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REVIEW ARTICLE

Bioprinting in cardiovascular tissue engineering: a review

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Abstract: Fabrication techniques for cardiac tissue engineering have been evolving around scaffold-based and scaffold-free approaches. Conventional fabrication approaches lack control over scalability and homogeneous cell distribution. Bioprinting provides a technological platform for controlled deposition of biomaterials, cells, and biological factors in an organized fashion. Bioprinting is capable of alternating heterogeneous cell printing, printing anatomical relevant structure and microchannels resembling vasculature network. These are essential features of an engineered cardiac tissue. Bioprinting can potentially build engineered cardiac construct that resembles native tissue across macro to nanoscale.

Keywords: Bioprinting, 3D printing, tissue engineering, cardiovascular tissue, scaffolds

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1. Introduction

As one of the world's leading causes of death, cardiovascular disease possesses a relatively large market in regenerative medicine industry. Due to medical advances, the mortality rates related to cardiovascular diseases such as acute coronary syndrome, congenital heart disease, uncontrolled hypertension and arrhythmias have decreased in the past decade[1]. Despite these improvements, deaths caused by heart failure still remain high with half of heart failure patients dying within five years after diagnosis[2]. The stagnation in heart failure mortality can be attributed to repeated episodes of cardiovascular diseases, even with previous intervention. This suggests a plausible scenario where the root of the problem has not been cured despite reduction in risk of heart failure in patients[1]. A plausible solution requires looking at the biological and architectural aspects of the native myocardium.

The human heart comprises of approximately two billion cells[3,4] with intricate myocardium architecture comprising of non-cardiomyocytes cells and fibrillary structure. The heart comprises of four distinct layers ranging from pericardium (outer), epicardium, myocardium, and endocardium (inner). Each distinct layer comprises of myocardial laminar sheets with muscular fibres of three to four cell layer thick[5]. These laminar layers, also known as sheetlets, re-orientates and contributes to wall thickening during systolic shortening[6]. Damaged myocardium cannot be restored on its own as native cardiomyocytes are limited in regenerative abilities. Hence, cardiac tissue engineering aims to provide biological solutions to restore the ill-function-

ing heart and has the largest potential in regenerative medicine[7].

A potential medical solution is through injecting cells at infarct site or via intracoronary route[8–11]. Clinical trials have been performed to show safety and feasibility of using cells as therapy. Cardiosphere-derived cells (CDC), cardiac stem cells and bone marrow cells have been injected to repair and regenerate the myocardium since stem/progenitor cells are capable of regeneration and differentiation[8–10]. For instance, cardiac stem cell can differentiate into myocyte, smooth muscle cell and endothelial cell lineages with potential of dividing into 4.2×10^{12} cells[12]. Increasing viable myocardium mass and promoting angiogenesis through paracrine effects have been postulated as the success factor in cell injection therapy[13,14]. However, injecting cells directly has limitations such as low cell survivability and low cell retention at injured site. Hence, fabricating a patch-like or scaffold as a vehicle for delivering cells is a potential solution to improve cell retention and survivability at infarct site.

The concept of creating engineered tissue from cells, biomaterials and biological molecules forms the fundamental of tissue engineering[15]. Tissue engineering is a field which applies the principles of engineering and life sciences to develop biological substitutes that can restore, maintain or improve tissue function[16]. Specifically, cardiac tissue engineering aims to provide biological solutions to restore failing hearts and has one of the largest potential in regenerative medicine[17].

With advancement of three-dimensional (3D) printing, also known as additive manufacturing, various techniques have been applied in producing patient-specific biological substitutes, ranging from orthopedic implants[18–19] to scaffolds for tissue engineering[14,20]. Coupled with computer-aided technology, customized patch-like scaffold can be designed using additive manufacturing for cell delivery.

In this paper, design considerations for cardiac tissue engineering will be highlighted. Conventional scaffold fabrication method in cardiac tissue engineering are discussed and evaluated. With the increase interest in bioprinting for tissue engineering, the application of bioprinting in cardiac tissue engineering is reviewed. Lastly, different research areas that will bridge the gap between engineered cardiac tissues with native cardiac tissue are discussed.

2. Conventional Engineering of Cardiac Tissues

2.1 Design Considerations

There are several factors to consider when designing and engineering cardiac tissue. Firstly, the engineered cardiac tissue physiological properties should be similar or close to human myocardium[21–22]. Specifically, their modulus should be between 0.2 to 0.5 MPa at end diastole with tensile strength of 3–15 kPa, contractile pressure of 2–4 mN/mm^2 and electrical propagation velocity of 25 cm/s.

Secondly, the engineered cardiac tissue should be compatible with the native in the aspect of heterogeneous cell population comprising of cardiomyocytes and non-cardiomyocytes[23]. Cardiomyocytes forms the minority of cell population in heart yet give rise to the bulk volume of the heart; while fibroblast dominates the non-cardiomyocyte population and is in direct contact with cardiomyocytes[23]. Other non-cardiomyocytes population includes endothelial cells, adipocytes and neurons. The benefits of cardiomyocytes and non-cardiomyocytes coupling include improvement in electrophysiology of engineered cardiac tissue and production of survival and trophic signals to cardiomyocytes[24,25].

Thirdly, the architectural features of native heart are different at various hierarchical levels. At the milli-scale level, aligned myofibers are induced by matrix anisotropy while across the transmural direction, varying spatial arrangement of myofibers is present[26]. In the micro-scale, vascularization is needed as support system for nutrient/waste exchange in highly densed native myocardium[27]. The design considerations are summarized in Figure 1.

