Chapter 9
Hydrogel as Bio-Ink for Organ Regeneration

Hemand Aravind, Blessy Joseph, and Sabu Thomas

Abstract An organ is an organ; nothing other can replace it or act as it. That is why there is an increasing medico-demand for tissue-engineered tissues and organs. Scientific world presently looking for alternative fabrication approaches to develop tissues and organs as conventional techniques is not capable of fabricating constructs with required structural, mechanical, and biological complexity. In such a condition, 3D bioprinting offers great potential to fabricate highly complex constructs with precise control of structure, mechanics, and biological matter, especially cells and extracellular matrix components. 3D bioprinting is an additive manufacturing approach that utilizes a "bioink" to fabricate devices and scaffolds in a layer-by-layer manner. 3D bioprinting allows printing of a cell suspension into a tissue construct with or without a scaffold support. The most common bioinks are cell-laden hydrogels, decellularized ECM-based solutions, and cell suspensions. In this chapter, an effort is taken to briefing hydrogels with particular focus on bioink design requirements. We also present the current state of the art in bioink design including the challenges and future directions.

Keywords Bio-fabrication · Tissue engineering · Regenerative medicine · Hydrogel · Cell printing · Extracellular matrix

1 Introduction

One of the promising and emerging multidisciplinary fields of bioengineering is tissue engineering, and its contribution comes majorly on two areas: (i) developing new methods to repair, regenerate, and replace damaged tissues and organs

H. Aravind
Department of Chemistry, Aromatic and Medicinal Plant Research Station, Eranakulam, Kerala, India

B. Joseph · S. Thomas (✉)
International and Inter University Centre for Nano Science and Nanotechnology, Mahatma Gandhi University, PD Hills (PO), Kottayam, Kerala 686 560, India
e-mail: sabuthomas@mgu.ac.in

J. Jose et al. (eds.), *Nano Hydrogels*, Gels Horizons: From Science to Smart Materials,
https://doi.org/10.1007/978-981-15-7138-1_9

and (ii) Constructing in vitro tissue models to better understand tissue development, disease development, and screen mode of action of new drugs [1–6]. In our changed social health status, more people are vulnerable to serious diseases which are directly affecting vital organs like kidney, liver, heart, pancreases, etc. In spite of latest advances in tissue engineering, there is a continuous lack of tissues and organs for transplantation and a shortage for tissue models for drug discovery and testing [7]. Extensively long waiting lists for organ transplantation exist all around the world. According to U.S. Department of Health and Human Services, as of June 2017, around 120,000 patients are in need of lifesaving organ transplant in the USA while only about 5200 donors are available. Also, while the number of transplants performed every year since 2003 has been somehow constant, the number of patients waiting at the year-end has been growing [8]. Under these circumstances, scientists are eager to find alternative ways to compensate for this shortage of organ. Unfortunately, conventional techniques, such as porogen-leaching, injection molding, and electrospinning, are generally recognized as the bottleneck due to limited control over scaffold architecture, composition, pore shape, size, and distribution [9–11]. 3D bioprinting is an actively studied method in tissue engineering since it shows effective control over scaffold fabrication and cell distribution. Printing resolution of 3D bioprinting techniques is 10–10,000 mm which is a wide range showing flexibility of bioprinting compared to other assembly methods such as molding and porous scaffolds. 3D bioprinting enables fabrication of scaffolds, devices, and tissue models with high complexity [9, 11–14]. 3D printing enables creation of tissues from commonly used medical images (such as X-ray, magnetic resonance imaging, and computerized tomography scan) using computer-aided design. Custom and patient-specific design, on-demand fabrication, high structural complexity, low-cost, and high-efficiency are some of the major advantages of 3D printing and such things making it very attractive for medicine [15, 16].

3D bioprinting is a technology to fabricate constructs from living cells with or without a carrier material in a layer-by-layer manner [9, 11, 12, 17, 18]. The material that is printed is referred to as a "bioink," which can be defined as an ink formulation that allows printing of living cells. Here, we would like to note that many of the biomaterial ink formulations are not suitable for cell printing. For instance, polycaprolactone (PCL) and poly(lactic acid) (PLA) are the most widely used biomaterials in 3D printing. However, they could only be printed at elevated temperatures in the form of a polymer melt or when dissolved in organic solvents as a polymer solution. Therefore, they are not considered as bioinks in this review, as both approaches are not suitable for live cell printing [19, 20]. In this paper, we discuss one among the most commonly used bioinks, cell-laden hydrogels [16, 21–23] and give the current state of the art in bioink design with challenges and future directions. A brief description and comparison of the bioprinting methods with particular focus on bioink design requirements are also given.

2 3D Bioprinting Technologies

3D printing technology is a promising innovative concept in tissue engineering; instead of using 2D structures with numerous limitations, nowadays scientists can use 3D scaffolds for cell studies. 3D bioprinting is comparatively manageable and cell friendly as it is required to allow cell printing, and of course this requirements restricted the number of 3D printing techniques that are appropriate for bioprinting (Fig. 1) [13, 24]. At present, the 3D printing technology available in industry can print a wide range of materials by using diverse ink formulations [16]. Fused deposition modeling (FDM) is a 3D printing technique pioneered in the 1990s by Stratasys, and presently, it is the trade mark of company. The company continues to be a leader in manufacturing 3D printers all over the world. Alternatively, the 3D printers that are based on this technology are also called as fused filament fabrication (FFF), plastic jet printing (PJP) or material extruding printers, which is the generic name for these 3D printers. Fused deposition modeling (FDM) is an extrusion-based printing and utilizes synthetic thermoplastics and their composites with ceramics and metals [25]. Because of its best performance at high temperature (140–250 °C) in melt state, it eliminates FDM as an option for bioprinting. Direct ink writing (DIW) is also an extrusion-based printing widely utilized in meso- and micro-scales and allows extrusion of high viscosity solutions, hydrogels, and colloidal suspensions [14]. DIW allows printing of cell suspensions and/or aggregates with or without a carrier. Inkjet printing is another technology for cell printing. The processing principle is deposition of polymeric solutions, colloidal suspensions, and cell suspensions, with relatively low viscosities [<10 cP (mPa s)] at relatively high shear rates (10^5–10^6 s^{-1}) in the form droplets (~50 μm in diameter) [26–29]. As compared to extrusion-based bioprinters, inkjet bioprinters are not readily available. Drop-on-demand printing that is one of the inkjet printing technologies enables the printing of complex and precise sections of living tissues or organs on the culture substrates utilizing cells and/or biomaterials as bioinks [30, 31]. Selective laser sintering utilizes metals, ceramics, polymers, and composites in powder form (10–150 μm in diameter) and is not suitable for bioprinting. In this technique, a directed laser beam locally melts either directly the powder or a polymeric binder onto the bed surface [32]. Layers of fresh powder are continuously supplied after each layer is created. Stereolithography (SLA) requires

