Multiplexing peptide nucleic acid therapies to destabilize bacterial biofilms in burn wound infections

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***Short Abstract* —The global health challenge of antimicrobial resistance (AMR) is exacerbated by biofilms, established bacterial colonies strengthened by diversified gene expression, tough extracellular matrices, and increased resistance to host and drug attack. Peptide nucleic acid (PNA)-based therapy is an intriguing alternative antimicrobial due to its high specificity, tolerance to degradation, and tunability. Single-target PNA and other specific small-molecule antibiotics apply evolutionary pressure that contributes to AMR. To mitigate AMR selection pressure, this work explores rational design of a PNA multiplex system targeting several fitness neutral biofilm genes to either disrupt or prevent a biofilm in an *in vitro* burn-wound model.**

***Keywords* — antisense therapeutic, peptide nucleic acid, multiplex, biofilm, burn wound model, antimicrobial resistance**

I. BACKGROUND & PURPOSE

ANTIMICROBIAL resistance (AMR) is emerging as a "silent pandemic," threatening modern treatments for bacterial infections around the world [1], [2]. While most therapeutics for bacterial infections are tested on planktonic bacteria, 60–80% of clinical bacterial infections form biofilms [3], [4]. Biofilms are organized bacterial colonies which show increased resistance to host immune responses and antimicrobial interventions as well as persistence that is characteristic of chronic wound infection, especially burn wounds [4], [5]. There is a crucial need for alternative, rationally designed therapies that address the unique challenges associated with biofilms.

Peptide nucleic acids (PNA) are gaining traction due to the molecules' tunable and highly specific sequence targeting as well as their tolerance to degradation [6]. There are many examples of efficacious PNA therapies targeting specific essential genes [7]–[9]; however, such specific targeting can deliver an evolutionary pressure similar to that of small-molecule antibiotics, thereby perpetuating conditions which foster AMR [9]. We investigate the rational design of PNA multiplex therapies targeting fitness neutral biofilm genes to either disrupt an established biofilm or prevent formation of biofilms by direct killing or resensitizing constituent bacteria to existing therapeutic interventions.

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II. METHODS & RESULTS

This work treats biofilms formed by clinical isolates of methicillin-resistant *Staphylococcus aureus, pseudomonas aeruginosa,* and multi-drug resistant *Escherichia coli* within an *in vitro* burn wound model. Burn wounds do not respond well to systemically administered antimicrobials because damaged vasculature impedes the achievement of therapeutic concentration at the wound site [5]. To ensure translatability, we maintain consistency with treatments for burns by covering mature biofilms with gauze coated in treatment-loaded ointment [5].

The severity of AMR infections demands that we explore new approaches and designs for smart therapies that are tested within the context of clinical infections. We will present biofilm viability data post-treatment to demonstrate efficacy of our multiplexed therapeutic against bacterial biofilms in an *in vitro* burn wound model to explore a new mediation strategy for chronic burn wound infections.

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