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Research paper

Development of advanced biantibiotic loaded bone cement spacers for arthroplasty associated infections

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ABSTRACT

The incidence increase of infections in patients with hip or knee implants with resistant pathogens (mainly some *S. coagulase-negative* and gram positive bacteria) demands advanced antibiotic loaded formulations. In this paper, we report the design of new biantibiotic acrylic bone cements for in situ delivery. They include a last generation antibiotic (daptomycin or linezolid) in combination with vancomycin and are performed based on a novel modification of the Palacos R® acrylic bone cement, which is based on two components, a liquid (methyl methacrylate) and a solid (polymeric phase). Hence, the solid component of the experimental formulations include 45 wt% of microparticles of poly(*D,L*-lactic-*co*-glycolic) acid, 55 wt% of poly(methyl methacrylate) beads and supplements (10 wt-% each) of antibiotics. These formulations provide a selective and excellent control of the local release of antibiotics during a long time period (up to 2 months), avoiding systemic dissemination. The antimicrobial activity of the advanced spacers tested against *S. aureus* shows that single doses would be enough for the control of the infection. In vitro biocompatibility of cements on human osteoblasts is ensured. This paper is mainly focused on the preparation and characterization of cements and the studies of elution kinetics and bactericidal effects. Developed formulations are proposed as spacers for the treatment of infected arthroplasties, but also, they could be applied in other antibiotic devices to treat relevant bone-related infection diseases.

1. Introduction

The infection associated to arthroplasties is caused by microorganisms that grow and form a "biofilm" (Van De Belt et al., 2001b) in which bacteria are wrapped and protected, achieving a strong adhesion to the surface of the implant (Van de Belt et al., 2001a). When the microorganism density is high, release of molecules that activate other microorganisms can occur contributing to the thickening of the biofilm, phenomenon that is known *quorum sensing* (Murray et al., 1998). In these cases the efficacy in the antibiotics therapy is drastically decreased. This reduction is also conditioned by the risk of systemic toxicity, which limits the therapeutic doses, and also by the bioavailability of the antibiotic to penetrate in the specific tissue. One of the currently used techniques to eradicate the infection is the two-stage arthroplasty using a temporal antibiotic loaded spacer (Masri et al., 1994). This technique involves the removal of the prosthesis, the filling of the infected cavity with the acrylic bone cement spacer (Anagnostakos et al., 2006) and the re-implantation of a new prosthesis within a period of time of 3 months (Cui et al., 2007; Peng et al., 2011). The acrylic bone cement spacer has demonstrated to reduce the rate of recurrent infection, probably

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because local antibiotic levels are much higher than those achieved by intravenous or oral therapy, avoiding even the entrance of the antibiotic in blood or urine, and resulting less toxic (Salvati et al., 1986). The implantation of osteo-articular prosthesis has increased noticeably because of the incidence of traumatic accidents and the increase of the average life time. This results in drastic increase of the number of implanted prosthesis and the average time of life of these devices. Also, this is the main cause of the appearance of infections associated to long term implants in such a way that frequently infections appear after 7–10 years, or even longer time of implantation.

The antibiotics currently incorporated to acrylic bone formulations are gentamicin, tobramycin, vancomycin and cephalosporin (Ensing et al., 2008; Lewis, 2009; Neut et al., 2006; Penner et al., 1996). Combinations of two antibiotics are widespread because of the synergistic effect produced on the elution characteristics and the antimicrobial activity of the cement (Penner et al., 1996). Antibiotic loaded poly(methyl methacrylate) (PMMA) bone cements have been applied since long in the treatment of infected arthroplasties and other bone diseases as those appearing in open fractures, infected fractures and chronic osteomyelitis. However, their prolonged used in clinical practice have made microorganisms become more and more resistant to them, i. e. methicillin-resistant *S. aureus* (MRSA). The prevalence of these increasingly resistant organisms is a major concern to achieve the therapeutic success and also because they can generate cross resistance. Therefore, in the latest years the use of last generation antibiotics is being explored, daptomycin and linezolid playing a relevant role. Daptomycin is a lipopeptide active against gram-positive bacteria including MRSA and *S. coagulase-negative*. It has a broad spectrum activity, scarce adverse effects and low risk of generating resistance (Critchley et al., 2003; Hall et al., 2004). Linezolid belongs to the group of oxazolidinones, and it is active against gram-positive organisms including resistant *enterococci* and MRSA (Stefani et al., 2010). It has been successfully used in the treatment of osseous infections such as osteomyelitis and infections associated with arthroplasties (Potoski et al., 2006) and cross resistance to linezolid of gram-positive germs is a rare phenomenon (Tenover et al., 2007; Vardakas et al., 2009).

