# In vivo assembly of endothelialized modules for tissue engineering

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Modular tissue engineering is a novel micro-scale technology to assemble uniform, scalable, functional constructs with an inherent vasculature[1]. The original premise was that the endothelial cell (EC) covered modules (sub-mm collagen gel containing a functional cell) would result in perfuseable channels due to the nonthrombogenic nature of the seeded EC[2]. Vessels form in vivo but the mechanism of vessel formation depends more on the consequences of remodeling and not apparently on the ability to perfuse EC lined channels.

In the omental pouch of an immune-suppressed (tacrolimus & atorvastatin) allogeneic outbred rats, transplanted GFP positive rat aortic endothelial cells migrated off the surface of the modules at day 3, and formed vessels in close proximity to modules at day 7. GFP-positive chimeric vessels (containing both host and donor cells) matured over time and by day 60, three types of GFP-positive vessels (venule-, arteriole- and capillary-like) could be seen (e.g., Fig. 1c). MicroCT indicated that by 21 days the new vasculature was at least in part connected to the host vasculature, although there was a portion that appeared leaky (a blood island?).

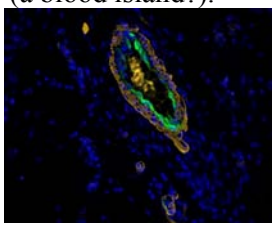

Figure 1: Transplanted GFP positive RAEC (green) formed arteriole-like vessels in immune-suppressed rats at day 60. Sections were co-stained for supporting smooth muscle cells (α-smooth muscle actin positive, orange) and counterstained with dapi (blue).

The addition of bone marrow derived mesenchymal stem cells MSC to the modules improved and acccelerated vessel formation consistent with known effects of these cells on pericyte formation and on modulating inflammation. With MSC there was a decrease in macrophage infiltration 14 days after implantation and the MSC develop SMA staining implying support for the newly formed vessels by acting as pericytes.

Implantation of adipose derived mesenchymal stem cells (ASC) with human microvascular EC (HMEC) 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 2). 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.

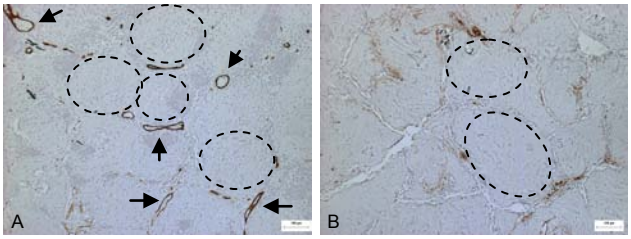


Figure 2: UEA-1 Lectin stained sections of ASC+HMEC modules (A) and HMEC-only modules (B) at day 7 (some modules highlighted by dashed lines). Arrows highlight some large UEA-1 stained vessels containing erythrocytes. Scale bar 100μm.

Modular tissue engineering results in vessels but exploration of different animal models and module systems suggest that the transplanted endothelial cells drive vascularization but that vascularization speed and maturity depends on the host immune, inflammatory and remodeling response as modulated by the behavior of the transplanted cells. The specifics of this mechanism remain to be clarified.

1. McGuigan AP, Sefton MV. PNAS, 103:11461, 2006
2. McGuigan AP, Sefton MV Biomaterials, 29: 2453-2463 (2008)

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