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Biomimetic Organic-Inorganic Nanocomposite Coatings for Titanium Implants: I. Preparation, Physicochemical and Mechanical Characterization

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Abstract. In the production of artificial bone and tooth implants, coating of the surfaces of hard, but bioinert materials (metals, polymers) with calcium phosphate crystals has been used to improve bioactivity and facilitate osteointegration. Recently low temperature methods, involving precipitation from aqueous solutions (biomimetic precipitation) including coprecipitation of specific organic macromolecules (growth hormones, enzymes, proteins) have been developed**.** In this paper an alternative approach is presented, which consists in first laying down a matrix consisting of polyelectrolyte multilayers (PE MLs) alternating with layers of amorphous calcium phosphate (ACP) particles and subsequently growing calcium phosphate crystals upon/within the multilayers. This attractive approach leads to the formation of a new class of true organic-inorganic nanocomposite coatings. In a previous communication we have shown preliminary results, which point to the feasibility of this approach [1]. Here we describe in detail the design, synthesis and characteristics of the thus obtained nanocomposite coatings.

Introduction

Many of the materials used for replacement and/or repair of bone and teeth, (metals, metal alloys, polymers) meet biomechanical requirements for load bearing applications, but they are mainly bioinert or biotolerant [2] and thus show poor or nonexistent interfacial bonding with the surrounding bone. To alleviate this problem, differently prepared surface coatings consisting of calcium phosphates have been applied for a long time. The development of biomimetic calcium phosphate coatings opened a way for the inclusion of biologically active molecules (drugs, growth hormones, etc.) into the system. So far this has been attempted by dissolving the macromolecules in the calcifying solution, and co-precipitating them with the inorganic phase [3]. However, macromolecules profoundly influence the properties of the nascent calcium phosphate precipitates, the quality and intensity of the effect depending on their type and concentration [4].

An attractive solution to this problem may be to first lay down an organic matrix that can include bioactive molecules, and then grow calcium phosphate crystals upon/within this matrix. Ideal candidates for the organic matrix are polyelectrolyte multilayers (PE ML's). These films are obtained by alternated deposition of oppositely charged polyelectrolytes, the main driving force being electrostatic attraction. Since the process involves adsorption from solution, in principle there are no restrictions of size and topology of the substrate [5]. In this paper we describe the principles of design and the physicochemical characteristics of novel organic-inorganic nanocomposite coatings for bone and/or tooth implants [1, 6] consisting of calcium phosphate crystals grown "*in situ*" upon and within PE ML, which were formed by alternate adsorption of biocompatible PE's (poly-l-lysine, PLL and poly-l-glutamic acid PGA) upon titanium substrates.

Materials and Methods

Nanocomposite coatings were prepared on chemically etched titanium plates by alternate adsorption of PE MLs and layers of ACP particles from HEPES/NaCl buffer solutions. PE MLs were built up by sequential deposition of poly-L-lysine (PLL) and poly-L-glutamic acid (PGA) while ACP particles were adsorbed from freshly prepared suspensions, obtained by rapid mixing of equal volumes of calcium chloride and sodium phosphate solutions. Finally the organic – inorganic layers were immersed into a metastable calcifying solution (*MCS)* to initiate and sustain "*in situ"* crystal growth of nano-crystalline apatite within the organic matrix. The *MCS* was prepared from calcium chloride and sodium phosphate solutions in HEPES/NaCl buffer and, if undisturbed, was metastable for at least 14 days. Multilayer build-up was followed by optical waveguide lightmode spectroscopy (OWLS), while the nanocomposites were visualized by SEM and characterized by EDX and thin layer XRD. Adherence of the coatings to the substrate was tested by the adhesive tape test according to ASTM D 3359-92a.

Results

The general formula of the nanocomposite was $(PLL/PGA)_n CaP[(PLL/PGA)_5 CaP]_m (PLL/PGA)_5$. Step – by step deposition of the initial PE ML followed by ACP particles, PE ML and so on as well as subsequent crystal growth could be followed by OWLS (Fig. 1) and mutual interactions of the polyelectrolyte and ACP particles could be demonstrated (not shown).

