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# Peritoneal pre-conditioning reduces macrophage marker expression in collagen-containing engineered vascular grafts

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ABSTRACT

Engineered vascular grafts have shown promise as arteriovenous shunts, but they have not yet progressed to clinical trials for coronary arteries <4 mm in diameter such as the coronary arteries. Control over initial biomaterial properties and remodeling are necessary to generate viable grafts. In this study, we blended collagen with a synthetic material, poly(ε-caprolactone), to modulate the post-grafting inflammatory response while avoiding aneurysmal-like dilation and failure that can occur with pure collagen grafts. We also used pre-implantation in an "*in vivo* bioreactor" to recruit autologous cells and improve patency after grafting. Electrospun conduits were pre-implanted within rat peritoneal cavities and then grafted autologously into abdominal aortae. Conduit collagen percentages and pre-implantation were tested for their impact on graft remodeling and patency. Burst pressures >2000 mmHg, reproducible expansion with systole/diastole, and maintenance of mechanical integrity were observed. More importantly, peritoneal pre-implantation reduced overall lipid oxidation, intimal layer thickness, and expression of an M1 macrophage marker. The condition with the most collagen, 25%, exhibited the lowest expression of macrophage markers but also resulted in a thicker intimal layer. Overall, the 10% collagen/PCL with peritoneal pre-implantation condition appeared to exhibit the best combination of responses, and may result in improved clinical graft viability.

**Statement of Significance**

This manuscript describes a rodent study to systematically determine the benefits of both pre-implantation in the peritoneal cavity and specific ratios of collagen on engineered vascular graft viability. We show that pre-implantation had a significant benefit, including decreasing the expression of macrophage markers and reducing lipid oxidation after grafting. This study is the first time that the benefits of peritoneal pre-implantation have been compared to an "off the shelf," directly grafted control condition. We also demonstrated the importance of specific collagen ratio on the response after grafting. Overall, we feel that this article will be of interest to the field and it has the potential to address a significant clinical need: a graft for coronary arteries <4 mm in diameter.



## 1. Introduction

Cardiovascular disease in small-diameter arteries is one of the main causes of death worldwide. Although autologous vessels (e.g. saphenous veins) are the gold standard for bypass grafting, they are not available in more than 30% of the patients due to previous harvest or systemic vascular disease [1,2]. Tissue engineered vascular grafts (TEVGs) are a promising option [3–5]. However, despite clinical trials using TEVGs as arterio-venous shunts, there are currently no TEVGs that have progressed to clinical trials for high pressure arteries with diameters <4 mm, such as the coronary and peripheral arteries [6]. In addition, heparin-modified expanded poly(tetrfluoroethylene)(ePTFE) grafts, such as the Gore Propaten® Vascular Graft, are not manufactured at <4 mm in diameter. Graft stenosis and aneurysmal-like dilation are among the most important challenges for these applications, and these challenges are closely tied to the scaffold properties. It is known that scaffolds can alter cell-signaling pathways, the inflammatory response, and graft viability through integrin-mediated mechanotransductive

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signaling [7,8], and even through degradation products of both natural and synthetic macromolecules [9,10].

Using natural materials for the scaffold has the benefit of enabling cell binding through integrin-binding domains and growth-factor binding through growth factor specific binding-sequences [11]. While quick enzymatic degradation and remodeling of these materials can help with integration [12,13], it can also lead to loss of structural integrity and aneurysmal-like dilation [6]. On the other hand, synthetic materials are known for having a more controlled, and often slow, degradation rate, but they can lack the ability to integrate with the host tissue. Blending natural and synthetic materials has been performed for TEVGs and can ideally provide the advantages of each component at appropriate ratios [14,15].

Most previous strategies have either suggested that there is a linear response with adding more collagen to engineered scaffolds or have not considered a range of compositions [16]. For example, if a scaffold consists of a higher ratio of collagen, then a greater cell exposure to collagen might be expected. Exposure of cells to collagen and its degradation products can modulate macrophage phenotype and promote a transition to an M2 phenotype by secretion of cytokines such as interleukin 10 (IL-10) after *in vivo* implantation [13,17,18]. It can also impact smooth muscle cell (SMC) phenotype by activation of integrin receptors, likely resulting in upregulation of the extracellular signal-regulated kinase (ERK) signaling pathway [19–21]. A few studies unrelated to vascular grafts have demonstrated that fibers blended with collagen are not homogenously distributed and mainly present collagen at the surface [22], which could suggest that further increasing collagen ratio in TEVGs might not have a significant impact on the cellular response. However, in our previous publication using electrospun blends of the synthetic material poly (ε-caprolactone) (PCL) and collagen, we showed that a 10% collagen condition promoted a higher expression of contractile markers (*Acta2* and *Cnn1*) than both lower and higher collagen compositions [23]. In addition, the mechanical properties of the scaffold remained unchanged after implantation unlike the lower and higher collagen compositions, suggesting that there is also a difference in the graft remodeling. Overall, these surprising results demonstrated that the impact of different ratios of collagen blended scaffolds require further investigation, and may be important for graft consistency and viability. In addition, it is important to study how the ratio will affect the outcome when these conduits are grafted into the artery with the important differences in the microenvironment that the cells are exposed to.

