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Controlled release of indomethacin from alginate–poloxamer–silicon carbide composites decrease *in-vitro* inflammation

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ABSTRACT

Composites of biomorphic silicon carbides (bioSiCs) and hydrogels are proposed in order to obtain materials able to load and release poor soluble drugs with application in bone pathologies therapy. Hydrogels composed by alginate and poloxamer were loaded with indomethacin, incorporated into the ceramics and crosslinked. The indomethacin release profile is dependent on the microstructure of the bioSiC selected. The loaded oak and sapelli bioSiCs composites have adequate release profiles to promote the decreasing of the secretion of pro-inflammatory cytokines in LPS stimulated macrophages, showing stronger anti-inflammatory effects than pine bioSiC composites. The released indomethacin is able to modulate the degradation of chondrocytes extracellular matrix and promote the formation of new collagen by osteoarthritic chondrocytes.

Particles derived from mechanical wear of biomorphic silicon carbides do not show high toxicity, being similar to the zirconia particles.

1. Introduction

Bone is a complex hierarchically organized tissue formed by an organic matrix, mainly collagen which is sequential mineralized with an inorganic component, hydroxyapatite (HAp), by the action of specific cells (Raghavan et al., 2013; Silverman et al., 2007). Due to this complex structure the development of new bone biomimetic materials has led to the production of new porous 3D composite systems formed by the combination of one or more than one organic components (natural or synthetic polymers) with an inorganic component. Composite systems should be able to meet all the physical and biological requirements for bone regeneration, combining the advantages of all components (Dessi et al., 2013).

These composite systems have been found to be adequate also for the release of growth factors as BMP-2 (Hernandez et al., 2012; Kim et al., 2012; MacDonald et al., 2011), VEGF (Suarez-Gonzalez et al., 2012), platelet derived growth factors (McCanless et al., 2012; Phipps et al., 2012) or drugs such as dexamethasone (Son et al., 2011, 2013), vancomycin (Ma et al., 2011; Thanyaphoo and Kaewsrichan, 2012) and gentamicin (Cai et al., 2011). In the last years, they have also been proposed for releasing a combination of therapeutic molecules such as BMP-2 and vancomycin (Doty et al., 2014), amikacin and gentamicin (Belcarz et al., 2013) or BMP-2 and VEGF (Shah et al., 2011). This new approach makes them an attractive alternative for the treatment of several bone pathologies.

Biomorphic silicon carbide ceramics (bioSiCs) obtained from natural resources (Gonzalez et al., 2009) have been shown to maintain the original structure of their precursors being highly porous and biocompatible materials (Diaz-Rodriguez et al., 2011) suitable for the regeneration and revascularization of tissues. The incorporation of a polymer component into their structure must increase their therapeutic value. The use of ionic crosslinking polymers such as alginate make possible to easily obtain a three dimensional network polymer through the addition of divalent ions (Drury and Mooney, 2003). Alginate hydrogels can exert the function of organic matrix suitable for cellular growth and encapsulation within the ceramic system and also facilitate the incorporation of drugs and growth factors in its three dimensional structure while modulate their release. Therefore, the combination of alginate hydrogels and bioSiCs can be presented as a promising strategy in the development of complex systems for tissue regeneration and controlled drug release.

The addition of synthetic block copolymers such as poloxamers or poloxamines to the hydrogel component should be able to increase the solubility of low aqueous solubility drugs. The ability of these polymers to form micelles in aqueous solution, where hydrophobic drugs can be incorporated, leads to an increase on

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their solubility and therefore makes possible their administration (Diaz-Rodriguez and Landin, 2012; Simoes et al., 2012). Furthermore, it was observed that poloxamines were able to stimulate the osteoblastic differentiation of adipose derived mesenchymal stem cells by themselves (Rey-Rico et al., 2011). The use of poloxamers has also been found to inhibit P-glycoprotein function decreasing the resistance of multidrug resistant (MDR) cell line (Kabanov et al., 2002, 2003).

Currently, the long-term stability of the prosthesis continues to be a challenge in the development of bone substitutes. Material wear debris is one of the main drawbacks associated to the use of artificial materials as implantable systems. The production of material particles may cause aseptic loosening and the activation of the surrounding macrophages leading to bone destruction and periprosthetic osteolysis (Gallo et al., 2008; Mahmoodi and Ghazanfari, 2011; St. Pierre et al., 2010). Several parameters modulate the inflammatory reaction caused by material particles such as their size (Kranz et al., 2009) or their chemical composition (Granchi et al., 2005; Nordsletten et al., 1996). Despite of that biomorphic silicon carbide has been previously described as a highly biocompatible material the inflammatory reactions caused by its potential debris have not been documented.

The aim of the present work is to develop composites able of loading by entrapment and release an anti-inflammatory drug, indomethacin. The development of composite systems formed by a natural (alginate) and a synthetic polymer (poloxamer) together with the biomorphic ceramic (silicon carbide) should allow the obtaining of an implant material suitable to load and release in a controlled way the hydrophobic drug in an enough amount to show an adequate anti-inflammatory effect on osteoarthritic chondrocytes.

