CHAPTER 7

Mechanical Stimulation and Biomimetic Scaffolds for Tissue Engineered Vascular Grafts

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Summary

Despite the early promise of tissue engineering, researchers have faced significant challenges in regenerating tissues with normal ultrastructure and function. In the present chapter, we will review the current state of research in manipulation of cellular mechanotransduction and the design of biomimetic tissue engineered matrices in the context of small diameter vascular graft tissue engineering. A variety of synthetic materials have been evaluated for use as vascular prostheses when suitable autologous tissue is unavailable. The two major synthetic graft materials are polytetrafluoroethylene or polyethylene terephthalate. However, their use is limited to high-flow/low resistance conditions, i.e., to > 6 mm ID vessels, because of their relatively poor elasticity and low compliance and their tendency to stimulate thrombosis and neointima formation. Tissue engineering represents a potential means to construct grafts in situations where autologous tissue is unavailable and current synthetic materials fail. While initial results with many of the tissue engineered vascular grafts (TEVGs) constructed to date are very encouraging, risk of thrombosis, hyperplasia, and mechanical failure have limited the general success of these grafts. The disparity in mechanical properties between TEVGs and native vessels is largely due to differences in the amount, composition, and microarchitecture of the extracellular matrix (ECM) produced by associated cells. Research into biomimetic scaffolds and mechanical preconditioning is aimed at improving ECM synthesis and organization in TEVGs.

KEYWORDS: Mechanical conditioning, Biomimetic scaffolds, Vascular tissue engineering.

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INTRODUCTION

Cardiovascular disease is the leading cause of death in the United States and claims more lives each year than the next five leading causes of death combined.[1] In 2000, coronary artery disease alone resulted in more than 20% of deaths in the United States and required approximately 500,000 coronary artery bypass graft surgeries. Similar trends in vascular disease and the need for bypass grafts are increasingly observed in industrialized nations worldwide.

At present, autologous saphenous veins or mammary arteries are preferred graft materials.[2] Unfortunately, approximately 10-20% of patients requiring coronary artery bypass grafts do not have suitable vessel material for grafting,[3, 4] either due to prior procedures or poor peripheral vessel health. As a result, alternative conduits constructed from synthetic materials, including a range of porous, woven, and knitted fabrics, have been investigated since the 1950's.[5] Of the examined materials, polytetrafluoroethylene or polyethylene terephthalate have seen the most widespread use due to clinical success in large diameter (ID > 6mm) graft applications. However, these synthetic grafts fail in small diameter applications (ID < 6 mm) due to resulting thrombosis and scar tissue formation.[6-10] Thus, a clinical need exists for alternative vascular prostheses. Tissue engineering represents a potential avenue to construct vascular grafts when autologous tissue is unavailable and conventional synthetic materials fail.[11]

Tissue engineering is generally defined as a tool that uses living cells to form or regenerate tissues, frequently using a scaffold to support, guide, and stimulate cells.[11] Most tissue engineered vascular graft (TEVG) strategies have focused on developing prostheses that mimic the structure, function, and physiologic environment of native vessels.[12-15] Normal arteries possess three distinct tissue layers: 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 (SMC) behavior, and homeostasis. The medial layer is composed of SMCs and circumferentially aligned elastic fibers and is considered to be primarily responsible for arterial cyclic distension under physiological loading conditions.[16] The adventitia is comprised of fibroblasts, connective tissue, microvasculature, and a neural network that regulates vasotone.

In developing a functional TEVG, regeneration of all of the vessel layers may prove to be necessary. However, at a minimum, an intimal and medial layer will likely be required to achieve long-term TEVG patency and mechanical integrity. Based on previous research, generating

functional TEVGs is expected to involve the following steps: 1) harvest of the desired cells, 2) potential genetic modification of isolated cells followed by *in vitro* expansion, 3) scaffold selection followed by cell seeding, 4) *in vitro* culture of the cell-containing scaffold (construct) under conditions designed to induce tissue formation, and 5) construct implantation in the patient. Each of these steps appears to critically impact the resultant graft, and a number of options exist at each phase of this process.

While initial TEVG results are very encouraging, a number of technical hurdles remain before TEVGs can be considered a viable vascular replacement option.[9, 10] The potential for thrombosis due to issues with retention of endothelial cells following implantation or to inappropriate endothelial cell function must be addressed.[10] In addition, the potential for burst or aneurysmal failure is a significant concern, since the mechanical strength of TEVGs is generally less than that of the arteries they replace and may not be maintained as the scaffold degrades. Thus a number of approaches are currently being investigated to address these issues. All of the strategies discussed above—cell source, genetic modification, scaffold materials, and culture conditions—will likely play a role in the fabrication of a clinically-relevant TEVG. The present work focuses on the impact of the selected scaffold and of construct culture conditions on resulting TEVG outcome.

