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Preparation and characterization of an electrodeposited calcium phosphate coating associated with a calcium alginate matrix

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A new way of optimizing osteoconduction of biomaterials is to bring to them biological properties. In this work, we associated a novel release system with an electrodeposited calcium phosphate (CaP) coated titanium alloy Ti6Al4V. The characterization of this material was performed by means of light microscopy, scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM) and X-ray energy dispersive spectroscopy (EDXS). The electrodeposited CaP coating was a tricalcium phosphate, and the release system was composed of microcapsules entrapped in an alginate film. We observed that the alginate matrix had a close contact with the coating. An intermediate layer containing calcium and phosphorus appeared at the interface between the alginate matrix and the CaP coating. These results allowed us to conclude that the association of two techniques, i.e. electrodeposition followed by deposition of a calcium alginate matrix, led to the elaboration of a new biomaterial.

1. Introduction

The compatibility and the stability of titanium implants used for dental and orthopaedic applications can be improved by coating with calcium phosphate (CaP). Generally, these coatings promote bone ingrowth during the first healing period, which leads to permanent fixation. The technique employed by many investigators for this purpose is plasma-spraying of calcium phosphate ceramic onto porous metal [1, 2]. However, this technique requires pure material powders and involves high temperatures which may alter the CaP structure. An alternative method to elaborate CaP coatings at low temperature and without the use of any kind of powder is electrodeposition. This method also allows the porosity and the thickness of the coatings to be controlled [3, 4].

In our laboratory, a technique based on electrodeposition is currently being used to produce calcium phosphate or hydroxyapatite (HA) coatings [5]. The process in general consists of a calcium phosphate electrodeposition using the electrolyte obtained by mixing solutions containing calcium and phosphorus.

The aim of the present work was to optimize the osteoconduction of the coated material by means of an association with a release system, in order to allow the sustained release of a peptide acting on bone cells recruitment.

This release system was mainly constituted of propylene-glycol alginate (PGA)—human serumalbumine (HSA) coated microspheres [6, 7] embedded in a Ca alginate film. The negatively charged groups carried by the alginate molecule can interact with oppositely charged ions and thus form three-dimensional networks. The gel-forming ability of alginate with $CaCl_2$ has been used in many applications, including immobilization of living cells [8, 9], and controlled release of drugs and macromolecules such as vaccines and peptides [10]. Recently, Jianqi *et al.* [11] recommended the use of calcium alginate matrices for guided bone regeneration. This method would prevent epithelium and fibrous tissue infiltration into bone defects.

Thus, the association of the two techniques, i.e. electrodeposition followed by deposition of a calcium alginate matrix allowed us to obtain a new biomaterial. Therefore, it seemed very important to analyse this material at a micrometric scale in order to investigate the interaction between the calcium phosphate coating and

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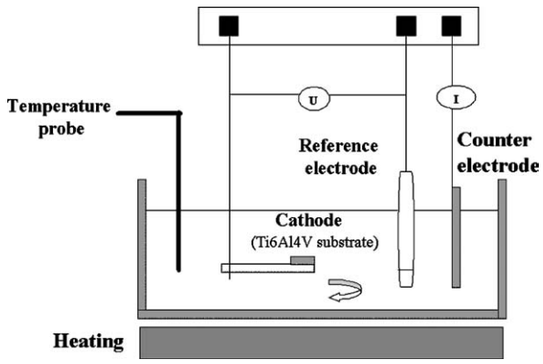


Figure 1 Schematic view of the electrolysis cell used to prepare calcium phosphate (CaP) coatings.

the calcium alginate matrix. These analyses were performed by scanning transmission electron microscopy (STEM), scanning electron microscopy (SEM) associated with X-ray energy dispersive spectroscopy (EDXS), and light microscopy.

2. Materials and methods

2.1. Sample characteristics

1. *Electrodeposition*. The substrate was a Ti6Al4V plate (1 cm^2). The surface was mechanically ground and blasted with Al_2O_3 grits in order to increase the roughness over 1 μm. Calcium phosphate coatings on Ti6Al4V were prepared by using an electrolyte composed of $Ca(NO_3)_2{\cdot}4H_2O$ (0.042 M) and $NH_4H_2PO_4$ (0.125 M) (Fig. 1). The coating process was carried out at 65 °C and with a current density $j = 2$ mA/cm^2 for 60 min. These experimental conditions led to a 5–6 μm coating thickness.