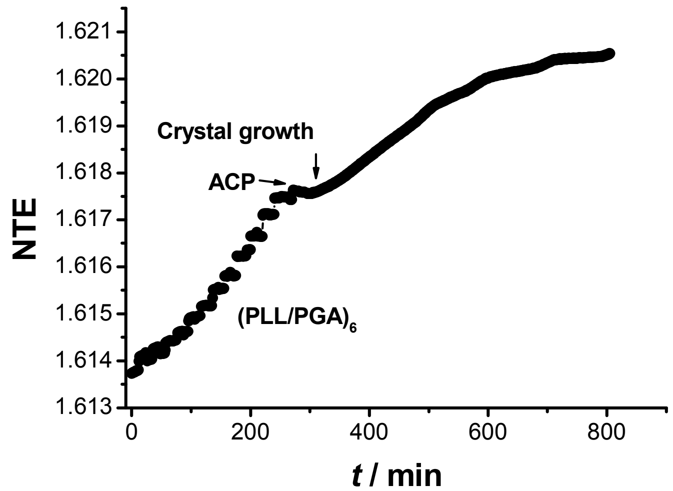
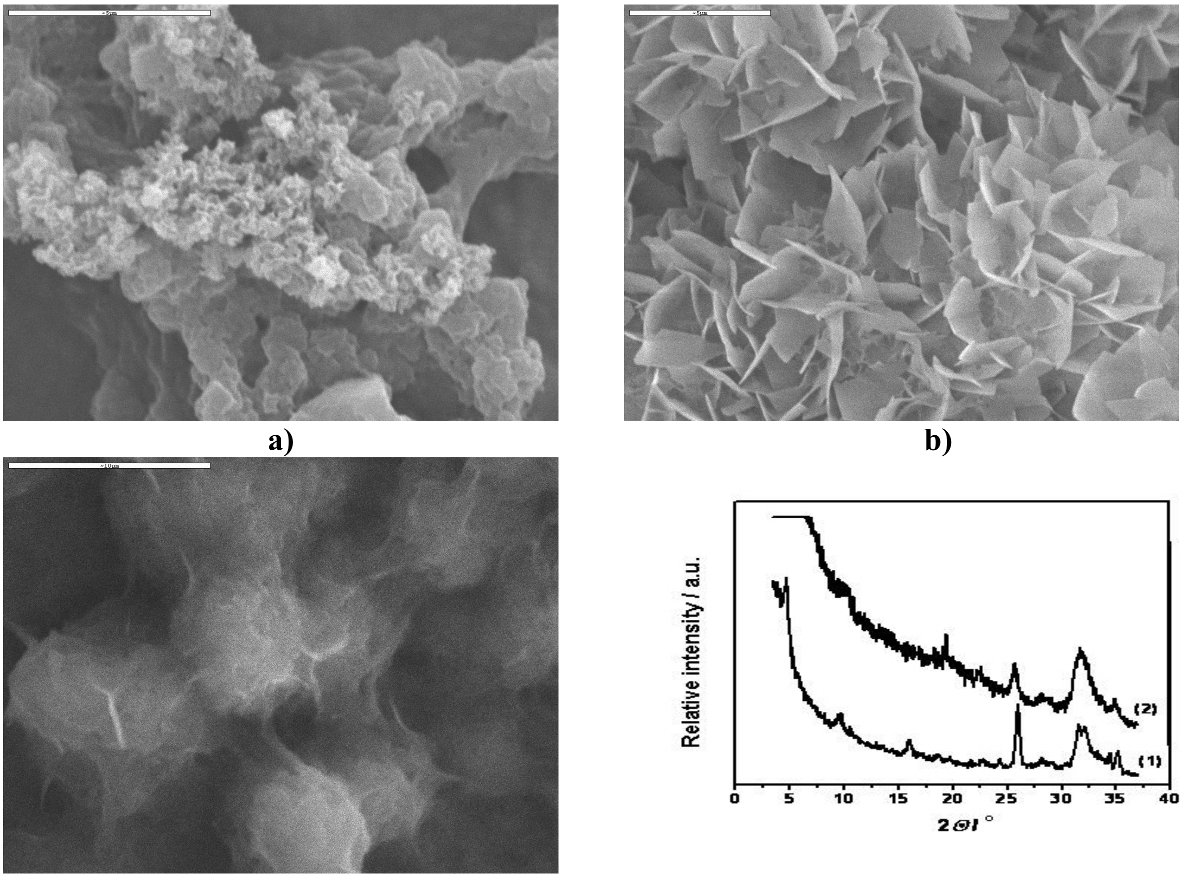


Figure 1. Changes of effective refractive indices of the transverse electric modes (NTE) upon deposition of $(PLL/PGA)_6$ ML , amorphous calcium phosphate (ACP) and upon crystal growth induced by metastable calcifying solution as followed by OWLS.