Additionally, the potential toxic effects of particles obtained from wear mechanical of biomorphic silicon carbides are evaluated.

2. Materials and methods

2.1. Preparation of composite systems

Three different types of biomorphic silicon carbide (bioSiC) samples were obtained as described elsewhere from oak (*Quercus robur*), pine (*Pinus pinnaster*) and sapelli (*Enthandrophragma cylindricum*). Disks of (6 mm × 2 mm) in diameter were sterilized by autoclaving at 121 °C for 20 min (Diaz-Rodriguez et al., 2014).

Two polymeric components were used; Pluronic F127® (PF127) that was kindly donated from BASF (Ludwigshafen, Germany) and sodium alginate (GRINDSTED® Alginate PH 155) that was purchased from Danisco (Copenhagen, Denmark).

Pluronic was dissolved in phosphate buffer (PBS) to achieve a final concentration of 2.5%. After its complete dissolution, indomethacin (2.38 mg/mL) and alginate (2%) were sequentially added. The final solution was autoclaved at 121 °C for 20 min.

30 μL of the polymeric solution was added on the bioSiC samples and crosslinked by the immersion of the loaded sample into a sterile solution of calcium chloride (Panreac; Barcelona, Spain) at a concentration of 20% for 10 s. After reticulation systems were washed twice with 2 mL of PBS for 10 s.

Crosslinked alginate–poloxamer beads prepared by dropping the polymeric solution into the calcium chloride solution were used as control.

2.2. Characterization of the polymeric component

The mechanical stability of the crosslinked and uncrosslinked polymeric systems was analyzed before and after the autoclaving process using a controlled stress rheometer (Rheolyst AR-1000N TA instruments, Surrey, UK). Ramps of temperature from 15 °C to 60 °C at 2 °C/min with an oscillatory stress of 0.1 Pa at 5 rad/s were carried out for all the samples.

Differential scanning calorimetric (DSC Q200, TA instruments, Surrey, UK) was used to evaluate the potential degradation of the polymeric chains during the autoclaving procedure. Ramps of temperature were carried out first from room temperature to −30 °C at 10 °C/min and then from this temperature to 50 °C at 10 °C/min.

2.3. Isolation of human osteoarthritic chondrocytes

Human osteoarthritic cartilages were provided by the Instituto de Ortopedia y Banco de Tejidos Musculoesqueléticos of the University of Santiago de Compostela. Pieces of the tissue were cut and placed into sterile tubes with trypsin and cell culture medium. The tissue was maintained into the solution at 37 °C for 30 min in order to kill the fibroblasts present in the extracts. Then, tissue samples were immersed in collagenase 1.5% in culture medium and kept overnight at 37 °C under mechanical stirring.

The dissolutions were centrifuged at 2500 rpm for 4 min. Cells were resuspended in DMEM supplemented with 10% of fetal bovine serum and 1% penicillin/streptomycin and cultured at 37 °C with 5% of CO_2 and 90% of relative humidity.

2.4. In vitro *release of indomethacin*

Loaded composite systems were immersed in 3 mL of phosphate buffer at 37 °C. At preset times the concentration of indomethacin was quantified by UV–visible spectrophotometry at 320 nm. All the experiments were done in triplicate.

2.5. Anti-inflammatory effect of indomethacin loaded composites

The anti-inflammatory effects of loaded indomethacin composites were evaluated in two cell types, extracted human osteoarthritic chondrocytes and a murine macrophage cell line (Raw 264.7).

The macrophage cell line was cultured in DMEM-F12HAM supplemented with 10% FBS and 1% penicillin/streptomycin and maintained at 37 °C with 5% of CO_2 and 90% relative humidity. Loaded composite systems were placed in 24-well plates, cultured with 100,000 cells per well and stimulated with lipopolysaccharide (LPS) at a concentration of 100 ng/mL.

The composites anti-inflammatory effect was evaluated after 24 and 72 h of culture by the quantification of prostaglandin E2 (PGE2) (Arbor), TNF-α (eBioScience), nitric oxide (Cayman) and IL-1α (eBioScience).

The composites cytotoxicity was analyzed by the quantification of lactate dehydrogenase (LDH) (Roche). Indomethacin at 100 μM, unloaded silicon carbide samples, and the polymeric solution were used as controls.

Composites were also cultured with osteoarthritic chondrocytes in 24-well plates at a density of 60,000 cells per well with 2 mL of supplemented DMEM. The effect on extracellular matrix synthesis of osteoarthritic chondrocytes was evaluated by the quantification of glycosaminoglycans (GAGs) and collagen (I–V) production after 15 days of culture.

Cell culture supernatants were centrifuged at 10,000 × *g* for 10 min. Two colorimetric assays were used, Blyscan (Biocolor) for GAGs and Sircol Collagen Assay kit (Biocolor) for collagen. Single cell culture medium was used as negative control and cells treated with indomethacin at a concentration of 100 μM equivalent to 100% of drug release were used as a positive control. Unloaded bioSiCs and crosslinked loaded and unloaded polymeric components were also used as controls.

Additionally, the concentration of inflammatory cytokine (IL-1β) and the secreted PGE2 were measured at 1, 2, 5 and