Ionic Liquids Deliver Antibiotic Into Skin and Neutralize Biofilm-Protected Pathogens

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Statement of Purpose: Treatment of bacterial biofilms has been identified as a serious and unmet medical need, as they account for ~80% of bacterial infections in humans and are 50-1000 times more resistant to antibiotics than their planktonic counterparts. Treatment is complicated further when biofilms develop in skin. This is because biofilms in skin are protected by the stratum corneum (SC), the outer layer of the skin, which significantly limits delivery of antibiotics, to the site of infection. The lack of treatment options is even more apparent for the treatment of infections in wounds where existing inflammation precludes the application of many topical formulations due to solvent induced irritation. In this work, we aimed to develop materials that could overcome the two main transport barriers to drug delivery for the treatment of skin infections – 1) the SC and 2) the bacterial biofilm, and provide compelling evidence for the use of Ionic Liquids (ILs) to safely enhance antibiotic delivery across skin and disrupt biofilms. Furthermore, we outline a comprehensive strategy for the rational-design and –selection of ILs for this purpose.

Methods: ILs are salts that are liquid at room temperature. The ability to modulate the cation and anion individually presents an advantageous framework for tuning multiple functions of the IL independently. A panel of twelve ILs was synthesized [1], applied neat on skin and biofilms, and assessed for anti-biofilm activity, dermal enhancement, and safety. ILs were rationally-designed by incorporating known chemical penetration enhancers. Transport of ^{3}H-cefadroxil (Moravek), an antibiotic, into skin was assessed using porcine skin in Franz diffusion cells. Biofilm activity was assessed using a modified MBEC plate assay. Potential for systemic toxicity was assessed by measuring cytotoxicity (LDH activity, Clontech) of ILs in dilute solution to normal human bronchial epithelial cells. Potential for skin irritation was measured using Epiderm tissues (MatTek). Clinical potential was validated using a biofilm-infected wound model performed by BioScience Laboratories Inc. The methods described allow rational- and iterative-screening of combinations of individual components for multi-functional material design.

Results: Transport of cefadroxil, an antibiotic, into skin was assessed, **Fig. 1A**. Specifically, IL-11, choline-geranate, enhanced delivery of antibiotic >16-fold into the viable tissue layers of the skin, where biofilm infections are likely to reside. Anti-biofilm activity was also assessed, **Fig. 1B**. In general, all ILs in the panel showed significant anti-biofilm activity against P. aeruginosa biofilms; moreover, several ILs, including IL-11, were more effective than bleach treatment. Surprisingly, however, ILs did not result in irritation typically observed with topical application of solvents or chemical penetration enhancers, **Fig. 1C**. Furthermore, IL-11, showed limited cytotoxicity (IC_{50} > 2 mM) suggesting toxicity in the event of systemic absorption, e.g. through an open wound, should be negligible. Clinical potential of the best candidate IL, IL-11 (choline-geranate), was validated in a biofilm-infected would model, **Fig. 1D**.

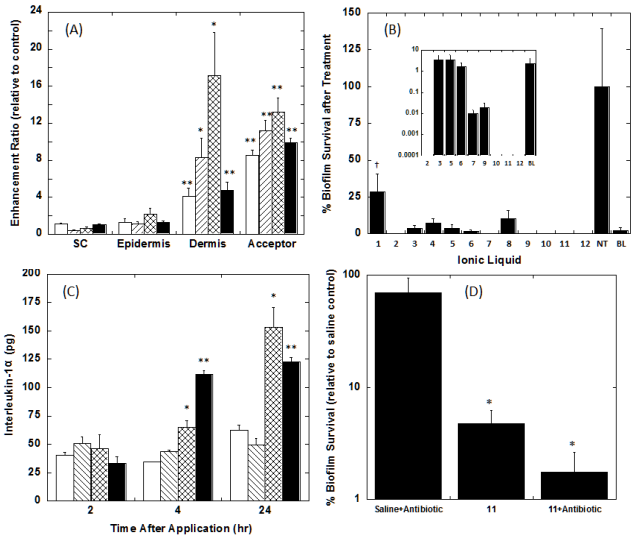

Fig. 1 – A: Enhancement of cefadroxil penetration into skin layers for four ILs; IL-11 (crosshatched). **B**: Anti-biofilm activity. Insert is log-scale. NT=no treatment; BL=bleach treatment **C**: Release of interleukin-1α, marker of irritation, from Epiderm tissues. PBS (open), IL-11 (hatched), CPE from IL-11 (crosshatched), 5% SDS (closed). **D**: Efficacy in a biofilm-infected wound model.

Conclusions: In this work, we provide a comprehensive strategy for the rational-design and –selection of ILs for biofilm disruption and enhanced antibiotic delivery into skin. Using this strategy, we demonstrate the use of ILs to reduce irritation of solvents and chemical penetration enhancers for topical drug delivery. Further, we identify a novel material (IL-11, choline-geranate) with nominal systemic toxicity and skin irritation potential, but with a dramatic ability to disrupt biofilms and treat biofilm infected wounds (>98% biofilm death after only 2 hours of treatment). This work validates the use of ILs for drug delivery, materials that show promise as a new arsenal against antibiotic resistant bacterial infections in skin.

References: [1] Zakrewsky M, et al. Proceedings of the National Academy of Sciences. 2014; 111:13313-8.