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Advances and future perspectives in 4D bioprinting

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Abstract

Three-dimensionally printed constructs are static and do not recapitulate the dynamic nature of tissues. Four-dimensional (4D) bioprinting has emerged to include conformational changes in printed structures in a predetermined fashion using stimuli-responsive biomaterials and/or cells. The ability to make such dynamic constructs would enable us to fabricate tissue structures that can undergo morphological changes. Furthermore, other fields (bioactuation, biorobotics, and biosensing) will benefit from developments in 4D bioprinting. Here, we discuss stimuli-responsive biomaterials as potential bioinks for 4D bioprinting. Natural cell forces can also be incorporated into 4D bioprinted structures. We introduce mathematical modelling to predict the transition and final state of 4D printed constructs. Different potential applications of 4D bioprinting are also described. Finally, we highlight future perspectives for this emerging technology in biomedicine.

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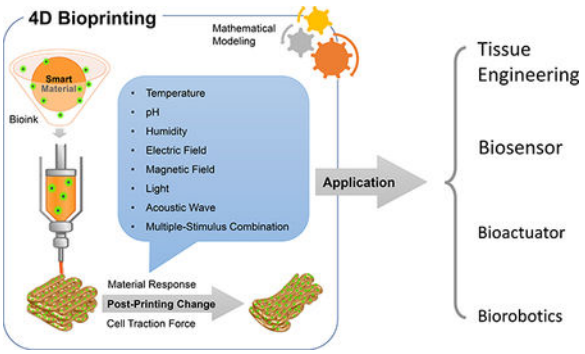
Graphical Abstract

Four-dimensional (4D) bioprinting has emerged to include conformational changes in printed structures in a predetermined fashion using stimuli-responsive biomaterials and/or cells. Here, different potential applications of 4D bioprinting are described. We also introduce mathematical modelling to predict the transition and final state of 4D printed constructs.



Keywords

4D bioprinting; Additive manufacturing; Bioinks; Stimuli-responsive biomaterials; Tissue engineering

1 Introduction

Three-dimensional (3D) printing (Figure 1) was first patented by Hull in 1986 [1]. Since then, numerous 3D printing techniques have been developed [2]. Later on, the concept of including cells into 3D printed constructs emerged leading to the development of 3D bioprinting [3] (Figure 1). Common 3D bioprinting technologies include inkjet, microextrusion, and laser-assisted printing approaches [4]. In inkjet 3D bioprinting, thermal, piezoelectric, or electromagnetic tools are used to deposit small bioink droplets through nozzle(s). Although inkjet printing is characterized by its short fabrication time and low cost, microextrusion became more common for 3D bioprinting because it can deposit cells at higher density and can work with a range of polymer viscosities [3]. In microextrusion 3D bioprinting, bioink is extruded through nozzle(s) either by mechanical methods [5]. In laser-assisted 3D bioprinting, laser energy is used to volatilize a sacrificial layer, propelling a payload to a receiving substrate (nozzle-free bioprinting) [6]. 3D bioprinting technologies have also been combined with microfluidic platform to precisely control bioink flow rate and to achieve multimaterial bioprinting at high and spatial resolution [7,8]. Therefore, it would be possible to print heterogeneous and biomimetic structures for tissue engineering applications [9–11].

Three-dimensional bioprinting technologies have been created complex tissue structures with precise control in an automated manner [12]. However, currently fabricated structures cannot precisely mimic the dynamic nature of tissues. Natural tissue regeneration and repair may involve conformational changes in the tissue structure [13]. Therefore, it is required to include time-dependence into 3D printed tissue constructs to recapitulate structural changes

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in tissues. To this end, four-dimensional (4D) bioprinting has been developed using stimuli-responsive biomaterials and cell traction forces to make structurally dynamic tissue constructs. Note that it is feasible to make 3D patterns of smart cell-laden constructs using conventional approaches (molding or lithography) rather than 3D bioprinting. However, 3D bioprinting provides faster fabrication, ability to make complex structures, and higher automation capability and reproduction. Herein, we define 4D bioprinting as 3D printing of cell-laden materials in which the printed structures would be able to respond to external stimulus or internal cell forces. This definition is more specific from Gao *et al.*'s one [14] who labeled the 4D bioprinting term not only to our statement but also to the cell maturation/functionality in 3D printed constructs with time (the geometry of constructs may not change). The development of 4D bioprinting followed the introduction of 4D printing (Figure 1) [15] in which multimaterials capable of transforming over time were printed. Since then, many works on 4D printing materials and technologies were reported [16–18]. However, 4D bioprinting still needs further development to adapt post-printing changes with cell behavior and function in a safe and predictable manner.

In this review paper, stimuli-responsive biomaterials are discussed for potential applications in bioinks for 4D bioprinting. Cell traction forces are also discussed as a source of post-printing changes. Mathematical modeling is presented as a tool to predict structural changes in printed materials. Applications of 4D bioprinting in tissue engineering, biosensing, bioactuators, and biorobotics are described. Finally, current challenges and outlook in this emerging research field are discussed. Our proposed outline is wider and covers more recent works compared to review papers on 4D bioprinting [14,19].

