3D Bioprinting of Cardiovascular Tissue Constructs: Cardiac Bioinks

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Introduction

Cardiovascular tissue bioprinting occupies a critical crossroads position between the fields of biomaterials engineering, cardiovascular biology, three-dimensional (3D) design and modeling, and biomanufacturing [1–4]. This complex area of research requires expertise from all these disciplines to provide a multidisciplinary approach that enables fabrication of functional and living tissues and organs, whether for basic science or translational research applications [5]. A major challenge that hampers this field is the lack of systematic characterization of the physical and chemical properties of hydrogel-based bioinks that are applicable to organ and tissue bioprinting [6–8]. Tailoring bioink properties to mimic the complex native tissue extracellular matrix (ECM) is of great importance and a slight divergence could result in pathological or loss of function manifests [9, 10].

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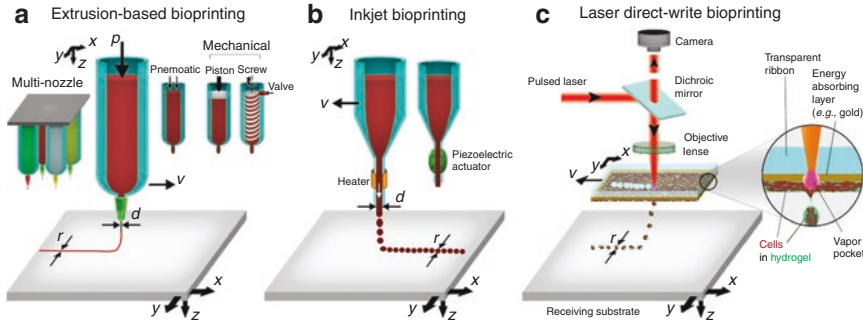e-mail: vahid.serpooshan@bme.gatech.edu

Fig. 4.1 Major methods that are available to bioprint tissue analogues. (**a**) Extrusion-based bio-printers use single- or multi-nozzle print heads, squeezing out the bioink using mechanical or pneumatic forces. (**b**) Droplet-based bioprinting uses thermal or piezoelectric forces to discharge droplets of bioinks. (**c**) Laser-based bioprinters use high-energy pulsed laser to eject bioink drop-lets from a donor layer onto the receiving substrate. (Adapted from [32])

Functional tissue bioprinting holds great promise to combine rationally designed biomaterials, functional cells, and macromolecules into 3D constructs that closely recapitulate the mechanical, structural, and functional microenvironment of native tissues [11]. With precise control over spatial arrangement of the cell-biomaterial architecture, 3D bioprinting can provide complex physiochemical and biological cues that are necessary for the maintenance and maturation of functional tissue analogues. To date, a range of bioprinting platforms have been used to create artifi-cial tissue constructs, such as extrusion-based [12–15], droplet-based [16–18], and laser-based printing [19–21] (Fig. 4.1).

Recent advances in in vitro tissue development has made 3D bioprinting an attrac-tive means for the next-generation regenerative medicine, specifically as a platform for tissue replacement to rescue failed organs in patient-specific therapies [6, 22–24]. Some of these applications include pancreatic tissue printing, to address loss of func-tion in diabetes [25, 26], bioprinted kidney tissue analogues that can be used instead of, or in conjunction with, dialysis in kidney failure therapies [27, 28] and cardiac tis-sue constructs which can be bioprinted to repair the damaged heart tissue post injury (e.g., myocardial infarction), or in the case of congenital heart diseases [29–31].

This chapter explores the critical parameters of hydrogel-based bioinks that are nec-essary for their successful application in functional cardiovascular tissue engineering, as tissue analogues for disease modeling and drug screening in vitro, or as implantable tissue grafts to treat a range of congenital and acquired cardiovascular diseases. We will explore the biophysical, biochemical, and biological considerations for the candidate bioinks that enable 3D bioprinting of functional cardiovascular constructs.

Types of Bioinks

Bioink can be defined as a printable biomaterial, based on naturally occurring or synthetically derived polymers (hydrogels), that can recreate some aspects of native tissue ECM [6, 7, 14]. In addition to the hydrogel component, bioinks

typically consist of cells and/or small molecules (e.g., growth or angiogenic factors) to enhance their bioactivity. Successful bioinks maintain (or promote) encapsulated cell survival, adhesion, proliferation, and function in vitro and ultimately in vivo. Some of the desirable features of bioinks include the ability to form stable filaments during printing and gentle crosslinking mechanisms, which allow for spatial control of hydrogel deposition while maintaining cell viability [7, 8, 15, 33]. Currently available bioink formulations are often based on existing hydrogel biomaterials, such as alginate [34, 35], fibrin [36, 37], hyaluronic acids [38, 39], and gelatin [13, 40–42]. These bioinks can be divided into several broad categories based on the specific crosslinking/solidification characteristics of their parent hydrogel (Table 4.1). Critical for a successful bioink, it should be able to incorporate and protect bioactive compounds within the formed hydrogel. This could enhance the bioink functional mimicry to native tissues and enhance the function of encapsulated cells [43].

To achieve an optimal bioink formulation and successfully print functional tissues, the choice of specific bioprinting process and post-print tissue culture, maintenance, and maturation is critically important [6, 7, 14]. Further, hydrogel properties such as viscosity, crosslinking mechanism, stiffness, mass transfer properties (e.g., diffusion and permeability), and biodegradability must be taken into consideration, depending on the specific application [6, 8, 15, 19, 44]. To generate a biomimetic niche that can support tissue functionality and cell maturation, the chosen bioink would need to also allow for specific chemical modifications such as small molecule conjugation and ECM proteins immobilization within the 3D printed construct.

