Neural Progenitor Cell Stabilization of 3D Fabricated Microvascular Networks

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Statement of Purpose: Understanding the neural progenitor cell (NPC) niche, which plays an indispensible role in regulating NPCs, would aid in the identification of therapeutic targets to control NPC behavior. A crucial feature of the niche is the anatomical and functional association of NPCs with surrounding vasculature. To better study this interaction, our lab has developed a neurovascular niche model using a unique 3D macroporous hydrogel system which supports the coculture of NPCs and brain endothelial cells (bECs) *in vitro* and the formation of functional, stable microvasculature *in vivo*. While NPCs improved vascular stability, they were not observed in the retrieved implants. We have now established using both the original and a modified NPC:BEC coculture (1:10 vs 2:1) that the vascular density of NPC supported networks peaks at nine weeks. Increasing the seeding density of NPCs in the modified coculture significantly increased the integration of graft BECs into the implant vessels (40.4 ± 2.6%) as compared with the original (24.1 ± 6.1%). Furthermore, the percentage of implant vessels incorporating graft bECs increased over time in both groups.

Methods: Macroporous hydrogels were synthesized by the addition of activated four-arm polyethylene glycol (PEG) (MW ~ 10,000 g/mol) to poly-L-lysine (PLL) (MW ~70-110kDa) in deionized water at a final ratio of 1:4 PEG hydroxyls to PLL free amines. The polymer solution was cast over a salt-leached polylactic-co-glycolic acid (PLGA) scaffold with 250-500µm pores and allowed to cure at room temperature. The PLGA scaffold was then removed by degradation. Hydrogel discs 5mm in diameter and 1mm thick were sterilized prior to cell seeding. For the original coculture control, gels were seeded at a ratio of 1:10 (100,000 NPCs:1 million BECs). For the modified coculture treatment group, gels were seeded at a ratio of 2:1 (2 million NPCs:1million BECs). BECs were a generous gift from Britta Engelhardt and NPCs were isolated from green fluorescent protein positive (GFP+) postnatal mouse brains. Four seeded hydrogels from the same study group were placed subcutaneously on the dorsal surface of a 6- to 8-week-old female C57BL/6 mouse. At 9 and 12 weeks, animals were injected retro-orbitally with biotinylated lectin prior to implant retrieval. Three representative implants per study group were randomly chosen (each from a different animal) for sectioning. 20-µm-thick cross sections were stained with hematoxylin and eosin for general pathology or immunostained with antibodies against mouse PECAM-1, Pymt, nestin, CD-15, glial fibrillary acidic protein, and neurofilament 200. Experiments were done in triplicate and data analyzed using a two-way ANOVA. The statistical significance threshold was $p < 0.05$.

Results: Hematoxylin and eosin staining demonstrated cellular morphology consistent with vasculogenesis and angiogenesis and lumenized structures containing red blood cells. Post fixation staining of the injected biotinylated tomato lectin revealed functional vessels at central aspects of the gel. Vessel density peaked at nine weeks in both groups, with some vascularity remaining at twelve weeks (Fig 1).

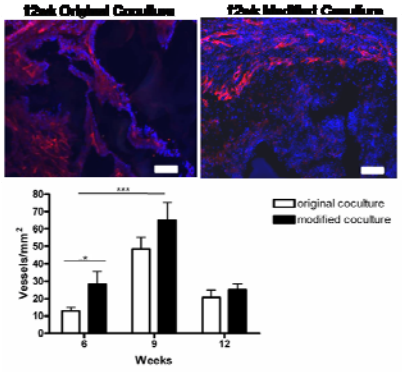

Figure 1. Implant vessel density. (Red = PECAM-1, Blue = DAPI, Scale = 100µm.)

In both the original and modified coculture groups graft ECs were found to contribute to implant vasculature (Fig 2). Interestingly, the modified coculture group demonstrated a marked enhancement in graft bEC integration. Additionally, the percentage of vessels incorporating graft ECs increased over time in both groups. .

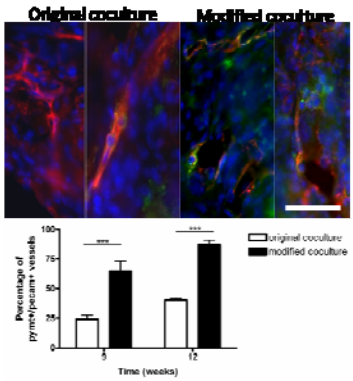

Figure 2. Graft bEC vessel integration. (Red = PECAM-1, Green = PyMT, Blue = DAPI, Scale = 50µm.)

Conclusions: Our 3D model of the neurovascular niche contains physically approximated cells that participate in crosstalk affecting cellular behavior. These data demonstrate a clear role for NPCs in affecting the behavior of the vascular niche component. NPCs support the stability of bEC derived microvascular networks *in vivo* to 9 weeks. Furthermore, increasing the number of NPCs in the construct significantly increased engraftment of bECs into the implant vessels. The amount of implant vessels bearing graft derived bECs also increased over time suggesting that vessels lacking graft ECs are more likely to regress. We have recently identified NPCs in the implant and have done initial studies to assess their behavior. This strategy also provides a platform for the growth of NPCs with additional niche components, such as astrocytes, to determine their role in niche interactions.