

BIOMIMETIC STRONTIUM-SUBSTITUTED HYDROXYAPATITE DEPOSITED ON CARBON FIBER SCAFFOLD FOR BONE REGENERATION

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Introduction

Due to their multi-scale organization, breathability and biocompatibility, carbon fiber cloths (CFC) offer new perspectives as tissue engineering, more specifically in the field of orthopaedic applications. Conversely, owing to their high bioactivity, osteoconductivity and biocompatibility, calcium phosphate (CaP) ceramics have received much attention and are clinically used either as coating or as scaffold in orthopaedics. Therefore, CaP-coated carbon fiber cloths and Sr-doped CaP coatings deposited on carbon fiber cloths are promising bioceramic materials in the field of bone regeneration. CaP and Sr-doped CaP coatings are deposited on carbon cloths by sono-electrodeposition process using cathodic polarization. The strontium substitution on the CaP structure is of interest since Sr²⁺ enhances the proliferation and differentiation of osteoblasts. Furthermore, Sr²⁺ inhibits the osteoclast activity and is currently used in osteoporosis treatment [1].

Materials and Methods

The carbon fiber cloth (CFC), referred as FM50K, is made of 8-12 µm diameter knitted fibers. The material is provided by Zorflex®. Coatings of xSr-CaP on CFC are obtained by sono-electrodeposition process using cathodic polarization [2-3]. The cathodic polarization of the carbon electrode is performed using a potentiostat/galvanostat (BioLogic VMP-2) with current density or constant potential (-1V/ Hg/Hg₂SO₄) for 6 hours. Sonication is applied during polarization in order to obtain a uniform and homogenous coating on the carbon fibers [2-4]. FTIR, XRD, HRTEM, SEM and ³¹P MAS NMR are performed to characterize the carbon cloth substrate and the composition, microtexture and structure of the CaP and Sr-CaP deposits. In vitro biological tests were performed with primary human osteoblasts (HOST) in order to assess the cell viability. For *in vivo* biological tests, a bone defect was created on femur of rats. Concerning the adsorption of drugs (aspirin), it was performed in each component of the biomaterials.

Results and Discussion

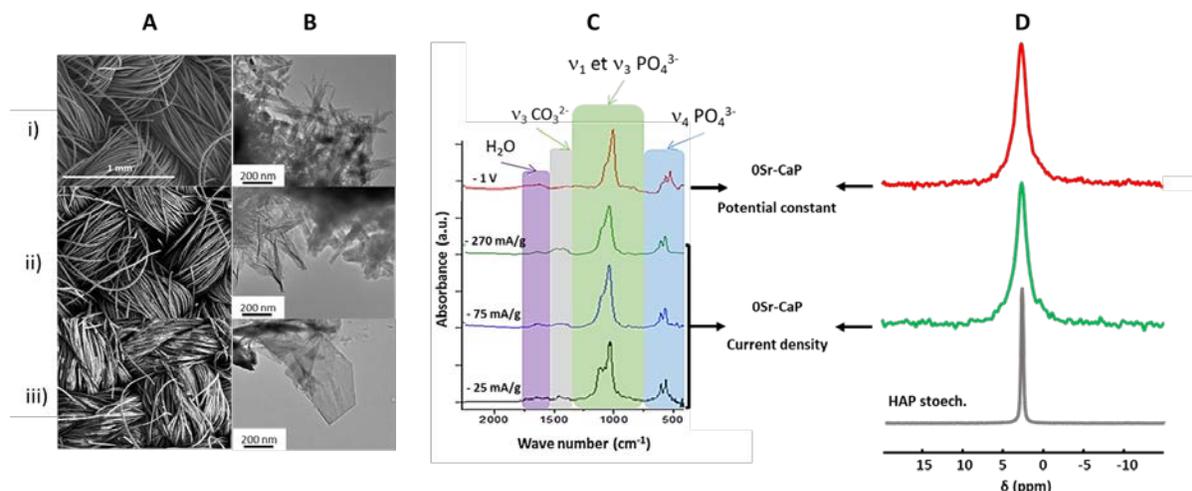


Figure 1. A: SEM micrographs of (i) pristine CFC; (ii) xSr-CaP coated CFC with homogeneous (as examples at 75 mA/g or -1V); and (iii) heterogeneous (as examples at 25 or 270 mA/g) coverage of the fibers.