2.2 Conventional Techniques in Cardiac Tissue Engineering

In order to satisfy the above mentioned requirement, various techniques have been used to engineer

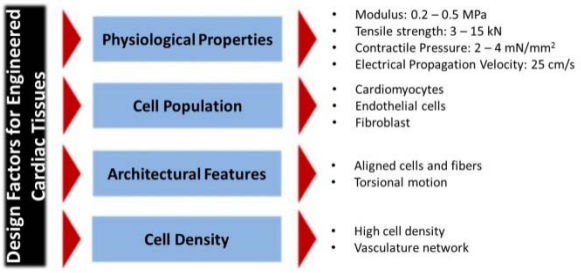

Figure 1. Design consideration in engineering cardiac tissue.

biomimetic cardiac tissues. Conventional fabrication techniques in cardiac tissue engineering can be broadly divided into scaffold-based or scaffold-free fabrication. A summary of different conventional fabrication techniques in cardiac tissue engineering is shown in Table 1.

(1) Scaffold-based Engineered Cardiovascular Tissues

Scaffolds are used for attachment and mechanical support for cardiac cells in scaffold-based approach of cardiac tissue engineering. In this approach, cells are seeded onto the scaffolds before going through tissue maturation. In scaffold fabrication, several methods, such as solvent casting[28–30], molding[31] and electrospinning[32–38], have been used. Features on the fabricated scaffolds can affect cell responses directly. For example, cellular alignment is shown by neonatal rat heart cells seeded on laser microblated polyglycerol sebacate scaffolds that have honeycomb microstructure[39].

In an alternative approach, cells are encapsulated within the biomaterials. Hydrogel has been widely used as the biomaterial to encapsulate cardiac cells[40,41]. Collagen ring casted with cardiac cell formed compact heart muscles and was grafted onto rat's epicardum[41,42]. To improve conductivity of engineered tissue, gold nanowires are mixed with alginate neonatal rat heart cells and fibroblasts casted into alginate scaffold containing the gold nanowires[43].

Another technique scaffold-based technique is to repopulate de-cellularized matrix with desired cell population. De-cellularization is a process to obtain cell-free scaffold from sacrificial tissue/organ through removal of xenogenic cells[44,45]. The technique is first used on heart valves and further improvised to repopulate a full heart[46]. A distinct advantage of de-cellularized matrix over hydrogel-based scaffold is retaining the native extracellular matrix. The composition and ratio of ECM proteins that have been isolated in decellularized matrix are site-specific[47].

Scaffold-based techniques discussed above are either laborious or lack repeatability. Firstly, structures casted out of molds are restricted by the design of master mold. The native structure of myocardium does not comprise of singular patterns. Instead, cardiomyocytes alignment varies across the transmural of myocardium[48]. The effectiveness in using molding approach to recapture complex native architecture is questionable.

Another major disadvantage of scaffold-based method is forming non-uniform macro-pore structure in casted scaffolds. An alternative to produce uniform pore size is to use computer aided technology to design scaffolds with defined pore structure and size[49]. Additive manufacturing techniques, such as selective laser sintering (SLS)[20] fabricate porous scaffold struture layer-by-layer with stiffness of fabricated scaffold similar to native human myocardium (0.2 mPa). Inkjet printing technique was also employed for indirect fabrication of scaffolds for tissue engineering[50].

Lastly, perfusion of decellularized matrix, which is currently limited to 70% of the original volume, still remain a challenge for this scaffolding approach[51]. Also, decellularized matrix repopulated with neonatal rat cardiomyocytes showed disarrayed arrangement with disorganized electrical propagation and decreased Connexion 43 expression[52]. However, decellularized matrix for cardiovascular tissue engineering remains optimistic with optimization in cell perfusion techniques and improvement on electromechanical properties of decellularized matrix during the fabrication process.

(2) Scaffold-free Engineered Cardiovascular Tissues

As the name suggests, scaffold-free approach does not make use of solid scaffolds, usually made from polylactic acid (PLA), polycaprolactone (PCL) and etc.,

Table 1. Summary of different fabrication techniques in engineered cardiac tissue

	Pre-fabricated Matrices	Cell Sheet	Decellularized Matrices	Cell Hydrogel Encapsulation (Casting)
Advantage	• Inherent structure guidance for cell orientation • Tunable mechanical properties	• Intact cell-cell and cell-matrix junction • Ability to beat spontaneously and synchronously • Vasculature network establishment for thick construct (300 – 800 μm)	• Native extracellular matrix retained	• Electrical coupling is not delayed • Left ventricular dilation is prevented
Disadvantage	• Non-homogeneous cell distribution through seeding	• Difficult to handle due to thin layers of cell sheets • Limited cell number generated	• Sacrificial tissue is required • Identification of ideal sacrificial tissue needed • Non-homogeneous distribution of re-entry cells	• Difficult to handle thick tissue assembly

as structural support for cell depositions. For example, cell sheet technology is capable of fabricating cellular compound without using solid scaffolds for structural support in the process. The process involves culturing cells on modified thermo-responsive polymer surface. With temperature change, the tissue culture is dislodged as an entire layer of cell with cell-cell junction intact. Primary neonatal rat cardiomyocytes, induced pluripotent stem cells, C2C12 mouse myoblasts have been fabricated as cell sheets[53–56].

As cell sheets can be transplanted in layers, cells retention at the infarcted sites is higher as compared to injecting cells. Furthermore, cell sheets provide minimally invasive solution as they are transplanted into the host tissue without sutures. Vascularization can be facilitated with specifically designed bioreactor[57].

However, delivering cell sheet for restoration of infarcted heart remains inconclusive. For instance, the number of cells in cell sheet is approximately 9.5×10^4[55] while number of damaged cells during infarction is approximated at 8×10^6[3]. The effectiveness of cell sheet in terms of replacing damaged cells requires further research since the difference is several magnitude lower.

3. Bioprinting: The New Paradigm in Engineering Cardiac Tissues

Bioprinting, a variant of additive manufacturing or 3D printing, uses computer-aided processes to pattern and assemble living and non-living materials with a prescribed two-dimensional (2D) or 3D organization[58]. However, bioprinting shall not be used interchangeably with 3D printing of inert materials[59]. Most importantly, bioprinting offers the advantage to control shape and material in multi-material printing. This unique capability of multi-material printing enables printing of anatomically relevant structures in fabricating cardiac tissues. Of the many bioprinting techniques, material jetting and material extrusion systems have been used to produce engineered cardiovascular models (Figure 1).

