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Plasma polymerized bioceramics for drug delivery: Do surface changes alter biological behaviour?

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ABSTRACT

One of the treatments for recurrent or complicated osteomyelitis is by local antibiotherapy mediated by suitable bone grafts. β–Tricalcium Phosphate (β–TCP) bioceramic is a resorbable bone graft. Its microporosity allows for incorporation of drugs, but a too fast release is often obtained. Complex strategies have been explored to obtain controlled drug release. In this work, plasma polymerization of a biocompatible polymer was investigated on β-TCP. Polyethyleneglycol (PEG)-like polymer coatings of different thickness were deposited on microporous β-TCP loaded with antibiotics. A highly hydrophobic surface was obtained despite the hydrophilicity of the PEG-like layer produced, which was associated to the roughness of the β-TCP substrate. The bioceramics nevertheless retained their suitable biological behavior with regard to human osteoblast cells. The microbiological activity of the antibiotics was preserved, and the coatings reduced the total amount of drug released as a function of the increasing plasma treatment time.

1. Introduction

Osteomyelitis is a bone infection. It can spring from different causes: i) from spreading of bacteria originating from an infection elsewhere in the body, ii) through an open wound over a bone or iii) after the exposure of bone to bacteria during a surgery or an injection around a bone. The most usual treatments involve administration of antibiotics, but sometimes surgery may be necessary, and whenever bone is damaged it requires removal and replacement with a bone graft [1]. Systemic antibiotic regimes are used for four to eight weeks depending on the pathogenic bacteria and the response of the patient. But with long delays in diagnosis or treatment, significant bone damage; or if the initial treatment is not effective, patients are more prone to reoccurrence and the condition can become chronic and difficult to eradicate [2,3].

The delivery and maintenance of therapeutic levels of antibiotic at the site of infection can be achieved by using implants or carriers that release antibiotics locally; these have significantly improved the treatment of osteomyelitis [4]. Many local antibiotic releasing systems have been developed in recent years, and several are available for clinical use, such as polymethylmethacrylate (PMMA) beads, collagen, apatite-wollastonite glass ceramic blocks, hydroxyapatite blocks, polylactide/polyglycolide implants, and polylactate polymers [3,5–7]. Among these PMMA rosary beads impregnated with gentamicin have been often employed in the clinics, but they have several disadvantages, especially the need to remove the foreign material surgically under general anaesthesia [5,6,8–10].

An ideal local carrier should be biocompatible and biodegradable, and in applications requiring bone substitution, it should be resorbable and able to promote bone formation. Many calcium phosphates (CaPs) have been widely employed as bone grafts and many of them are in the market. Among them, β–tricalcium phosphate (β–TCP) is an interesting bioceramic obtained at high temperatures with higher solubility than i.e. hydroxyapatite and that has been widely employed in reconstructive surgery [11] due to its resorbability and ability to promote new bone formation [12–14].

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β-TCP has been evaluated as drug delivery system of different drugs. For example, in the release of gentamicin fast release was obtained in the first day and different strategies, more or less complex were evaluated to control this release [15]. Different works have relied in producing composite materials from β-TCP or other CaPs and biocompatible polymers (such as chitosan, gelatin, etc.) [16,17]; to improve different features of the material, among which to modulate drug release from the matrices. However, this approach usually involves complex processing stages. Plasma polymerization is a versatile technique for the deposition of films with functional properties suitable for a wide range of applications [18,19]. Although plasma polymerization is a well-studied field, its application to bioceramics is rather recent. In a recent work, we proposed plasma polymerization of a hydrophilic polyethyleneglycol (PEG)-like layer as a dry method allowing to coat β-TCP [20]. In that work, the coating produced allowed to successfully modify drug release kinetics, avoiding burst release, and slowing down the initial rate of release. However, the coatings produced by plasma polymerization led to very hydrophobic surfaces. This change in wettability could have a critical impact on the biological behaviour of these bioceramics.

Herein, we aim at investigating in-depth the origins of this unexpected hydrophobicity, with a particular focus on its effects on biological behaviour. Therein, β-TCP has been subjected to plasma polymerization to obtain PEG-like coatings, by using single and multi- step coatings, and the modified surfaces have been characterized. To ascertain the barrier effect of the coatings developed, the release of two different antibiotics (gentamicin and ampicillin) has been evaluated from these bioceramics, and their microbiological behaviour has been studied after the plasma polymerization process undergone by the samples.

