

Set 1

Can clearance be achieved?

- K20 scaffold loaded for 24 hrs
- 9 animals/treatment group (3 animals/time point)
- Time point: 28d, 56d, or 84d
- Groups: infected and uninfected (PBS) exposed to scaffold saturated with vancomycin or PBS (treated vs. untreated).
- X Rays taken every 21 days
- Bacteriology: soft tissue
- uCT done on formalin fixed bones

Set 2

How much vancomycin is needed for clearance?

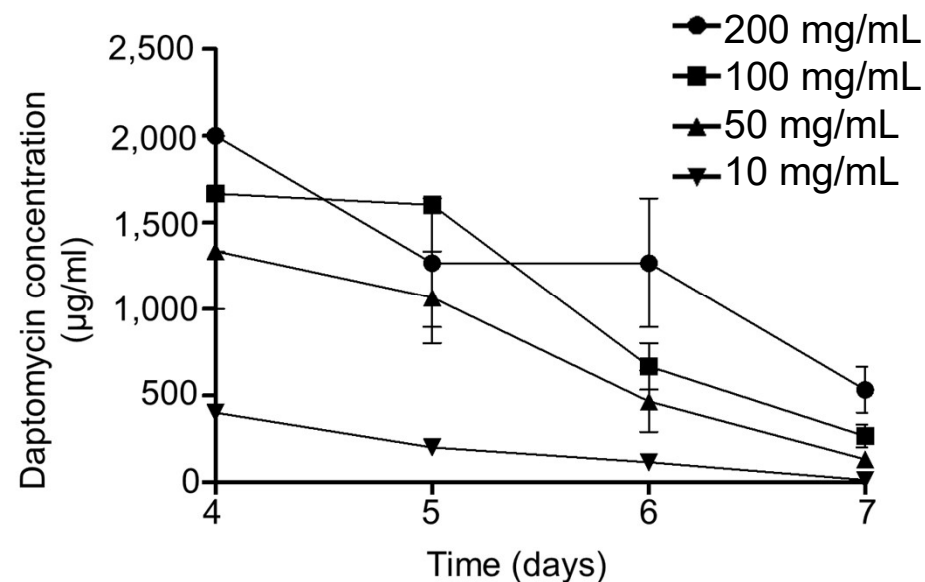
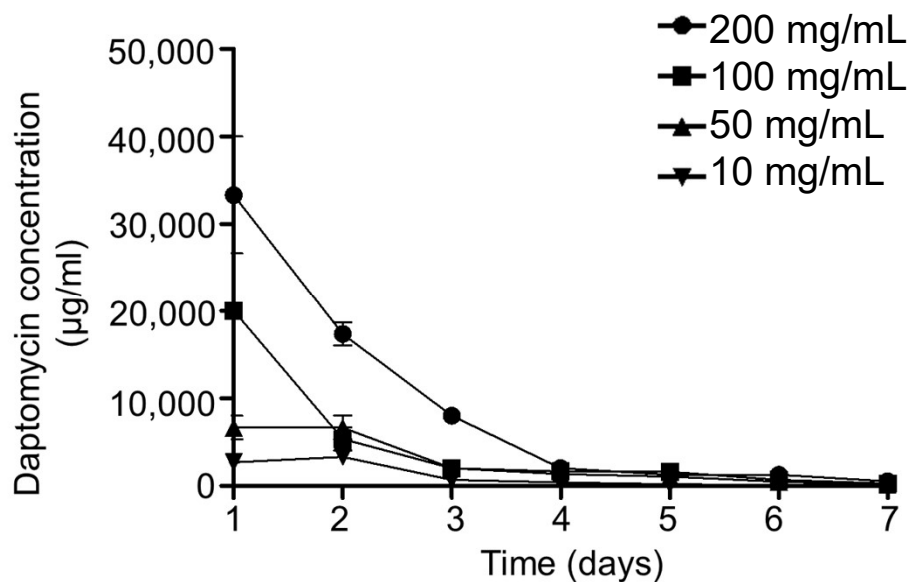
- K20 scaffold loaded for 1 hr
- 3 animals/treatment group
- Time point: 14d
- Groups: infected and exposed to scaffold with 0, 25, 50, 75, or 100 mg/mL vancomycin.
- X Rays taken at infection and harvest
- Bacteriology: soft tissue and bone/scaffold homogenates
- uCT not done