SCAFFOLDS FOR VASCULAR TISSUE ENGINEERING

TEVG scaffolding is intended to provide initial mechanical support and integrity and to direct cell behavior as neotissue is produced. TEVG efforts have explored a variety of scaffolds, including synthetic materials such as poly(lactic-co-glycolic acid)[12] and polyglycolic acid (PGA)[17, 18] and natural materials such as collagen[17, 19, 20] and fibrin[21, 22]. Natural scaffolds materials are often comprised of typical extracellular matrix (ECM) components and thus provide many of the biochemical signals necessary for the cell. However, the difficulties that are often involved in natural scaffold processing, the potential for disease transmission, and the often poor mechanical properties of natural scaffolds have led some groups to concentrate on the development of synthetic biomaterials as TEVG scaffolds. Although degradable synthetic polymers usually lack desired cell stimuli, they generally offer greater control over scaffold structural and mechanical properties as well as degradation rate than natural materials.[23]

An alternative path is the development of biomimetic synthetic scaffolds which combine the specific cell-material interactions provided by natural materials with the control over material properties and ease of processing offered by synthetic polymers. As such, biomimetic derivatives of synthetic macromer polyethylene glycol (PEG) are currently being studied as vascular tissue engineering scaffolds.[14] Aqueous solutions of acrylate-derivatized PEG can be rapidly polymerized into complex geometries in direct contact with cells and tissues[24, 25] (Figure 1). Similar to many other synthetic materials, the mechanical properties of hydrogels can be tuned over a broad range by manipulation of PEG molecular weight and concentration (Table 1). PEG-based materials are also intrinsically resistant to protein adsorption and cell adhesion, in contrast to most other synthetic materials, which adsorb a range of bioactive proteins from serum. Thus, unmodified PEG hydrogels present a "blank slate" [26-28], essentially devoid of biological interactions, to cells.

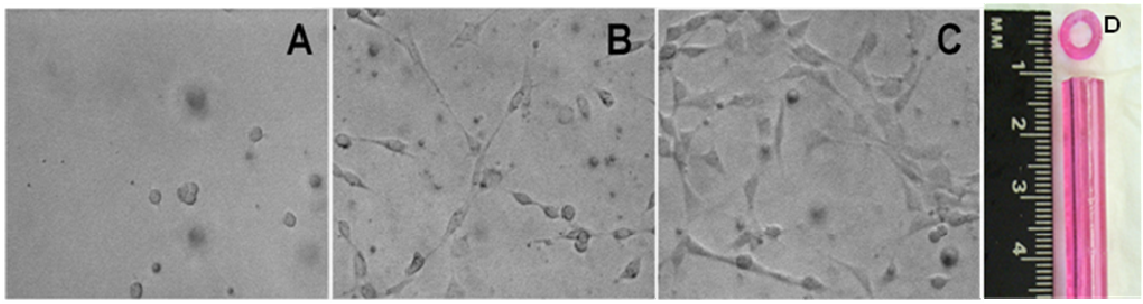

Fig. 1. Demonstration of the ability to modulate PEG hydrogel bioactivity and to create geometrically complex PEG hydrogel scaffolds. PEG hydrogels with **(A)** 0, **(B)** 0.5, and **(C)** 1 μmol/mL cell adhesive RGDS peptide. **(A)** Cells do not spread onto a pure PEG hydrogel; it is, thus, resistant to serum protein adsorption, presenting a "biological blank slate" to cells in absence of modification. **(B)** and **(C)**: As the amount of acrylate-derivatized cell adhesive peptide RGDS tethered to the PEG network increases, cell adhesion and spreading increases. Thus, the biochemical landscape of PEG hydrogels can be tuned by controlling the identities and concentrations of added biochemical moieties. **(D)** PEG hydrogels can be readily prepared as seamless tubular grafts by pouring the PEG precursor solution into a cylindrical mold and polymerizing.

