Poly(N-acryloyl-D-aminoalanine) shows anti-*Staphylococcus aureus* biofilm activity using both *in vitro* and *in vivo* models

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Treating bacterial infections is becoming extremely challenging due to the rise of antibiotic-resistant pathogens. Many of these infections persist by forming biofilms, which are complex, three-dimensional structures that protects bacteria from immune defences and antibiotics. These biofilms are embedded in a self-produced matrix composed of polysaccharides, extracellular DNA (eDNA), proteins, and lipids, making them difficult to eliminate with standard antibiotics. Polymer-based strategies, such as poly(N-acryloyl-D-diaminopropionic acid) (PDap), have shown promise in disrupting *S. aureus* biofilms by directly targeting peptidoglycan (PG), an essential component of the bacterial cell wall.

To evaluate the antibiofilm activity of PDap's, 48 hour-cultured *S. aureus* biofilms were grown in tryptic soy broth (TSB) on titanium disks and then treated for three days with either vancomycin (50 mg/mL), PDap (200 mg/mL), or PDap loaded with vancomycin, with treatments refreshed every 24 hours. Bacterial viability was measured using colony-forming unit (CFU) assays, while biofilm integrity was analysed using Syto9 staining and confocal laser scanning microscopy (CLSM). Vancomycin alone had no effect on reducing bacterial CFUs, while PDap alone achieved a 2-log reduction in CFU ($p = 0.0255$). The highest bacterial reduction was observed with PDap loaded with vancomycin, which resulted in a 3-log CFU reduction ($p < 0.001$).

48 hours-old *S. aureus* biofilms grown on titanium disks were implanted subcutaneously in mice (two disks per animal). Experimental groups included: *a)* untreated, *b)* vancomycin (2 mg), *c)* PDap (600mg/ml), and *d)* PDap loaded with vancomycin. Treatments were administered as a single application directly onto the biofilm before closing the skin pocket. At the end of the study (day 4 post-implantation), the implants were processed for CFU and CLSM. Vancomycin treatment showed a 3-log CFU reduction ($p < 0.0001$), with similar reductions observed in the PDap group ($p = 0.0017$) and the PDap loaded with vancomycin group ($p = 0.0089$). CLSM imaging showed a substantial reduction in biofilm area for the vancomycin-loaded PDap group ($p = 0.0194$) compared to untreated controls, demonstrating that this combination not only disrupted biofilm structure but also enhanced antibiotic penetration, resulting in improved bacterial clearance.

These findings highlight the potential of PDap-based therapies, particularly when used in combination with antibiotics such as vancomycin, as an effective approach for treating biofilm-associated infections, including those caused by antibiotic-resistant *Staphylococcal* strains.