A Model for Bone-Biomaterial Infections: *Pseudomonas aeruginosa* Infection Inhibits Bone Regeneration in PEG-Hydrogels Functionalized with BMP-2 and Collagen-mimetic Peptide Without Accelerating Gel Degradation Time

Christopher T. Johnson, Susan M. Lehman, and Andrés J. García
Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA.
Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA.

Introduction: Implant-associated infections are a significant clinical problem accounting for over 1 million infections per year. Infection typically leads to device failure and complete removal of the implant. Bone fractures and non-union defects are injuries that often require surgical intervention where biomaterials, bone grafting, and protein therapeutics (BMP-2) delivery are used to correct the defect. These injuries account for over 600,000 cases and $5 billion dollars in costs. Of these, approximately 10% result in bacterial infection. *Pseudomonas aeruginosa* is a significant clinical pathogen capable of developing widespread antibiotic resistance. These factors provides motivaiton to develop a model capable of investigating how infection affects regenerative medicine therapies.

Currently, few robust models are availible to study how pathogenic bacteria interact with regenerative therapies. Our lab has recently developed a poly (ethylene glycol) based hydrogel to deliver BMP-2 and integrin specific peptides to facilitate bone regeneration in a critically-sized radial segmental defect in a mouse[1]. The main objective of this study is to develop a model that evaluates how a clinical isolate of *Pseudomonas aeruginosa* interacts with regenerative medicine implants.

Materials and Methods:

PsAer-9 contaminated GFOGER/BMP-2 hydrogel preparation: PEG-MAL (20 kDa MW, Laysan Bio) was reacted with GGYGGGPG$(GPP)_5$GFOGER$(GPP)_5$GPC (GFOGER) and rhBMP-2 (R&D Systems). Hydrogels were cross-linked with GCRDVPMSMRGGDRCG peptide (AAPPTEC). Non-sterile gels were inoculated with 5.0e5 colony forming units (CFU)/mL of the *Pseudomonas aeruginosa* clinical isolate PsAer-9 (CDC). Sterile gels did not contain bacteria.

Segmental bone defects: Male C57-BL were operated on to remove a 2.5mm segmental defect of the radius, resulting in a non-healing, critical-sized bone defect. PEG-GFOGER hydrogels with rhBMP-2 were implanted with and without PsAer-9 contamination. Micro-computed tomography was used to monitor bone regeneration at 0, 4, and 8 weeks.

In vivo gel degradation: GFOGER was labeled with the near infra-red dye, vivoTag 680 (Perkin Elmer). PEG-GFOGER hydrogels with rhBMP-2 were synthesized with labeled GFOGER. Sterile and contaminated hydrogels were implanted as above. Hydrogel degradation was monitored using fluorescence molecular tomography (Perkin Elmer), performed at days 0, 1, 3, 6, and 14.

Necropsy and implant processing: At 14 days post implantation, contaminated implants were explanted and processed. CFU per implant was determined through the plate count method performed on the processed implants.

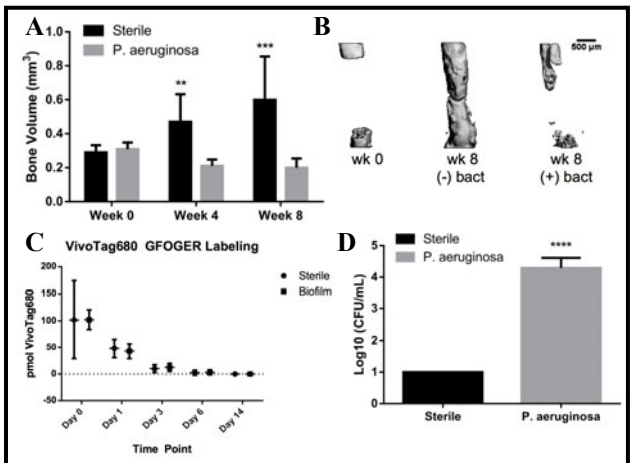

Figure 1: A) Bone volume over time of contaminated and sterile gels **B)** Representative images **C)** Gel degradation **D)** Bacterial count at day 14.

Results: ***PsAer-9 infection and bone healing:*** Hydrogels seeded with 5.0e5 CFU/mL PsAer-9 significantly inhibit healing as assessed by bone volume. Over the course of 8 weeks, infected animals saw an overall reduction in bone volume whereas sterile hydrogels produced a significant increase and defect healing (Figure 1A). Representative microCT reconstructions are shown in Figure 1B.

PsAer-9 does not increase hydrogel degradation rate: When GFOGER was labeled with vivoTag 680 and monitored using FMT imaging for 14 days, no significant difference in PEG-hydrogel degradation time was observed (Figure 1C). At day 14, implants were removed and processed for PsAer-9 presence. Figure 1D shows bacterial persistence of 2.5e4 CFU/mL, an order of magnitude less than the original inoculum.

Conclusions: The work presented here provides a model to study biomaterials associated implant infection. These data demonstrate that bacterial infection of therapeutic hydrogels by PsAer-9 inhibits bone regeneration using a mouse radial segmental defect model. The therapeutic potential of the implants is not compromised by increased gel degradation. Bacteria are present 14 days after gel implantation suggesting biofilm formation and protection from the host immune response. The platform developed here provides a robust method for evaluating the mechanism by which healing is inhibited as well as potential therapies to restore the implant's therapeutic properties.

References:

1. Shekaran et al. Biomaterials (Under Review)

Acknowledgements: This work is supported by NIH grants R01 AR062368 and R01 AR062920.

Disclosures: The authors have nothing to disclose

©2014 Society for Biomaterials