Films of varying methacrylic acid content modulate gene expression in dTHP1 and endothelial cells

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Statement of Purpose: Advanced wound dressings, functional tissue constructs and biomedical devices would benefit from a vascularised foreign body response to promote healing and their integration. Poly(methacrylic acid-co-methyl methacrylate) beads (polyMAAcoMMA beads) promote wound healing and angiogenesis in normal and diabetic animal models compared to inert controls of poly methyl methacrylate (Martin DC. J Biomed Mater Res A. 2010;93:484). Interestingly, gene expression of inflammatory factors and osteopontin, but not typical angiogenic growth factors, were significantly modulated during polyMAAcoMMA bead-promoted wound healing illustrating the complexity of the system (Fitzpatrick L. Biomaterials. 2011;32:895). The focus with this work is to understand what it is about the methacrylic acid (MAA) in polyMAAcoMMA that causes the changes in gene expression and ultimately angiogenesis and to devise strategies for incorporating similar material in medical devices. The aims of this research are to develop new MAA-materials, as potential device coatings, and to use them as tools to identify cells and factors important in MAA associated angiogenesis.

Methods: Isodecyl acrylate (IDA) is copolymerized with MAA or methyl methacrylate (MMA, as a control) via free radical polymerization to produce water-insoluble polymers that are cast as films to create a library of materials. The comonomer content, which is varied from 20-40%, is verified by NMR and the film chemistry is verified by XPS and titration. A macrophage cell-line (dTHP1, activated with phorbol-12-myristate-13-acetate) and endothelial cells (HUVEC) representative of wound healing environments are grown on the films. Morphology (antibody staining) and gene expression (qPCR) are investigated to determine the influence of polymer content on cell behaviour.

Results and discussion: A polymer library was created with the 20-40% (± 3%) MAA or MMA and was cast on glass coverslips or glass petri dishes with tetrahydrofuran to produce coatings that had swollen thicknesses as high as 25 microns, depending on MAA content. The average film roughness varied up to 0.114 ± 0.015 μm for 40% MAA, but became smoother upon swelling in aqueous solution.

Activated dTHP1 cells were cultured on films for up to 96 hours. On 40% MAA films, there was a significant decrease in osteopontin (OPN) and increase in interleukin 1β (IL1β), interleukin 6 (IL6) and tissue necrosis factor α (TNFα) gene expression (relative to the IDA-co-MMA, 40% MMA, control) (Figure 1) as observed in previous studies with angiogenic polyMAAcoMMA beads (Fitzpatrick L. Biomaterials. 2011;32:895). In addition, CXCL12, hypoxia inducible factor 1α and fibroblast growth factor 2 expression were increased (not shown) which may influence cell migration and angiogenesis.

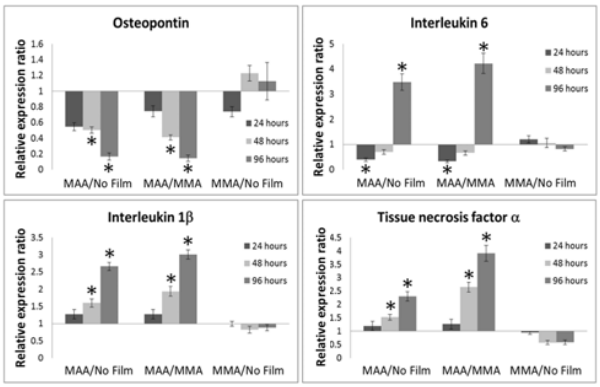

Figure 1. Gene expression in dTHP1 cells grown on 40% copolymer films of MAA or MMA with IDA (±SEM, n=3, * = 0.67>ratio>1.5 and Cq' $p<0.05$).

MAA content affected gene expression (Figure 2) demonstrating that screening of the MAA formulation is possible. Increased MAA content resulted in higher inflammatory and angiogenic gene expression in dTHP1 cells.

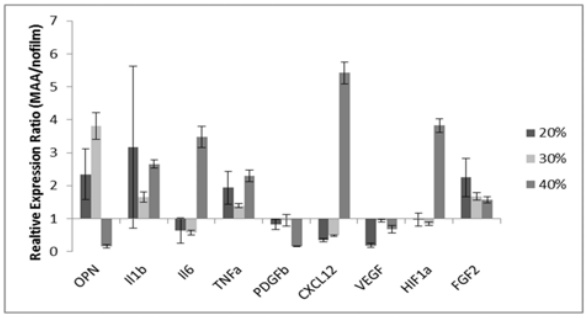

Figure 2. Gene expression of dTHP1 cells grown on MAAcoIDA films of varying content (±SEM, n=3).

HUVECs adhered to the films and showed VE-cadherin staining indicative of tight gap junctions. On higher content MAA films, the HUVEC had increased expression of OPN, CXCR4 and matrix metallopeptidase (MMP) 9 (not shown), which may influence migration required for angiogenesis.

Conclusions: Gene expression screening of cells grown on polymer coatings made from the copolymerization of IDA with different monomers will enable insight into the effect of material composition on cell behaviour. Importantly in the context of this study, MAA has been further demonstrated to elucidate an inflammatory and angiogenic response with macrophage and endothelial cells as previously noted with 45% polyMAAcoMMA (Fitzpatrick L. Biomaterials. 2011;32:895). Current and future studies are focusing on the investigation of effects in vivo.