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Relevant insight of surface characterization techniques to study covalent grafting of a biopolymer to titanium implant and its acidic resistance

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ABSTRACT

Peri-implant bacterial infections are the main cause of complications in dentistry. Our group has previously proposed the attachment of chitosan on titanium implants via a covalent bond to improve its antibacterial properties while maintaining its biocompatibility. A better knowledge of the coating preparation process allows a better understanding of the bioactive coating in biological conditions. In this work, several relevant characterization techniques were used to assess an implant device during its production phase and its resistance in natural media at different pH. The titanium surface was functionalized with 3-aminopropyltriethoxysilane (APTES) followed by grafting of an organic coupling agent; succinic anhydride, able to form two covalent links, with the substrate through a Ti-O-Si bond and the biopolymer through a peptide bond. Each step of the coating synthesis as well as the presence confirmation of the biopolymer on titanium after saliva immersion was followed by FTIR-ATR, SEM, EDS, 3D profilometry, XPS and ToF-SIMS analyses. Results allowed to highlight the efficiency of each step of the process, and to propose a mechanism occurring during the chitosan coating degradation in saliva media at pH 5 and at pH 3.

1. Introduction

Synthesis and improvement of biomaterials are challenging goals to increase performances of implantology cares [1]. Among usual biomaterials, metallic implants based on titanium and its alloys are preferred, essentially because of their biocompatibility, high corrosion resistance and suitable mechanical properties [2,3]. However, titanium implants present a main weakness linked to its inability to avoid establishment and proliferation of bacteria [4]. Actually, according to previous studies, the implant surface itself constitutes a preferential site for bacterial adhesion [5] and in oral implantology, this infection is a worsen factor leading to peri-implantitis [6]. This is defined as an inflammatory disease that induces the loss of supporting bone in the tissues surrounding a functional implant [7] and it represents the most common reason for implant failure. Hence, in order to obtain sufficient integration of dental implant, it is necessary both, to inhibit biofilm formation and to improve the adhesion between metal implant and peri-implant tissues [8]. Functionalization of titanium implant with bioactive molecules could thus be a pertinent solution to enhance integration of titanium [9–11].

Among interesting biomolecules, such as hyaluronic acid and collagen [12,13], chitosan (CS) appears as a prime candidate to improve dental materials due to its non-toxicity, biodegradability and biocompatibility suitable for biomedical application [14]. Many studies have demonstrated the antibacterial properties of chitosan against gingival pathogens [15,16]. It is a polysaccharide composed of *N*-acetyl-*D*-glucosamine and *D*-glucosamine units with β binding at positions 1 and 4. It is obtained from deacetylation of chitin [17], one of the most abundant polysaccharides in nature after cellulose. For example, it can be found in exoskeletons of crustaceans and insects as well as in some fungi and microorganisms [18].

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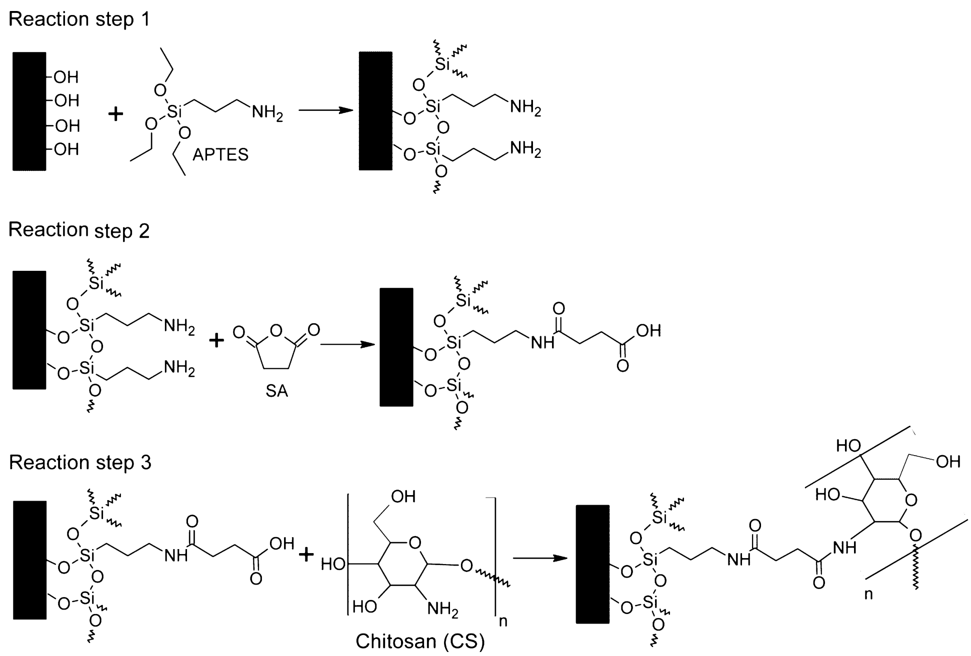

Fig. 1. Scheme of chitosan immobilization on titanium surface using APTES-SA coupling agent method.

