, J.U. Sommer, A. Janke, S. Zakharchenko, L. Ionov, Reversible thermosensitive biodegradable polymeric actuators based on confined crystallization, Nano Lett. 15 (3) (2015) 1786–1790.
[62] P. Fratzl, F.G. Barth, Biomaterial systems for mechanosensing and actuation, Nature 462 (7272) (2009) 442–448.
[63] R. Elbaum, L. Zaltzman, I. Burgert, P. Fratzl, The role of wheat awns in the seed dispersal unit, Science 316 (5826) (2005) 884–886.
[64] M. Dai, O.T. Picot, J.M. Verjans, L.T.D. Haan, A.P. Schenning, T. Peijs, C.W. Bastiaansen, Humidity-responsive bilayer actuators based on a liquid-crystalline polymer network, ACS Appl. Mater. Interfaces 5 (11) (2013) 4945–4950.
[65] L.T. de Haan, J.M. Verjans, D.J. Broer, C.W. Bastiaansen, A.P. Schenning, Humidity-responsive liquid crystalline polymer actuators with an asymmetry in the molecular trigger that bend, fold, and curl, J. Am. Chem. Soc. 136 (30) (2014) 10585–10588.
[66] M. Jamal, S.S. Kadam, R. Xiao, F. Jivan, T.M. Onn, R. Fernandes, T.D. Nguyen, D.H. Gracias, Bio-Origami hydrogel scaffolds composed of photocrosslinked peg bilayers, Adv. Healthc. Mater. 2 (8) (2013) 1042–1050.
[67] L. Zhang, H. Liang, J. Jacob, P. Naumov, Photogated humidity-driven motility, Nat. Commun. 6 (2015) 7429.
[68] K. Zhang, A. Geissler, M. Standhardt, S. Mehlhase, M. Gallei, C. Marie Thiele, Moisture-responsive films of cellulose stearoyl esters showing reversible shape transitions, Sci. Rep. 5 (2015) 11011.
[69] O.V. Borisova, L. Billon, R.P. Richter, E. Reimhult, O.V. Borisov, pH- and electro-responsive properties of poly(acrylic acid) and poly(acrylic acid)-block-poly(acrylic acid-grad-styrene) brushes studied by quartz crystal microbalance with dissipation monitoring, Langmuir 31 (27) (2015) 7684–7694.
[70] H. Okuzaki, T. Kuwabara, K. Funasaka, T. Saido, Humidity-sensitive polypyrrole films for electro-active polymer actuators, Adv. Funct. Mater. 23 (36) (2013) 4400–4407.
[71] S. Li, K. Shu, C. Zhao, C. Wang, Z. Guo, G. Wallace, H.K. Liu, One-step synthesis of graphene/polypyrrole nanofiber composites as cathode material for a biocompatible zinc/polymer battery, ACS Appl. Mater. Interfaces 6 (19) (2014) 16679–16686.
[72] N. Sood, A. Bhardwaj, S. Mehta, A. Mehta, Stimuli-responsive hydrogels in drug delivery and tissue engineering, Drug Deliv. 23 (3) (2016) 758–780.
[73] Y. Wang, Programmable hydrogels, Biomaterials 178 (2018) 663–680.
[74] R.A. Green, S. Baek, L.A. Poolewarren, P.J. Martens, Conducting polymer-hydrogels for medical electrode applications, Sci. Technol. Adv. Mater. 11 (1) (2010) 014107.
[75] E. Song, J.W. Choi, Conducting polyaniline nanowire and its applications in chemiresistive sensing, Nanomaterials (Basel) 3 (3) (2013) 498–523.
[76] E. Fantino, I. Roppolo, D. Zhang, J. Xiao, A. Chiappone, M. Castellino, Q.Q. Guo, C. Fabrizio Pirri, J. Yang, 3D printing/interfacial polymerization coupling for the fabrication of conductive hydrogel, Macromol. Mater. Eng. 303 (4) (2018) 1700356.
[77] S. Ahadian, R. Obregón, J. Ramónazcón, G. Salazar, H. Shiku, M. Ramalingam, T. Matsue, Carbon nanotubes and graphene-based nanomaterials for stem cell differentiation and tissue regeneration, J. Tissue Eng. Regen. Med. 16 (9) (2016) 8862–8880.
[78] J. Ramón-Azcón, S. Ahadian, R. Obregón, H. Shiku, M. Ramalingam, T. Matsue, Applications of carbon nanotubes in stem cell research, J. Biomed. Nanotechnol. 10 (10) (2014) 2539–2561.