Cardiac Bioink Characteristics

Printability Printing resolution is dependent on the volume of deposited layer. To maintain a fine balance between thin prints (high resolution) and cell viability, the bioink should generate relatively low shear stress levels under modest pressures [33, 45, 46]. Shape fidelity at high-resolution prints is critical for building up functional tissue analogues, particularly for organs that are highly vascularized and have complex tissue organization such as the heart [8, 15, 40, 41]. To maintain fidelity, bioinks should have low reflow rate during the printing process and facile crosslinking steps and culture conditions (Fig. 4.2). This requires the ability of printed construct to be self-supporting at the macroscale, ideally with little to no supporting materials. While some support bioinks might be required to maintain complex/hollow shapes during printing, they would have to be either incorporated as a functional component of the tissue analogue or allow for full removal post-printing (i.e., sacrificial inks such as pluronic). This is particularly important in bioprinting of cardiovascular tissues, considering the remarkably high blood vessel density in the tissue (about 160 capillaries per mm^2 of myocardial tissue [47]). For cardiac tissue bioprinting, therefore, successful fabrication of self-standing and stable, hollow channels at diameters ranging from micrometers (capillaries) to centimeters (arteries) would be a major challenge. These perfusable vascular

Table 4.1 Classification of hydrogel bioinks based on crosslinking or solidification mechanism

Hydrogel formation	Ionic interactions	Temperature based	Time dependent	Photoinitiator based	Chemical initiator
Advantages	Physiological conditions	Wide stiffness range	Gentle crosslinking	Biodegradable	Biodegradable
	Stability in vitro and in vivo	Physiological conditions	Easily modifiable	Wide range of stiffness	Wide range of stiffness
	Gentle cell recovery	Great sacrificial hydrogel	Biodegradable	Easily modifiable	Well-established bioink formulations
Disadvantages	Not always xeno-free	Damage encapsulated cells	Not always xeno-free	Light-based cell damage	Not always xeno-free
	Limited modification ability	Printing resolution can be low	Not chemically defined	Chemically defined	Limited gas and nutrient diffusion
	Limited biodegradability	Can induce immune responses	Printing resolution can be low	Xeno-free	Endotoxin contamination
Bioink examples	Alginate; Dextran	Pluronic; PNIPAM*	Matrigel; HyStem	PEGDA**; GELMA***	Gelatin based; silk-based

* PNIPAM - Poly(N-isopropylacrylamide)
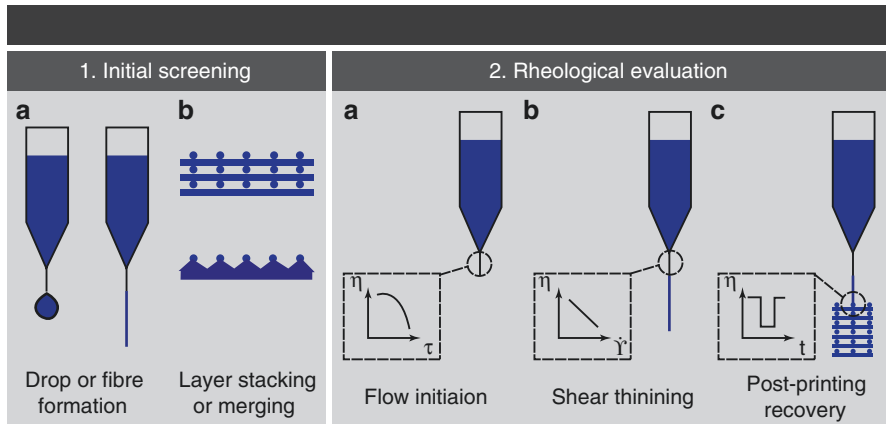** PEGDA - Poly(ethylene glycol) diacrylate
*** GelMA - Methacrylated gelatin

Fig. 4.2 Schematic demonstration of the typical approach to assess bioink printability. **1**: Initial screening of bioink formulations to (**a**) establish fiber versus droplet formation, and (**b**) successfully stack multiple layers without fusing between the layers. **2**: Rheological evaluations are performed to determine (**a**) the flow initiation and yield stress properties, (**b**) degree of shear thinning, and (**c**) recovery from shear thinning after printing. (Adapted from [14])

cardiac constructs can provide an invaluable platform for drug screening [27, 48, 49] and disease modeling studies [2, 27, 48, 50] in vitro, and a new generation of cardiac patch devices for in vivo regenerative therapies [38, 51, 52].

Post-print Processing Post-print processes are required to achieve the adequate print fidelity, as bioinks often require crosslinking [7, 53, 54]. Introducing crosslinking agents via an additional liquid phase may be detrimental to the shape fidelity. Therefore, employing a crosslinking step via long-wave ultraviolet (UV) radiation [53, 55] or visible light [40, 56] exposure at appropriate durations and intensities would be beneficial. Other types of post-print processes have often been used. For instance, promising results have been shown for salt-based post-processing after the constructs were printed [57–59]. Regardless of the chosen process, the structural and chemical stability of the initial print are critical to maintain reproducibility of engineered tissues. One challenge with light-based crosslinking is, however, the possible cell damage due to UV light irradiation [60]. This can be mitigated by combining or replacing UV with other more cytocompatible post-processing methods. Some alternative processes include aerosolized salt solution spray [61], incubation at elevated temperatures (>30 °C) [62], and enzymatic reactions [63] (Table 4.1). Additionally, less reliance upon ionic-based crosslinking may mitigate the precipitation or *salting-out* of adjunct proteins [64].

A successful cardiac bioprint will rely on a fast-acting crosslinking reagent with negligible cytotoxicity, while capable of retaining high printing fidelities. UV-crosslinked hydrogels, such as methacrylate modified gelatin (gelMA), have been extensively used for cardiac tissue printing as these hydrogels can generate

stable yet relatively soft matrices that mimic the physiomechanical and biochemical properties of the native heart tissue [5, 65]. GelMA crosslinking via UV light occurs at relatively short times and moderate intensities (~2 minutes and between 1 and 20 mW/cm^2), which could help to avoid excessive cell damage and death [16, 65].