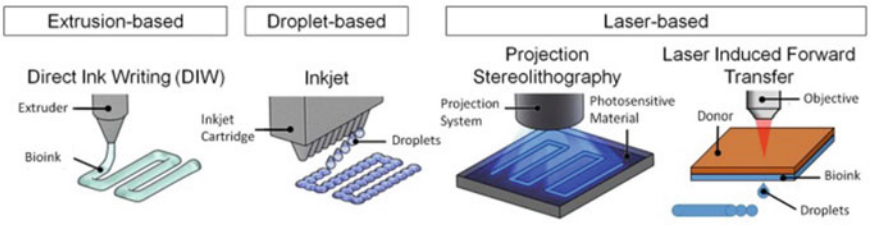

Fig. 1 3D bioprinting techniques for bioprinting of tissues and organs. Figure with permission from Miller and Burdick [43]. Copyright 2016, American Chemical Society reproduced

a viscous photocurable polymer solution or a prepolymer, which is exposed to a directed light (such as UV or laser) to spatially cross-link the solution [33]. SLA could potentially be considered for printing live cells as long as a cell-laden prepolymer formulation is used and the photocuring takes place in a mild, cell-friendly condition, which is the two major issues for SLA in bioprinting [34–36]. When 3D printing technologies are considered for bioprinting, the most commonly used technologies are DIW and inkjet printing [13, 14]. In addition to these technologies, laser-induced forward transfer (LIFT) is also shown to be suitable for bioprinting [37–42]. In this technique, ink solution is coated onto a glass slide and coated with a laser absorption layer (metal or a metal oxide). Laser is directed to the laser absorption layer with an ablation spot size between 40 and 100 μm in diametercreating a local pressure to eject the ink layer to the substrate.

3 Bioink Design

An ultimate bioink formulation should gratify certain material and biological requirements. Material properties are printability, mechanics, degradation, and functionalizability. Biological requirements mainly include biocompatibility, cytocompatilibilty, and bioactivity. When material properties are considered, printability is the most important constraint. Printability encompasses two parts: (i) the processability of the bioink formulation and (ii) the print fidelity associated with the mechanical strength of the printed construct to self-sustain a 3D structure post-printing. Depending on the printing process, printability could potentially involve solution viscosity, surface tension, and cross-linking properties. Viscosity is a crucial parameter for a bioink formulation as it affects both the print fidelity and cell encapsulation efficiency. High viscosity polymer solutions are less likely to flow easily so that the printed structure could hold its shape at longer times post-printing. However, they require higher pressures to flow, limiting the gage size and smallest achievable print size (mainly for DIW). In this regard, Tirella et al. [44] investigated the processing window for alginate hydrogels using pressure-assisted microfabrication (DIW technique). They successfully developed a 3D phase diagram showing the interplay between bioink viscosity, print velocity, and applied pressure to obtain high print reliability [44]. The bioink formulation is preferred to have a tunable viscosity to be compatible with different bioprinters. For instance, bioinks for inkjet or droplet-based bioprinters have viscosity values close to 10 mPa s [29]; the viscosity of bioinks for extrusion-based DIW bioprinting ranges from 30 to 6×10^7 mPa s [13, 14, 45]; for laser-assisted bioprinting, the bioink viscosity is in the range of 1–300 mPa s [45, 46]. For high viscosity bioinks used in extrusion and droplet-based print, the shear-thinning feature is desired to compensate for the high shear stress associated with high viscosity. The overall mechanics, i.e., achievable stiffness, is important not only to create self-supporting constructs but also to control and direct cellular behavior. Degradation is important for the functional amalgamation of the printed construct in vivo by enabling cells to gradually replace the construct with their ECM. Both the bioink and

the degradation products should not contain materials that induce inflammatory host response when implanted. Functionalizability is required to incorporate biochemical cues, i.e., bioactivity, to direct cellular behavior, such as adhesion, migration, and differentiation. In addition to biocompatibility and cytocompatibility, high cell viability, both prior- and post-printing, is crucial for the ink formulation. In addition to bioink design, a recent study showed the importance of the print substrate for live cell inkjet printing. In this work, computational and experimental studies confirmed that the stiffness of the print substrate directly influences the impact forces acting on the droplet, which affects the overall cell survival [47]. Below we will discuss the commonly used bioinks including current state of the art in ink design.

4 Established Bioinks

The most commonly used bioinks for tissue and organ printing are cell-laden hydrogels, decellularized extracellular matrix (dECM)-based solutions, and cell suspensions (Fig. 2). Cell-laden hydrogels are particularly attractive due to their tunable properties and their ability to recapitulate the cellular microenvironment [48]. ECM-based bioink formulations or decellulerized tissue inks are an emerging field due to their inherent bioactivity and ease of formulation into a printable bioink [49]. Cell suspension inks based on cell aggregates are a viable option to create scaffold-free biological constructs [50, 51].

5 Cell-Laden Hydrogels

Cell-laden hydrogels are the most commonly used bioinks as they can be easily formulated for extrusion-based (DIW), droplet-based (inkjet), and laser-based (SLA and LIFT) bioprinting technologies. Cell-laden hydrogel bioink formulations utilize natural hydrogels such as agarose, alginate, chitosan, collagen, gelatin, fibrin, and hyaluronic acid (HA), as well as synthetic hydrogels such as pluronic (poloxamer) and poly(ethylene glycol) (PEG), or blends of both. Natural hydrogels offer inherent bioactivity except for agarose and alginate and display a structural resemblance to ECM. For instance, fibrin and collagen hydrogels with inherent filamentous structure display strain-stiffening property, mimicking the nonlinear elastic behavior of the soft tissues in our body [54, 55]. Synthetic hydrogels permit but do not promote cellular function, yet there are many ways to tether bioactive cues into synthetic hydrogels [56]. When compared to natural hydrogels, synthetic hydrogels generally offer tunable mechanical properties. Many natural polymers (such as gelatin and HA) have functionalizable backbone side chains enabling them to be functionalized with chemical moieties to induce cross-linking (chemical- and/or photo-cross-linking) or additional bioactivity [57]. Blends of synthetic and natural polymers have been used to develop mechanically tunable hydrogels with user-defined bioactivity. Finally, the

