Biomaterial Screening for Lymphatic Regeneration

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Statement of Purpose: Lymph nodes are often removed during surgery for cancer and the wound site is subsequently irradiated at approximately 1 month post-surgery. A consequence of surgery and radiation is the formation of a fibrotic scar at the site of injury that causes poor lymphatic regeneration. This frequently results in secondary lymphedema of the limb due to impaired lymphatic drainage. Our ultimate goal is to develop a biomaterial replacement that 1) reduces the formation of obstructive scar tissue and 2) improves lymphatic regeneration across the site of injury. In this work, we have developed rational *in vitro* and *in vivo* assays to screen candidate materials for 1) lymphatic cell attachment-infiltration and 2) structural stability and degradation behavior (material must be stable post radiation treatment to facilitate cell and matrix therapy).

Methods: ***Fabrication of Materials.*** First round candidate materials were hydrogels (composed of methylcellulose, agarose, dextran, and chitosan), fibrin, collagen, and composites of these materials, created as previously described.[1] In addition to these, fibrin scaffolds were synthesized using PMMA spheres sintered in templates and infiltrated with a fibrinogen solution followed by thrombin for polymerization. PMMA spheres were then dissolved using acetone and scaffolds were rinsed in ethanol and stored in PBS.[2] ***In Vitro Testing.*** Candidate biomaterials were evaluated for cell attachment and infiltration *in vitro* by culturing Human Dermal Lymphatic Endothelial Cells (HDLECs) three days on candidate materials and imaging fluorescently with a calcein stain. Material degradation rates were determined by an *in vitro* dissolution test where the biomaterials were incubated at 37° in PBS and lyophilized at varying time points for a change in mass calculation[3]. ***Murine Foreleg Model.*** Axillary lymph nodes were removed from the right foreleg of balb/c mice and Indocyanine Green (IC-Green) dye was injected into each forepaw to visualize regenerating lymphatic vessels.

Results: ***In Vitro Material Testing.*** Calcein stained fluorescent images of HDLECs cultured on hydrogels, fibrin, and fibrin scaffolds demonstrated that HDLECs had attached and infiltrated into the fibrin-based materials more effectively than into the polysaccharide hydrogels, with a large density of cells seen within the fibrin gel and along the pores of the fibrin scaffold (Fig. 1) (Images for collagen scaffold and hydrogel composites are not shown). Fibrin scaffolds were examined by FE-SEM imaging, which also showed that cells infiltrated the pores of the scaffold. FE-SEM images also showed that the pores were roughly the same size and interconnected (data not shown). An *in vitro* dissolution assay was performed

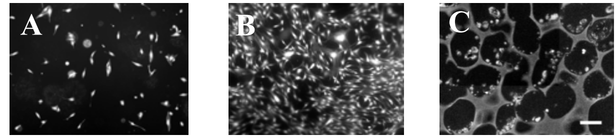

Figure 1. HDLECs were seeded on candidate materials and fluorescent images of calcein stained cells were collected at three days. (A)Representative Hydrogel material; (B)Fibrin Gel; (C)Fibrin Scaffold. Scale bar is 100 micrometers. Note that (C) was captured from a thin section to aid in visualization of the pores, whereas images A and B were collected from whole mounts.

on all material and material composites. These tests demonstrated that the fibrin gel and fibrin scaffold had degraded 69% and 57%, respectively by day 10. ***Murine Foreleg Model.*** We are developing a murine foreleg model of lymphangiogenesis that can be used to screen candidate materials for their ability to increase lymphatic regeneration. IC-Green dye is used to visualize lymphangiogenesis in the mouse foreleg. IC-Green dosing and administration into live animals was optimized to 2.5uL of 5mg/mL dye per injection. With this approach, we are able to visualize and assess the function of regenerating lymphatics, *in vivo* (Fig. 2). This model can be employed in the future to test the ability of implanted candidate biomaterials to increase lymphangiogenesis in a realistic animal model.

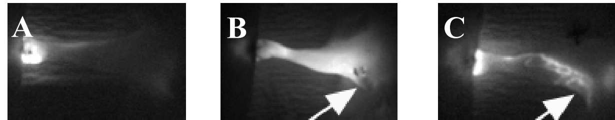

Figure 2. Lymphatic vessel regeneration during wound repair. Axillary lymph nodes were removed from the mouse underarm and lymphatic vessels in the arm were visualized via indocyanine green lymphangiography. (A)In pre-operative controls, lymph tracer flows through a single lymphatic vessel in the foreleg. (B)In contrast, at day 0 post-surgery, lymphatic tracer spreads throughout the entire arm outside of lymphatic vessels. (C)At day 15, lymphatic flow through new lymphatic vessels is evident. Wound region identified by white arrow.

Conclusions: *In vitro* and i*n vivo* testing of candidate biomaterials demonstrated our ability to measure degradation rate, cell attachment and infiltration, and lymphangiogenesis. The i*n vivo* murine foreleg model will add important information about these parameters as well as flow conductivity and scar mitigation in an *in vivo* environment. These methods will allow us to screen candidate materials to develop a suitable biomaterial for reducing scarring and promoting lymphatic regeneration following axillary lymph node dissection.

References:

[1] (Martin et al, J. Neural Eng, 2008. 5: 221-231)
[2](Linnes et al. Biomaterials, 2007. 28: 5298-5306)
[3](Osathanon et al. Biomaterials, 2008. 29: 4091-409)

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