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Bioactivity and electrochemical behavior of hydroxyapatite-silicon-multi walled carbon nano-tubes composite coatings synthesized by EPD on NiTi alloys in simulated body fluid

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ABSTRACT

In order to improve the surface bioactivity of NiTi bone implant and corrosion resistance, hydroxyapatite coating with addition of 20 wt% silicon, 1 wt% multi walled carbon nano-tubes and both of them were deposited on a NiTi substrate using a cathodic electrophoretic method. The apatite formation ability was estimated using immersion test in the simulated body fluid for 10 days. The SEM images of the surface of coatings after immersion in simulated body fluid show that the presence of silicon in the hydroxyapatite coatings accelerates in vitro growth of apatite layer on the coatings. The Open-circuit potential and electrochemical impedance spectroscopy were measured to evaluate the electrochemical behavior of the coatings in the simulated body fluid at 37 °C. The results indicate that the compact structure of hydroxyapatite-20 wt% silicon and hydroxyapatite-20 wt% silicon-1 wt% multi walled carbon nano-tubes coatings could efficiently increase the corrosion resistance of NiTi substrate.

1. Introduction

The surface of NiTi alloys for application as the bone implant plays an important role in bioactivity and corrosion behavior. The human body fluid acts as the relatively corrosive environment for metallic implant [1]. The NiTi shape memory alloys as the bone implant have been developed because of their mechanical properties which are close to bone [2]. However, during implanting, releasing corrosive products such as Ni^{1+} ions may induce an allergic reaction on the surroundings tissues [3]. On the other hand, it is well known that the NiTi surface is bio-inert [4]. The bio-inert surface is encapsulated by a fibrous tissue and isolated from surrounding bone when implanted in the human body [5]. Therefore it is necessary to coat the surface of NiTi implant to improve its bioactivity and corrosion resistance. The bioactive materials can chemically bond to bone structure through the formation of a bone like apatite layer on its surface by immersion in the simulated body fluid (SBF) [6].

Bohner et al. [7] indicated that the results of immersion test in SBF are not adequate to confirm the in vivo bone bioactivity while Kokobo et al. [5] reported that it is useful to predict the in vivo bone bioactivity, reduce the number of animals and the duration of test in the in vivo experiment. In order to determine the bioactivity behavior of bone implant materials, cell culture experiments should be done on the candidate materials. The concentration of various ions in the SBF is similar to human blood plasma but not included proteins [5]. In vitro behavior of different implants in the SBF has attracted great attention by researchers. Kokubo et al. [8] evaluated the bioactivity of Ti and its alloys by investigation of apatite formation on their surfaces in the SBF. They showed that releasing alkali ions from the surface of heat treated Ti alloys in the SBF induced the apatite layer formation. Also, Chen et al. [4] demonstrated that hydroxyapatite (HA) layer can spontaneously precipitate on chemically treated NiTi in SBF. They exhibited that the HA coating increased the rate of proliferation for osteoblast cells. In the other work, Fujibayasi et al. [9] compared the in vivo bone ingrowth and in vitro apatite formation on the biomaterials. They indicated that the newly formed bone on the biomaterials surface in the in vivo studies is proportion to their apatite formation ability in the in vitro condition, thus using immersion test in the SBF before implantation can save time and cost of animal experiments. The biological response of hydroxyapatite/Ti-6Al-4V composite coatings in SBF showed that some calcium-phosphate phases dissolve partially after soaking in the SBF. Then bone like apatite crystals nucleate and grow all over the coating [6].

The biological reactions can occur between the implant surface and the surrounding tissues in the early stage of implantation. The surface engineering of biomaterials changes the biological response of materials, in which, the main mechanical properties of the substrate are maintained and the surface properties are improved [10]. The bioactive ceramic of HA with general formula of $Ca_{10}(PO_4)_6(OH)_2$ is the best candidate of coating materials on the NiTi implant. It can promote the

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growth of new bone on its surface due to its ability in the various bone regeneration [11]. However, it has been reported that the fracture often occurs inside the coating or substrate-coating interface over time when the pure HA coated implant is replaced in living tissue [12]. One method to improve the mechanical behavior of the HA-based coatings is introduction of bioactive reinforcement to them. One of the best candidate for bioactive reinforcement component is Si. Because it is one of the most important elements in the initial stage of bone growth and the essential trace elements in the biological processes. The composite of HA and Si can improve the bioactivity of biomaterials [13]. Also using carbon nano-tubes (CNTs) as natural bone collagen fibers in HA-based coating can be a promising composite for application as bone implant [14]. There are contradictory data about the in vitro and in vivo toxicity of CNTs. The toxicity of unpurified CNTs is more than pristine CNTS. The chemical functionalization of CNTs results in non-toxic effect in the biological environment [15]. Thus in this investigation, CNTs were functionalized by exposure to HNO_3 vapor as reported in our previous paper [16]. The same authors of this paper developed HA-Si-MWCNTs composite coatings synthesized by electrophoretic deposition (EPD) on NiTi alloys [16,17]. The mechanical properties of these coatings and interaction of human mesenchymal stem cells (hMSCs) with their surfaces were reported with more details elsewhere [2]. In this paper, for the first time in literature, we report the ability of these coatings in apatite formation and electrochemical properties of them in the SBF.

2. Materials and methods

The Ni rich NiTi alloy with chemical composition of 55.68 wt% Ni, <500 pm C, <500 pm O and balance Ti, was used as a substrate. At first, the NiTi rod was cut into dimensions of $5 \times 5 \times 2$ mm^3 and grinded up to 1000 mesh. Four different coatings of HA, HA-20 wt% Si, HA-20 wt% Si-1 wt% MWCNTs and HA-1 wt% MWCNTs were developed on NiTi specimens using cathodic EPD. The NiTi specimens were sand blasted and then etched in the solution of 10 cm^3 hydrofluoric acid, 40 cm^3 nitric acid and 50 cm^3 distilled water before EPD coating.