2 Potential bioinks for 4D bioprinting

Stimuli-responsive materials undergo conformational changes in response to specific triggers, such as temperature, pH, humidity, electricity, magnetic field, light, acoustics, or a combination of these stimuli [20,21]. Stimuli-responsive materials have great potential to be used as bioinks for 4D bioprinting. Printability and biocompatibility of these materials are key parameters for their successful use in 4D bioprinting. Moreover, safety of stimulation procedure to cell and tissue constructs should be evaluated. In this section, we discuss some examples of stimuli-responsive materials, which can be used in 4D bioprinting.

2.1 Temperature-responsive materials

Temperature-responsive materials can change their physicochemical properties under temperature changes. The most extensively studied temperature-responsive materials are poly(N-isopropylacrylamide) (PNIPAM)-based polymers [22], which have a relatively low critical solution temperature (LCST) of ~32°C. Below this temperature, polymeric chains are in extension mode and thereby the polymer exists in solution phase. When temperature is above the LCST, polymeric chains are retracted turning the polymer into gel. Although temperature-responsive materials were mainly used for drug delivery, they can potentially be used in 4D bioinks. The biocompatibility of these materials has already been confirmed in cell-based studies [23,24]. There are also some reports on printability of temperature-responsive materials. For example, PNIPAM-based hydrogels were shown to be printable

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[25]. However, further investigations are required to control swelling behavior of such hydrogels for cell encapsulation studies. In general, the phase transition of temperature-responsive materials should not also adversely affect cell viability and structural stability of printed constructs. An interesting area of research would be the combination of temperature-responsive materials with other cell-laden biomaterials in multi-material bioprinting [26].

2.2 pH-responsive materials

pH-responsive materials respond to pH changes in their surroundings and show structural and/or chemical changes [27]. These materials contain chemical groups (*e.g.*, carboxyl, pyridine, sulfonic, phosphate, and tertiary amines) that release or accept protons when pH changes. Such materials have been used to develop pH-responsive constructs for release of biomolecules [28–30]. Biomolecules loaded in these materials can be released in a tailored fashion as a response to pH changes of the surrounding environment. Alginate has extensively been used in 3D bioprinting approaches [31,32]. Therefore, alginate-based pH-sensitive materials have potential to be used in 4D bioinks. To this end, biocompatibility of these materials should be evaluated. pH-responsive materials may also release some compounds in cell culture medium during the phase change process. These compounds should not significantly affect cell behavior and function.

2.3 Humidity-responsive materials

Humidity-responsive materials can swell up or shrink and thereby change their shape and size with the variation of humidity [33]. These materials provide a safe and facile transition process for cell-based structures with potential application in 4D bioinks [34]. However, swelling/shrinking degree of humidity-responsive materials should be precisely controlled in the transition procedure to keep the integrity of printed structures. Humidity-responsive materials have already been used in 4D printing. In a remarkable work, Gladman *et al.* developed a bioink comprised of stiff cellulose fibrils embedded in a soft acrylamide matrix [29]. Following the printing, the acrylamide monomer was photopolymerized resulted in a composite hydrogel with high swelling capacity. The 4D printed hydrogels were able to undergo localized and anisotropic swelling due to alignment of the cellulose fibrils in the constructs.

2.4 Electric field-responsive materials

Electric field-responsive materials are often polyelectrolyte hydrogels that can swell, shrink, erode or bend in response to an electrical field. Electro-responsive hydrogels have been used in biomedical applications, such as in drug delivery [35], engineering electro-responsive tissues [36], and making soft actuators [37]. Some hydrogels can be electro-responsive after being doped with conductive polymers, such as polyaniline, polypyrrole (PPy), and polythiophene [38]. Biocompatibility [39] and 3D printing [40] of polyaniline-based materials have been reported. Conductive polymers have also shown actuation under applying voltage [41]. Therefore, hybrid hydrogel-polyaniline materials can potentially be used in 4D bioinks. For instance, Fantino *et al.* fabricated 3D electro-active hydrogels using 3D printing of poly(ethylene glycol) (PEG) followed by PPy interfacial polymerization in the PEG matrix to create a conductive phase [42]. In another work, Zhao *et al.* made 3D

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printed electrodes of PPy for interdigitated supercapacitors [43]. The developed printing technologies should be modified to be compatible with cells.

Carbon-based nanobiomaterials (*e.g.*, carbon nanotubes (CNTs) and graphene) have also been used in making electro-responsive materials [44,45]. For example, Servant *et al.* developed a graphene-based macroporous scaffold with high electrical, mechanical, and thermal properties. They showed that drugs were released from the graphene scaffold in an on/off fashion upon the application of a low electrical voltage [46]. Graphene has been used in inks to fabricate 3D printed constructs with electroconductive properties [47–49]. Therefore, graphene-based nanomaterials have potential to be used in bioinks for 4D bioprinting. Other carbon-based nanobiomaterials, such as CNTs can also provide electro-responsive bioinks for 4D bioprinting. There are also some studies on printability of CNTs. For example, Shin *et al.* synthesized bioinks of single-walled CNTs in hyaluronic acid (HA) to fabricate 3D constructs [50] (Figure 2). In general, carbon-based nanobiomaterials provide high surface area and ease of functionalization with different functional groups. Therefore, they can be combined with a range of biomaterials to make bioinks with different physicochemical properties.

