Poly(methacrylic acid – co – methyl methacrylate) beads, but not poly(methyl methacrylate) beads, increased blood vessel counts and Sonic Hedgehog expression in diabetic wound healing.

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Statement of Purpose: Previous studies have shown that poly(methacrylic acid –co- methyl methacrylate) bead treatment of large (1.5 cm x 1.5 cm) wounds improved wound healing and wound bed vascularity in diabetic mice compared to untreated wounds, while poly(methyl methacrylate) (PMMA) beads did not[1]. However, little is known about the host response at a cellular or molecular level to these two biomaterials that underpins the differences reported in the wound bed morphology. Quantitative real-time PCR was used to analyze gene expression in the wound tissue of diabetic mice treated with MAA, PMMA or no beads to assess differences in the molecular level response.

Methods: *Bead Preparation*: Endotoxin-free MAA beads (150 – 250 μm diameter, 45 mol% methacrylic acid) were donated by Rimon Therapeutics Ltd (Toronto, Canada). PMMA beads (same diameter, endotoxin free; Polysciences, Warrington, PA) were used as a "non-bioactive" control material. *Wounding Assay*: Two small full-thickness skin wounds (7.5 mm x 7.5 mm) were created on the backs of 8-12 week old male $Lepr^{db/db}$ mice (Jackson Laboratories, Bar Harbor, ME). Either 7 mg of MAA beads or 7 mg of PMMA beads were applied topically to the wound beds, or the wounds were left untreated (blank). All wounds were left undressed and scabs were allowed to form. Mice were sacrificed on post-operative day 4 and 7. *Blood Vessel Counts:* The number of CD31-positive blood vessels in six 300 μm x 300 μm areas within the granulation tissue were averaged for each animal (n = 4). *Real-time PCR*: Total RNA was isolated from wound tissue was excised at day 4 and 7, and was used for cDNA synthesis. Fold-change in gene expression was calculated from real-time PCR data using the Pfaffl equation with GAPDH as an endogenous control (n = 4). A gene was considered differentially expressed if the fold-change in mRNA expression was greater than ± 1.5.

Results: At day 7, wounds treated with MAA beads had significantly more blood vessels than wounds treated with PMMA beads or untreated wounds ($p \leq 0.008$), Figure 1. No significant difference was found between PMMA and control wounds ($p = 0.064$). This result was consistent with previous experiments using a large wound model[1].

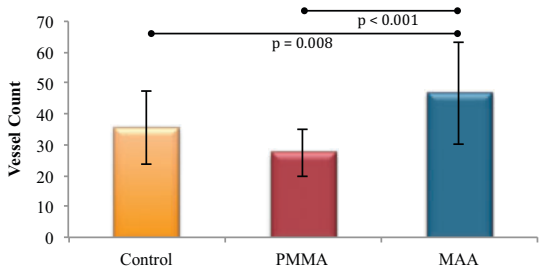

Figure 1. Blood vessel counts for day 7 granulation tissue

Total RNA was isolated from the wound tissue, and the expression of genes involved in wound healing and angiogenesis was analyzed by real-time PCR, Figure 2. The most notable results were the increased expression of sonic hedgehog (Shh) and the decreased expression Cxcl10 in MAA-treated wound tissue compared to untreated and PMMA-treated wounds on day 4 and 7. PMMA also downregulated Cxcl10 on day 7 compared to control but not to the same extent as MAA. Furthermore, wounds treated with MAA generally expressed more Pdgfb, Il-6 and Il-1b compared to untreated wounds, while PMMA treatment resulted in lowered expression.

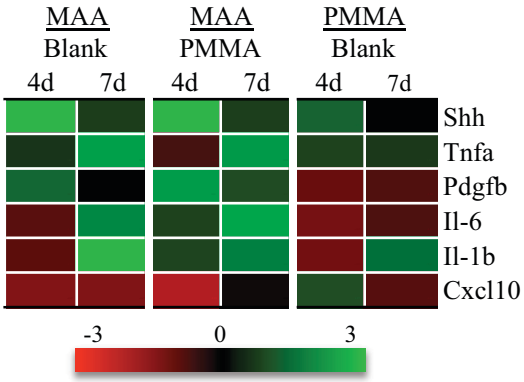

Figure 2. Fold-change of genes differentially regulated in wounds treated with MAA beads on day 4 and 7, compared to PMMA-treated and untreated wounds.

The increased expression of Shh and decreased expression of Cxcl10 in MAA wounds may have important implications in wound healing and vascularization of the wound bed. Recent studies indicated that Shh plays a role in angiogenesis by indirectly stimulating expression of pro-angiogenic growth factors[2]. Exogenous Shh was also shown to accelerate wound healing in diabetic mice by increasing the vascularity of the wound[3]. Cxcl10 is a pro-inflammatory chemokine that inhibits new vessel formation[4], and also induces regression of newly formed vessels [5]; thus lowering the expression of Cxcl10 may be beneficial.

Conclusions: The increased blood vessel density and improved wound healing observed with MAA beads are due, in part, to the increased expression of Shh and other pro-angiogenic factors (Pdgfb, Il-6, Il-1b) and decreased expression of angiogenic inhibitor Cxcl10. Conversely, PMMA beads, which did not increase blood vessel density, downregulated Pdgfb, Il-6 and Il-1b and did not modulate the expression of Shh or Cxcl10.

References: (1) Martin DC. J. Biomed. Mater. Res. 2010;93:484-492. **(2)** Pola R. Nat Med 2001;7:706-711 **(3)** Asai J, Circulation. 2006;113:2413-2424, **(4)** Streiter RM, Biochem Biophys Res Commun 1995;210:51-57. **(5)** Bodnar RJ. J Cell Sci 2009;122:2064-2077.