ELUTION OF GENTAMICIN SULPHATE FROM BONE CEMENT AND POLYMER COATED Ti6Al4V

+* Vass, S A; *Hippensteel, E A; *Suresh, S; *Salvati, L.
+*DePuy Orthopaedics, Inc., Warsaw, IN
svass@dpyus.jnj.com

INTRODUCTION
The incidence of infection following orthopedic surgery is very low, however it is still one of the greatest areas of concern for both the surgeon and the patient. Post surgical infections are the major source of morbidity and mortality. In an effort to protect against implant infections most surgeons administer systemic antibiotics for 24–48 hrs post surgery. However, this does not address the need for localized antibiotics at the implantation site. Systemic antibiotics cannot reach the high concentrations needed at the site of implantation, and thus, can are typically ineffective.

Currently, the antibiotic loaded bone cement is the primary method utilized to supply localized drug. This method has led to a significant decrease in the reoccurrence of infection following revision surgeries. The antibiotic is gradually released from the cement and can provide locally higher concentrations than systemic therapy. An alternative to bone cement using a gentamicin sulphate incorporated PLGA coating has been developed for applications where PMMA is not the best option. This system could provide localized drug release from ingrowth structures and could mean less OR preparation for surgeons. The purpose of this study was to compare the elution and osteoblast response to gentamicin sulfate eluted from commercially available bone cement and a novel PLGA coating containing gentamicin sulfate.

METHODS
Sample Description - Three types of T6A14V (Ti) disks were used (1” in diameter, 0.25” height). One sample type consisted of a 20-gram Al2O blasted (GB) Ti disk spray coated with a combination of gentamicin sulfate (GS) and PLGA. The second sample type was a GB Ti disk that contained GS only, while the third sample type was a GB Ti disk with PLGA only. In addition to the Ti/drug samples specimens were also prepared using antibiotic bone cement (DePuy GMV Smart Set®).

Elution Protocol - One disk of each sample type was placed separately in 50mL centrifuge tube filled with 15mL deionized water (elution medium) and placed in a 37°C incubator. At each time point, the disks were removed from solution and placed in new tubes with fresh (15mL) water. Samples were removed for analyses at 15min, 1hr, 4hrs, 24hrs and 48hrs. Gentamicin concentrations were evaluated using liquid chromatography-mass spectroscopy (LC/MS). A Waters Q-ToF-Micro LC/MS hybrid quadrupole time-of-flight system, including a Waters Alliance 2795 Separations Module LC was used. MassLynx software was used for data acquisition, interpretation and analysis. Sample separation was obtained using a reversed phase ACE-111-1003 C18 column. Tandem MS (LC/MS/MS) was performed inducing fragmentation of the main precursor ion m/z 478.3. Two daughter ions (m/z 322.218 and 157.147) were chosen in order to allow for greater selectivity.

Cell Culture - In this study, the effect of a continual dose of GS was compared to a burst release (48 hours) on osteoblast-like cells in vitro. Bone marrow cells were prepared from the femora of Wistar rats according to established protocols. Antibiotics and anti-fungals were omitted to make sure the bone cell response was due only to the admission of GS to the media. 10mL of the cell suspension was added to the media, mixed and stored in a humidified incubator at 37°C and 5% CO2. For the continual supply medium, GS was added to the media at a concentration of 0 (control), 70 or 230µg/ml to their respective flasks with every media change. The burst release only received GS supplemented medium for media changes at 24 and 48 hours. Cultures were maintained for 11, 13, and 15 days before staining for alkaline phosphatase (ALP) activity. Bone mineralization was detected using Von Kossa (VK) staining and imaged with digital photography.

RESULTS and DISCUSSION
The LC/MS/MS elution showed the ability to sustain drug release for at least 4 hours (Fig. 1) when using gentamicin and PLGA coated disks (P/G-1). Disks with only gentamicin coating (G-1) released the drug in the first 15 minutes with no evidence of drug at 1 hour. The PLGA coated (P-1) disks were used as a control. The combination of drug and PLGA shows promising results as a localized drug delivery system. The initial GS burst for PLGA coated disks was approximately 230µg/ml compared to GMV at 100µg/ml. GMV GS elution occurs only from the surface due to the lack of degradation of bone cement. PLGA degrades at a much higher rate and will ensure total release of GS.

The in vitro model used for this study represented the effect of a continuous supply as well as burst release of GS to the osteoblast-like cells and surrounding environment. 230µg/ml (PLGA+GS) GS and 70µg/ml (GMV) GS were added to the culture media to emulate the initial (15min) GS eluted from the two sample types. The VK stained flasks from the continual doses (Fig. 2) showed that the control (A) had a greater number of nodules than the 70µg/mL (B) and 230µg/mL (C) flasks at 11, 13 and 15 days in culture (15 day data shown in Fig. 2). As GS concentration increased, the amount of calcified bone decreased. Positive ALP stains for all three doses at all time points confirmed that the osteoblast-like cells were proliferating and differentiating, but were unable to mineralize bone. VK stained flasks from the burst release doses (Fig. 3) showed the control (A), 70µg/mL (B) and 230µg/mL (C) flasks all had a similar number of nodules at 11, 13 and 15 days in culture (15 day data shown in Fig. 3). This indicated that the GS concentration supplied for the first 24 and 48 hours did not affect cellular activity through 15 days in culture. In addition, the 48hr supply of GS did not adversely affect the cells, as indicated by positive ALP staining for all three doses at all time points (data not shown). The in vitro model used for this study represented the effect of an initial burst of GS over a 48 hour time period, similar to the in vivo elution of GS from bone cement.

SUMMARY
Data presented here shows that the localized delivery of GS can be an effective alternative for bone cement drug delivery. It is possible to construct a drug delivery system that has a burst effect to that observed for antibiotic bone cement. Results clearly demonstrated that a 48 hour burst release of gentamicin sulphate does not exhibit any adverse effects on osteoblast-like cell activity in vitro.