2.5 Magnetic field-responsive materials

Magnetic field-responsive materials are comprised of magnetic micro- or nanoparticles, including ferromagnetic or paramagnetic particles, which are able to respond to magnetic fields [51]. For instance, Zhang *et al.* combined mesoporous Fe_3O_4 nanoparticles with PEG to develop a magnetic field-responsive material for controlled release of doxorubicin (DOX) [52]. Lalitha *et al.* developed a self-assembled supramolecular gel with magnetic field-responsiveness and self-healing properties [53]. Such magnetically responsive materials can potentially be used in bioinks for 4D bioprinting.

Some magnetic field-responsive materials have already been used in inks for 3D printing or cell-based studies. For example, Kokkinis *et al.* developed a poly(urethane acrylate) oligomers with modified alumina platelets, which were responsive to low magnetic field. Local orientation and alignment of the platelets in the printed structure was tunable using a magnetic field [54]. In another study, Tasoglu *et al.* showed that PEG hydrogels were assembled under magnetic field by the aid of paramagnetism of free radicals in the gels [55]. The same research group used gelatin methacryloyl (GelMA) or cell-laden GelMA submerged in a stable free-radical solution to paramagnetize hydrogels allowing their assembly under the influence of magnetic field. They showed that vitamin E (as free radical scavenger) erased the magnetic signature of the microgels [56]. In another study, it was shown that mesenchymal stem cells loaded with magnetic nanoparticles formed 3D cell aggregates under a magnetic field [57]. Similarly, embryonic stem cells loaded with iron oxide nanoparticles were attached together and formed embryoid bodies when exposed to magnetic field (Figure 3) [58]. Such magnetic field-responsive behavior can be used in 4D bioprinting applications to manipulate cell-laden printed structures in a safe and rapid manner.

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2.6 Light-responsive materials

Photo-responsive materials represent an attractive group of biomaterials that can be used to develop 4D bioprinted constructs. These moieties can capture the optical signal using photochromic molecules and then convert the photoirradiation to a photoreaction in the biomaterials [59]. Photo-responsive biomaterials can be activated in relatively wide wavelength ranges including UV, infrared (IR), and near-IR. IR radiation has low absorbency by living tissues compared to UV light and therefore, it provides lower phototoxicity and higher tissue penetration [60].

Light was first integrated with 4D printing technology as an external stimulus to change the color of printed objects [61]. In one example, polymeric chains including azo compounds deformed under the influence of UV light leading to color change of the polymer from white to purple. Light was also used to change the shape of printed materials. For example, Wei *et al*. investigated shape-memory properties of printed polylactic acid induced by UV light [62]. The printed polymer was able to change its configuration. In another study, Wang *et al*. printed HA hydrogels modified with aldehyde or hydrazide with high structural fidelity [63]. Due to dynamic hydrazone bonds in the printed hydrogel, further stiffening of hydrogel was done using light-induced thiolene reaction. An interesting potential of such photo-responsive hydrogels would be to direct stem cell differentiation and fate in a mechanically dynamic microenvironment.

Photo-responsive property of biomaterials can be used to induce photodegradation of the biomaterials [64]. Adding photodegradable moieties (*e.g.*, coumarin or o-nitrobenzyl ether groups) to hydrogels is a useful approach to tune biodegradation rate of hydrogels [65]. Due to relative biocompatibility and spatial control of light, photodegradation of biomaterials can be obtained in the presence of cells, which provides an asset in making the dynamic tissue culture. Therefore, 4D bioinks based on photo-responsive biomaterials would be useful to mimic the dynamic nature of ECM degradation.

2.7 Acoustic-responsive materials

Acoustic waves may induce physical or chemical changes in a material, particularly at high energies [66]. We recently showed that acoustic waves were utilized to pattern cells in an accurate, rapid, and contactless manner [67]. Acoustic waves have also been used for spatiotemporal control on drug release from hydrogels. For example, Huebsch *et al*. developed alginate-based acoustic-responsive hydrogels [68]. This acoustic-responsive hydrogel has potential to be used in bioinks for 4D bioprinting as alginate has extensively been used as ink material for 3D bioprinting [69]. Four-dimensional bioprinted constructs made of acoustic-responsive bioinks would be able to undergo morphological changes in a safe and non-invasive approach to cells.

2.8 Multiple stimuli-responsive materials

Multiple stimuli-responsive materials have recently been developed to achieve smart and multifunctional materials with precise control over material responsiveness to various stimuli [70]. Commonly used combinatorial stimuli-responsive materials employ dual stimuli of temperature and pH [71–73], magnetic field and temperature [74], pH and

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magnetic field [75], and near-IR and pH [76]. These materials have potential to be used in 4D bioinks to recapitulate the multifunctional and complex nature of the ECM. Multiple stimuli-responsive materials have mainly been used for drug release. For example, Jalili *et al.* developed injectable PNIPAM-co-acrylamide hydrogels capable of releasing DOX in a temperature and magnetic field dependent manner [77]. Multiple stimuli-responsive hydrogels for drug delivery and release have been reviewed elsewhere [78–81]. Such materials can be used in 4D bioinks. However, stimulation parameters should vary in biologically relevant ranges without deleterious effect on cell viability and function.