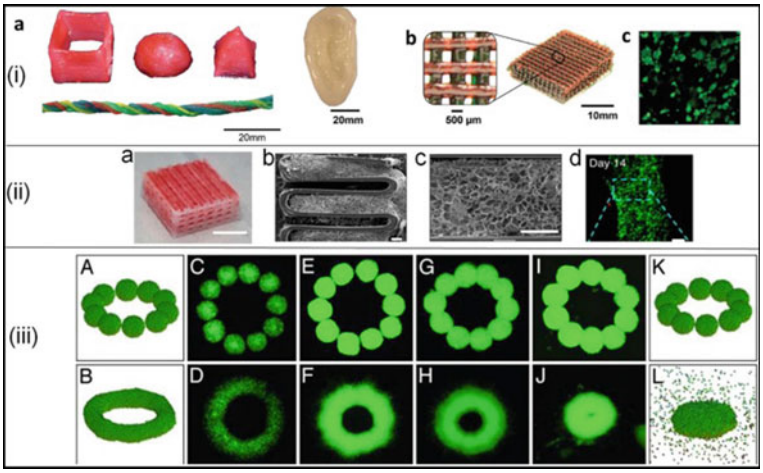

Fig. 2 **i** 3D printed constructs in various forms (**a**, **b**) using poly(ethylene glycol)–alginate–nanoclay hydrogels. Red food dye was incorporated into some of the bioink formulations for visibility. Live/dead assay of cells (**c**) in a collagen infused mesh from (**b**). Reprinted with permission from Hong et al. [52]. Copyright 2015, John Wiley and Sons. **ii** Tissue construct printed from decellularized extracellular matrix (dECM) (**a**), SEM images of hybrid constructs from dECM supported with polycaprolactone framework (**b**, **c**), and fluorescent images of cells (**d**). Scale bars are 5 mm for (**a**), 400 μm for (**b**, **c**), and 100 μm for (**d**). Adapted with permission from Pati et al. [49]. Copyright 2014, Nature Publishing Group. **iii** Cell aggregate (500-μm average diameter) configurations in simulations (**A**, **B**, **K**, **L**) and experiments. **C**–**J** correspond to cell aggregates embedded in a neurogel with RGD fragments (**C**, **D**) and collagen gels of concentration 1.0 mg/ml (**E**, **F**), 1.2 mg/ml (**G**, **H**), and 1.7 mg/ml (**I**, **J**). Figure adapted with permission from [53]. Copyright 2004, National Academy of Sciences

mechanical properties and/or bioactivity can also be tuned by incorporating small amounts of nanoparticles into bioink formulation [58].

Usually, all hydrogel bioink formulations require printing of a polymer solution followed by subsequent cross-linking. This requires a highly viscous polymer solution (polymer wt% >3%) and rapid cross-linking to develop self-supporting structures. There are two forms of cross-linking: physical and chemical cross-linking. Physical cross-linking is a non-chemical approach that utilizes hydrophobic interactions, ionic interactions, and hydrogen bonding. Chemical cross-linking relies on the formation of covalent bonds, which could be a radical polymerization (such as photo-cross-linking) or Michael-type addition reaction. The chemically cross-linked hydrogels form a mechanically robust network as compared to the physically cross-linked hydrogels, which is particularly important for the stem cell behavior including differentiation [59, 60]. Recently, hydrogels have been defined as two- or multi-component systems consisting of a three-dimensional network of polymer chains and water that fills the space between macromolecules. Depending on the properties of the polymer (polymers) used, as well as on the nature and density of the network joints, such structures in an equilibrium can contain various amounts

of water; typically in the swollen state, the mass fraction of water in a hydrogel is much higher than the mass fraction of polymer. In practice, to achieve high degrees of swelling, it is common to use synthetic polymers that are water-soluble when in non-cross-linked form.

Hydrogels may be synthesized in a number of "classical" chemical ways. These include one-step procedures like polymerization and parallel cross-linking of multifunctional monomers, as well as multiple step procedures involving synthesis of polymer molecules having reactive groups and their subsequent cross-linking, possibly also by reacting polymers with suitable cross-linking agents. The polymer engineer can design and synthesize polymer networks with molecular-scale control over structure such as cross-linking density and with tailored properties, such as biodegradation, mechanical strength, and chemical and biological response to stimuli [61].

Pluronic and PEG are the most common synthetic polymers for bioprinting. Pluronic, a poloxamer-based triblock copolymer composed of two hydrophobic groups between a water-soluble group, has been widely used in extrusion-based bioprinting as it gels at room temperature but flows at temperatures below 10 °C. However, it is not very stable and erodes within hours. Thus, it is generally used as a supporting material [62]. Lewis Lab took an advantage of this property and printed pluronic within a photopolymerizable hydrogel to create micro-channels [63]. Müller et al. [64] developed an acrylated pluronic to create UV cross-linked stable gels post-printing [64]. The most common forms of PEG for bioinks are PEG-diacrylate (PEG-DA) and PEG-methacrylate, which are suitable for extrusion-based, droplet-based, and laser-based printing technologies [52, 65, 66]. PEG is hydrophilic and not adhesive to proteins and cells; therefore, it requires blending with other natural polymers or functionalization with biochemical cues. It is possible to form strong robust hydrogels using PEG-based polymers. For instance, Hockaday et al. [67] printed aortic valve geometries using PEG-DA hydrogels blended with alginate and achieved tenfold range in elastic modulus from ~5 to ~75 kPa [67]. Hong et al. [52] reported D printing of tough and biocompatible, cell-laden PEG–alginate–nanoclay hydrogels infused with collagen [68]. Rutz et al. [69] developed partially cross-linked PEG-based multi-material bioink formulations with tunable viscosity to enhance print fidelity and secondary cross-linking ability to stabilize the constructs [69].

Alginate is one of the most commonly used natural polymers to formulate bioinks for inkjet and DIW printing. For inkjet printing, calcium chloride is jetted onto alginic acid solution [70]. For extrusion-based printing, alginate is printed as a viscous solution, and the constructs are exposed to $CaCl_2$ solution to induce post-printing cross-linking. Alginate is not cell adhesive, thus it is generally blended with other natural polymers (e.g., gelatin and fibrinogen) to induce cell adhesion and biological activity [71–75]. Note that the majority of the natural polymers are used as a component of bioink formulation. HA and gelatin that have been utilized extensively in the form of functionalized polymers thus fall into the synthetic polymer category, which is discussed below.