[79] C.W. Foster, M.P. Down, Y. Zhang, X. Ji, S.J. Rowleyneale, G.C. Smith, P.J. Kelly, C.E. Banks, 3D Printed graphene based energy storage devices, Sci. Rep. 7 (2017) 42233.
[80] J.H. Kim, W.S. Chang, D. Kim, J.R. Yang, J.T. Han, G.W. Lee, J.T. Kim, S.K. Seol, 3D printing of reduced graphene oxide nanowires, Adv. Mater. 27 (1) (2015) 157–161.
[81] S.R. Shin, R. Farzad, A. Tamayol, V. Manoharan, P. Mostafalu, Y.S. Zhang, M. Akbari, S.M. Jung, D. Kim, M. Comotto, N. Annabi, F.E. Al-Hazmi, M.R. Dokmeci, A. Khademhosseini, A bioactive carbon nanotube-based ink for printing 2D and 3D flexible electronics, Adv. Mater. 28 (17) (2016) 3280–3289.
[82] A. Servant, V. Leon, D. Jasim, L. Methven, P. Limousin, E.V. Fernandezpacheco, M. Prato, K. Kostarelos, Graphene-based electroresponsive scaffolds as polymeric implants for on-demand drug delivery, Adv. Healthc. Mater. 3 (8) (2015) 1334–1343.
[83] Y. Zhang, D. Zhai, M. Xu, Q. Yao, H. Zhu, J. Chang, C. Wu, 3D-printed bioceramic scaffolds with antibacterial and osteogenic activity, Biofabrication 9 (2) (2017) 025037.
[84] B. Huang, C. Vyas, I. Roberts, Q.A. Poutrel, W.H. Chiang, J.J. Blaker, Z. Huang, P. Bartolo, Fabrication and characterisation of 3D printed MWCNT composite porous scaffolds for bone regeneration, Mater. Sci. Eng. C Mater. Biol. Appl. 98 (2019) 266–278.
[85] E.M. Goncalves, F.J. Oliveira, R.F. Silva, M.A. Neto, M.H. Fernandes, M. Amaral, M. Vallet-Regi, M. Vila, Three-dimensional printed PCL-hydroxyapatite scaffolds filled with CNTs for bone cell growth stimulation, J. Biomed. Mater. Res. B Appl. Biomater. 104 (6) (2016) 1210–1219.
[86] K. Dan, M.J. Tan, M.P. Prabhakaran, B.Q.Y. Chan, S.S. Liow, S. Ramakrishna, X.J. Loh, Biocompatible electrically conductive nanofibers from inorganic-organic shape memory polymers, Colloids Surf. B Biointerfaces 148 (2016) 557–565.
[87] G. Filipcsei, I. Csetneki, A. Szilágyi, M.J. Zrínyi, Magnetic field-responsive smart polymer composites, Oligom.Polym. Compos. Mol. Imprint. 206 (1) (2007) 137–189.
[88] K. Ulbrich, K. Hola, V. Subr, A. Bakandritsos, J. Tucek, R. Zboril, Targeted drug delivery with polymers and magnetic nanoparticles: covalent and noncovalent approaches, release control, and clinical studies, Chem. Rev. 116 (9) (2016) 5338–5431.
[89] Q.I. Zhang, J. Liu, K. Yuan, Z. Zhang, X. Zhang, X. Fang, A multi-controlled drug delivery system based on magnetic mesoporous $Fe_3O_4$ nanopaticles and a phase change material for cancer thermo-chemotherapy, Nanotechnology 28 (40) (2017) 405101.
[90] F.H. Zhang, L.L. Wang, Z.C. Zheng, Y.J. Liu, J.S. Leng, Magnetic programming of 4D printed shape memory composite structures, Compos. A – Appl. Sci. Manuf. 125 (2019), doi:10.1016/j.compositesa.2019.105571.
[91] L.Q. Ren, B.Q. Li, Z.Y. Song, Q.P. Liu, L. Ren, X.L. Zhou, Bioinspired fiber-regulated composite with tunable permanent shape and shape memory properties via 3d magnetic printing, Compos. B – Eng. 164 (2019) 458–466.
[92] D. Wang, S. Zheng, H. Liu, J. Tang, W. Miao, H. Wang, Y. Tian, H. Yang, L. Jiang, A magnetic gated nanofluidic based on the integration of a superhydrophilic nanochannels and a reconfigurable ferrofluid, Adv. Mater. 31 (7) (2019) e1805953.
[93] L. Zhang, X. Zuo, S. Li, M. Sun, H. Xie, K. Zhang, J. Zhou, L. Che, J. Ma, Z. Jia, F. Yang, Synergistic therapy of magnetism-responsive hydrogel for soft tissue injuries, Bioact. Materr. 4 (2019) 160–166.
[94] K. Lalitha, Y.S. Prasad, V. Sridharan, C.U. Maheswari, G. John, S. Nagarajan, A renewable resource-derived thixotropic self-assembled supramolecular gel: magnetic stimuli responsive and real-time self-healing behaviour, RSC Adv. 5 (95) (2015) 77589–77594.
[95] C. Adine, K.K. Ng, S. Rungarunlert, G.R. Souza, J.N. Ferreira, Engineering innervated secretory epithelial organoids by magnetic three-dimensional bioprinting for stimulating epithelial growth in salivary glands, Biomaterials 180 (2018) 52–66.
[96] G.R. Souza, H. Tseng, J.A. Gage, A. Mani, P. Desai, F. Leonard, A. Liao, M. Longo, J.S. Refuerzo, B. Godin, Magnetically bioprinted human myometrial 3D cell rings as a model for uterine contractility, Int. J. Mol. Sci. 18 (4) (2017) e683.
[97] V. Du, N. Luciani, S. Richard, G. Mary, C. Gay, F. Mazuel, M. Reffay, P. Menasché, O. Agbulut, C. Wilhelm, A 3D magnetic tissue stretcher for remote mechanical control of embryonic stem cell differentiation, Nat. Commun. 8 (1) (2017) 400.
[98] G.R. Souza, J.R. Molina, R.M. Raphael, M.G. Ozawa, D.J. Stark, C.S. Levin, L.F. Bronk, J.S. Ananta, J. Mandelin, M.M. Georgescu, J.A. Bankson, J.G. Gelovani, T.C. Killian, W. Arap, R. Pasqualini, Three-dimensional tissue culture based on magnetic cell levitation, Nat. Nanotechnol. 5 (4) (2010) 291–296.
[99] T. Sun, Q. Shi, Q. Huang, H. Wang, X. Xiong, C. Hu, et al., Magnetic alginate microfibers as scaffolding elements for the fabrication of microvascular-like structures, Acta Biomater. 66 (2018) 272–281.
[100] A. Tocchio, N.G. Durmus, K. Sridhar, V. Mani, B. Coskun, R. El Assal, U. Demirci, Magnetically guided self-assembly and coding of 3D living architectures, Adv. Mater. 30 (4) (2018) 1–15.
[101] S. Tasoglu, C.H. Yu, V. Liaudanskaya, S. Guven, C. Migliaresi, U. Demirci, Magnetic levitational assembly for living material fabrication, Adv. Healthc. Mater. 4 (10) (2015) 1469–1476 1422.
[102] V.A. Parfenov, E.V. Koudan, E.A. Bulanova, P.A. Karalkin, F.D. Pereira, N.E. Norkin, A.D. Knyazeva, A.A. Gryadunova, O.F. Petrov, M.M. Vasiliev, M.I. Myasnikov, V.P. Chernikov, V.A. Kasyanov, A.Y. Marchenkov, K. Brakke, Y.D. Khesuani, U. Demirci, V.A. Mironov, Scaffold-free, label-free and nozzle-free biofabrication technology using magnetic levitational assembly, Biofabrication 10 (2018) 034104.