2. *Alginate matrix*. A 3% (w/w) sodium alginate (Manucol DH, Kelco international.) solution was prepared. Then the solution was poured onto the coated metal plate in dishes and dried for 24 h at 37 °C. In order to achieve gelation, a 10% calcium chloride solution was added to the film and the contact was maintained for 3 h. After rinsing with water, the film was dried again for 4 h at 37 °C.

2.2. Light microscopy

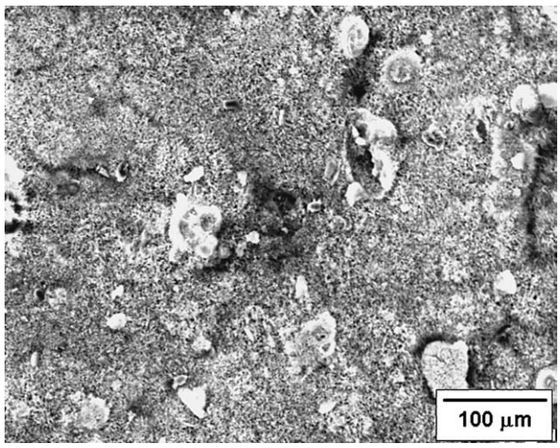
The morphology and thickness of the calcium alginate matrix were studied using a light microscope (OLYMPUS BH2, combined with a digital camera OLYMPUS DP50). The calcium alginate matrix was removed from the Ti6Al4V substrate. At this stage, the samples were only composed of calcium phosphate associated with the alginate matrix. Samples were embedded in Tissue-Tek® glue (O.C.T. Compound, Miles) and thin sections (2 μm) were prepared at −20 °C using a cryotome (Reichert-Jung 2700 Frigocut).

2.3. Scanning electron microscopy (SEM)

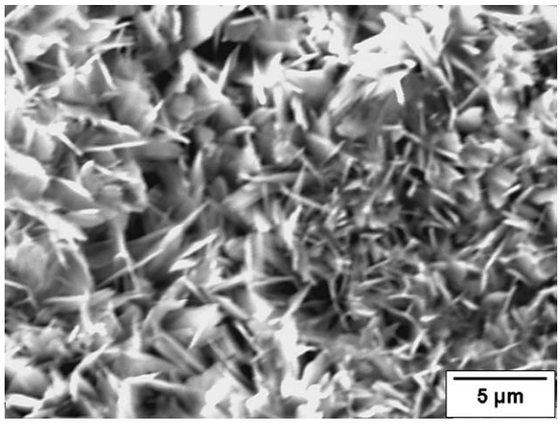
The morphology of the Ca/P coatings was observed in secondary electron mode using a LaB_6 electron microscope (JEOL JSM 5400 LV); the primary beam energy was 15 keV with a primary beam current close to 40 μA. The specimens were coated with a conductive layer of carbon in a sputter coater to avoid charging effects. The Ca/P ratio was measured by X-ray microanalysis.

2.4. Scanning transmission electron microscopy (STEM)

For STEM observation, the specimens were embedded in an epon resin (Agar Scientific). The Ti6Al4V alloy was carefully removed. At this stage, the samples were only composed of calcium phosphate associated with the alginate matrix embedded in resin. Finally, ultrathin sections (∼100 nm) were cut with a diamond knife using an ultramicrotome and collected on copper grids. The sections were coated with a conductive layer of carbon in a sputter coater to avoid charging effects. They were observed using a scanning transmission microscope STEM (Philips CM3O) operating at a voltage of 100 kV. X-ray analysis was performed using energy dispersion X-ray spectroscopy (EDXS). The detector was a Si(Li) diode equipped with an ultrathin window.



(a)



(b)

Figure 2 Secondary electrons micrograph of the electrodeposited CaP coating. (a) ×200 and (b) ×3500.

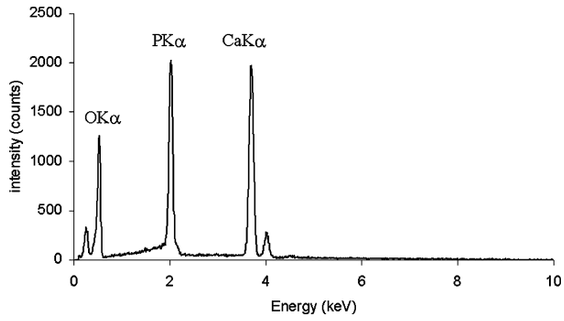


Figure 3 X-ray spectrum of the CaP coating (primary beam energy Eo = 10 keV acquisition time $t = 200$ s).