Gelatin is commonly used in the form of gelatin methacryloyl (GelMA)-based hydrogel for DIW [76, 77]. Lim et al. [74] recently reported a visible light photo-cross-linking system to minimize the oxygen inhibition in photopolymerized GelMA hydrogels [74]. They reported higher print fidelity and cell viability for ruthenium/sodium persulfate visible photo-initiator as compared to UV photo-initiator Igracure 2959. Similar to gelatin, HA has been modified in many ways to create cell-laden bioinks [78–80]. For instance, Burdick Lab reported HA-based supramolecular hydrogels cross-linked by cyclodextrin–adamantane host–guest interactions, which are capable of shear-thinning and self-healing [78]. The non-covalent bonds allow direct writing of inks into support gels. HA hydrogels were developed to display both shear-thinning behavior due to guest–host bonding and stabilization post-printing via UV-induced covalent cross-linking [80]. Supramolecular hydrogels are particularly attractive for extrusion-based printing as they could flow under shear and self-heal immediately after printing, leading to high print fidelity. In addition to guest–host bonding, self-assembling peptides [81] and polypeptide–DNA hydrogels [82] are other emerging candidates for bioink design.

6 Nanoparticle-Reinforced Hydrogels

Nanocomposite hydrogels are found to be more superior to conventional hydrogels in terms of stability, mechanical strength, and stiffness. Hybrid ink of monomer (*N*-acryloyl glycinamide) (NAGA) and nanoclay (Laponite XLG) was developed by Zhai et al. suitable for 3D printing. The printed pregel NAGA-clay fine-tuned to form PNAGA clay composite hydrogel scaffold by polymerizing with UV light radiation. The prepared scaffolds supported osteogenic differentiation of primary rat osteoblast (ROB) cells. Moreover they facilitated the regeneration of new bone in tibia defects of rats [83]. Water immersion studies were performed, and it was concluded that even after immersion for months, the scaffold was stable and did not show further swelling activity. Thus, it was confirmed that addition of nanoclay into PNAGA had no influence on hydrogen bonding interactions. The mechanical properties was analyzed in terms of bearing external load investigated by pressing the sample with car wheel and hand folding as shown in Fig. 3.

Nanoparticles show great promise for purification and removal of toxins. This strategy have been used to fabricate toxification device that mimic liver lobule microstructure. The matrix poly(ethylene glycol) diacrylate (PEGDA) allows the efficient trapping of toxins while polyacetylene nanopartciles sense and attract toxins [84]. The ability to produce patient-specific implants is a major attraction of 3D printing or additive manufacturing. It has enabled the design of complex architectures required for hearing aids. A team of researchers developed bionic ear using alginate hydrogel matrix (Fig. 4). The matrix pre-seeded with viable chondrocytes was then 3D printed along with silver nanoparticle-infused silicone solutions. The printed bionic ear possessed enhanced auditory sensing for radio frequency reception

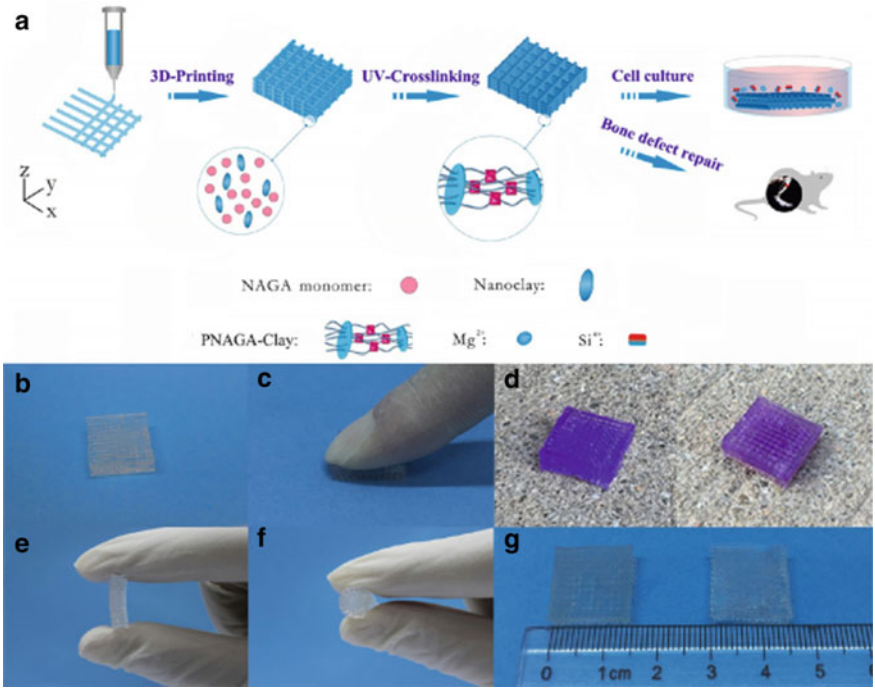

Fig. 3 Procedure of 3D-printing PNAGA-clay scaffold (**a**) and photographs of PNAGA20%-clay scaffolds showing their ability to resist finger compression (**b** and **c**), car wheel pressing (**d** left and right denote before and after pressing), and hand folding (**e** and **f**). The scaffold is very stable even after immersing in water for a long time (**g** and left and right denote before and after water immersion for 3 months). The scaffolds used for the car wheel pressing experiment were stained with gentian violet. Reproduced with permission from [83]. Copyright © 2017, American Chemical Society

and moreover the printing process had no adverse effect on the viability of the cells [85].

Hydrogels based on cellulose are widely known for their availability, biocompatibility, and efficient cell encapsulation [86]. Nanocellulose containing hydrogels have shown good cell growth and cell viability. NFC-alginate bioink was used to print auricular constructs along with chondrocytes [87]. The chondrocyte cells proliferated and cartilage specific extracellular matrix was seen around the cells.

