Title: Human Breast Milk Enhances Cellular Proliferation in Cornea Wound Healing

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Purpose: Corneal epithelial defects from ulcers, trauma, or surgery heal as new epithelial cells grow centripetally from the limbus and replenish the epithelium. Corneal wound healing requires cell signaling molecules. However, a topical treatment with these components is not available. Human breast milk (HBM) offers a potential, novel treatment as it contains bioactive molecules important in epithelial cell healing. This study seeks to investigate the potential of HBM in cornea wound healing.

Methods: Balb/C mice, 8-12 wks old, were anesthetized prior to creating a 2mm central cornea epithelial defect. Mice were randomly assigned to a treatment group: HBM, ophthalmic ointment containing neomycin, polymyxin B, dexamethasone (RxTx), or saline and treated 4x/day for 2 days. Wound area was quantified by fluorescein and ImageJ at 0, 8, 24, and 48h post wounding and eyes used for histology, RT-qPCR, and ELISA.

Results: Wounded corneas treated with HBM demonstrated increased re-epithelialization at 8h post injury compared to RxTx and saline treatments. ELISA showed significantly higher Ki67 in HMB treated eyes vs. saline control at 8h (p=0.0278). Additionally, immunohistology revealed more Ki67 positive cells in the HBM group compared to saline at 8h and 24h (p=0.0063 8h; p=0.00072 4h). For inflammatory analysis, HBM group IL-1β levels were similar to the saline group, and higher than RxTx treated eyes (p<0.05). Immunohistochemical staining for CD11b (macrophage marker) revealed HBM-treated eyes had significantly more positive cells vs. saline. RT-qPCR of limbal stem cell markers (LESCs) revealed upregulation of Integrin αV at 8h with HBM vs. saline.

Conclusions: HBM treatment on corneas with debridement of epithelium demonstrated improved healing, cellular proliferation, and upregulation of the LESC gene transcript, integrin αV, after wounding. Future studies could investigate LESC response to different signaling molecules in HBM to better understand the efficacy of this potential therapy.