Table 1. The dependence of scaffold mechanical properties and experienced strain (at 120/80 mm Hg pulsatile pressures) on PEG hydrogel composition. Adapted from Hahn et al, 2006.[14]

Formulation	Modulus (kPa)	UTS (kPa)	Strain (%)
100 mg/mL 3.4 kDa	92.1 ± 2.7	67.0 ± 6.7	6.2 ± 0.4
100 mg/mL 6 kDa	81.2 ± 1.2	69.8 ± 8.2	6.4 ± 0.5
200 mg/mL 6 kDa	140.4 ± 5.3	101.7 ± 12	2.9 ± 0.4
100 mg/mL 10 kDa	48.4 ± 1.7	66.2 ± 11	10.9 ± 1.3
200 mg/mL 10 kDa	76.3 ± 2.0	69.8 ± 4.3	3.6 ± 0.4

Although the inability of cells to interact with pure PEG hydrogels may at first appear undesirable for tissue engineering applications, this property allows for the controlled introduction of bioactivity.[29, 30] For example, the acrylate-derivatized cell adhesive peptide RGDS is often covalently bonded to PEG hydrogels to introduce defined levels of cell-material interactions in tissue engineering applications[14, 31] (Figure 1). In addition, PEG-based materials have been rendered bioactive by inclusion of proteolytically degradable peptides into the polymer backbone[32] and by grafting cell-specific adhesion peptides[33] or growth factors[34] into the hydrogel network during the photopolymerization process. PEG hydrogels that mimic many of the properties of collagen have been recently developed.[35, 36] The ability to spatially and systematically tune and control PEG hydrogel biochemical and biomechanical properties over a broad range is expected to permit exploration of scaffold property impact on resulting TEVG outcome toward identification of optimal scaffold properties.

The photoactivity of acrylate-derivatized PEG macromers allows the principles of photopatterning to be applied to PEG hydrogels. Thus, the incorporation of bioactivity into a PEG hydrogel network can be tightly controlled in both 2D and 3D via simple mask-based or laser-scanning photopatterning (Figure 2).[29, 30, 37] Also, desirable for TEVG applications, PEG based hydrogels permit the ready formation of multi-layered scaffolds by successive polymerizations of PEG macromer solutions containing desired cell interaction moieties and/or cell types (Figure 2). The ability to tailor the microscale biochemical and biomechanical properties of 3D scaffolds is anticipated to be important to the regeneration of complex, multi-layered tissues such as arteries.

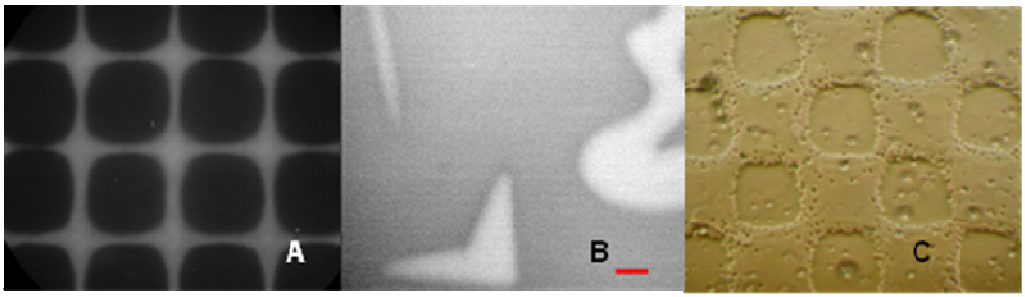

Fig. 2. Demonstration of the ability to spatially control the microscale biochemical landscape of PEG hydrogels and to create multi-layered gels. **(A, B)** Grayscale fluorescent images of PEG hydrogels patterned with fluorescent acrylate-derivatized cell adhesion peptide RGDS using conventional photolithographic and laser scanning patterning techniques, respectively. **(C)** A multi-layered PEG hydrogel in which a second 3D layer was formed in rectangular patches using a photomask. In each hydrogel layer, different biochemical ligands and cells can be entrapped.

IN VITRO CULTURE CONDITIONS

Following cell seeding within the selected scaffold, an *in vitro* culture period is usually needed to allow for neotissue formation and development of appropriate mechanical and functional characteristics. During the culture period, the construct should receive necessary chemical and/or mechanical signals for cells to synthesize proteins and to remodel their environment such that the construct develops into a functional graft with mechanical properties similar to native vessels. Thus, the use of culture media supplements and/or physiological mechanical conditioning has been explored to improve TEVG outcome.