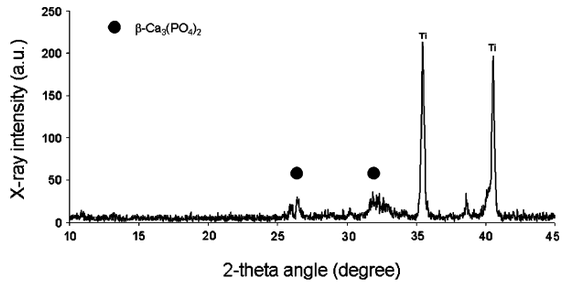


Figure 4 X-ray diffraction spectrum of the electrodeposited CaP coating.

3. Results and discussion

3.1. Characterization of electrodeposited CaP

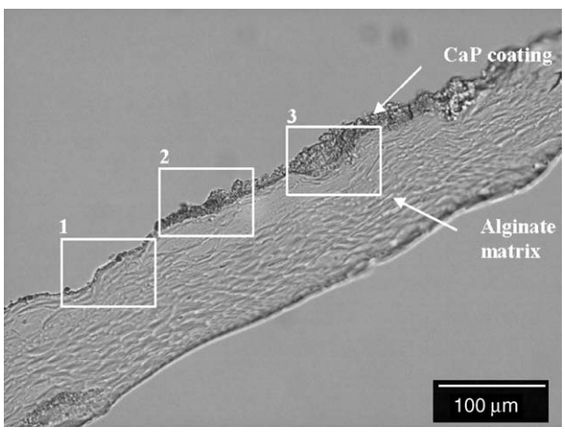
The SEM micrograph clearly shows that the electrodeposited coating presented a needle-like morphology (Fig. 2). The porosity of the coating seems to be very low (Fig. 2(a)). The X-ray spectrum of the coating corresponding to the observed zone is presented in Fig. 3. The measured intensities of Ca K_α and PK_α peaks allow us to determine the concentration values of calcium and phosphorus. The Ca/P concentration ratio thus obtained was about 1.5. This value corresponds to a tricalcium phosphate [$Ca_3(PO_4)_2$]. This result was confirmed by the X-ray diffractogram (XRD) presented in Fig. 4. Indeed, the peaks at 26° and 32° are characteristic of tricalcium phosphate [12].

3.2. Characterization of alginate matrix associated with electrodeposited CaP

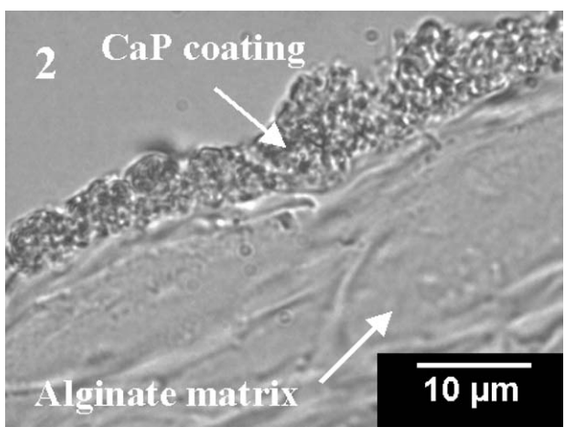
(3.2.a)a. Light microscopy

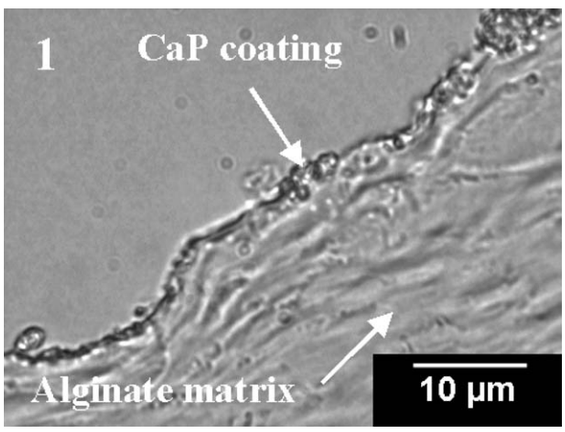
(3.2.a)1. Morphology

The light micrograph of Fig. 5 shows a global view of a section of the calcium phosphate coating in contact with the calcium alginate matrix. The section in different regions revealed a variation of the CaP coating thickness (zones 1, 2, 3, Fig. 5). This thickness



