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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/mm² 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 densified 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

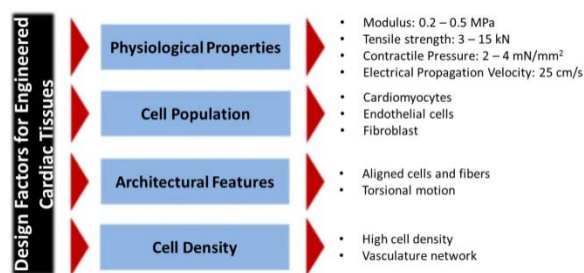


Figure 1. Design consideration in engineering cardiac tissue.

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 epicardium^[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 de-cellularized 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 structure 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	<ul style="list-style-type: none"> Inherent structure guidance for cell orientation Tunable mechanical properties 	<ul style="list-style-type: none"> Intact cell-cell and cell-matrix junction Ability to beat spontaneously and synchronously Vasculature network establishment for thick construct (300 – 800 μm) 	<ul style="list-style-type: none"> Native extracellular matrix retained 	<ul style="list-style-type: none"> Electrical coupling is not delayed Left ventricular dilation is prevented
Disadvantage	<ul style="list-style-type: none"> Non-homogeneous cell distribution through seeding 	<ul style="list-style-type: none"> Difficult to handle due to thin layers of cell sheets Limited cell number generated 	<ul style="list-style-type: none"> Sacrificial tissue is required Identification of ideal sacrificial tissue needed Non-homogeneous distribution of re-entry cells 	<ul style="list-style-type: none"> 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 ink-jetting, 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 advan-

tage 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 infarction. 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 bio-printed 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

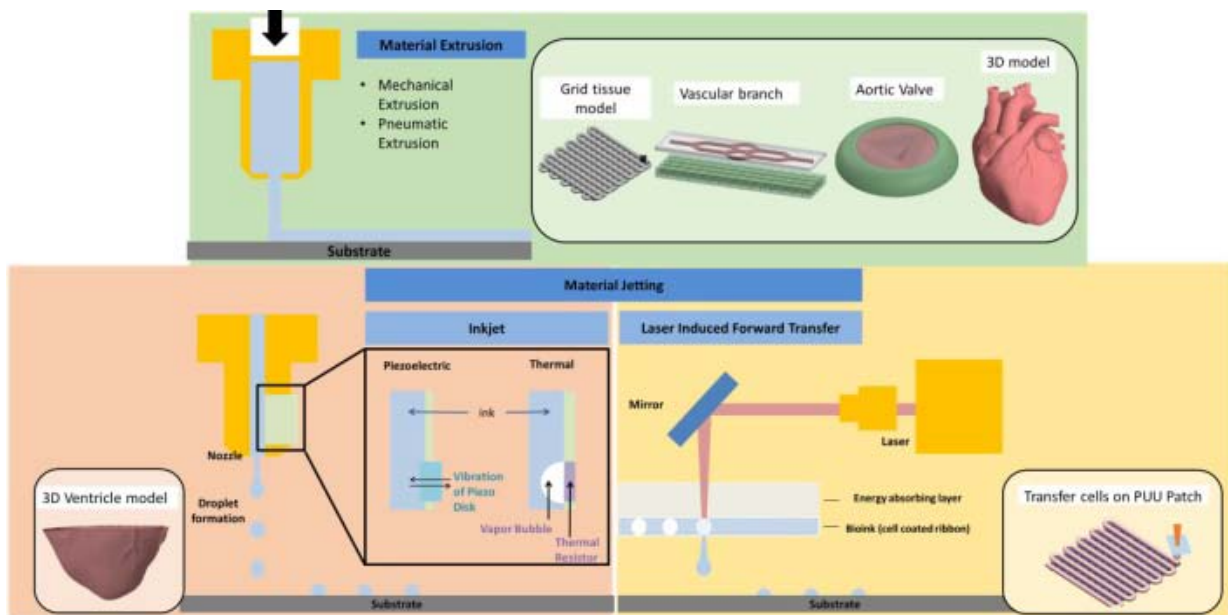


Figure 2. Schematic representation of current state-of-the-art bioprinted cardiovascular and cardiac-related tissue.

