Release Kinetics and Efficacy of a Broad-spectrum Antimicrobial Peptide from Poly(Methyl Methacrylate) Bone Cement

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Statement of Purpose: Poly(Methyl Methacrylate) (PMMA)-based bone cements are the gold standard in orthopedics for post-surgical void filling, implant anchoring, and bone reconstruction procedures. However, a major drawback of PMMA cement is the propensity for bacterial colonization and biofilm development on its surface post-implantation. To prevent and treat infection, antibiotics are routinely added to PMMA, but often fail to clear the infection and may lead to antibiotic resistance. Therefore, non-antibiotic antimicrobial alternatives are sought. Antimicrobial peptides (AMPs) are a class of small proteins which act as a first line of host defense against pathogens. AMPs have been shown to be less likely than traditionally used antibiotics to elicit bacterial resistance. Our group has previously engineered AMPs for the purpose of metallic implant coating and integration into dental adhesive formulations. Here, we evaluated a novel 11 amino acid AMP (AMP2) for use in clearing orthopedic infection via local delivery from PMMA by comparing its release kinetics and post-release efficacy to vancomycin. We hypothesized that incorporated AMP2 will release from PMMA in quantities greater than vancomycin and will retain its antibacterial efficacy after release.

Materials and Methods: AMP2 was synthesized using our established protocols and lyophilized until use. Minimum bactericidal concentrations (MBCs) of both vancomycin and AMP2 were tested before PMMA incorporation against *S. aureus* (SA), *S. epidermidis*, and *P. aeruginosa* using microbroth dilution assays. A subsequent Alamar Blue assay was performed to differentiate between bacteriostatic and bactericidal activity. Three cement groups were formulated: Control PMMA (CON) with no additives, AMP2-incorporated PMMA (AMP2), and vancomycin-incorporated PMMA (VANC). Both AMP2 and vancomycin were added to PMMA so that they comprised 1.87% of the resultant composites by weight, and curing cements were molded into 6x12 mm cylinders for testing. Drug elution into 2.5 mL of phosphate-buffered saline was collected over 21 days analyzed via HPLC. The MBCs of released AMP2 and vancomycin were tested against SA and SE. Finally, the mechanical strength of all groups was tested after 21 days, following ASTM protocol.

Results: Efficacy testing of AMP2 showed broad-spectrum bactericidal activity against gram-negative and gram-positive bacteria (Table 1). Both AMP2 and vancomycin were able to elute from PMMA over the course of 21 days. Total AMP2 elution was shown to be significantly greater than vancomycin ($p<0.05$) (Figure 1). AMP2 post-release activity was shown to be less efficacious than non-incorporated AMP2, while post-release vancomycin retained full efficacy ($p<0.05$). Quantitatively, AMP2 was only 11% as efficacious against both SA and SE after releasing from PMMA when compared to control AMP2 based upon MBC values. No fragmentation of AMP2 was seen based upon the HPLC chromatograph, however, a retention time shift was observed after release from PMMA. No significant difference in either compressive strength or modulus of AMP2- or vancomycin-incorporated PMMA from control was observed after 21 days ($p<0.05$).

Bacterial Strain	AMP2 (µg/mL)	Vancomycin (µg/mL)	Gentamicin (µg/mL)
Staphylococcus Aureus	9.4	1.5	N/A
Staphylococcus Epidermidis	0.5	3.0	N/A
Pseudomonas Aeruginosa	32.0	N/A	0.5

Table 1. Minimum bactericidal concentrations (MBCs) of AMP2 as compared to common antibiotics. n=3

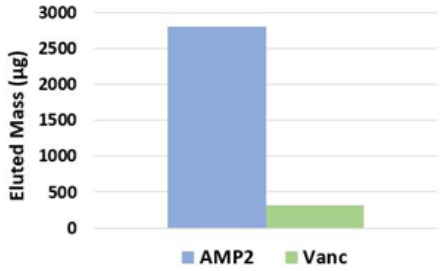

Figure 1. Cumulative eluted mass of AMP2 and vanc over the first 5 days. n=10

Conclusions: The ability of AMP2 to be efficacious against both gram-negative and gram-positive bacteria makes it a great candidate for treating orthopedic infection without high concern for resistance. While its incorporation into PMMA did not reduce the strength of the cement, its reduction in efficacy upon release is problematic. A conformational change in the AMP is likely upon interaction with PMMA and will be subject to future analysis in order to better stabilize the AMP for local delivery from PMMA. Given the shift in retention time as detected by the HPLC for AMP2 after PMMA release, functional group analysis on the exterior of the folded peptide will be our next logical step. Given AMP2's superior elution kinetics, it shows future promise for local delivery from PMMA or other orthopedic biomaterials.