

## Delivery of Pharmaceutical Agents to Orthopedic Defects from Modified Calcium Sulfate Implants

J. Amber Jennings, R. Matthew Crews, Jonathan D. McCanless, Scott P. Noel, Benjamin T. Reves, J. Tracy Watson\*, Warren O. Haggard \*Orthopaedic Surgery Department, St. Louis University, University of Memphis, Department of Biomedical Engineering

**Statement of Purpose:** Advantages of calcium sulfate for use as a bone graft substitute include osteoconductivity, complete resorption, and the ability to incorporate pharmaceutical agents during fabrication. However, fast degradation rate, drainage issues, and a lack of osteoinductivity limit its utility as a bone graft substitute. Drug elution from calcium sulfate typically occurs in a large initial burst.<sup>1</sup> For optimal clinical effectiveness in complex bone fractures, the elution profile of particular agents must be enhanced—longer sustained release of antibiotics is necessary and delayed availability of growth factors during the healing process is needed. Coating calcium sulfate pellets with chitosan has been shown to decrease the initial burst level of an antibiotic<sup>2</sup> and slow the dissolution rate of pellets.<sup>3</sup> In this study, *in vitro* elution profiles of an antibiotic, tobramycin, as well as an osteoinductive growth factor, BMP-2, from coated implants were compared to those from non-coated implants. Also, *in vivo* degradation and new bone formation were evaluated in rat tibial defects for 6 weeks.

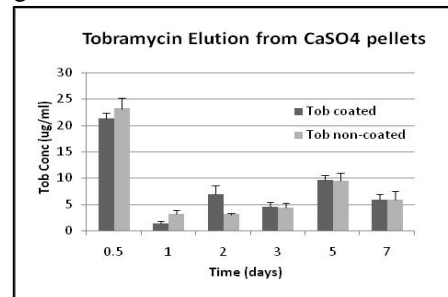
**Methods: Implant fabrication.** Cylindrical implants 1.9 mm in diameter and 2 mm in height were obtained by aseptically casting unaccelerated type V hemihydrate calcium sulfate powder (Wright Medical, Arlington, TN) with 0.23 ml sterile deionized water. For antibiotic-containing pellets, 52 mg of sterile tobramycin powder (4wt% loading) was added before water addition. Water with 4.35 mg/ml of recombinant human BMP-2 (Genetics Institute, Cambridge, MA) was used to cast pellets with a theoretical loading of 10 µg/pellet. Modified pellets were dipped in a solution of 3 wt%, 81% DDA chitosan (AgraTech, Goose Creek, SC) in 1% acetic acid and dried five times to produce a uniform coating.

**Elution.** Elution was conducted as previously described, measuring tobramycin levels with TDxFLx<sup>3</sup> and BMP-2 levels with ELISA.

**Tibial Defect.** Round defects in the proximal tibia measuring 2.4 mm in diameter were created in male Wistar rats (Harlan) using sterile customized drill bits and a Rotex™ 782 dental handpiece with saline irrigation. Cylindrical implants were press-fit into defects. Implant groups included 1) plain CaSO<sub>4</sub> pellets, 2) CaSO<sub>4</sub> pellets + 4% tobramycin, 3) coated CaSO<sub>4</sub> pellets +4% tobramycin, 4) coated CaSO<sub>4</sub> pellets + 4% tobramycin + BMP-2, and 5) empty defect.

**Results:** *In vitro* results demonstrate an initial burst release of tobramycin, followed by significantly different release profiles at 24 and 48 hours. Elution is extended through day 7 (Fig 1). At 48 hours, coated implants elute more tobramycin. In contrast to the tobramycin elution profile, preliminary ELISA results indicate that more BMP was eluted from coated implants than non-coated implants. BMP-2 elution is still being investigated; however, low recovery of eluted protein indicates a possible interaction of BMP-2 with either CaSO<sub>4</sub> or

tobramycin which may interfere with antibody-based detection. Within the rat tibial defect, at six weeks the majority of the unmodified calcium sulfate implants had degraded, while degradation of coated implants was significantly delayed. At six weeks intact coatings of chitosan are evident in histological sections. Implants containing BMP-2 produced more osteoid within the healing bone.



**Fig.1** Graphical representation of tobramycin elution over a 7 day study. Data for coated and non-coated pellets is shown. Data is reported in µg/ml concentration. (n=5)

**Table 1. *In vivo* bone response effects.**

	Empty	CaSO <sub>4</sub>	CaSO <sub>4</sub> + tobra	coated CaSO <sub>4</sub> + tobra	coated CaSO <sub>4</sub> + +BMP-2+ tobra
• Bone healing	+	++	++	-	-
• Osteoid formation	+	+	+	-	++
• Slowed Degradation	-	-	-	++	++

**Conclusions:** *In vitro* antibiotic elution results are consistent with previous studies of chitosan-coated calcium sulfates.<sup>3</sup> Because of possible interaction between BMP-2 and implant materials, concentrations of BMP-2 in eluates are underrepresented in ELISA tests. The presence of increased osteoid in defects with implants containing BMP-2 indicates that BMP-2 is eluted from implants at a level sufficient to induce bone formation. Chitosan coatings delayed the degradation of CaSO<sub>4</sub> *in vivo*, which is in agreement with previous *in vitro* dissolution studies.<sup>3</sup> The coated implants degraded slower than normal bone healing, so these implant materials affected the bone healing rate. In future studies, these slower degrading materials will be tested in critical-sized defects, where slower degradation could be advantageous. Additionally, the composition of the chitosan coating can be altered to increase degradation rate.

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### References:

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