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BIOPRINTING FOR CARDIOVASCULAR TISSUE ENGINEERING

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ABSTRACT: Heart disease is the leading cause of death worldwide. Advanced therapeutic approach such as tissue engineering provides alternative treatment options and model testing for cardiovascular diseases. Some design considerations when engineering cardiac tissue includes inducing cell alignment, attaining high cell density and culturing heterogeneous population of cells within a construct. Bioprinting, a computer-assisted technology, can potentially achieve the abovementioned considerations. The purpose of this review is to 1) highlight the prevalence of cardiovascular disease 2) identify design factors in engineering a cardiovascular tissue and describe conventional fabrication methods in achieving these engineered construct 3) discuss and analyze the role of bioprinting in engineering cardiovascular tissue

KEYWORDS: Bioprinting, Tissue Engineering, Cardiovascular Tissue Engineering, Scaffold Fabrication

CARDIOVASCULAR DISEASE
Cardiovascular disease, one of the world’s leading cause of death, possess a relatively large market in the field of regenerative medicine. In the past decade, medical advances have decreased mortality rates related with cardiovascular diseases such as acute coronary syndromes, valvular and congenital heart disease, uncontrolled hypertension and arrhythmias (Braunwald (2013). However, death rate from heart failure still remains high with 50% of heart failure patient dying within 5 years of diagnosis (Go et al. (2014). One possible reason attributing to the stagnated improvement of heart failure’s mortality could be explained by repeated episodes of cardiovascular diseases despite previous intervention. This suggests a plausible case where risk of heart failure in patients have been reduced but the root of problem has not be cured (Braunwald (2013). 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 functioning heart and has the largest potential in regenerative medicine (Tillman, Hardin-Young, Shannon, Russell, & Parenteau (2013).

ENGINEERING CARDIAC TISSUE

DESIGN CONSIDERATION
Several factors are considered when engineering a cardiac tissue (Figure 1). Firstly, engineered cardiac tissue should have similar mechanical properties (Modulus: 0.2 – 0.5 MPa at end diastole; Tensile Strength: 3 – 15 kPa; Contractile force: 2 – 4 mN/mm²) and electrical propagation velocity: 25 cm/s) as human myocardium (Iyer, Chiu, Reis, & Radisic (2011; Jawad et al. (2007). Secondly, the native heart which comprises of heterogeneous cell population – both
cardiomyocytes and non-cardiomyocytes (Kohl, Camelliti, Burton, & Smith (2005). Thirdly, the architectural features of native heart differ at different hierarchical level. At milliscale level, matrix anisotropy induces aligned myofibers while having varying spatial arrangement across transmural direction (Nakatani (2011); In the micrometer scale, high cell density of native myocardium requires support from vascularization (Vunjak-Novakovic, Lui, Tandon, & Chien (2011). Different fabrication techniques have been used to engineer biomimetic cardiac tissue that satisfies the different requirements. Conventional fabrication techniques in cardiac tissue engineering can be broadly divided into two categories, namely scaffold and scaffold-free fabrication.

**SCAFFOLD-BASED ENGINEERED CARDIOVASCULAR TISSUE**

In scaffold-based approach, solid scaffold are used as basis for cardiac cells attachment and mechanical support. These fabrication processes usually require two-step process, i) forming scaffold ii) seeding cardiac cells. Several methods have been employed to construct scaffolds, namely, solvent casting (LeBlon et al. (2013; Pego et al. (2003; Sin et al. (2010), molding (Neal et al. (2013), and electrospinning (Fleischer et al. (2013; Kai, Prabhakaran, Jin, & Ramakrishna (2011; Kharaziha et al. (2013; Orlova, Magome, Liu, Chen, & Agladze (2011; Prabhakaran, Nair, Kai, & Ramakrishna (2012; Shevach, Maoz, Feiner, Shapira, & Dvir (2013; Xu et al. (2013). One major disadvantage of such method is the inability to produce uniform macropore structure in casted scaffold. An alternative to produce uniform pore size is to use computer aided design to fabricate scaffolds with defined pore structure and controlled stiffness (Chua, Yeong, & Leong (2005; W.-Y. Yeong, Chua, Leong, Chandrasekaran, & Lee (2005; W. Y. Yeong et al. (2010)

**SCAFFOLD-FREE ENGINEERED CARDIOVASCULAR TISSUE**

Scaffold-free approach does not use solid scaffolds e.g. poly lactic acid (PLA), polycaprolacton (PCL), etc to provide structural base for subsequent deposition of biological materials. Cell sheet technology has the ability to retain cells at the injured site as compared to individual cell suspension during cell injection (Miki et al. (2012; Shimizu, Yamato, Kikuchi, & Okano (2003). Moreover, cell sheet can be transplanted without sutures and represents a potential for minimally invasive solution. One limitation of such technology is the limited cell number generated from each cardiac cell sheet are significantly less than the amount of cardiomyocytes lost (approximately 9.5x10⁴ cells in cell sheet versus 8 x 10⁶ heart cells lost in rat during infarction) (Adler, Friedburg, Herget, Neuberger, & Schwalb (1996; Miki et al. (2012).

