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Antiresorption implant coatings based on calcium alendronate and octacalcium phosphate deposited by matrix assisted pulsed laser evaporation

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ABSTRACT

The integration of an implant material with bone tissue depends on the chemistry and physics of the implant surface. In this study we applied matrix assisted pulsed laser evaporation (MAPLE) in order to synthesize calcium alendronate monohydrate (a bisphosphonate obtained by calcium sequestration from octacalcium phosphate by alendronate) and calcium alendronate monohydrate/octacalcium phosphate composite thin films on titanium substrates. Octacalcium phosphate coatings were prepared as reference material. The powders, which were synthesized in aqueous medium, were suspended in deionised water, frozen at liquid nitrogen temperature and used as targets for MAPLE experiments. The transfer was conducted with a KrF* excimer laser source ($\lambda = 248$ nm, $\tau_{FWHM} \leq 25$ ns) in mild conditions of temperature and pressure. XRD, FTIR and SEM analyses confirmed that the coatings contain the same crystalline phases as the as-prepared powder samples. Osteoblast derived from stem cells and osteoclast derived from monocytes of osteoporotic subjects were co-cultured on the coatings up to 14 days. Osteoclast displayed significantly reduced proliferation and differentiation in the presence of calcium alendronate monohydrate, pointing to a clear role of the coatings containing this bisphosphonate on inhibiting excessive bone resorption. At variance, osteoblast production of alkaline phosphatase and type I pro-collagen were promoted by the presence of bisphosphonate, which also decreased the production of interleukin 6. The positive influence towards osteoblast differentiation was even more enhanced in the composite coatings, thanks to the presence of octacalcium phosphate.

1. Introduction

Bisphosphonates (BPs) are widely used for the treatment of specific disorders of bone metabolism associated with conditions of bone loss, such as osteoporosis, Paget's disease and bone metastases [1–3]. The central carbon atom of BPs binds two phosphonate groups and two covalently bound sidechains, R1 and R2 (Figure S1). It has been shown that the affinity for biological apatite is increased when R1 is a hydroxyl group [4,5]. Moreover, the presence of a nitrogen atom in R2 sidechain enhances the anti-osteoporotic potency and influences bone affinity [6–8]. The relevant antiresorption action of amino-BPs (N-BPs) is related to their inhibition of farnesyl diphosphate synthase, a major enzyme in the mevalonate pathway. The inhibition prevents the prenylation of small GPTases signaling proteins and, as a consequence, interferes with many osteoclast activities, eventually leading to cell apoptosis [4]. BPs also display anti-angiogenic properties [7] and anti-tumoral activity [8]. As a matter of fact, BPs have been shown to prevent and delay skeletal complications in patients with bone metastases from solid tumors or osteolytic lesions from multiple myeloma [9–10]. The major limits of BPs oral administration are poor bioavailability and development of gastrointestinal disorders [11]. On the other hand, adverse side effects, namely osteonecrosis of the jaw and atypical fractures, have been recently reported in patients receiving high doses by intravenous formulation [12–14]. Alternative modes of administration, such as local release at specific bone sites, could allow to reduce the doses of systemic administration and prevent adverse side effects [15]. We recently showed that alendronate (AL), one of the most potent N-BPs, can sequester calcium from insoluble calcium phosphates and yield precipitation of a crystalline BP, calcium alendronate monohydrate, CaAL• H_2O [16,17]. This compound, both alone and especially when associated to octacalcium

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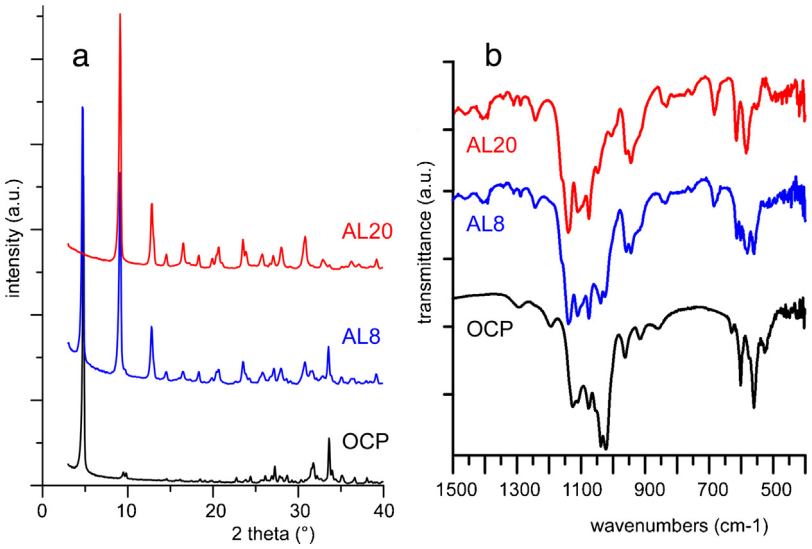

Fig. 1. X-ray diffraction patterns (a) and FT-IR spectra (b) of crystalline powders obtained at increasing AL concentration in solution.

phosphate (OCP), exhibits a remarkable inhibition of osteoclast proliferation and activity, whereas it enhances osteoblast differentiation [17].