7 Summary and Future Perspectives

3D printing has a strong potential to become a common fabrication technique in medicine as it enables fabrication of modular and patient-specific scaffolds and devices, and tissue models, with high structural complexity and design flexibility

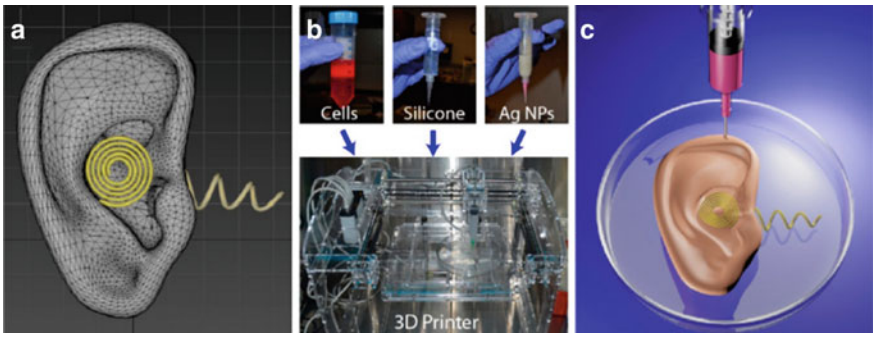

Fig. 4 Three-dimensional interweaving of biology and electronics via additive manufacturing to generate a bionic ear. (**a**) CAD drawing of the bionic ear. (**b**) (top) Optical images of the functional materials, including biological (chondrocytes), structural (silicone), and electronic (AgNP-infused silicone) used to form the bionic ear. (Bottom) a 3D printer used for the printing process. (**c**) Illustration of the 3D printed bionic ear. Reproduced with permission from [85]. Copyright © 2013, American Chemical Society

[5, 9, 62, 88–91]. There is a significant interest in designing novel bioink formulations toward the goal of achieving the "ideal" bioink for each bioprinting technology [45]. Cell-laden hydrogels are the most common bioinks, offering novel strategies including multi-material printing, shear-thinning capability, and sequential cross-linking toward self-supporting constructs. Decellularized extracellular matrix (dECM)-based bioinks provide an alternative approach utilizing decellularized tissues, yet the processing of decellularized tissue increases the cost of the bioinks. Cell aggregate printing enables direct printing of cells into tissue constructs, but the size of these constructs is currently limited as the process requires large quantities of cells. In addition to bioink development, there is also need for bioprinters with high resolution, which is particularly important to develop vascularized constructs. Considering future perspectives, supramolecular hydrogels with reversible cross-linking mechanism [79] and stimuli-responsive materials for biomimetic 4D printing [92] are potentially the most interesting candidates for bioink design. Four-dimensional (4D) printing is an emerging as a fascinating method to fabricate stimuli-responsive 3D structures with wide applications in organ engineering and tissue regeneration. The concept introduced in 2013 has gained much popularity, and several hydrogel-based inks have been developed for 4D printing. Much of the works focus on altering the shape of the 3D printed materials in response to temperature change or water absorption [93]. 4D printing may provide a novel platform for biomedical studies of functional synthetic tissues and organs. Significant efforts are required to design highly robust hydrogels having shape memory effect. Development of such hydrogels are still in the infant stage. There are still many hurdles to overcome when considered for biomedical applications, since biocompatibility and biodegradability is a major concern when tissue engineering is concerned. Finally, there are still many regulatory challenges to move the 3D bioprinted constructs into clinic.

References

1. Griffith LG, Naughton G (2002) Tissue engineering—current challenges and expanding opportunities. Science (80–). https://doi.org/10.1126/science.1069210
2. Benam KH, Dauth S, Hassell B, Herland A, Jain A, Jang K-J, Karalis K, Kim HJ, MacQueen L, Mahmoodian R, Musah S, Torisawa Y, van der Meer AD, Villenave R, Yadid M, Parker KK, Ingber DE, Engineered in vitro disease models. Annu Rev Pathol. https://doi.org/10.1146/annurev-pathol-012414-040418
3. Tibbitt MW, Rodell CB, Burdick JA, Anseth KS (2015) Progress in material design for biomedical applications. Proc Natl Acad Sci. https://doi.org/10.1073/pnas.1516247112
4. Nguyen D, Hägg DA, Alma F, Ekholm J, Brants C, Kalogeropoulos T, Zaunz S, Concaro S, Brittberg M, Lindah A, Gatenholm P, Enejder A (2017) Cartilage tissue engineering by the 3D bioprinting of iPS cells in a nanocellulose/alginate bioink. Sci Rep 7:1–10. https://doi.org/10.1038/s41598-017-00690-y
5. Zhang YS, Duchamp M, Oklu R, Ellisen LW, Langer R, Khademhosseini A (2016) Bioprinting the cancer microenvironment. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.6b00246
6. Langer R, Vacanti J (1993) Tissue engineering. Science (80–):260, 920–926. https://doi.org/10.1126/science.8493529
7. Bajaj P, Schweller RM, Khademhosseini A, West JL, Bashir R (2014) 3D biofabrication strategies for tissue engineering and regenerative medicine. Annu Rev Biomed Eng. https://doi.org/10.1146/annurev-bioeng-071813-105155
8. https://optn.transplant.hrsa.gov (n.d.)
9. Murphy SV, Atala A (2014) 3D bioprinting of tissues and organs. Nat Biotechnol. https://doi.org/10.1038/nbt.2958
10. Groen N, Guvendiren M, Rabitz H, Welsh WJ, Kohn J, De Boer J (2016) Stepping into the omics era: opportunities and challenges for biomaterials science and engineering. Acta Biomater. https://doi.org/10.1016/j.actbio.2016.02.015
11. Shafiee A, Atala A (2016) Printing technologies for medical applications. Trends Mol Med. https://doi.org/10.1016/j.molmed.2016.01.003
12. Mandrycky C, Wang Z, Kim K, Kim DH (2016) 3D bioprinting for engineering complex tissues. Biotechnol Adv. https://doi.org/10.1016/j.biotechadv.2015.12.011
13. Ozbolat IT, Moncal KK, Gudapati H (2017) Evaluation of bioprinter technologies. Addit Manuf. https://doi.org/10.1016/j.addma.2016.10.003
14. Ozbolat IT, Hospodiuk M (2016) Current advances and future perspectives in extrusion-based bioprinting. Biomaterials. https://doi.org/10.1016/j.biomaterials.2015.10.076
15. Guillemot F, Mironov V, Nakamura M (2010) Bioprinting is coming of age: report from the international conference on bioprinting and biofabrication in bordeaux (3B'09). In: Biofabrication. https://doi.org/10.1088/1758-5082/2/1/010201
16. Guvendiren M, Molde J, Soares RMD, Kohn J (2016) Designing biomaterials for 3D printing. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.6b00121
17. Dababneh AB, Ozbolat IT (2014) Bioprinting technology: a current state-of-the-art review. J Manuf Sci Eng. https://doi.org/10.1115/1.4028512
18. Cui H, Nowicki M, Fisher JP, Zhang LG (2017) 3D bioprinting for organ regeneration. Adv Healthc Mater. https://doi.org/10.1002/adhm.201601118
19. Jose RR, Rodriguez MJ, Dixon TA, Omenetto F, Kaplan DL (2016) Evolution of bioinks and additive manufacturing technologies for 3D bioprinting. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.6b00088
20. Munaz A, Vadivelu RK, St John J, Barton M, Kamble H, Nguyen NT (2016) Three-dimensional printing of biological matters. J Sci Adv Mater Dev. https://doi.org/10.1016/j.jsamd.2016.04.001
21. Levato R, Visser J, Planell JA, Engel E, Malda J, Mateos-Timoneda MA (2014) Biofabrication of tissue constructs by 3D bioprinting of cell-laden microcarriers. Biofabrication. https://doi.org/10.1088/1758-5082/6/3/035020

