Biomimetic Strategies in Vascular Tissue Engineering

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INTRODUCTION

Cardiovascular disease remains the leading cause of death in the United States, claiming more lives each year than the next five leading causes of death combined (American Heart Association National Center for Health Statistics). Coronary heart disease caused more than one in every five American deaths in 2000 and required approximately 500,000 coronary artery bypass graft surgeries (CABGs) that year. Bypass grafting is also used in the treatment of aneurysmal disease or trauma. At present, surgeons use autologous tissue and synthetic biomaterials as vascular grafts. Transplantation of autologous tissue has the best outcome in applications such as CABG because synthetic grafts, such as those made from expanded polytetrafluoroethylene or polyethylene terepthalate, fail in small diameter applications (ID < 6 mm) due to formation of blood clots and scar tissue. However, autologous tissue is limited in supply, either due to prior procedures or peripheral vascular disease. Recent advances in tissue engineering provide hope that new blood vessel substitutes may one day be fabricated for small diameter applications, such as CABG, where treatment options are often severely limited.

TISSUE ENGINEERING

Tissue engineering refers to the application of engineering principles to the design of tissue replacements, usually formed from cells and biomolecules. Tissue engineered products are already commercially available for skin and cartilage. Typically, an engineered tissue is

formed by harvesting a small sample of the patient's cells, expanding them in culture, then seeding the cells onto a scaffold material. Scaffold materials are intended to define the size and shape of the new "tissue" and to provide mechanical support for the cells as they synthesize the new tissue. Scaffolds are usually biodegradable synthetic polymers. The cell-seeded scaffolds can either be implanted into the patient, with tissue formation occurring in situ, or cultured further in vitro to achieve properties more similar to normal tissue before implantation. This culture period is often carried out in a bioreactor to provide appropriate mechanical conditioning during tissue formation.

Most tissue engineering strategies attempt to create small caliber vascular grafts by closely mimicking the structure, function, and physiologic environment of native vessels. Normal arteries possess three distinct tissue layers (Figure 1): the intima, media, and adventitia. The intima consists of an endothelial cell monolayer, which prevents platelet aggregation and regulates vessel permeability, vascular smooth muscle cell behavior, and homeostasis. Within the medial layer, smooth muscle cells (SMCs) and elastin fibers are aligned circumferentially, contributing the majority of the vessel's mechanical strength (Wight 1996). Finally, the adventitial layer contains fibroblasts, connective tissue, the microvascular supply, and a neural network that regulates the vasotone of the blood vessel. The re-creation of some or all of the vessel layers and their properties may result in the development of a patent, functional vascular graft. In all likelihood, an intima and media will be required to achieve any degree of success. As described above, the general concept for creating a tissue-engineered vascular graft (TEVG) usually involves the harvest of desired cells, cell expansion in culture, cell seeding onto a scaffold, construct culture in an environment that induces tissue formation, and implantation of

the construct back into the patient. Many options exist at each step of this process and each must be carefully considered.

While efforts to create TEVGs remain in an early developmental stage, several problems potentially exist. Graft patency is still threatened by thrombosis, in all likelihood due to issues with retention of endothelial cells after implantation or with alterations in endothelial cell function after culture in vitro. Also, the possibility of burst failure after implantation in the physiological flow environment raises concern since the consequences would be catastrophic. Mechanical properties of TEVGs are generally observed to be lower than those of native arteries, and thus a number of approaches have begun to address these issues. All of the strategies discussed above—cell source, genetic modification, scaffold materials, and culture conditions—will likely impact the fabrication of an optimal, clinically-useful TEVG.

Cell Sources for Vascular Tissue Engineering

The development of a functional TEVG is likely to require the construction of an intima and media composed of endothelial and smooth muscle cells. Limitations imposed by immunogenicity will probably require the use of autologous cells, so the majority of studies to date have utilized differentiated smooth muscle and endothelial cells isolated from harvested blood vessels. Issues with donor site morbidity and the performance of these cell types in the engineered tissues have led to the consideration of alternative cell sources. Recent advances in stem cell biology offer hope for suitable progenitors that can be effectively differentiated into endothelial and smooth muscle cells for use in vascular tissue engineering.

