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Endothelialization and the bioactivity of Ca-P coatings of different Ca/P stoichiometry electrodeposited on the Nitinol superelastic alloy

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ABSTRACT

An alternative approach to improve the cardiovascular stents with less restenosis than drug eluting stents, involves an improvement in endothelialization of implants. In this study, the bio compatibility of the modified Ti-50.9Ni alloy was investigated. At the first step, a thermo-chemical surface modification process was used to control the Ni release of the alloy. XPS and Raman analysis revealed that the surface of the alloy contains titanium dioxide after the modification process. According to the Ni release test, this surface condition has a good durability in Ringer's solution and offers a standard range to the leached Ni. At the next step, porous Ca-P films were electrodeposited on the modified surface. The results of endothelial cell culture on the coated samples revealed that the Ca-P coating, which has the highest value of Ca/P ratio shows the best result. The coating revealed a moderately wettable surface with a water contact angle of 53.3°. According to Ca content analysis of the cell culture medium, this coating has the lowest amount of Ca as a result of minimum solubility of the coating. In the other Ca-P coatings with lower Ca/P ratios, the solubility of coatings results in the detachment of the cells. Also nanoindentation and SEM studies revealed that the low stiffness in the calcium deficient coating can result in the failure of the coating as a result of the tensions created by the cells.

1. Introduction

NiTi alloys have considered as an important biomaterial since several years ago [1,2]. In most other metallic implants, the elastic module mismatch between the implant and surrounding tissues results in the implantation failure [3]. The low difference between the elastic modulus of NiTi alloys and that of the human tissues makes the alloys a suitable candidate for implant materials [4]. Also the superelastic property of the alloys makes them exclusive for special applications such as self expanding cardiovascular stents [5].

There are some problems in the application of these alloys as implants especially because of the Ni release and the bio inert surface. Not only does the nickel ion lead to allergic symptoms, but it also weakens the proliferation of cells in the NiTi surface [6,7]. Also the bio inert surface leads to the formation of a nonadherent layer at the surface of implants and failing of the implantation [8–11].

Although calcium-phosphate (Ca-P) based compounds offer a good medium for living body cells growth, they cannot be used as bulk materials in implants because of the poor mechanical properties. Using Ca-P based coatings on the surface of metallic implants is an effective method for achieving the both advantages of metallic implants and Ca-P compounds [12,13].

Different methods have been introduced for the deposition of Ca-P compounds [12–18]. Electrocrystallization is one of the effective methods for the deposition of these compounds in low temperature with controlled thickness, crystallinity and morphology [18].

Calcium phosphate coatings have demonstrated to favor the hard tissue integration in orthopedic implants [19,20]. On the other hand, there are some ideas for the application of these coatings on the cardiovascular stents. These types of coatings can be used in drug eluting stents. In spite of the polymer coated stents, Ca-P coatings do not increase the platelet activation in circulating human blood [21].

In drug eluting stents, the growth of smooth muscle cells is inhibited inside the vessels by a controlled drug releasing technique. Although this technique would be helpful at the initial stages of stenting, preventing endothelial cell growth can result in other problems such as restenosis over time. Accordingly, as an alternative approach, improving the endothelialization of the stents is the subject of recent researches [22,23].

Despite the progress made towards Ca-P deposition, the influence of the coating properties in controlling the adhesion and proliferation of endothelial cells is still poorly understood. There are some reports on the bioactivity of calcium phosphates which have characterized some other kinds of cells and deposition methods. For example, it was

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shown that the Ca/P molar ratio of bioglasses has a significant effect on the fibroblast cells growth [24]. In the field of bone implants, because of the structural similarity between bone and calcium deficient hydroxyapatite, most studies have been focused on the calcium deficient hydroxyapatite [25]. Even though, there are some reports which show that the best growth condition for osteoblasts occurs at Ca/P ratio around 1.7 in bulk Ca-P materials [26,27].

The main objective of this study is investigating the potential of Ca-P coatings in endothelialization of superelastic Nitinol alloys. For this purpose, the effect of Ca/P ratio on the biological properties of the coated samples was investigated. The chemical and nanomechanical tests were carried out on the coatings to justify the observed in-vitro behaviors.

2. Experimental details

2.1. Preparation of specimens

In this work, NiTi rod with nominal composition of 50.9% Ni was used as substrates. The rods with the diameter of 13 mm were sliced into 1 mm disks. The surface of the samples was abraded with different grades of SiC papers from P80 to P600 grit and then was etched in an acid solution of 1 HF-4 NHO_3-5 H_2O for 4 min, and finally was soaked in distilled boiling water for 20 min. After each step, specimens were cleaned in acetone and then rinsed with deionized water. For the heat treatment process, the specimens were encapsulated in a glass tube after purging and vacuuming the high purity Ar gas inside the tube for several times. The heat treatment carried out at 470 °C for 30 min.

2.2. Electrochemical deposition

The electrodeposition was performed in an individual cell using a regular three electrode configuration in which NiTi alloy served as the cathode and a platinum mesh acts as the anode. Mechanical stirring of the electrolyte was controlled at a speed of 150 rpm for all depositions. Fig. 1 shows a schematic representation of the electrochemical cell which demonstrates the relative position of each component in the cell. The selected positions result in a uniform stream of the electrolyte from the anode to the cathode surface.