Set 3

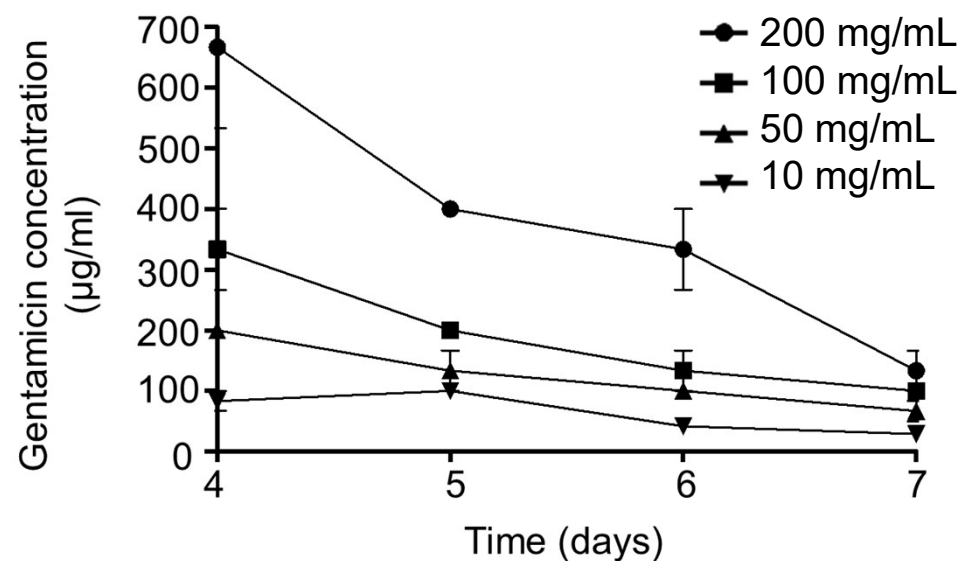
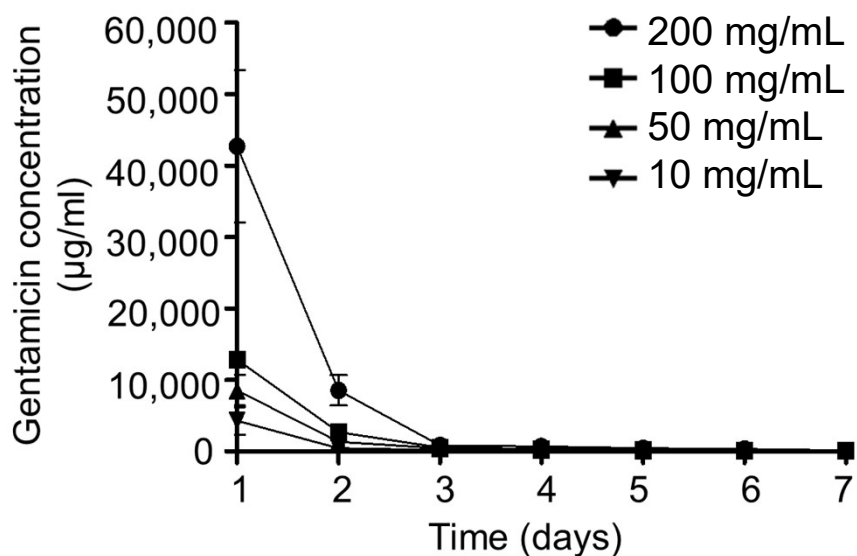
Is the S1 formulation effective?

- S1 scaffold loaded for 1 hr
- 3 animals/treatment group
- Time point: 14 d
- Groups: infected and exposed to scaffold saturated with 100 mg/mL vancomycin or PBS
- X Rays taken at infection and harvest
- Bacteriology: soft tissue and bone/scaffold homogenates
- uCT done with frozen bone protocol