Genetic Modification of Vascular Cells

Genetic engineering of vascular cells ex vivo may provide an effective strategy to improve graft properties for tissue engineering applications. The leading cause of vascular graft failure has been attributed to thrombosis, or blod clot formation. The seeding of small-diameter vascular graft constructs with cells genetically engineered to secrete anti-thrombotic factors may improve graft patency rates. For example, the use of a platelet aggregation inhibitor, nitric oxide (NO), has been shown to be promising. Using an ex vivo approach, bovine smooth muscle cells liposomally-transfected with nitric oxide synthase III (NOS III) and GTP cyclohydrolase, which produces a cofactor essential for NOS activity, were grown as monolayers on plastic slides or biomaterials of interest and then placed in a parallel plate flow chamber. Whole blood was introduced into the flow chamber to assess platelet adherence to the cell monolayers. The number of platelets that adhered to the NOS-transduced SMCs was significantly lower than those bound to mock-transduced SMCs and similar to the numbers adhered to cultured endothelial cells (Scott-Burden et al., 1996).

Improving the mechanical properties of TEVGs has also been a goal of numerous research efforts, and genetic modification of cells used to seed the TEVG may prove beneficial. The mechanical properties of a TEVG will be related in large part to the extracellular matrix (ECM) that forms, both its composition and structure. ECM crosslinking can result from the enzymatic activity of lysyl oxidase (LO) (Aeschlimann et al. 1991) and may be a means to improve mechanical properties of the TEVG. LO, a copper-dependent amine oxidase, forms lysine-derived crosslinks in connective tissue, particularly in collagen and elastin (Rucker et al. 1998). A gene therapy strategy has demonstrated the enhancement of mechanical properties of tissue engineered collagen constructs using vascular smooth muscle cells transfected with LO

(Elbjeirami et al. 2003). The elastic modulus and ultimate tensile strength of collagen gels seeded with LO-transfected SMCs nearly doubled as compared to gels seeded with mock-transfected SMCs. These enhanced mechanical properties resulted from increased ECM crosslinking rather than increased amounts of ECM, changes in the ECM composition, or increased cellularity. This strategy may ultimately enhance mechanical characteristics of TEVGs and minimize in vitro culture times prior to implantation.

Scaffolds for Vascular Tissue Engineering

Tissue engineers have had to select between natural (i.e., collagen; Weinberg and Bell, 1986) and synthetic (i.e., polyglycolic acid; Niklason et al., 1999) polymer scaffolds. Each has its advantages and also its issues. Ideally, one would want specific cell-material interactions like those between cells and collagen while also having control over material properties and ease of processing that come with synthetic polymers. Therefore, biomimetic derivatives of polyethylene glycol (PEG) currently are being studied as scaffolds for vascular tissue engineering. PEG-based materials are hydrophilic, biocompatible, and intrinsically resistant to protein adsorption and cell adhesion (Merrill et al., 1983; Gombotz et al., 1991). Thus, PEG essentially provides a "blank slate," devoid of biological interactions, upon which the desired biofunctionality can be built. Aqueous solutions of acrylated PEG can be rapidly photopolymerized in direct contact with cells and tissues (Sawhney et al., 1994; Hill-West et al., 1994), allowing an easy method of cell seeding. Furthermore, PEG-based materials can be rendered bioactive by inclusion of proteolytically degradable peptides into the polymer backbone (West et al., 1999) and by grafting adhesion peptides (Hern et al., 1998) or growth factors (Mann et al., 2001a) into the hydrogel network during the photopolymerization process. Recently, PEG

hydrogels that largely mimic the properties of collagen have been developed (Gobin and West, 2002; Mann et al., 2001b). Also, the elastin-derived peptide VAPG has been shown to be specific for SMC adhesion, and PEG hydrogels modified with this adhesive peptide rather than RGDS supported adhesion and growth of vascular SMCs but not fibroblasts or platelets (Gobin et al., 2003). Moreover, bioactive molecules like TGF-β may be covalently incorporated into scaffolds to induce protein synthesis by vascular SMCs. TGF-β has been reported to stimulate expression of several matrix components, including elastin, collagen, fibronectin, and proteoglycans (Lawrence et al., 1994; Amento et al., 1991). TGF-β covalently immobilized to PEG-based hydrogels significantly increased collagen production of vascular SMCs seeded within these scaffold materials (Mann et al., 2001). Mechanical testing of these engineered tissues also determined that the elastic modulus was higher in TGF-β tethered PEG scaffolds than PEG scaffolds without TGF-β, indicating that material properties for TEVGs may be improved using this technology. A cell-seeded graft formed from this biomimetic hydrogel scaffold is shown in Figure 2. These types of bioactive materials may allow one to capture the advantages of a natural scaffold, such as specific cell-material interactions and proteolytic remodeling in response to tissue formation, while also having the benefits of a synthetic material, namely the ease of processing and the ability to manipulate mechanical properties.

