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Porous orthopedic steel implant as an antibiotic eluting device: Prevention of post-surgical infection on an ovine model

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ABSTRACT

Traumatology and orthopedic surgery can benefit from the use of efficient local antibiotic-eluting systems to avoid bacterial contamination of implanted materials. In this work a new percutaneous porous-wall hollow implant was successfully used as a local antibiotic-eluting device both *in vitro* and *in vivo*. The implant is a macroporous 316L stainless steel filter tube with a nominal filtration cut-off size of 200 nm with one open end which was used to load the synthetic antibiotic linezolid and an opposite blind end. The antibiotic release kinetics from the device on a simulated biological fluid under *in vitro* conditions demonstrated an increased concentration during the first five days that subsequently was sustained for at least seven days, showing a kinetic close to a zero order release. Antibiotic-loaded implants were placed in the tibia of four sheep which were trans-surgically experimentally infected with a biofilm forming strain of *Staphylococcus aureus*. After 7 and 9 days post infection, sheep did not show any evidence of infection as demonstrated by clinical, pathological and microbiological findings. These results demonstrate the capability of such an antibiotic-loaded implant to prevent infection in orthopedic devices *in vivo*. Further research is needed to assess its possible use in traumatology and orthopedic surgery.

1. Introduction

Traumatology and orthopedic surgery use several types of percutaneous and internal devices aimed to repair, fix or reconstruct damaged bones. To avoid bacterial contamination of these devices, the use of prophylactic systemic antibiotics (usually cefazolin, ceftazidime, cefuroxime or linezolid) before and/or after surgery is required (Bratzler and Houck, 2004), but sterile conditions during surgery are not always guaranteed. In fact, bacterial infections, mostly caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*, are a common problem after implantation (Crockarell et al., 1998). The slime, which is a component of the bacterial biofilm matrix produced by some strains of those bacteria, can adhere to the implant surface protecting them from the action of antibiotics and tissue macrophages, thus originating an inflammatory and infectious process in the bone (osteomyelitis) which can also causes damage to adjacent tissue. Biofilm infections are difficult to eradicate (Costerton et al., 1987; Gristina, 1994; Gracia et al., 1998). Once these infections are established, a systemic treatment of intravenously administered culture-specific antibiotics is needed. When the antibiotic therapy is unsuccessful, surgical interventions with debridement of the adjacent implant tissue and prosthesis re-implantation may be required, with all the risks and associated costs involved with these procedures (Bernard et al., 2004).

In this context, both traumatology and orthopedic surgery may potentially benefit from the use of an on-site, efficient local drug-eluting device to help prevent bacterial colonization while providing a sustained local antibiotic concentration above the minimal inhibitory concentration (MIC) but still under the systemic toxicological threshold, and at the same time, minimizing the collateral side effects characteristic of systemic administration (Perez et al., 2011). Drug-eluting implants used in traumatology and in orthopedic surgery include: antibiotic-loaded bone cements and fillers used to prevent osteomyelitis or cements loaded with osteoinductive molecules such as growth factors to favor the osseointegration of the implant (Garvin et al., 1994). A disadvantage of using antibiotic-loaded bone cements is that the necessary antibiotic concentration is frequently at least 10 times the MIC,

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well above the biofilm eradication concentration for the involved pathogen; therefore high concentrations of antibiotics are used (Soundrapandian et al., 2007). Moreover, the elution of the drug depends on the surface area and porosity of the cement, which is difficult to control and reproduce, because the antibiotic diffuses trough the defects or cracks that are formed during the cement drying process. This diffusion is very fast, not controlled and the pharmacokinetic and pharmacodynamics are usually not known and difficult to reproduce, since the cement defects are unpredictable and non-repetitive. Other disadvantage of using antibiotic-loaded bone cements is that a second surgery is necessary to remove them. Also, antibiotic loaded bone cements cannot be used when hydroxyapatite coated prostheses are employed (Mclaren, 2004). To date, despite antibiotic-loaded bone cements being used worldwide for more than three decades for prophylaxis and treatment in orthopedics, the FDA has only approved the use of bone cements with gentamicin or tobramycin as antibiotic delivery vehicles (Hanssen, 2004).

The design of efficient local drug-eluting devices could be an alternative to deliver therapeutic dosages at target tissues, minimizing undesired side effects and reducing costs associated with traditional systemic delivery (Englert et al., 2007). In this work we present the use of a hollow macroporous steel implant as a local antibiotic eluting device. This model is relevant in trauma because the usual clinical practice in major bone fractures involves the use of percutaneous fixation devices (e.g., Ilizarov frames, Montecelli type, pins, screws, rods) and some of these devices could potentially be transformed into a hollow structure without comprising their mechanical properties to additionally provide them with localized drug delivery ability. This concept has been tested by implanting an antibiotic-loaded porous-wall stainless steel reservoir in the ovine tibia through the skin as an external fixation device, and immediately challenged by an experimental local infection with a biofilm forming strain of *S. aureus.* Adult sheep are an appropriate model as they offer the advantage of having a similar body weight to that of humans together with long bones with suitable dimensions for implantation of human prostheses and implants, a circumstance which is not feasible in smaller species such as rabbits or small dog breeds (Newman et al., 1995). This work represents an *in vivo* demonstration of our original *in vitro* proof-of-concept previously reported (Perez et al., 2011).

