Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Pediatric cardiovascular grafts: historical perspective and future directions

Stanley Heydrick[1], Erin Roberts[2,3], Jaeyun Kim[1], Sitaram Emani[3] and Joyce Y Wong[1,2]

Tissue-engineered cardiovascular patches, cardiac valves, and great vessels are emerging solutions for the surgical treatment of congenital cardiovascular abnormalities due to their potential for adapting with the growing child. The ideal pediatric cardiovascular patch/graft is non-thrombogenic, phenotypically compatible, and matches the compliance and mechanical strength of the native tissue, both initially and throughout growth. Bottom-up tissue engineering approaches, in which three-dimensional tissue is built layer-by-layer from scaffold-less cell sheets *in vitro*, offer an exciting potential solution. Cell source variability, sheet patterning, and scaffold-less fabrication are promising advantages offered by this approach. Here we review the latest developments and next steps in bottom-up tissue engineering targeted at meeting the necessary design criteria for successful pediatric cardiac tissue-engineered grafts.

Addresses
[1] Department of Biomedical Engineering, Boston University, 44 Cummington Mall, Boston, MA 02215, USA
[2] Division of Materials Science and Engineering, Boston University, 15 St. Mary's Street, Boston, MA 02215, USA
[3] Cardiac Surgery, Boston Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA

Corresponding author: Wong, Joyce Y (jywong@bu.edu)

Current Opinion in Biotechnology 2016, **40**:119–124

This review comes from a themed issue on **Tissue, cell and pathway engineering**

Edited by **April Kloxin** and **Kyongbum Lee**

For a complete overview see the Issue and the Editorial

Available online 2nd April 2016

http://dx.doi.org/10.1016/j.copbio.2016.03.013

Introduction

Tissue engineering is a multi-interdisciplinary medical field in which a combination of state-of-the-art biology and engineering is used to build replacement tissues or organs, or help restore their function. Viable, engineered tissues are particularly needed in the treatment of congenital heart diseases, which are estimated to occur in as many as 1% of live births [1]. Congenital heart conditions differ from most adult cardiovascular disease (e.g. atherosclerosis or thrombosis) in that they generally arise from anatomical abnormalities, including cardiac septal defects, valve defects, and/or structural abnormalities in the great arteries and veins. As a result, many of these conditions require surgical solutions that involve the implantation of patches, cardiac valves, and portions of great vessels.

While autografts taken from other parts of the body remain the gold standard for use in surgical reparation of pediatric cardiovascular anomalies, there is frequently a limited availability of adequately sized autograft tissue from pediatric patients. Xenograft or allograft patches/vessels are often used instead, but these can cause an unacceptably high rate of immune complications if they are not decellularized, and decellularization often reduces durability. Artificial cardiac septum patches, vessel grafts, and valves composed of non-immunogenic polymers such as Gore-tex (expanded-polytetrafluoroethylene or ePTFE), or Dacron (polyethylene terephthalate or PET), have also been developed to fill this void. However, these materials are somewhat pro-thrombotic, do little to promote cardiac/vessel regeneration or strengthen damaged tissue, and can cause maladaptive remodeling due to the mechanical and compliance mismatch [2–5]. More importantly, Dacron and Gore-tex patches and grafts are incapable of growing with the child, often necessitating either a delay in surgery until the child reaches adulthood or multiple surgeries over the course of childhood. There is thus growing interest in the development of living tissue-engineered cardiovascular patches and grafts.

Structural and biological design considerations for cardiovascular patches and grafts

From a structural standpoint, the ideal cardiovascular patch/graft would be non-thrombogenic and match the compliance and mechanical strength of the cognate native tissue. This is especially true in tissue-engineered blood vessel (TEBV) grafts intended for systemic arterial applications, where high compliance is required for durability [6], and high mechanical strength is required to prevent fraying at sutures and vessel bursting under physiological blood pressures. The compliance and tensile strength of native cardiovascular tissues reside largely in their highly organized structural scaffold of collagen and elastic fibers, with collagen fibers providing the bulk of the tissue's structural strength, and elastin fibers the bulk of its compliancy. An equally important but often overlooked structural consideration is functional replication of the basement membranes upon which

cells in native tissues rest. Basement membranes are composed of numerous protein fibrils and fibers interwoven within a hydrated network of glycosaminoglycan chains whose inherent attachment points, together with mechanical and biochemical stimuli, help maintain the health of the attached cells [7,8]. Combined, the structural scaffold and basement membrane comprise the extracellular matrix (ECM). Because cells in native tissues dynamically maintain and remodel their ECM according to environmental cues [9•], promotion of cellular health through their interactions with basement membranes is crucial for the long-term patency of engineered tissue patches and grafts.

