

Novel Combination Treatment of Protease, DNase I, and Antibiotics for Biofilm-Involved *Staphylococcus epidermidis* Infections

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Objectives

- Determine the presence of eDNA and eProteins in *Staph. epi.* biofilms
- Find optimal concentration of Protease and DNase I for biofilm degradation
- Develop a novel, effective, and clinically practical method using combination of Protease+DNase I+Antibiotics to treat biofilm-involved *Staph. epi.* infections
- Evaluate the effectiveness of the novel treatment method in enhancing the bactericidal effect of antibiotics

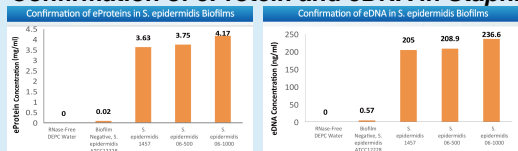
Materials

- Research conducted at Jiaotong University Renji Hospital, Shanghai, China
- Staph. epi.* 06-500 and 06-1000: 2 clinical isolates, biofilm-forming, methicillin-resistant
- ATCC12228 and *S.p.*1457 used as negative and positive controls, respectively
- Protease K, DNase I obtained in standard powder from YeaSen Bio. Shanghai
- Gentamicin, Penicillin, Vancomycin, and Linezolid from Sigma Aldrich

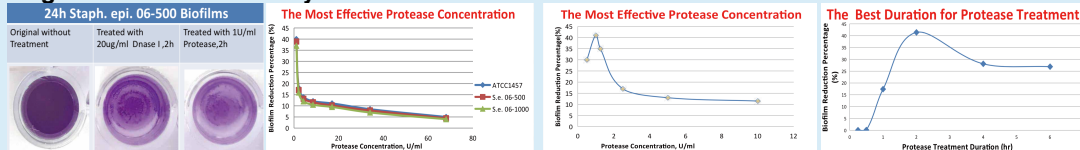
Procedures

- Biofilms grown in 96 well plates with TSB
- Microplate Reader, Synergy 2, at 570/630 nm wavelength used to semi-quantitate biomass of biofilms stained with 0.1% Crystal Violet
- Nanodrop 2000 for nucleic acid and protein quantitation
- Protease and/or DNase I for biofilm degradation trials
- Antibiotics were used in concentration of 50 x planktonic MIC
- 4 steps for eDNA and eProtein isolation: scrape biofilm, homogenize, centrifuge, and aspirate supernatant
- CFU determined by 10^7 serial dilution and plating of TSB agar

Confirmation of eProtein and eDNA in *Staph. epi.* Biofilms

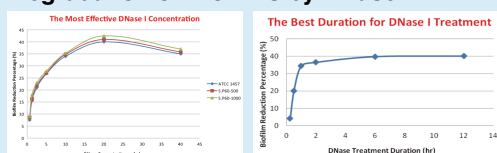


Degradation of Biofilms by Protease



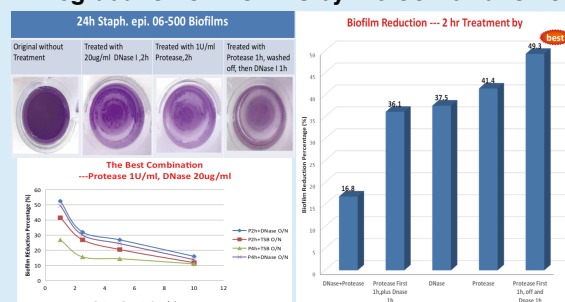
- Protease has visible effect on reducing *Staph. epi.* biofilm biomass
- Best Protease concentration for biofilm degradation is 1U/mL. As protease concentration increases, degradation efficacy decreases
- Best treatment duration is 2 hours

Degradation of Biofilms by DNase I



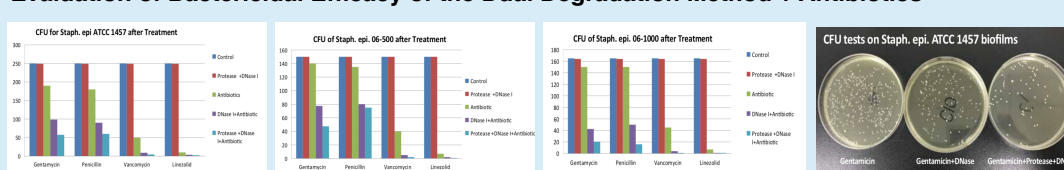
- The best DNase I concentration is 20 µg/mL
- The best duration is 12 hours

Degradation of Biofilms by the Combination of Protease + DNase I



- Biofilm reduction efficacy is ranked by "Protease First 1h, washing off and DNase 1h" > "Protease" > "DNase" > "Protease First 1h, plus DNase" > "DNase+Protease same time"
- "Protease First 1h, washing off and DNase 1h" has highest biofilm reduction percentage of 49.3, which is significantly higher than the other treatments ($p < 0.01$, Student's T-test)
- Adding DNase overnight significantly increases the efficacy of biofilm reduction ($P < 0.01$)
- Pre-treatment of protease (1U/ml) for 2 hours, washing off, and then adding DNase (20ug/ml) for 12 hours - the dual degradation method achieved the highest biofilm reduction percentage (52.5%).

Evaluation of Bactericidal Efficacy of the Dual Degradation Method + Antibiotics



- The combination treatment of the dual degradation method (Protease+DNase I) and antibiotics has the highest bactericidal efficacy. >91% for Vancomycin, >90% for Linezolid VS. antibiotics only ($P < 0.001$, paired T-test).

Discussion - Why Novel?

- Current treatment of biofilm-involved *Staph. epidermidis* infections relies solely on antibiotics - far less effective than the combination treatment method
- Use of Protease + DNase I + antibiotics to combat biofilm-involved infections has not been done before
- Determined effectiveness of combination method in treating biofilm-involved *Staph. epidermidis* infections

Conclusion

This study provides scientific data to support the effectiveness of the novel combination treatment (Protease + DNase I + Antibiotic) in treating biofilm-involved *Staph. epi.* Infections by reducing biofilm biomass and enhancing the bactericidal efficacy of antibiotics. It will enable healthcare personnel to more successfully counter such infections.