A poly(butyl methacrylate-co-methacrylic acid) tissue engineering scaffold with pro-angiogenic potential *in vivo*

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Statement of Purpose: One of the challenges of tissue engineering is addressing the diffusional limitation imposed on nutrient and waste transfer. As cells cannot survive more than approximately 200µm from a blood vessel[1], hypoxia and cell death are apparent in many scaffold-based approaches to tissue engineering which often results in failure of the engineered tissue. Stimulation of blood vessel growth upon scaffold implantation, resulting in enhanced perfusion, is expected to help overcome the diffusional limitation and assure tissue survival and long-term success of the tissue engineered construct *in vivo*.

Forming a vascular network depends on angiogenesis, the growth of new blood vessels from a preexisting microvascular bed. Site-specific delivery of angiogenic growth factors (ie.VEGF) from a tissue engineered device is one approach to stimulate localized vessel recruitment. An alternative and relatively unexplored approach uses the biomaterial itself as an agonist of the angiogenic response without the use of exogenous growth factors. Previous work in our laboratory has demonstrated the angiogenic potential of copolymers of methacrylic acid (MAA) in a wound healing model *in vivo*[2]. In the present work we evaluate the pro-angiogenic potential of a non-degradable poly(butyl methacrylate-co-methacrylic acid) (BMA-MAA) tissue engineering scaffold.

Methods: Porous BMA-MAA copolymer scaffolds (45mol% MAA) were fabricated via an *in situ* polymerization solvent casting/salt leaching technique, using fused salt particles as the porogen, described elsewhere[3]. Scaffolds disks (6mm x 2mm) were implanted subcutaneously in the dorsum of male CD31 mice (6-8 weeks old, Charles River Laboratories, MA). For each implantation time (7, 21, 30d) mice were implanted with both a BMA-MAA (test) and BMA homopolymer (control) scaffold. Upon termination of the experiment the mice were euthanized, the implants were recovered from the subcutaneous pockets and fixed, cut and stained for hematoxylin and eosin (H+E) and vonWillebrand Factor (FVIII). The level of angiogenesis in the tissue invading the porous scaffolds was quantified using a microvessel density (MVD) count technique adapted from tumour literature[4].

Results: BMA-MAA (test) and BMA (control) scaffolds (porosity ~ 90%, pore size 100-650µm) implanted subcutaneously in mice displayed progressive tissue penetration into the scaffolds over 30 days with complete tissue infiltration achieved after 21 days, indicative of the open-pore nature of the scaffolds. Microvessel density (MVD) counts of tissue penetrating the pores of the BMA-MAA scaffold at post-operative days 21 and 30 were found to be significantly higher than those of the BMA control scaffold ($p<0.001$) (Figure 1). Photomicrographs of FVIII stained sections of the implanted BMA-MAA scaffolds show many stained blood vessels in close proximity to the polymer (Figure 2).

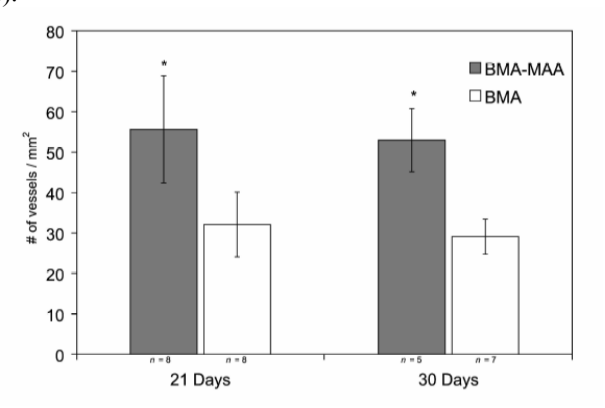

Figure 1

Microvessel densities of implanted BMA-MMA scaffolds. Values represent mean ± standard deviation; * represents statistical significance relative to the BMA conrol ($p < 0.001$); *n* shown on graph.

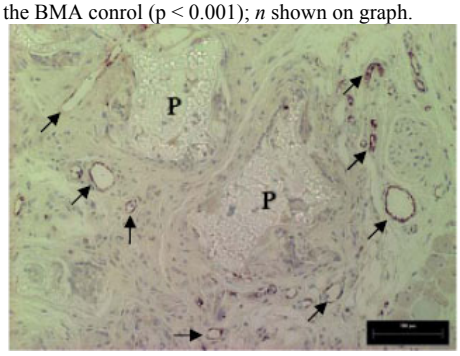

Figure 2

A large number of FVIII stained vessels can be seen in the invading tissue in close proximity with the BMA-MAA copolymer scaffold (P) after 21d. Arrows highlight some stained vessels. Scale bar = 100µm.

Conclusions: In this study, a BMA-MAA tissue engineering scaffold was fabricated and evaluated for its ability to enhance angiogenesis in the invading host tissue. Scaffolds implanted subcutaneously in mice revealed a high number of FVIII stained blood vessels in tissue with close proximity to the BMA-MAA polymer. Microvessel Density counts revealed a statistically higher number of counted vessels in the tissue invading the pores of the BMA-MAA scaffolds compared to a BMA control. These results suggest that BMA-MAA is a pro-angiogenic biomaterial.

References: **1.** Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000;407:249-257. **2.** Gorbet M, Eckhaus A, Lawson-Smith R, May M, Sefton MV. Material-induced angiogenesis in impaired wound healing. Wound Repair Regen 2003:A14 (Abstract). **3.** Butler, M.J., Sefton, M.V. A poly(butyl methacrylate-co-methacrylic acid) tissue engineering scaffold with proangiogenic potential *in vivo*. J. Biomed. Mat. Res., Part A 2007 (in press). **4.** Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. N Engl J Med 1991;324:1-8.