Bone & Joint Research

Supplementary Material

10.1302/2046-3758.101.BJR-2019-0193.R3

Supplementary Methods

Other methods of periprosthetic joint infection (PJI) induction tested

All animal work was carried out according to the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC) and Animal Care and Use Review Office (ACURO)-approved protocols. In the development of this model, each experimental condition was tested using five 5 to ten 10 New Zealand White Rabbits (female, 2 kg to 4 kg): three 3 to five 5 animals were harvested at 5 and 28 days after surgery. Induction of anaesthesia was achieved by administering Ketamine at 30 mg/kg subcutaneous (SQ) and Xylazine 2 mg/kg SQ, and animals were then moved to 2% isoflurane inhalation anaesthesia for the remainder of the procedure. Intubation was utilized to assist in maintaining the correct plane of anaesthesia. The left hind limb of the animals was clipped and disinfected with Chlorohexidine surgical scrub rotating with 70% isopropyl alcohol and then the surgical area was sprayed with 5% povidone-iodine. All veterinary supplies were purchased from Henry Schein (Dublin, Ohio, USA). After anaesthetization, the left femur, sterilely prepped, and the bone were exposed by a small incision. In all groups, a bone tunnel was created in the exposed femoral condyle using a 1.2 mm or 1.6 mm tungsten carbide drill bit depending on

screw size. The bone tunnel entrance was located within the capsule (exposed to synovial fluid), on the medial epicondylar surface with the bone tunnel positioned transversely across the medial femoral condyle penetrating deep into the cancellous (trabecular) bone. After creation, the bone tunnel was dried and treated using one of the following:

(1) Intra-articular injection with headed screw (intra-articular HS): An aseptic screw was placed into the bone tunnel without bacterial inoculation and the wound was closed. Before closure of the superficial skin layer, a 0.1 mL inoculum of 2 x 10^6 colony-forming units (CFU)/mL or 2 x 10^7 CFU/mL in phosphate-buffered saline (PBS) was injected into the joint space. [Note: In all cases, the aseptic screw used was a 1.5 mm × 6.4 mm flat head stainless-steel screw (#0; McMaster-Carr, Elmhurst, Illinois, USA), unless otherwise indicated.]

(2) Intraosseous injection with headed screw (intraosseous HS): Here, a 1 μL inoculum of 1×10^9 CFU/mL in PBS was injected into the bone tunnel before placement of the aseptic screw.

(3) Intraosseous injection with encapsulated bacteria and headed screw (intraosseous mGL): Before placement of the aseptic screw into the bone tunnel, a 1 μL droplet of methacrylated gelatin and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) (provided by Dr. Hang Lin, at University of Pittsburgh) resuspended in PBS containing 2×10^5 CFU was placed into the bone tunnel, following which the inoculum was photo-illuminated at 395490 nm to photopolymerize the bioscaffold.

(4) Intraosseous injection with headless screw (intraosseous HLS): Aa 1 μL inoculum of 1×10^9 CFU/mL in PBS was injected into the bone tunnel before a stainless-steel hex-wrench set screw (2 mm × 4 mm; McMaster-Carr) was placed in the defect. The screw was counter-sunk and bone wax used to seal the bone.

(5) Aseptic screw: An aseptic screw was placed into the bone tunnel without bacterial inoculation and the wound was closed layer-by-layer without any bacterial inoculation.

After surgery, all animals received Ketoprofen (2 mg/kg, twice daily) and buprenorphine (0.05 mg/kg, twice daily) or Torbugesic (0.5 mg/kg, three times a day) and cefazolin treatment (25 mg/kg, twice daily) as follows. Animals in the initial 'control aseptic group' (screw with head, no inoculation) received no antibiotics. During the 'screw with head, intraosseous group' test, we administered antibiotics to the last animal and discovered that, while the animal succumbed to infection, it survived longer. For all subsequent cohorts, we treated all animals with cefazolin beginning at three 3 days after surgery and continuing up to ten days, as needed, until the animals were no longer febrile or until humane endpoint determinations were made (based upon weight loss and pain score). The number of animals receiving antibiotic treatment for each group is indicated in Supplementary Table i. We were able to repeat a second control aseptic group (headless screw) with the same antibiotic treatment, the data for which are shown in Supplementary Table i and reported in this manuscript. Postoperative radiographs were taken every week, and blood was collected at 3, 5, 7, 14, 21, and 28 days after surgery.

