(LR-002) Natural Biomaterial-based Dressing for Chronic Wound Healing

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Abstract

Introduction: Diabetic chronic wounds are decreasing the quality of life for millions as the number of diabetic patients is increasing worldwide. In terms of accelerating diabetic wound healing, chitosan has proven to be effective to decrease inflammation, increase fibroblast proliferation, and possess hemostatic properties, which are compromised in diabetic wounds. As a consequence, diabetic conditions can lead to chronic and non-healing skin wounds, and further complications such as osteomyelitis and amputation. Current treatments include debridement, topical platelet-derived growth factor treatment, wound dressings, negative pressure, and hyperbaric chamber therapy. We developed a relatively low-cost polyelectrolyte complex (PEC) film dressing made of chitosan and polygalacturonic acid (PgA), and tested it for its ability to accelerate diabetic wound healing in a diabetic animal model.

Methods: Chitosan and PgA solutions were prepared overnight at 37°C, and combined in a 60:40 mass ratio. The mixture was sonicated and then poured into a circular mold to dry (41°C) in ambient air for 32-36 hours. Following hair removal, full-thickness wounds, 10 mm in diameter, were created on the dorsum of diabetic male BKS. Cg-Dock7m+/+ Leprdb/J mice. The wounds were covered with a piece of PEC film that extended a few millimeters outside the wound area. Control animals received fibrin gel instead. All wounds were covered with Tegaderm and monitored. Wounds were photographed at regular time intervals and sacrificed at the endpoint on post-wounding day 42. Wound tissue samples were collected for histological analysis.

Results: In vivo testing showed that PEC films accelerated diabetic wound closure, such that on post-wounding 42-day, PEC-treated wounds had fully closed, while controls treated with fibrin gel were only 50% closed. In addition, hair regrowth was accelerated in skin regions under the PEC films, as compared to fibrin gel controls.

Discussion: In summary, the PEC film was shown to accelerate both the closure of full-thickness wounds in a diabetic mouse model, as well as hair regrowth around the scar area. Therefore, the PEC film made of chitosan and PgA is a promising avenue that warrants further study for the treatment of diabetic wounds.

Methods and Materials

Making the Film

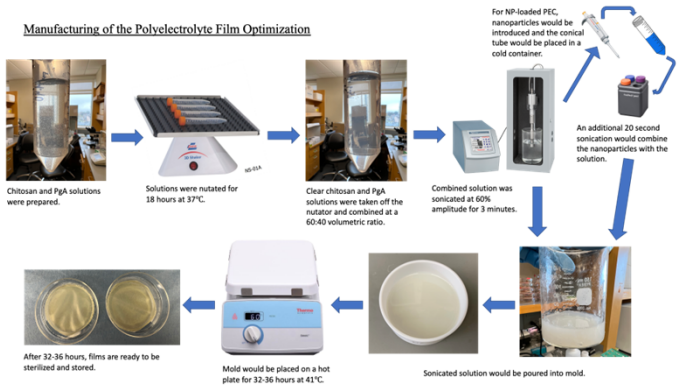

Figure 1: Schematic shows the progressions of steps followed to manufacture Chi-PgA PEC films. PgA = polygalacturonic acid, NP = nanoparticle, PEC = Polyelectrolyte complex

In Vivo Study

To investigate the effects of Chi-PgA PEC films and Chi-PgA PEC films as a delivery mechanism for vRAGE-ELP in vivo, 10 mm full-thickness wounds in diabetic mice were treated topically with fibrin gel (FT Gel), fibrin gel with vRAGE-ELP (FT+ vRAGE-ELP), PEC film (PEC), and PEC film with vRAGE-ELP (PEC + vRAGE-ELP) (**Figure 2**). Each mouse was photographed at day 0, day 3, day 7 and every 7 days after this point for 6 week. Images were analyzed using ImageJ and wound closure percentage was calculated and graphed. After the 6 week study, the mice were sacrificed and tissue samples were collected and sent to Rutgers University Pathology Services.

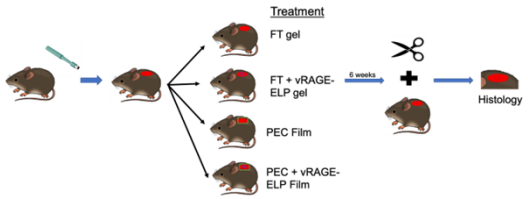

Figure 2: Schematic of the wound closure study.