3.1 Material Jetting

This involves droplet displacement of material. Methods using this technique are piezoelectric/thermal inkjetting, and laser induced forward transfer (LIFT)[60–61]. Density of cell printed can be controlled by overprinting over a specific area. Moreover, different cell types can be printed within a construct. One distinct advantage of LIFT over other orifice-based techniques, such as inkjet and extrusion, is the ability to print materials of wide viscosity range. Hence, high cell loading density (10^8 cells/mL) can be printed using LIFT.

3.2 Material Extrusion

This technique is a combination of an automated robotic system for extrusion and a dispensing system for fluids[61]. The dispensing system can extrude hydrogel from the nozzles, producing defined structures. The automated robotic system for extrusion printing is generally powered by either a pneumatic[62–70] or a mechanical pump[71,72]. These pumps act by applying a positive pressure on the hydrogel causing it to flow out of the nozzle. Additionally, valves can be placed at the nozzle to create droplets by regulating the flow of the hydrogel within the syringe[73].

3.3 Future Prospect

The current state-of-the-art involves several research groups using bioprinting for construct containing patterned heterogeneous cells, structures with complex shape and microchannels for vascularization (Figure 2 and Table 2). However, the differences between bioprinted cardiac tissue compared to native cardiac tissue still require further research, as shown in Figure 3.

(1) Cell Source for Bioprinting

The ideal cell type that can be used for engineering *in vivo* solutions for cardiovascular diseases has not been identified. Furthermore, the protocol for cell isolation from endocardium, epicardium and cardiac fibroblast have not been developed while the relationship between cardiomyocytes and non-cardiomyocytes has not been established[80]. These are intrinsic biological questions that remain to be answered in the field of cardiac tissue engineering. Due to the limitation in vascularization, fabrication of full thickness cardiac tissue has not been achieved. A vascular network is needed to facilitate nutrient transportation in an engineered cardiac tissue due to high loss of cardiomyocytes during infraction. However, conventional methods in fabricating engineered heart tissue showed limited advancement in this prospect.

(2) Micro / Nano Features

Conventional approaches have achieved cell alignment of myocytes at planar level through the understanding of cell-material interactions. The cell alignment can be conformed and guided using defined spaces as shown by cell alignment along the spun fi-

bers with nanoscale features formed by electrospinning[81]. Microscale geometries confine and direct cell growth towards anisotropic direction. However, these methods fall short in describing cell behavior in native environment. Therefore, there is a need to design 3D engineered tissue in order to utilize and integrate the previous findings into a 3D perspective.

(3) Functioning Vasculature Network

Sooppan *et al.* demonstrated the perfusion and anastomosis of a microchannel printed using polydimethylsiloxane (PDMS)[82]. The integration of bioprinted vascular network with host tissue still remains hopeful. One of the major concerns is with the choice of biomaterials. It is critical to ensure that the lumen of vasculature network does not collapse while the stiffness does not impede nutrient and waste transportation across the network. Moreover, with computer-based technology, vascular system for complex organ manufacturing can be simulated and printed within a bioprinted construct[83].

(4) Material Formulation

Apart from designing materials to improve print fidelity in bioprinting, materials for engineering cardiac tissue is needed to capture the physiological and functional properties of native cardiac tissues. Nanocomposite hydrogel and electronics printing can be engineered

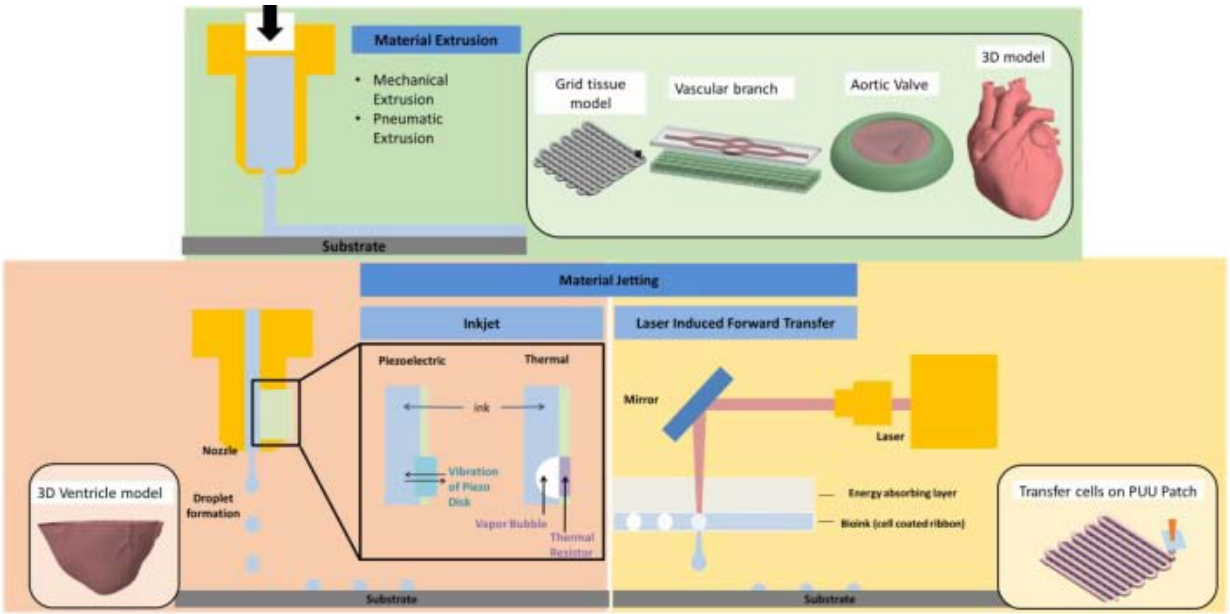

Figure 2. Schematic representation of current state-of-the-art bioprinted cardiovascular and cardiac-related tissue.

