Testing the Efficacy of the Purgo Pouch for Biofilm Eradication in the Spine

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DISCLOSURES

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INTRODUCTION

One major issue with spine infection treatment is biofilm eradication. A biofilm is a structured community of bacteria most commonly found attached to solid surfaces, within a matrix of self-produced extracellular polymeric substances (EPS). Broad-spectrum systemic antibiotics, one of the clinical standards of care for spinal infections, are highly effective at killing off planktonic bacteria, which are individual bacteria or small clusters free-floating in a liquid medium. However, the bacteria can aggregate into a biofilm, causing phenotypical changes such as antibiotic resistance–especially at sub-lethal concentrations in systemic treatments. With enough phenotypical changes, persister cells often develop within this biofilm and can reseed the infection even after it appears to have been cleared. Such resilient bacteria are difficult to truly clear from the body and often require higher concentrations of antibiotics than result from a systemic antibiotic treatment. However, using this higher concentration of antibiotics would be physiologically toxic. Additionally, with advanced infections, the vasculature at the site can become compromised, blocking the route through which systemic antibiotics could reach the bacteria. A device that could locally deliver antibiotics to the infection site would attain the high concentrations to kill the resistant biofilms, minimize systemic toxicity, and not require healthy vasculature for administration. One such device, the Purgo Pouch, was developed by Purgo Scientific®. The pouch is a refillable device that functions via passive diffusion to allow antibiotics to be released at a rate-controlled across its membrane.

We hypothesize that the Purgo Pouch, used in conjunction with the systemic antibiotic treatment, will result in a statistically significant lower bioburden at the treatment endpoints than the systemic antibiotic treatment alone.

METHODS

Staphylococcus Aureus strain 6538 is first cultured on agar plates for 24 hours. After which, 1 mL of a 0.5 McFarland standard of the bacteria is loaded into a modified CDC bioreactor filled with 500 mL of 100% modified BHI broth and etched titanium spinal fusion rods. The bacteria proliferate for 24 hours, and we flow the bioreactor with fresh 10% modified BHI broth for another 24 hours. We quantify the bacteria on dilution agar plates using three rods as controls to verify the inoculation quantity. With another one of the rods, we surgically implant it in the lumbar spine of a sheep model, using a pedicle screw and a locking cap. During this process, we scrape a small amount of the biofilm from the rod and place it on the top few threads of the pedicle screw. If the treatment includes a Purgo Pouch, we put it after the spinal hardware is fully implanted. The sheep receive treatments for either 14 or 30 days according to the following groups: systemic antibiotics only (750 mg levofloxacin and 600 mg rifampin), 352 mg tobramycin only via Purgo Pouch, systemic antibiotics and tobramycin via the Purgo Pouch, and negative and positive controls. The current study plan is to do four sheep in each group–two male and two female–for 36 total sheep. After euthanasia, we collect the rod and locking cap as well as gather tissue and bone samples. With these samples, we quantify the bacterial bioburden of *Staphylococcus Aureus* 6538 and use Scanning Electron Microscopy (SEM) to view any potential biofilm or bacteria remaining on a section of the spinal fusion rod. We claim our detection limit to be a minimum of 1 colony-forming unit (CFU) per 1 mL of sample without dilution. We also collect a spine section that includes the vertebra where we implanted the hardware and the two adjacent vertebrae. From this sample, we use Micro-CT to visualize bone remodeling across the groups and analyze the histology.

After more microbiological data is gathered and processed, two-sided T-tests will be performed between each group to detect if there is a statistically significant difference across treatments for each sample type.

RESULTS

While this is an ongoing study, we have processed preliminary micro-CT data on four sheep and microbiology data on 13 sheep. The micro-CT data show that sheep receiving systemic antibiotics only demonstrated visible bone resorption, while sheep receiving a locally delivered antibiotic through the Purgo Pouch did not show visible bone resorption (Figure 1). Additionally, our initial data show that for each sample type, the sheep receiving the pouch with systemic antibiotics had a lower average bioburden than sheep receiving systemic antibiotics only (Figure 2).

DISCUSSION

Our initial microbiological data show that the systemic antibiotic treatment mostly clears the infection by the treatment endpoint of 30 days. However, the surgical site of this treatment group appears to have more necrotic tissue when compared to the groups treated with the Purgo Pouch with or without Systemic Antibiotics. Moving forward, we will perform 14-day treatment windows to analyze the infection when we believe it to be most severe. We want to demonstrate that the Purgo Pouch with systemic antibiotics clears the infection more rapidly than systemic antibiotics alone.

CLINICAL RELEVANCE

It is estimated that vertebral osteomyelitis affects 26,170 to 65,400 people annually in the United States and that the rate of spinal infections is increasing[1].

REFERENCES

1. Gandhi S. M.D. and Schulder M. M.D. *"Spinal Infections." American Association of Neurological Surgeons*, 15 Apr. 2024.

IMAGES AND TABLES

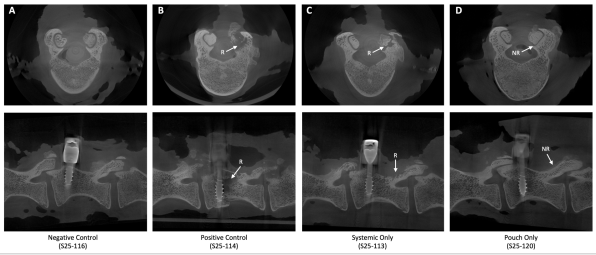

FIGURE 1. Micro-CT Images of Bone Resorption from Four Different Sheep

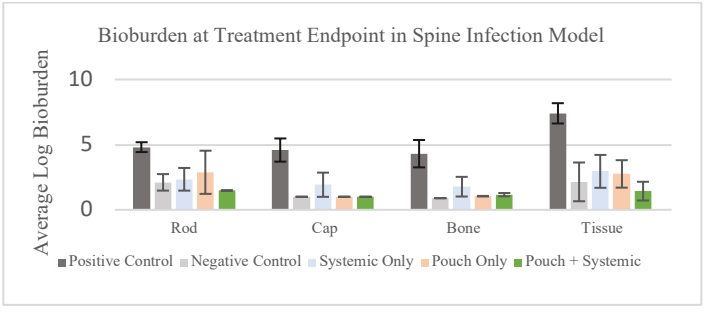

FIGURE 2. Average Log Bioburden of *Staphylococcus Aureus* 6538 Across Treatment Groups