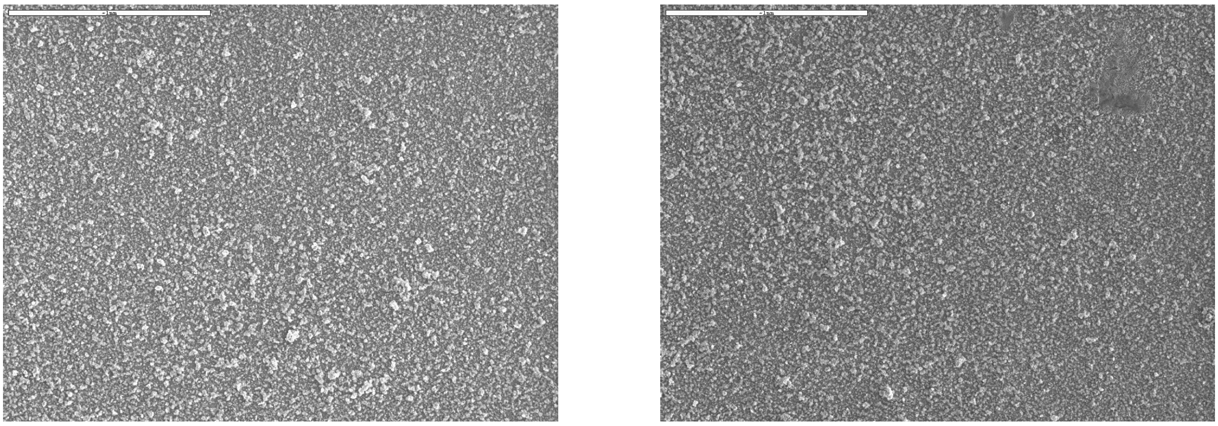
The optimum number of initially deposited PLL/PGA bilayers, which ensured good coverage of the etched titanium plates [6], was found to be $n = 10$. SEM micrographs of a $(PLL/PGA)_{10}ACP[(PLL/PGA)_5ACP]_5$ coating show spherulitic aggregates of ACP particles covering the plates (Fig. 2.a). When the top layer of the coating was calcium phosphate, upon immersion into *MSS,* relatively large crystals, perpendicularly oriented towards the substrate, grew from its surface (Fig. 2.b). Thin layer XRD confirmed that the obtained crystals are octacalcium



a) **b)** **c)** **d)**

Figure 2. SEM micrographs of a) $(PLL/PGA)_{10}ACP[(PLL/PGA)_5ACP]_5$, b) $(PLL/PGA)_{10}OCP[(PLL/PGA)_5OCP]_5$ and c) $(PLL/PGA)_{10}CaP[(PLL/PGA)_5CaP]_5(PLL/PGA)_5$ coatings. d) Thin layer XRD of (1) $(PLL/PGA)_{10}OCP[(PLL/PGA)_5OCP]_5$ and (2) $(PLL/PGA)_{10}CaP[(PLL/PGA)_5CaP]_5(PLL/PGA)_5$ coatings.

phosphate (OCP) (Fig. 2.d). However, good cellular response was only obtained from coatings containing a final PLL/PGA multilayer (Fig. 2.c), rather than a final calcium phosphate layer (see [7]). SEM micrographs of such coatings show relatively smooth surfaces, while EDX spectra show peaks characteristic of Ca, P , O, C and N and Ti with average atomic ratios C/Ti = 208.8 ± 46.4 and Ca/P = 1.46 ± 0.09. Thin layer XRD spectra of this coating showed characteristic peaks of apatite (Fig 2.d). The adhesive tape test showed good adherence of the material to the substrate surface (Fig 3.).



a) b)

Figure 3. SEM micrographs of $(PLL/PGA)_{10}CaP[(PLL/PGA)_5CaP]_2$ a) before and b) after tape test.

Discussion and Conclusions

The strategy of biological systems in building organic-inorganic composite systems like bone, enamel or dentine is to lay down an organic matrix, which then controls the deposition and growth of biological mineral [8]. Our novel way of producing organic-inorganic nanocomposite coatings closely mimics this strategy i.e. we first deposit a polyelectrolyte multilayer film, alternating with layers of ACP and subsequently induce growth of OCP crystals within such structures [6]. The advantages of this novel procedure are numerous:

- The preparation procedure of the composite closely imitates the mode of biomineralization.
- Since the deposition of PE MLs is largely independent of the type and topology of the substrate, it is possible to apply the coatings to a wide variety of bioinert implant materials.
- Bioactive molecules (e.g. growth hormones, drugs and other) can be co-adsorbed into the organic matrix, instead of coprecipitating them with the inorganic phase
- Because of "*in situ*" crystal growth the coating is tightly anchored to the substrate, exhibiting satisfactory mechanical stability.
- The coating procedure is simple, energy saving and environmentally friendly.

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