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# Human beta-defensin 3: a novel inhibitor of *Staphylococcus*-Produced biofilm production. Commentary on "Human β-defensin 3 inhibits antibiotic-resistant Staphylococcus biofilm formation"

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With the precipitous increase in the use of surgical implants, the rate of implant-associated infection has followed. Of the estimated 3 million surgical implants placed annually within the United States, roughly 1 in 20 eventually develops an associated nosocomial infection [1]. Often times these infections mandate implant debridement and potentially replacement. Repeated surgical intervention not only confers additional morbidity to the patient, but also treatment of implant-associated infection incurs a significant cost burden on the health care system [1]. *Staphylococcal* spp., including antibiotic-resistant strains of *S. aureus* and *S. epidermidis*, are responsible for approximately two-thirds of these infections. *Staphylococcal* infections notoriously involve the formation of biofilm, a thin layer of slime along the surgical implant that contains colonies of bacteria densely adherent a scaffolding matrix of host-derived and bacteria-derived proteins [2]. Properties of the biofilm itself, including poor antibiotic penetration into the matrix and even potential inhibition of antibiotics themselves, often render typical intravenous antibiotic treatments ineffective and thus necessitate operative intervention [1]. Over and above the antimicrobial effects of biofilm, the evolution of multidrug-resistant strains of bacteria poses additional challenges for the treatment of these infections. Although antibiotics exist which adequately treat blood-borne infections of these virulent bacteria, their treatment course often requires an extended therapy with the potential for adverse effects.

Previous investigations [3–5] have demonstrated novel therapeutic potential within a class of medication known as antimicrobial peptides. Through their contribution to defensin–bacterium bonding because of their strong positive charge [6], these endogenous peptides have been shown to provide broad spectrum antimicrobial activity at noncytotoxic concentrations. Human β-defensin 3 (hβd-3), one such peptide secreted by epithelial skin cells and cells within apocrine sweat glands, has shown particular promise in the treatment of wound infections within both diabetic and burn animal models [7,8]. Although hβd-3 has demonstrated strong bactericidal effects against methicillin-resistant *S. aureus* (MRSA)



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[4,5,9] and efficacy in the treatment of biofilm formation by methicillin-sensitive *S. aureus* [10], its antimicrobial and antibiofilm efficacy against MRSA biofilm formation and methicillin-resistant *S. epidermidis* (MRSE) has yet to be elucidated.

In a paper recently published in the *Journal of Surgical Research,* Zhu et al. [11] outline a series of experiments designed to test the antimicrobial and antibiofilm efficacy of the hβd-3 on two differentMRSE and MRSA. The authors observed the effects of hβd-3 on several phases of biofilm formation: initial bacterial adhesion to the titanium surface, bacterial biofilm production, and maturation of the biofilm. After determination of the specific minimum inhibitory concentration of hβd-3 for each strain, titanium plates with the three bacterial cultures were initially treated with an increasing dose–response of hβd-3 ranging from 0.5 to 6 minimum inhibitory concentration. A significant dose–response reduction of intact bacterial colonies on the titanium surfaces was observed with increasing hβd-3 treatments compared with control. This dose-dependent effect was subsequently observed at the 12 and 30-h time points, suggesting that hβd-3 maintains its antibacterial and antibiofilm properties even when used to treat densely adherent bacteria already producing biofilm *ad libitum.* In addition to light microscopy, confocal laser scanning microscopy and scanning electron microscopy were used to qualitatively confirm these findings.

Real-time polymerase chain reaction was also used to quantify the level of expression of biofilm-associated genes within the *S. epidermidis* bacterial strain and the antibiotic resistance–associated gene within the *S. aureus* bacterial strain. Treatment with hβd-3 resulted in significantly decreased expression of icaA and icaD gene expression (genes responsible for biofilm production) and significant upregulation of icaR expression (a transcriptional repressor of the ica operon expression), resulting in a significant net attenuation of biofilm production. Additionally, through its reduction in MecA expression, the gene responsible for conferring antibacterial resistance to gram-positive bacteria, hβd-3 also demonstrates a mechanism to prevent the further development of multidrug-resistant bacterial strains.

Through their model, Zhu *et al.* successfully demonstrated the therapeutic efficacy of hβd-3 by genetically inhibiting *Staphylococcus* adherence to, and biofilm formation along, *in vitro* titanium plating. In addition, this antimicrobial peptide inhibited expression of the mecA gene, effectively reducing the bacterial resistance of MRSA and MRSE. Although this finding clearly has great therapeutic potential in the field of implantation of orthopedic and spinal hardware, hβd-3 and other therapeutics within the class of antimicrobial peptides could prove beneficial in the treatment of temporary or permanent vascular catheter-induced gram-positive bacteremia that may otherwise require catheter removal. Although additional *in vivo* studies in animal models and potential clinical trials in human volunteers will be necessary to determine an adequate dosing regimen, it appears that this class of therapeutics may provide the antimicrobial action against gram-positive bacteria without the significant risk of nephrotoxicity posed by vancomycin. One limitation of their investigation, which the authors addressed within the manuscript, involves the rapid pharmacokinetic degradation of hβd-3 by both host and bacterially secreted proteases [12,13]. Further investigations are warranted to better elucidate the proper delivery and drug maintenance methods. Nevertheless, in a world of antibacterial over use and the subsequent evolution of ever

increasingly virulent bacterial strains, hβd-3 and the class of antimicrobial peptides provide a novel weapon for our antimicrobial armamentarium.

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