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Design of calcium phosphate scaffolds with controlled simvastatin release by plasma polymerisation

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ABSTRACT

Calcium Phosphates (CaPs) have excellent bone regeneration capacity, and their combination with specific drugs is of interest because it allows adding new functionalities. In CaPs, drug release is mainly driven by diffusion, which is strongly affected by the porosity of the matrix and the drug–material interaction. Therefore, it is very difficult to tune their drug release properties beyond their intrinsic properties. Furthermore, when the CaPs are designed as scaffolds, the increased complexity of the macrostructure further complicates the issue.

This work investigates for the first time the use of biocompatible plasma-polymers to provide a tool to control drug release from drug-loaded CaP scaffolds with complex surfaces and intricate 3D structure. Two different CaPs were selected displaying great differences in microstructure: low-temperature CaPs (Calcium-deficient hydroxyapatite cements, CDHA) and sintered CaP ceramics (β-Tricalcium Phosphate, β-TCP). The deposition of PCL-co-PEG (1:4) copolymers on CaPs was achieved by a low pressure plasma process, which allowed coating the inner regions of the scaffolds up to a certain depth. The coating covered the micro and nanopores of the CaPs surface and produced complex geometries presenting a nano and micro rough morphology which lead to low wettability despite the hydrophilicity of the copolymer. Plasma coating with PCL-co-PEG on scaffolds loaded with Simvastatin acid (potentially osteogenic and angiogenic) allowed delaying and modulating the drug release from the bone scaffolds depending on the thickness of the layer deposited, which, in turn depends on the initial specific surface area of the CaP.

1. Introduction

Calcium Phosphates (CaP) are excellent candidates in bone replacement due to their similarity to the mineral phase of bone and potential resorbability [1,2]. Their combination with different types of drugs allows providing them with additional functionalities, in addition to their excellent osteoconductivity and osteogenicity. However, in CaPs a part of the loaded drug often remains trapped in the matrix [3,4]. This could be partly solved by introduction of macroporosity to the material, by production of different kinds of scaffolds [5,6]. In any case – both in bulk materials as in scaffolds – the performance of these ceramic matrices as drug delivery systems is tightly linked to their inherent porosity and pore size distribution features, which is dependent on the fabrication method followed [3]. Thus, whereas biomimetic calcium phosphate cements exhibit a high porosity, ranging from the nanometric to the micrometric scale, the porosity in sintered CaP ceramics tends to be micrometric in size.

Thus, a common problem is that drug release cannot be tuned beyond their intrinsic capacity, which is related with their porosity. Therein, adapting the release profile to specific pathologies requiring a certain rate of delivery poses a problem. Coating of the

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material's surface could be a solution. However, once the ceramic matrices are loaded with drugs, coating by conventional wet methods can lead to loss of drug from the material to the coating media.

In those cases, a promising novel approach to tune the drug release kinetics can be found in low temperature plasma technologies. Low temperature plasma, herein plasma, can be defined as a particular state of a gas or mixture of gases containing a mixture of ions, free radicals, electrons, excited molecules, UV and visible radiation that preserves electrical neutrality. This reactive medium can modify the first nanometres of the surface of the material without altering its bulk properties. Roughly, the three main effects of plasmas on the surface of a material are: i) functionalization or grafting (covalent bonding of new chemical species); ii) etching (removal of surface material); and iii) thin film deposition (deposition of thin layers).

These effects may be employed to tailor drug release; Recent works [7,8] have shown that surface functionalization of polyamide 6.6 fibres can improve the amount of anti-inflammatories and lipolithic agents released due to improved interaction of the materials with the surrounding media.

Another approach which can be used to slow down the drug release profile is by creation of thin films by plasma polymerisation on the surface of the materials, acting as barrier for its release. Coating by plasma polymerization refers to the deposition of polymer films due to the excitation of an organic monomer in the gas state and subsequent deposition and polymerization of the excited species on the surface of a substrate [9].

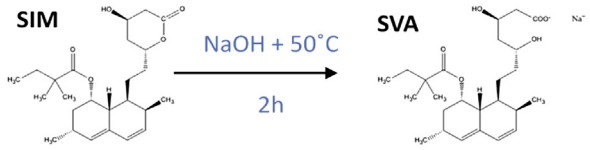
Few works have investigated plasma polymerization to produce an overlayer to control the release kinetics of drugs placed on the surface of solid carrier surfaces. Vasilev et al. have coated Vancomycin contained in nanoporous anodic aluminium oxide with allyllamine plasma layers [10]. In another work, quartz surfaces were employed as supports for a first n-heptylamine plasma polymer layer, to allow suitable wettability of Levofloxacin which was subsequently coated with a second n-heptylamine layer [11]. Similarly, quartz surfaces were employed as supports for model dye molecules (Methylene Blue) or anticancer agents (Cisplatin) and were plasma coated with multilayered biodegradable Poly caprolactone-co-polyethyleneglycol (PCL-co-PEG) coatings [12]. Both authors have shown that by gradually increasing the barrier layer thickness with plasma polymerization deposition time, the amount of drug released diminished. In all cases, simple model surfaces have been employed.

