Development of Gentamicin Containing Bioactive Macroporous Injectable Calcium Phosphate Cement

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Statement of Purpose: Studies have shown that bone graft substitutes (BGS) may be used as a carrier of antibiotics in vitro and in vivo [1]. Local antibiotic delivery is a good alternative to systemic delivery as a high dose may be achieved at the site of infection while keeping systemic levels of antibiotics low [2]. Incorporating antibiotics into biodegradable void fillers may allow the surgeon to treat the infection and heal the bone defect in a single surgery. It was hypothesized that a novel, macroporous injectable calcium phosphate cement (MCPC™) can be used to deliver therapeutically significant amount of antibiotics locally. Previous studies have analyzed the release profile of gentamicin from MCPC™ [3]. Recent changes have been made to improve the mixing, and handling properties of MCPC™. The in vitro activity of gentamicin released from this second formulation has been analyzed via broth microdilution [4]. This study analyzes the gentamicin concentration from the samples used in the in vitro activity study and compares the release profile relative to the first formulation.

Methods: The MCPC™ was provided by Biomatlante (Vigneux de Bretagne, France). The cement was prepared by mixing the liquid component with the dry component. To prepare cement samples containing gentamicin sulfate, (Sigma-Aldrich, St. Louis, MO) powdered gentamicin was first added to the dry powder component before mixing with the liquid component. The gentamicin loading was 3% by weight based on the total weight of the cement components. Cylindrical molds (6dia x 12L mm) were created with an average cylinder weight of 600 ± 25 mg, resulting in an average gentamicin concentration of 18.035 ± 0.749 mg per cylinder. The cement samples were allowed to set for either one hour or 24 hours prior to placement in the release medium, 50 mM phosphate buffer of pH 7.4. Each cement sample was immersed in 5 ml of release medium at rest in an incubator set at 37˚C. Complete removal of the supernatants, and replacement with fresh phosphate buffer, were performed regularly up to 28 days. The gentamicin concentrations in the supernatant were measured using a High-Performance Liquid Chromatography (HPLC) procedure with ultraviolet (UV) detection with some modification [5].

Results: Initial release of gentamicin was slightly affected by the cement setting time. By 28 days, about 50% of the gentamicin was released from the cements that were set for either 1 hour or 24 hours respectively (Figure 1).

Previous data showed the Minimum Inhibitory Concentration (MIC) for the specific gentamicin lot used in these studies to be 0.5 µg/mL [4]. Based on the results from the previous in vitro activity data (broth microdilution) in conjunction with the current analytical data, the actual MIC was calculated at each time point (Figure 2). At all timepoints, gentamicin concentrations in the PBS were significantly higher than the MIC for a reference strain *S. aureus*, reported to be in the range of 0.12 – 1 µg/ml[6].

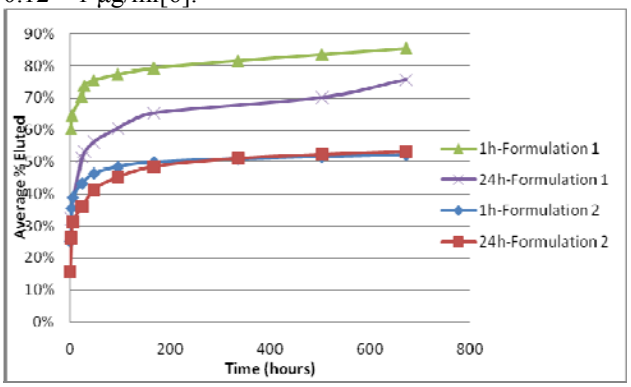
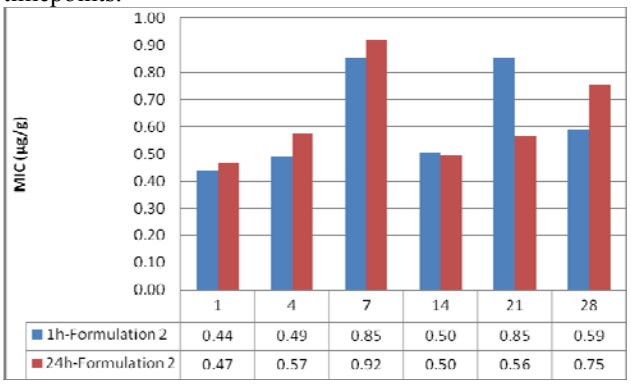


Figure 1. Weight % of gentamicin released from two MCPC™ cement formulations cured for two different timepoints.



	1	4	7	14	21	28
1h-Formulation 2	0.44	0.49	0.85	0.50	0.85	0.59
24h-Formulation 2	0.47	0.57	0.92	0.50	0.56	0.75

Figure 2. Average MIC for samples. MIC were calculated based on the average HPLC concentration divided by the average dilution factor (n=3).

Conclusions: The gentamicin release rate of the second formulation is different from the first formulation. The first formulation exhibited a burst release and released relatively more gentamicin by day 28 when compared to the second formulation. The in vitro release study has demonstrated that the MCPC™ was able to release a therapeutically significant amount of gentamicin up to 28 days. The results have shown that the gentamicin remains biologically active over the time frame of the experiment. Given the sensitivity of the assay (± one 2-fold dilution), gentamicin activity appears stable throughout the 28 days. The MCPC™ appears effective when used as resorbable bone substitute for the release of gentamicin. In addition, the release kinetics can be controlled by adjusting the formulation.

References:

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