Integrating principles of developmental biology in tissue engineering of heart valves

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'The ideal heart valve replacement would not only be biocompatible, readily available and incredibly durable, but also have the potential for growth.'

Approximately eight out of every thousand infants are born with congenital heart disease. The majority of these defects involve malformations or absence of the pulmonary valve and main pulmonary arteries. Although repair early in life is possible, the procedure often requires the use of a replacement valve. Current replacement options include prosthetic and bioprosthetic valves; however, these replacements have limited durability and are subject to calcification, thrombosis and a lack of growth potential [1]. Children, therefore, face an ongoing morbidity due to the limitations of these replacement valves and must endure multiple follow-up operations in order to place progressively larger valves to accommodate their growth. Tissue engineering offers the potential to create a living valve with the capacity for growth and self-repair, and which is resistant to infection. Tissue engineering is defined as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve function [2]. This approach is based on seeding autologous cells onto biodegradable scaffolds or decellularized biological matrices in order to provide temporary structural support and organization until the cells synthesize their own extracellular matrix. Previous studies by our group and others have demonstrated *in vivo* results culminating in the creation of single-valve leaflets [3], vascular grafts [4,5] and trileaflet-valved conduits [6–11]. These tissue-engineered heart valve (TEHV) structures have been created using vascular cells [3,4,6–9], umbilical blood-derived endothelial progenitor cells [11] and noncirculating bone marrow-derived mesenchymal stem cells [12,13] as cell sources. Our group

and others have also demonstrated results in preliminary *in vivo* experiments. However, many questions remain unanswered:

- What is the optimal cell source?
- How will the scaffold material influence tissue growth and allow favorable scaffold cell and extracellular matrix interactions?
- What *in vitro* conditions provide the most cell growth?
- What is the timeframe for *in vivo* maturation?

The ideal heart valve replacement would not only be biocompatible, readily available and incredibly durable, but also have the potential for growth [14]. Although the ultimate goal of TEHV is to recapitulate the matrix and cells found in the native tissue, variabilities exist within the potential strategies and sources of cells. A widely accepted paradigm of tissue engineering comprises of a scaffold that is preseeded with cells, followed by an *in vitro* stage of tissue formation typically conducted in a bioreactor (that recapitulates a physiological metabolic and mechanical environment) and, following subsequent implantation of the construct, an *in vivo* stage of tissue growth and remodeling [15].

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Unresolved issues

Cell source

Moving towards clinical applications, less invasively accessible cells, such as circulating and umbilical-derived endothelial progenitor cells [11] and bone marrow-derived mesenchymal stem cells [12,13] have shown promising results for TEHVs. Although their pluripotency and multipotency potential is enormous, we still do not fully understand how soluble hormones, extracellular matrix proteins, other cells and physical factors control stem cell fate switching in distinct tissue microenvironments. This information is crucial before we can develop clinically applicable TEHVs using stem

cells as cell sources [16]. Recently, it was demonstrated that approximately 10–20% of the cells that make up adult valves have hematopoietic origins. This suggests that stem cells may be able to repopulate valvular tissues and recapitulate valvular cell phenotypes. However, where to target along the continuous spectrum of valvular cell phenotypes and how to stimulate the desired mature phenotype remains unknown [17,18].

Scaffold material

The tissue-engineered biomaterial scaffolds need to be biocompatible and bioresorbable and should be designed for optimized scaffold architecture (pore size, morphology and surface topography) in order to enhance oxygen diffusion and support cell and tissue growth. In addition, they must also exhibit the desired degradation rate and suitable mechanical and structural properties comparable with the native tissue they replace, from the time of implantation through the lifetime of the recipient. This implies central requirements of relatively constant mechanical properties despite potentially considerable tissue remodeling and scaffold resorption [15]. Therefore, should we fabricate biomaterials to construct microenvironments that mimic embryonic organs, healing wounds or developmentally active inducer tissues [16]? Powerful methods of fabricating tissue-engineering scaffolds could include [16,19,20]:

- Engineering the surface topography of these scaffolds at the micrometer and nanometer scales to produce optimized cellular responses;
- Developing injectable self-assembling nanomaterials;
- Incorporating biological signals into the nanostructured scaffolds to create healing microenvironments that harness the body's own stem cells, tissue-specific chemical factors and cell-generated traction forces.

Electrospun nanofibrous scaffolds have also been shown to exhibit highly mechanical properties that resemble native pulmonary heart valve cusps [21].

In vitro conditioning

Mechanical and biological stimulation during *in vitro* tissue development are utilized in TEHV for improving tissue formation, organization and cellular function. The bioreactor exposes the developing tissue to mechanical conditioning, such as flow, shear stress and/or

strain. One type of bioreactor used in the construction of TEHVs is the pulse duplicator, which provides pressure and flow to the developing TEHV, subsequently promoting mechanical strength and cellular organization in the engineered tissues [6,22]. However, there is increasing evidence that hemodynamics regulate valvulogenesis [23]. That is, mechanical forces may play a role in the maturation of developing a TEHV. Should we focus on engineering adult valve structures and then strengthen them through mechanical conditioning or, instead, preferably create immature developmental tissue and stimulate maturation by using the embryonic hemodynamic regime [18]?

In vivo maturation

We and others have provided proof-of-concept studies demonstrating growth and remodeling of the engineered construct to a trilaminar structure, resembling the normal architecture and biology of cardiac valves, which functions satisfactorily *in vivo* for more than 4–8 months following implantation [6,9,13]. The *in vivo* mechanical environment presumably contributes to the ingrowth and tissue remodeling, and results in normal valve architecture [15,24]. Can these tissue-engineered structures remain viable, sustain the wear and tear of normal circulation over a lifetime and grow in pediatric patients [14]? There are numerous challenges to overcome before this technology can be translated to a clinical setting [25].

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Future perspective

Developmental biology approach in TEHV

In embryonic cardiac valves, explants of endocardial cushion tissue undergo endothelial to mesenchymal transdifferentiation when valve endothelial cells invade the cardiac jelly (extracellular matrix). Functional cardiac valve formation involves dynamic and continuing interactions between cells and the hyaluronan-rich ‘cardiac jelly’, which are necessary for the proper chamber separation and valve-leaflet development. Therefore, it appears likely that the spatial and temporal presence of hyaluronan with specific proteoglycans and growth

factors will promote the switch in cell signaling machinery that facilitates the maturation of heart valves [26–28]. Biochemical and biomechanical signals that guide normal fetal cardiac valve development will likely have similar effects during the formation of TEHV. Manipulation of these signals can favorably influence the development of TEHV formed from cells and biodegradable scaffolds. By identifying and engineering microenvironmental cues provided in embryonic cardiac valve development, we can perhaps recapitulate these programmed morphogenetic events and guide cellular behavior toward the formation of appropriate tissue for a normal cardiac valve. The fundamental principles of developmental processes will advance our understanding of the factors affecting *in vitro* TEHV development and how this translates into the *in vivo* performance of engineered heart

valves. If the TEHV can become a reality, it will significantly change the treatment paradigm for children with congenital heart diseases.

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