However, coating of complex surfaces in 3D scaffold structures and being able to fine tune drug release from them is far from being obvious. Therefore, it is the main aim of this work to show the potential of plasma polymerization in drug modulation from complex ceramic scaffolds for bone regenerative applications. These scaffolds were obtained by foaming of a calcium phosphate cement, and contained different levels of micro and nanoporosity, depending on the subsequent treatment applied, namely low-temperature setting to yield Calcium Deficient Hydroxyapatite (CDHA) or high-temperature sintering to produce β-Tricalcium Phosphate (β-TCP). Given the great differences on the surface texture of both ceramic materials, in the first stage, we investigated the deposition of biocompatible PCL-co-PEG polymer layers on 2D discs. Subsequently, the level of complexity was increased introducing 3D scaffolds, and the coating of the surface and the penetration of the plasma-polymers within the scaffolds was ascertained. To evaluate the possibilities of these layers on drug release, Simvastatin acid (SVA), was incorporated to the CaP scaffolds. In local drug delivery to the bone site from CaPs, different drugs are of interest [3]. SVA has shown potential action on bone and blood vessel formation [13,14], which is of clear interest in views of improving bone healing. Thus, to tailor the SVA release from the scaffolds, plasma polymerisation of a biocompatible and biodegradable copolymer of PCL-co-PEG was evaluated as barrier layer in different conditions, and its potential effect on modulating the drug release kinetics was assessed.

2. Experimental

2.1. Materials

ε-Caprolactone (ε-CL, Purity: 97%, MW: 114, Empirical formula: $C_6H_{10}O_2$), and the cyclic esther monomer Diethylene glycol dimethyl ether (DEGME, Purity: 99.5%, MW: 134.17, linear formula: $(CH_3OCH_2CH_2)_2O$) were purchased from Sigma Aldrich, France and used in this study without further purifications. Simvastatin (≥97%, MW:418.57, Sigma–Aldrich) was used as the precursor of Simvastatin acid (SVA), which was prepared according to [15].



(1)

α-TCP was used as starting material for the preparation of the powder phase of the cement, and was obtained by heating in a furnace (Hobersal CNR-58) in air, an appropriate mixture of calcium hydrogen phosphate ($CaHPO_4$, Sigma Aldrich) and calcium carbonate ($CaCO_3$, Sigma–Aldrich) at 1400 °C for 15 h followed by quenching in air. α-TCP powder was milled in an agate ball mill (Pulverisette 6, Fritsch GmbH) with 10 balls (d = 30 mm) for 15 min at 450 rpm and blended with 2%wt of precipitated hydroxyapatite (PHA)(HA; BP-E341, Merck, Germany), which was added as a seed. The liquid phase employed in the preparation of cements consisted of a 2.5% solution of Na_2HPO_4 (Panreac) in MilliQ water, while the liquid phase used to prepare scaffolds was a solution of 1 wt% of Polysorbate 80, herein Tween80 (Polysorbate 80, Sigma Aldrich, USA) in distilled water. 10 wt% Pluronic F-127 (Sigma Aldrich, USA) was blended with the solid phase in the preparation of the scaffolds.

2.2. Preparation of low temperature and sintered CaP ceramic discs and scaffolds

CaP discs were used for the characterization of the polymer layer obtained. In the first place, CaP cements were prepared with a liquid-to-powder (L/P) ratio of 0.35 mL/g. The powder phase was mixed with the liquid phase in a mortar for about 1 min and then transferred into disc moulds of 2 × 15 mm. Samples were allowed to set in Ringer's solution (0.9% NaCl) for 7 days to obtain Calcium-Deficient Hydroxyapatite (CDHA) discs.

To obtain β-Tricalcium Phosphate (β-TCP) ceramic discs, the CDHA samples were sintered in an oven (Hobersal), in air, by heating for 2.5 h up to 400 °C, and then for 2.20 h up to 110 °C where samples were maintained for 9 h. Cooling was achieved naturally.

Calcium phosphate scaffolds were prepared by foaming, using a L/P ratio of 0.55 mL/g. The powder phase was a mixture of α-TCP containing 2 wt% of PHA and 10 wt% Pluronic F-127. The liquid phase was a solution of 1%wt of Tween80 in water. The foams were injected into moulds (6 mm diameter × 12 mm height) and allowed to set as described. To obtain β-TCP scaffolds, the CDHA scaffolds were sintered applying the same protocol used for the β-TCP ceramic discs.