Controlled Delivery of HB-EGF Via Coacervate Accelerates Healing of Diabetic Wounds

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Statement of Purpose: In pursuit of a regenerative therapy for diabetic wounds, we developed a heparin-based coacervate delivery system to provide sustained release of heparin-binding EGF-like growth factor (HB-EGF) within the wound bed. We evaluated this therapy in full-thickness excisional wound model using polygenic type 2 diabetic mice. We found the HB-EGF coacervate to enhance numerous processes in the wound healing cascade, including re-epithelialization, granulation tissue deposition, wound contraction, and angiogenesis. These results demonstrate the potential of controlled growth factor delivery as a promising therapy for diabetic wounds.

Methods: PEAD was synthesized as previously described. The coacervate was loaded with fluorescein and imaged by fluorescence microscopy. Heparin was initially combined with recombinant human HB-EGF, then PEAD was added to form the coacervate. HB-EGF release from the coacervate was determined using ELISA. The effects of the HB-EGF coacervate on normal and diabetic keratinocytes were assessed using an in vitro scratch wound migration assay. Full-thickness excisional wounds were made on the backs of NONcNZO10/LtJ mice (Jackson Labs) using a 5mm round skin biopsy punch. Three groups were tested: Saline, 1µg Free HB-EGF, and 1µg HB-EGF coacervate. Treatments were applied immediately after wounding in 10µl saline. Animals were sacrificed on day 3, 7, 14, and 28 (n=6-8 wounds). Wound re-epithelialization was assessed by immunofluorescence (IF) staining for cytokeratin. Keratinocyte proliferation in vivo was evaluated by IF co-staining for Ki-67 and cytokeratin. Collagen deposition was analyzed in Picosirius Red stained wound sections by polarized light microscopy. Wound contraction was measured in H&E stained wounds. Neo-vascularization was observed by IF co-staining for vWF and α-SMA.

Results: PEAD is a strong polycation containing arginine linked to a biodegradable polyester backbone. When combined in saline, PEAD and heparin self-assemble into micron-sized liquid coacervate droplets. HB-EGF is incorporated into the coacervate by pre-binding to heparin at a loading efficiency greater than 92% and release is slow and sustained over 10d (data not shown). Diabetic human epidermal keratinocyte (DHEK) migration was inhibited compared to normal keratinocytes (NHEK), however this deficiency was rescued by 10ng/ml HB-EGF. At higher concentrations, free HB-EGF oversaturated cell receptors, resulting in no significant effect on DHEK migration. However the coacervate reduces the concentration of HB-EGF cells are exposed to and thus significantly stimulated cell migration at these high doses (data not shown).

HB-EGF coacervate resulted in significantly better healing outcomes of diabetic wounds compared to saline control and free HB-EGF in the following analyses: (1) Increased keratinocyte migratory capacity observed in vitro manifested as accelerated re-epithelialization on day 3 and 7 (Fig. 1A). (2) Wound contraction was increased on day 14 and 28 (Fig. 1B). (3) Proliferative capacity of migrating keratinocytes was maintained (Fig. 1C) and significantly more proliferating cells were observed in the basal epidermis (Fig. 1D). This is agreement with the effect of delivered HB-EGF on keratinocyte proliferation in vitro previously reported. (4) Collagen deposition in the granulation tissue was increased on day 14 and significantly enhanced by day 28 (Fig. 1E). (5) Stable collagenous matrix allowed infiltration of vascular cells and resulted in improved mature vascularization of the neo-dermis by day 28 (Fig. 1F).

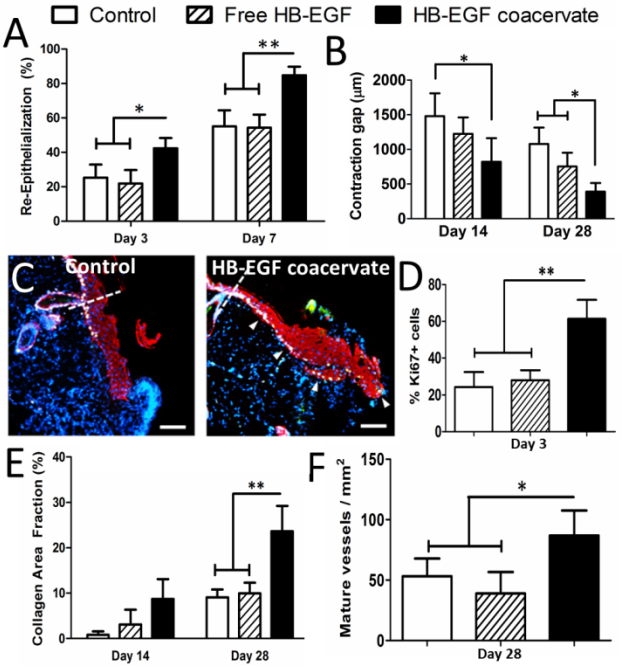

Figure 1. A) Percent re-epithelialization on day 3&7. B) Contraction gap. C) Co-staining of cytokeratin (red) and Ki-67 (green) on day 3. Bars=100µm. D) Ki-67+ keratinocytes in neo-epidermis. E) Percent area positive for collagen in the granulation region. F) Number of mature vessels in the neo-dermis. *P<0.05, **P<0.01.

Conclusions: The cocacervate effectively loads, protects, and releases HB-EGF slowly over a relevant timeframe. HB-EGF coacervate can stimulate human keratinocytes to overcome the phenotypical detriment of their diabetic condition. HB-EGF coacervate improved multiple healing outcomes of diabetic wounds, including re-epithelialization, collagen deposition, wound contraction, and re-vascularization.