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Animal models of cardiovascular disease as test beds of bioengineered vascular grafts

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The last two decades have seen many advances in regenerative medicine, including the development of tissue engineered vessels (TEVs) for replacement of damaged or diseased arteries or veins. Biomaterials from natural sources, as well as synthetic polymeric materials have been employed in engineering vascular grafts. Recently, cell-free grafts have become available, opening new possibilities for the next generation, off-the-shelf products. These TEVs are first tested in small or large animal models, which are usually young and healthy. However, the majority of patients in need of vascular grafts are elderly and suffer from comorbidities that may complicate their response to the implants. Therefore, it is important to evaluate TEVs in animal models of vascular disease in order to increase their predictive value and learn how the disease microenvironment may affect the patency and remodeling of vascular grafts. Small animals with various disease phenotypes are readily utilizable due to the availability of transgenic or gene knockout technologies and can be used to address mechanistic questions related to vascular grafting. On the other hand, large animal models with similar anatomy, hematology and thrombotic responses to humans have been utilized in a preclinical setting. We propose that large animal models with certain pathologies or age range may provide more clinically relevant platforms for testing TEVs and facilitate the clinical translation of tissue engineering technologies by increasing the likelihood of success in clinical trials.

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Introduction

Cardiovascular disease claims 801,000 deaths in the United States annually, according to the 2017 AHA (American Heart Association) statistic. The estimated global healthcare cost for cardiovascular disease by year 2030 is projected to exceed $1044 Billion USD. One of the most prevalent conditions among heart diseases is coronary artery blockage (45.1% of all

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cardiovascular ailments), leading to heart attacks or stroke [1]. The established surgical remedy involves the harvest of an autologous vein or artery, which is then transplanted to ''bypass'' the diseased region of the coronary artery, restoring regular blood flow back into the heart. Harvesting an autologous vessel from an elderly person, or patients suffering from other complications such as hypertension, diabetes, or previous bypass or shunt procedures, often does not yield viable graftable vessel segments [1]. This prompts for alternate TEVs to be made readily available ''off-the-shelf'' [2–4]. In addition to their application for the treatment of coronary artery disease, TEVs can also be employed to treat congenital heart disease, peripheral artery diseases, chronic vein insufficiency as well as their utilization as dialysis shunts for treatment of kidney diseases, especially for diabetic patients. Existing technologies utilize cell-based approaches and bio-reactor processing which are time-consuming and synthetic products which may result in unfavorable outcomes, due to lack of development of biological function [3,5].

An ideal TEV should develop biological function, and be similar in structure and mechanical properties to the native blood vessel into which it is implanted. Remodeling of the TEV should result in a lumen with a confluent monolayer of endothelial cells, surrounded by a medial wall containing collagen, elastin and circumferentially aligned smooth muscle, capable of contractile function and control of blood flow. A major challenge facing most research groups is the correct choice of an animal model where the TEVs may be tested. Small animal models such as mice allow genetic manipulation, thereby providing a suitable platform to study cellular and molecular mechanisms underlying the remodeling processes. However, larger animal models provide a more physiologically relevant platform to study graft remodeling, due to physiological similarities with humans. That being said, hematologic and hemostatic processes such as clotting cascades, secondary thrombogenic events, and cell infiltration and migration patterns in different species may vary from those in humans. Processes such as inflammation and immune responses must also be considered when making the choice of the animal model.

Biomaterials for vascular tissue engineering

The choice of biomaterial is critical for the construction of a successful TEV (Tissue Engineered Vessel). The appropriate porosity, allowing for cell migration and supporting natural ECM (extracellular matrix) secretion, is important to graft patency as is a healthy endothelial layer. A canine study, which examined the effects of biomaterial and endothelial cell seeding revealed that simply applying endothelial lining in dacron and e-PTFE (expanded-Polytetrafluoroethylene) grafts was not a deciding factor for patency, but, the biomaterial used to construct the vessel played a significant role [6]. The study results concluded that isodiametric naturally harvested scaffolds performed best to maintain patency [6]. Graft porosity allows for cell adherence as well as migration and diffusion of important healing factors critical to the remodeling responses [7–9]. In fact, porous biomaterials might allow for capillary ingrowth, leading to transmural endothelialization of the grafts [10]. Similarly, the lumen of perforated Gore-tex® implanted in the canine aorta was lined with host endothelial cells faster as compared to solid grafts [7]. Healing and stabilization of implanted grafts mostly occurs through fibroblast transmural migration. In fact, when the tissue surrounding the implant site was preserved and rewound around the porous TEV, fibroblast migration from perivascular tissue enhanced patency [8]. Although natural biomaterials provide sufficient porosity and mechanical strength, synthetic biomaterials can be tuned more easily by electrospinning or 3D printing techniques to make off-the-shelf customizable grafts.

Synthetic materials

The main advantage of using synthetic materials is the abundance in availability, relative ease in controlling their desired mechanical properties, porosity and cell adhesiveness, as well as quality control leading to reduced batch-to-batch variability. Electrospinning polymers with desired natural biomaterials yields very good materials for TEVs. For example, electrospinning poly-L-lactide/PCL copolymer with collagen yielded high tensile strength and proved to promote cell migration and adhesion [11]. Likewise, by manipulating inter-nodal distances of polymer fibers within PTFE grafts, transmural endothelial cell migration leading to capillary formation could be achieved *in vivo* [10]. It has been observed that the size of fibers used for electrospinning has been shown to control porosity and the surface are available for cell adhesion. Specifically, electrospinning with microfibers increases porosity, while spinning with nanofibers enables perfusion flow through the graft material leading to increased cell density, as tested *in vitro* in a bioreactor [12].

The function of synthetic biomaterials has been enhanced by functionalization with biological active signals such as growth factors. For example, immobilizing VEGF (Vascular Endothelial Growth Factor) on PTFE grafts using standard EDC chemistry and HAS (Human Serum Albumin) electrostatic linkages enabled endothelial cell migration *in vivo* [13]. Interestingly, Wu et al. employed the biodegradability and cell homing capacity of polyglycerol sebacate elastomer to create a ''template'' for neoartery formation *in vivo* [14]. Although synthetic biomaterials are a convenient option for tissue engineering advances, natural biomaterials possess natural ECM for enhanced host cell invasion and remodeling.

Natural biomaterials

Natural biomaterials include fibrin, collagen, and hyaluronic acid as well as decellularized tissues e.g. decellularized arteries that maintain their ECM composition and mechanical

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