3 Cell traction forces

A biologically driven stimulation to use in 4D printing is through the action of cells in printed constructs. Cells can generate traction forces in their matrix. Cell traction forces originate from intracellular actin polymerization and actomyosin interactions, which occur normally in various physiological processes [82]. In 4D printing, these forces were collectively employed from multiple cells and direct at the periphery of the cellular constructs. Here, cell culture materials should be flexible and adhesion between the cells and materials should be strong enough to preserve cellular attachment under traction forces. Cell origami is based on cell traction forces by which cells generate 3D structures from two-dimensional (2D) surfaces by folding elements in pre-defined shapes. In one study, Kuribayashi-Shigetomi *et al.* cultured cells on microplates and then folded them to make 3D microstructures using the cell traction forces [83]. By changing the geometry of the patterned 2D microplates, they obtained various cell-laden structures after folding. Cell traction forces provide a natural stimulus for 4D bioprinting. Cell phenotype, cell-cell communication, and cell density and alignment can largely affect cell traction forces and need to be optimized for controlled conformations in printed structures.

4 Mathematical modeling

Mathematical modeling may predict printing process and final state of printed materials upon applying stimulus. In particular, modeling is able to provide useful information about desired shape, properties, and function of printed materials [84]. Therefore, mathematical modeling can significantly reduce time and cost of experiments to optimize 4D bioprinted structures. There are four core elements involved in modeling of 4D printed materials including print path, desired (final) shape, ink properties, and stimulus properties [85]. Two different strategies have been used in such mathematical modeling, forward problem and inverse problem approaches [34]. In forward problem approach, final shape of material is unknown while in inverse problem method print path is unknown. Forward problem approach deals with fundamental concepts and mechanisms of 4D printing technology. On the other hand, inverse problem approach is an application-oriented strategy.

There are several reports on using mathematical modeling in 4D printing. For example, Raviv *et al.* implemented a spring-mass mathematical model as a forward problem approach to identify the final structure of printed shape-memory materials [86]. In their simulation, the material was assumed to have an elastic behavior, which could be extendable to biomaterials in 4D bioprinting. In another study, Yu *et al.* proposed a quantitative analysis to

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evaluate thermo-responsive and shape-memory polymers in energy storage and release during the shape-memory cycle [19]. The model was rather simple. However, the concept could be extended to polymers with complicated structures. Mathematical modeling has also been used to quantify hydrogel properties in 4D printing. For instance, Gladman *et al.* used an inverse problem approach to identify print path of hydrogels [34]. The hydrogels were deformed after the printing due to hydrogel swelling. In another work, Kwok *et al.* performed a design optimization technique for 4D printing of origami and kirigami structures [87]. Despite the rather novel use of mathematical modeling in 4D printing, modeling studies have been able to provide some insights in 4D printing. Mathematical modeling can further be used to collect and digitize complex tissue structures in the design of printed structures. Finding mathematical relationships between external or internal stimuli and tissue structure and function would be greatly helpful to engineer tissue constructs in a dynamic and precise manner. In a broader view, such information can be used to understand the principles of structural biology, cell biology, tissue morphogenesis, and disease modeling. It would be also useful to further develop modeling approaches to include cells and cell-biomaterial interactions in printing and post-printing processes.

5 Applications

Four-dimensional bioprinting has tremendous potential for diverse biomedical applications, such as tissue engineering, biosensors, bioactuators, and biorobotics. In general, the aim of 4D bioprinting is to fabricate smart and multifunctional materials for these applications. Such materials would be able to increase functionality of currently used materials. Different potential applications of 4D bioprinting are discussed in this section.

5.1 Tissue engineering

Four-dimensional bioprinting is needed to achieve such dynamic processes toward fabricating hierarchically complex and dynamic tissues. An example would be 4D bioprinting of shape-memory and cell-laden scaffolds. The printed constructs can then adapt themselves with conformational changes occurring in the constructs. A recent work also showed the application of shape-memory scaffolds in minimally invasive delivery of functional tissues [88]. Such scaffolds can be fabricated using printing technologies in a facile, rapid, and controllable manner, which accelerates regenerative potential of shape-memory scaffolds. In another potential application of 4D bioprinting in tissue regeneration, delivery of cells in narrow spaces within the body (*e.g.*, subretinal space) may be envisaged using *in situ* unfolding of 4D bioprinted scaffolds. The self-folding process can be done in the presence of biological moieties or as a response to external stimuli *in vivo* [89]. Bioelectronic and biodegradable devices can also be integrated with scaffolds to control such delivery procedures in a wireless and accurate manner [90,91]. In another work, cell traction forces were utilized to make endothelialized tubes for 4D printed structures in a biomimetic way [83]. In general, such smart constructs would enable us to communicate with the natural environment of the body in an effective way and more importantly utilize the natural forces or stimuli to obtain desired changes in the constructs.