Table 2. Bioprinted cardiovascular and cardiac-related tissue

Technique		Resolution	Advantages	Disadvantages	Application	Reference
Material Extrusion		100–2000 µm	• Wide choice of materials • Able to control material extrusion by modifying needle tip	• Resolution limited by cell viability • Clogging of needle tip	Heterogeneous aortic valve conduits	[64]
					Tissue model	[74]
					Vascular branches	[75]
					Vascular tubular grafts	[76]
					3D model	[77]
Material Jetting	Laser-Induced Forward Transfer	10–100 µm	• Single cell resolution • High cell loading density (10^8 cells/mL) • Any viscosity of loading material • Contact-less printing minimizing cross contamination risk	• Long preparation process • Complex instrumentation for precise control needing materials with optical property	Cardiac patch containing heterogeneous cell population	[78]
	Inkjet	100 µm	• Picoliter droplets gives high resolution • Contact-less printing minimizing cross contamination risk	• Limited to low viscous materials	3D construct of half heart	[79]

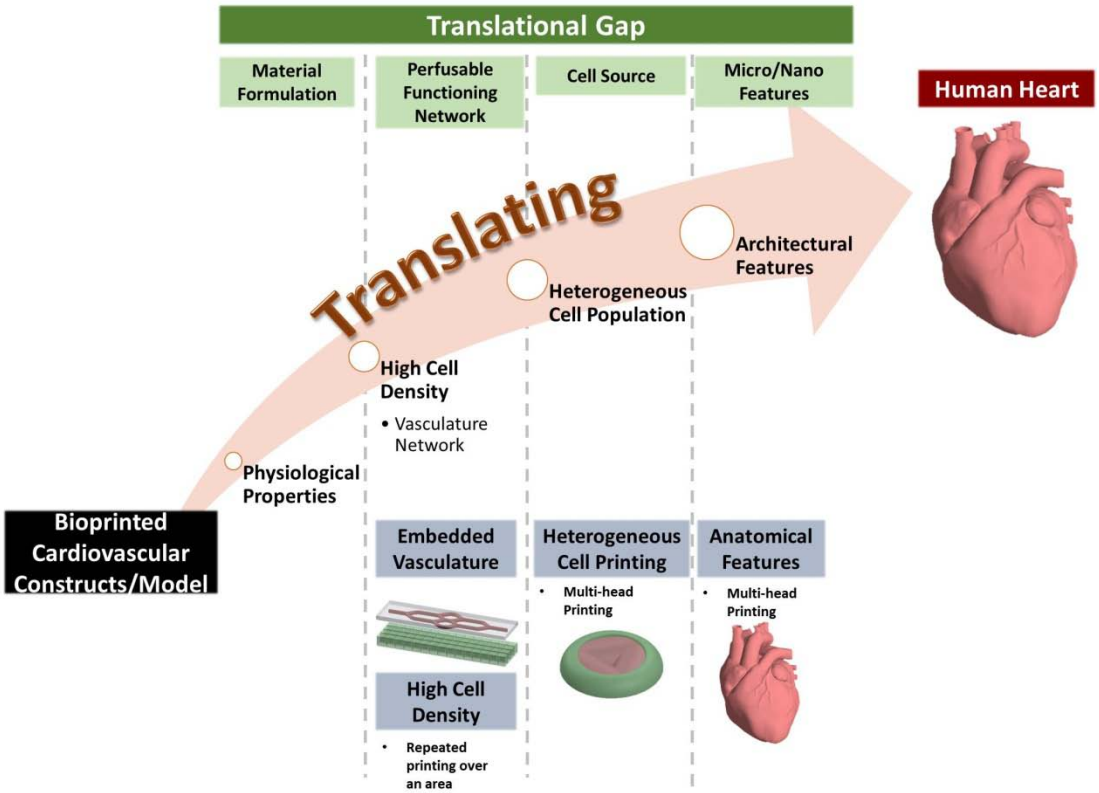

Figure 3. Future prospect of cardiac tissue engineering using bioprinting.

onto cardiac patches to enhance electrical properties of engineered cardiac construct[84,85]. Hydrogel's architecture and microenvironment can be tuned to illicit certain cellular response[86,87]. Oxygen-rich hydrogel can be engineered to sustain high metabolic demand of engineered cardiac tissue before angiogenesis occurs[88,89].

4. Conclusion

Bioprinting remains as a powerful tool for engineers and scientists to produce engineered biological construct from virtual designs. Future engineered cardiovascular tissue will see an integration of lessons learnt from decade of cardiovascular tissue engineering and precision from bioprinting, to print constructs with close resemblance of a native myocardium. In essence, bioprinting serve as a platform to provide macro and microscale engineering while engineering material formulation and cell biology to initiate nanoscale responses — bringing about an engineered myocardium with multiscale resemblance of native tissue.

Conflict of Interest and Funding

The authors declare no conflict of interest.

References

1. Braunwald E, 2013, Heart failure. *JACC: Heart Failure*, vol.1(1): 1–20. http://dx.doi.org/10.1016/j.jchf.2012.10.002
2. Go A S, Mozaffarian D, Roger V L, *et al.*, 2014, Heart disease and stroke statistics—2014 update: a report from the American Heart Association. *Circulation*, vol.129: e28–e292. http://dx.doi.org/10.1161/01.cir.0000441139.02102.80
3. Adler C P, Friedburg H, Herget G W, *et al.*, 1996, Variability of cardiomyocyte DNA content, ploidy level and nuclear number in mammalian hearts. *Virchows Archiv*, vol.429: 159–164. http://dx.doi.org/10.1007/bf00192438
4. Laflamme M A and Murry C E, 2011, Heart regeneration. *Nature*, vol.473: 326–335. http://dx.doi.org/10.1038/nature10147
5. Hunter P, 2009, Cardiovascular mechanics, in Binder M, Hirokawa N and Windhorst U (eds), *Encyclopedia of Neuroscience*, Springer, Berlin Heidelberg, 564–567. http://dx.doi.org/10.1007/978-3-540-29678-2_824
6. Ferreira F P, Kilner P J, McGill L A, *et al.*, *In vivo* cardiovascular magnetic resonance diffusion tensor imaging shows evidence of abnormal myocardial laminar orientations and mobility in hypertrophic cardiomyopa-

thy. *Journal of Cardiovascular Magnetic Resonance*, vol.16(87).
http://dx.doi.org/10.1186/s12968-014-0087-8

