Novel Hydrogel Delivering Gentamicin Controls Orthopaedic Infection in Rabbit Model

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Disclosures:
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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.

Carl Nelson and colleagues developed a standardized model in the late 1990’s that has been used by other investigators as a model of orthopaedic infection. This model was employed by Nelson, et al, to evaluate a poly-anhydride based bead delivery system, “Biodel”® in 1997. Mikos, et al, employed this model to evaluate the efficacy of PLGA microspheres delivering tobramycin in 2004.

Nelson showed a cure rate of 4/16 animals (25%) with debridement +iM gentamicin sulfate, and 15/16 (93%) with Biodel beads at 20 wt% gentamicin sulfate. Mikos showed a 25% (2/8) cure rate in the debridement alone group, and a cure rate of 37.5% (3/8) with PLGA microsheres alone. An additional parenteral 25 mg/kg of cefazolin was delivered IV 2x per day post-operatively to selected groups in the Mikos study, increasing the success in controlling the infection to 75% (6/8) when combined with the PLGA microspheres or 5/8 (63%) when combined with the ortho-set®. Mikos concluded that only the PLGA+tobramycin+iv cefazolin group was statistically different from the debridement alone, with a 25% vs 75% cure rate. In this study, we report on the effectiveness of our novel hydrogel, loaded with gentamicin, at controlling infection/osteomyelitis in a modified Nelson model in rabbits.

Methods: A copolymers of [poly(NIPAAm-DBLA-JAAm)] were synthesized by free radical polymerization with 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.

16 white New Zealand female rabbits were divided into two groups, 8 to receive debridement only, and 8 to receive debridement plus gel with gentamicin. Animals were initially anesthetized using ketamine/xylazine and maintained free breathing on 2-3% isoflurane during the course of the procedure. A 2cm long incision was made along the radius on the right fore-limb of each animal, separating muscles along fascial planes to reach the animal’s radius. The periosteum was stripped from the radius using a scalpel and dissector. An approximate 1.25 inch length of the radius was removed using a small-toothed saw blade. An 0.045” k-wire was placed inside the length of the bone, and a micropipette was used to introduce 50 uL of a 10^8 colony 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.

All subjects showed the presence of major abscess after 4 weeks of incubation. All debridements were completed by a trained orthopaedic surgeon with experience in infection management. The surgeon was blinded during the debridement as to the treatment that the specimen was to receive, and animals were completed in random order. Initially, an incision was made in the skin and underlying tissue, and any pus present was drained. Subsequently, infected tissue was removed using a scalpel. Radius was resected using rongeurs until there was no evidence of pus remaining in the canal (termed paprika sign). A section of 0.045” k-wire spanning the remaining radius was then placed in the wound. This represents a departure from the Nelson and Mikos models which do not leave any foreign material behind after debridement, and renders the model more difficult to effect a cure in. Based on intent to treat, the wound was either closed with 3-0 prolene, or filled with 30 wt% PNDJ gel containing 50mg/mL gentamicin sulfate (33 mg/mL gentamicin at 66% activity by mass), and then closed with 3-0 prolene. Volume of the debrided space ranged from 300-800 uL, for a total dose per animal of ~9.9-26.4 mg of active gentamicin. Animals were allowed to survive for 4 weeks post debridement. Cultures were routinely taken for all subjects at two time points: post debridement, and post treatment. Post debridement, the k-wire, surrounding tissue, and any pus were carefully collected on a sterile swab supplied by the laboratory. Care was taken in the approach and handling of the tissue by the operating surgeon to avoid any contamination.
of the site prior to or after culture. Immediately post euthanasia, samples of the k-wire, suture and surrounding tissue were similarly collected in a sterile environment to avoid contamination. All samples were sent to IDEXX reference laboratory for independent verification of culture status. Any reported culture negatives were maintained for 21 days to ensure the absence of small colony variants before they were termed “negative” cultures.

**Results:** 16/16 animals had active infections as confirmed by culture after inoculation. Two of the subjects formed draining sinuses between the infection and the debridement procedure. Abscesses produced by the procedure were large and originated from involucrum formed around the retained radius fragment (Figure 1).

Post debridement, 0/8 (0%) of animals who received debridement alone and had a replaced k-wire were cured, with culture results ranging from 2+ to 4+ for *Staphylococcus aureus*. 8/8 (100%) animals which received hydrogel were culture negative after 21 day negative culture retention (Table 1). Gentamicin delivered by the hydrogel reliably prevented re-infection after debridement (p<0.001). Tissues which had received hydrogel showed apposition onto the k-wire remaining in the wound, whereas tissues that did not receive hydrogel had loose k-wires present, and several had large regions of pus present 4 weeks after surgery.

**Discussion:** Taken together with the results obtained by Mikos and Nelson, this data indicates to us that there is an interaction between the degree of debridement, the placement of, and the dosage of the local delivery vehicle. Successful treatment should require debridement of infected tissue such that fluid will reach all remaining contaminated surfaces, combined with adequate concentrations of antimicrobial in delivered fluid to affect MBEC (minimum biofilm eradication concentration) for sufficient time to remove residual bacteria/biofilm. This study supports that an **in situ**, conformal drug delivery system which is rapidly resorbed, but provides several days of high flux, large volume release coverage will have a meaningful impact on orthopaedic infection rates.

**Significance:** PNDJ hydrogel shows promise for managing active infection through local delivery in fractures or revision arthroplasty.
Acknowledgments:

References:

Figure 1: Infected Subject at Time of Surgery. A) Large abscess formed subcutaneously in the tissues of the rabbit fore-limb. B) Involucrum and K-wire with tissues mid-debridement inferior to the abscess.

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