Silk Microneedle Formulation of a Non-lytic Peptide for Klebsiella pneumoniae Biofilm Eradication and Wound Infection Treatment

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Introduction

- *Klebsiella pneumoniae* is a dangerous pathogen that has gained much notoriety due to its extreme rate of resistance development.
- We have shown that proline rich antimicrobial peptides can decrease the capsulation of *K. pneumoniae* and disrupt the biofilms formed by these species and the potential of Bac7 (1-35) to decrease dissemination of hypervirulent *K. pneumoniae* from an *in vivo* biofilm wound infection.
- Silk fibroin-based biomaterials are approved by the US Food and Drug Administration (FDA) for delivery systems and are an understudied delivery vehicle for HDPs.
- The multiple formats of silk fibroin biomaterials increases its versatility as a vehicle for HDPs to allow for *in vivo* delivery through different routes, including both topical and systemic delivery.
- Importantly, the tunable biodegradability can be capitalized to allow for long-term HDP delivery, which is a major limitation impeding clinical applications. However, optimization of host defense peptide delivery vector release warrants iterative formulation testing using *in vivo* models.
- Here we reveal the microneedle patch and microparticle liquid formulation of Bac7 (1-35) using silk fibroin-based biomaterials to show efficacy against MDR *K. pneumoniae* using *in vitro* and *in vivo* delivery.

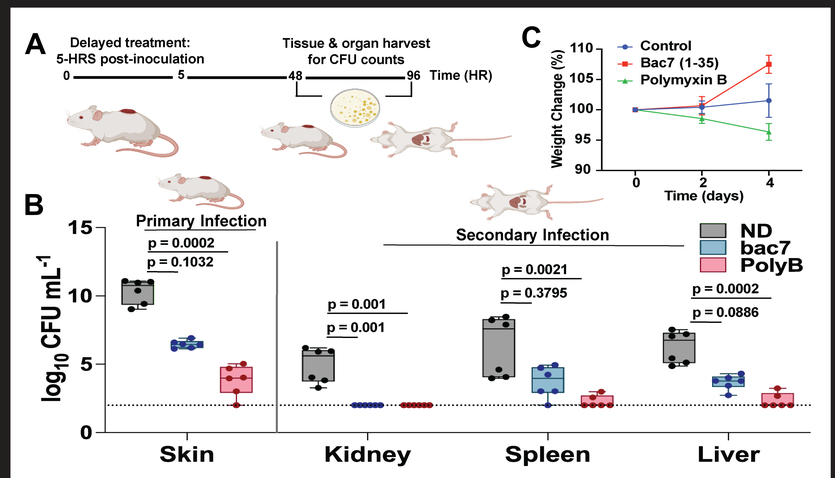

Figure 3. Bac7 (1-35) reduces disemination in an *in vivo* biofilm model. A murine skin abscess infection model was used to assess a single dose 5 hours after infection (**A**). Scarified skin areas were enumerated at day 2 and day 4 (only day 4 shown)(**B**). Mouse weight was recorded throughout the experiment (**C**). Significance determined using One-way Anova (n=6) with Dunnetts correction to obtain p-values.

