Scaffold Mesh Size and Mechanical Properties and Their Effects on TEVG Outcome

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Blood vessel replacements are frequently necessary in the treatment of advanced atherosclerosis, aneurysmal and peripheral vascular disease, and vascular trauma. In the US alone, roughly 1.4 million patients undergo operations requiring arterial prostheses each year(1). Autologous saphenous veins and mammary arteries are currently the preferred graft materials. However, the availability of tissue of appropriate dimensions is limited, with fewer than 10% of patients having suitable autologous tissue(2), and donor site morbidity is a significant complication in these procedures. When autologous tissue is unavailable, synthetic materials (mainly Dacron and polytetrafluoroethylene) are frequently used for the treatment of peripheral vascular disease, but their use is limited to high-flow/low resistance conditions(3, 4), i.e., to > 6 mm ID vessels, because of their thrombogenicity, relatively poor elasticity, and low compliance(5). Tissue engineering represents a potential means to construct functional grafts that could be used in vascular replacement procedures where autologous tissue is unavailable and synthetic materials fail(1).

Since the medial layer is the primary load bearing layer of the arterial wall, much of the previous research in tissue engineered vascular grafts (TEVGs) has focused on developing a bioartificial medial layer(6, 7). While initial results with many of the medial TEVGs constructed to date are very encouraging, a number of technical hurdles remain before TEVGs can be considered a viable vascular replacement option(8). The potential for aneurysmal failure is a significant concern, since the mechanical integrity of TEVGs is generally less than that of the arteries they replace and since the mechanical integrity of the engineered grafts may not be maintained with time. In addition, many TEVG studies have been plagued in the *in vivo* setting by thrombosis and intimal hyperplasia(8). To address the potential for aneurysmal failure, researchers have attempted to create TEVGs with mechanical properties that approach those of native tissue via the use of media additives(9-11) and mechanical stimulation(12). However, the composition and properties of the scaffold material and the resulting effects on TEVG outcome have not been extensively examined.

In the present study, we systematically explore the impact of matrix mesh size and mechanical properties on TEVG outcome using polyethylene glycol (PEG)-based hydrogels as a model scaffold material. PEG has several properties which make it a desirable vessel replacement material. In addition to its biocompatibility, it has been demonstrated to be non-thrombogenic(13), significantly reducing the potential for hyperplasia and thrombosis. Diacrylate-derivatized PEG (PEGDA) macromers readily dissolve in aqueous solution, forming an optically transparent, low viscosity mixture that is photopolymerizable in the presence of cells(13, 14). Thus, seamless, mechanically isotropic(15) cylindrical constructs with homogenously seeded cells(16-18) can be readily formed by pouring a solution of photoactive PEG macromers, cells, and photoinitiator into an appropriately shaped mold and applying light. The photoactivity of PEGDA combined with its intrinsic resistance to cell and protein adhesion results in a biological “blank slate” which can be modified in a controlled manner to contain bioactive moieties(19). In addition, PEG hydrogels are highly elastic, which is important in the vasculature where tissues must maintain their form in response to prolonged mechanical stress, and their mechanical properties and mesh size can be tuned over a wide range by varying the PEG composition of the scaffold.

In this work, pig aortic smooth muscle cells (SMCs) were encapsulated at $0.5x10^6$ cells/mL in the following precursor solutions, each containing 2 μmol/mL acryloyl-PEG-RGDS:

(1) 10 (w/v) % 10000 Da PEGDA, (2) 20 (w/v) % 10000 Da, (3) 30 (w/v) % 20000 Da and cultured in the DMEM supplemented with 10% FBS, 100 mg/L, 100 mU/mL penicillin, and 100 mg/L streptomycin composites in a humidified incubator maintained at 5% CO_2 / 37 ºC. After 8 weeks of culture, the constructs were examined biochemically, biomechanically, and histologically. Correlation of the biochemical results with the mechanical properties and mesh size of the matrices should allow for the improved selection of materials for TEVG applications.

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