2. Material and methods

2.1. Materials

Calcium hydrogen phosphate ($CaHPO_4$, Sigma-Aldrich C7263) and calcium carbonate ($CaCO_3$, Sigma-Aldrich C4830) were used as raw materials for the synthesis of β–tricalcium phosphate (β-$Ca_3(PO_4)_2$, β–TCP). Sodium phosphate dibasic (Na_2HPO_4, Sigma-Aldrich) was used in solution as accelerant in the synthesis of calcium deficient hydroxyapatite (CDHA) used as a precursor of β–TCP. Ampicillin sodium salt (371.39 g/mol; 50 mg/ml in water), and gentamicin sulphate (477.6 g/mol, 50 mg/ml in water) provided by Sigma- Aldrich were selected as antibiotics for loading β–TCP ceramics (Supplementary material Fig. S1). Diethylene glycol dimethyl ether (Diglyme, anhydrous, 99.5%, Sigma Aldrich) $(CH_3OCH_2CH_2)_2O$ was used as precursor for plasma polymerization. Phosphate buffer saline (PBS), pH 7.4, was prepared from PBS Tablets (Gibco, Lifetechnologies™, UK) and Milli-Q® deionized water. Agar bacteriological (Scharlau S.A., Spain) and Brain Heart Infusion Broth (BHI Broth) (Scharlau S.A., 02–599, Spain) were used to prepare the bacteriological culture media of Staphylococcus aureus (S. aureus), (Culture Collection University of Göteborg (CCUG 15915), Sweden).

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2.2. β-TCP synthesis

Microporous β–TCP discs were obtained by thermal treatment of calcium deficient hydroxyapatite (CDHA), which was in turn obtained through the setting reaction of an α–TCP calcium phosphate cement. α–TCP was obtained by solid state reaction of a 1:2 M mixture of calcium hydrogen phosphate and calcium carbonate at 1400 °C. A cement was produced by blending α–TCP with a solution of sodium phosphate dibasic at 2.5% (w:w) at liquid to powder ratio of 0.65. The mixture was put in a disc-shaped mould and allowed to set immersed in water for 7 days to obtain CDHA, as described in [21]. The former discs were sintered at 1100 °C to obtain microporous β–TCP discs of 2 mm thickness 12 mm ø. This allowed obtaining a 100% β-TCP material, according to XRD (not shown).

2.3. Plasma polymerization

Plasma polymerization of β–TCP discs was performed using low-pressure radio-frequency plasma (13.56 MHz) (Standard Femto Plasma System, Diener, Germany) with a cylindrical glass chamber. Diglyme was used as source of ethylene oxide monomers to obtain a PEG-like coating on β–TCP [22]. Unloaded or antibiotic-loaded β -TCP discs were placed in the centre of the reactor. To enhance the polymerization process a short surface activation step with O_2 (5.0 sccm, 0.40 mbar, 150 W) was performed for 60 s. The subsequent polymerization process consists in introducing Diglyme in the plasma reactor by bubbling a carrier gas (Ar) through the liquid monomer. The polymerization treatment performed in continuous mode (15 sccm, 1.70 mbar, 150 W) for 10 min is labelled as plasma polymerization (PP10). Repetition of the polymerization cycle in the same conditions described, without removing the samples from the reactor was designed for 20 min, 30 min or 40 min. Polymerizations were performed on each side of the β–TCP materials, and the corresponding samples were referenced as PP20, PP30 and PP40 respectively (Fig. 1).

2.4. Surface topography

Topography of untreated and plasma polymerized β–TCP discs were studied by Scanning Electron Microscopy using a Zeiss Neon 40 cross-beam workstation with Gemini SEM column for sample observation. Samples were carbon-coated before SEM observation. Observations were carried out at 5.0 kV working voltage. Coupled-Energy-Dispersive X-ray spectroscopy (EDX) equipment (INCAPentaFETx3 detector, 30 mm^2, ATW2 window) was also used for in situ elemental analysis of the surface of a cross-section of plasma-polymerized β–TCP to determine the depth of the effects of plasma treatment on the surface of the bioceramic materials.

2.5. Wetting properties

Determination of the wettability of the β–TCP surfaces, to compare the untreated with the PEG-coated ceramics by plasma polymerization was done by static and dynamic contact angle measurements. A Contact Angle System OCA15 (Dataphysics, Germany) was used with the SCA20 Software (Dataphysics, Germany) to analyse the images acquired with a CCD. In static, 10 μL water droplet were deposited on the β–TCP surface

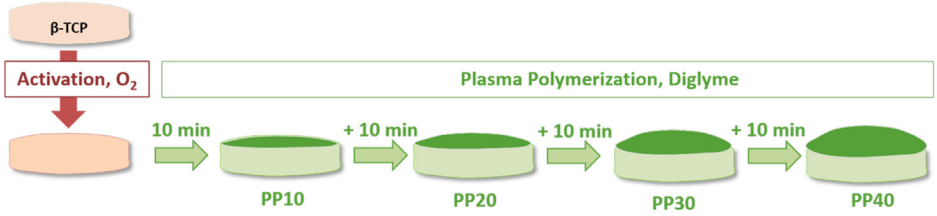

Fig. 1. Scheme of the experimental layout employed to obtain PEG-like coatings on β-TCP discs. Low pressure plasma: 1st activation of the surface by O_2 plasma and 2nd plasma polymerization Ar as carrier with Diglyme monomer, with 10 min sequential treatments named as plasma polymerization for 10 min (PP10), 20 min (PP20), 30 min (PP30) and 40 min (PP40).