The living, cellular components of cardiovascular patches and grafts would ideally be derived from the patient, that is, autologous, in order to limit immune complications. They should also be capable of growing and remodeling or repairing the implant in a manner identical to their 'native' counterparts *in vivo*. Moreover, cardiomyocytes or smooth muscle cells (SMCs) in engineered cardiovascular structures should also be able to contract or relax as they are modulated by electrical and endocrine/paracrine signals. Finally, it is crucial that engineered tissues be receptive to neovascularization, since there is typically insufficient oxygen diffusion in thick vascular patches or grafts [10].

Two basic strategies have been employed to meet these ideal design considerations: 'top down' and 'bottom up' tissue engineering approaches. While each has advantages and disadvantages, this review will only briefly touch on top-down tissue engineering and will focus primarily on bottom-up approaches.

Top-down approaches

In the 'top-down' approach, the bioengineer seeds vascular and/or other cells on/into a biocompatible (artificial or natural) scaffold; in some applications the patient's body is expected to seed the scaffold. The seeded cells are expected to attach to and grow within the scaffold, and eventually replace it by synthesizing sufficient ECM to provide the necessary structural strength and compliance. While beyond the scope here, tissue engineering utilizing this approach continues to be extensively investigated (reviewed in [11••,12]) and has led to some clinical successes in the surgical treatment of congenital heart diseases, including the routine production and implantation of decellularized-tissue cardiac and arterial valves to correct structural anomalies [13–16], and experimental implantation of pulmonary grafts to alleviate the insufficient pulmonary perfusion seen with Tetralogy of Fallot or single ventricle abnormalities [17–19].

However, even routinely implanted scaffold-based engineered tissues such as cardiac and arterial valve grafts fail after 10–15 years [20,21], and the design of these types of engineered tissues has yet to provide the fine control of cell-to-biomaterial and intercellular adhesion, intercellular communication, and organized basement membrane secretion necessary for consistent long term patency. Patency is further diminished by deficient vascularization often found in scaffold-based engineered tissues [22]. Addressing these deficiencies is especially crucial in pediatric patients, where the engineered tissue would ideally last a lifetime.

Bottom-up approaches

Bottom-up approaches, in which three-dimensional tissue is built layer-by-layer from scaffold-less cell sheets *in vitro*, offer an exciting alternative to top-down approaches. Cardiac and vascular tissues are particularly amenable to this approach because they have an inherently layered structure as seen in Figure 1. Moreover, since cell sheets secrete their own ECM, it is theoretically possible to generate fully cellularized tissues whose non-cellular components can be manipulated to closely reflect those in native tissue. Finally, cellular orientation can be controlled by patterning the substrate a cell sheet is grown on. This latter advantage makes the bottom-up approach uniquely capable of generating engineered tissues that recapitulate the alternating axes of elongation seen in smooth muscle layers of major arteries [23].

One key to the bottom-up approach has been the continued development of scaffold-less cell sheet technology, in which two-dimensional cell sheets are first grown in culture and then detached from the culture substrate for use in larger tissue constructs as summarized in Figure 2. The development of novel methods to safely detach cell sheets from the cell culture substrates (e.g. plasticware) they are grown on has significantly enabled this approach. In the most commonly used method, cell sheets are grown on plasticware coated with the temperature-responsive polymer poly(*N*-isopropylacrylamide) (P(NIPAAm)). P(NIPAAm) is a dense, hydrophobic film at 37 °C that undergoes a reversible conformation change below 32 °C to a swollen, hydrophilic state [24]. Mature cell sheets easily anchor to P(NIPAAm) in the hydrophobic conformation under 37 °C culture conditions, but spontaneously detach from it as it changes phase upon cooling to room temperature. The 'gentleness' of this method ensures there is little cellular damage, with preservation of cell–cell contact, cellular patterning and the attached ECM. A number of other less-widely used methods, including electro-responsive, photo-responsive, pH-responsive, and magnetic detachment systems have also been developed; these have been extensively reviewed by Patel and Zhang [25••]. Our laboratory has also recently developed a novel sacrificial-substrate detachment system in which cell sheets are grown on tyramine-conjugated polymers of carboxymethyl cellulose (CMC-ty) and alginate (Al-ty) (manuscript in preparation and patent submitted). Because these carbohydrate