[103] A.R. Abdel Fattah, S. Mishriki, T. Kammann, R.P. Sahu, F. Geng, I.K. Puri, 3D cellular structures and co-cultures formed through the contactless magnetic manipulation of cells on adherent surfaces, Biomater. Sci. 6 (3) (2018) 683–694.
[104] E. Turker, N. Demircak, A. Arslan-Yildiz, Scaffold-free three-dimensional cell culturing using magnetic levitation, Biomater. Sci. 6 (7) (2018) 1745–1753.
[105] S. Tasoglu, D. Kavaz, U.A. Gurkan, S. Guven, P. Chen, R. Zheng, U. Demirci, Hydrogels: paramagnetic levitational assembly of hydrogels, Adv. Mater. 25 (8) (2013) 1137–1143.
[106] S. Tasoglu, C.H. Yu, H.I. Gungordu, S. Guven, T. Vural, U. Demirci, Guided and magnetic self-assembly of tunable magnetoceptive gels, Nat. Commun. 5 (2014) 4702.
[107] Y. Qin, J. Chen, Y. Bi, X. Xu, H. Zhou, J. Gao, Y. Hu, Y. Zhao, Z. Chai, Near-infrared light remote-controlled intracellular anti-cancer drug delivery using thermo/pH sensitive nanovehicle, Acta Biomater. 17 (2015) 201–209.
[108] A. Bagheri, H. Arandiyan, C. Boyer, M. Lim, Lanthanide-Doped upconversion nanoparticles: emerging intelligent light-activated drug delivery systems, Adv. Sci. (Weinh) 3 (7) (2016) 1500437.
[109] M. Nakayama, W.Q. Lim, S. Kajiyama, A. Kumamoto, Y. Ikuhara, T. Kato, Y. Zhao, Liquid-crystalline hydroxyapatite/polymer nanorod hybrids: potential bioplatform for photodynamic therapy and cellular scaffolds, ACS Appl. Mater Interfaces 11 (19) (2019) 17759–17765.
[110] S.A. Kumar, M. Alonzo, S.C. Allen, L. Abelseth, V. Thakur, J. Akimoto, Y. Ito, S.M. Willerth, L. Suggs, M. Chattopadhyay, B. Joddar, A visible light-cross-linkable, fibrin-gelatin-based bioprinted construct with human cardiomyocytes and fibroblasts, ACS Biomater. Sci. Eng. 5 (9) (2019) 4551–4563.
[111] Y. Luo, X. Lin, B. Chen, X. Wei, Cell-laden four-dimensional bioprinting using near-infrared-triggered shape-morphing alginate/polydopamine bioinks, Biofabrication 11 (4) (2019) 045019.
[112] H.T. Cui, S.D. Miao, T. Esworthy, S.J. Lee, X. Zhou, S.Y. Hann, T.J. Webster, B.T. Harris, L.G. Zhang, A novel near-infrared light responsive 4D printed nanoarchitecture with dynamically and remotely controllable transformation, Nano Res. 12 (6) (2019) 1381–1388.
[113] H. Wei, Q. Zhang, Y. Yao, L. Liu, Y. Liu, J. Leng, Direct-write fabrication of 4D active shape-changing structures based on a shape memory polymer and its nanocomposite, ACS Appl. Mater. Interfaces 9 (1) (2017) 876–883.
[114] X. Kuang, K. Chen, C.K. Dunn, J. Wu, V.C.F. Li, H.J. Qi, 3D printing of highly stretchable, shape-memory and self-healing elastomer toward novel 4D printing, ACS Appl. Mater. Interfaces 10 (8) (2018) 7381–7388.
[115] T.E. Brown, K.S. Anseth, Spatiotemporal hydrogel biomaterials for regenerative medicine, Chem. Soc. Rev. 46 (21) (2017) 6532–6552.
[116] D.R. Griffin, A.M. Kasko, Photodegradable macromers and hydrogels for live cell encapsulation and release, J. Am. Chem. Soc. 134 (31) (2012) 13103–13107.
[117] C.K. Arakawa, B.A. Badeau, Y. Zheng, C.A. Deforest, Multicellular vascularized engineered tissues through user-programmable biomaterial photodegradation, Adv. Mater. 29 (37) (2017) 201703156.
[118] K.S. Lim, B.J. Klotz, G.C.J. Lindberg, F.P.W. Melchels, G.J. Hooper, J. Malda, D. Gawlitta, T.B.F. Woodfield, Visible light cross-linking of gelatin hydrogels offers an enhanced cell microenvironment with improved light penetration depth, Macromol. Biosci. 19 (6) (2019) e1900098.
[119] S.M. Naseer, A. Manbachi, M. Samandari, P. Walch, Y. Gao, Y.S. Zhang, F. Davoudi, W. Wang, K. Abrinia, J.M. Cooper, A. Khademhosseini, S.R. Shin, Surface acoustic waves induced micropatterning of cells in gelatin methacryloyl (GelMA) hydrogels, Biofabrication 9 (1) (2017) 015020.
[120] K. Melde, E. Choi, Z. Wu, S. Palagi, T. Qiu, P. Fischer, Acoustic fabrication via the assembly and fusion of particles, Adv. Mater. 30 (3) (2018), doi:10.1002/adma.201704507.
[121] N. Huebsch, C.J. Kearney, X. Zhao, J. Kim, C.A. Cezar, Z. Suo, D.J. Mooney, Ultrasound-triggered disruption and self-healing of reversibly cross-linked hydrogels for drug delivery and enhanced chemotherapy, Proc. Natl. Acad. Sci. USA 111 (27) (2014) 9762–9767.
[122] C. Bouyer, P. Chen, S. Guven, T.T. Demirtas, T.J. Nieland, F. Padilla, U. Demirci, A bio-acoustic levitational (BAL) assembly method for engineering of multilayered, 3D brain-like constructs, using human embryonic stem cell derived neuro-progenitors, Adv. Mater. 28 (1) (2016) 161–167.
[123] G. Kocak, C. Tuncer, V. Bütün, pH-Responsive polymers, Polym. Chem. 8 (1) (2016) 144–176.
[124] M. Nadgorny, A. Ameli, Functional polymers and nanocomposites for 3D printing of smart structures and devices, ACS Appl. Mater. Interfaces 10 (21) (2018) 17489–17507.
[125] M. Nadgorny, Z. Xiao, C. Chen, L.A. Connal, Three-dimensional printing of pH-Responsive and functional polymers on an affordable desktop printer, ACS Appl. Mater. Interfaces 8 (42) (2016) 28946–28954.
[126] C. Chen, M.L. Zhao, R.K. Zhang, G. Lu, C.Y. Zhao, F. Fu, H.T. Sun, S. Zhang, Y. Tu, X.H. Li, Collagen/heparin sulfate scaffolds fabricated by a 3D bioprinter improved mechanical properties and neurological function after spinal cord injury in rats, J. Biomed. Mater. Res. A 105 (5) (2017) 1324–1332.
[127] V. Lee, G. Singh, J.P. Trasatti, C. Bjornsson, X. Xu, T.N. Tran, S.S. Yoo, G. Dai, P. Karande, Design and fabrication of human skin by three-dimensional bioprinting, Tissue Eng. C Methods 20 (6) (2014) 473–484.