Bioreactors for Mechanical Conditioning

In vivo, the pulsatile nature of blood flow imposes radial pressure upon the vessel wall, which subjects SMCs within the medial layer to cyclic strain. Thus, a great deal of research has examined SMC behavior in response to cyclic stretch and found such stimuli important in the fabrication of vascular tissue, particularly with respect to ECM synthesis and tissue organization.

For example, SMCs seeded on purified elastin membranes and exposed to two days of cyclic stretching (10 percent beyond the resting length) have been shown to align perpendicular to the direction of applied strain and to incorporate hydroxyproline into protein three to five times more rapidly than stationary controls, indicating increased collagen synthesis in response to strain (Leung et al., 1976).

Because of the profound effects of cyclic strain on SMC orientation, ECM production, and tissue organization, preculture of vascular graft constructs in a pulsatile flow bioreactor system may help recreate the natural structure of native vessels and allow one to better achieve the mechanical properties required of the construct. A schematic of a typical pulsatile flow bioreactor system is shown in Figure 3. The mechanical stimuli from pulsatile flow could generate the cyclic strain necessary to alter ECM production, thereby creating a histologically organized, functional construct with satisfactory mechanical characteristics for implantation. To develop a blood vessel substitute, Niklason et al. cultured PGA constructs in a pulsatile blow bioreactor generating 165 beats per minute (bpm) and five percent radial strain (Niklason et al, 1999). The pulse frequency of this system was chosen to mimic a fetal heart rate, believed to possibly provide optimal conditions for new tissue formation. However, most mechanical conditioning investigations mentioned above conducted strain studies at 60 bpm, more representative of an adult heart rate, with promising outcomes. Therefore, the optimal bioreactor culture conditions for the development of a TEVG remain to be elucidated. Nevertheless, such a system shows promise for the production of a blood vessel substitute with the necessary mechanical and biochemical components.

CONCLUSION

In the past couple of decades, a great deal of progress on TEVGs has been made. Still, many challenges remain and are currently being addressed, particularly with regard to the prevention of thrombosis and the improvement of graft mechanical properties. In order to develop a patent TEVG that grossly resembles native tissue, required culture times in most studies exceed eight weeks. Even with further advances in the field, TEVGs will likely not be used in emergency situations because of the time necessary to allow for cell expansion, ECM production and organization, and attainment of desired mechanical strength. Furthermore, TEVGs will probably require the use of autologous tissue to prevent an immunogenic response, unless advances in immune acceptance render allogenic and xenogenic tissue use feasible. TEVGs have not yet been subjected to clinical trials, which will determine the efficacy of such grafts in the long term. Finally, off the shelf availability and cost will become the biggest hurdles in the development of a feasible TEVG product.

Although many obstacles still exist in the effort to develop a small diameter TEVG, the potential benefits of such an achievement are exciting. In the near future, a non-thrombogenic TEVG with sufficient mechanical strength may be developed for clinical trials. Such a graft will have the minimum characteristics of biological tissue necessary to remain patent over a time period comparable to current vein graft therapies. As science and technology advance, TEVGs may evolve into complex blood vessel substitutes. TEVGs may become living grafts, capable of growing, remodeling, and responding to mechanical and biochemical stimuli in the surrounding environment. These blood vessel substitutes will closely resemble native vessels in most every way, including structure, composition, mechanical properties, and function. They will possess vasoactive properties, able to dilate and constrict in response to stimuli. Close mimicry of native

blood vessels may ultimately aid in the engineering of other tissues dependent upon vasculature to sustain function. With further understanding of the factors involved in cardiovascular development and function combined with the foundation of knowledge already in place, the development of TEVGs should one day lead to improved quality of life for those with vascular disease and other life-threatening conditions.

