In Vivo Evaluation of Enzymatically Degradable Thiol-ene Hydrogels Formed *in situ* Designed to Promote and Accelerate the Natural Wound Healing Response

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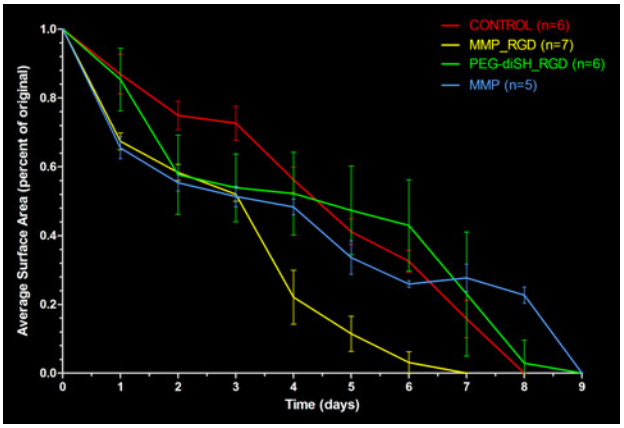
Statement of Purpose: Enzymatically degradable hydrogels that can be formed *in situ* provide an excellent opportunity to promote and accelerate the natural wound healing process. By applying a degradable hydrogel designed to serve as a synthetic extracellular matrix to the wounded area, native cells are presented with an immediate functional scaffold to which they can adhere, infiltrate, remodel and degrade over the course of normal wound healing. Thiol-ene hydrogels were identified and selected for this study because of their exceptional biological compatibility and unique coupling chemistry, which occurs between a thiol (R-SH) containing molecule and a carbon, carbon double bond "ene". Thiol-ene chemistry provides exceptional flexibility and the ability to incorporate additional bioactive and/or bioresponsive components into the final matrix.

Methods: Degradable and non-degradable thiol-ene hydrogels were designed to promote and accelerate full thickness wound healing. 4-arm-PEG-tetra-norbornene (ave MW 20kDa) was used as the "ene" component in all hydrogels tested. The "thiol" containing monomer varied based on experimental group. Degradable gels incorporated a dicystine MMP degradable peptide cross linker (KKCGGPQGIWGQGCKK), whereas HS-PEG-SH (ave MW 3400 Da) was used as the crosslinker in non-degradable gels. In addition a short bioactive peptide, fibronectin mimic CRGDS [250 uM] was co-polymerized into degradable and non-degradable hydrogels via thiol-ene chemistry. Monomers were prepared in a 1:1 stoichiometric ratio with respect to "thiol" and "ene" function groups. 10 wt% monomer solutions were prepared in PBS with 0.1 wt% photoinitiator 4-(2-hydroxyethoxy) phenyl-(2-hydroxyl-2-propyl)ketone (I2959 Ciba). Hydrogels were photopolymerized *in situ* using a UV lamp (365 nm, 6.3 mW/cm^2 for 3min).

Four full thickness wounds (d = 5mm) were made on the back of Swiss Webster female mice (Chales River Inc). Thiol-ene monomer solutions were applied to wounded areas and photopolymerized *in situ*. Study groups are as follows: MMP degradable gel, MMP degradable gel (+) CRGDS, HS-PEG-SH non-degradable gel (+) CRGDS, control group received no treatment. Quantification: Wounds were measured (max x and y diameter) daily. Mice (n=2) from each group were sacrificed at days 3, 5, 9; wounds were excised for histological analysis. An exclusion criteria was applied on a per animal basis due to excessive chewing and scratching of wounds.

Results: All hydrogels were successfully polymerized *in situ*, adhered and remained in place until day 4-6 post wounding. No visible signs of inflammation or irritation were observed throughout the duration of study and all mice healed within 9 days of surgery.



In the figure above, wound healing (average unhealed area expressed as percent of original wound area) was tracked vs. time for all experimental groups and control. In the untreated control, wound margins contracted readily during initial scab formation (day 1-2), followed by a plateau period with little change in the exterior dimensions of the wound. Day 3-5 represent a second increase of visible rate of wound contraction; here the initial scab is lost exposing healed and unhealed skin. Following loss of the initial scab, any unhealed tissue experiences a secondary scabbing event (day 5-6). The secondary scab, continually contracts (day 6-8) until the wound is completely healed (day 8). Wounds treated with a degradable hydrogel that incorporated a cell adhesion peptide (MMP_RGD) healed significantly faster than control. Interestingly only 25% of the original unhealed wound area remains when the hydrogel treatment was shed (MMP_RGD, day 4), in comparison 45% of the wound remains unhealed in the control when the initial scab was lost (Control, day 5). Alone, inclusion of a cell adhesion peptide or use of a degradable network was not sufficient to promote or accelerate wound healing (PEG-diSH_RGD, MMP). Histological analysis of the cellularity and extraellular matrix of the wound environment is underway and should provide insight into the influence of the material chemistry on the cellular aspects of the wound healing mechanism.

Conclusions: Two fundamental requirements for biomaterials designed to promote wound healing have been identified. In addition the ability to tailor thiol-ene hydrogels to meet these needs is demonstrated. Only when a hydrogel containing a cellular adhesion peptide was combined with a MMP degradable cross linker was a significant increase in the rate of wound healing observed. This observation relates to the need for native cells to be able to adhere, infiltrate, remodel and degrade the synthetic matrix, in order to promote and accelerate the natural wound healing process.

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