2. Materials and methods

2.1. In vitro experiment: study of linezolid kinetics by using a macroporous steel implant

Hollow macroporous 316L stainless steel porous filter tubes were purchased from Mott Corporation (Farmington, USA). Sections of this material were mechanized to be used as implants with one open end (used to load the antibiotic) and a blind end welded on the opposite side. Each implant has a porous length of 25.4 mm and an outside diameter of 6.35 mm with a wall thickness of 1.6 mm. The nominal filtration cut-off size of the stainless steel filter is 200 nm showing a volumetric porosity of 17%. The design of this implant allows diffusion from the inner space through the porous wall (Fig. 1). The inner volume of the hollow implant was carefully loaded with 95–120 mg of lyophilized commercial linezolid (Zyvox®). According to the manufacturer, each mL of the pharmacological solution contains 2 mg of linezolid, 45.7 mg of glucose and 0.38 mg of sodium (as sodium hydroxide). The injection also contains sodium citrate, citric acid anhydrous, hydrochloric acid and water for injections.

In vitro desorption rates were evaluated by immersing at 37 °C under stirring linezolid loaded implants in 100 mL of simulated body fluid (SBF), prepared according to the method described by Kokubo et al. (1990) using the following molar concentration: $142Na^+:5K^+:1.5Mg^{2+}:2.5Ca^{2+}:148.8Cl^-:4.2HCO_3^-:1HPO_4^{2-}$, because such ionic media promotes bone formation in bioactive glasses. Linezolid release rate was evaluated in independent triplicate experiments by measuring drug concentration in SBF using 251 nm UV–vis spectrophotometry at fixed time intervals.

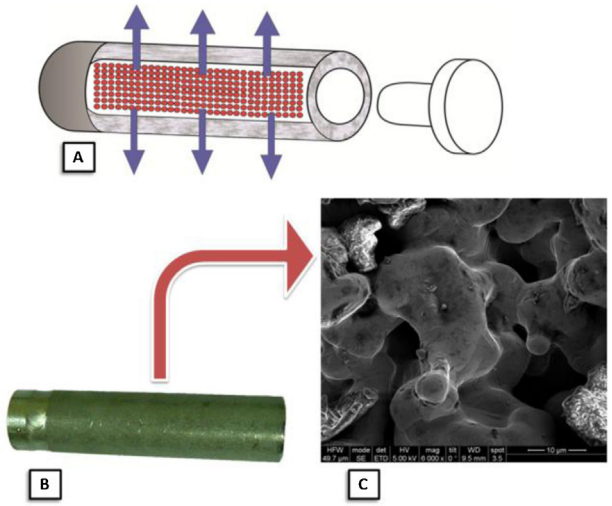

Fig. 1. Insert A: scheme of the drug release device. Note the permeation through the porous wall (arrows). Insert B: macroporous 316L stainless steel implant. Insert C: scanning electron microscopy (SEM) view of the porous wall.

2.2. In vivo experiment: macroporous steel implant as an antibiotic eluting device against an experimental infection

All procedures in the *in vivo* experiment were carried out under Project License PI14/12 approved by the Ethic Committee for Animal Experiments from the University of Zaragoza. The care and use of animals were performed according to the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 86/609 of the protection of animals used for experimental and other scientific purposes.

Four adult ewes and one adult neutered ram with weights of 40–45 kg, were used in this study. Animals were distributed into two groups. Group A consisted of four healthy female sheep (A1, A2, A3, A4) and Group B consisted of the control ram (B1). Group A was implanted with the above-described macroporous steel devices loaded with linezolid to evaluate the implant efficiency as a preventive element against an experimental infection. Group B was implanted with the same device without antibiotic. Both groups were percutaneously infected immediately after surgery by using a biofilm forming strain (ATCC 6538) of *S. aureus*.

2.3. Surgical procedure and experimental infection

The implants were loaded with 95–120 mg of lyophilized commercial linezolid (Zyvox®) in sterile conditions. At day 0, animals were first premedicated with xylazine (0.1 mg/kg) and five minutes later anesthetized with ketamine hydrochloride (2 mg/kg), both intravenously. Sheep were then intubated using intravenous propofol (2–4 mg/kg) to facilitate the procedure. Animals were maintained under general anesthesia with isoflurane (0.5–3% to effect) in oxygen delivered with a rebreathing anesthesia circuit. Lactated Ringer's solution was administered via an intravenous catheter throughout the surgical procedure at a rate of approximately 15 mL/kg h. One implant per animal was inserted percutaneously at the medium third of the medial side of the right