Mitigation of MRSA infection *in vivo* by dual local controlled delivery of anti-infective agents

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Statement of Purpose: Surgical site infection (SSI) during orthopedic procedures is a feared surgery failure mechanism. SSI results in the attachment of microorganisms onto the implant surface. Extracellular matrix is secreted and a biofilm forms on the implant surface which is impenetrable to antibiotics. MRSA is one causative microorganism and its prevalence in the healthcare setting is increasing. The antibiotic vancomycin is used to treat methicillin resistant staphylococcus aureus (MRSA) infections, yet effective dosing is difficult to achieve because of the high minimum inhibitory concentration (MIC) against MRSA. Previous studies have shown its effectiveness when released from orthopedic implants caused by methicillin sensitive *S. aureus*[1]. Before, we also demonstrated the effectiveness of vancomycin in tandem with the adjuvant farnesol against MRSA *in vitro* [2]. The key advance was local delivery in the right ratio of two very dissimilar molecules, the adjuvant being poorly bioavailable. In the present study, we now investigated the effectiveness of the vancomycin-farnesol sol-gel film on mitigating MRSA infection *in vivo*. In our first report, we rely on the differences in bone morphology between experimental and control groups. The control groups included sol-gel releasing vancomycin, sol-gel without vancomycin (no treatment) and vancomycin intravenous injection. The bone morphology was assessed macroscopically (clinical observation) with micro CT, and histologically and immunohistologically for evidence of osteomyelitic changes.

Methods: Silica sol-gel thin film preparation- Silica based sols were prepared using methods previously described [2]. Briefly, the sols were prepared with the mixture of water, 1 N HCl, ethanol with tetraethyl orthosilicate (TEOS). The formulation of 20 wt% vancomycin/30 wt% farnesol was prepared based on the previous *in vitro* data demonstrating greatest effect on MRSA.

MRSA preparation and animal model- Male, adult Sprague-Dawley rats were used. The intramedullary canal of the femur was drilled from the intercondylar notch in the knee and reamed with needles for further cannulation. 10^3 CFU of MRSA (ATCC #33591) was injected into the canal. Following the MRSA inoculation, the implant was fitted into the canal. The muscle and skin were sutured to close the incision. For the vancomycin control injection group, following inoculation and implantation with sol-gel (no treatment) rods, a solution of vancomycin (4 mg/mL) was injected intravenously into the tail vein immediately following surgery and once daily 3 days post-surgery. This concentration was selected to reflect the amount of 10X the daily quantity of vancomycin in the plasma released from the sol-gel with vancomycin. The rats were housed for 2 or 4 weeks, upon which they were sacrificed, and femora were collected for further observation and analysis.

Micro CT, histological and immunohistological analysis- The implants were removed from each right femur (n=5 per group per time-point) and fixed prior to scanning using a Scanco 75T micro CT at high resolution. For histological and immunohistological analysis, the femora (n=3 per group per time-point) were fixed, sectioned, and embedded in plastic blocks.

Results: Clinical observation and micro CT images revealed pronounced changes in the distal femora of groups which received sol-gel (no treatment) with and without vancomycin injection (data not shown). Micro CT morphometric data revealed that the Trabecular Connective Density and Cortical Bone Volume/Total Volume (BV/TV) was higher in groups which received sol-gel implants with vancomycin alone or vancomycin-farnesol, thus retaining most of its morphology following inoculation and implantation (Figure 1A-B) at 4 weeks.

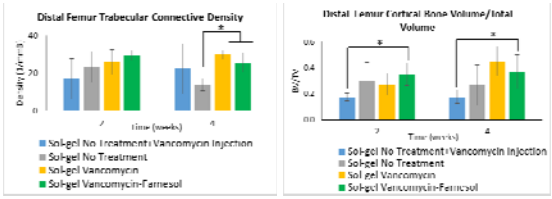

Fig. 1: A) Trabecular Connective Density, B) BV/TV

Fig. 2: Histology micrograph at 4 weeks. A) Sol-gel no treatment, B) Sol-gel vancomycin-farnesol.

Figure 2A shows a micrograph of a sol-gel (no treatment) sample and reveals microscopic evidence of osteomyelitis with peritoneal bone apposition and the presence of inflammatory infiltrate, osteoclasts and macrophages. These findings could not be observed in the sol-gel vancomycin-farnesol specimen (Figure 2B), but, significantly, excellent bone to implant contact existed.

Conclusions: In this study, focusing on micro CT analysis the rods coated with sol-gel silica with vancomycin with or without farnesol were shown not to produce osteomyelitic changes associated with MRSA. Analysis of the data using other methods is ongoing to reveal any differences between the controlled release of vancomycin, and vancomycin and farnesol.

[1] Adams, C.S., et al., Journal of Orthopaedic Research, 2009. 27(6): p. 701-709. [2] Bhattacharyya, S., et al., Biomaterials, 2014. 35(1): p. 509-17.