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Delivery of a Novel Connexin-43 Mimetic Peptide Enhances Wound Healing

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The ability to safely and quickly close wounds and lacerations is an area of need in regenerative medicine, with implications toward healing a wide range of tissues and wounds. Using a number in vivo injury models, our lab has investigated a newly developed peptide capable of promotion of wound healing and epithelial regeneration. The alpha-carboxy terminus 1 (αCT1) peptide is a 25 amino acid peptide from the C-terminus of connexin 43 (Cx43), modified to promote cellular uptake. Previous studies applying αCT1 to excisional wounds in porcine models produced tissues having an overall reduced level of scar tissue and decreased healing time. Early studies in our laboratory applying αCT1 to excisional wounds in rats resulted in tissue having an overall reduced level of scar formation and decreased healing time, compared to controls. Both αCT1 alone and in combination with bone marrow stromal cells (BMSCs) were used in the treatment of excisional wounds. The interplay between the two agents was investigated and demonstrated that stem cells in addition to αCT1 proved to be very effective in closing wounds quicker and with less scar tissue present (Fig.1).

Recently we have investigated methods of enhancing the delivery method of the αCT1 to various wounds. Microencapsulation of the αCT1 peptide using an alginate polymer provides a novel time-release formula for the peptide. In our rat corneal wound model, we delivered αCT1 both directly, in a concentrated pluronic solution, and in a sustained system, using polymeric alginate-poly-l-ornithine (A-PLO) microcapsules. Cell toxicity analysis showed minimal cell-loss with microcapsule treatment. Measurement of wound healing using histology and fluorescence microscopy indicated significant reduction in healing time of αCT1 microcapsule treated rat corneas compared with controls (88% vs. 38%). RT-PCR analysis of treated corneas showed an initial up regulation followed by down regulation of the gene keratin-19 (Krt19). Zonula occluden 1 (ZO-1) showed an opposite down regulation followed by an up regulation whereas Cx43 showed a biphasic response. Inflammatory indexes demonstrated a reduction in the inflammation of corneas treated with αCT1 microcapsules when compared with pluronic gel vehicle (Fig. 2). Finally, we employed a scratch wound assay to investigate the mechanism of action of αCT1 peptide. We examined scratched BMSCs for a number of genes involved in wound healing. Krt19 was again upregulated in contrast to Cx43, ZO-1, Snail 2, and JamA which all showed a down regulation of transcript expression. In total these results begin to lay the foundation for future therapeutic application of αCT1 as a beneficial wound healing treatment.

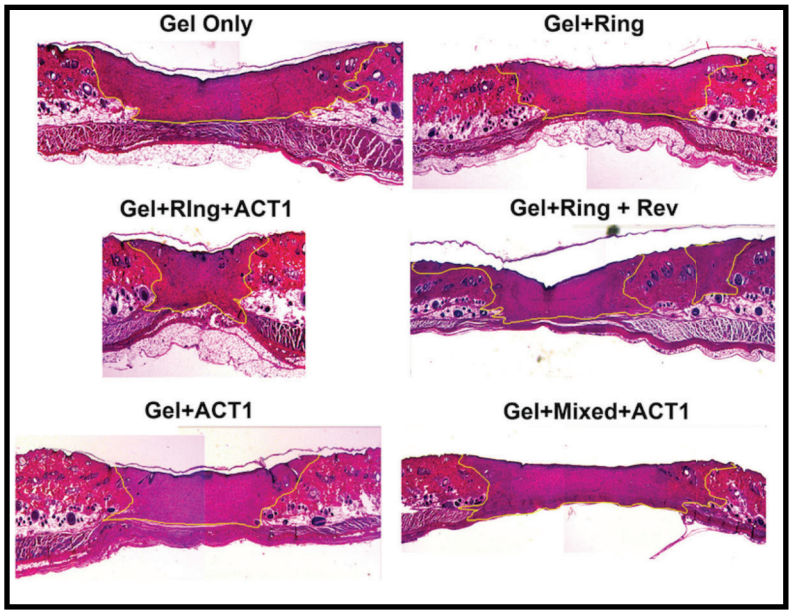

Figure 1. Excisional skin wounds 30 days post surgery stained for the presence of scar tissue. Skin wounds were treated with a regime of αCT1 alone and in combination with BMSCs. The treatment of αCT1 in combination with Stem cells grown to form a toroidal (ring) showed significant improvement compared with the other treatments.

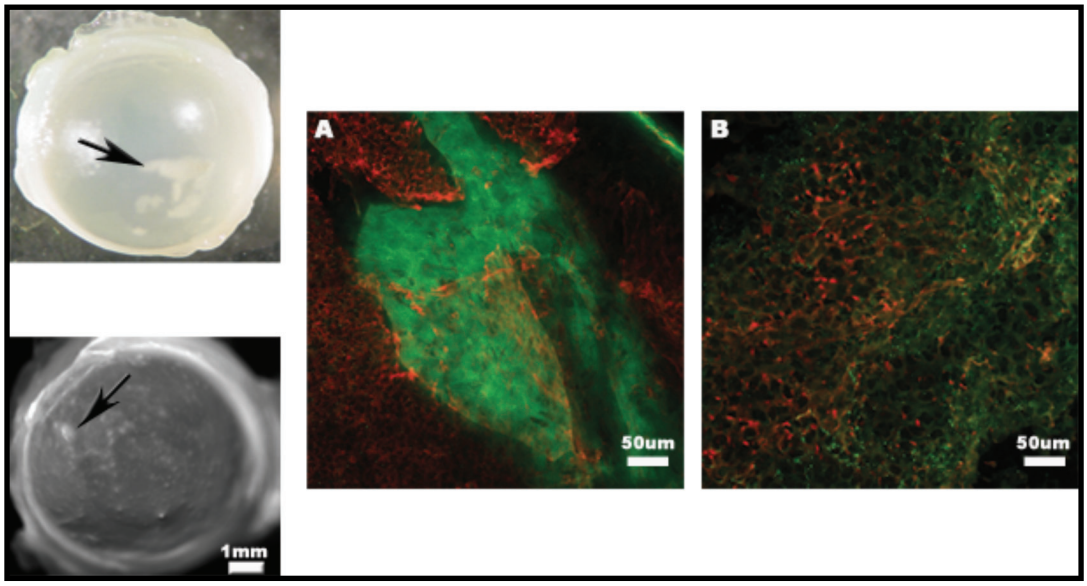

Figure 2. The inflammatory response of the rat cornea to the ACT1 A-PLO microcapsule treatment. Images are shown in both the upper left and lower left of the figure showing two representative portions of the affected tissue of the cornea at 30 days. (**A**) Confocal image at a 20x magnification of the affected tissue in a 3 day microcapsule treated cornea. Green-TNF-α, Red-smooth muscle actin. (**B**) 20x magnification of the affected tissue in a 3 day microcapsule treated cornea. Green-ITAC, Red-smooth

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