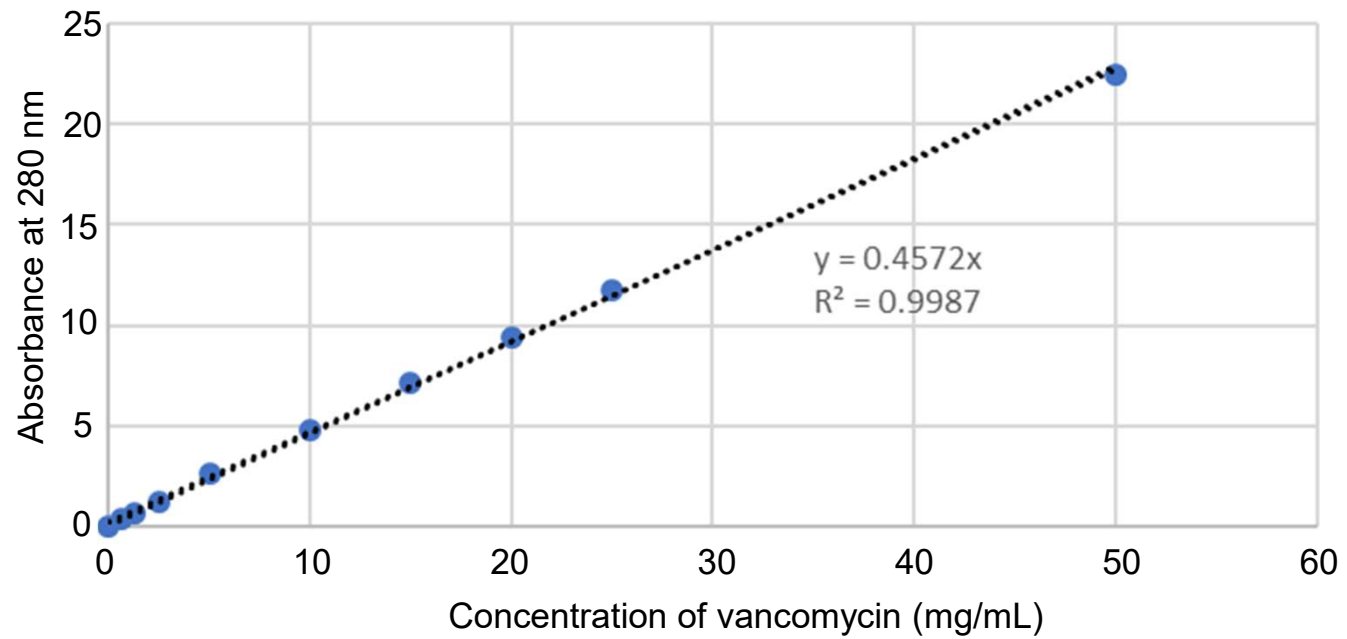
Supplementary Figure S1. Animal Experiment Progression Schematic. The animal experiments were performed in three sets, each aimed at a different question. Improvements to the protocol and differences between the sets are noted.



