Cotransplantation of Adipose-Derived Stem Cells and Microvascular EC Using a Modular Tissue Engineering Approach Produces Vascularized Adipose Tissue *In Vivo*

Mark J. Butler, Kimberly A. Woodhouse, Michael V. Sefton,
Institute of Biomaterials and Biomedical Engineering, and Department of Chemical Engineering & Applied Chemistry,
University of Toronto, Toronto, Ontario, Canada

Statement of Purpose: Tissue engineering approaches to repair soft tissue deficits have been unsuccessful because they fail to maintain implant volume *in vivo* long-term[1]. This is likely due to lack of rapid vascularization upon implantation resulting in ischemia and resorption of the transplanted tissue. Modular tissue engineering enables the creation of uniform, scaleable and most importantly vascularized constructs. Here, sub mm-sized collagen hydrogel rods ("modules") are embedded with human Adipose-derived Stem Cells (ASC) and surface seeded with Human Microvascular Endothelial Cells (HMEC). Many modules randomly packed together can be implanted at the site of a soft tissue defect to restore normal cosmesis. The endothelialized lining of the modules have the potential to rapidly remodel and anastamose with the host vasculature upon implantation to avoid ischemic conditions, while the embedded ASC may differentiate into fat cells thus creating a functional fat pad. Here we investigate the effect of cotransplantation of primary human ASC and HMEC on modular implant revascularization *in vivo,* and the potential to create a volume-stable fat pad.

Methods: Collagen (bovine type I) hydrogel modules were prepared by mixing 10^6 cells/mL of ASC (passage 2, undifferentiated) obtained from the stromal-vascular fraction of abdominal fat tissue obtained from patients undergoing elective surgery and injected into the lumen of PE tubing which is subsequently cut after collagen gelation to produce cylindrical modules. HMEC, obtained from the same fat source as the ASC and separated by CD31+ magnetic bead separation (Miltenyi Biotech), were subsequently seeded onto the surface of the modules. Modules were implanted subcutaneously into the dorsum of SCID mice (~250 modules/implant via injection with 18G needle) and explanted at various timepoints (3, 7, 21, 30, 60, 90 days) to observe HMEC survival/remodelling and ASC differentiation *in vivo* by histology. The dimensions of resulting fat pads were measured with calipers at explant to demonstrate volume maintenance. MicroCT was used to assess vessel perfusion.

Results: Implantation of ASC+HMEC collagen modules in SCID mice revealed that HMEC surrounding the modules containing ASC were found to detach from the module surface and organize into vessels (many containing erythrocytes) as early as day 3, in contrast to the HMEC-only control where the HMEC would remain on the module surface and staining would diminish by day 7 suggesting cell death (Figure 1). MicoCT analysis of the explants demonstrated that host vasculature had penetrated and perfused the ASC+HMEC module construct by day 21, while the HMEC-only control remained avascular (Figure 2). Long-term studies (60, 90d) were conducted to assess the effect of transplanted HMEC on ASC differentiation into fat and volume maintenance of the fat pad. Oil Red O staining revealed the accumulation of intracellular lipid by day 60 and extensive fat accumulation by day 90 in the ASC+HMEC module implant (Figure 3). Volume measurements demonstrated that the fat pad made of the ASC+HMEC modules were larger and persisted longer than modules without HMEC. ASC+HMEC modules persisted up to 90d.

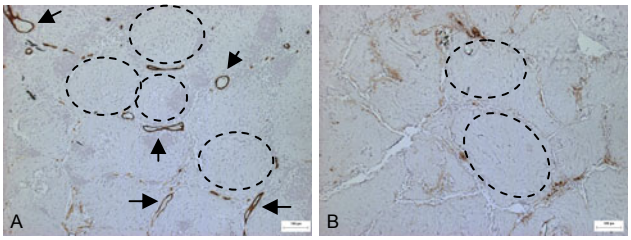

Figure 1: UEA-1 Lectin stained sections of ASC+HMEC modules (A) and HMEC-only modules (B) at day 7 (some modules highlighted by dashed lines). Arrows highlight some large UEA-1 stained vessels containing erythrocytes. Scale bar 100µm.

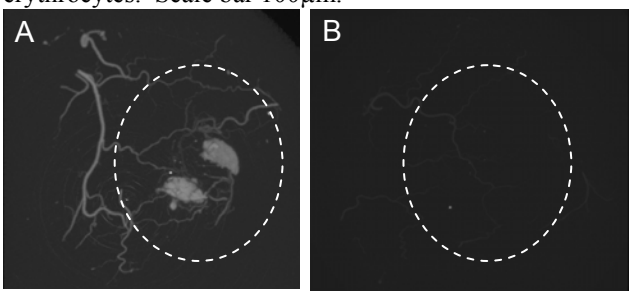

Figure 2: MicroCT image of ASC+HMEC modules (A) and HMEC-only modules (B) at day 21 under mouse skin. Region of implant highlighted with dashed line.

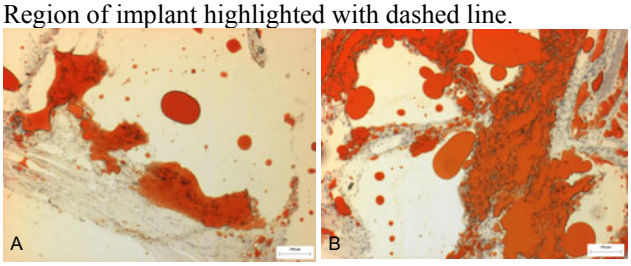

Figure 3: Oil Red O staining of ASC+HMEC module sections at day 60 (A) and 90 (B).

Conclusions: Modular tissue engineering has the potential to create volume-stable vascularized adipose tissue. Current studies have demonstrated that embedded ASC have an anti-apoptotic and angiogenic effect on transplanted HMEC to support implant vascularisation. This early vascularisation supports ASC survival and differentiation to create a volume-persistent fat pad.

References: 1. Cho SW, et al. Engineering of volume-stable adipose tissues. Biomaterials 2005;26:3577-3585.