22. Jakus AE, Rutz AL, Shah RN (2016) Advancing the field of 3D biomaterial printing. Biomed Mater. https://doi.org/10.1088/1748-6041/11/1/014102
23. Panwar A, Tan LP (2016) Current status of bioinks for micro-extrusion-based 3D bioprinting. Molecules. https://doi.org/10.3390/molecules21060685
24. Ozbolat IT, Peng W, Ozbolat V (2016) Application areas of 3D bioprinting. Drug Discov Today. https://doi.org/10.1016/j.drudis.2016.04.006
25. Turner BN, Strong R, Gold SA (2014) A review of melt extrusion additive manufacturing processes: I. Process design and modeling. Rapid Prototyp J. https://doi.org/10.1108/RPJ-01-2013-0012
26. Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR (2003) Organ printing: computer-aided jet-based 3D tissue engineering. Trends Biotechnol. https://doi.org/10.1016/S0167-7799(03)00033-7
27. Wilson WC, Boland T (2003) Cell and organ printing 1: protein and cell printers, Anat Rec Part A Discov Mol Cell Evol Biol. https://doi.org/10.1002/ar.a.10057
28. Nakamura M, Kobayashi A, Takagi F, Watanabe A, Hiruma Y, Ohuchi K, Iwasaki Y, Horie M, Morita I, Takatani S (2006) Biocompatible inkjet printing technique for designed seeding of individual living cells. Tissue Eng. https://doi.org/10.1089/ten.2005.11.1658
29. Gudapati H, Dey M, Ozbolat I (2016) A comprehensive review on droplet-based bioprinting: past, present and future. Biomaterials. https://doi.org/10.1016/j.biomaterials.2016.06.012
30. Nishiyama Y, Nakamura M, Henmi C, Yamaguchi K, Mochizuki S, Nakagawa H, Takiura K (2009) Development of a three-dimensional bioprinter: construction of cell supporting structures using hydrogel and state-of-the-art inkjet technology. J Biomech Eng. https://doi.org/10.1115/1.3002759
31. Choi WS, Ha D, Park S, Kim T (2011) Synthetic multicellular cell-to-cell communication in inkjet printed bacterial cell systems. Biomaterials. https://doi.org/10.1016/j.biomaterials.2010.12.014
32. Shirazi SFS, Gharehkhani S, Mehrali M, Yarmand H, Metselaar HSC, Adib Kadri N, Osman NAA (2015) A review on powder-based additive manufacturing for tissue engineering: selective laser sintering and inkjet 3D printing. Sci Technol Adv Mater. https://doi.org/10.1088/1468-6996/16/3/033502
33. Skoog SA, Goering PL, Narayan RJ (2014) Stereolithography in tissue engineering. J Mater Sci Mater Med. https://doi.org/10.1007/s10856-013-5107-y
34. Elomaa L, Pan CC, Shanjani Y, Malkovskiy A, Seppälä JV, Yang Y (2015) Three-dimensional fabrication of cell-laden biodegradable poly(ethylene glycol-co-depsipeptide) hydrogels by visible light stereolithography. J Mater Chem B. https://doi.org/10.1039/c5tb01468a
35. Wang Z, Abdulla R, Parker B, Samanipour R, Ghosh S, Kim K (2015) A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks. Biofabrication. https://doi.org/10.1088/1758-5090/7/4/045009
36. Morris VB, Nimbalkar S, Younesi M, McClellan P, Akkus O (2017) Mechanical properties, cytocompatibility and manufacturability of chitosan: PEGDA hybrid-gel scaffolds by stereolithography. Ann Biomed Eng. https://doi.org/10.1007/s10439-016-1643-1
37. Barron JA, Spargo BJ, Ringeisen BR (2004) Biological laser printing of three dimensional cellular structures. Appl Phys A Mater Sci Process. https://doi.org/10.1007/s00339-004-2620-3.
38. Barron JA, Wu P, Ladouceur HD, Ringeisen BR (2004) Biological laser printing: A novel technique for creating heterogeneous 3-dimensional cell patterns. Biomed Microdevices. https://doi.org/10.1023/B:BMMD.0000031751.67267.9f
39. Ringeisen BR, Kim H, Barron JA, Krizman DB, Chrisey DB, Jackman S, Auyeung RYC, Spargo BJ (2004) Laser printing of pluripotent embryonal carcinoma cells. Tissue Eng. https://doi.org/10.1089/107632704323061843
40. Hopp B, Smausz T, Kresz N, Barna N, Bor Z, Kolozsvári L, Chrisey DB, Szabó A, Nógrádi A (2005) Survival and proliferative ability of various living cell types after laser-induced forward transfer. Tissue Eng. https://doi.org/10.1089/ten.2005.11.1817