Printed Tissue Stability and Controlled Degradation Swelling and contraction of hydrogels upon crosslinking and during tissue culture could be detrimental to the printed construct fidelity and cellular interactions/functions [66]. This can also alter the final mechanical properties of the bioink and skew its 3D arrangement [67, 68]. Controlled degradation and remodeling of bioprinted construct, along with secretion of ECM proteins by encapsulated cells, are critical for engineering a biomimetic tissue microenvironment [69]. However, printed tissue breakdown and remodeling, in an uncontrolled manner, can severely limit its translational applications, as they could cause implant detachment and failure in vivo [70, 71]. To alleviate these challenges, extensive research has focused on the development of new bioink formulations with tunable degradation profile, to enable cell-mediated tissue remodeling, while maintaining the construct integrity [72–79]. Particularly in the case of cardiac tissue constructs, a significant degree of matrix remodeling by bioprinted cells is necessary to achieve the intercellular connectivity and the remarkably high cell packing density of the native heart tissue [50, 80–83]. To that end, gelMA-based bioinks are specifically favorable as they are both biodegradable and chemically defined.

Mass Transfer Properties of Cardiac Bioinks Incorporating functional vascularization is a critical aspect of tissue bioprinting to generate and maintain large (clinically relevant) tissue constructs [84–93]. This is particularly important for bioengineered cardiac constructs, considering the remarkably high vascular density in the native myocardial tissue (approximately one capillary per cardiomyocyte) [94, 95]. With passive perfusion alone, the maximum thickness of a viable 3D tissue construct is usually around 100–250 μm [96, 97] before vascularization is required. This distance can potentially be extended to about 600 μm in bioprinted constructs that can be prevascularized prior to cell seeding. If the construct's pore size is large enough to allow for more effective passive diffusion, a similar effect can occur [98–100].

Tissue-Specific and Chemically Modifiable Bioinks The ability to incorporate tissue- and cell type-specific materials into the bioink is critically important for achieving in vivo-like functionality. Modification chemistry can provide the small molecules and ECM factors that are specific to the tissue/organ microenvironment [73, 78, 101]. For this purpose, covalent conjugation, or similar levels of immobilization, would be an effective approach to recapitulate the specific tissue cues for bioprinted cells and initiate self-directed environmental remodeling. Keeping the modification chemistry and bioink preparation steps simple would also be significantly beneficial by cutting down on preparation time and equipment and material expenses and by enhancing batch-to-batch consistency [6]. Furthermore, the hydrogel bioink should

be xeno-free or consist of entirely chemically defined components, to facilitate trans-
lational use in regenerative medicine and to enhance the reliability for use in in vitro
assays, such as drug screening and disease modeling [3].

To generate a bioink that is supportive to cardiac cells and recapitulates the
organ/tissue-specific niche, high-throughput analysis techniques, such as transcrip-
tome analysis (RNA-Seq) and proteomics can be used to characterize the native
cardiac tissue ECM. For instance, bone morphogenic proteins (BMP2/BMP4) and
Wnt inhibitors (IWP2) are known to play key roles in generation of early cardio-
myocytes in vitro [102–110]. Incorporating certain concentrations of these factors
in the tissue generation pipeline (e.g., in cardiac bioink) may promote the regenera-
tive capacity of printed constructs. Further, functionalizing the bioink with ECM
proteins, such as cadherins, connexins, and collagen, can be used to promote cell
attachment, migration, and remodeling [86, 111–116]. ECM proteins coupled with
secreted small molecules such as tumor necrosis factor alpha (TNFα), interleukin
(IL)-1, IL-6, transforming growth factor beta (TGFβ), angiotensin II, and endothe-
lin 1 can also help promote tissue maturation and vascularization in cardiac con-
structs (Fig. 4.3) [117, 118].

Tunable Mechanical Properties Altering biomechanical characteristics of the
bioinks can be achieved via initial or secondary crosslinking processes. Such modi-
fications can provide the specific mechanical cues to encapsulated cells and pro-
mote desired cellular functionalities [14, 119]. The ability to independently tune
chemical and mechanical properties of these hydrogels is critically important. For
example, crosslinking of hydrogel matrices can tune their stiffness [120–123], while
conjugation of various ECM proteins and small molecules can independently pro-
vide biochemical cues to the cells [7, 124]. Mechanical properties play a major role
in successful application of bioprinted cardiac constructs, as these tissues require
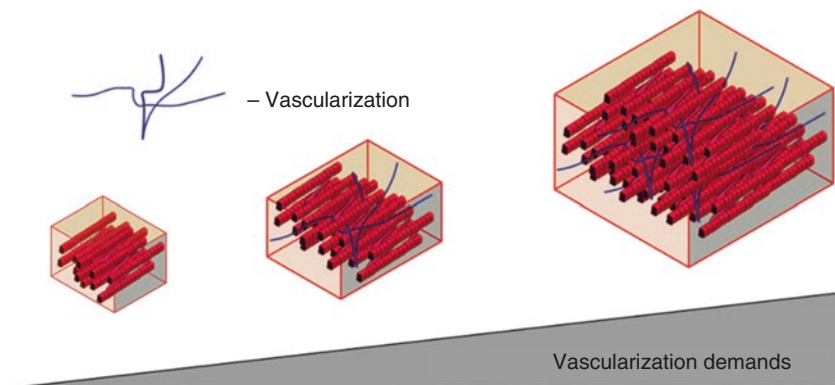strictly regulated stiffness values to exhibit proper functionality both in vitro and