Results

Figure 3: a. Wound Closure Over Time b. Wound Closure Percentage Over Time

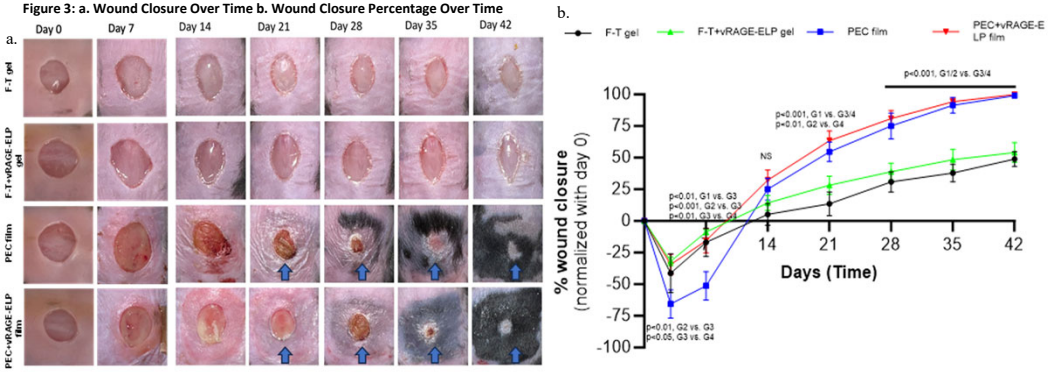

Figure 3: a. Representative images show the skin wounds in diabetic mice over 42 days at weekly time points. PEC film treatments with or without nanoparticles are observed to accelerate wound closure in diabetic mice, especially around day 21. The blue arrows indicate wound contraction/closing for the PEC groups. The appearance of hair is also observed starting day 21 in PEC film and PEC+vRAGE-ELP groups only. By day 42, PEC film and PEC+vRAGE-ELP Film groups have healed wounds while the 2 gel groups still have open wounds. **b.** Shows wound closure percentage over time in which mice treated with PEC film and PEC film + vRAGE demonstrated significantly faster wound closure by day 21. Data are represented as mean ± SEM (n=6/each group). Data are analyzed statistically using repeated measures of two-way ANOVA followed by post hoc Tukey's HSD test. G1-F-T gel; G2-FT+vRAGE-ELP gel; G3-PEC film; G4- PEC+vRAGE-ELP film; NS-not significant.

By day 35, the two PEC groups (PEC and PEC+vRAGE-ELP) had over 90% wound closure while the two gel groups (FT gel and FT gel+vRAGE-ELP) were only about 37-48% closed at the same time point. On day 42, both PEC groups had wound closures of 100% while the fibrin gel group was closed 50% and the FT gel + vRAGE-ELP group, 54%.

Figure 4: a. Histology Samples Post-Wound Closure Study b. Effect of PEC Films on Hair Growth

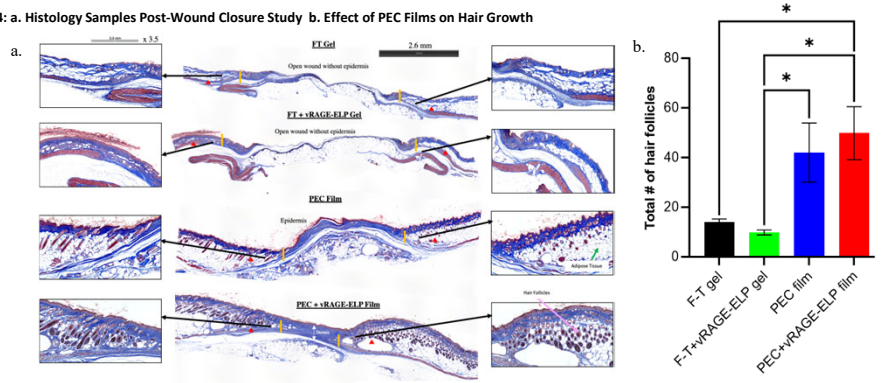

Figure 4: a. Images show histological samples of the wound post 6 week wound closure study. The scale for larger images in the middle is 2.6mm represented by the black bar while the scale for high magnification images is 900 um represented by the gray bar. **b.** Graph shows the effect of PEC films on hair growth in diabetic wounds. The total number of hair follicles in increased significantly from the FT gel group as compared to the PEC ($p<0.05$) and PEC+vRAGE-ELP ($p<0.05$) as well as between the FT+vRAGE to the PEC ($p<0.05$) and PEC-vRAGE-ELP ($p<0.05$). Data Represented as mean ± SEM with n=6. *$p<0.05$

The addition of vRAGE-ELP in the gel and PEC groups does show a trend for slight improvement compared to their respective controls for both wound healing and hair growth but overall, the benefit was not significant.

Conclusions/Future Direction

In vivo, study results suggest that PEC film treatment was effective in accelerating wound healing and increased hair growth in diabetic mice as compared to the control treatment with histology images confirming this claim. Future studies should test with an increased dosage of vRAGE-ELP to confirm if vRAGE-ELP can contribute to accelerated diabetic wound healing in mice and provide results that correlate to previous studies done with this nanoparticle. In vivo testing of the PEC films can also be expanded with normal mice (non-diabetic) to potentially prove a treatment for all wound healing.

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