41. Doraiswamy A, Narayan RJ, Lippert T, Urech L, Wokaun A, Nagel M, Hopp B, Dinescu M, Modi R, Auyeung RCY, Chrisey DB (2006) Excimer laser forward transfer of mammalian cells using a novel triazene absorbing layer. Appl Surf Sci. https://doi.org/10.1016/j.apsusc.2005.07.166
42. Koch L, Kuhn S, Sorg H, Gruene M, Schlie S, Gaebel R, Polchow B, Reimers K, Stoelting S, Ma N, Vogt PM, Steinhoff G, Chichkov B (2010) Laser printing of skin cells and human stem cells. Tissue Eng Part C Methods. https://doi.org/10.1089/ten.tec.2009.0397
43. Miller JS, Burdick JA (2016) Editorial: special issue on 3D printing of biomaterials. ACS Biomater Sci Eng 2:1658–1661. https://doi.org/10.1021/acsbiomaterials.6b00566
44. Tirella A, Orsini A, Vozzi G, Ahluwalia A (2009) A phase diagram for microfabrication of geometrically controlled hydrogel scaffolds. Biofabrication. https://doi.org/10.1088/1758-5082/1/4/045002
45. Hölzl K, Lin S, Tytgat L, Van Vlierberghe S, Gu L, Ovsianikov A (2016) Bioink properties before, during and after 3D bioprinting. Biofabrication. https://doi.org/10.1088/1758-5090/8/3/032002
46. Guillotin B, Souquet A, Catros S, Duocastella M, Pippenger B, Bellance S, Bareille R, Rémy M, Bordenave L, Amédée J, Guillemot F (2010) Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. Biomaterials. https://doi.org/10.1016/j.biomaterials.2010.05.055
47. Tirella A, Vozzi F, De Maria C, Vozzi G, Sandri T, Sassano D, Cognolato L, Ahluwalia A (2011) Substrate stiffness influences high resolution printing of living cells with an ink-jet system. J Biosci Bioeng. https://doi.org/10.1016/j.jbiosc.2011.03.019
48. Fedorovich NE, Alblas J, de Wijn JR, Hennink WE, Verbout AJ, Dhert WJA (2007) Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing. Tissue Eng. https://doi.org/10.1089/ten.2006.0175
49. Pati F, Jang J, Ha DH, Won Kim S, Rhie JW, Shim JH, Kim DH, Cho DW (2014) Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun. https://doi.org/10.1038/ncomms4935
50. Forgacs G, Foty RA (2004) Biological relevance of tissue liquidity and viscoelasticity. In: Deutsch A, Howard J, Falcke M, Zimmermann W (eds) Function and regulation of cellular systems. Birkhäuser, Basel, pp 269–277
51. Marga F, Neagu A, Kosztin I, Forgacs G (2007) Developmental biology and tissue engineering. Birth Defects Res C Embryo Today. https://doi.org/10.1002/bdrc.20109
52. Wüst S, Müller R, Hofmann S (2015) 3D Bioprinting of complex channels—effects of material, orientation, geometry, and cell embedding. J Biomed Mater Res Part A. https://doi.org/10.1002/jbm.a.35393
53. Jakab K, Neagu A, Mironov V, Markwald RR, Forgacs G (2004) Engineering biological structures of prescribed shape using self-assembling multicellular systems. Proc Natl Acad Sci. https://doi.org/10.1073/pnas.0400164101
54. Gardel ML, Shin JH, MacKintosh FC, Mahadevan L, Matsudaira P, Weitz DA (2004) Elastic behavior of cross-linked and bundled actin networks. Science (80–). https://doi.org/10.1126/science.1095087.
55. Storm C, Pastore JJ, MacKintosh FC, Lubensky TC, Janmey PA (2005) Nonlinear elasticity in biological gels. Nature. https://doi.org/10.1038/nature03521
56. Guvendiren M, Burdick JA (2013) Engineering synthetic hydrogel microenvironments to instruct stem cells. Curr Opin Biotechnol. https://doi.org/10.1016/j.copbio.2013.03.009
57. Burdick JA, Prestwich GD (2011) Hyaluronic acid hydrogels for biomedical applications. Adv Mater. https://doi.org/10.1002/adma.201003963
58. Ribeiro M, de Moraes MA, Beppu MM, Garcia MP, Fernandes MH, Monteiro FJ et al (2015) Development of silk fibroin/nanohydroxyapatite composite hydrogels for bone tissue engineering. Eur Polym J, 66–77
59. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA et al (n.d.) Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nat Mater, 518–526

60. Khetan S, Guvendiren M, Legant WR, Cohen DM, Chen CS, Burdick JA (2013) Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels. Nat Mater. https://doi.org/10.1038/nmat3586
61. Burkert S, Schmidt T, Gohs U, Dorschner H, Arndt KF (2007) Cross-linking of poly(*N*-vinyl pyrrolidone) films by electron beam irradiation. Radiat Phys Chem. https://doi.org/10.1016/j.radphyschem.2007.02.024
62. Kang HW, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A (2016) A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. Nat Biotechnol. https://doi.org/10.1038/nbt.3413
63. Wu W, Deconinck A, Lewis JA (2011) Omnidirectional printing of 3D microvascular networks. Adv Mater. https://doi.org/10.1002/adma.201004625
64. Müller M, Becher J, Schnabelrauch M, Zenobi-Wong M (2015) Nanostructured pluronic hydrogels as bioinks for 3D bioprinting. Biofabrication. https://doi.org/10.1088/1758-5090/7/3/035006
65. Cui X, Breitenkamp K, Finn MG, Lotz M, D'Lima DD (2012) Direct human cartilage repair using three-dimensional bioprinting technology. Tissue Eng Part A. https://doi.org/10.1089/ten.tea.2011.0543
66. Hribar KC, Soman P, Warner J, Chung P, Chen S (2014) Light-assisted direct-write of 3D functional biomaterials. Lab Chip. https://doi.org/10.1039/c3lc50634g
67. Hockaday LA, Kang KH, Colangelo NW, Cheung PYC, Duan B, Malone E, Wu J, Girardi LN, Bonassar LJ, Lipson H, Chu CC, Butcher JT (2012) Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. Biofabrication. https://doi.org/10.1088/1758-5082/4/3/035005
68. Hong S, Sycks D, Chan HFA, Lin S, Lopez GP, Guilak F, Leong KW, Zhao X (2015) 3D printing: 3D printing of highly stretchable and tough hydrogels into complex, cellularized structures. Adv Mater. https://doi.org/10.1002/adma.201570182
69. Rutz AL, Hyland KE, Jakus AE, Burghardt WR, Shah RN (2015) A multimaterial bioink method for 3D printing tunable, cell-compatible hydrogels. Adv Mater. https://doi.org/10.1002/adma.201405076
70. Boland T, Tao X, Damon BJ, Manley B, Kesari P, Jalota S, Bhaduri S (2007) Drop-on-demand printing of cells and materials for designer tissue constructs. Mater Sci Eng C. https://doi.org/10.1016/j.msec.2006.05.047
71. Xu T, Baicu C, Aho M, Zile M, Boland T (2009) Fabrication and characterization of bio-engineered cardiac pseudo tissues. Biofabrication. https://doi.org/10.1088/1758-5082/1/3/035001
72. Jia J, Richards DJ, Pollard S, Tan Y, Rodriguez J, Visconti RP, Trusk TC, Yost MJ, Yao H, Markwald RR, Mei Y (2014) Engineering alginate as bioink for bioprinting. Acta Biomater. https://doi.org/10.1016/j.actbio.2014.06.034
73. Zhao Y, Yao R, Ouyang L, Ding H, Zhang T, Zhang K, Cheng S, Sun W (2014) Three-dimensional printing of Hela cells for cervical tumor model in vitro. Biofabrication. https://doi.org/10.1088/1758-5082/6/3/035001
74. Lim KS, Schon BS, Mekhileri NV, Brown GCJ, Chia CM, Prabakar S, Hooper GJ, Woodfield TBF (2016) New visible-light photoinitiating system for improved print fidelity in gelatin-based bioinks. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.6b00149
75. Pan T, Song W, Cao X (2016) 3D bioplotting of gelatin/alginate scaffolds for tissue engineering: influence of crosslinking degree and pore architecture on physicochemical properties. J Mater Sci Technol 32:889–900 (2016)
76. Bertassoni LE, Cardoso JC, Manoharan V, Cristino AL, Bhise NS, Araujo WA, Zorlutuna P, Vrana NE, Ghaemmaghami AM, Dokmeci MR, Khademhosseini A (2014) Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels. Biofabrication. https://doi.org/10.1088/1758-5082/6/2/024105
77. Loessner D, Meinert C, Kaemmerer E, Martine LC, Yue K, Levett PA, Klein TJ, Melchels FPW, Khademhosseini A, Hutmacher DW (2016) Functionalization, preparation and use of cell-laden gelatin methacryloyl-based hydrogels as modular tissue culture platforms. Nat Protoc. https://doi.org/10.1038/nprot.2016.037