[128] D.E. Clarke, C.D.J. Parmenter, O.A. Scherman, Tunable pentapeptide self--assembled beta-Sheet hydrogels, Angew. Chem. Int. Ed. Engl. 57 (26) (2018) 7709–7713.

[129] M.S.M. Eldin, E.A. Kamoun, M.A. Sofan, S.M. Elbayomi, l -Arginine grafted alginate hydrogel beads: a novel pH-sensitive system for specific protein delivery, Arab. J. Chem. 8 (3) (2015) 355–365.

[130] M. Kashi, F. Baghbani, F. Moztarzadeh, H. Mobasheri, E. Kowsari, Green synthesis of degradable conductive thermosensitive oligopyrrole/chitosan hydrogel intended for cartilage tissue engineering, Int. J. Biol. Macromol. 107 (Pt B) (2018) 1567–1575.

[131] P. Mukhopadhyay, K. Sarkar, S. Bhattacharya, A. Bhattacharyya, R. Mishra, P.P. Kundu, pH sensitive N-succinyl chitosan grafted polyacrylamide hydrogel for oral insulin delivery, Carbohydr. Polym. 112 (2) (2014) 627–637.

[132] Y. Xu, J. Han, Y. Chai, S. Yuan, H. Lin, X. Zhang, Development of porous chitosan/tripolyphosphate scaffolds with tunable uncross-linking primary amine content for bone tissue engineering, Mater. Sci. Eng. C Mater. Biol. Appl. 85 (2018) 182–190.

[133] L.A. Hockaday, K.H. Kang, N.W. Colangelo, P.Y.C. Cheung, B. Duan, E. Malone, J. Wu, L.N. Girardi, L.J. Bonassar, H. Lipson, C.C. Chu, J.T. Butcher, Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds, Biofabrication 4 (3) (2012) 035005.

[134] A.G. Tabriz, M.A. Hermida, N.R. Leslie, W. Shu, Three-dimensional bioprinting of complex cell laden alginate hydrogel structures, Biofabrication 7 (4) (2015) 045012.

[135] Z. Ren, Y. Zhang, Y. Li, B. Xu, W. Liu, Hydrogen bonded and ionically crosslinked high strength hydrogels exhibiting $Ca^{2+}$-triggered shape memory properties and volume shrinkage for cell detachment, J. Mater. Chem. B 3 (30) (2015) 6347–6354.

[136] T. Bai, Y. Han, P. Zhang, W. Wang, W. Liu, Zinc ion-triggered two-way macro-/microscopic shape changing and memory effects in high strength hydrogels with pre-programmed unilateral patterned surfaces, Soft Matter. 8 (25) (2012) 6846–6852.

[137] W. Nan, W. Wang, H. Gao, W. Liu, Fabrication of a shape memory hydrogel based on imidazole–zinc ion coordination for potential cell-encapsulating tubular scaffold application, Soft Matter. 9 (1) (2012) 132–137.

[138] T. Jungst, W. Smolan, K. Schacht, T. Scheibel, J. Groll, Strategies and molecular design criteria for 3D printable hydrogels, Chem. Rev. 116 (3) (2016) 1496–1539.

[139] A. Skardal, A. Atala, Biomaterials for integration with 3-D bioprinting, Ann. Biomed. Eng. 43 (3) (2015) 730–746.

[140] A. Kirillova, R. Maxson, G. Stoychev, C.T. Gomillion, L.J. Ionov, 4D Biofabrication using shape-morphing hydrogels, Adv. Mater. 29 (46) (2017), doi:10.1002/adma.201703443.

[141] P. Camacho, H. Busari, K.B. Seims, P. Schwarzenberg, H.L. Dailey, L.W. Chow, 3D printing with peptide-polymer conjugates for single-step fabrication of spatially functionalized scaffolds, Biomater. Sci. 7 (10) (2019) 4237–4247.

[142] P.J. Skrzeszewska, L.N. Jong, F.A. de Wolf, M.A. Cohen Stuart, d.G.J. Van, Shape-memory effects in biopolymer networks with collagen-like transient nodes, Biomacromolecules 12 (6) (2011) 2285–2292.

[143] Q. Hao, M. Ghodousi, Y. Du, C. Grun, H. Bae, Y. Peng, A. Khademhosseini, DNA-directed self-assembly of shape-controlled hydrogels, Nat. Commun. 4 (9) (2013) 2275.