Fig. 4.3 The demand for effective vascularization in vitro increases with tissue construct size and
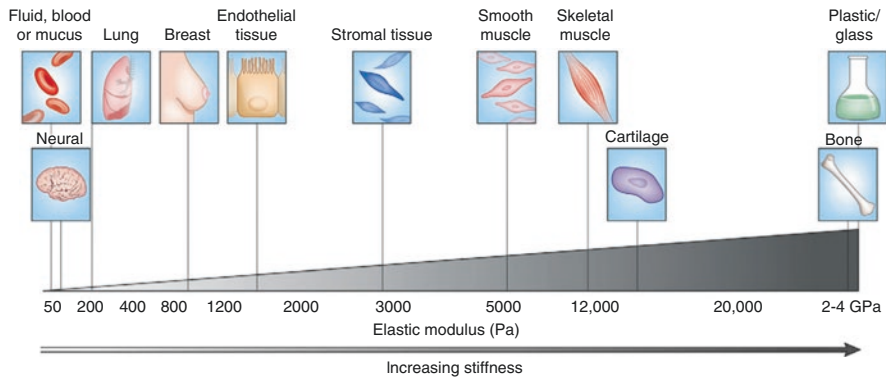complexity

Fig. 4.4 Range of ECM stiffness required for proper organ development and functionality of various organs and tissues. (Adapted from [127])
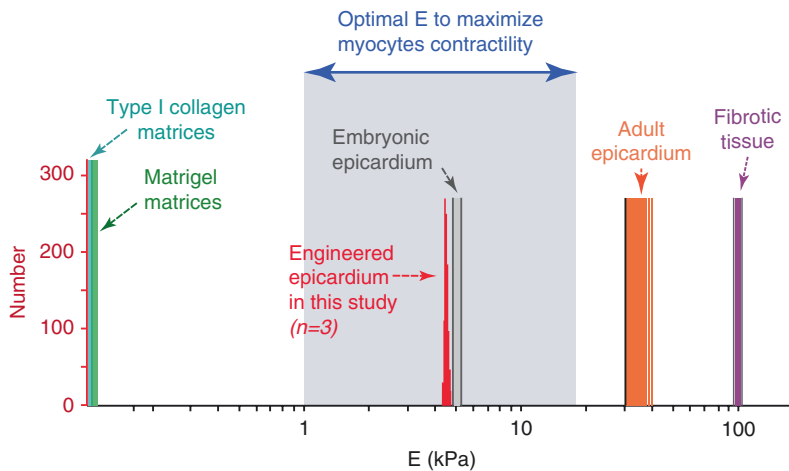

Fig. 4.5 Demonstration of varying range of stiffness for various cardiac tissues and tissue engineering hydrogels. Optimal range of stiffness, resulting in maximum contractile work of cardiac myocytes, is highlighted in blue. (Adapted from [126])

in vivo (Fig. 4.4) [32, 82, 123, 125, 126]. The post-print crosslinking mechanisms that are used for most hydrogel bioinks allow for generating tissues with a relatively broad range of stiffness. Therefore, bioprinting technology holds a great potential for manufacturing a wide variety of functional tissues.

It has been shown by different groups that cardiomyocytes exhibit maximal contractile function on matrix stiffnesses ranging from 1 to 16 kPa [51, 82, 115, 123, 126]. Thus, an optimal cardiac-specific bioink may be expected to show elastic modulus within this range (Fig. 4.5) [126]. Incorporating large numbers of nondividing cardiomyocytes in bioinks can compromise their mechanical properties and

printing fidelity (resolution and stability). To address this issue, cardiomyocytes can be combined with proliferative cardiac precursor/stem cells, cardiac, and vascular cells (endothelial and smooth muscle cells) and be encapsulated in the bioink at remarkably lower cell densities [29, 128–130]. Multiplication, differentiation, and maturation of these multilineage cardiac cell populations in bioprinted constructs, in association with controlled matrix degradation and remodeling, can lead to generation of myocardial mimetic tissue constructs at appropriate cell density and configuration. The use of stem cell sources for cardiac tissue printing may require additional examination and characterization to avoid incomplete or undesirable cell differentiation (e.g., tumor formation or reduced functionality of the engrafted tissue) that could impact the clinical application of the printed constructs [131–133].

Outlook and Conclusions

To maintain cell viability and functionality, hydrogel-based bioinks must fulfill several key biophysical and biochemical requirements, before, during, and post-printing processes. These parameters, together with the need for a functional vascular network in the construct, are critical for generating high fidelity cardiac tissue analogues. Initial and/or secondary crosslinking processes would allow for better control over the chemical and mechanical cues within the 3D constructs and therefore, enable reconstructing diverse tissue microenvironments. Preparing commercially available *cardiac bioink kits* that can be optimized for a specific tissue bioprinting would be a significant advancement in the field, especially if different chemical and mechanical properties of the hydrogels could be decoupled and independently tuned. A balance must be obtained between cardiac bioink crosslinking degree, stiffness, and biodegradation to allow for bioprinted cells to remodel their microenvironment. This is a critical step toward achieving enhanced cardiac cell connectivity, maturation, and function. Additionally, keeping bioink synthesis and modification chemistry robust and simple would be highly beneficial for wider appeal to researchers in the field.

In summary, cardiac bioprinting aims to generate clinically applicable, cardiac tissue analogues that can replace damaged/diseased tissue in vivo or be used as biomimetic platforms in vitro to model various diseases. Recent advances in bioprinting technologies have enabled fabrication of complex, patient-specific, tissue architectures at an organ-relevant spatial resolution, while supporting viability and function of multiple cell types. However, there remain some challenges for the clinical application of bioprinted cardiac constructs. Development of new cardiac-specific bioinks, using tailored biomaterials and precisely tuned selection of macromolecules, could be a great step forward toward clinical bioprinting. New methods are also needed to incorporate functional, multiscale vascular networks within printed constructs that can be perfused to maintain functionality of large-scale tissue constructs. Further, enhanced temporal and spatial resolutions in the new generation of bioprinters can help engineering more advanced cardiac tissue substitutes for regenerative medicine.