Postoperative animal care

After surgery, rabbits were monitored twice a day for 28 days. For infected rabbits, body temperature was taken every day before analgesic treatment until they got back to normal and then checked once a week until day 28. Body weight was checked twice a week until day 28. The rabbits' daily diet was supplemented with Critical Care (Oxbow Animal Health, Omaha, Nebraska, USA) and animal-specific nutrient enrichment throughout the study.

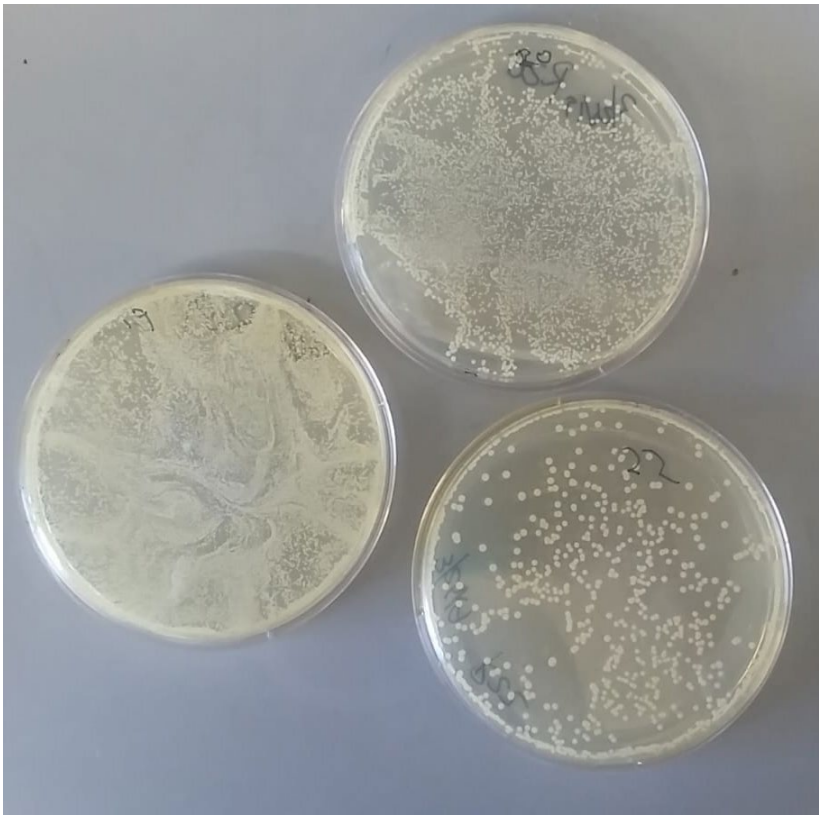
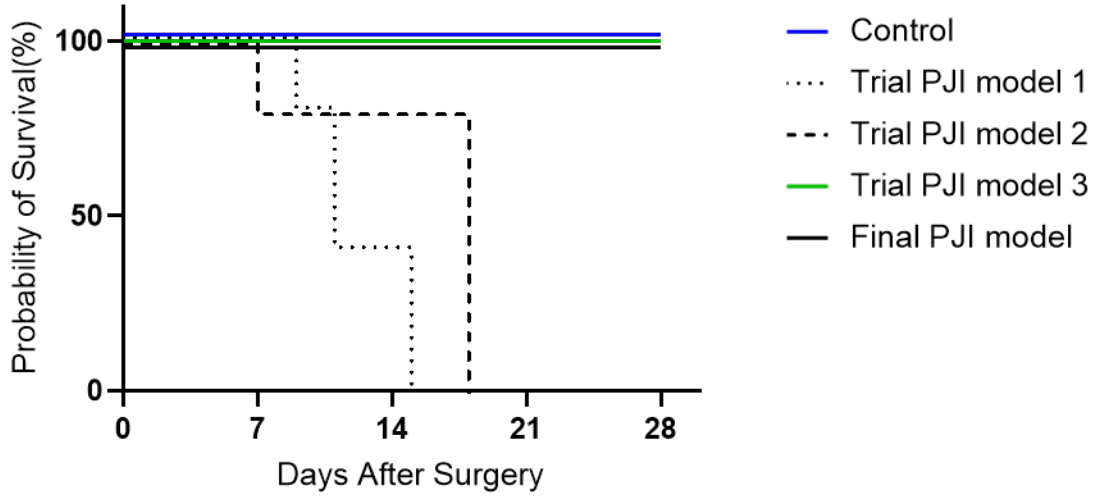