[144] W. Guo, C.H. Lu, R. Orbach, F. Wang, X.J. Qi, A. Cecconello, D. Seliktar, I. Willner, pH-stimulated DNA hydrogels exhibiting shape-memory properties, Adv. Mater. 27 (1) (2015) 73–78.

[145] M.E. Todhunter, N.Y. Jee, A.J. Hughes, M.C. Coyle, A. Cerchiari, J. Farlow, J.C. Garbe, M.A. LaBarge, T.A. Desai, Z.J. Gartner, Programmed synthesis of three-dimensional tissues, Nat. Methods 12 (10) (2015) 975–981.

[146] J. Kim, Y. Park, G. Tae, K.B. Lee, S.J. Hwang, I.S. Kim, I. Noh, K. Sun, Synthesis and characterization of matrix metalloprotease sensitive-low molecular weight hyaluronic acid based hydrogels, J. Mater. Sci. Mater. Med. 19 (11) (2008) 3311–3318.

[147] N. Broguiere, F. Formica, G. Barreto, M. Zenobi-Wong, Sortase a as a cross-linking enzyme in tissue engineering, Acta Biomater. 77 (2018) 182–190.

[148] J.B. Costa, J. Silva-Correia, J.M. Oliveira, L.R. Rui, Fast setting silk fibroin bioink for bioprinting of patient-specific memory-shape implants, Adv. Healthc. Mater. 6 (22) (2017) 1701021.

[149] L.P. Yan, J.M. Oliveira, A.L. Oliveira, R.L. Reis, R. Medicine, Core-shell silk hydrogels with spatially tuned conformations as drug-delivery system, J. Tissue Eng. Regen. Med. 11 (11) (2016) 3168–3177.

[150] C.A. Mandon, L.J. Blum, C.A. Marquette, Adding biomolecular recognition capability to 3D printed objects, Anal. Chem. 88 (21) (2017) 10767–10772.

[151] N.J. Castro, C. Meinert, P. Levett, D.W. Hutmacher, Current developments in multifunctional smart materials for 3D/4D bioprinting, Curr. Opin. Biomed. Eng. 2 (2017) 67–75.

[152] A.S. Gladman, E.A. Matsumoto, R.G. Nuzzo, L. Mahadevan, J.A. Lewis, Biomimetic 4D printing, Nat. Mater. 15 (4) (2016) 413–418.

[153] J. Malda, J. Visser, F.P. Melchels, T. Jüngst, W.E. Hennink, W.J.A. Dhert, J. Groll J, D.W. Hutmacher, 25th Anniversary article: engineering hydrogels for biofabrication, Adv. Mater. 25 (36) (2013) 5011–5028.

[154] B. Lanfer, F.P. Seib, U. Freudenberg, D. Stamov, T. Bley, M. Bornhäuser, C. Werner, The growth and differentiation of mesenchymal stem and progenitor cells cultured on aligned collagen matrices, Biomaterials 30 (30) (2009) 5950–5958.

[155] M. Costantini, S. Testa, P. Mozetic, A. Barbetta, C. Fuoco, E. Fornetti, F. Tamiro, S. Bernardini, J. Jaroszewicz, W. Święszkowski, M. Trombetta, L. Castagnoli, D. Seliktar, P. Garstecki, G. Cesareni, S. Cannata, A. Rainer, C. Gargioli, Microfluidic-enhanced 3D bioprinting of aligned myoblast-laden hydrogels leads to functionally organized myofibers in vitro and in vivo, Biomaterials 131 (2017) 98–110.

[156] J. Koffler, W. Zhu, X. Qu, O. Platoshyn, J.N. Dulin, J. Brock, L. Graham, P. Lu, J. Sakamoto, M. Marsala, S.C. Chen, M.H. Tuszynski, Biomimetic 3D-printed scaffolds for spinal cord injury repair, Nat. Med. 25 (2) (2019) 263–269.

[157] M. Betsch, C. Cristian, Y.Y. Lin, A. Blaeser, J. Schöneberg, M. Vogt, E.M. Buhl, H. Fischer, D.F. Duarte Campos, Incorporating 4D into bioprinting: real-time magnetically directed collagen fiber alignment for generating complex multilayered tissues, Adv. Healthc. Mater. 7 (21) (2018) e1800894.

[158] S. Miao, H. Cui, M. Nowicki, S.J. Lee, J. Almeida, X. Zhou, W. Zhu, X. Yao, F. Masood, M.W. Plesniak, M. Mohiuddin, L.G. Zhang, Photolithographic-stereolithographic-tandem fabrication of 4D smart scaffolds for improved stem cell cardiomyogenic differentiation, Biofabrication 10 (3) (2018) 035007.

[159] S. Miao, M. Nowicki, H. Cui, S.J. Lee, X. Zhou, D.K. Mills, L.G. Zhang, 4D anisotropic skeletal muscle tissue constructs fabricated by staircase effect strategy, Biofabrication 11 (3) (2019) 035030.

[160] G. Villar, A.D. Graham, H. Bayley, A tissue-like printed material, Science 340 (6128) (2013) 48–52.

[161] M.J. Booth, V.R. Schild, A.D. Graham, S.N. Olof, H. Bayley, Light-activated communication in synthetic tissues, Sci. Adv. 2 (4) (2016) e1600056.

[162] A.K. Miri, A. Khalilpour, B. Cecen, S. Maharjan, S.R. Shin, A. Khademhosseini, Multiscale bioprinting of vascularized models, Biomaterials 198 (2019) 204–216.

[163] C. Norotte, F. Marga, L. Niklason, G. Forgacs, Scaffold-Free vascular tissue engineering using bioprinting, Biomaterials 30 (30) (2009) 5910–5917.

[164] R.J. Morrison, S.J. Hollister, M.F. Niedner, M.G. Mahani, A.H. Park, D.K. Mehta, R.G. Ohye, G.E. Green, Mitigation of tracheobronchomalacia with 3D-printed personalized medical devices in pediatric patients, Sci. Transl. Med. 7 (285) (2015) 285ra264.

