Bioreactor Protocols and Scaffolds for Engineering Muscularized Blood Vessels

[1,2,3]Masood A. Machingal, [2]Heather P.M. Merritt, [2,3] Brent G. Witten, [2]Andrew B. Christian, [2,3]Luke P. Neff, [2,3]Matthew Stern, [1,2,3]Mark Van Dyke, [2,3]Randolph Geary and [1,2,3]George J. Christ

[1] Virginia Tech - Wake Forest University School of Biomedical Engineering and Sciences, Winston Salem, NC;

[2] Wake Forest Institute for Regenerative Medicine, [3] Wake Forest University School of Medicine, Winston Salem, NC

Introduction

Implantation of large engineered skeletal muscle for volumetric muscle loss will require an upstream and downstream (from site of implantation) vasculature. Limitations of synthetic scaffolds and potential unavailability and morbidity associated with use of suitable autologous vessels for this purpose has led up to pursue the exploration of tissue engineering methods to develop arterial substitutes. We are pursuing the development of tissue engineered blood vessels (TBEV) using decellularized scaffolds seeded with Smooth muscle cells (SMC) cultured from sheep arterial explants. We have recently shown that bioreactor preconditioning of decellularized arterial scaffolds seeded with SMC accelerates the formation of a significant muscle layer that is capable of calcium mobilization in response to cellular depolarization (Yazdani et al., 2008). However limited migration of SMC into the medial layer of the arterial scaffolds still remains a major problem in this approach. In this study we explore the possibility of seeding using three alternative porous scaffolds (decellularized venous scaffolds (DVS), PCL -Collagen sheet roll up (PCL-C) and porcine acellular muscle matrix scaffolds (PAMM)) with SMC and determine the role of bioreactor preconditioning in attachment, proliferation and migration of SMC in these scaffolds.

In this regards, autologus saphanous veins have been routinely used in surgical procedures as a graft material and recent evidence (Schaner et al., 2004, Martin et al., 2005) has suggested that decellularized venous scaffolds (DVS) are mechanically suitable as a scaffolding material for blood vessel tissue engineering. However, seeding of decellularized vein scaffolds with SMC has not been reported in the literature, which forms the basis of this preliminary investigation of SMC seeding on DVS for developing muscularized blood vessels.

Our lab has previously demonstrated that electrospun PCL-Collagen scaffolds can be developed for vascular applications (Lee et al., 2007; Lee et al., 2008) that are suitable for vascular reconstruction in vivo (Tillman et al., 2009). However these grafts do have limitations of SMC migration into the scaffold wall and hence may take longer time for graft remodeling in vivo. In this study, we evaluate the possibility of seeding SMC on a thin PCL -Collagen sheet (PCL-C) and roll it up to make a tubular construct. In addition, our lab has also recently developed PAMM scaffolds from decellularized extracellular matrix of skeletal muscles (Stern et al., 2009), which due to it's porous nature promises to be a suitable scaffold for vessel tissue engineering applications.

We hypothesize that SMC seeded on porous DVS, PAMM and PCL-C scaffolds may have better migration and distribution of SMC within the scaffold as compared to that of an arterial scaffold. We also hypothesize that cyclic strain in a bioreactor will improve cellular in growth of SMC into the medial layer of the scaffold. The long term goal is to develop TEBV using scaffolds that have a uniform SMC population in the scaffold wall, with physiological characteristics similar to that of native vessels.

