Remodelling of an Endothelialized Modular Construct *In Vivo*

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Statement of Purpose

Modular tissue engineering is a novel micro-scale technology to assemble constructs with the potential for uniform cell density, scalability, mixed cell populations and vascularisation. Functional cells are embedded in sub-millimetre sized collagen rods, and the outside surface is covered with endothelial cells. These modules are then assembled in situ or within a larger "container" to create a porous structure in which the interstitial gaps are lined with endothelium and permit blood perfusion[1]. With different embedded cells, this type of construct can be used for numerous tissue engineering applications.

Here we assessed the extent of remodelling that occurred when endothelialized modules (no embedded cells) are implanted in a nude rat. We hypothesized that endothelial cell (EC) covered modules transplanted in the omentum will form channels in situ that permit blood perfusion. To prolong EC survival, we utilized clodronate liposomes to temporarily delete peritoneal macrophages[2] and report here on the consequences of such treatment.

Materials and Methods

Neutralized bovine Type 1 collagen (3.1 mg/mL, Cohesion Technologies) was drawn into sterile 0.71 mm ID polyethylene tubing (Intramedic Inc.) and gelled at 37°C for 45 minutes. The tubing was cut into 1 mm long pieces, collagen cylinders were separated and dynamically seeded with human umbilical vein endothelial cells (HUVEC, Cambrex Corporation) at a concentration of 2.0x 10^6 cells/3 m tubing for 30 min and incubated for 7 days (modules contract to ~600um x 400um size)[1].

5 week old male nude (athymic) rats were treated (or not) with 1 mL of clodronate liposome formulation (10 mg clodronate/mL, Gift from Dr. Nico Van Rooijen, Netherlands) 1 day and 4 days prior to surgery. Animals were anesthetised and modules in PBS were delivered to a surgically created omental pouch. At 3, 7, 14, and 21 days after implantation, animals (n=5) were sacrificed and the omental pouch explanted into 4% neutral buffered formalin. Tissue was fixed for 48 hrs, embedded in paraffin wax, routinely processed and stained for: H&E; Masson trichrome; rat CD68 (macrophage); and UEA-1 lectin (Human EC).

Results and Discussion

Trichrome staining showed that HUVEC covered modules remodelled significantly less than collagen only modules (without EC) and formed channels in situ that persisted up to 21 days after transplant in both untreated and treated rats. CD68 staining indicated severe macrophage infiltration near the modules in untreated rats at all time points (Figure 1a – Day 3). In clodronate treated rats, there was little macrophage presence at Day 3 (Figure 1b) and full repopulation beginning at Day 7.

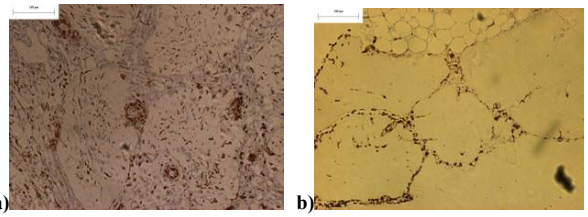

Figure 1: Macrophage (CD68) infiltration is reduced with clodronate treatment a) untreated b) clodronate treated [3 days]

In untreated rats, HUVECs (UEA-1 positive cells) were present along the modules at Day 3, sparse at Day 7 and absent by Day 14. Interestingly, in clodronate treated rats, HUVECs were confluent on module surface at Day 3 and by Day 7, HUVEC derived lumens could be seen within modular channels (Figure 2a). Some of the lumens even indicated the presence of erythrocytes (Figure 2b), suggesting that perfusion had occurred. However, by Day 14 a majority of HUVEC derived lumens disappeared.

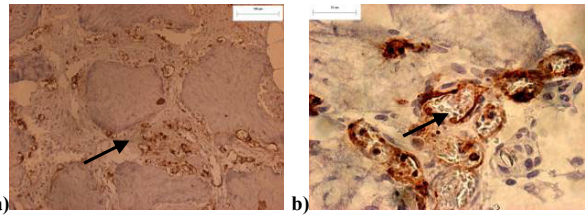

Figure 2: HUVEC (UEA-1) lined channels in clodronate treated rats a) 100x b) 400x [7 days]

Nude rats maintain a strong innate immune response capable of xenogeneic rejection as evident by limited HUVEC survival after 3 days in untreated rats. Clodronate liposomes induce macrophage apoptosis and this treatment delayed macrophage mediated EC attack. Hence, transplanted HUVECs matured and formed lumens capable of blood perfusion within modular channels until 7 days. However, macrophages repopulate the omentum a week after clodronate treatment[2], which resulted in HUVEC loss by Day 14.

Conclusions

Endothelialized module implants in an omental pouch show that transplanted HUVEC can form lumens within in situ created channels for 7 days. These results were our first indication that this micro scale technology is capable of forming pseudo vascular networks *in vivo*. We are exploring syngeneic rat EC transplants to further alleviate adverse immune responses to form stable and functional vessels.

References

1. McGuigan AP, Sefton MV. PNAS. 2006; 103:11461
2. Biewenga J. et al Cell Tissue Res. 1995; 280:189

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