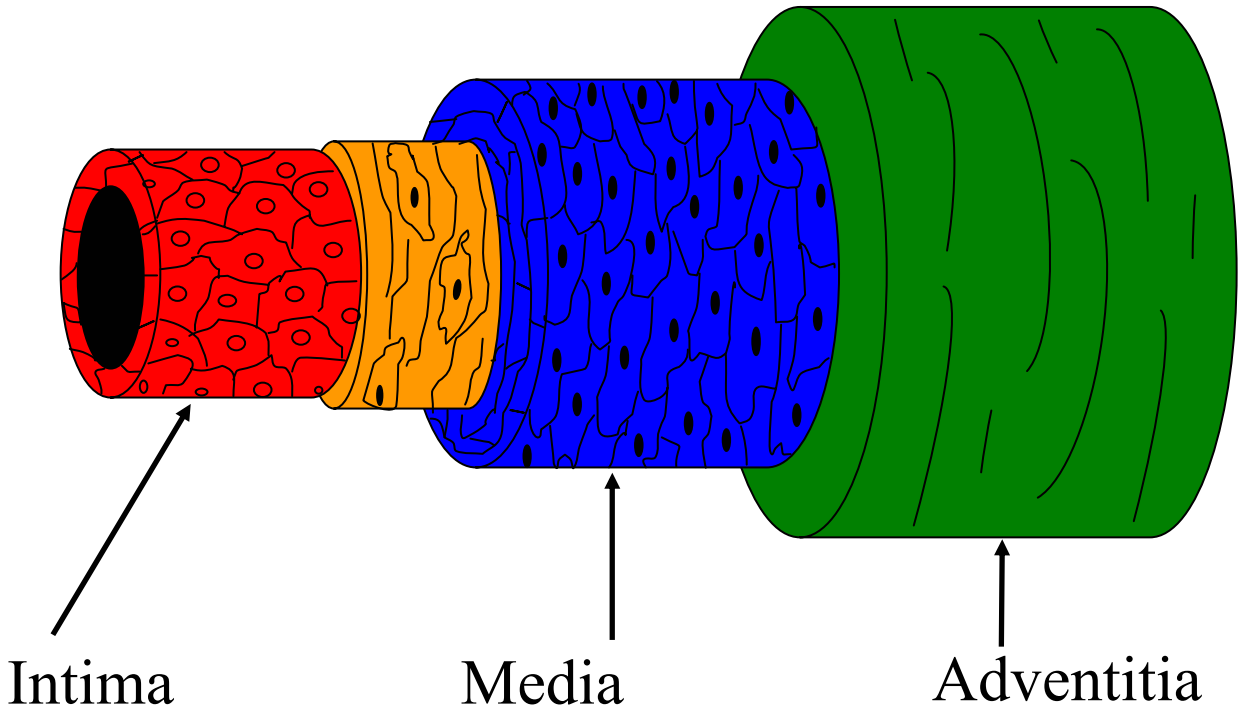

Figure 1: The arterial wall is composed of three distinct layers: the intima, media, and adventitia. The intima, composed of endothelial cells, provides a nonthrombogenic surface. In the medial layer, smooth muscle cells and elastin fibers align circumferentially and provide mechanical integrity and contractility. The outer adventitia is a supportive connective tissue.

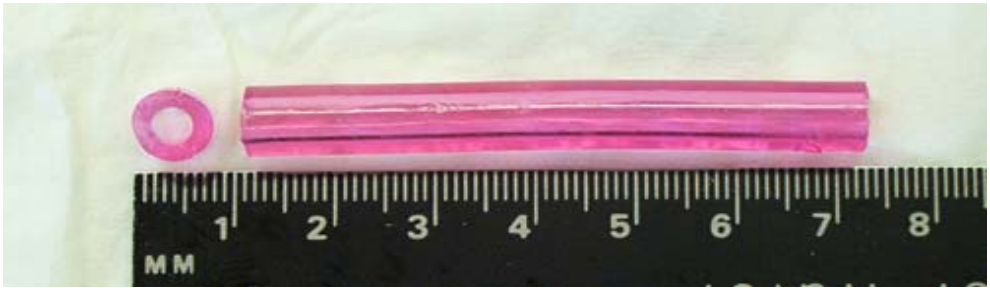

Figure 2: A PEG-based scaffold seeded with smooth muscle cells and endothelial cells ready for insertion into a bioreactor for in vitro culture of a TEVG (left, standing upright; right, laying on side). The cell-seeded scaffold is formed via photopolymerization, so the dimensions are easily tailored for a given application and cells are homogeneously seeded throughout the material. The scaffold is designed to degrade in response to cellular proteolytic activity during tissue formation.