In our previous study, we implanted vascular conduits into the rat peritoneal cavity to use it as a model system to investigate the impact of composition on the inflammatory response but also as part of an "*in vivo* bioreactor" strategy to generate vascular grafts. This peritoneal TEVG strategy has been used to provide cells with components of a physiologic microenvironment as an alternative to a traditional *in vitro* bioreactor [24–28]. One advantage is that the autologous cells recruited directly to a scaffold implanted within the peritoneal cavity can circumvent the need to expand primary cells with *in vitro* culture, avoiding associated phenotypic changes. These previous studies demonstrated promise in animal models and included detailed expression profiling to characterize the recruited cells, but they did not provide the beneficial properties from blended natural and synthetic grafts [24–27]. In addition, no direct comparison had previously been performed with off the shelf direct implants to determine what specific benefits peritoneal pre-implantation can provide to address the concern of graft stenosis.

In this study, electrospun conduits of different compositions – 0, 10, and 25% (w/w) collagen/PCL – were implanted in the rat peritoneal cavity as an "*in vivo* bioreactor" to recruit autologous cells. Conduits were subsequently grafted into the abdominal aorta of the same rat to investigate the impact of composition and peritoneal cavity pre-implantation on graft remodeling after being grafted into the arterial microenvironment. After grafting, changes in luminal diameter and percent expansion were monitored over time using ultrasound imaging. Grafts were removed and analyzed for thickness of intimal hyperplasia, accumulation of oxidized lipid species, recruited cells phenotype, and extracellular matrix (ECM) composition after 6 weeks of grafting. Lipid oxidation was assessed because reactive oxygen species (ROS) generated during inflammation can oxidize lipids and accumulation of oxidized lipids can prevent vascular regeneration by inhibiting endothelial cell migration [29]. Our results demonstrate benefits of peritoneal pre-implantation and the 10% collagen condition. They also demonstrate important considerations with some of the markers (e.g., von Willebrand Factor) commonly used to characterize TEVG viability.

## 2. Materials and methods

### 2.1. Materials

All lab supplies were purchased from Fisher Scientific (Pittsburgh, PA) unless specified otherwise. High-molecular weight poly(ε-caprolactone) with an inherent viscosity of 1.2 dL/g in chloroform was purchased from Lactel Absorbable Polymers (Pelham, Al). Lyophilized collagen type 1 from calf skin was purchased from Elastin Products Company (Owensville, MO). All antibodies were from Abcam (Cambridge, MA) except CD80 was purchased from Biolegend (San Diego, California). All primers were designed and purchased from Life Technologies (Grand Island, NY) with the exception of primers for *Rpl13a* and *Gapdh*, which were from Real Time Primers (Elkins Park, PA).

### 2.2. Conduit production and characterization

#### 2.2.1. Electrospinning

Zero percent, 10%, and 25% blends of collagen/PCL (w/w) were electrospun onto a 1.6 mm outer diameter stainless steel mandrel to produce small diameter conduits with controlled fiber diameters, as described previously [23]. Electrospinning was performed with a 22 gauge needle using a 15 kV potential, a throw distance of 10 cm, and a syringe flow rate of 0.8 mL/h. Fiber diameter was kept consistent between conditions by varying the solution concentration in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) from 12 to 14 wt% for the 100% PCL and the collagen/PCL blend conditions. In previous work, similar changes in solution concentrations did not lead to changes in pore area – as measured from 2-D SEM images – as long as the fiber diameters were kept similar [23]. The consistent thickness of the conduits and random orientation of the fibers throughout the drum circumference was achieved using controllable lateral movement and slow rotation (<100 rpm) [30]. Ethanol was used after electrospinning to remove the constructs from the rod. Conduits were cut into 1 cm long segments, and stored in a desiccator until use. Ends of the conduits were closed using solvent-bonding prior to implantation to prevent cell and tissue deposition inside the lumen.

#### 2.2.2. Conduit characterization

Average fiber diameter and average pore area were determined from scanning electron microscopy (SEM) images using a JEOL 6380-LV (Peabody, MA), as described previously [23]. Images were analyzed using ImagePro Plus® software (Media Cybernetics, Bethesda, MD). For analysis of sample surfaces after peritoneal pre-implantation, the conduits were dried using critical point dry-

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