Global View (GV)





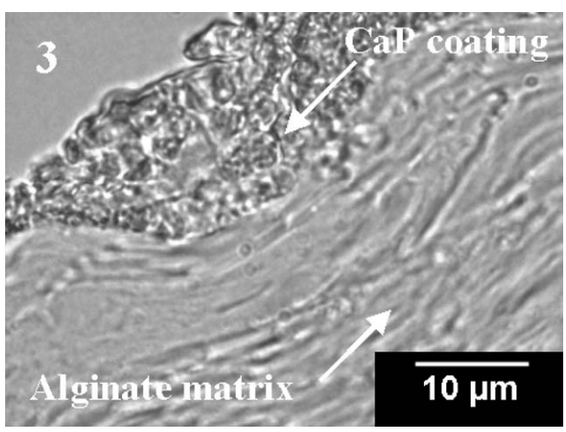


Figure 5 Light micrograph of the calcium phosphate coating associated to the calcium alginate matrix: (GV) global view, (1) Light micrograph showing the selected area (no. 1): the CaP thickness is about 1–2 μm, (2) Light micrograph showing the selected area (no. 2): the CaP thickness is about 5–6 μm, (3) Light micrograph showing the selected area (no. 3): the CaP thickness is about 10–15 μm.

TABLE I Thickness measurements of alginate matrix deduced from light micrographs

	Specimen 1		Specimen 2	
	Zone 1	Zone 2	Zone 1	Zone 2
Thickness (μm)	114.2	123.6	114.5	113.1
	114.7	122.1	114.3	114.3
	113.9	122.1	114.0	114.7
Mean thickness (μm)	114.3	122.6	114.3	114.0
Standard deviation (μm)	0.4	0.9	0.2	0.9

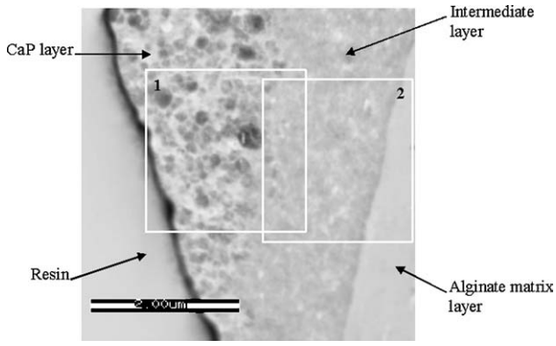


Figure 6 STEM micrograph of the calcium phosphate coating associated to the calcium alginate matrix.

variation is mainly due to the specimen preparation when removing the Ti6Al4V substrate. Nevertheless, a close contact was observed between the coating and the calcium alginate matrix (zoom of zone 3 presented in Fig. 5).

(3.2.a)2. Matrix thickness measurements

Two samples of films directly prepared on glass dishes were investigated for thickness measurements. In each sample, two zones were analysed three times. Table I shows the obtained values. The calcium alginate matrix presented a mean thickness of 116 ± 3 μm. This thickness knowledge is important for a future optimization of the release system.

(3.2)b. STEM analysis

The STEM micrograph shows direct contact between the coating and the matrix (Fig. 6). Three layers were observed: an internal CaP layer, an interface layer and an external Ca alginate layer. The CaP layer (left zone in Fig. 7) was composed of calcium and phosphorus elements (spectrum in Fig. 8) and presented a crystalline structure. The interface layer, about 2 μm thick (right zone in Fig. 7), was homogeneous without any crystal structure. The third layer (right zone in Fig. 9) corresponded to the calcium alginate matrix (spectrum in Fig. 10). The transformation of the CaP coating near the calcium alginate matrix (disappearance of crystals in the interface layer) was probably due to the migration of species such as Ca or Cl ions from the matrix to the CaP coating. This interface layer seemed to play an important role with regard to the

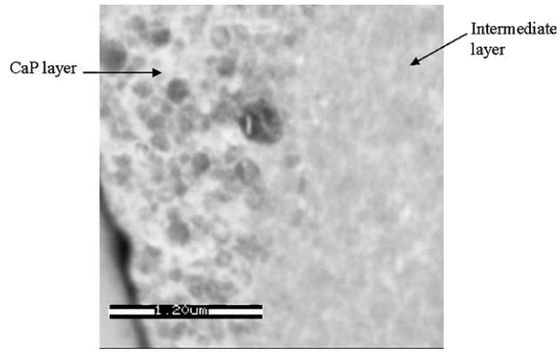


Figure 7 STEM micrograph showing the selected area (no. 1) of Fig. 6.