Fig. a. Representative bacterial cultures to confirm the presence of viable bacteria at 28 days after inoculation.

Table i. Comparison of preliminary models in the development of the final chronic periprosthetic joint infection model.

Group	Implant	Bacterial injection	Number of bacteria, CFUs	Number of animals with antibiotic Rx	Bone wax sealing	Signs of illness	Survival rate	PJI development
Control	Headless screw	No	None	5/5	-	-	100 (5/5)	No
Trial PJI model 1	Screw with head	Intra-articular	2×10^6 in 100 µL of PBS	5/5	-	+	0 (0/5)	No
			2×10^5 in 100 µL of PBS	5/5	-	+	0 (0/5)	No
Trial PJI model 2	Screw with head	Intraosseous	1×10^6 in 1 µL of PBS	5/5	-	+	0 (0/5)	No
Trial PJI model 3	Screw with head	Intraosseous	2×10^5 in 1 µL of photocrosslinkable gel	1/5	-	+/-	100 (5/5)	No
Final PJI model	Headless screw	Intraosseous	1×10^6 in 1 µL of PBS	5/5	+	+	100 (5/5)	Yes

PJI, periprosthetic joint infection; PBS, phosphate-buffered saline.



Number at Risk

Days	0	7	9	11	15	18	28
Control	5	5	5	5	5	5	5
Trial PJI model 1	5	5	5	4	2	0	0
Trial PJI model 2	5	5	4	4	4	4	0
Trial PJI model 3	5	5	5	5	5	5	5
Final PJI model	5	5	5	5	5	5	5

Fig. b. Kaplan-Meier survival plots of the four periprosthetic joint infection (PJI) models tested as well as the control group.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	In Methods "Surgical groups and procedures" including each animal (also clarified in legends of
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	In Methods "Surgical groups and In previous work, we observed:
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Not included in the text: Animals that showed signs of There were no exclusions Described in Methods "Surgical groups and
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	In Methods "Surgical groups and procedures: animals Confounders were not specifically controlled.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	All surgical. research and veterinary staff were aware of group
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Described clearly in In Methods Radio-translucency (directly related to
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	See Methods "Statistical Assumptions were met
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	In Methods "Surgical groups and Animals had to be in good health in order
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Described in Methods Described in Materials Described in Materials Described in Materials
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	See Resutls section and accompanying Enter details here

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	See Abstract subsectio
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	See Introduction See Introduction paragraphs 3 and 4
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	See Introduction paragr
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	See Methods "Ethical review committee statement
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	See Methods "Postoper
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	See Methods "Postoperative animal care" None reported The humane endpoints are not explicitly stated in the
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	See Results organized by experimental outcome See Discussion paragraph 5
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Relation to human condition is made in Discussion paragraph 3
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	This is a an animal study. Protocol identifiers are listed in Methods "Ethical
Data access	20	Provide a statement describing if and where study data are available.	Data are currently store
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	Conflict of Interest forms are submitted. Statement that no See Acknowledgements