References

1. Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol. 2014;32(8):773–85.
2. Wang Z, et al. 3D bioprinted functional and contractile cardiac tissue constructs. Acta Biomater. 2018;70:48–56.
3. Ji S, Guvendiren M. Recent advances in bioink design for 3D bioprinting of tissues and organs. Front Bioeng Biotechnol. 2017;5:23.
4. Suntornnond R, et al. A mathematical model on the resolution of extrusion bioprinting for the development of new bioinks. Materials (Basel). 2016;9(9):756.
5. Jang J. 3D bioprinting and in vitro cardiovascular tissue modeling. Bioengineering (Basel). 2017;4(3):71.
6. Gopinathan J, Noh I. Recent trends in bioinks for 3D printing. Biomater Res. 2018;22:11.
7. Holzl K, et al. Bioink properties before, during and after 3D bioprinting. Biofabrication. 2016;8(3):032002.
8. Ouyang L, et al. Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. Biofabrication. 2016;8(3):035020.
9. Smith L, Cho S, Discher DE. Mechanosensing of matrix by stem cells: From matrix heterogeneity, contractility, and the nucleus in pore-migration to cardiogenesis and muscle stem cells in vivo. Semin Cell Dev Biol. 2017;71:84–98.
10. Geckil H, et al. Engineering hydrogels as extracellular matrix mimics. Nanomedicine. 2010;5(3):469–84.
11. Mandrycky C, et al. 3D bioprinting for engineering complex tissues. Biotechnol Adv. 2016;34(4):422–34.
12. Chung JHY, et al. Bio-ink properties and printability for extrusion printing living cells. Biomater Sci. 2013;1(7):763–73.
13. Liu W, et al. Coaxial extrusion bioprinting of 3D microfibrous constructs with cell-favorable gelatin methacryloyl microenvironments. Biofabrication. 2018;10(2):024102.
14. Paxton N, et al. Proposal to assess printability of bioinks for extrusion-based bioprinting and evaluation of rheological properties governing bioprintability. Biofabrication. 2017;9(4):044107.
15. Zhao Y, et al. The influence of printing parameters on cell survival rate and printability in microextrusion-based 3D cell printing technology. Biofabrication. 2015;7(4):045002.
16. Stratesteffen H, et al. GelMA-collagen blends enable drop-on-demand 3D printablility and promote angiogenesis. Biofabrication. 2017;9(4):045002.
17. Graham AD, et al. High-resolution patterned cellular constructs by droplet-based 3D printing. Sci Rep. 2017;7(1):7004.
18. Gudapati H, Dey M, Ozbolat I. A comprehensive review on droplet-based bioprinting: past, present and future. Biomaterials. 2016;102:20–42.
19. Dababneh AB, Ozbolat IT. Bioprinting technology: a current state-of-the-art review. J Manuf Sci Eng-Trans ASME. 2014;136(6):061016.
20. Entsfellner K, et al. First 3D printed medical robot for ENT surgery – application specific manufacturing of laser sintered disposable manipulators. 2014 IEEE/RSJ international conference on Intelligent Robots and Systems (Iros 2014), 2014. p. 4278–83.
21. Zhang Z, et al. Effects of living cells on the bioink printability during laser printing. Biomicrofluidics. 2017;11(3):034120.
22. Leberfinger AN, et al. Concise review: bioprinting of stem cells for transplantable tissue fabrication. Stem Cells Transl Med. 2017;6(10):1940–8.
23. Malyala SK, Kumar YR, Rao CSP. Organ printing with life cells: a review. Mater Today-Proc. 2017;4(2):1074–83.
24. Datta P, Ayan B, Ozbolat IT. Bioprinting for vascular and vascularized tissue biofabrication. Acta Biomater. 2017;51:1–20.
25. Ravnic DJ, Leberfinger AN, Ozbolat IT. Bioprinting and cellular therapies for type 1 diabetes. Trends Biotechnol. 2017;35(11):1025–34.