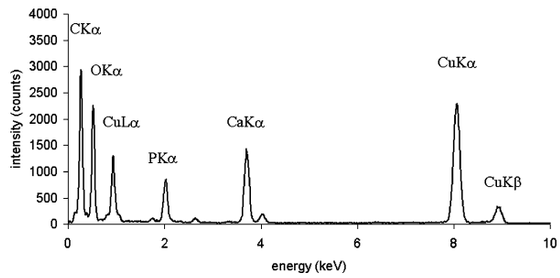


Figure 8 X-ray spectrum of the CaP layer (left zone of Fig. 7).

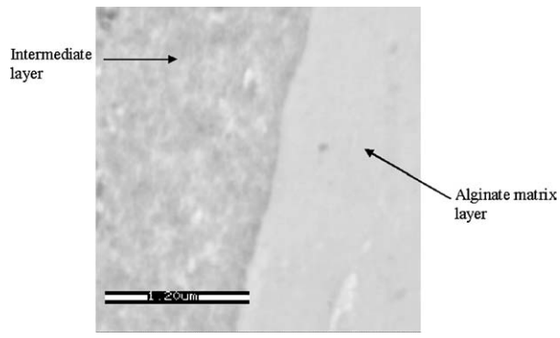


Figure 9 STEM micrograph showing the selected area (no. 2) of Fig. 6.

bonding between the coating and the calcium alginate matrix.

The main advantage of the matrix consists of the possibility of obtaining a slow release drug delivery system by inclusion of microspheres. With this aim, PGA-HSA microcapsules produced by the transacylation method would be useful as biocompatible carriers. The method consists of coating alginate beads with a membrane made of covalently linked alginate and human serumalbumin. The beads thus obtained are formed of two parts: a gel core which allows a slow release of bioactive molecules, and an external membrane used as a mechanical protection and as an additional controlling agent for the drug diffusion [7–9, 13]. It should be emphasized that the biocompatibility of these particles has already been demonstrated by *in vivo* and *in vitro* studies [8, 9]. The next step of our study will be to determine the release kinetics of a specific peptide helping

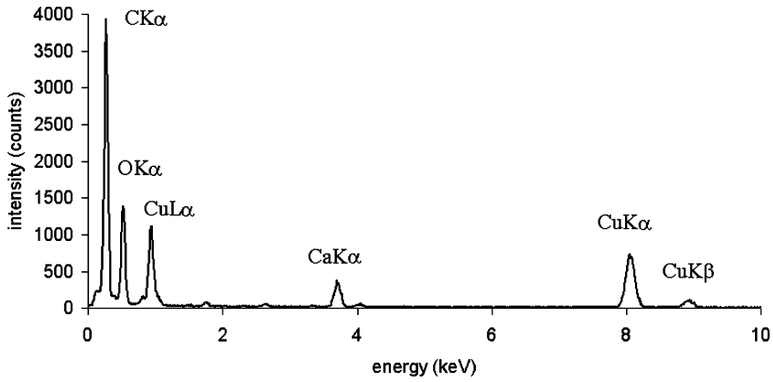


Figure 10 X-ray spectrum of the calcium alginate matrix (right zone of Fig. 9).

bone production from microcapsules embedded in the alginate film.

4. Conclusion

A new biomaterial was prepared by the association of two techniques, i.e. electrodeposition and calcium alginate matrix formation. The structural and physicochemical characterization of this material showed that it was composed of three superimposed layers, an alginate matrix, an interface layer and a CaP layer. Each of these layers is expected to play an important role during bone implantation. The film layer will be used to control the release of the bioactive molecule. The CaP layer will encourage the bone ingrowth and the intermediate layer will provide the bonding between the two other layers.

Microcapsules will be embedded in the film layer. The next steps will consist of release kinetics measurements from the embedded microcapsules as well as biocompatibility and osteoconduction studies of the new biomaterial.

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