Crosslinking Agents For The Development Of An Infected Chronic Wound Model

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Introduction:

The development and characterization of chronic wound models in animals have historically been an active area of research [1]. While many of these published models have demonstrated a short-term delay in granulation and early wound closure, the time to total re-epithelialization is generally the same as acute control wounds, or delayed to a negligible degree. Furthermore, none of these models are "rescued" through surgical intervention/debridement, which is generally considered the standard treatment in human patients.

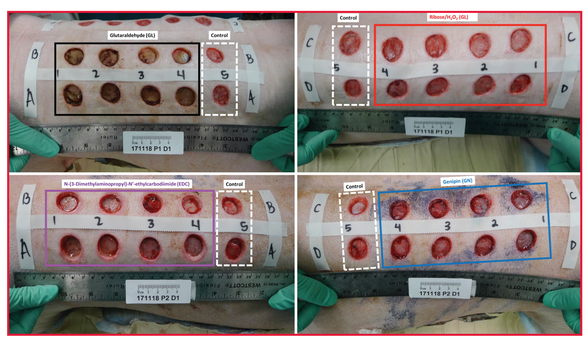
Our overall hypothesis is that animal models fail to generate chronic wounds because laboratory animals do not live long enough to accumulate the wide-spread connective tissue damage and crosslinking that occurs in humans through intrinsic and diabetic aging. With this hypothesis in mind, we created acute wounds and then simulated aging-related tissue damage through the short-term application of a chemical crosslinking agent. These prior studies demonstrated a ~350% delay in healing of 5% glutaraldehyde (GL) cross-linked wounds, which was correctable with surgical debridement [2].

Based on the established correlation of both wound duration and biofilm with wound chronicity, we have set out to challenge and expand the crosslinked chronic wound model by adding bacterial infection. We hypothesized that the cytotoxicity of GL would present a barrier to bacterial survival, therefore we screened 3 non-cytotoxic crosslinkers [3] for their potential effectiveness in preventing wound closure and maintaining a multispecies bacterial infection in comparison to 5% GL positive controls and non-crosslinked negative controls.

Methods:

On Day 0, twenty full-thickness 2cm dorsal wounds were created on the dorsum of two Yorkshire-cross pigs

- Eight wounds on the first pig were treated with 5% glutaraldehyde (GL) and 8 were treated with 1mg/mL ribose in 1% hydrogen peroxide (R-HP)
- On pig 2, 8 wounds were treated with >97% N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and 8 were treated with 1mg/mL genipin (GN). Crosslinkers were washed away with sterile saline prior to dressing
- Four untreated wounds on each pig served as untreated controls



On Day 1, all wounds were inoculated with 10^6 CFU/mL *S. epidermidis, P. aeruginosa* and *Fusobacterium* in a 1:1:0.5 ratio

Wounds were covered with non-adherent dressings and healing was measured with calipers on days 1, 4, 7, 11 & 13. Wounds were biopsied and selectively cultured on days 1, 4 & 13. to enumerate CFU/gram for total bacteria, *S. epidermidis*, and *P. aeruginosa*.

Results:

Wound Healing.

From Day 7 through the end of the study, the wounds treated with EDC and GL showed the greatest delay in wound healing compared to GN, R-HP & controls ($p<0.05$; ANOVA with Tukey post-hoc). Healing rates for EDC and GL were not significantly different from each other. GN treated wounds were delayed compared to R-HP ($p<0.05$) but were not significantly different from control. Treatment with R-HP did not result in healing rates different from untreated control wounds. See Figure 1

Wound Bioburden.

EDC-treated and GL wounds also showed the greatest levels of bacteria, with total bioburden and *Pseudomonas* reaching >8log at days 4 and 13, while *Staphylococcus* showed 7log at day 4 with reduction to 5log at day 13. Surprisingly, the expected cytotoxicity of the GL did not appear to negatively impact the growth of these two species in this model. A trend in relative bacterial predominance by *Pseudomonas* was observed in all wounds. See Figure 2

Conclusion:

Contrary to our hypothesis that GL would not allow the growth of bacteria in the crosslinked chronic wound model, persistence of both species was observed. These data show that both GL and EDC delayed healing better than GN and R-HP and maintained high bioburden of clinically relevant bacteria.

These data support the hypothesis that crosslinking and inoculation of the acute wound shows promise as a stable infected chronic wound model that may be useful for studies of debridement and other therapies.

Figure 1

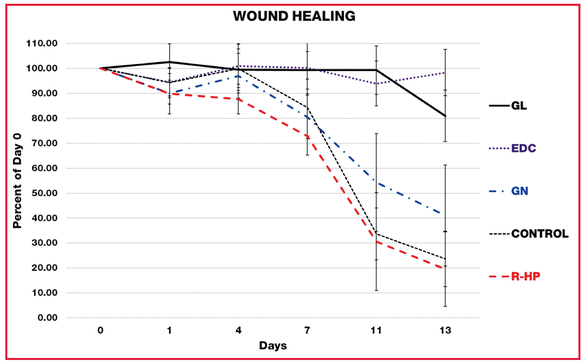
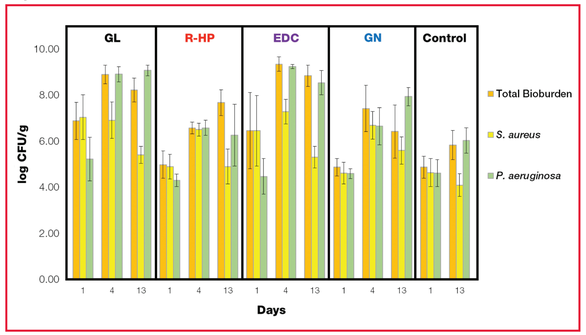


Figure 2



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