CERAMENT Bone Void Filler Impregnated with Gentamicin Increases Bone Formation and Decreases the Rate of Detectable Infection After Debridement in a Rat Model of Osteomyelitis

Aleksey Dvorzhinskiy1, Giorgio Perino, MD1, Robert Chojnowski1, Marjolein C.H. van der Meulen, PhD2,1, F. Patrick Ross, PhD1, Mathias P.G. Bostrom, MD1, Xu Yang, MD1.

1Hospital for Special Surgery, New York, NY, USA, 2Cornell University, Ithaca, NY, USA.

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Introduction: Osteomyelitis is an infectious disease of the bone, most commonly caused by methicillin-sensitive Staphylococcus aureus (1). In most cases, acute osteomyelitis is responsive to intravenous antibiotics but in certain situations can require surgical debridement due to the poor vascular capacity of necrotic bone (2). Proper debridement can leave a large defect that must be filled to prevent the recurrence of infection and improve mechanical stability. Autograft is the gold standard void filler but limited bone stock and donor site morbidity preclude its use in many situations (1). A synthetic alternative which retains the bone-building qualities of autograft while also being cheap and abundant would benefit orthopaedic practice. CERAMENT™|BONE VOID FILLER (CBVF) is an FDA cleared injectable bone substitute which consists of hydroxyapatite and calcium sulfate. The advantage of this substance over other bone cements is that it is osteoconductive and facilitates its own replacement by new bone ingrowth. Unfortunately, this product is contraindicated in infected environments because of its propensity to act as a foreign body and serve as a nidus of infection.

In this study we utilized a new type of CBVF which is impregnated with gentamicin: CERAMENT™|G (C-G). This new formulation would serve to retain the bone generating capacity of the original CBVF while also exhibiting anti-microbial action and allowing for use in osteomyelitic environments. The aim of this study was to test the hypothesis that C-G would improve new bone growth and decrease infection rate after debridement as compared with 1) CBVF and 2) no void filler in a rat osteomyelitis model.

Methods:

Animal Model and Study Design: Cement plugs with or without gentamicin were made with a cylindrical mold (diameter 3mm, depth 2.78mm) in an aseptic environment (Bonesupport AB, Lund, Sweden). Sixty adult Sprague Dawley rats weighing ~450 grams were injected with 1.5 x 10^6 CFU of S. aureus (ATCC 29213, gentamicin-sensitive) into a drill hole (diameter 3 mm, depth 3 mm) at 3 mm distal to the growth plate in the right anteromedial tibia. After three weeks, infection was confirmed radiographically, the osteomyelitic defect was debrided under anesthesia, and filled with either: 1) C-G, 2) CBVF, or 3) left blank (n = 20/group). Six weeks after the second surgery, the rats were sacrificed.

Bacterial Enumeration: Standard sterile technique was used for all tissue collection procedures. The right tibias and surrounding muscle of all animals were harvested immediately after sacrifice. The drill hole was exposed and the tibia was cut transversely 5 mm distal to the infection site to expose the bone marrow to sonication. The samples were then placed in 10 mL of phosphate-buffered saline and vortexed and sonicated vigorously for 5 minutes. The tibias were then removed from the sonicate and placed in 10% of neutral buffered formalin for subsequent microCT and histological analysis. The
sonicate was serially diluted and plated on 5% Sheep Blood, Tryptic Soy Agar. The plates were incubated at 37°C and the colonies were counted after 48 hours. Plates with 30-300 colonies were counted and multiplied by the respective dilution factor for the given plate, as per standard microbiological protocol (3).

**Microcomputed Tomography (microCT):** The right tibia of each animal was scanned by microCT at a resolution of 15 µm. A region of interest (ROI) corresponding to the size of the original defect was chosen and centered on the middle of the defect. A global threshold was used to segment mineralized tissue from cement, water and soft tissue. Bone volume fraction (BV/TV) of the region of interest was analyzed for each sample.

**Histology:** After microCT analysis all samples underwent decalcification, processing, and embedding in paraffin. The defect was sectioned axially at the level of the center of the drill hole and stained with Hematoxylin and Eosin. Samples were scanned using a slide scanner (Aperio, Buffalo Grove, IL) Infection in a given sample was assessed by recording the incidence of a significant neutrophil response (microabscess) as judged by a blinded attending pathologist at our institution (G. Perino).

**Statistical Analyses:** Differences in microCT and histological BV/TV between treatments were assessed with one-way ANOVA followed by a Student-Newman-Keuls post-hoc method. Proportions of infected samples were compared using Chi-square analysis (histology) and a Fisher’s exact test (culture) on a 2x3 contingency table. The level of significance was p < 0.05.

**Results:**

(*: p<0.05)

**Bacterial Enumeration:**
- **Positive cultures found in (Fig 1A):**
  - 30% of animals treated with CBVF
  - 25% of animals treated with no void filler
  - 0% of animals treated with C-G (*)

**Histology:**
- **Neutrophil reaction in (Figs 1B and 2):**
  - 50% of animals treated with CBVF
  - 35% of animals treated with no void filler
  - 0% of animals treated with C-G (*)

**MicroCT (Fig. 3):**
- **The BV/TV of the ROI in:**
  - C-G treated rats was 24% greater than CBVF treated rats (*)
  - C-G treated rats was 94% greater than rats treated with no void filler (*)
  - CBVF treated rats was 56% greater than rats treated with no void filler (*)
- **The BV/TV of the ROI in neutrophil negative samples was:**
  - 41% greater than rats with a neutrophil reaction (N+) in the CBVF treated group (*)
  - 116% greater in C-G treated rats than rats treated with no void filler (*)
  - 94% greater in CBVF treated rats than rats treated with no void filler (*)
Discussion:
In this study, we demonstrated that CERAMENT™ G decreased the rate of infection and increased new bone growth as compared with both CBVF (w/o gentamicin) and no void filler in a debrided osteomyelitic environment. In animals without evidence of infection, significant differences were not found between the bone growth exhibited by CBVF and C-G. Both were superior to the infected CBVF and infected/non-infected empty groups. Although CBVF improved bone growth, the level of continued
infection in these animals (50%) continues to support the contraindication of this treatment for osteomyelitic defects.

Owing to the low rate of positive cultures seen in all three groups, significant differences in bacterial counts were not found among treatments. The non-destructive method used to extract organisms may have prevented the complete retrieval of bacteria from the bone. Additionally, even if more aggressive methods of extraction were used, these may not reflect the severity of infection over the entire course of the disease. An animal treated without antibiotics may still eventually clear the offending organism but the temporary presence of infection in the drill hole will delay the process of defect repair and thus result in a decreased level of bone growth.

**Significance:**
The treatment of chronic osteomyelitis often leaves a large critical defect which requires a void filler. Current void fillers are inadequate because of donor site morbidity, expense, or because of their propensity to act as a nidus of infection. This study supports the future use of CERAMENT™| G as an inexpensive, readily available void filler which can be used in osteomyelitic environments after debridement.

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