Bone Healing is Unaffected By PNDJ Hydrogel At Bone-Implant Interface

Ryan McLemore¹, Derek Overstreet, PhD², Brent Vernon, PhD³, Alexander C. McLaren, MD⁴.
¹Banner Good Samaritan Medical Center, Phoenix, AZ, USA, ²Sonoran Biosciences, Chandler, AZ, USA, ³Arizona State University, Tempe, AZ, USA.

Disclosures:
R. McLemore: 4; Sonoran Biosciences. 5; Astellas Pharmaceuticals. D. Overstreet: 4; Sonoran Biosciences. B. Vernon: 4; Sonoran Biosciences. A.C. McLaren: 4; Sonoran Biosciences. 5; Astellas Pharmaceuticals.

Introduction:

Prosthetic joint infections (PJIs) are caused by sessile, biofilm-forming bacteria. These bacteria are resistant to many antimicrobials, with MIC 100 to 1000-fold greater concentrations than those required to kill the same organism in a planktonic state. Treatment of PJI requires thorough removal of diseased tissues (debridement) and is augmented by local administration of antimicrobials. Currently, local antimicrobial delivery is done by placing high-dose antimicrobial-loaded bone cement (ALBC) in the wound following debridement. In a second procedure, ALBC is removed and a new permanent implant is inserted. Hydrogels based on the temperature-responsive polymer PNDJ (poly(N-isopropylacrylamide-co-dimethyl-γ-butyrolactone acrylate-co-Jeffamine® M-1000) have unique properties not found in current clinical drug delivery systems. PNDJ hydrogels are in situ gelling, allowing for complete implant coverage. They provide tunable, partition-controlled release of hydrophilic antimicrobials and re-dissolve completely via hydrolysis with minimal generation of acid groups. This study is intended to evaluate if the presence of these gels will inhibit the healing response at the bone/implant interface, or if these gels might be compatible with use as part of cementless revision.

Methods:

Two copolymers of [poly(NIPAAm-DBLA-JAam)] were synthesized by free radical polymerization with 91.2 mol% N-isopropylacrylamide (NIPAAm), 1.7 mol% Jeffamine M-1000 acrylamide (JAam) and 7.1 mol% (R)-(+)R-acryloyloxy-β,β-dimethyl-γ-butyrolactone (DBLA) (PNDJ 15) or 90.1 mol% N-isopropylacrylamide (NIPAAm), 2.9 mol% Jeffamine M-1000 acrylamide (JAam) and 7.0 mol% (R)-(+)R-acryloyloxy-β,β-dimethyl-γ-butyrolactone (DBLA) (PNDJ 22). Both polymers had weight average molecular weight (Mw) in the range of 35-45 kDa. Polymers were sterilized by ethylene oxide gas sterilization and stored at -20°C until use. Solutions were prepared by dissolving PNDJ15 or PNDJ22 at 30 wt% in sterile 150 mM phosphate buffered saline (PBS) (pH 7.4) at 4°C. After dissolution, gentamicin sulfate powder was mixed into the solutions a using a sterile spatula until homogeneously distributed. In vitro degradation was assessed via serial cloud point measurements.

A transcortical press-fit implant model in female New Zealand white rabbits (7-8 lb) was used to study bone healing, based on the work of Linder and Linskogg. Each rabbit was anesthetized, and had a 1.5 cm incision made over the lateral aspect of the left distal femur. The incision was carried down through fascial and muscle layers. The periosteal layer was then elevated, exposing the bone. A 4.5
mm drill bit was used to gently remove the cortex from the lateral aspect of the left distal femur. A defect similar to the size of the titanium implant was then created using a 3.5 mm drill bit, approximately 8 mm in depth. The defect was filled with one of 5 materials: PNDJ15-H, PNDJ15-L, PNDJ22-H, PNDJ22-L, or PBS. H indicates a high dose of gentamicin sulfate (50 mg/mL) and L indicates a low dose (5 mg/mL). Hydrogels were delivered using a 1 cc syringe fitted with an 18G needle. Hydrogels were injected, pressed into the defect using the thumb, defects were filled again prior to implant insertion to further drive the gel into interstices in the nearby bone. Prior to insertion of the implant, the implant was also dip-coated in gel to ensure complete surface coverage of the implant. A total volume of about 300 μL was used in each gel site. The coated implant was driven into the defect with a dowel attached to a hand chuck until the top (4.7 mm diameter face) of the implant was flush with the bone surface. The same procedure was repeated at the lateral aspect of the left proximal tibia through the same incision, and then repeated on the right leg in a similar fashion for a total of 4 implants per rabbit. Post-operative pain was managed with buprenorphine.

Eight weeks post-operatively, the rabbits were euthanized under anesthesia by sodium pentobarbital injection with thoracotomy as a secondary method of euthanasia. The distal femur and proximal tibia were harvested, fixed in 10% formalin, and sent for histological processing. Samples were embedded in polymethylmethacrylate prior to sectioning. Sections were taken along the length of each implant and stained with toluidine blue or hematoxylin and eosin.

**Results:**
PNDJ22 hydrogel has an *in vitro* degradation time of 28 days and PNDJ15 has an *in vitro* degradation time of 56 days at 37°C in PBS. Bone healing was visible primarily between the teeth of the implants. The quality of bone healing at the implant interface for all formulations is generally indistinguishable from that of PBS controls at 8 weeks post-surgery (Figure 1). Cortical bone was observed to heal around the implants in the femur, whereas primarily cancellous bone was observed around the implants in the tibia.

**Discussion:**
The results of this study indicate that neither PNDJ degradation time nor gentamicin dose affected bone ingrowth and healing rate for the materials tested. There was concern that the high viscosity of the material or acidity/toxicity produced by high concentrations of gentamicin sulfate might prevent normal bone healing to Ti-6Al-4V surfaces under compression. We observed *in vitro* degradation times of 4 and 8 weeks for the PNDJ hydrogel formulations used *in vitro*. These times are likely shorter in vivo because of the effects of proteins on polymer LCST, termed “salting out”. These degradation rates appear to be adequately rapid to not inhibit bone ingrowth in this model. Ingrowth in an animal model will tend to over-estimate ingrowth in a human subject, and the implants tested in this experiment, while creating compressive force to spur bone healing, are minimally load bearing, and not placed to prevent instability. Despite these limitations, the results are encouraging that an *in situ* gelling, viscous, anti-infective gel will be able to be successfully resorbed without inhibiting the normal healing of the surrounding bone.

**Significance:**
PNDJ hydrogels can be
successfully resorbed by the body and hydrolyze at expected rates in vivo. The survival and ingrowth of nearby bone indicate that this hydrogel may be suitable for inclusion around ingrowth surfaces or implants. Further study in models that incorporate instability is necessary.

Acknowledgments:
We gratefully acknowledge DACT at Arizona State University for animal care, Histion (Everett, WA, USA) for histological processing, Elizabeth Lee for assisting with the degradation study, and James Fraser and Keith Jarbo for assisting with surgery.

References:

\[\text{Figure 1: Results of Bone Healing Study. A and B show sections stained with toluidine blue collected from specimens immediately after insertion. C shows a section from an implant coated with PSS. D shows an implant coated with PNDU15H- the slowest degrading and highest drug loaded formulation tested in the study.}\]