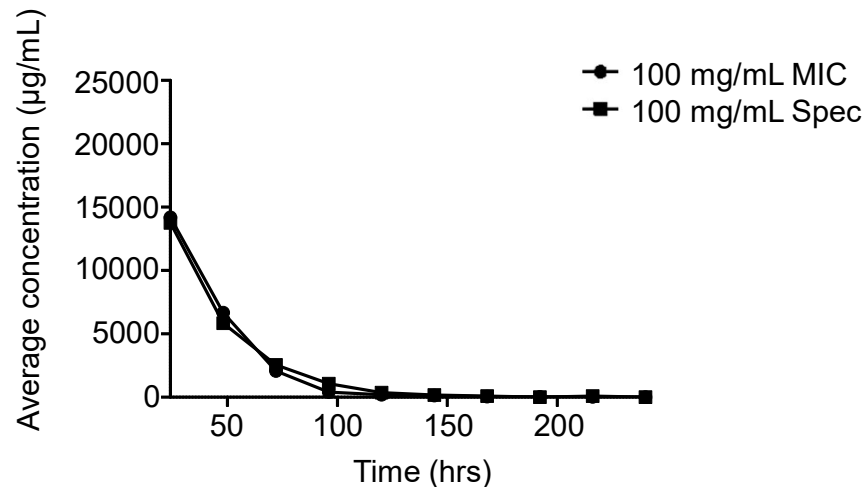
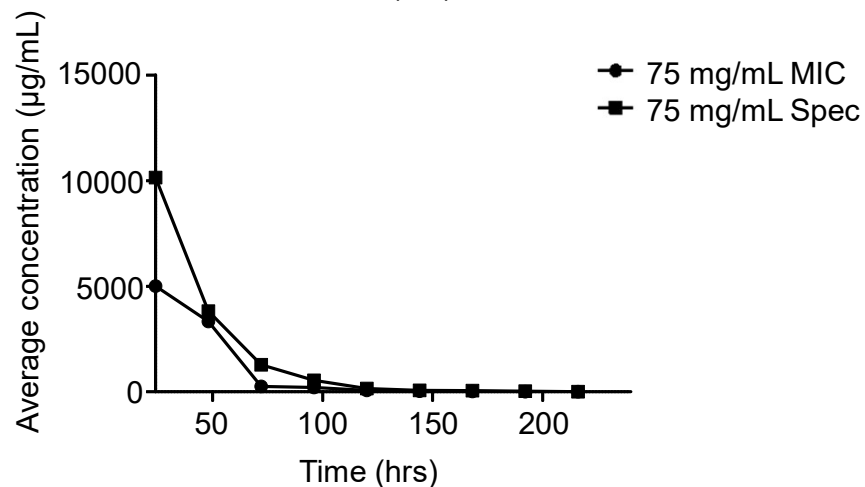
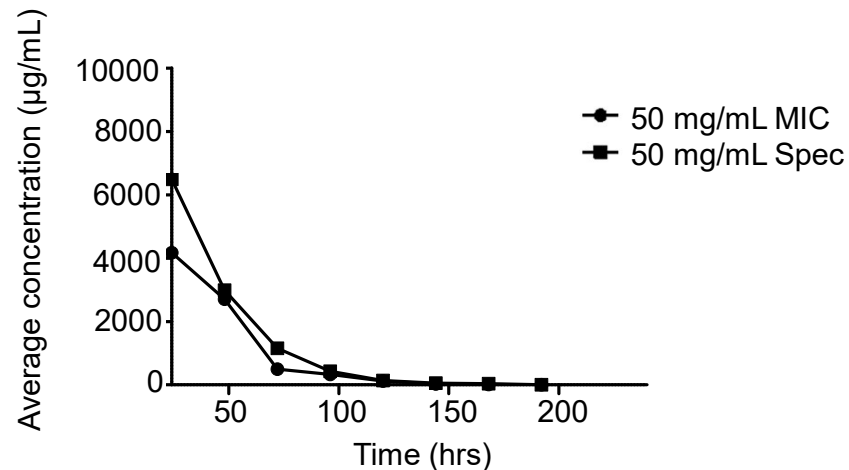
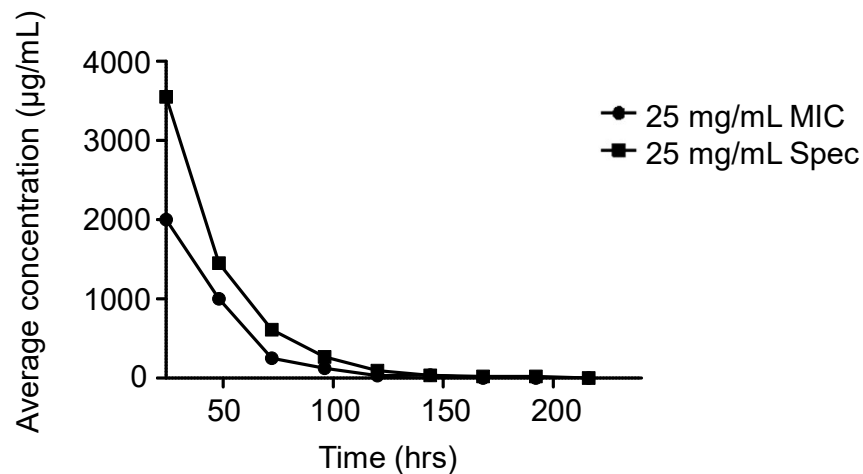
Supplementary Figure S2. Uptake and elution of daptomycin as a function of antibiotic concentration. The K20 scaffold was exposed to solutions of daptomycin at the indicated concentrations for 24 hours. Scaffolds were then removed and placed in PBS without antibiotic. Scaffolds were then removed and placed in PBS without daptomycin. The buffer was replaced with fresh antibiotic-free buffer at the indicated time points and the approximate amount of active daptomycin determined using a broth microdilution bioassay. Left: the results from a 7-day elution period. Right: Exploded view of the data focusing only on the later time points.



Supplementary Figure S3. Uptake and elution of gentamicin as a function of antibiotic concentration. The K20 scaffold was exposed to solutions of gentamicin at the indicated concentrations for 24 hours. Scaffolds were then removed and placed in PBS without antibiotic. Scaffolds were then removed and placed in PBS without gentamicin. The buffer was replaced with fresh antibiotic-free buffer at the indicated time points and the approximate amount of active gentamicin determined using a broth microdilution bioassay. Left: the results from a 7-day elution period. Right: exploded view of the data focusing only on the later time points.



Supplementary Figure S4. Correlation between concentration of active vancomycin absorbance at 280 nm. A solution of vancomycin was freshly prepared in PBS at the indicated concentrations and the absorbance at 280 nm measuring as a function of antibiotic concentration.



Supplementary Figure S5. Correlation between bioassay and spectrophotometric assay for vancomycin. The K20 scaffold formulation was saturated in a solution containing the indicated amounts of vancomycin for 1 hour at room temperature. Scaffolds were then removed and placed in PBS without vancomycin. The buffer was replaced with fresh antibiotic-free buffer at the indicated time points and the approximate amount of active vancomycin at each sampling point determined by bioassay (MIC) and spectrophotometric analysis (Spec).