Sonic Hedgehog Pathway Activation in Non-Diabetic Wounds Treated with Poly(Methacrylic Acid-co-Methyl Methacrylate) Beads

Alexandra Lisovsky[1], Michael V. Sefton[1,2]
[1]Institute of Biomaterials and Biomedical Engineering, [2]Department of Chemical Engineering and Applied Chemistry
University of Toronto, Toronto, Canada

Statement of Purpose: This work focuses on the cellular and molecular mechanisms of angiogenesis in the presence of a bioactive biomaterial - poly(methacrylic acid-co-methyl methacrylate) (polyMAA-co-MMA or MAA). It was previously shown that direct application of MAA beads promoted angiogenesis and subsequent wound healing in diabetic mice (Martin DC. J Biomed Mater Res A. 2010;93:484-492); however, the molecular and cellular mechanisms of angiogenesis in the presence of MAA beads are largely unknown. Knowledge of these mechanisms can potentially be used to advance the development of wound dressings and the design of other tissue constructs where vascularization is important. Preliminary gene expression studies in diabetic wounds treated with MAA beads revealed the upregulation of a potent pleiotropic gene - Sonic hedgehog (Shh) (Fitzpatrick LE. Biomaterials.2012;33(21):5297-307), which is vital in adult angiogenesis (Pola R. Nat Med. 2001;7:706-711) and wound healing (Asai J. Circulation. 2006; 113:2413-2424). Hence, we investigated the role of the Shh pathway as a potential mechanism responsible for angiogenesis in the presence of MAA, in the absence of diabetes.

Methods: Large (1.5x1.5 cm) full thickness wounds were created on the dorsum of non-diabetic male Shh-eGFP-Cre/Ptch1-lacZ CD1 mice (n≥5) and 20 mg of MAA beads (150-250 μm in diameter, 45 mol% methacrylic acid) or control poly(methyl methacrylate) beads (PMMA, 150-250 μm in diameter, 100 mol% methyl methacrylate) were applied evenly over the wounds, or the wounds were left untreated. Tissue was explanted 4 and 7 days post-surgery and processed for histological and differential gene expression (qRT-PCR) studies.

Results: MAA beads were previously shown to promote angiogenesis in diabetic mice (Martin DC. J Biomed Mater Res A. 2010;93:484-492). Here, we demonstrated that MAA treatment also improved angiogenesis in granulation tissue of healthy non-diabetic mice at day 4 (p≤0.05) and day 7 (p<0.001) (Figure 1).

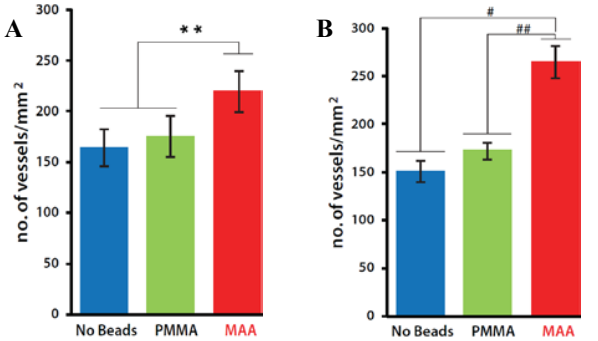

Figure 1. MAA increased average vessel density at days 4 (A) and 7 (B) in non-diabetic Shh-eGFP-Cre/Ptch1-lacZ CD1 mice (±SEM, n≥7) ##p<0.001, **p≤0.05

The transgenic animal model in which Shh and Ptch1 (Shh receptor) were co-expressed with two reporter genes, GFP and lacZ respectively, was used to detect and quantify cells expressing these proteins. Shh+ cells (GFP+ cells) were quantified within the healthy tissue surrounding the wound bed and are in the process of being quantified in the granulation tissue. The total number of Shh+ cells increased in the tissue surrounding the wound at day 4 (p<0.05) (Figure 2A) and at day 7 (p<0.07). The density of high intensity GFP+ cells increased in the same tissue at day 4 (p<0.05) suggesting that not only the number of Shh-expressing cells increased but the cells also produced higher quantities of Shh protein. Morphological examination of Shh+ cells suggested that potential source of Shh *in vivo* were hair follicle, immune, fibroblast-like cells and cells associated with adipose tissue and neuromas.
Differential gene expression analysis of wound tissue demonstrated that MAA treatment increased expression of Shh, Ptch1 and Gli2 in comparison to both controls (p<0.05) and Gli3 (Figure 2B) in comparison to PMMA (p<0.1) at day 7. Ptch1 is a product of Shh pathway (Stanton BZ. Mo Biosyst. 2010; 6:44-54); thus, its upregulation confirmed the pathway activation. Gli2 is implicated in embryonic folliculogenesis (Mill P. Genes & Development. 2003;17:282-294) and Gli3 is the main transcription factor that regulates Shh associated angiogenesis (Renault M. Circ Res. 2009;105:818-826).

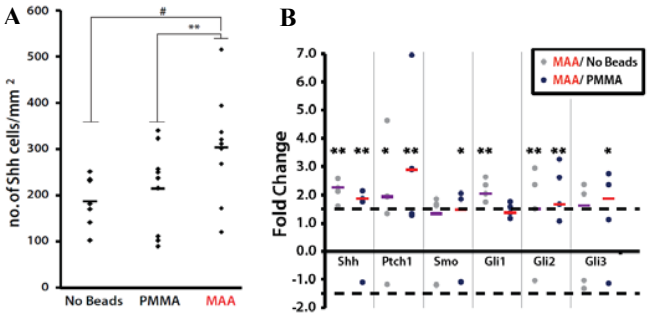

Figure 2. (A) MAA increased expression of Shh protein in the healthy tissue surrounding the wound at day 4 (n≥7). (B) MAA modulated Shh signaling pathway in wound tissue at day 7 (n=5) (±SEM) #p<0.01,**p<0.05, *p<0.1

Conclusions: In the wound healing study, in non-diabetic animals, MAA treatment increased vessel density and expression of Shh, Ptch1, Gli2 and Gli3 in the wound tissue, and Shh+ cell density in the tissue surrounding the wound bed suggesting activation of the Shh signaling pathway and its involvement in MAA-mediated angiogenesis. Studies with transgenic animal models are on-going to confirm the importance of the Shh pathway modulation as a mechanism responsible for angiogenesis in the presence of MAA.