7. Tillman B, Hardin-Young J, Shannon W, *et al.*, 2013, Meeting the need for regenerative therapies: translation-focused analysis of U.S. regenerative medicine opportunities in cardiovascular and peripheral vascular medicine using detailed incidence data. *Tissue Engineering Part B: Reviews*, vol.19(2): 99–115.
http://dx.doi.org/10.1089/ten.teb.2011.0678
8. Makkar R R, Smith R R, Cheng K, *et al.*, 2012, Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): A prospective, randomised phase 1 trial. *The Lancet*, vol.379(9819): 895–904.
http://dx.doi.org/10.1016/S0140-6736(12)60195-0
9. Bolli R, Chugh A R, D'Amario D, *et al.*, 2011, Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet*, vol.378(9806): 1847–1857.
http://dx.doi.org/10.1016/S0140-6736(11)61590-0
10. Lin Y D, Yeh M L, Yang Y J, *et al.*, 2010, Intramyocardial peptide nanofiber injection improves postinfarction ventricular remodeling and efficacy of bone marrow cell therapy in pigs. *Circulation*, vol.122: S132–S141.
http://dx.doi.org/10.1161/CIRCULATIONAHA.110.939512
11. Malliaras K, Makkar R R, Smith R R, *et al.*, 2014, Intracoronary cardiosphere-derived cells after myocardial infarction: evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction). *Journal of the American College of Cardiology*, vol.63(2): 110–122.
http://dx.doi.org/10.1016/j.jacc.2013.08.724
12. Bolli R, Chugh A R, D'Amario D, *et al.*, 2011, Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *The Lancet*, vol.378(9806): 1847–1857.
http://dx.doi.org/10.1016/S0140-6736(11)61590-0
13. Cheng K, Blusztajn A, Shen D, *et al.*, 2012, Functional performance of human cardiosphere-derived cells delivered in an *in situ* polymerizable hyaluronan-gelatin hydrogel. *Biomaterials*, vol.33(21): 5317–5324.
http://dx.doi.org/10.1016/j.biomaterials.2012.04.006
14. Wiria F E, Leong K F, Chua C K, *et al.*, 2007, Poly-ε-caprolactone/hydroxyapatite for tissue engineering scaffold fabrication via selective laser sintering. *Acta Biomaterialia*, vol.3(1): 1–12.
http://dx.doi.org/10.1016/j.actbio.2006.07.008
15. Langer R and Vacanti J P, 1993, Tissue engineering. *Science*, vol.260(5510): 920–926.
http://dx.doi.org/10.1126/science.8493529
16. Yeong W Y, Chua C K, Leong K F, *et al.*, 2004, Rapid prototyping in tissue engineering: challenges and potential. *Trends in Biotechnology*, vol.22(12): 643–652.
http://dx.doi.org/10.1016/j.tibtech.2004.10.004
17. Tillman B, Hardin-Young J, Shannon W, *et al.*, 2013, Meeting the need for regenerative therapies: translation-focused analysis of U.S. regenerative medicine opportunities in cardiovascular and peripheral vascular medicine using detailed incidence data. *Tissue Engineering Part B: Reviews*, vol.19(2): 99–115.
http://dx.doi.org/10.1089/ten.teb.2011.0678
18. Sing S L, An J, Yeong W Y, *et al.*, 2016, Laser and electron-beam powder-bed additive manufacturing of metallic implants: a review on processes, materials and designs. *Journal of Orthopaedic Research*, vol.34(3): 369–385.
http://dx.doi.org/10.1002/jor.23075
19. Sing S L, Yeong W Y, Wiria F E, *et al.*, 2016, Characterization of titanium lattice structures fabricated by selective laser melting using an adapted compressive test method. *Experimental Mechanics*, vol.56(5): 735–748.
http://dx.doi.org/10.1007/s11340-015-0117-y
20. Yeong W Y, Sudarmadji N, Yu H Y, *et al.*, 2010, Porous polycaprolactone scaffold for cardia tissue engineering fabricated by selective laser sintering. *Acta Biomaterialia*, vol.6(6): 2028–2034.
http://dx.doi.org/10.1016/j.actbio.2009.12.033
21. Jawad H, Ali N N, Lyon A R, *et al.*, 2007, Myocardial tissue engineering: a review. *Journal of Tissue Engineering and Regenerative Medicine*, vol.1(5): 327–342.
http://dx.doi.org/10.1002/term.46
22. Iyer R K, Chiu L L, Reis L A, *et al.*, 2011, Engineered cardiac tissues. *Current Opinion in Biotechnology*, vol.22(5): 706–714.
http://dx.doi.org/10.1016/j.copbio.2011.04.004
23. Kohl P, Camelliti P, Burton F L, *et al.*, 2005, Electrical coupling of fibroblasts and myocytes: relevance for cardiac propagation. *Journal of Electrocardiology*, vol.38(4): 45–50.
http://dx.doi.org/10.1016/j.jelectrocard.2005.06.096
24. Camelliti P, Green C R, LeGrice I, *et al.*, 2004, Fibroblast network in rabbit sinoatrial node: structural and functional identification of homogeneous and heterogeneous cell coupling. *Circulation Research*, vol.94: 828–835.
http://dx.doi.org/10.1161/01.RES.0000122382.19400.14
25. Hirsch E, Nagai R and Thum T, 2014, Heterocellular signalling and crosstalk in the heart in ischaemia and heart failure. *Cardiovascular Research*, vol.102(2): 191–193.
http://dx.doi.org/10.1093/cvr/cvu073
26. Nakatani S, 2011, Left ventricular rotation and twist: why should we learn? *Journal of Cardiovascular Ultrasound*, vol.19(1): 1–6.
http://dx.doi.org/10.4250/jcu.2011.19.1.1