Methods

SMC were obtained by primary culture of sheep femoral artery explants and grown (upto passage 8) in DMEM containing 10% fetal bovine serum and 1% Antibiotics and Antimycotics. SMC were characterized with specific SMC markers

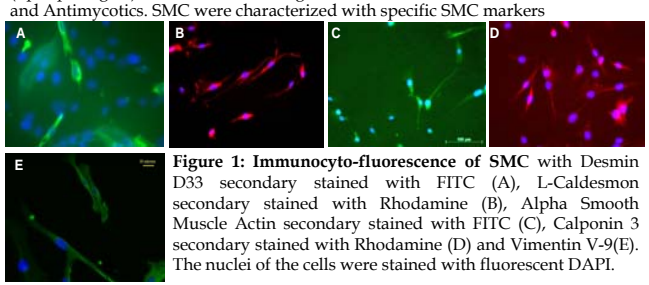
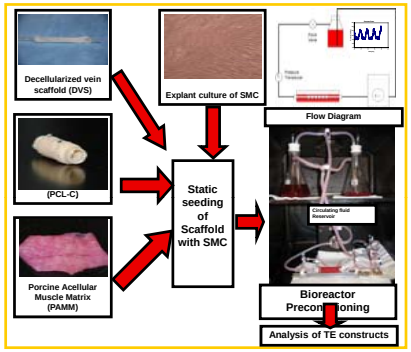


Figure 1: Immunocyto-fluorescence of SMC with Desmin D33 secondary stained with FITC (A), L-Caldesmon secondary stained with Rhodamine (B), Alpha Smooth Muscle Actin secondary stained with FITC (C), Calponin 3 secondary stained with Rhodamine (D) and Vimentin V-9(E). The nuclei of the cells were stained with fluorescent DAPI.

Methods (continued)

Preparation of Scaffolds : Porcine saphenous veins were decellularized by cleaning the veins of excess tissues and washing them with a decellularization solution (10 ml Triton X100, 3.4 ml ammonium hydroxide and 490 DI water) for three days, followed by a three day rinse with water. After freeze-drying and sterilization with ethylene oxide, the scaffolds were seeded with explant derived SMC and placed in a bioreactor for preconditioning for two weeks. PAMM scaffolds were prepared as described (Stern et al., 2009) and PCL-Collagen scaffolds prepared as described (Choi et al., 2008).

Static Seeding and Bioreactor preconditioning: Sterilized scaffolds were rehydrated and kept in DMEM containing 10% FBS and 1% Antibiotics and Antimycotics overnight at 37^{0}C and then the outer surfaces of the scaffold were seeded with SMC concentrations optimized for each scaffold and kept in the incubator for 5 days. Bioreactor Preconditioning: PAMM and PCL-C rollup scaffolds were connected to the small vessel ring bioreactor system described in (Yazdani et al., 2009a) and preconditioned with pulsatile flow (60 beats per minute/80-120 mm Hg pressure) to produce a five percent cyclic strain on the scaffold. DVS scaffolds were connected to the modified bioreactor where the preconditioning situations varied with one set of samples preconditioned with a steady flow through its lumen and another set of samples receiving pulsatile flow (60 beats per minute/80-120 mm Hg pressure) to produce a five percent cyclic strain on the scaffold. Frozen sections of all samples were made and stained with H&E and DAPI staining.



Results

Bioreactor preconditioning improves SMC Attachment and migration on DVS

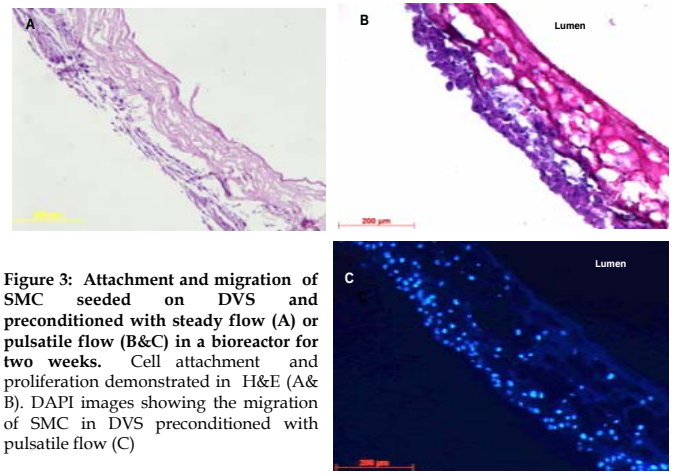


Figure 3: Attachment and migration of SMC seeded on DVS and preconditioned with steady flow (A) or pulsatile flow (B&C) in a bioreactor for two weeks. Cell attachment and proliferation demonstrated in H&E (A& B). DAPI images showing the migration of SMC in DVS preconditioned with pulsatile flow (C)

