Effectiveness of a Dual Drug Delivery Calcium Sulfate, Chitosan-Calcium Phosphate Bone Scaffold.

Heather Doty, Warren Haggard, Harry Courtney, Joel Bumgardner
Biomaterials Applications of Memphis (BAM) laboratories at the UT-UM Joint Biomedical Engineering Program, Memphis, TN

Statement of Purpose: Infections resulting from wound contaminations can lead to delayed healing, high medical expenses, increased site morbidity and patient mortality. Studies have found as much as 65-70% of open musculoskeletal wounds can be contaminated with microorganisms.[1,2] Therefore, there is a need for orthopedic scaffolds to be drug delivery vehicles in addition to having good wound healing properties. This study investigated known scaffold materials: chitosan, calcium phosphate (CaP) and calcium sulfate (CaS) as a drug delivery system for dual antibiotic release. The goal of this study was to extend the antibiotic release of two commonly used orthopedic drugs, vancomycin and amikacin, from the scaffold material for up to six weeks at a level that would abate and kill bacterial species.

Methods: *Composite Bead Fabrication*: A co-precipitation method was employed for the fabrication of the composite chitosan-CaP beads.[3] Solution of 3.57 wt% chitosan (80% DDA, Prim-Ex), 0.1 M $CaCl_2$, 0.06 M NaH_2PO_4, (Ca:P ratio = 1.67) in 2 wt% acetic acid was dripped into a solution of 20% NaOH, 30% methanol, 50% water (pH=13) to precipitate beads. After 24 hours (hrs) the beads were neutralized, frozen (-20 ^oC) and lyophilized in a 2.5 liter Labconco freeze-dryer for 48 hrs. *Amikacin and Vancomycin Loading*: Approximately 80 mg of composite beads were loaded in 1.5 mL of a 10 mg/mL solution of amikacin at room temperature (RT) for 24 hrs. The beads were removed and placed in either clean vials with 2 mL of PBS at 37^oC or mixed with CaS to form pellets. A vancomycin solution was added to CaS powder to yield 2% (w/w) of vancomycin in the pellet. Three scaffolds were analyzed (n=5): A = amikacin loaded beads, V = vancomycin loaded CaS and VA = amikacin loaded beads encased with vancomycin CaS. *Pellet Fabrication and Elution*: The pellets were made from 0.6g of α-hemihydrate CaS and 0.24mL dI H_2O. Composite beads were mixed into some CaS samples prior to setting. Dried scaffolds in 1.5mL PBS were sampled at 1, 5, 12, 24, 48, 96, 84, 168, 336, 480, 648, 816 and 1008 hrs. The PBS solution was completely refreshed after each elution time point. The samples were analyzed via a fluorescent immunoassay with the TDx machine (Abbott Diagnostics, Abbott Park, IL). *Inhibition of Growth and Bactericidal Assay:* Sterile tubes were prepared with 1.75 ml of Trypticase soy broth (TSB), 200 µl eluate sample or 200 µl PBS and 25-50 µl of *S.aureus* Cowan I or *P. aeruginosa* ATCC 27317 grown to ~2x10^6 CFU or 25-50 µl PBS for blanks. Samples were grown over night (ON) at 37 °C and the absorbance at 530 nm was recorded. All samples which contained no growth were diluted 10, 100 and 1000 times, and 100 µl was plated on TSB plates and incubated ON at 37 °C.

Results: The scaffold (VA) loaded with both drugs showed the longest elution profile. Release was above the vancomycin MIC for 6 wks and the amikacin MIC for 27days compared with the other scaffolds loaded with one antibiotic.

Table 1: Vancomycin and amikacin elution from scaffold material for 6 weeks

	Amikacin (µg/mL)		Vancomycin (µg/mL)	
Hrs	VA	A	VA	V
1	227± 21	534 ± 48	2970 ± 150	4250 ± 1790
5	176 ± 22	193 ± 9	1900 ± 186	2410 ± 206
12	158 ± 16	56.2 ± 1.9	1370 ± 144	2050 ± 93
24	111 ± 7	22.0 ± 0.3	1260 ± 70.7	1880 ± 104
48	68.5 ± 12.7	11.6 ± 0.3	1340 ± 116	1730 ± 109
96	29.2 ± 4.4	5.44 ± 0.16	1580 ± 108	626 ± 84
168	14.9 ± 2.2	3.00 ± 0.06	1200 ± 114	113 ± 13
336	9.71 ± 0.72	2.04 ± 0.06	525 ± 114	26.7 ± 3.7
480	6.26 ± 0.48	2.00 ± 0.10	148 ± 34	7.20 ± 0.77
648	4.13 ± 0.32	1.69 ± 0.11	46.1 ± 9.51	4.53 ± 0.39
816	3.19 ± 0.32	2.00 ± 0.30	16.1 ± 3.29	3.22 ± 0.39
1008	3.31 ± 0.30	1.84 ± 0.12	8.33 ± 1.42	3.70 ± 0.11

Vancomycin in combination with amikacin (VA) inhibited bacterial growth up to 34 days and was bactericidal for 27days. Vancomycin (V) alone inhibited and killed bacteria for 14 days. Amikacin (A) was less effective against *P. aeruginosa* for both samples with inhibition through 24hrs and bactericidal only for the first hour.

Table 2: Inhibitory/bactericidal effect of vancomycin and amikacin against *S. aureus* and *P.aeruginosa* respectively.

	S. aureus				*P. aeruginosa*			
	Inhib.		Bact.		Inhib.		Bact.	
Hrs	V	VA	V	VA	A	VA	A	VA
1	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	+
12	-	-	-	-	-	-	+	+
24	-	-	-	-	-	-	+	+
48	-	-	-	-	+	+	+	+
96	-	-	-	-	+	+	+	+
168	-	-	-	-	+	+	+	+
336	-	-	-	-	+	+	+	+
480	+	-	+	-	+	+	+	+
648	+	-	+	-	+	+	+	+
816	+	-	+	+	+	+	+	+
1008	+	+	+	+	+	+	+	+

Conclusions: The scaffold containing both antibiotics exhibited extended drug release and bacterial abatement and killing of *S.aureus* for over 3weeks. The scaffold was not as effective against *P. aeruginosa,* most likely due to the smaller amount of amikacin loaded into the scaffold. It appears that there may be interactions between vancomycin, amikacin and the scaffold material, which contributed to the extension of the drug elution profile. Future studies will investigate the loading and controlled delivery of multiple other factors from scaffolds that may be helpful for bacterial abatement and bone growth.

References:

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2. Zalavras CG. Infect Dis Clin North Am. 2005;19:915-929.
3. Chesnutt BM. J Biomed Mater Res A. 2007;82A:343-353.