27. Vunjak-Novakovic G, Lui K O, Tandon N, *et al.*, 2011, Bioengineering heart muscle: a paradigm for regenerative medicine. *Annual Review of Biomedical Engineering*, vol.13: 245–267. http://dx.doi.org/10.1146/annurev-bioeng-071910-124701
28. LeBlon C E, Pai R, Fodor C R, *et al.*, 2013, *In vitro* comparative biodegradation analysis of salt-leached porous polymer scaffolds. *Journal of Applied Polymer Science*, vol.128(5): 2701–2712. http://dx.doi.org/10.1002/app.38321
29. Sin D, Miao X G, Liu G, *et al.*, 2010, Polyurethane (PU) scaffolds prepared by solvent casting/particulate leaching (SCPL) combined with centrifugation. *Materials Science & Engineering C: Materials for Biological Applications*, vol.30(1): 78–85. http://dx.doi.org/10.1016/j.msec.2009.09.002
30. Pego A P, Siebum B, Van Luyn M J A, *et al.*, 2003, Preparation of degradable porous structures based on 1,3-trimethylene carbonate and D,L-lactide (co)polymers for heart tissue engineering. *Tissue Engineering*, vol.9(5): 981–994. http://dx.doi.org/10.1089/107632703322495628
31. Neal R A, Jean A, Park H, *et al.*, 2013, Three-dimensional elastomeric scaffolds designed with cardiac-mimetic structural and mechanical features. *Tissue Engineering Part A*, vol.19(5-6): 793–807. http://dx.doi.org/10.1089/ten.tea.2012.0330
32. Kharaziha M, Nikkhah M, Shin S R, *et al.*, 2013, PGS: Gelatin nanofibrous scaffolds with tunable mechanical and structural properties for engineering cardiac tissues. *Biomaterials*, vol.34(27): 6355–6366. http://dx.doi.org/10.1016/j.biomaterials.2013.04.045
33. Orlova Y, Magome N, Liu L, *et al.*, 2011, Electrospun nanofibers as a tool for architecture control in engineered cardiac tissue. *Biomaterials*, vol.32(24): 5615–5624. http://dx.doi.org/10.1016/j.biomaterials.2011.04.042
34. Xu B, Li Y, Fang X, *et al.*, 2013, Mechanically tissue-like elastomeric polymers and their potential as a vehicle to deliver functional cardiomyocytes. *Journal of the Mechanical Behavior of Biomedical Materials*, vol.28: 354–365. http://dx.doi.org/10.1016/j.jmbbm.2013.06.005
35. Prabhakaran M P, Nair A S, Kai D, *et al.*, 2012, Electrospun composite scaffolds containing poly(octanediol-co-citrate) for cardiac tissue engineering. *Biopolymers*, vol.97(7): 529–538. http://dx.doi.org/10.1002/bip.22035
36. Kai D, Prabhakaran M P, Jin G, *et al.*, 2011, Guided orientation of cardiomyocytes on electrospun aligned nanofibers for cardiac tissue engineering. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol.98(2): 379–386. http://dx.doi.org/10.1002/jbm.b.31862
37. Shevach M, Maoz B M, Feiner R, *et al.*, 2013, Nanoengineering gold particle composite fibers for cardiac tissue engineering. *Journal of Materials Chemistry B*, vol.1(39): 5210–5217. http://dx.doi.org/10.1039/c3tb20584c
38. Fleischer S, Feiner R, Shapira A, *et al.*, 2013, Spring-like fibers for cardiac tissue engineering. *Biomaterials*, vol.34(34): 8599-8606. http://dx.doi.org/10.1016/j.biomaterials.2013.07.054
39. Engelmayr G C Jr, Cheng M, Bettinger C J, *et al.*, 2008, Accordion-like honeycombs for tissue engineering of cardiac anisotropy. *Nature Materials*, vol.7: 1003–1010. http://dx.doi.org/10.1038/nmat2316
40. Zhang D, Shadrin I Y, Lam J, *et al.*, 2013, Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes. *Biomaterials*, vol.34(23): 5813–5820. http://dx.doi.org/10.1016/j.biomaterials.2013.04.026
41. Zimmermann W H, Fink C, Kralisch D, *et al.*, 2000, Three-dimensional engineered heart tissue from neonatal rat cardiac myocytes. *Biotechnology and Bioengineering*, vol.68(1): 106–114. http://dx.doi.org/10.1002/(SICI)1097-0290(20000405)68:1%3C106::AID-BIT13%3E3.0.CO;2-3
42. Zimmermann W H, Melnychenko I, Wasmeier G, *et al.*, 2006, Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Natural Medicine*, vol.12: 452–458. http://dx.doi.org/10.1038/nm1394
43. Dvir T, Timko B P, Brigham M D, *et al.*, 2011, Nanowired three-dimensional cardiac patches. *Nature Nanotechnology*, vol.6: 720–725. http://dx.doi.org/10.1038/nnano.2011.160
44. Dohmen P M, Scheckel M, Stein-Konertz M, *et al.*, 2002, *In vitro* hydrodynamics of a decellularized pulmonary porcine valve, compared with a glutaraldehyde and polyurethane heart valve. *International Journal of Artificial Organs*, vol.25: 1089–1094.
45. Juthier F, Vincentelli A, Gaudric J, *et al.*, 2006, Decellularized heart valve as a scaffold for *in vivo* recellularization: deleterious effects of granulocyte colony-stimulating factor. *Journal of Thoracic and Cardiovascular Surgery*, vol.131(4): 843–852. http://dx.doi.org/10.1016/j.jtcvs.2005.11.037
46. Ott H C, Matthiesen T S, Goh S K, *et al.*, 2008, Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nature Medicine*, vol.14: 213–221. http://dx.doi.org/10.1038/nm1684
47. Song J J and Ott H C, 2011, Organ engineering based on decellularized matrix scaffolds. *Trends in Molecular Medicine*, vol.17(8): 424–432. http://dx.doi.org/10.1016/j.molmed.2011.03.005
48. Li Z and Guan J, 2011, Hydrogels for cardiac tissue en-