26. Woliner-van der Weg W, et al. A 3D-printed anatomical pancreas and kidney phantom for optimizing SPECT/CT reconstruction settings in beta cell imaging using (111)In-exendin. EJNMMI Phys. 2016;3(1):29.
27. Homan KA, et al. Bioprinting of 3D Convoluted Renal Proximal Tubules on Perfusable Chips. Scientific Reports. 2016;6:34845.
28. Zhang YS, et al. 3D bioprinting for tissue and organ fabrication. Ann Biomed Eng. 2017;45(1):148–63.
29. Ong CS, et al. Biomaterial-free three-dimensional bioprinting of cardiac tissue using human induced pluripotent stem cell derived cardiomyocytes. Sci Rep. 2017;7:4566.
30. Cheung DYC, Duan B, Butcher JT. Chapter 21 – Bioprinting of cardiac tissues. In: Atala A, Yoo JJ, editors. Essentials of 3D biofabrication and translation. Boston: Academic; 2015. p. 351–70.
31. Lee S, et al. 3D bioprinted functional and contractile cardiac tissue constructs. Tissue Eng A. 2017;23:S96.
32. Serpooshan V, et al. Bioengineering cardiac constructs using 3D printing. J 3D Print Med. 2017;1(2):123.
33. Nair K, et al. Characterization of cell viability during bioprinting processes. Biotechnol J. 2009;4(8):1168–77.
34. Axpe E, Oyen ML. Applications of alginate-based bioinks in 3D bioprinting. Int J Mol Sci. 2016;17(12):1976.
35. Duan B, et al. 3D Bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. J Biomed Mater Res A. 2013;101(5):1255–64.
36. Lee YB, et al. Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture. Exp Neurol. 2010;223(2):645–52.
37. Kolesky DB, et al. 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. Adv Mater. 2014;26(19):3124–30.
38. Gaetani R, et al. Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/ hyaluronic acid patch preserves cardiac function after myocardial infarction. Biomaterials. 2015;61:339–48.
39. Luo Y, et al. Injectable hyaluronic acid-dextran hydrogels and effects of implantation in ferret vocal fold. J Biomed Mater Res B Appl Biomater. 2010;93b(2):386–93.
40. Lim KS, et al. New visible-light photoinitiating system for improved print fidelity in gelatin-based bioinks. ACS Biomater Sci Eng. 2016;2(10):1752–62.
41. Liu W, et al. Extrusion bioprinting of shear-thinning gelatin methacryloyl bioinks. Adv Healthc Mater. 2017;6(12). https://doi.org/10.1002/adhm.201601451.
42. Yin J, et al. 3D bioprinting of low-concentration cell-laden gelatin methacrylate (GelMA) bio-inks with a two-step cross-linking strategy. ACS Appl Mater Interfaces. 2018;10(8):6849–57.
43. Levato R, et al. Biofabrication of tissue constructs by 3D bioprinting of cell-laden microcarriers. Biofabrication. 2014;6(3):035020.
44. Duan B. State-of-the-art review of 3D bioprinting for cardiovascular tissue engineering. Ann Biomed Eng. 2017;45(1):195–209.
45. Blaeser A, et al. Controlling shear stress in 3D bioprinting is a key factor to balance printing resolution and stem cell integrity. Adv Healthc Mater. 2016;5(3):326–33.
46. Forget A, et al. Mechanically tunable bioink for 3D bioprinting of human cells. Adv Healthc Mater. 2017;6(20). https://doi.org/10.1002/adhm.201700255.
47. Khazaei M, Salehi E. Myocardial capillary density in normal and diabetic male rats: effect of bezafibrate. Res Pharm Sci. 2013;8(2):119–23.
48. Vanderburgh J, Sterling JA, Guelcher SA. 3D printing of tissue engineered constructs for in vitro modeling of disease progression and drug screening. Ann Biomed Eng. 2017;45(1):164–79.
49. Zhang YS, et al. Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip. Biomaterials. 2016;110:45–59.
50. Serpooshan V, et al. Use of bio-mimetic three-dimensional technology in therapeutics for heart disease. Bioengineered. 2014;5(3):193–7.

51. Serpooshan V, Ruiz-Lozano P. Ultra-rapid manufacturing of engineered epicardial substitute to regenerate cardiac tissue following acute ischemic injury. Methods Mol Biol. 2014;1210:239–48.
52. Serpooshan V, Wu SM. Patching up broken hearts: cardiac cell therapy gets a bioengineered boost. Cell Stem Cell. 2014;15(6):671–3.
53. Bartnikowski M, et al. Tailoring hydrogel viscoelasticity with physical and chemical crosslinking. Polymers. 2015;7(12):2650–69.
54. Schoenmakers DC, Rowan AE, Kouwer PHJ. Crosslinking of fibrous hydrogels. Nat Commun. 2018;9:2172.
55. Jung J, Oh J. Influence of photo-initiator concentration on the viability of cells encapsulated in photo-crosslinked microgels fabricated by microfluidics. Dig J Nanomater Biostruct. 2014;9(2):503–9.
56. Zhou D, Ito Y. Visible light-curable polymers for biomedical applications. Sci China-Chem. 2014;57(4):510–21.
57. Akar E, Altinisik A, Seki Y. Preparation of pH- and ionic-strength responsive biodegradable fumaric acid crosslinked carboxymethyl cellulose. Carbohydr Polym. 2012;90(4):1634–41.
58. Fekete T, et al. Synthesis and characterization of superabsorbent hydrogels based on hydroxyethylcellulose and acrylic acid. Carbohydr Polym. 2017;166:300–8.
59. Segura T, Chung PH, Shea LD. DNA delivery from hyaluronic acid-collagen hydrogels via a substrate-mediated approach. Biomaterials. 2005;26(13):1575–84.
60. Boerma M, et al. Microarray analysis of gene expression profiles of cardiac myocytes and fibroblasts after mechanical stress, ionising or ultraviolet radiation. BMC Genomics. 2005;6:6.
61. Johnson DM, et al. Polymer spray deposition: a novel aerosol-based, electrostatic digital deposition system for additive manufacturing. NIP Digit Fabric Conf. 2016;2016(1):129–33.
62. Wu Z, et al. Bioprinting three-dimensional cell-laden tissue constructs with controllable degradation. Sci Rep. 2016;6:24474.
63. Irvine SA, et al. Printing cell-laden gelatin constructs by free-form fabrication and enzymatic protein crosslinking. Biomed Microdevices. 2015;17(1):16.
64. Najdanovic-Visak V, et al. Salting-out in aqueous solutions of ionic liquids and K(3)PO(4): aqueous biphasic systems and salt precipitation. Int J Mol Sci. 2007;8(8):736–48.
65. Wang X, et al. Gelatin-based hydrogels for organ 3D bioprinting. Polymers (Basel). 2017;9(9):401.
66. Ersumo N, Witherel CE, Spiller KL. Differences in time-dependent mechanical properties between extruded and molded hydrogels. Biofabrication. 2016;8(3):–035012.
67. Bueno VB, et al. Synthesis and swelling behavior of xanthan-based hydrogels. Carbohydr Polym. 2013;92(2):1091–9.
68. Rahali K, et al. Synthesis and characterization of nanofunctionalized gelatin methacrylate hydrogels. Int J Mol Sci. 2017;18(12):2675.
69. Swinehart IT, Badylak SF. Extracellular matrix bioscaffolds in tissue remodeling and morphogenesis. Dev Dyn. 2016;245(3):351–60.
70. Tosun Z, Villegas-Montoya C, McFetridge PS. The influence of early-phase remodeling events on the biomechanical properties of engineered vascular tissues. J Vasc Surg. 2011;54(5):1451–60.
71. Velasco MA, Narvaez-Tovar CA, Garzon-Alvarado DA. Design, materials, and mechanobiology of biodegradable scaffolds for bone tissue engineering. Biomed Res Int. 2015;2015:729076.
72. Fenn SL, Oldinski RA. Visible light crosslinking of methacrylated hyaluronan hydrogels for injectable tissue repair. J Biomed Mater Res B Appl Biomater. 2016;104(6):1229–36.
73. Burkoth AK, Burdick J, Anseth KS. Surface and bulk modifications to photocrosslinked polyanhydrides to control degradation behavior. J Biomed Mater Res. 2000;51(3):352–9.
74. Coletta DJ, et al. (∗) Bone regeneration mediated by a bioactive and biodegradable extracellular matrix-like hydrogel based on elastin-like recombinamers. Tissue Eng Part A. 2017;23(23–24):1361–71.