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5.2 Biosensors

Four-dimensional bioprinting can provide novel avenues for development of biosensors to monitor cell behavior and function. Three-dimensional printing technologies have already been used to make biosensors. For example, Gomez *et al.* used a two-photon stereolithography approach to fabricate microcantilever-based biosensors using molecularly imprinted polymers [92]. Similarly, Credi *et al.* used stereolithography technique to make cantilevers containing magnetic nanocomposites [93]. These cantilevers can be used to study cell behavior and function. For example, Cui *et al.* bioprinted mouse myoblasts (C2C12 cell line) on tiny cantilevers for biosensing applications. The myotubes on the cantilevers responded synchronously to electrical stimulation and their contraction rate was changed as exposed to exogenous chemical toxin (veratridine) [94]. In general, physical activity of cells and metabolites derived from them can be used as triggers for bioprinted biosensors. Biosensor components should also be biocompatible and do not interfere with physiological activities of cells.

5.3 Bioactuators and biorobotics

Numerous examples of 4D printed actuators or robots have been reported. For example, Bakarich *et al.* fabricated mechanically strong alginate/PNIPAM hydrogels, which could actuate while heating or cooling in a range between 20 and 60 °C [95]. Moreover, Zarek *et al.* printed an airway stent having a temporary shape, which was deployed back into permanent shape as a result of an increase in temperature [96]. López-Valdeolivas *et al.* prepared cross-linkable liquid crystal polymers as inks and used them to print stimuli-responsive structures. The printed elastomers were able to undergo shape changes in response to thermal stimulus and served as actuators or soft-robots [97]. Nishiguchi *et al.* used a direct laser writing method to generate sophisticated and controlled folding of thermosensitive hydrogels [98]. The designed hydrogels were capable of biomimetic actuation. The laser writing method can also be utilized to obtain desired structures of self-folding constructs, which can spontaneously curve, fold, or roll-up as exposed to external stimuli (Figure 4). Self-folding constructs can be made using PEG with different molecular weight and concentrations [99] or using two different polymers with distinct hydrophobicity [100]. Grippers were also fabricated using 3D printing of soft multimaterials [101] (Figure 5). These printed structures have great potential to be used as biorobots.

Cells or tissues can be incorporated into printed structures to provide mechanical forces for bioactuators or biorobots [102]. We recently developed self-actuating cardiac muscles on a scaffold with flexible microelectrodes [103]. To enhance the mechanical integrity and electrical conductivity of the robots, gold microelectrodes were also added to the scaffold. After maturation of cardiomyocytes, the cardiomyofiber organization was observed leading to the self-actuating movement. The emergence of 4D bioprinting is a great opportunity to fabricate next generation of smart, biomimetic, and multifunctional bioactuators or biorobots [104]. However, one problem with biorobots is their sole reliance on cell function, which may not work reliably and consistently. To solve this problem, different control mechanisms have been proposed including the use of light for controllable movement of genetically engineered cells [105,106], the use of electrical stimulation for controlled cell contractions

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[107,108], and the use of selective stimulation via neuromuscular junctions in skeletal muscle tissues [109,110].

6 Current Challenges and Outlook

Four-dimensional bioprinting has great potential to fabricate dynamic cellular structures for biomedical applications. Cell traction forces and stimuli-responsive biomaterials can be employed to produce 4D constructs. However, there are still some challenges related to 4D biofabrication, which can be summarized in the following questions: Does the printing process affect the ability of cell-laden biomaterials to respond to stimuli? Do cells affect the responsiveness of stimuli-responsive biomaterials? Does material dynamics affect viability of cells? These fundamental questions need to be investigated prior to wide applications of 4D bioprinting materials and technologies.

It would be interesting to use stimuli-responsive materials in 4D bioprinted structures to interact with host tissues for facile and robust integration of printed constructs with the native microenvironment. However, local tissue environment might be harsh (especially in pathological conditions with inflammation). Moreover, printed tissues will be surrounded with the body's immune system. Therefore, studies are needed to investigate interactions of printed tissues with the native tissues in a smart and controllable manner.

Most stimuli-responsive materials can respond to only one type of stimulus. However, cellular activities in the human body are complicated and regulated by multiple stimuli, such as self-regulation, neuro-regulation, and humoral regulation [14]. Therefore, 4D bioprinted constructs that are able to respond to multiple physiological signals are desirable for use in biomedical applications *in vivo*. Biological design principles can be utilized to accelerate the development of new generation of smart materials with unparalleled functionalities and properties [111]. In addition to developing and assessing such novel biomaterials *in vitro*, their efficiency and function should also be evaluated in animal models and clinical studies.

With the expansion of research in nanobiomaterials and nanotechnology [45,112,113], further advancement would be the production of smart nanomaterials capable of interacting with cells and tissues in cellular and molecular level. In general, nanobiomaterials provide facile functionalization with different biomolecules ranging from proteins, peptides, and small soluble factors [44,114]. Advances in 4D bioprinting will allow the development of smart nanomaterial-based constructs with capability to release biomolecules from them [115,116]. Smart and functional nanobiomaterials can be used to mimic the ECM by incorporating growth factors in 4D printed structures. These factors can modulate the inflammatory response after tissue implantation or help in tissue maturation and functionality *in vitro*.

