Surface preparation. The pins were ultrasonicated in 10% Decon 90 (Decon Laboratory Ltd., Hove, United Kingdom), for 20 minutes. An air gun was used to clear debris from within the pores of the implants before and after ultrasonication in 10% Decon 90. This was followed by ultrasonication in industrial methylated spirit for 20 minutes. Coatings were produced by immersion of the implant into a 0.13 M calcium phosphate monobasic solution with 100 mg of $AgNO_3$ added per litre of solution to produce hydroxyapatite with silver (HAAg) coatings. The implant to be coated acted as a cathode and was attached to the negative terminal of a direct current Dual Power Supply 6010D (Peak Tech, Ahrensburg, Germany). A platinum ring was used as the anode.[1] A current density of 58 mA/cm^2 was applied for 270 seconds.[2] Implants were sterilised with dry heat at 160°C for one hour. Fibronectin was adsorbed onto the porous flanges by immersing implants into a 3.5 ml solution of phosphate buffered saline containing 2745 ng of fibronectin for 1.5 hours.

Surface topography, coating thickness and elemental composition. Scanning electron microscopy (SEM) was used for surface analysis, with six areas on three implants visualised. Additionally, SEM was used to measure the thickness of hydroxyapatite (HA) and HAAg coatings on the outer surface of the implant and within the central pores after embedding samples in LR White Resin (London Resin Company, Reading, United Kingdom) (four areas on two implants). The embedded samples were cut longitudinally through the centre with the Exakt diamond edge saw (Mederex, Frome, United Kingdom) and polished with grinding paper in ascending order of grit sizes and MD polishing cloth (Struers Ltd, Rotherham, United Kingdom),with OP-S colloidal silica suspension (Struers, Ballerup, Denmark) and 30% hydrogen peroxide (VWR International Ltd., Lutterworth, United Kingdom). A ruler was used to measure the coating thickness. Energy-dispersive X-ray spectroscopy analysis was performed to determine the Ca/P ratio of HA coatings and to measure the atomical percentage of silver within coatings (three implants, six areas per implant).

Premedication and anaesthesia. A dose of 0.2 mg/kg of 2% xylazine (Bayer HealthCare, Berkshire, United Kingdom) was administered as premedication. Induction of anaesthesia was achieved with 2 mg/kg of intravenous ketamine hydrochloride (Fort Dodge Animal Health Ltd., Southampton, United Kingdom) and 2.5 mg of intravenous midazolam (Roche Products Ltd, Hertfordshire, United Kingdom). The animals underwent endotracheal intubation and 5 mL cephalexin (Schering-Plough Animal Health, Welwyn Garden City, United Kingdom) was administered as prophylactic antibiotic cover on induction. Anaesthesia was maintained with inhaled 2% isoflurane. Peri-operative analgesia was maintained with fentanyl transdermal patches (75 µm/hour) (Duragesic, Janssen Pharmaceuticals, Raritan, New Jersey.

Surgical procedure. Both hindlegs were shaved and prepared with povidone iodine scrub and solution. Implants were inserted along the length of the tibias under aseptic conditions. A separate 15 mm longitudinal skin incision over the anteromedial subcutaneous aspect of the tibia was made per implant. A periosteal elevator was used to expose the bone. A 4.2 mm hole was drilled through both cortices. The pins were inserted with the flanges positioned sub-dermally. The skin and subcutaneous tissues were closed with interrupted 2-0 vicryl sutures. Opsite spray dressing, mepitel, gauze and a crepe bandage were used to dress the wounds. The position of implantation of each pin type along the tibia was changed in a rotational manner for each animal in order to account for the usual reduction in soft-tissue thickness from the proximal to the distal end of the tibia.

Post-operative course. Cephalexin (900 mg in 5 ml) was administered intramuscularly once daily for three days post-operatively. A fentanyl patch was used as analgesia and was replaced two days after surgery. The wounds were reviewed and re-dressed weekly. At four weeks post-operatively, the hindlegs were amputated following euthanasia with 0.7 mL/kg of intravenous pentobarbitone sodium. The implants were removed *en bloc*, with care taken not to disrupt the interface between the soft tissue and the implant.

Histological sectioning. A longitudinal section was taken through the centre of each specimen using an Exakt E310 diamond-edged band saw (Mederex). Sections were prepared and were ground to a thickness of 70 µm and polished using an Exact-Micro-Grinding System (Mederex). Sections were stained with toluidine blue and paragon prior to visualisation with a Zeiss microscope (Zeiss KS300 3.0, Imaging Associates, Thame, United Kingdom).

References

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