B: TEM micrographs of CaP deposits: (i) needle-like microtexture obtained at $cd \geq 100$ mA/g; (ii) mixture of plate-like and needle-like microtextures obtained at 75 mA/g; and (iii) plate-like microtexture obtained at $cd \leq 50$ mA/g and -1V

C: FTIR spectra of xSr-CaP deposits and D: ^{31}P MAS NMR spectra of deposits obtained at constant potential (red), current densities from 25 to 270 mA/g (green) and spectrum of stoichiometric hydroxyapatite (grey).

Morphology and chemical composition of CaP deposits show a strong dependence with the water electrolysis regime (**Figure 1.**), which is governed by the current density or the constant potential applied at the negative electrode. Deposits analysis by TEM (**Figure 1.B.**) show that for applied low current densities and constant potential, a plate-like morphology is observed, whereas a needle-like morphology is observed for high current densities. At intermediate current density, both morphologies are observed (**Figure 1.B.ii.**). It clearly appears that two different types of CaP morphologies can be obtained and FTIR analyses allow identifying the CaP deposit chemical compositions (**Figure 1.C.**). For current densities ranging from 50 to 270 mA/g and constant potential, spectra are characteristic of carbonated calcium-deficient hydroxyapatite (CDA). At a current density of 25 mA/g, the presence of weak adsorption bands observed at 916, 1025 and 1193 cm^{-1} are characteristic of octacalcium phosphate (OCP). Whatever the applied current density or the potential constant, quantitative ^{31}P MAS NMR spectra are characteristic of CDA (**Figure 1.D.**). In adjusting the experimental conditions (constant potential of -1V), electrolyte composition and concentration ($\text{Sr}/(\text{Ca} + \text{Sr})$ ratio), Sr^{2+} can be incorporated in CDA phase. Substituted Sr^{2+} is evidenced in: - FTIR by the shift of PO_4^{3-} peak, - X-ray diffraction by the shift of (211) reflection and ^{31}P MAS NMR by shift of the characteristic CDA peak. In TEM, it is seen that the substitution does not modify the microtexture, the xSr-CDA particles keep the plate-like microtexture [3].

In vitro biological tests, performed in using primary human osteoblasts, highlighted the cell viability, especially on xSr-CaP biomaterials. Indeed, a strong interaction with cells and an increase of cell density and proliferation is emphasized with an increase of strontium in the CDA structure, pointing out that Sr^{2+} promotes osteoblast activity.

For *in vivo* biological tests, a bone defect was performed on thighbones of rats. The application of

CDA/ CFC biomaterials on the bone defect showed a major acceleration of the bone regeneration, in particular for xSr-CaP/CFC biomaterials, validating their potential as bioactive materials for bone regeneration. The adsorption of drugs (aspirin) in each component of the biomaterials was studied, providing new medical properties to the hybrid biomaterials.

Conclusions

Three different types of CaP deposits obtained through variation of electrochemical parameters were synthesised. At low current densities and at constant potential (-1V), the deposit consists in a biomimetic plate-like carbonated calcium-deficient hydroxyapatite (CDA), while at higher current densities the synthesis leads to a needle-like carbonated CDA. At intermediate current density, a mixture of plate-like and needle-like carbonated CDA is deposited. A thorough investigation of the CaP characteristics has shown that the deposition mechanism depends on the water electrolysis rate. The sono-electrodeposition is shown to be a versatile process allowing tuning the microtexture of the CaP coatings, thereby bringing new insights in the development of such biomaterials for bone repair. The cell viability on xSr-CaP, performed in using primary human osteoblasts, shows a strong interaction with cells and an increase of cell density and proliferation with strontium increase in the CDA structure, pointing out that Sr^{2+} promotes osteoblast activity [2]. The first in vivo tests done by performing a defect in femur rats in order to validate the potential of these biomimetic xSr-CaP/CFC as bioactive scaffold for bone regeneration.

Acknowledgment

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