Novel Matrix Metalloproteinase-Inhibiting Polymers for the Treatment of Chronic Skin Wounds

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Statement of Purpose: Rimon Therapeutics develops synthetic therapeutic polymers (Theramers™) that combine bioactivity with a broad range of desirable mechanical and physical properties. The MI Theramer™ is a novel, zinc-binding polymer that modulates the activity of matrix metalloproteinases (MMPs). Excessive proteolytic cleavage of extracellular matrix and growth factors resulting from elevated levels of MMPs is believed to contribute to the impaired healing associated with chronic, non-healing wounds. Therefore, we have conducted pre-clinical studies to demonstrate the efficacy of MI Theramer™ microspheres for the treatment of chronic skin wounds.

Methods: Crosslinked poly(methylmethacrylate-co-methacrylic acid) microspheres were produced by suspension polymerization in a $CaCl_2/H_2O$ solution. The methacrylic acid functional groups were then derivatized to hydroxamic acids via a mixed anhydride intermediate to generate MI Theramer beads. Hydroxamic acid functionalization was characterized by X-ray photoelectron spectroscopy (XPS).

In vitro MMP-inhibitory efficacy was demonstrated using a number of techniques including a DQ™ gelatin-fluoroscein/MMP-2 assay, ELISA, and chromogenic substrate assays. MMP inhibition in both model solutions and chronic wound fluid extracts was assessed. *In vivo* inhibition of MMP activity was investigated in a mouse implant model. Crosslinked gelatin tubes were implanted subcutaneously (with or without 50 mg of MI Theramer beads) in male Balb/c mice. The gelatin tubes were explanted at 7 and 11 days, photographed for visual assessment and transferred to PBS to extract MMPs. Extract MMPs (MMP-2 and MMP-9) were quantified using gelatin zymogram densitometry.

Results / Discussion: XPS analysis showed increasing hydroxamic acid derivatization (by N content) with increasing initial methacrylic acid content of the microspheres. In general, approximately 20% of the surface methacrylic acid groups were converted to hydroxamic acid.

Activity measurements of MMP-2 solutions after exposure to MI Theramer microspheres and buffer extracts from MI Theramer showed no inhibition by the extract. This indicates that the inhibitory effect of the material is not a result of an extractable, soluble compound but rather the material itself. In addition, a dose-dependent inhibition of MMP-2 activity was measured for MI Theramer™ beads (Figure 1). The MMP-inhibitory effect of the beads was also demonstrated in human chronic wound fluid extracts.

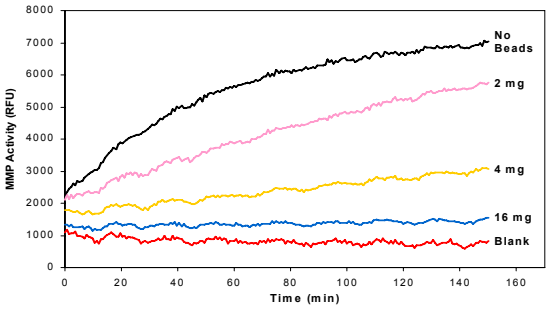

Figure 1 - Increasing inhibition of MMP-2 with increasing MI Theramer mass incubated in a model MMP-2 solution. Measured by gelatin-FITC assay.

Examination of crosslinked gelatin tubes after explantation at 7 days post-implantation showed marked differences depending on the presence of MI Theramer (Figure 2). Gelatin tubes co-implanted with MI Theramer microspheres maintained their pre-implanted appearance and mechanical integrity while the gelatin tubes implanted without MI Theramer developed a marked opacity and diminished mechanical integrity. Densitometric analysis of gelatin zymograms of extracted wound fluids also showed significant reductions in both the pro- and active forms of MMP-2 and MMP-9.

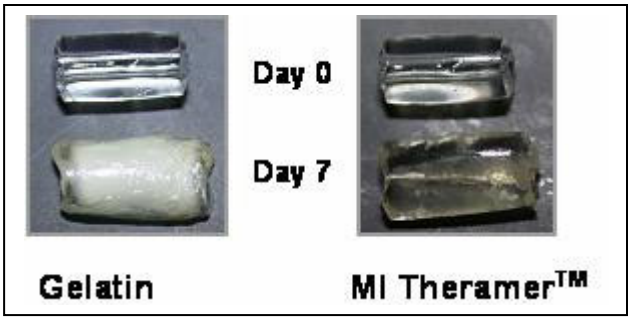

Figure 2 – Photographs showing visual differences in explanted gelatin tubes at day 7 with (picture on right) and without (picture on left) co-implanted MI Theramer™.

Conclusions: MI Theramer™ beads are able to inhibit MMP acitivity and reduce solution MMP concentrations in both model solutions and human wound fluid extracts. The inhibitory activity is a result of MMP binding to the MI Theramer™ rather than the release of any soluble compounds that may have undesirable systemic effects. The MMP inhibitory capacity of MI Theramer™ has a demonstrated in vivo functional effect (i.e. reduction in degradation of implanted gelatin) that is expected to translate into the promotion of chronic wound healing.