Media Additives

A range of media additives have been investigated for their impact on TEVG outcome.[12, 18, 21] SMCs grown in cultures supplemented with ascorbate synthesized three times as much collagen as SMCs incubated without ascorbate.[38, 39] Unfortunately, ascorbate also decreased elastin production by up to 25% over four weeks in culture.[38] TGF-β has been reported to stimulate expression of several matrix components, including elastin, collagen, fibronectin, and proteoglycans.[40-42] Combined, TGF-β1 and ascorbate have been shown not only to increase net SMC collagen deposition and fibril thickness but also to increase elastin production[21], improving graft mechanical properties. Other media additives including PDGF and copper sulfate have been explored to enhance TEVG ECM synthesis and crosslinking.[12, 43]

Bioreactors for Mechanical Conditioning

In vivo, the pulsatile nature of blood flow subjects SMCs within the medial layer to cyclic stretch and transmural shear. Mechanical stretching of TEVGs *in vitro* has been shown to have profound effects on cell phenotype,[44, 45] orientation,[45] and ECM deposition.[46-47] Thus, investigators have begun to exploit the ability of SMCs to sense and respond to mechanical stimuli to improve the mechanical strength of the resulting construct.

To develop a blood vessel substitute, Niklason et al.[12] cultured PGA constructs over thin walled silicone sleeves in a pulsatile bioreactor generating 165 beats per minute (bpm) and 5% radial strain. The pulse frequency of this system was chosen to mimic a fetal heart rate, believed to possibly provide optimal conditions for new tissue formation. More recently, tubular collagen

constructs seeded with SMCs were cultured over thin-walled silicone sleeves and exposed to regulated intraluminal pressures for eight days. The 10% cyclic (60 bpm) distension induced by the applied pressure caused SMCs and collagen fibers to align circumferentially, resulting in enhancement of the scaffold mechanical properties.[48] This model system was also used to investigate the increased capacity for encapsulated SMCs to remodel their environment following mechanical stimulation.[49]

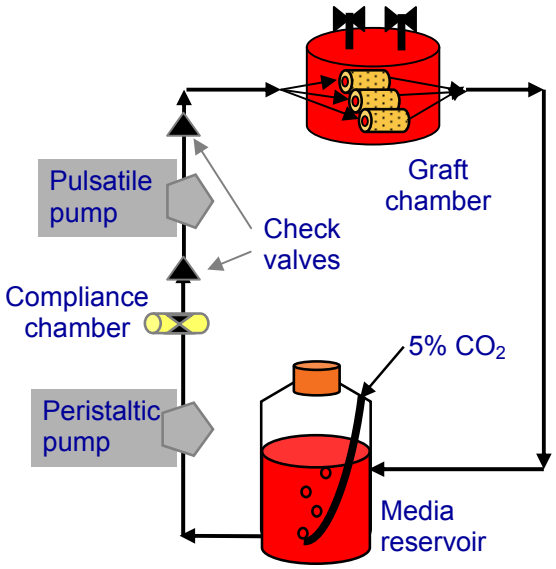

Fig. 3. Pulsatile flow bioreactor schematic. A peristaltic pump draws media from a reservoir and creates the desired flow rate. The compliance chamber removes pulsation induced by the peristaltic pump from the flow stream, permitting the desired pulsatile waveform to be imposed by the pulsatile pump. This system has been designed so that media never contacts pump head components, significantly reducing the potential for contamination. The resulting flow stream is channeled through constructs which, in contrast to most bioreactors, are not insulated from the shear flow by a silicone sleeve.

These analyses indicate that cyclic strain may be critical for improved TEVG outcome. However, further studies are needed to identify optimal bioreactor culture conditions for TEVGs. Towards this end, a novel pulsatile flow bioreactor was recently designed to allow for examination of the separate and combined effects of shear and pulsatile stimuli (both fetal and adult) on TEVG outcome (Figure 3).[14] When this custom reactor is combined with PEG-based hydrogel scaffolds, a highly versatile platform is created for the systematic exploration of the impact of scaffold properties and applied mechanical stimuli on TEVG outcome.[14] For example, the impact of hydrogel modulus and crosslinking density on TEVG outcome can be studied

independently of experienced strain, pulsatile waveform, and shear by appropriately selecting the composition of the hydrogel precursor solution (Table 1). Studies using this flexible bioreactor system should greatly enhance our ability to identify optimal scaffold and culture conditions for TEVGs.

CONCLUSION

The past twenty years have seen significant progress toward the development of clinically-useful TEVGs. Still, many challenges remain and are currently being addressed, particularly with regard to the prevention of thrombosis and the improvement of graft mechanical properties. A number of variables can be manipulated to improve TEVG outcome, including cell source, cell gene expression, scaffold properties, and construct culture conditions. Systematic investigation of the effects of specific scaffold properties and applied mechanical stimuli on TEVG outcome should permit the optimization of TEVG preparation.

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