[165] J. Park, Y.V. Kalinin, S. Kadam, C.L. Randall, D.H. Gracias, Design for a lithographically patterned bioartificial endocrine pancreas, Artif. Organs 37 (12) (2013) 1059–1067.

[166] T. Wang, L. Chen, T. Shen, D. Wu, Preparation and properties of a novel thermo-sensitive hydrogel based on chitosan/hydroxypropyl methylcellulose/glycerol, Int. J. Biol. Macromol. 93 (Pt A) (2016) 775–782.

[167] Y. Cai, J. Cao, C. Xu, J. Zhou, Thermo-responsive behaviors and bioactivities of hydroxybutyl chitosans prepared in alkali/urea aqueous solutions, Carbohydr. Polym. 215 (2019) 90–98.

[168] C. Li, K. Wang, X. Zhou, T. Li, Y. Xu, L. Qiang, M. Peng, Y. Xu, L. Xie, C. He, B. Wang, J. Wang, Controllable fabrication of hydroxybutyl chitosan/oxidized chondroitin sulfate hydrogels by 3D bioprinting technique for cartilage tissue engineering, Biomed. Mater. 14 (2) (2019) 025006.

[169] A. Parameswaran-Thankam, C.M. Parnell, F. Watanabe, A.B. RanguMagar, B.P. Chhetri, P.K. Szwedo, A.S. Biris, A. Ghosh, Guar-Based injectable thermoresponsive hydrogel as a scaffold for bone cell growth and controlled drug delivery, ACS Omega 3 (11) (2018) 15158–15167.

[170] J. Yang, R. van Lith, K. Baler, R.A. Hoshi, G.A. Ameer, A thermoresponsive biodegradable polymer with intrinsic antioxidant properties, Biomacromolecules 15 (11) (2014) 3942–3952.

[171] J.P. Chen, M.J. Tsai, H.T. Liao, Incorporation of biphasic calcium phosphate microparticles in injectable thermoresponsive hydrogel modulates bone cell proliferation and differentiation, Colloids Surf. B Biointerfaces 110 (2013) 120–129.

[172] X. Chen, Y. Zhao, S. Geng, R.J. Miron, Q. Zhang, C. Wu, Y. Zhang, In vivo experimental study on bone regeneration in critical bone defects using PIB nanogels/boron-containing mesoporous bioactive glass composite scaffold, Int. J. Nanomed. 10 (2015) 839–846.

[173] C. Zhao, Z. Zeng, N.T. Qazvini, X. Yu, R. Zhang, S. Yan, Y. Shu, Y. Zhu, C. Duan, E. Bishop, J. Lei, W. Zhang, C. Yang, K. Wu, Y. Wu, L. An, S. Huang, X. Ji, C. Gong, C. Yuan, L. Zhang, W. Liu, B. Huang, Y. Feng, B. Zhang, Z. Dai, Y. Shen, X. Wang, W. Luo, L. Oliveira, A. Athiviraham, M.J. Lee, J.M. Wolf, G.A. Ameer, R.R. Reid, T.C. He, W. Huang, Thermoresponsive citrate-based graphene oxide scaffold enhances bone regeneration from BMP9-Stimulated adipose-derived mesenchymal stem cells, ACS Biomater. Sci. Eng. 4 (8) (2018) 2943–2955.

[174] J. Wu, K. Zheng, X. Huang, J. Liu, H. Liu, A.R. Boccaccini, Y. Wan, X. Guo, Z. Shao, Thermally triggered injectable chitosan/silk fibroin/bioactive glass nanoparticle hydrogels for in-situ bone formation in rat calvarial bone defects, Acta Biomater. 91 (2019) 60–71.

[175] L. Vojtova, L. Michlovska, K. Valova, M. Zboncak, M. Trunec, K. Castkova, M. Krticka, V. Pavlinakova, P. Polacek, M. Dzurov, V. Lukasova, M. Rampichova, T. Suchy, R. Sedlacek, M.P. Ginebra, E.B. Montufar, The effect of the thermosensitive biodegradable plga(-)peg(-)plga copolymer on the rheological, structural and mechanical properties of thixotropic self-hardening tricalcium phosphate cement, Int. J. Mol. Sci. 20 (2) (2019), doi:10.3390/ijms20020391.

[176] A.S. Azevedo, M.J. Sa, M.V. Fook, P.I. Neto, O.B. Sousa, S.S. Azevedo, M.W. Teixeira, F.S. Costa, A.L. Araujo, Use of chitosan and beta-tricalcium phosphate, alone and in combination, for bone healing in rabbits, J. Mater. Sci. Mater. Med. 25 (2) (2014) 481–486.

[177] M. Dessi, A. Borzacchiello, T.H. Mohamed, W.I. Abdel-Fattah, L. Ambrosio, Novel biomimetic thermosensitive beta-tricalcium phosphate/chitosan-based hydrogels for bone tissue engineering, J. Biomed. Mater. Res. A 101 (10) (2013) 2984–2993.