Four-dimensional bioprinted constructs can benefit from advances made in wireless communication and data storage devices. These devices can be integrated with stimuli-responsive materials to develop novel generation of multifunctional and smart cellular-based constructs for applications in tissue regeneration and drug release. A recent example showed proper coupling of electronics and sensor technology for monitoring physical activities in

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the body using bioengineered systems [117]. Future works would provide precise control on function of 4D bioprinted constructs *in vivo* in minimally invasive manner. These works will open new ways for follow-up and care of implanted devices, tissues or organs in a personalized way.

Four-dimensional bioprinting materials and technologies should be translated into products to be useful in solving real-world problems. Scalability, manufacturing, affordability, and ease of application for end users are major hurdles in the commercialization of 4D bioprinting. These issues should be considered at early stages of development process to enable the translation of 4D bioprinting. The use of simple and portable bioprinters [118] could be interesting in 4D bioprinting by which smart constructs are fabricated *in situ* and then the body environment would play the stimuli role. Some 4D bioprinted constructs depend on cell traction forces and thereby often have low production yield or reproducibility. In addition, there is a trade-off between the complexity of 4D bioprinted constructs and manufacturability. Therefore, continuous production of such constructs should be limited to few processing steps. There might also be some issues related to validation and cryopreservation of printed tissues in large-scale production.

7 Conclusions

Four-dimensional bioprinting has recently emerged to confer conformational changes in printed structures in a controllable manner using stimuli-responsive biomaterials and/or cells. Such dynamic constructs would enable us to fabricate dynamic tissue structures that can undergo morphological changes in a predetermined way. Four-dimensional bioprinting may find other biomedical applications, such as in bioactuation, biorobotics, and biosensing. Stimuli-responsive biomaterials and/or cell traction forces can provide potential bioinks for 4D bioprinting. Mathematical modelling is a useful technique to predict the transition and final state of 4D printed constructs. Taken together, 4D bioprinting has a promising future as a powerful technology to mimic the dynamic and hierarchical organization of native cellular structures.

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Abbreviations:

4D	four-dimensional
3D	three-dimensional
UV	ultraviolet
PNIPAM	poly(N-isopropylacrylamide)
LCST	low critical solution temperature

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PPy	polypyrrole
PEG	poly(ethylene glycol)
CNTs	carbon nanotubes
HA	hyaluronic acid
DOX	doxorubicin
GelMA	gelatin methacryloyl
IR	infrared
2D	two-dimensional
PS	polystyrene
AA-MA	methacrylated alginate
PBS	phosphate-buffered saline
EDTA	ethylenediaminetetraacetic acid

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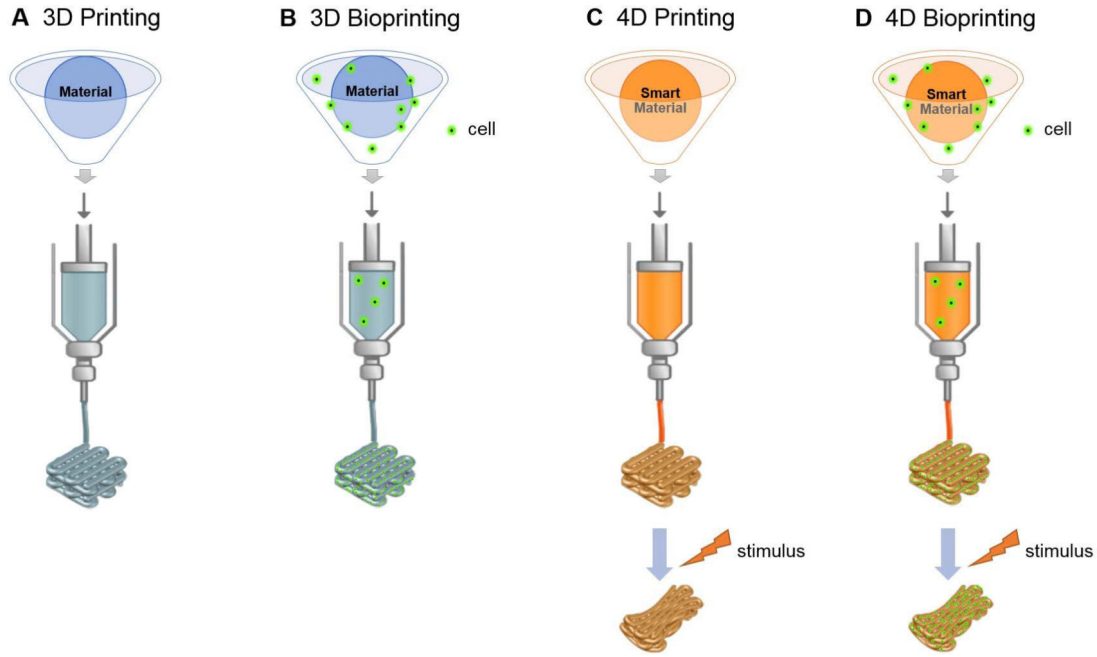

Figure 1.

