

## Screening of the Biological Interactions of Methacrylic Acid Polymers

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**Statement of Purpose:** Advanced wound dressings, functional tissue constructs and biomedical devices would benefit from a vascularized foreign body response to promote healing and their integration. Poly(methacrylic acid-co-methyl methacrylate) beads (MAA beads) promoted functional angiogenesis with improved wound healing in diabetic mice<sup>1</sup> and improved skin grafting in Wistar rats<sup>2</sup>. Interestingly, the gene expression of inflammatory factors and osteopontin, but not typical angiogenic growth factors, were significantly modulated during MAA bead-promoted wound healing.<sup>3</sup> The aim of this work is to develop coatable copolymers to screen cell, protein, and tissue (in vivo) interactions with MAA-based polymer biomaterials to identify the cells and factors important in MAA associated angiogenesis.

**Methods:** Isodecyl acrylate (IDA) was copolymerized with MAA or methyl methacrylate (MM, as a control) via free radical polymerization to produce water-insoluble polymers that were cast as films to create a library of materials. The MAA or MM content was varied from 20-40%, as verified by NMR, XPS and titration. Minimal differences in roughness were noted among the different polymers.

Cell interactions were screened by growing macrophage-like cells (dTHP1, activated with phorbol-12-myristate-13-acetate) on the films of varying content and then comparing their viability (Alamar Blue) and gene expression profiles (qPCR). Protein interactions were screened by using shotgun mass spectrometry to identify proteins that adsorbed to the polymers from fresh serum and by using ELISA to measure complement activation. Biological interactions were histologically assayed after the subcutaneously insertion of silicone sheet disks coated with the different films into diabetic mice (db/db, male BKS.Cg-m<sup>+/+</sup> Lepr(db)/J).

**Results:** As shown in Figure 1, different gene expression was noted on dTHP1 cells grown on films of varying MAA content in comparison to controls of matched MM content. The MAA content of polymers with a threshold between 30-40% MAA, independent of roughness, influenced cellular interactions shifting the cells to express significantly more inflammatory and angiogenic genes.

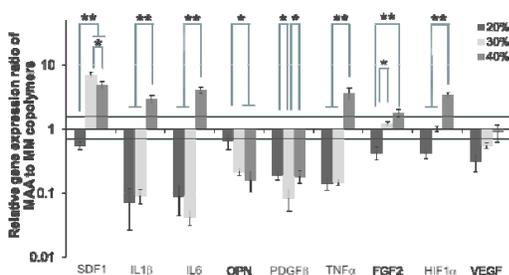


Figure 1. Gene expression changes in dTHP1 cells grown on MAA copolymers (\*= $p < 0.05$ , \*\*= $p < 0.005$ ).

Over 30-40 different proteins adsorbed to MAA and PMMA beads with some similar proteins adsorbing in different magnitudes (Figure 2). Complement proteins preferentially adsorbed to MAA beads but complement activation was 70% lower in serum exposed to MAA beads compared to PMMA beads ( $p = 0.0107$ , 3 donors). MAA adsorbed a different spectrum of proteins than “inert” polymers such as PMMA presumably resulting in altered complement activation which may in turn alter the local healing environment in vivo. The presence of different proteins at the biomaterial surfaces may initiate different events during wound healing and angiogenesis.

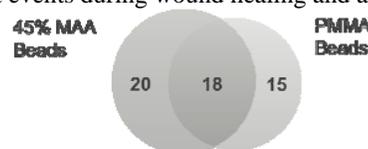


Figure 2. Mass spectrometry identified 20 unique proteins that adsorbed to MAA beads and 15 unique proteins that adsorbed to the control PMMA beads.

Polymer-coated silicone disks implanted subcutaneously into diabetic mice had a higher vascular healing response when coated with 40% MAA compared to 20% MAA and uncoated controls. This suggests that MAA promotes composition-dependent vascularization.

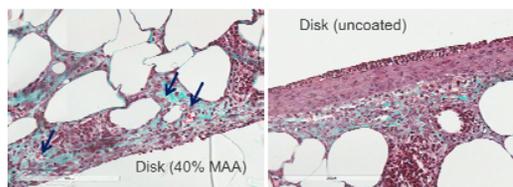


Figure 3. Vessels (arrows) are shown near surface of 40% MAA coated disks (arrows); the uncoated disks exhibit an avascular foreign body response (db/db mice, subcutaneous, day 7, trichrome stain).

### Conclusions:

MAA influenced cell behavior, protein interactions and tissue reactions in a composition-dependent manner to promote increased in vivo vascularization. MAA copolymers altered the behavior of dTHP1 cells to express increased angiogenic and inflammatory genes. MAA copolymer beads adsorbed different plasma proteins which resulted in lower complement activation. These phenomena may alter the wound healing environment in vivo causing the resulting histological features. Future studies will assess the in vivo reactions in further detail.

### References:

- 1) Martin DC, et al. J Biomed Mater Res A. 2010;93:484
- 2) Eckhaus AA, et al. Plast Reconstr Surg. 2008;122:1361
- 3) Fitzpatrick L, et al. Biomaterials. 2011;32:895