**In Vitro Study of Gentamicin Release from Strontium Containing Hydroxyapatite Bone Cement**

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INTRODUCTION:
Osteomyelitis is a complication of bacterial infections in the bone usually occurred after implantation. A combination of surgical debridement and antibiotic prescription is the standard remedy. Local drug delivery is usually more preferable to give a high dosage in treating some local problems without systemic toxicity. Incorporation of antibiotics into bone cements provides local delivery of antibiotics at the osteomyelitic site with better efficiency than systemic injection. PMMA has been the most widely used bone cement, but concerns over this material have been arisen to its very high setting temperature and lack of osteoconductivity. Our group has developed Sr-HA bone cement which was proved to be osteoconductive with lower setting temperature. Currently, there are several commercial PMMA bone cements loaded with antibiotics. We hypothesized that gentamicin loaded Sr-HA bone cement would act similarly in vitro but better gentamicin release efficiency than gentamicin loaded PMMA cement available in the market.

METHODS:
Release kinetics of gentamicin were studied by fluorometric method and its antibacterial actions on *Staphylococcus aureus*. DePuy’s SmartSet Endurance™ PMMA bone cements (with and without 1g based gentamicin) were compared to our Sr-HA bone cement loaded with the same amount of gentamicin. These cements were fabricated into 0.5g cylinders with 6mm diameter and immersed in 25mL phosphate buffered saline (PBS) set on a shaker at 37ºC for a period of 30 days. PBS samples were collected 60, 120 and 180 minutes on day 0 and every day for fluorescent and diffusion measurements.

Gentamicin was derivatized by fluorescamine and analyzed by a spectrofluorometer at excitation wavelength 395nm and emission wavelength 485nm. Antibacterial action of gentamicin eluted into PBS was tested by modified Kirby-Bauer antibiotic testing. One hundred microliters of PBS samples were pipetted into 9mm diameter holes on BHI agar spread with *s. aureus*. The sizes of the clear zone were quantified to estimate gentamicin concentrations. Direct short term release effects from the cements were also studied with *s. aureus* growth inhibition with cement inserted into BHI agar. Images were taken of these diffusion tests for measurement of clear zones.

MG-63 was used to study the in vitro effects of gentamicin loaded cements on osteoblast proliferation, alkaline phosphatase activity and mineralization. Cements were cut into plates of 2mm thick and 14.2mm diameter and seeded with 20000 cells per plate. MTT and ALP assays were done on day 3, 7, 14 and 21. Mineralization was studied on day 21 by alizarin red S stain and imaged with stereo microscope.

RESULTS SECTION:
Gentamicin loaded in Sr-HA bone cement released much greater amount and lasted longer over the period of study. Clear zones caused by gentamicin in PBS from PMMA and Sr-HA cements are shown in figure 1. Sr-HA cement gave out bigger clear zones presented in figure 1 in comparison to PMMA cement showing more gentamicin released in Sr-HA cement. The cumulative releases calculated by fluorometric method and diffusion are shown in figure 2. Gentamicin was released from PMMA during the first few days only while it was still detectable after 30 days in Sr-HA cement. Only 1.5-2.8% gentamicin was released from PMMA cement in the first few days. Gentamicin continued to release from Sr-HA cement over the period of study and reached 26-29% by day 30.

Direct diffusion test was intended to mimic the environment of bone cements in bone. Clear zones were clearly seen on *s. aureus* spread agar within the first few days of insertion. Gentamicin was shown to elute from both PMMA and Sr-HA bone cements. The sizes of the clear zone were bigger in Sr-HA cement than PMMA cement, so more gentamicin was released from Sr-HA cement.

Sr-HA cement loaded with gentamicin was shown not to cause any cytotoxic effects on MG-63. No serve effects on proliferation were reported within the range of concentrations of gentamicin loaded in both Sr-HA and PMMA cements. However, the alkaline phosphatase activity was reduced slightly in Sr-HA cement at higher gentamicin concentrations reported. Mineralization was enhanced on Sr-HA cement compared to traditional PMMA cement.

**Figure 1. Diffusion study with PBS soaked with Sr-HA and PMMA cements with and without gentamicin.**

**Figure 2. Cumulative gentamicin release over 30 days calculated with fluorometric method and diffusion.**

DISCUSSION:
This Sr-HA bone cement demonstrates a great potential to deliver gentamicin over traditional PMMA cement loaded with the same amount of gentamicin. Although there is a slight difference in the results calculated from the fluorometric method and diffusion study, Sr-HA cement follows the same trend of released several times more gentamicin than PMMA cement. Our result of only a small portion of gentamicin eluted from PMMA is consistent with earlier study (Lewis & Janna, 2004). The high gentamicin release rate from Sr-HA cement is favorable in minimizing the chance of prolonged antibiotic exposure in the case of PMMA. Moreover, the high efficiency of Sr-HA cement as a drug carrier can reduce the dosage of antibiotics required. The better release rate in Sr-HA cement should be caused by the hydrophilic monomer used.

Not only greater gentamicin release characteristic, but the great bioactivity also favors Sr-HA bone cement. At the infected site, the readiness of osteoblasts attachment, proliferation and mineralization on the bone cement is as important as bacteria eradication. Enhancement in mineralization from Sr-HA bone cement re-confirms it as an attractive material with better bioactivity over PMMA cement. No cytotoxic effects caused by the relatively high local concentration of gentamicin in Sr-HA explore the chance of future Sr-HA bone cement applications to deliver antibiotics.

**REFERENCE:**