Table 2. Bioprinted cardiovascular and cardiac-related tissue

Technique	Resolution	Advantages	Disadvantages	Application	Reference
Material Extrusion	100–2000 μm	<ul style="list-style-type: none"> • Wide choice of materials • Able to control material extrusion by modifying needle tip 	<ul style="list-style-type: none"> • 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	10–100 μm	<ul style="list-style-type: none"> • Single cell resolution • High cell loading density (10⁸ cells/mL) • Any viscosity of loading material • Contact-less printing minimizing cross contamination risk 	<ul style="list-style-type: none"> • Long preparation process • Complex instrumentation for precise control needing materials with optical property 	Cardiac patch containing heterogeneous cell population	[78]
				Inkjet	100 μm

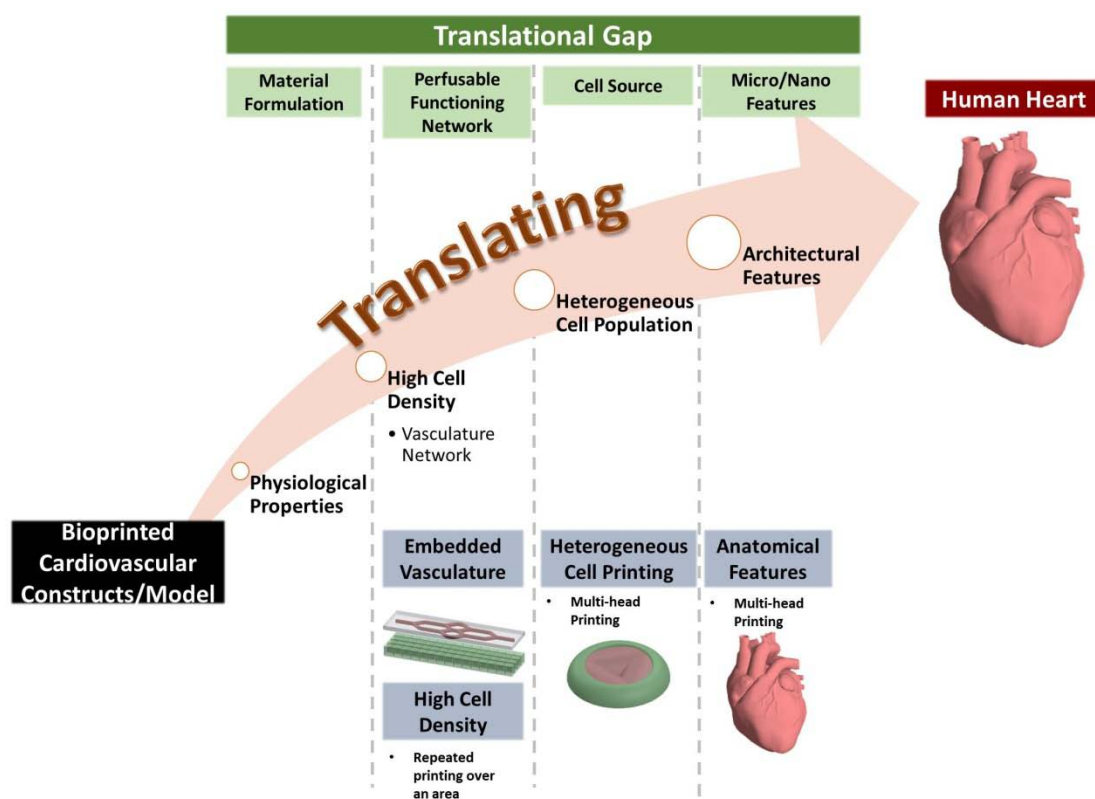


Figure 3. Future prospect of cardiac tissue engineering using bioprinting.

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.

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