[178] C.D. Moreira, S.M. Carvalho, H.S. Mansur, M.M. Pereira, Thermogelling chitosan-collagen-bioactive glass nanoparticle hybrids as potential injectable systems for tissue engineering, Mater. Sci. Eng. C Mater. Biol. Appl. 58 (2016) 1207–1216.
[179] T.T. Demirtas, G. Irmak, M. Gumusderelioglu, A bioprintable form of chitosan hydrogel for bone tissue engineering, Biofabrication 9 (3) (2017) 035003.
[180] X. Liu, B. Yu, Q. Huang, R. Liu, Q. Feng, Q. Cai, S. Mi, In vitro BMP-2 peptide release from thiolated chitosan based hydrogel, Int. J. Biol. Macromol. 93 (Pt A) (2016) 314–321.
[181] G. Chang, Y. Cai, X. Kong, G. Han, J. Yao, Development and characterization of injectable chitosan-based hydrogels containing dexamethasone/rhBMP-2 loaded hydroxyapatite nanoparticles, Mater. Lett. 93 (1) (2013) 312–315.
[182] N. Nafee, M. Zewail, N. Boraie, Alendronate-loaded, biodegradable smart hydrogel: a promising injectable depot formulation for osteoporosis, J. Drug Target 26 (7) (2018) 563–575.
[183] S. Miao, P. Wang, Z. Su, S. Zhang, Vegetable-oil-based polymers as future polymeric biomaterials, Acta Biomater. 10 (4) (2014) 1692–1704.
[184] S. Miao, W. Zhu, N.J. Castro, J. Leng, L.G. Zhang, Four-dimensional printing hierarchy scaffolds with highly biocompatible smart polymers for tissue engineering applications, Tissue Eng. C Methods 22 (10) (2016) 952–963.
[185] S. Miao, W. Zhu, N.J. Castro, M. Nowicki, X. Zhou, H. Cui, J.P. Fisher, L.G. Zhang, 4D printing smart biomedical scaffolds with novel soybean oil epoxidized acrylate, Sci. Rep. 6 (2016) 27226.
[186] F. Pati, T.H. Song, G. Rijal, J. Jang, S.W. Kim, D.W. Cho, Ornamenting 3D printed scaffolds with cell-laid extracellular matrix for bone tissue regeneration, Biomaterials 37 (2015) 230–241.
[187] H. Kang, Z. Hou, Q.H. Qin, Experimental study of time response of bending deformation of bone cantilevers in an electric field, J. Mech. Behav. Biomed. Mater. 77 (2018) 192–198.
[188] B. Tandon, J.J. Blaker, S.H. Cartmell, Piezoelectric materials as stimulatory biomedical materials and scaffolds for bone repair, Acta Biomater. 73 (2018) 1–20.
[189] J. Jacob, N. More, K. Kalia, G. Kapusetti, Piezoelectric smart biomaterials for bone and cartilage tissue engineering, Inflamm. Regen. 38 (2018) 2, doi:10.1186/s41232-018-0059-8.
[190] Y. Tang, C. Wu, Z. Wu, L. Hu, W. Zhang, K. Zhao, Fabrication and in vitro biological properties of piezoelectric bioceramics for bone regeneration, Sci. Rep. 7 (2017) 43360, doi:10.1038/srep43360.
[191] A.S. Neto, J.M.F. Ferreira, Synthetic and marine-derived porous scaffolds for bone tissue engineering, Materials 11 (9) (2018) E1702.
[192] G.D. Barabaschi, V. Manoharan, Q. Li, L.E. Bertassoni, Engineering pre-vascularized scaffolds for bone regeneration, Adv. Exp. Med. Biol. 881 (2015) 79–94.
[193] B.P. Dos Santos, B. Garbay, M. Fenelon, M. Rosselin, E. Garanger, S. Lecommandoux, H. de Oliveira, J. Amedee, Development of a cell-free and growth factor-free hydrogel capable of inducing angiogenesis and innervation after subcutaneous implantation, Acta Biomater. (2019), doi:10.1016/j.actbio.2019.08.028.
[194] C.D. Devillard, C.A. Mandon, S.A. Lambert, L.J. Blum, C.A.J.B.J. Marquette, Bioinspired multi-activities 4D printing objects: a new approach toward complex tissue engineering, Biotechnol. J. 13 (12) (2018) e1800098.
[195] S. Miao, H. Cui, M. Nowicki, L. Xia, X. Zhou, S.J. Lee, W. Zhu, K. Sarkar, Z. Zhang, L.G. Zhang, Stereolithographic 4D bioprinting of multiresponsive architectures for neural engineering, Adv. Biosyst. 2 (9) (2018), doi:10.1002/adbi.201800101.
[196] I. Apsite, J.M. Uribe, A.F. Posada, S. Rosenfeldt, S. Salehi, L. Ionov, 4D biofabrication of skeletal muscle microtissues, Biofabrication (2019), doi:10.1088/1758-5090/ab4cc4.
[197] T.J. Esworthy, S.D. Miao, S.J. Lee, X. Zhou, H.T. Cui, Y.Y. Zuo, L.J.G. Zhang, Advanced 4D-bioprinting technologies for brain tissue modeling and study, Int. J. Smart Nano Mater. 10 (3) (2019) 177–204.
[198] D. Grinberg, S. Siddique, M.Q. Le, R. Liang, J.F. Capsal, P.J. Cottinet, 4D Printing based piezoelectric composite for medical applications, J. Polym. Sci. B – Polym. Phys. 57 (2) (2019) 109–115.
[199] E. Tomaskovic-Crook, P. Zhang, A. Ahtiainen, H. Kaisvuo, C.Y. Lee, S. Beirne, Z. Aqrawe, D. Svirskis, J. Hyttinen, G.G. Wallace, J. Travas-Sejdic, J.M. Crook, Human neural tissues from neural stem cells using conductive biogel and printed polymer microelectrode arrays for 3D electrical stimulation, Adv. Healthc. Mater. 8 (15) (2019) e1900425.
[200] M. Ali, A.K. Pr, J.J. Yoo, F. Zahran, A. Atala, S.J. Lee, A photo-crosslinkable kidney ECM-Derived bioink accelerates renal tissue formation, Adv. Healthc. Mater. 8 (7) (2019) e1800992.
[201] Y. Wang, Y. Miao, J. Zhang, J.P. Wu, T.B. Kirk, J. Xu, D. Ma, W. Xue, Three--dimensional printing of shape memory hydrogels with internal structure for drug delivery, Mater. Sci. Eng. C Mater. Biol. Appl. 84 (2018) 44–51.