gineering. *Polymers*, vol.3(2): 740–761.
http://dx.doi.org/10.3390/polym3020740

49. Chua C K, Yeong W Y and Leong K F, 2005, Rapid prototyping in tissue engineering: a state-of-the-art report, in *Virtual Modelling and Rapid Manufacturing: Advanced Research in Virtual and Rapid Prototyping. Proceedings of the 2nd International Conference on Advanced Research in Virtual and Rapid Prototyping, Leiria, Portugal, 28 September–1 October, 2005*, 19–27.
50. Yeong W Y, Chua C K and Leong K F, *et al.*, 2005, Development of scaffolds for tissue engineering using a 3D inkjet model maker, in *Virtual Modelling and Rapid Manufacturing: Advanced Research in Virtual and Rapid Prototyping. Proceedings of the 2nd International Conference on Advanced Research in Virtual and Rapid Prototyping, Leiria, Portugal, 28 September–1 October, 2005*, 115–118.
51. Shimizu T, 2014, Cell sheet-based tissue engineering for fabricating 3-dimensional heart tissues. *Circulation Journal*, vol.78(11): 2594–2603.
http://doi.org/10.1253/circj.CJ-14-0973
52. Yasui H, Lee J K, Yoshida A, *et al.*, 2014, Excitation propagation in three-dimensional engineered hearts using decellularized extracellular matrix. *Biomaterials*, vol.35(27): 7839–7850.
http://dx.doi.org/10.1016/j.biomaterials.2014.05.080
53. Shimizu T, Yamato M, Kikuchi A, *et al.*, 2003, Cell sheet engineering for myocardial tissue reconstruction. *Biomaterials*, vol.24(13): 2309–2316.
http://dx.doi.org/10.1016/S0142-9612(03)00110-8
54. Hata H, Bar A, Dorfman S, *et al.*, 2010, Engineering a novel three-dimensional contractile myocardial patch with cell sheets and decellularised matrix. *European Journal of Cardio-Thoracic Surgery*, vol.38(4): 450–455.
http://dx.doi.org/10.1016/j.ejcts.2010.02.009
55. Miki K, Uenaka H, Saito A, *et al.*, 2012, Bioengineered myocardium derived from induced pluripotent stem cells improves cardiac function and attenuates cardiac remodeling following chronic myocardial infarction in rats. *Stem Cells Translational Medicine*, vol.1(5): 430–437.
http://dx.doi.org/10.5966/sctm.2011-0038
56. Hasegawa A, Haraguchi Y, Shimizu T, *et al.*, 2015, Rapid fabrication system for three-dimensional tissues using cell sheet engineering and centrifugation. *Journal of Biomedical Materials Research Part A*, vol.103(12): 3825–3833.
http://dx.doi.org/10.1002/jbm.a.35526
57. Sakaguchi K, Shimizu T, Horaguchi S, *et al.*, 2013, *In Vitro* engineering of vascularized tissue surrogates. *Scientific Reports*, vol.3: 1316.
http://dx.doi.org/10.1038/srep01316
58. Guillemot F, Mironov V and Nakamura M, 2010, Bioprinting is coming of age: report from the International Conference on Bioprinting and Biofabrication in Bordeaux (3B'09). *Biofabrication*, vol.2(1): 010201.
http://dx.doi.org/10.1088/1758-5082/2/1/010201
59. Ozbolat I T and Hospodiuk M, 2016, Current advances and future perspectives in extrusion-based bioprinting. *Biomaterials*, vol.76: 321–343.
http://dx.doi.org/10.1016/j.biomaterials.2015.10.076
60. Xu T, Zhao W, Zhu J M, *et al.*, 2013, Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology. *Biomaterials*, vol.34(1): 130–139.
http://dx.doi.org/10.1016/j.biomaterials.2012.09.035
61. Guillemot F, Guillotin B, Fontaine A, *et al.*, 2011, Laser-assisted bioprinting to deal with tissue complexity in regenerative medicine. *MRS Bulletin*, vol.36(12): 1015–1019.
http://dx.doi.org/10.1557/mrs.2011.272
62. Lee H, Ahn S, Bonassar L J, *et al.*, 2013, Cell-laden poly(varepsilon-caprolactone)/alginate hybrid scaffolds fabricated by an aerosol cross-linking process for obtaining homogeneous cell distribution: fabrication, seeding efficiency, and cell proliferation and distribution. *Tissue Engineering Part C: Methods*, vol.19(10): 784–793.
http://dx.doi.org/10.1089/ten.tec.2012.0651
63. Billiet T, Gevaert E, De Schryver T, *et al.*, 2014, The 3D printing of gelatin methacrylamide cell-laden tissue-engineered constructs with high cell viability. *Biomaterials*, vol.35(1): 49–62.
http://dx.doi.org/10.1016/j.biomaterials.2013.09.078
64. Duan B, Hockaday L A, Kang K H, *et al.*, 2013, 3D bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. *Journal of Biomedical Materials Research: Part A*, vol.101(5): 1255–1264.
http://dx.doi.org/10.1002/jbm.a.34420
65. Fedorovich N E, Wijnberg H M, Dhert W J *et al.*, 2011, Distinct tissue formation by heterogeneous printing of osteo- and endothelial progenitor cells. *Tissue Engineering Part A*, vol.17(15–16): 2113–2121.
http://dx.doi.org/10.1089/ten.TEA.2011.0019
66. Huang Y, He K and Wang X, 2013, Rapid prototyping of a hybrid hierarchical polyurethane-cell/hydrogel construct for regenerative medicine. *Materials Science and Engineering: C. Materials for Biological Applications*, vol.33(6): 3220–3229.
http://dx.doi.org/10.1016/j.msec.2013.03.048
67. Ozbolat I T, Chen H and Yu Y, 2014, Development of 'Multi-arm Bioprinter' for hybrid biofabrication of tissue engineering constructs. *Robotics and Computer-Integrated Manufacturing*, vol.30(3): 295–304.
http://dx.doi.org/10.1016/j.rcim.2013.10.005
68. Shim J M, Lee J S, Kim J Y, *et al.*, 2012, Bioprinting of a mechanically enhanced three-dimensional dual cell-laden construct for osteochondral tissue engineering us-

ing a multi-head tissue/organ building system. *Journal of Micromechanics and Microengineering*, vol.22(8): 085014.
http://dx.doi.org/10.1088/0960-1317/22/8/085014

