In Vitro Activity of Gentamicin Released from Macroporous Injectable Calcium Phosphate Cement

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Introduction

Bioactive cements formulated with calcium phosphates are important synthetic bone graft substitutes for treating bone deficits owing to the ability to be injected through a small cannula and harden in situ, providing immediate structural stability and strength at fracture sites. Recent advancement in formulation development have improved this bioactive cement's resorption profile to facilitate the formation of interconnected macroporous structure, paving the way for cellular invasion and new bone growth. It is a common practice for surgeons to mix antibiotics with bone grafts when treating infected bone defects or preventing infection from surgery (1). Local delivery of antibiotics is pharmacologically more effective and safer. Bioactive cements have been shown to be ideal carriers for antibiotics for local delivery if properly formulated (2). A novel, high strength calcium phosphate cement has been specifically engineered to possess macroporosity and resorbability for optimal cell adhesion, cell migration, and bone formation. It was hypothesized that this macroporous injectable calcium phosphate cement (MCPC™) can be used to deliver therapeutically significant amount of antibiotics locally. The purpose of the study was to use gentamicin as a model antibiotic to evaluate its bioactivity after release from MCPC™.

Methods

The MCPC™ was provided by Biomatlante (Vigneux de Bretagne, France). The cement was prepared by mixing the liquid component with the dry powder component until homogenous. To prepare cement samples containing antibiotic, gentamicin sulfate powder (Sigma-Aldrich, St. Louis, MO) was 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. Cylindrical samples were created with a mold and the cement samples were allowed to set for either one hour or 24 hours prior to placement in the release medium. Six gentamicin loaded cements were prepared for each set time. Bone cements without gentamicin were similarly prepared (n=6 for each set times), and were used as controls. Each cement sample (6 mm x 12 mm) was immersed in 5 ml of 50 mM phosphate buffer of pH 7.4, and release was allowed to occur at rest in an incubator set at 37˚C. Complete removal of the supernatants were performed and replaced with fresh phosphate buffer regularly up to 28 days. The biological activity of the released gentamicin was assessed by two different in vitro methods: agar diffusion and broth microdilution with 5 x 10^5 CFU/mL (final concentration) of *Staphylococcus aureus* ATCC 29213 *(S. aureus)* as the inoculum.

Broth Microdilution – Elution from twelve cement samples (3 from each group) was tested at 1, 4, 7, 14, 21 and 28 days. Serial two fold dilutions were prepared for each elution from the gentamicin loaded cement samples. The minimum inhibitory concentration (MIC) of fresh gentamicin sulfate solution was determined using the same lot of antibiotic. The final concentrations tested included 8 – 0.0625 µg/mL in serial two fold dilutions. After incubation for 20 hours, the highest dilution with no visible growth was recorded representing the MIC. Positive control wells were also included consisting of inoculated wells with elution from control samples. Sterility control wells were included containing uninoculated broth with elution from control samples.

Agar diffusion – Twelve cement samples (3 from each group) were incubated at 37˚C for 1 day, 14 days, and 28 days (n=1 for each timepoint) in phosphate buffer without removal of the supernatants. In addition, the twelve cement samples used for the broth microdilution were tested at 28 days. *S. aureus* was used to inoculate plates by dipping a sterile cotton-wool swab into the suspension and spreading over the entire surface of the plate. Three cylindrical samples were placed per plate and incubated at 37˚C in air for 20 hours. The zones of inhibition were measured to the nearest millimeter.

Results

Broth Microdilution – Gentamicin released from the bioactive cement maintained its ability to inhibit bacterial growth of *S. aureus* throughout the 28 days. The MIC for gentamicin sulfate solution was determined to be 0.5 µg/mL. The control elution showed no inhibitory bacterial effect in the positive control wells indicating that the cement does not have an observable antibacterial effect. The sterility control wells had no visible growth indicating that the samples were sterile.

Agar diffusion – All the gentamicin loaded cement samples had zonal inhibitions of at least 35 mm. In contrast, none of the control cement samples inhibited growth.

Conclusion

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 MCPC™ appears effective when used as a resorbable bone substitute for the release of gentamicin.

References

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