PAMM scaffolds seeded with SMC shows cellular attachment & proliferation

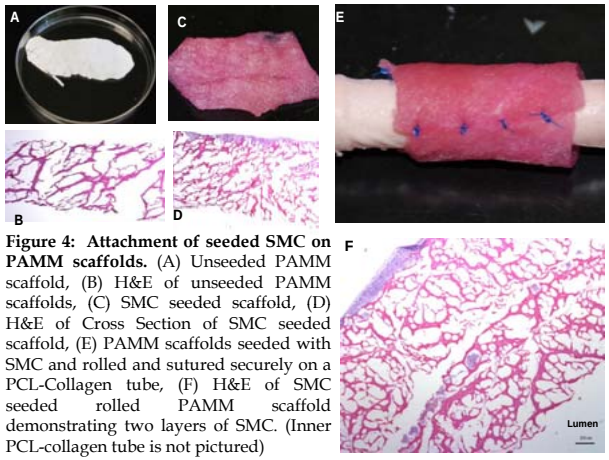


Figure 4: Attachment of seeded SMC on PAMM scaffolds. (A) Unseeded PAMM scaffold, (B) H&E of unseeded PAMM scaffolds, (C) SMC seeded scaffold, (D) H&E of Cross Section of SMC seeded scaffold, (E) PAMM scaffolds seeded with SMC and rolled and sutured securely on a PCL-Collagen tube, (F) H&E of SMC seeded rolled PAMM scaffold demonstrating two layers of SMC. (Inner PCL-collagen tube is not pictured)

SMC seeded PCL-Collagen sheets rolled up to create muscularized vessels

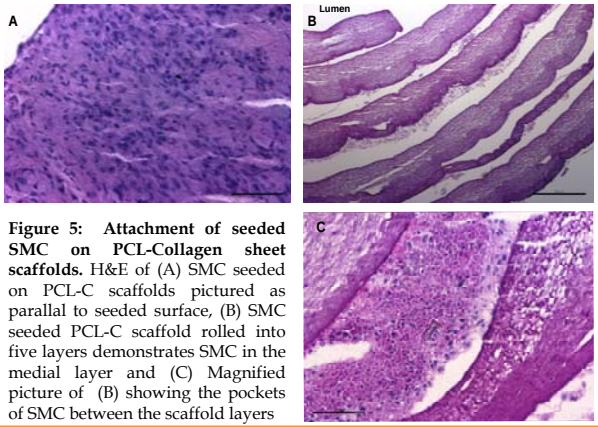


Figure 5: Attachment of seeded SMC on PCL-Collagen sheet scaffolds. H&E of (A) SMC seeded on PCL-C scaffolds pictured as parallal to seeded surface, (B) SMC seeded PCL-C scaffold rolled into five layers demonstrates SMC in the medial layer and (C) Magnified picture of (B) showing the pockets of SMC between the scaffold layers

Conclusion

In this study, we demonstrate that seeded SMC adhere and proliferate on DVS. We also demonstrate that cyclic preconditioning using pulsatile flow improves SMC migration and integration/ingrowth into the medial layer of the scaffold. In addition to this, our preliminary observations shows that SMC attach and grow on PAMM and PCL-C rollup scaffolds. While further optimization of seeding and bioreactor protocols for the use of these scaffolds for TEBV is required, these preliminary data seem encouraging. The long term goal is to develop TEBV that mimic the architecture and physiological characteristics of native arteries.

Acknowledgements : Mandy Lockard, Cathy Mathis, Jay Barrett (WFIRM)

VIRGINIA TECH WAKE FOREST UNIVERSITY
School of Biomedical Engineering and Sciences

Wake Forest Institute *for* REGENERATIVE MEDICINE