In this work, we explored the possibility to deposit thin films of OCP at different contents of CaAL• H_2O on titanium (Ti) substrates in order to get coatings able to offer a suitable interface for bone tissues thanks to the presence of OCP, and to provide a local availability of BP. In fact, although metallic materials, such as Ti, fulfill the requirements of a successful orthopaedic implants, in terms of strength, toughness and resistance to wear and corrosion [18], incomplete osteointegration and stress shielding are the main causes of bone implant failures [19]. Coating the metallic surface with a thin film of calcium phosphate provides improved implant fixation to hard tissues [20,21]. Among the numerous different methods available to coat metallic substrates [20], Matrix-Assisted Pulsed Laser Evaporation (MAPLE) allows transferring a variety of compounds, including organic molecules and proteins, with an accurate control of thickness and stoichiometry [22–24]. Herein, we applied MAPLE to transfer and deposit composite crystals at different OCP and CaAL• H_2O content directly on Ti substrates in order to grow coatings with improved properties. This is the first attempt to synthesize in situ CaAL• H_2O–OCP coatings as an inorganic–organic assembling system by MAPLE technique. The biological performance of the coatings was investigated through in vitro tests carried out using co-cultures of osteoblast derived from stem cells and osteoclast derived from monocytes of osteoporotic subjects.

2. Materials and methods

2.1. Synthesis and characterization of crystalline powders

OCP was synthesized by dropwise addition of 0.04 M $Ca(CH_3COO)_2 \cdot H_2O$ (250 ml) over a period of 60 min into a phosphate solution (750 ml) containing $Na_2HPO_4 \cdot 12H_2O$ (5 mmol) and $NaH_2PO_4 \cdot H_2O$ (5 mmol) at a starting pH of 5. The reaction was carried out at 70 °C with smooth mechanical stirring. The precipitate was stored in contact with the mother solution for 10 min, filtered, repeatedly washed with bidistilled water and dried at 37 °C.

Reaction of OCP and AL was carried out in bidistilled water in the presence of two different concentrations of sodium alendronate trihydrate (Chemos), that is 8 and 20 mM. Resulting solid samples have been labeled AL8 and AL20, respectively. The reaction was performed on 500 mg OCP/250 ml solution at 30 °C, stirring for 72 h. Then the products were centrifuged at 5000 rpm for 10 min, repeatedly washed with double distilled water and dried at 37 °C.

X-ray diffraction (XRD) analysis was carried out by means of a PANalytical X'Pert PRO powder diffractometer equipped with a fast X'Celerator detector. CuKα radiation was used (λ = 0.15418 nm, 40 mA, 40 kV). For phase identification, the 2θ range was investigated from 3 to 40 degrees with a step size of 0.067° and time/step of 100 s.

BP content was determined spectrophotometrically via complex formation with Fe(III) ions using a Varian Cary50Bio instrument (λ = 290 nm) [25].

For infrared absorption analysis, spectra were recorded by a Shimadzu 8400S instrument in the 1500–400 cm^{-1} range, with a resolution of 4 cm^{-1} and a total of 50 scans/experiment.

Morphological investigation was performed by scanning electron microscopy (SEM) using a Philips XL20 instrument operating at 15 kV. The samples were sputter-coated with Au before.

2.2. Deposition and characterization of coatings

Disk-shaped Ti substrates (12 mm diameter and 0.5 mm thick) were mechanically polished and submitted to chemical etching before use as collectors. They were clean in ultrasonic sequential baths of acetone, alcohol and deionized water. For the preparation of one target used in MAPLE experiments, deionized H_2O based solution containing 0.12 g of OCP powder was suspended in 10 ml and subsequently stirred, homogenized and frozen in a special copper holder at 77 K in liquid nitrogen. The holder containing the solid target was then mounted inside a vacuum chamber. The schematic of the setup is presented in Figure S2. During exposure to multipulse laser irradiation, the target was maintained frozen by continuous liquid nitrogen flow inside a supporting cooler device and continuously rotated with 80 rpm to avoid drilling and improve the overall quality of the deposited films. A pulsed KrF* laser source (λ = 248 nm, $\tau_{FWHM} \leq 25$ ns) operating at 5 Hz was employed for the irradiation of the target. 20,000 subsequent pulses were applied at an incident laser fluence of 0.73 J cm^{-2} for the synthesis of each assembly (the corresponding pulse energy was of 280 mJ). The deposition was carried out in a residual pressure of 13 Pa. The substrate was facing the target at a separation distance of ~4 cm, while its temperature was kept constant at 30 °C during deposition. Iden-