75. Du JZ, et al. Synthesis and characterization of photo-cross-linked hydrogels based on biodegradable polyphosphoesters and poly(ethylene glycol) copolymers. Biomacromolecules. 2007;8(11):3375–81.
76. Jeon O, et al. The effect of oxidation on the degradation of photocrosslinkable alginate hydrogels. Biomaterials. 2012;33(13):3503–14.
77. Kocen R, et al. Viscoelastic behaviour of hydrogel-based composites for tissue engineering under mechanical load. Biomed Mater. 2017;12(2):025004.
78. Prestwich GD, et al. Controlled chemical modification of hyaluronic acid: synthesis, applications, and biodegradation of hydrazide derivatives. J Control Release. 1998;53(1–3):93–103.
79. Shin H, et al. In vivo bone and soft tissue response to injectable, biodegradable oligo(poly(ethylene glycol) fumarate) hydrogels. Biomaterials. 2003;24(19):3201–11.
80. Badylak SF, et al. The use of extracellular matrix as an inductive scaffold for the partial replacement of functional myocardium. Cell Transplant. 2006;15:S29–40.
81. Eitan Y, et al. Acellular cardiac extracellular matrix as a scaffold for tissue engineering: in vitro cell support, remodeling, and biocompatibility. Tissue Eng Part C Methods. 2010;16(4):671–83.
82. Serpooshan V, et al. The effect of bioengineered acellular collagen patch on cardiac remodeling and ventricular function post myocardial infarction. Biomaterials. 2013;34(36):9048–55.
83. Wang B, et al. Myocardial scaffold-based cardiac tissue engineering: application of coordinated mechanical and electrical stimulations. Langmuir. 2013;29(35):11109–17.
84. Dvir T, et al. Prevascularization of cardiac patch on the omentum improves its therapeutic outcome. Proc Natl Acad Sci U S A. 2009;106(35):14990–5.
85. Hirt MN, Hansen A, Eschenhagen T. Cardiac tissue engineering: state of the art. Circ Res. 2014;114(2):354–67.
86. Kreutziger KL, et al. Developing vasculature and stroma in engineered human myocardium. Tissue Eng Part A. 2011;17(9–10):1219–28.
87. Lux M, et al. In vitro maturation of large-scale cardiac patches based on a perfusable starter matrix by cyclic mechanical stimulation. Acta Biomater. 2016;30:177–87.
88. Mannhardt I, Marsano A, Teuschl A. Perfusion bioreactors for prevascularization strategies in cardiac tissue engineering. In: Holnthoner W, et al., editors. Vascularization for tissue engineering and regenerative medicine. Cham: Springer International Publishing; 2017. p. 1–14.
89. Montgomery M, Zhang B, Radisic M. Cardiac tissue vascularization: from angiogenesis to microfluidic blood vessels. J Cardiovasc Pharmacol Ther. 2014;19(4):382–93.
90. Pagliari S, et al. A multistep procedure to prepare pre-vascularized cardiac tissue constructs using adult stem sells, dynamic cell cultures, and porous scaffolds. Front Physiol. 2014;5:210.
91. Sarig U, et al. Pushing the envelope in tissue engineering: ex vivo production of thick vascularized cardiac extracellular matrix constructs. Tissue Eng Part A. 2015;21(9–10):1507–19.
92. Schaefer JA, et al. A cardiac patch from aligned microvessel and cardiomyocyte patches. J Tissue Eng Regen Med. 2018;12(2):546–56.
93. Vunjak-Novakovic G, et al. Challenges in cardiac tissue engineering. Tissue Eng B-Rev. 2010;16(2):169–87.
94. Johansson B, et al. Myocardial capillary supply is limited in hypertrophic cardiomyopathy: a morphological analysis. Int J Cardiol. 2008;126(2):252–7.
95. Mohammed SF, et al. Coronary microvascular rarefaction and myocardial fibrosis in heart failure with preserved ejection fraction. Circulation. 2015;131(6):550–9.
96. Rouwkema J, et al. Supply of nutrients to cells in engineered tissues. Biotechnol Genet Eng Rev. 2010;26:163–78.
97. Liu J, et al. Monitoring nutrient transport in tissue-engineered grafts. J Tissue Eng Regen Med. 2015;9(8):952–60.
98. McMurtrey RJ. Analytic models of oxygen and nutrient diffusion, metabolism dynamics, and architecture optimization in three-dimensional tissue constructs with applications and insights in cerebral organoids. Tissue Eng Part C Methods. 2016;22(3):221–49.

