Accelerated Re-epithelialization of Skin Wounds Using Epidermal Growth Factor Coacervate

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Statement of Purpose: Growth factor therapies are highly investigated for accelerating the body's natural wound healing process; however, clinical translation is often severely limited by mode of delivery. Heparin-binding EGF-like growth factor (HB-EGF) is directly involved in the process of re-epithelialization. It induces keratinocyte proliferation and migration into the wound site and is also implicated in fibroblast motility and granulation tissue formation. We have developed a unique growth factor delivery system comprised of a synthetic polycation (PEAD) and native heparin which self-assemble by charge attraction to form a liquid coacervate. The coacervate loads HB-EGF, protects it from degradation, and slowly releases it over time. The objective of this work was to test the ability of controlled delivery of HB-EGF using the coacervate to promote wound closure in a splinted rodent model that mimics human wound healing by re-epithelialization.

Methods: PEAD was synthesized as previously described (Chu H. PNAS. 2011;108:13444–49). Heparin was initially combined with HB-EGF, then PEAD was added to form the coacervate. The coacervate was loaded with fluorescein and imaged by fluorescence microscopy. HB-EGF release from the coacervate was determined using ELISA. The effects of the EGF coacervate on cell migration and proliferation *in vitro* were assessed using primary human epidermal keratinocytes. The HB-EGF coacervate was then evaluated in a splinted full-thickness excisional wound model using C57BL6/J mice. Four groups included Saline, Vehicle (coacervate alone), Free HB-EGF, and HB-EGF coacervate. Analysis included gross wound closure over 17 days, H&E staining for general histology, cytokeratin IHC staining for epithelialization, and MTS staining for collagen content in granulation tissue. IF staining of wounds after 7 days was performed for markers of cell proliferation, Ki-67, and cell migration, β4 integrin. Angiogenesis in the granulation tissue after 17 days was evaluated by IF staining for CD31 and α-SMA.

Results: Fluorescent imaging revealed that the coacervate contained spherical droplet diameters ranging from 10-500nm. Release of HB-EGF from the coacervate was slow and sustained for at least 10 days with low initial burst release. Free and coacervate HB-EGF significantly accelerated keratinocyte migration in scratch wounds *in vitro* (Fig.1a). The coacervate also mitigated the inhibited proliferation seen at high concentration of free HB-EGF (Fig.1b). HB-EGF coacervate accelerated closure of mouse wounds at all timepoints while free HB-EGF showed no difference from controls (Fig.1c). In HB-EGF coacervate-treated wounds, high collagen content in granulation tissue was observed after 7 days and a thick, protective epidermis. Co-localization of proliferation and migration markers well beyond the wound margin indicated that migrating cells still retained their proliferative capacity (Fig.1d). A notably greater presence of endothelial cells was observed, some co-localized with mural cells indicating nascent vessel formation (Fig.1d).

Conclusions: Controlled delivery of HB-EGF significantly accelerated wound closure within 17 days by comprehensive healing which included expedited re-epithelialization, improved granulation tissue formation, and angiogenesis. Epithelial cell migratory capacity was improved and proliferative capacity was uninhibited. These results suggest that coacervate-based delivery of HB-EGF may serve as a new therapy for accelerating the healing of cutaneous wounds. Future work includes a diabetic rodent model as well as a porcine model.

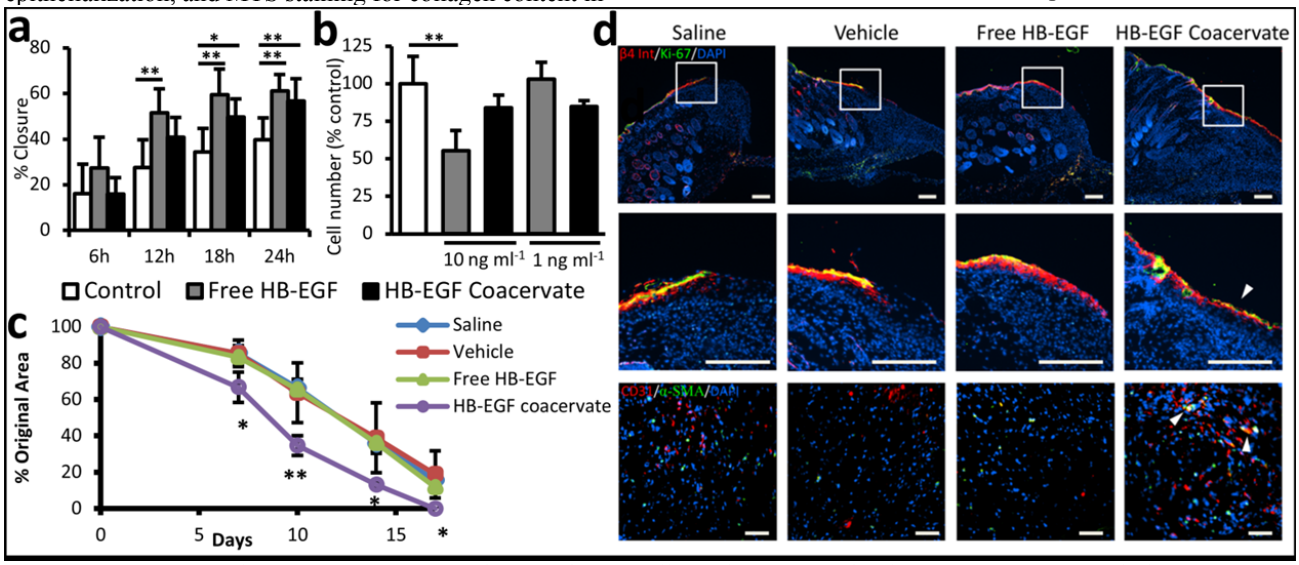

Figure 1. a) Percent closure of *in vitro* scratch wounds. **b)** Cell number after 4 d culture. **c)** Mouse wound closure over 17 days. **d) Row 1-** IF staining for integrin β4 (red) and Ki-67 (green), **Row 2-** High magnification of Row 2, **Row 3-** IF staining for endothelial cells (CD31; red) and mural cells (α-SMA; green). Co-localization: red+green=yellow. (*p<0.05, **p<0.01)