Three different conditions were used for the electrodeposition of calcium-phosphate based coatings. In the (A) condition, an electrolyte solution of 0.042 M $Ca(NO_3)_2 \cdot 4H_2O$ and 0.025 M $NH_4H_2PO_4$ and 6 ml/l H_2O_2 was prepared and used. The pH of the electrolyte was 4.3 and the temperature of the electrolyte was maintained at 70 ± 1 °C using

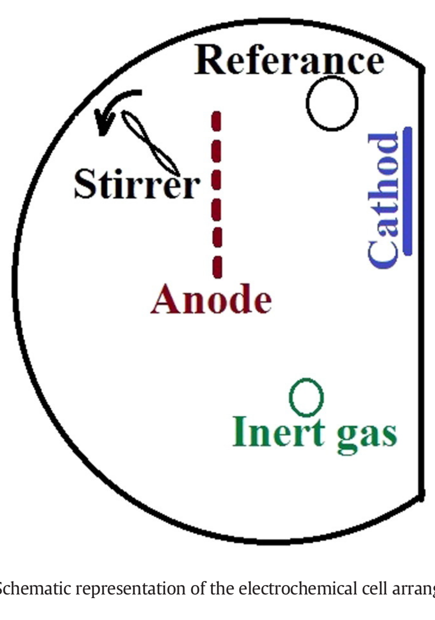

Fig. 1. Schematic representation of the electrochemical cell arrangement.

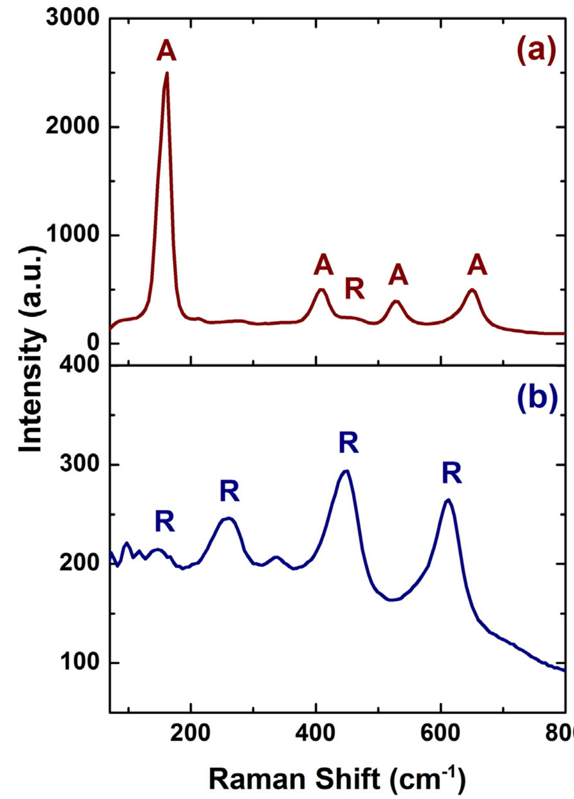

Fig. 2. Raman spectra of the Nitinol alloy after the heat treatment process for a) abraded and b) chemically treated samples.

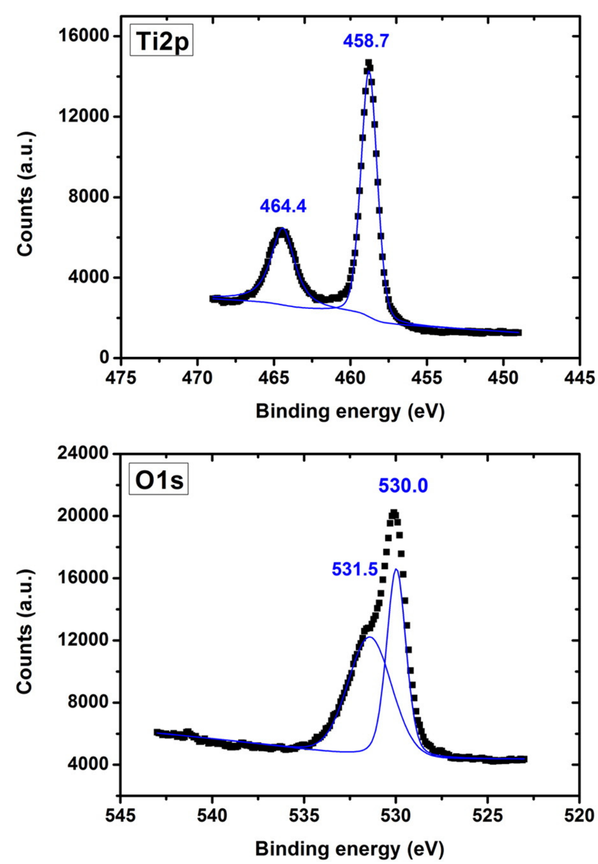

Fig. 3. The high resolution XPS spectra for Ti2p and O1s of the modified NiTi alloy.