**Testing of a Bioactive, Moldable Bone Graft Substitute in an Infected, Critically-Sized Defect Model**

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## Statement of Purpose

Large bony defects, often resulting from high energy traumas, such as explosions, are difficult to treat due to their high variability in their size, complexity, and location. Many of these wounds become infected, and the presence of a chronic infection can have a major effect on healing of the bone and the overall health of the patient. The traditional method of treating these wounds has remained relatively unchanged since World War I and requires debridement of the wound followed by bone grafting and systemic antibiotics (Med Sci Monit 16:BR24, 2010). Current research focuses on developing bone graft substitutes which are biodegradable, biocompatible, and bioactive. In the present work, a moldable calcium sulfate (CS) bone filler capable of delivering bioactive molecules was tested in a rigorous infected segmental defect rat model.

## Methods

Bone filler samples were prepared using pre-dried cores and a moldable outer shell in order to provide a physical barrier for the delayed release of simvastatin while releasing antibiotics. Cores containing 97 wt% CS and 3 wt% simvastatin acid were dried at 40°C for at least 24 hours prior to implantation. Shells were composed of 75 wt% CS, 10 wt% hyaluronan (HY), and 15 wt% vancomycin loaded poly(lactic-co-glycolic acid) (PLGA) microspheres. After attaching a fixation plate to the femur of a Sprague-Dawley rat with six K-wires, a 6mm segmental defect was created using a surgical burr. A *Staphylococcus aureus* (*S. aureus:* ATCC 25923, $10^5$ CFU) soaked gelatin sponge was implanted in the defect site in order to create a chronic infection. After two weeks, the wound was surgically debrided and the simvastatin/vancomycin loaded CS bone filler implanted. Rats were euthanized at 4 and 12 weeks post-implantation and the resulting femurs analyzed using microcomputed tomography (microCT), non-decalcified histology, and torsional mechanical testing.

## Results and Discussion

In vitro release studies showed a prolonged release of vancomycin over the course of around 50 days and a delayed release of simvastatin from the bone graft substitute as can be seen in Figure 1. An established biofilm of *S. aureus* was successfully created in the critically sized defects of the rat femurs. This infection proved to be detrimental to the rats' health, and without treatment often resulted in the need for euthanasia. Rats treated with a month of systemic antibiotics showed no outward signs of infection during the drug treatment but became progressively worse shortly after discontinuation. Rats treated with the drug-loaded composite bone filler material, while surviving better than those without treatment still showed some signs of infection. Figure 2 shows microCT images comparing infected femurs treated with and without simvastatin after 4 weeks. Although significant bone formation in the defect sites at four weeks was not expected due to the delayed release and action of simvastatin on BMP upregulation, early signs of new bone can be seen in the treated femur images.

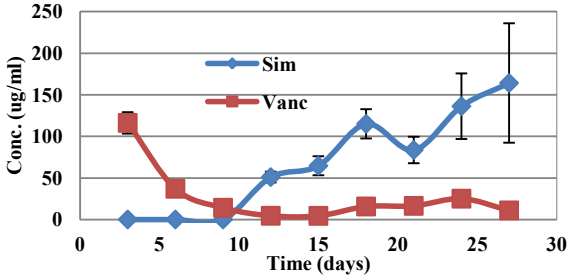


Figure 1: *In vitro* release profile showing prolonged release of vancomycin and delayed release of simvastatin.

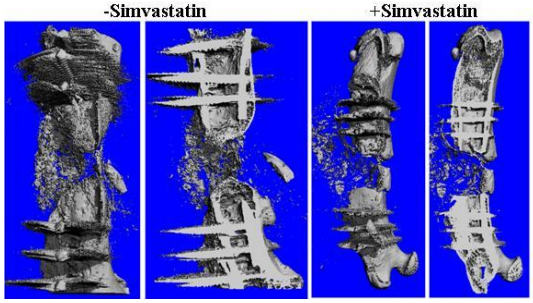


Figure 2: MicroCT images comparing treated and untreated infected critically sized defects in rats.

## Conclusions

Prolonged release of antibiotics is critical to treatment of infections and the delayed release of osteogenic drugs avoids drug waste and adverse healing in an infected environment. Traditional methods of treating massive infections are not an effective or efficient way to handle a chronic infection such as was studied here. One limitation of this study was that the lack of repeated debridements or removal the colonized hardware. Systemic delivery of antibiotics was unable to eradicate the infection, which soon grew out of control once treatment stopped. The local delivery of antibiotics from a bone grafting substitute was shown to slow down the progression of the infection and keep it in under control but was unable to obliterate it completely. Novel methods of eliminating biofilms need to be investigated for future therapies to allow for better bone regeneration.

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