99. Lovett M, et al. Vascularization strategies for tissue engineering. Tissue Eng Part B Rev. 2009;15(3):353–70.
100. Phelps EA, Garcia AJ. Engineering more than a cell: vascularization strategies in tissue engineering. Curr Opin Biotechnol. 2010;21(5):704–9.
101. Hoch E, et al. Chemical tailoring of gelatin to adjust its chemical and physical properties for functional bioprinting. J Mater Chem B. 2013;1(41):5675–85.
102. Chen WP, Wu SM. Small molecule regulators of postnatal Nkx2.5 cardiomyoblast proliferation and differentiation. J Cell Mol Med. 2012;16(5):961–5.
103. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. J R Soc Interface. 2011;8(55):153–70.
104. Ghazizadeh Z, et al. Transient activation of reprogramming transcription factors using protein transduction facilitates conversion of human fibroblasts toward cardiomyocyte-like cells. Mol Biotechnol. 2017;59(6):207–20.
105. Braam SR, et al. Inhibition of ROCK improves survival of human embryonic stem cell-derived cardiomyocytes after dissociation. Ann N Y Acad Sci. 2010;1188:52–7.
106. Cagavi E, et al. Functional cardiomyocytes derived from Isl1 cardiac progenitors via Bmp4 stimulation. PLoS One. 2014;9(12):e110752.
107. Cai CL, et al. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. Dev Cell. 2003;5(6):877–89.
108. Degeorge BR Jr, et al. BMP-2 and FGF-2 synergistically facilitate adoption of a cardiac phenotype in somatic bone marrow c-kit+/Sca-1+ stem cells. Clin Transl Sci. 2008;1(2):116–25.
109. Hao J, et al. Dorsomorphin, a selective small molecule inhibitor of BMP signaling, promotes cardiomyogenesis in embryonic stem cells. PLoS One. 2008;3(8):e2904.
110. Serpooshan V, et al. Nkx2.5+cardiomyoblasts contribute to cardiomyogenesis in the neonatal heart. Sci Rep. 2017;7(7):12590.
111. Bax NA, et al. Matrix production and remodeling capacity of cardiomyocyte progenitor cells during in vitro differentiation. J Mol Cell Cardiol. 2012;53(4):497–508.
112. Fan D, et al. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. Fibrogenesis Tissue Repair. 2012;5(1):15.
113. Hanson KP, et al. Spatial and temporal analysis of extracellular matrix proteins in the developing murine heart: a blueprint for regeneration. Tissue Eng Part A. 2013;19(9–10):1132–43.
114. Lindsey ML, et al. A novel collagen matricryptin reduces left ventricular dilation post-myocardial infarction by promoting scar formation and angiogenesis. J Am Coll Cardiol. 2015;66(12):1364–74.
115. Suhaeri M, et al. Cardiomyoblast (h9c2) differentiation on tunable extracellular matrix microenvironment. Tissue Eng Part A. 2015;21(11–12):1940–51.
116. Ungerleider JL, et al. Fabrication and characterization of injectable hydrogels derived from decellularized skeletal and cardiac muscle. Methods. 2015;84:53–9.
117. Porter KE, Turner NA. Cardiac fibroblasts: at the heart of myocardial remodeling. Pharmacol Ther. 2009;123(2):255–78.
118. Nian M, et al. Inflammatory cytokines and postmyocardial infarction remodeling. Circ Res. 2004;94(12):1543–53.
119. Diamantides N, et al. Correlating rheological properties and printability of collagen bioinks: the effects of riboflavin photocrosslinking and pH. Biofabrication. 2017;9(3):034102.
120. Duong H, Wu B, Tawil B. Modulation of 3D fibrin matrix stiffness by intrinsic fibrinogen-thrombin compositions and by extrinsic cellular activity. Tissue Eng Part A. 2009;15(7):1865–76.
121. Kaiser NJ, Coulombe KLK. Physiologically inspired cardiac scaffolds for tailored in vivo function and heart regeneration. Biomed Mater. 2015;10(3):034003.
122. Throm Quinlan AM, et al. Combining dynamic stretch and tunable stiffness to probe cell mechanobiology in vitro. PLoS One. 2011;6(8):e23272.
123. Lee S, et al. Contractile force generation by 3D hiPSC-derived cardiac tissues is enhanced by rapid establishment of cellular interconnection in matrix with muscle-mimicking stiffness. Biomaterials. 2017;131:111–20.

124. Lee JP, et al. N-terminal specific conjugation of extracellular matrix proteins to 2-pyridinecarboxaldehyde functionalized polyacrylamide hydrogels. Biomaterials. 2016;102:268–76.
125. Serpooshan V, et al. Chapter 8 – 4D printing of actuating cardiac tissue. In: Al'Aref SJ, et al., editors. 3D printing applications in cardiovascular medicine. Boston: Academic; 2018. p. 153–62.
126. Wei K, et al. Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. Nature. 2015;525(7570):479–85.
127. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis Model Mech. 2011;4(2):165–78.
128. Gao L, et al. Myocardial tissue engineering with cells derived from human-induced pluripotent stem cells and a native-like, high-resolution, 3-dimensionally printed scaffold. Circ Res. 2017;120(8):1318–25.
129. Giacomelli E, et al. Three-dimensional cardiac microtissues composed of cardiomyocytes and endothelial cells co-differentiated from human pluripotent stem cells. Development. 2017;144(6):1008–17.
130. Pati F, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun. 2014;5:3935.
131. Weissman IL. Normal and neoplastic stem cells. Novartis Found Symp. 2005;265:35–50; discussion 50–4, 92–7
132. Knappe N, et al. Directed dedifferentiation using partial reprogramming induces invasive phenotype in melanoma cells. Stem Cells. 2016;34(4):832–46.
133. Clement F, et al. Stem cell manipulation, gene therapy and the risk of cancer stem cell emergence. Stem Cell Investig. 2017;4:67.