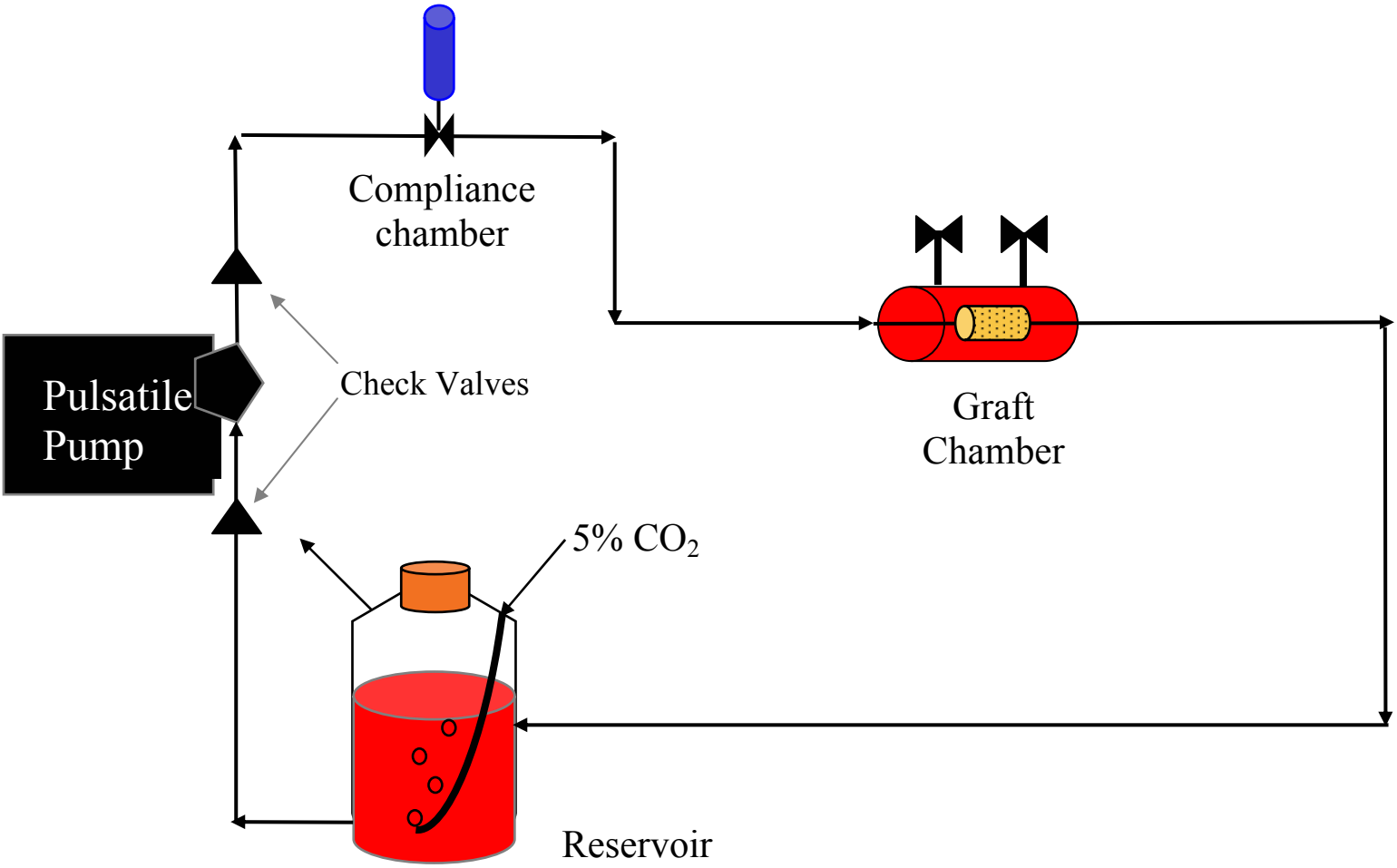

Figure 3: Diagram of a typical pulsatile flow bioreactor for culture of tissue engineered vascular grafts. The pulse frequency and amplitude can be controlled via the pump, and alteration of the mechanical properties of the scaffold material can control the resultant strain environment.

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