In the past years, several strategies were attempted to enhance antibiotic elution from acrylic formulations such as alteration of the cement composition and antibiotic loading (Anagnostakos and Kelm, 2009). Some formulations introduced soluble components in the solid phase to increase porosity and hence facilitate elution of the drug (Kuechle et al., 1991). Addition of 25% dextran to a commercial formulation greatly facilitated elution of daptomycin, vancomycin and amikacin. (Kuechle et al., 1991). Release modulators of lactose and hydroxypropylmethylcellulose (HPMC) have been added to the solid phase of gentamicin loaded self-curing bone cements (Virto et al., 2003), controlling elution by the amount of the modulator (Frutos et al., 2010). Loading PMMA self-curing formulations with soluble fillers such as xylitol, glycine (McLaren et al., 2006), sucrose and erythritol (McLaren et al., 2007), enhanced PMMA permeability and elution kinetics. Partially biodegradable acrylic composites containing PMMA/poly(ε-caprolactone) (PCL) beads were prepared for vancomycin release in non-load bearing graft applications (Méndez et al., 2002). Porosity of self-curing systems increased by using only 50% of the prescribed amount of monomer and permeability enhanced by incorporation of gel-forming polymeric filler, i.e. poly(vinyl pyrrolidone) or HPMC. Reduction of the amount of monomer was crucial to obtain a release antibiotic improvement and all biodegradable fillers almost tripled the amount of gentamicin release (Rasyid et al., 2009).

In this work, we consider both the enhancement of antibiotic release of cements and the incorporation of latest generation antibiotics used in clinical practice, that, up to our knowledge has not been reported so far, for application as antibiotic loaded acrylic bone cement spacers in the treatment of recurrent infections.

Therefore, this paper deals with the preparation of advanced acrylic bone cement spacers formulated with biodegradable microparticles of poly(*D,L*-lactic-*co*-glycolic) acid copolymer (PLGA) and loaded with combinations of antibiotics, one them being of last generation (daptomycin or linezolid). The paper also reports on in vitro release kinetics and biodegradation. The in vitro antimicrobial activity of formulations was studied using *S. aureus* as a gram-positive microorganism. Finally, cytotoxicity of cements was tested using cell cultures of human osteoblasts and standardized protocols.

2. Materials and methods

2.1. Materials

Palacos R® (Heraeus Medical, Germany) was used as commercial acrylic bone cement. PLGA with *D,L*-lactide:glycolide molar ratio 50:50 and 0.45-0.60 dl/g intrinsic viscosity in chloroform (0.1%) at 25 °C (Resomer® RG504) was purchased from Evonik. Linezolid (Pfizer, Peapack, USA), daptomycin (Cubist Pharmaceutical Inc, USA), vancomycin (Lab. Normon, Spain), poly (vinyl alcohol) (PVA, Sigma-Aldrich), dichloromethane (Scharlau) and phosphate buffered saline solution pH 7.4 (PBS, Sigma-Aldrich) were used as received.

2.2. Preparation and characterization of PLGA microparticles

500 mg of PLGA were dissolved in 30 mL of dichloromethane and the solution was added drop by drop over a PVA aqueous solution (0.25 wt%) immersed in a water/ice bath (0 °C) under stirring (1000 rpm) and applying an ultrasounds probe. After 1 h, the microparticles suspension was centrifuged at 12000 rpm for 10 min. The supernatant was removed and the microparticles were washed with distilled water and centrifuged. Three cycles of washing and centrifugation were applied and finally, the solid was lyophilized. Particle size distributions were obtained by a Laser Diffraction Particle Size Analyser Coulter LS230 (Beckman, Electronics, USA), small volume module plus, connected with a software LS32. Approximately, 10–15 mg of sample were dispersed in 2 mL of distilled water at room temperature. Three measurements were taken and values averaged. The morphology of the microparticles was examined by Scanning Electron Microscopy (SEM) using a Hitachi SU8000 FE-SEM apparatus at accelerating voltage range of 0.5–5.0 kV. Samples were prepared by deposition of the sample directly over discs (10 mm diameter and 0.1 mm thickness) and were coated with gold palladium alloy for analysis.

2.3. Antibiotic loaded acrylic bone cements formulation and characterization

Two biantibiotic loaded acrylic bone cement spacers were formulated by partial substitution of the acrylic copolymer of Palacos R® by PLGA microparticles (45 wt%) and supplementation of daptomycin or linezolid in combination of vancomicyn (10 wt% of each). Additionally and for interpretation of results, acrylic bone cements based on plain Palacos R® containing one or two antibiotics were prepared as well as cement formulations containing the microparticles in absence of antibiotics or in presence of a single last generation antibiotic. The compositions and names of all the cements appear in Table 1. Cement preparation was performed according to international standard instructions (ISO-5833, 2002-05-01) by mixing the corresponding liquid and solid phases in a bowl with a spatula under atmospheric