Schematic of different printing technologies (3D, 3D bioprinting, 4D, and 4D bioprinting) using conventional materials, cells, and smart materials. Cells are involved in bioprinting technologies. We defined 4D bioprinting as 3D printing of cell-laden materials in which the printed structures would be able to respond to external stimulus due to stimuli-responsive bioinks or internal cell forces.

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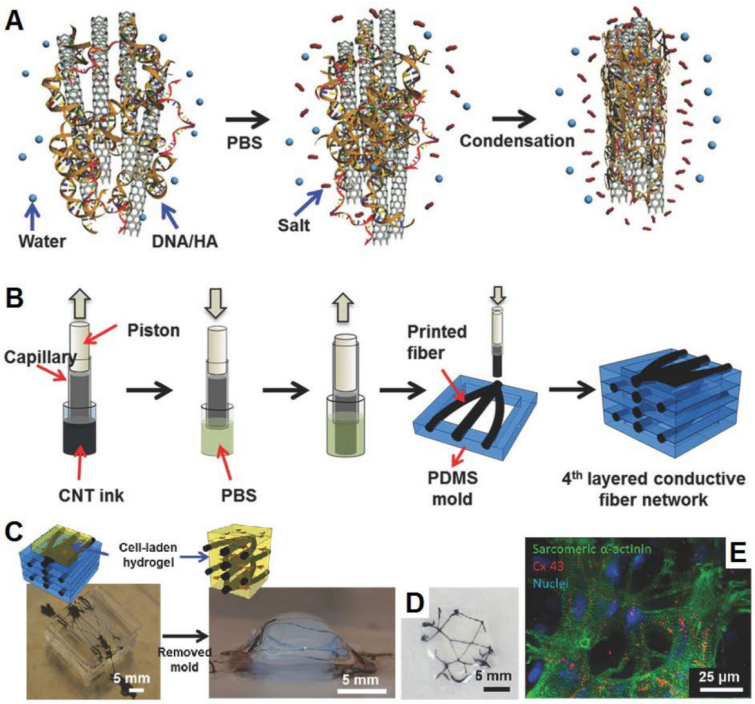

Figure 2.

Multimaterial-based ink preparation and deposition process to make electrically conductive and cell-laden structures. i) Schematic diagram for making DNA/HA-coated single-walled CNT inks. ii) Schematic of 3D printing steps to fabricate conductive fibers. iii) Schematic diagram indicating the connection of printed fibers into GelMA hydrogels. iv) Top view of printed fiber incorporated in GelMA hydrogels. v) Encapsulated cardiomyocytes in GelMA hydrogels with 3D stacked CNT fibers on day 10 of culture. Immunostaining was done for sarcomeric α-actinin (green), cell nuclei (blue), and Cx-43 (red). Reproduced with permission from Shin *et al.* [50].

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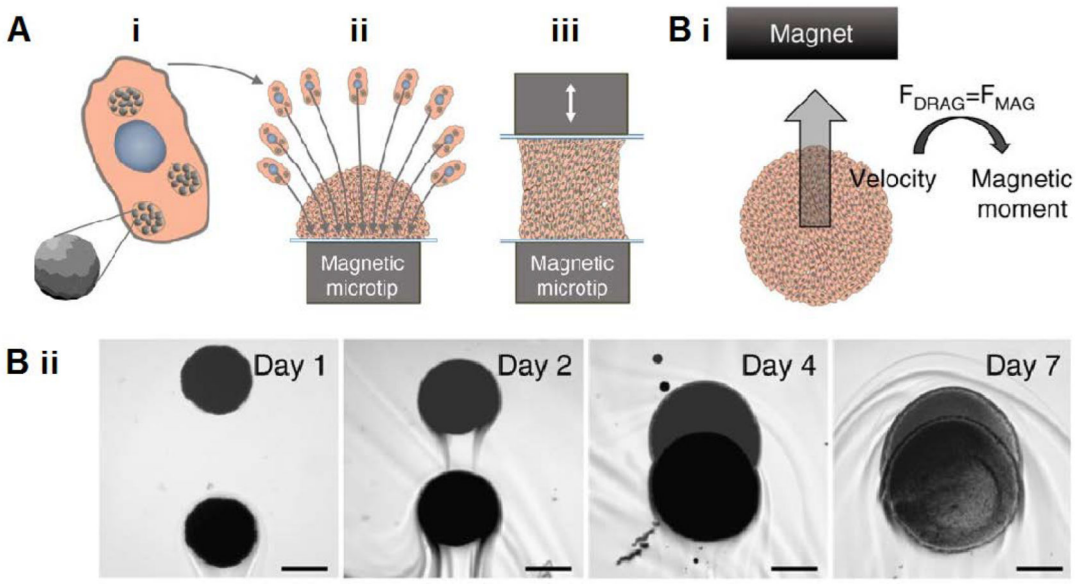

Figure 3.

Making cell aggregates triggered by magnetic field. (a) The use of intracellular magnetic particles and external magnetic field to control embryonic stem cell aggregation. (b) Photographs (i) and illustration (ii) of stem cell aggregate movement under the influence of magnetic field. Reproduced with permission from Du *et al.* [58].

