FibriLysins as next-generation endolysins targeting multicellular staphylococcal communities

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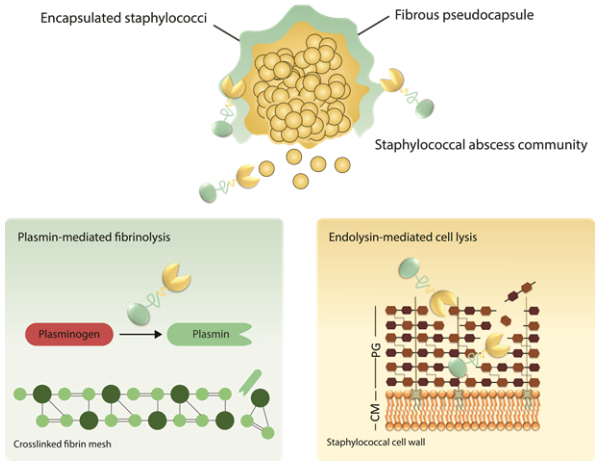
Besides being a commensal (30% of the population is a carrier), *Staphylococcus aureus* is also an abundant pathogen: half of the infections in high-income countries are attributed to antimicrobial resistance involve either *S. aureus* or *E. coli*. This complicated relationship has led to the development of several virulence factors enabling *S. aureus* to persist in the host, often resulting in the formation of multicellular communities such as staphylococcal abscess communities (SACs) or biofilms. These communities are difficult to treat with (the currently available) small-molecule antibiotics, amongst others due to a diverse metabolism of the bacteria in these communities as well as a physical barrier in which fibrin is a key compound. Therefore, we developed the FibriLysin concept. We fused endolysins, bacteriophage-derived peptidoglycan-degrading enzymes known to be performant against metabolically inactive cells, to a plasminogen activator. The latter converts human plasminogen into plasmin, leading to local fibrinolysis of the biofilm matrix or SAC, while the endolysin moiety is able to degrade the peptidoglycan layer, resulting in rapid osmotic lysis. Starting from a library of different variants, screening for these key activities allowed to select a lead variant, FL-028. The latter was tested in specific *in vitro* abscess and biofilm models showed promising results in which FibriLysins outperform the standard-of-care (vancomycin), resulting in a 3.4 log and 2.0 log reduction respectively. After elaborate toxicity screening, a fracture-related infection model in mice confirmed the strength of the FibriLysin technology as no multicellular staphylococcal communities could be



Schematic overview of the FibriLysin technology. By combining a plasminogen activator and an endolysin, multicellular communities can be completely disrupted.

retrieved in the FL-028 treatment group while bone healing was not affected. As a result, this technology has been extensively validated preclinically, and further steps include initial contacts with regulators to efficiently compose a regulatory dossier and pinpointing key data that might be lacking facilitating regulatory affairs.