Mesenchymal Stem Cells Enhanced Endothelial Regeneration and Reduced Fibrosis on Bioengineered Vascular Graft

Winston Elliott[a], Krishna Madhavan[a], Eric Monnet [b], Wei Tan[a,c]

[a]Department of Mechanical Engineering, University of Colorado at Boulder. [b]Department of Clinical Sciences, Colorado State University. [c]Departments of Pediatrics and Bioengineering, University of Colorado at Denver.

Statement of Purpose: Autologous vessels are the gold standard for small-diameter (<6 mm) vascular bypass; however, many patients lack suitable autologous tissues due to diseases or prior vein harvest. As an alternative, synthetic vascular grafts made from bioinert synthetic materials are often in use. The high long-term failure rate of these materials in the replacement of small vessels is known to be associated with the lack of physiological signals to vascular cells causing adverse intima hyperplasia, inflammation and fibrosis. Aimed at constructing engineered tissue to guide vascular regeneration *in vivo*, we have developed bilayered scaffolds that provide not only proper mechanical support and compliant construct, but also environments for stem cell seeding and proliferation. Importantly, vascular regeneration requires specific mechanical and biological signals to perpetuate development of stem cells into a specific cell lineage. To that end, we have demonstrated functions of bone marrow-derived mesenchymal stem cells on the nanofiber-based grafts using a large animal (sheep) model, and we further integrate nanofibrous coating and patterning strategies into the bilayered model to define the mechanical and biomolecular microenvironments for the stem cells seeded on the grafts.

Methods: We have developed a biodegradable graft, consisting of a bilayered scaffold with a nanofibrous heparin-impregnated poly ε-caprolactone (PCL) inner layer and a porous collagen-based outer layer (Fig 1A). The graft lumen is uniformly seeded with allogeneic MSCs collected from the bone marrow of sheep. Both seeded and unseeded grafts possess compliance (4.5% per 100mmHg) comparable to native blood vessels, and burst strength (680-800 mmHg) and permeability (500-600 ml/cm^2.min) values suitable for implantation. Grafts were implanted as carotid artery interposition grafts in the senescent sheep which are characterized with low regeneration rate (Fig 1B). Ultrasonic imaging of the grafts was taken every week to evaluate graft patency and flow in the graft. Grafts were explanted after one month and analyzed with immunofluorescent staining.

Results: The interposition grafts in sheep remained patent for one month, the latest time point tested. Acellular grafts (n=4) had significant outward remodeling mainly by proliferation and migration of adventitial fibroblasts, and had no PECAM+ cells on the lumen. MSC-seeded grafts (n=4) showed significant reduction in the fibrotic process, and PECAM+ cells were found on the lumen, suggesting the possibility of MSCs in regenerating endothelial-like cells.

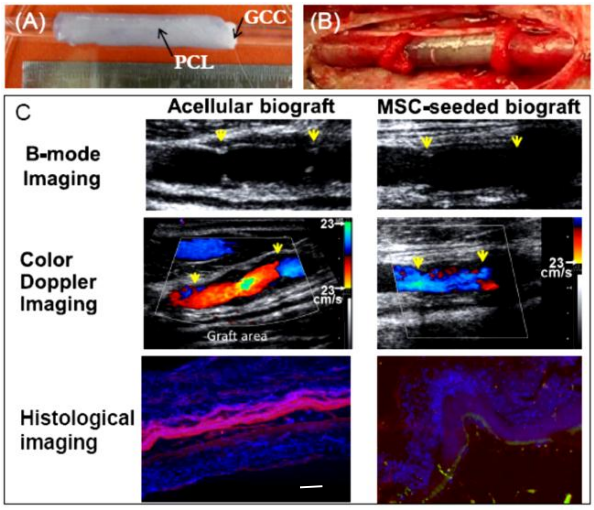

Fig 1. (A) Nanofibrous bilayered graft; (B) Implantation of the graft as an interpositional graft of a carotid artery of sheep; (C) Ultrasonic imaging of the morphology of grafts and the flow in the grafts after 3 weeks of implantation. Histological sections of explanted grafts were triple-stained with DAPI (nuclei, blue), anti-PECAM (green) and anti-smooth muscle actin (red). Yellow arrows point at the graft-artery anastomosis.

Conclusion: Allogeneic MSCs appeared to be non-immunogenic and have anti-fibrotic and pro-regenerative potential in sheep. Using nanofibrous coating and molecule-impregnated nanofibers, we further design the graft materials with tuned mechanical and biomolecular environments to improve vascular regeneration from the stem cells.