In order to determine the bioactive behavior of the coated specimens, they were immersed in the SBF for 10 days at 37 °C. The protein-free and acellular SBF was prepared according to Kokopo's recipe [18]. Table 1 illustrates the amount of required salts to produce 1 l of SBF. The prepared SBF was buffered to pH 7.4 at 37 °C by adding 1 M Hydrochloric acid and tris (hydroxymethyl aminomethane). The volume of required SBF for immersion of each specimen was calculated using the following Eq. (1) [19]:

$$S/V = 0.05\ \text{cm}^{-1} \quad (1)$$

where S is exposed surface area and V is the SBF volume. The SBF was regularly refreshed every day to stabilize its ion concentration and pH value. After exposing for 10 days, the specimens were removed from the SBF and washed with distilled water and then dried in desiccator for 24 h. In order to characterize the apatite growth, the surface morphology and elemental analysis of specimens were studied using SEM (Quanta FEG650, FEI™, USA) equipped with energy dispersive X-ray spectroscopy (EDS) at high vacuum of 1.4×10^{-5} mbar. Due to the low electrical conductivity of the deposited layer on the specimens, they were covered with a layer of Iridium using Sputter Coater Emitech K575X operating at 60 mA, 1 min, 2×10^{-2} mbar and 2 cycles. Also, FTIR analysis (4600, Unicam™, USA) was performed to identify the surface of coatings after immersion in the SBF for 10 days. Finally, the electrochemical behavior was evaluated by electrochemical impedance spectroscopy (EIS) of IVIUM device for the coated and uncoated NiTi specimens in the SBF at 37 °C. A three-electrode cell was used consisting of an Ag/AgCl electrode (reference electrode), a platinized platinum electrode (auxiliary electrode) and a working electrode (the specimen). At first, open circuit potential (OCP) was recorded for 3600 s. Then, the EIS measurements were performed at OCP condition with surface area of 1 cm^2 for the working electrode, the frequency range of 100 kHz down to 10 mHz and voltage amplitude of 5 mV at 253 points. The impedance spectra was reported in the format of Nyquist and Bode plots and modeled by ZSimp Win 3.21 software as equivalent circuit (EC). An EC is a set of the circuit elements that provides the physical and electrical features of electrochemical interface [17].

Table 1
The amount of materials needed to prepare 1000 ml of SBF [18].

Salt	Amount	Purity%	Formula weight
NaCl	8.036 g	99.5	58.44
$NaHCO_3$	0.352 g	99.7	84.01
KCl	0.225 g	99.0	74.56
$K_2HPO_4 \cdot 3H_2O$	0.230 g	99.0	228.23
$MgCl_2 \cdot 6H_2O$	0.311 g	99.0	203.30
1 M Hal	40 ml	–	–
$CaCl_2$	0.293 g	98	110.98
Na_2SO_4	0.072 g	99.0	142.04
Tris	6.063 g	99.8	121.14
1 M HCl	0.2–0 ml	–	–

3. Results and discussion

3.1. Surface morphology and elemental analysis

As can be seen in Fig. 1, different magnification of the SEM images of NiTi and point EDS analysis were respectively used to determine the morphology and atomic ratio of $\frac{Ca}{P}$ in the deposited crystallites on their surface in the SBF after 10 days immersion. The spherical particles in the approximate diameters of 50–100 nm nucleate and grow on the NiTi substrate, but does not cover the whole surface of the NiTi specimen after immersion in the SBF for 10 days. The EDS results reveal these particles are bone-like apatite crystallites with $\frac{Ca}{P}$ ratio of 1.40.

The different magnification of SEM images and point EDS analysis were shown in Figs. 2–5 for HA, HA-1 wt% MWCNTs, HA-20 wt% Si, HA-20 wt% Si-1 wt% MWCNTs respectively after immersion in the SBF for 10 days. Fine bone-like apatite crystallites cover the whole surface of HA coating. The Higher magnification shows that these crystallites on the HA coating are plate and have the petal-like shape with the maximum thickness of 30 nm in compared to the spherical shape of deposited crystallites on the NiTi substrate. The $\frac{Ca}{P}$ atomic ratio of deposited crystallites is increased from 1.40 for the NiTi substrate to 1.50 for the HA coating which is closer to the chemical composition of HA in the human bone structure. The surface morphology of the HA-1% MWCNTs coating are similar to the HA coating after immersion in the SBF for 10 days. The deposited petal-like crystallites on the HA-1% MWCNTs coating mainly demonstrate two-dimensional and intertwined growth with approximate thickness of 50 nm. The $\frac{Ca}{P}$ atomic ratio of 1.56 for the HA-1% MWCNTs indicates that the presence of 1% MWCNTs in the HA coating does not have negative influence on the ability of apatite crystal growth in the in vitro conditions.

The HA-20% Si coating demonstrates different behavior in compared to the HA and HA-1% MWCNTs coatings after immersion in the SBF. It can be seen in Fig. 4 that deposited crystallites grow side by side with three-dimensional and spherical morphology. The diameter of spherical deposits grow up to 15 μm on the some surface areas during 10 days in the SBF (Fig. 4a). These crystallites independently nucleate and reach to each other. Therefore there is no typical grain boundaries between them observed in crystal structure. The SEM images with higher magnification in Figs. 4b and c depict the formation of planer petal-like apatite with relatively large size and maximum thickness of 60 nm. The $\frac{Ca}{P}$ atomic ratio for the crystallites of the HA-20% Si coating is about 1.67 that is precisely equal to the $\frac{Ca}{P}$ ratio of available HA in the bone structure.