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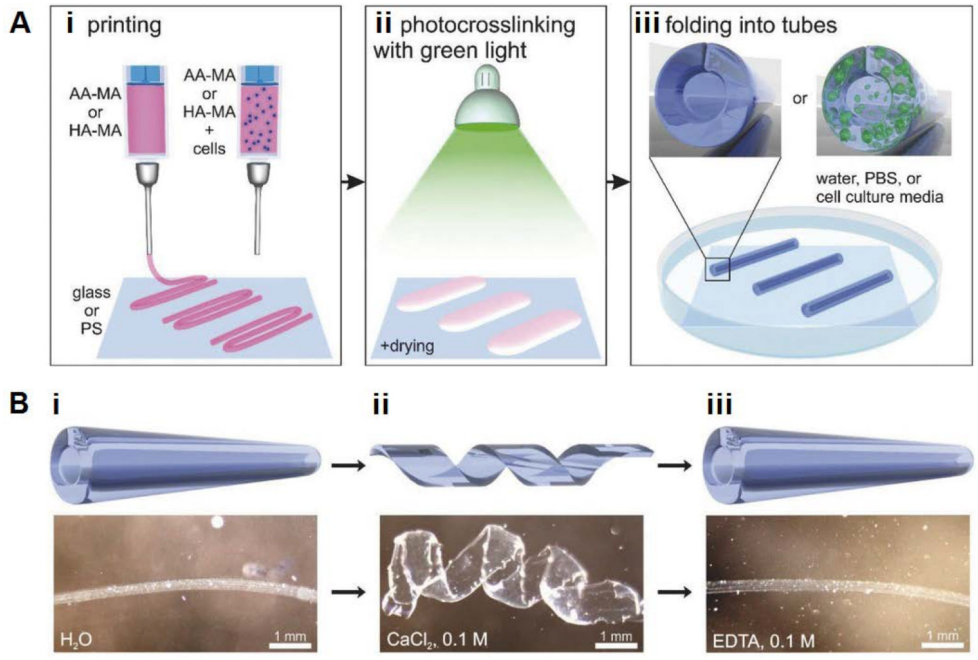

Figure 4.

4D biofabrication of cell-laden biomaterials. (i) 4D bioprinting of cell-laden self-folding hydrogel-based tubes using methacrylated alginate (AA-MA) or HA-MA on different substrates (glass or polystyrene (PS)). Green light (530 nm) was used for mild drying of structures. Instant folding into tubes was obtained upon immersion of crosslinked films in water, phosphate-buffered saline (PBS), or cell culture media. (ii) The tube responsiveness (cartoons (upper panel) and representative photographs (lower panel)) in water (1), same tube immersed in $CaCl_2$ solution (2), which led to an additional crosslinking of alginate with Ca^{2+} ions and complete unfolding of the tube, and folded tube immersed in ethylenediaminetetraacetic acid (EDTA) solution (3), where EDTA bound the Ca^{2+} ions from the alginate, leading to refolding of the film into a tube [119].

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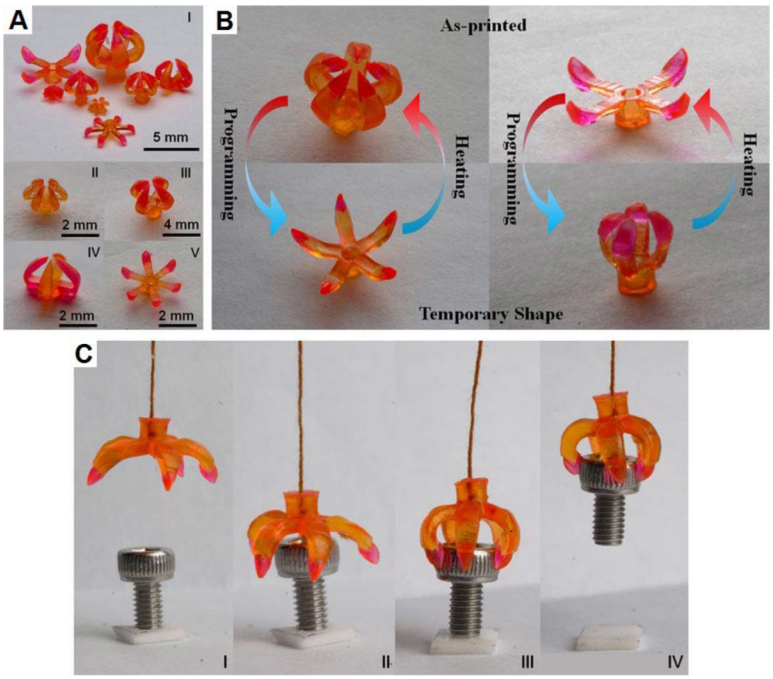

Figure 5.

4D printed grippers as biorobots. (a) Multimaterial grippers were fabricated with different designs. (b) The demonstration of the transition between as-printed and temporary shapes of multimaterial grippers. (c) The snapshots of the process of grabbing an object. Reproduced with permission from Ge *et al.* [101].