Another scaffold-free fabrication technique removes xenogenic cells from the matrix and repopulated through perfusion of desired cell type. However, perfusion of decellularized matrix is limited 70% of original volume. Homogeneous and high seeding cell density still remain elusive (Shimizu (2014). Moreover, decellularized matrix repopulated with neonatal rat cardiomyocytes showed disorganized electrical propagation with disarray cardiomyocytes and decreased expression of Connexion 43 (Yasui et al. (2014).

**BIOPRINTING: THE NEW PARADIGM IN ENGINEERING CARDIOVASCULAR TISSUE**
Conventional fabrication processes discussed in the earlier section mostly describe planar construction of engineered heart tissue. 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 (Li & Guan (2011). The ability to control shape and material via multi-material printing is the main advantages offered by bioprinting. Previously, tubular and planar film were bioprinted for tissue engineering purpose (Lee & Yeong (2014; Ng, Yeong, & Naing (2014; E. Y. S. Tan & Yeong (2015; Y. S. E. Tan & Yeong (2014). The potential in using 3D bioprinting technology for fabricating engineered cardiac tissue is in the control of the geometrical shape or anatomically relevant structures (Duan, Hockaday, Kang, & Butcher (2013). Another significant bioprinted construct was the vasculature network demonstrated by Kolesky and co-worker (Kolesky et al. (2014).

### Table 1 Bioprinted cardiovascular and cardiac-related tissue

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<tr>
<th>Technique</th>
<th>Cells</th>
<th>Biomaterials</th>
<th>Application</th>
<th>Ref</th>
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<tr>
<td>Cell laden alginate/gelatin gel extruded into disc mold</td>
<td>Porcine Aortic Valve Interstitial, Human Root Smooth Muscle Cells/mL</td>
<td>Gelatin Type A and alginate</td>
<td>Heterogeneous aortic valve conduits</td>
<td>(Duan et al. (2013)</td>
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<tr>
<td>Cell/Alginate construct extruded</td>
<td>Human fetal cardiomyocyte progenitor</td>
<td>Sodium Alginate</td>
<td>Tissue model</td>
<td>(Gaetani et al. (2012)</td>
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<tr>
<td>Sequentially extruding multiple inks</td>
<td>Human neonatal dermal fibroblasts, Human umbilical vein endothelial cell 10T1/2 fibroblast cells</td>
<td>Silicone elastomer, Pluronic F127, Gelatin methacrylate</td>
<td>Vascular Branches</td>
<td>(Kolesky et al. (2014)</td>
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<tr>
<td>Cells transferred onto cardiac patch via Laser-Induced Forward Transfer</td>
<td>Human Mesenchymal Stem Cells, Human Umbilical Vein Endothelial Cells</td>
<td>Polyester Urethane Urea</td>
<td>Cardiac Patch containing heterogeneous cell populations</td>
<td>(Gaebel et al. (2011)</td>
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### FUTURE PROSPECT

**Cell source for bioprinting** The ideal cell type used for engineering in vivo solutions to cardiovascular diseases has not been identified. Moreover, isolation of cells from endocardium, epicardium and cardiac fibroblast have not been established Vunjak Novakovic, Eschenhagen, and Mummery (2014). The relation between cardiomyocytes and non-cardiomyocytes still remains a fundamental biological question. The high loss of cardiomyocytes during infarction requires a vascular network that facilitates nutrient transportation in an engineered cardiac tissue. Conventional methods show limited advancement in terms of vascularization.

**Cell alignment in bioprinted constructs** In terms of cell alignment, the understanding of cell-material interaction has been acquired through conventional fabrication approaches to achieve
aligned myocytes at planar level. The essence of cell alignment lies in conforming and guiding cells using defined spaces. Nanoscale features such as electrospinning guide cell alignment along spun fibers (Chanthakulchan, Koomsap, Parkhi, & Supaphol (2015); Microscale geometries confine and direct cell growth towards anisotropic direction. However, these methods vaguely describe cell behaviour in native environment. To utilize such knowledge and integrate into a three dimensional perspective, an engineered tissue with three dimensionality design is required.

At current state-of-technology, several research groups have used bioprinting for patterning heterogeneous cell construct, printing shape complex structure (i.e. anatomical resemblance of the heart), and constructing microchannels for vascularization. Bioprinting remains as a powerful tool in forming constructs based on virtual designs. This tool will allow engineers and scientists to integrate lessons learnt from decade of cardiovascular tissue engineering into a bioprinted cardiovascular tissue with close resemblance to the native tissue.

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64


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