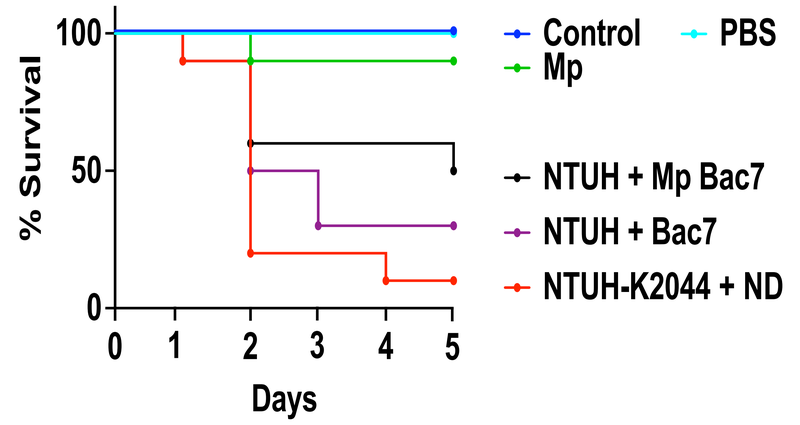

Figure 6. *Galleria mellonella* wax worm survival comparision of Bac7 and silk-fibrion microparticles formulated Bac7. The figure shows the percent survival of *Galleria mellonella* infected with 1 x 10^6 CFU hypervirulent Kp NTUH-K2044. Control groups recieved no injection, PBS groups recieved 5 µL 1x PBS or 5µL microparticles formulated with Bac7 to a final concentration of 5 mg mL^{-1}. The inoculated groups are labeled NTUH + treatment (No Drug= ND), microsphere formulated Bac7 (Mp Bac7), and Bac7 without formulation at 5 mg mL^{-1}. Survival was monitored for 5 days.

Results

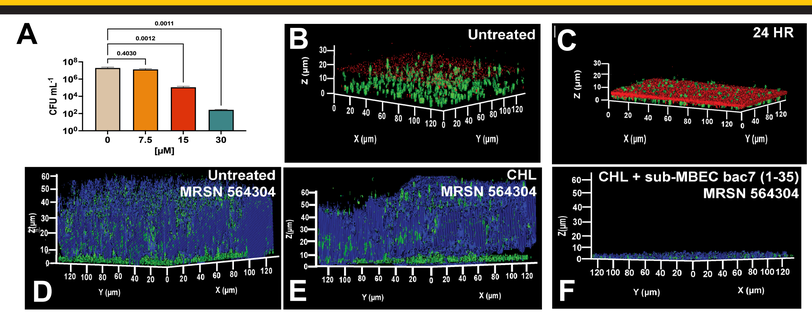

Figure 1. Biofilm disruption by PrAMPs. The figures show CFU/mL (**A**) with bac7 (1-35) treatment of biofilms and confocal z-stack images with GFP-expressing hvKp NTUH K2044 (green cells) stained with TxRed concanavalinA tagged lectin (red polysacchride) showing no treatment (**B**), and bac7 (1-35) 24 HR treatment (**C**). MRSN 564304 confocal z-stack image with SYTO9 (green cells) and calcofluor white (blue polysaccharide) with no treatment (**D**), 198 µM Chloramphenicol (CHL) (**E**), or 198 CHL + 7.5 µM bac7 (1-35) (**F**).

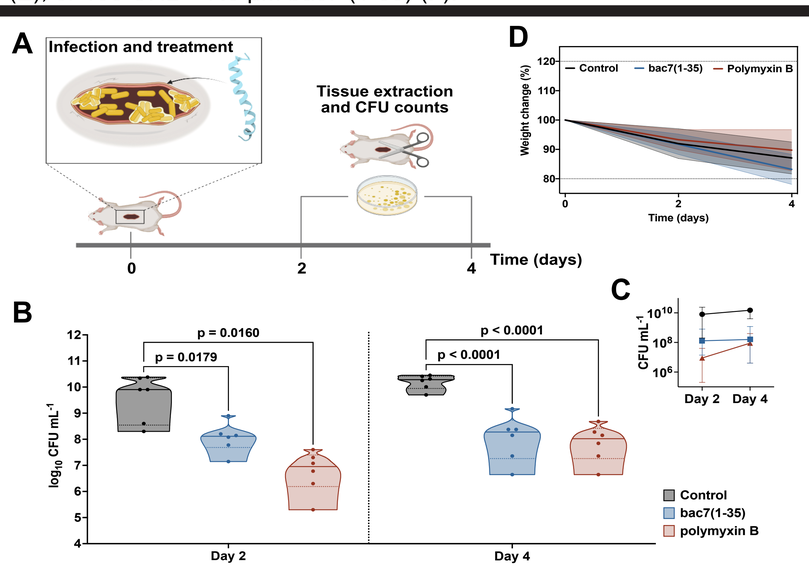

Figure 2. Bac7 (1-35) decreases viability in a acute wound model of infection. Procedure of skin abscess infection (**A**) Day 2 and 4 bacterial recovery from the skin abscess (**B**). Comparison between day 2 and day 4 recovery (**C**). Mouse weight changes over the 4 day experiment (**D**). Significance determined using One-way Anova (n=6) with Dunnetts correction to obtain p-values.