78. Highley CB, Rodell CB, Burdick JA (2015) Direct 3D printing of shear-thinning hydrogels into self-healing hydrogels. Adv Mater. https://doi.org/10.1002/adma.201501234
79. Rodell CB, MacArthur JW, Dorsey SM, Wade RJ, Wang LL, Woo YJ, Burdick JA (2015) Shear-thinning supramolecular hydrogels with secondary autonomous covalent crosslinking to modulate viscoelastic properties in vivo. Adv Funct Mater. https://doi.org/10.1002/adfm.201403550
80. Ouyang L, Highley CB, Rodell CB, Sun W, Burdick JA (2016) 3D printing of shear-thinning hyaluronic acid hydrogels with secondary cross-linking. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.6b00158
81. Raphael B, Khalil T, Workman VL, Smith A, Brown CP, Streuli C, Saiani A, Domingos M (2017) 3D cell bioprinting of self-assembling peptide-based hydrogels. Mater Lett. https://doi.org/10.1016/j.matlet.2016.12.127
82. Li C, Faulkner-Jones A, Dun AR, Jin J, Chen P, Xing Y, Yang Z, Li Z, Shu W, Liu D, Duncan RR (2015) Rapid formation of a supramolecular polypeptide-DNA hydrogel for in situ three-dimensional multilayer bioprinting. Angew Chem Int Ed. https://doi.org/10.1002/anie.201411383
83. Zhai X, Ma Y, Hou C, Gao F, Zhang Y, Ruan C, Pan H, Liu WW (2017) 3D-printed high strength bioactive supramolecular polymer/clay nanocomposite hydrogel scaffold for bone regeneration, ACS Biomater Sci Eng 3:1109–1118
84. Gou M, Qu X, Zhu W, Xiang M, Yang J, Zhang K, Wei Y, Chen S (2014) Bio-inspired detoxification using 3d-printed hydrogel nanocomposites. Nat Commun. https://doi.org/10.1038/ncomms4774
85. Mannoor MS, Jiang Z, James T, Kong YL, Malatesta KA, Soboyejo WO, Verma N, Gracias DH, McAlpine MC (2013) 3D printed bionic ears. Nano Lett. https://doi.org/10.1021/nl4007744
86. Blessy Joseph NK, Maria HJ, Thomas S (2018) Nanocellulose: health care applications. In: Mishra M (ed) Encyclopedia of polymer applications. CRC Press, pp 1829–1852
87. Martínez H, Schwarz S, Rotter N, Gatenholm P (2016) Bioprinting 3D bioprinting of human chondrocyte-laden nanocellulose hydrogels for patient-specific auricular cartilage regeneration. Bioprinting 1–2:22–35. https://doi.org/10.1016/j.bprint.2016.08.003
88. Jang J, Yi HG, Cho DW (2016) 3D printed tissue models: present and future. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.6b00129
89. Jang J, Kim TG, Kim BS, Kim SW, Kwon SM, Cho DW (2016) Tailoring mechanical properties of decellularized extracellular matrix bioink by vitamin B2-induced photo-crosslinking. Acta Biomater. https://doi.org/10.1016/j.actbio.2016.01.013
90. Jang J, Park HJ, Kim SW, Kim H, Park JY, Na SJ, Kim HJ, Park MN, Choi SH, Park SH, Kim SW, Kwon SM, Kim PJ, Cho DW (2017) 3D printed complex tissue construct using stem cell-laden decellularized extracellular matrix bioinks for cardiac repair. Biomaterials. https://doi.org/10.1016/j.biomaterials.2016.10.026
91. Kuo CY, Eranki A, Placone JK, Rhodes KR, Aranda-Espinoza H, Fernandes R, Fisher JP, Kim PCW (2016) Development of a 3D printed, bioengineered placenta model to evaluate the role of trophoblast migration in preeclampsia. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.6b00031
92. Gladman AS, Matsumoto EA, Nuzzo RG, Mahadevan, L, Lewis JA (2016) Biomimetic 4D printing. Nat Mater 15. https://doi.org/10.1038/NMAT4544
93. Bakarich SE, Gorkin R, In Het Panhuis M, Spinks GM (2015) 4D printing with mechanically robust, thermally actuating hydrogels. Macromol Rapid Commun 36:1211–1217. https://doi.org/10.1002/marc.201500079