Various strategies such as electrodeposition of alginate/chitosan layer-by-layer on substrates [19], the use of catechol groups [20] and dopamine-glutaraldehyde system [21] have been already reported to bond chitosan to titanium. Another widespread approach consists in using organosilane as coupling agent such as triethoxysilylbutyraldehyde (TESBA) [22,23], triethoxysilylpropylsuccinic anhydride (TESPSA) [24] or 3-aminopropyltriethoxysilane (APTES) associated with glutaraldehyde [25–27].

Even though efforts have been made to reduce the number of steps using new organically modified silane, this approach still remains a multistep synthesis requiring a fine control of each stage. Hence, it appears very important to analyze, control and understand each step of the bioactive coating preparation to improve further the grafting and the aging of the bioactive coating [28]. Different types of techniques could be used such as attenuated total reflectance-Fourier transform infrared (FTIR-ATR), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), 3D profilometry, X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) in static and dynamic modes. Recent studies focused on the surface characterization, especially on the interface between chitosan coating and metal support [26,29]. Nevertheless up to now, no study combining both bulk and outermost surface characterization techniques presented above were reported in the literature.

Furthermore, when dental implantology applications are aimed, a key factor concerns the chitosan degradation in terms of matrix composition, film thickness and resistance on titanium after immersion in saliva media. Oral cavity can observe consequent pH variation, function of the patient eating and drinking as well as its personal buccal health. Even though chitosan is stable in neutral pH, it becomes soluble/degradable in aqueous acid media. Consequently, an important challenge consists in synthesizing a chitosan-based dental implant resistant at low pH values. Previous work has already highlighted acidic resistance of chitosan film on hydroxyapatite substrate [30]. Our group has also recently proved the acidic pH resistance of grafted chitosan using TESPSA as coupling agent [24,31].

In this study, we propose a new procedure to graft chitosan on pure titanium T40 consisting in the successive use of two coupling agents. To react with the Ti support, APTES was employed as silane source to ensure (1) a covalent bond with the substrate (Ti-O-Si) and (2) the formation of double peptide bond between amino groups from both APTES and chitosan, it was associated with succinic anhydride. Reports on APTES use as coupling agent [25,26] can already be found but always in association with glutaraldehyde, which is toxic [32]. Furthermore, study of bioactive coating after acidic saliva immersion is presented here. Accurate analyzes of all prepared surfaces, meaning after each grafting step and immersion in acidic saliva media at pH 3 and pH 5 might be a reliable method of an implant device prior any biological tests.

In this work, a combination of several relevant surface characterization techniques was used, to precisely analyze the functionalization of titanium substrates with chitosan, via a covalent bonding. These techniques are necessary to highlight and well-understand the production process of a bioactive coating and also to follow precisely the matrix modification of coating following immersion in acidic solutions. Indeed, these techniques permit direct analysis of the samples and provide information about outermost surface at nanoscale. Efforts were made to precisely outline and analyze characteristic ionic fragments and chemical groups of each reactant in order to clearly define references, which will be monitored along the grafting progression. It should be pointed out that these results could be useful not only for our study but also in other applications using these compounds, such as study of SAMs in microelectronics field and food applications.

2. Experimental

2.1. Chemical functionalization of titanium

Titanium (Ti) disks (18 mm diameter) were supplied by Global D (France). Samples were cleaned first in an ultrasonic bath with ethanol/acetone solution (v/v, 50/50) for 20 min. Then, the surface was decontaminated and oxidized in fresh piranha solution (sulfuric acid/hydrogen peroxide, v/v, 70/30) for 10 min, rinsed in deionized water and dried at room temperature. This treatment has usually two roles, i.e. surface decontamination and chemical oxidation etching by the formation of surface hydroxyl groups [33]. The thus-cleaned surface is labeled PiTi. Silanation of the surface was achieved by immersion of the sample in a solution of APTES