[202] J.W. Su, W.X. Gao, K. Trinh, S.M. Kendere, E.T. Pulatsu, C. Zhang, A. Whittingto, M.S. Lin, J. Lin, 4D printing of polyurethane paint-based composites, Int. J. Smart Nano Mater. 10 (3) (2019) 237–248.
[203] D. Kim, T. Kim, Y.G. Lee, 4D Printed bifurcated stents with kirigami-inspired structures, J. Vis. Exp. (149) (2019), doi:10.3791/59746.
[204] M. Bodaghi, W.H. Liao, 4D printed tunable mechanical metamaterials with shape memory operations, Smart Mater. Struct. 28 (4) (2019), doi:10.1088/1361-665X/ab0b6b.
[205] L. Wu, J. Virdee, E. Maughan, A. Darbyshire, G. Jell, M. Loizidou, M. Emberton, P. Butler, A. Howkins, A. Reynolds, I.W. Boyd, M. Birchall, W. Song, Stiffness memory nanohybrid scaffolds generated by indirect 3D printing for biologically responsive soft implants, Acta Biomater. 80 (2018) 188–202.
[206] S.J. Bryant, F.J. Vernerey, Programmable hydrogels for cell encapsulation and neo-tissue growth to enable personalized tissue engineering, Adv. Healthc. Mater. 7 (1) (2018), doi:10.1002/adhm.201700605.
[207] M. Invernizzi, S. Turri, M. Levi, R. Suriano, Processability of 4D printable modified polycaprolactone with self-healing abilities, Mater. Today – Proc. 7 (2019) 508–515.
[208] X. Zhao, C.X. Yang, L.G. Chen, X.P. Yan, Dual-stimuli responsive and reversibly activatable theranostic nanoprobe for precision tumor-targeting and fluorescence-guided photothermal therapy, Nat. Commun. 8 (2017) 14998.
[209] F. Zhang, N. Zhang, H.X. Meng, H.X. Liu, Y.Q. Lu, C.M. Liu, Z.M. Zhang, K.Y. Qu, N.P. Huang, Easy applied gelatin-based hydrogel system for long-term functional cardiomyocyte culture and myocardium formation, ACS Biomater. Sci. Eng. 5 (6) (2019) 3022–3031.
[210] Q. Tang, T.N. Plank, T. Zhu, H. Yu, Z. Ge, Q. Li, L. Li, J.T. Davis, H. Pei, Self-Assembly of metallo-nucleoside hydrogels for injectable materials that promote wound closure, ACS Appl. Mater Interfaces 11 (22) (2019) 19743–19750.
[211] D. Tukmachev, O. Lunov, V. Zablotskii, A. Dejneka, M. Babic, E. Sykova, S. Kubinova, An effective strategy of magnetic stem cell delivery for spinal cord injury therapy, Nanoscale 7 (9) (2015) 3954–3958.
[212] W.S. Yun, J.S. Choi, H.M. Ju, M.H. Kim, S.J. Choi, E.S. Oh, Y.J. Seo, J. Key, Enhanced homing technique of mesenchymal stem cells using iron oxide nanoparticles by magnetic attraction in olfactory-injured mouse models, Int. J. Mol. Sci. 19 (5) (2018), doi:10.3390/ijms19051376.
[213] I.R. Konig, O. Fuchs, G. Hansen, E. von Mutius, M.V. Kopp, What is precision medicine? Eur. Resspir. J. 50 (4) (2017), doi:10.1183/13993003.00391-2017.
[214] M.E. Gomes, M.T. Rodrigues, R.M.A. Domingues, R.L. Reis, Tissue engineering and regenerative medicine: new trends and directions-a year in review, Tissue Eng. B Rev. 23 (3) (2017) 211–224.
[215] P. Rychter, E. Pamula, A. Orchel, U. Posadowska, M. Krokborkowicz, A. Kaps, N. Smigielgac, A. Smola, J. Kasperczyk, W. Prochwicz, P. Dobrzynski, Scaffolds with shape memory behavior for the treatment of large bone defects, J. Biomed. Mater. Res. A 103 (11) (2015) 3503–3515.
[216] D. Zhang, O.J. George, K.M. Petersen, A.C. Jimenezvergara, M.S. Hahn, M.A. Grunlan, A bioactive "self-fitting" shape memory polymer scaffold with potential to treat cranio-maxillo facial bone defects, Acta Biomater. 10 (11) (2014) 4597–4605.
[217] L.F. Tseng, J. Wang, R.M. Baker, G. Wang, P.T. Mather, J.H. Henderson, Osteogenic capacity of human adipose-derived stem cells is preserved following triggering of shape memory scaffolds, Tissue Eng. A 22 (15–16) (2016) 1026–1035.
[218] R.M. Baker, L.F. Tseng, M.T. Iannolo, M.E. Oest, J.H. Henderson, Self-deploying shape memory polymer scaffolds for grafting and stabilizing complex bone defects: a mouse femoral segmental defect study, Biomaterials 76 (2016) 388–398.
[219] X. Liu, K. Zhao, T. Gong, J. Song, C. Bao, E. Luo, J. Weng, S. Zhou, Delivery of growth factors using a smart porous nanocomposite scaffold to repair a mandibular bone defect, Biomacromolecules 15 (3) (2014) 1019–1030.
[220] D. Raviv, W. Zhao, C. Mcknelly, A. Papadopoulou, A. Kadambi, B. Shi, S. Hirsch, D. Dikovsky, M. Zyracki, C. Olguin, R. Raskar, S. Tibbits, Active printed materials for complex self-evolving deformations, Sci. Rep. 4 (2014) 7422.
[221] C.R. Kruse, M. Singh, S. Targosinski, I. Sinha, J.A. Sørensen, E. Eriksson, K. Nuutila, The effect of pH on cell viability, cell migration, cell proliferation, wound closure, and wound reepithelialization: in vitro and in vivo study, Wound Repair. Regen. 25 (2) (2017) 260–269.
[222] S. Tibbits, 4D Printing: multi-Material shape change, Archit. Des. 84 (1) (2014) 116–121.
[223] S. Wu, J. Zhang, R.B. Ladani, A.R. Ravindran, A.P. Mouritz, A.J. Kinloch, C.H. Wang, Novel electrically conductive porous PDMS/Carbon nanofiber composites for deformable strain sensors and conductors, ACS Appl. Mater. Interfaces 9 (16) (2017) 14207–14215.