69. Snyder J E, Hamid Q, Wang C, *et al.*, 2011, Bioprinting cell-laden matrigel for radioprotection study of liver by pro-drug conversion in a dual-tissue microfluidic chip. *Biofabrication*, vol.3(3): 034112.
http://dx.doi.org/10.1088/1758-5082/3/3/034112
70. Wang X H, Yan Y N, Pan Y Q, *et al.*, 2006, Generation of three-dimensional hepatocyte/gelatin structures with rapid prototyping system. *Tissue Engineering*, vol.12(1): 83–90.
71. Skardal A, Zhang J and Prestwich G D, 2010, Bioprinting vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral polyethylene glycol tetracrylates. *Biomaterials*, vol.31(24): 6173–6181.
http://dx.doi.org/10.1016/j.biomaterials.2010.04.045
72. Visser J, Peters B, Burger T J, *et al.*, 2013, Biofabrication of multi-material anatomically shaped tissue constructs. *Biofabrication*, vol.5(3): 035007.
http://dx.doi.org/10.1088/1758-5082/5/3/035007
73. Lee W, Lee V, Polio S, *et al.*, 2009, Three-dimensional cell-hydrogel printer using electromechanical microvalve for tissue engineering. in *TRANSDUCERS 2009 — 2009 International Solid-State Sensors, Actuators and Microsystems Conference*, 2230–2233.
http://dx.doi.org/10.1109/SENSOR.2009.5285591
74. Gaetani R, Doevendans P A, Metz C H, J. *et al.*, 2012, Cardiac tissue engineering using tissue printing technology and human cardiac progenitor cells. *Biomaterials*, vol.33(6): 1782–1790.
http://dx.doi.org/10.1016/j.biomaterials.2011.11.003
75. Kolesky D B, Truby R L, Gladman A S, *et al.*, 2014, 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Advanced Materials*, vol.26(19): 3124–3130.
http://dx.doi.org/10.1002/adma.201305506
76. Norotte C, Marga F S, Niklason L E, *et al.*, 2009, Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials*, vol.30(30): 5910–5917.
http://dx.doi.org/10.1016/j.biomaterials.2009.06.034
77. Hinton T J, Jallerat Q, Palchesko R N, *et al.*, 2015, Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Science Advances*, vol.1: e1500758.
http://dx.doi.org/10.1126/sciadv.1500758
78. Gaebel R, Ma N, Liu J, *et al.*, 2011, Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. *Biomaterials*, vol.32(35): 9218–9230.
http://dx.doi.org/10.1016/j.biomaterials.2011.08.071
79. Xu T, Baicu C, Aho M, *et al.*, 2009, Fabrication and characterization of bio-engineered cardiac pseudo tissues. *Biofabrication*, vol.1(3): 035001.
http://dx.doi.org/10.1088/1758-5082/1/3/035001
80. Vunjak-Novakovic G, Eschenhagen T and Mummery C, 2014, Myocardial tissue engineering: *in vitro* models. *Cold Spring Harbor Perspectives in Medicine*, vol.4.(3): a014076.
http://dx.doi.org/10.1101/cshperspect.a014076
81. Chanthakulchan A, Koomsap P, Parkhi A A, *et al.*, 2015, Environmental effects in fibre fabrication using electrospinning-based rapid prototyping. *Virtual and Physical Prototyping*, vol.10(4): 227–237.
http://dx.doi.org/10.1080/17452759.2015.1112411
82. Sooppan R, Paulsen S J, Han J, *et al.*, 2016, *In vivo* anastomosis and perfusion of a three-dimensionally-printed construct containing microchannel networks. *Tissue Engineering Part C: Methods*, vol.22(1): 1–7.
http://dx.doi.org/10.1089/ten.TEC.2015.0239
83. Liu L B and Wang X H, 2015, Creation of a vascular system for organ manufacturing. *International Journal of Bioprinting*, vol.1(1): 77–86.
http://dx.doi.org/10.18063/IJB.2015.01.009
84. Dvir T, Timko B P, Brigham M D, *et al.*, 2011, Nanowired three-dimensional cardiac patches. *Nature Nanotechnology*, vol.6(11): 720–725.
http://dx.doi.org/10.1038/nnano.2011.160
85. Xu L, Gutbrod S R, Bonifas A P, *et al.*, 2014, 3D multifunctional integumentary membranes for spatiotemporal cardiac measurements and stimulation across the entire epicardium. *Nature Communications*, vol.5: 3329.
http://dx.doi.org/10.1038/ncomms4329
86. Wang S, Lee J M and Yeong W Y, 2015, Smart hydrogels for 3D bioprinting. *International Journal of Bioprinting*, vol.1(1): 3–14.
http://dx.doi.org/10.18063/IJB.2015.01.005
87. Dixon J E, Shah D A, Rogers C, *et al.*, 2014, Combined hydrogels that switch human pluripotent stem cells from self-renewal to differentiation. *Proceedings of the National Academy of Sciences*, vol.111(15): 5580–5585.
http://dx.doi.org/10.1073/pnas.1319685111
88. Lee H Y, Kim H W, Lee J H, *et al.*, 2015, Controlling oxygen release from hollow microparticles for prolonged cell survival under hypoxic environment. *Biomaterials*, vol.53: 583–591.
http://dx.doi.org/10.1016/j.biomaterials.2015.02.117
89. Farris A L, Rindone A N, Grayson W L, 2016, Oxygen delivering biomaterials for tissue engineering. *Journal of Materials Chemistry B*, vol.4(20): 3422–3432.
http://dx.doi.org/10.1039/C5TB02635K