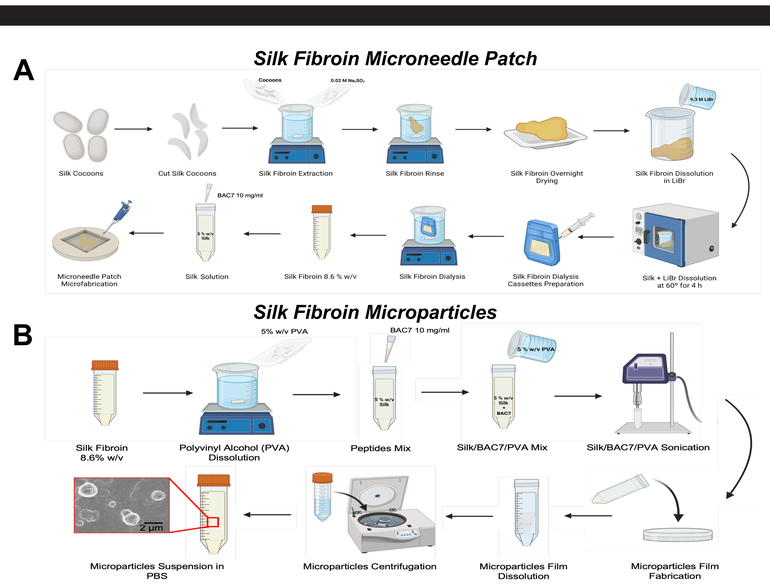

Figure 4. Formulation of Bac7 (1-35) in silk fibroin delivery vehicles. The figures show the process of encapsulating Bac7 into a microneedle patch (**A**) or microparticles suspended in PBS (**B**).

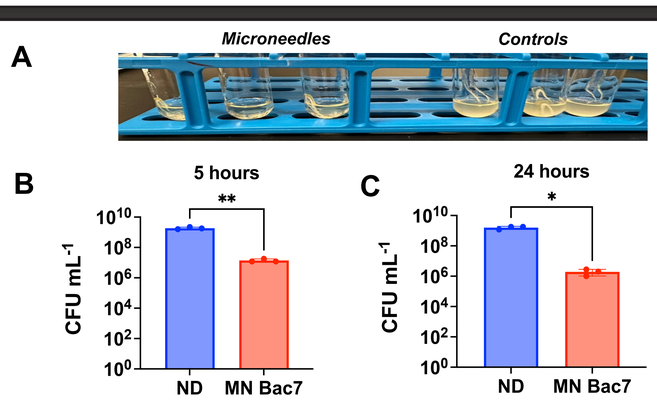

Figure 5. *In vitro* testing of silk-fibroin microneedles towards *K. pneumoniae*. The figures show the *in vitro* testing of silk fibrion microneedle formulation of Bac7. Microneedles were loaded with 600 µg of Bac7 and incubated in 2 mL of either Mueller Hinton Broth/PBS or MHB without buffering. Bacteria were inoculated at 1 x 10^6 CFU mL^{-1}. Aliquots were spot plated for enumeration at HR-5 and -24 and photos are showing 24-HR growth (**A**). Viability after 5- (**B**) and 24-HR (**C**) is graphed as CFU mL^{-1} with error shown as +/- SEM and p-values generated using T-test. * <0.01 and ** <0.001

Conclusions

- PrAMPs offer a solution for MDR *K. pneumoniae* showing *in vivo* biofilm disruption and decreased dissemination to the vital organs.
- We formulated our peptide for delivery using a microneedle patch and a microparticle liquid silk-fibroin biomaterial.
- We show *in vitro* the microneedles can release the encapsulated peptide to kill MDR *K. pneumoniae*.
- Formulation in silk fibroin microspheres reveals superior efficacy compared to the unformulated Bac7 (1-35) delivery to rescue *G. mellonella* from a leathal *K. pneumoniae* infection.
- Future work will be optimization of release of the peptide for prolonged delivery of *in vivo* MDR *K. pneumoniae* infections.

References

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