Electrochemical impedance spectroscopy study of the nucleation and growth of apatite on chemically treated pure titanium

C.X. Wang*, M. Wang

School of Mechanical and Production Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

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Abstract

Bone-like apatite formed on the surface of pure titanium pretreated with NaOH solution after having been immersed in simulated body fluid (SBF). In the present study, electrochemical impedance spectroscopy (EIS) measurement was used to investigate the nucleation and growth of apatite on chemically treated pure titanium immersed in the SBF solution. An appropriate equivalent circuit model was constructed to describe the nucleation and growth of apatite. It was found that EIS is a useful method for investigating the nucleation and growth of bone-like apatite on pure titanium pretreated with NaOH solution. It may quantify apatite growth on pretreated titanium substrates. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Electrochemical impedance spectroscopy; Alkaline treatment; Pure titanium; Bone-like apatite; Nucleation and growth

1. Introduction

Calcium phosphate ceramics, such as hydroxyapatite (HA)-coated titanium and its alloys, are among the most promising implant materials for orthopedic and dental applications. However, problems such as low bond strength between the coating and the substrate and nonuniformity across the thickness of the coating are often encountered with these coatings [1].

Recently, it has been reported that chemically treated titanium can induce bone-like apatite formation in vitro and in vivo [2–4], which means that titanium and its alloys have potential bioactivity. Treatment with a NaOH solution produces a sodium titanate gel layer on titanium surface. The gel layer has the ability to induce formation of bone-like apatite during immersing in simulated body fluid (SBF) and, thus, is considered bioactive. The gel layer can initiate apatite nucleation on itself. Once apatite nucleation occurs, it spontaneously grows by taking calcium and phosphate ions from the surrounding environment.

The qualitative observation of nucleation and growth of apatite on pretreated pure titanium could be done using conventional methods such as X-ray diffraction (XRD), scanning electron microscope (SEM), etc. The main objective of this study was focused on a quantitative investigation of the nucleation and growth of apatite by using electrochemical impedance spectroscopy (EIS) measurements.

* Corresponding author. Tel.: +65-790-5332; fax: +65-791-1859.
E-mail address: mcxwang@ntu.edu.sg (C.X. Wang).
2. Materials and methods

2.1. Surface treatment of titanium substrates

A commercially available pure titanium substrate (discs of dimensions $15 \times 3$ mm) was mechanically polished and ultrasonically cleaned with acetone and alcohol. These discs were soaked in 5.0 M NaOH solution at 60 °C for 24 h, then gently washed with distilled water and finally dried at 37 °C for 24 h.

2.2. Immersing of pretreated substrates in simulated body fluid (SBF)

An acellular simulated body fluid (SBF) with pH 7.4 and ion concentrations (in mM: Na$^+$ 142.0, K$^+$ 5.0, Mg$^{2+}$ 1.5, Ca$^{2+}$ 2.5, Cl$^-$ 147.8, HCO$_3^-$ 4.2, HPO$_4^{2-}$ 1.0, SO$_4^{2-}$ 0.5) nearly equal to those of human blood plasma was previously proposed by Kokubo et al. [5], and has been extensively confirmed to reproduce the in vivo apatite formations on bioactive materials [5,6]. The SBF was prepared by dissolving reagent grade chemicals of NaCl, NaHCO$_3$, KCl, K$_2$HPO$_4$·3H$_2$O, MgCl$_2$·6H$_2$O, CaCl$_2$ and Na$_2$SO$_4$ into distilled water and buffering at 7.40 with tris-hydrochloromethyl-amminomethane ((CH$_2$OH)$_3$-CNH$_3$) and hydrochloric acid at 36.5 °C. After the alkaline treatment, the treated titanium substrates were immersed in SBF. At regular intervals, the specimens were removed from SBF, washed with distilled water and acetone and dried at room temperature. Some of the specimens were used for microstructural characterization and the others were used for electrochemical impedance spectroscopy measurements.

2.3. Microstructural characterizations

X-ray diffraction (XRD) was employed to analyze the structure of titanium substrate, gel layer and bone-like apatite. A thin-film X-ray diffractometer (XRD, Rigaku X-ray diffractometer) was used. The morphologies of the specimens were examined under scanning electron microscopy (SEM, JEOL JSM 5600LV).

2.4. Electrochemical impedance spectroscopy (EIS) measurements

The electrochemical impedance spectroscopy (EIS) measurements were made using a lock-in amplifier (Model 5210, EG&G Instrument) coupled to a Potentiostat–Galvanostat System (Model 273A, EG&G Parc.), which was connected to a three-electrode electrochemical cell. A platinum foil was used for the auxiliary electrode and Ag/AgCl (saturated KCl) for the reference electrode.
as counter electrode and a saturated calomel electrode (SCE) was used as a reference electrode. The treated titanium specimens were used as the working electrodes. EIS spectra were obtained at open-circuit potential of the specimens in SBF, with an amplitude of 10 mV. The frequency span was from 100 KHz down to 1 mHz. Data registration and analysis were performed on an interfaced computer. The spectra were then interpreted using the nonlinear least square fitting procedure developed by Boukamp [7]. The quality of fitting to the equivalent circuit was judged firstly by the chi-square value, and secondly by the error distribution vs. frequency comparing experimental with simulated data [7].

3. Results

3.1. Scanning electron microscopy

Fig. 1 shows scanning electron microscopy (SEM) micrographs of the surfaces of titanium substrates that were soaked in 5.0 M NaOH solution at 60 °C for 24 h in comparison to the untreated titanium.

![SEM micrographs of the surfaces of pretreated titanium substrates immersed in the SBF solution at 36.5 °C at regular intervals: (a) 1 week, (b) 3 weeks.](image)

Fig. 2. SEM micrographs of the surfaces of pretreated titanium substrates immersed in the SBF solution at 36.5 °C at regular intervals: (a) 1 week, (b) 3 weeks.

![TF-XRD pattern of titanium substrate treated with 5.0 M NaOH at 60 °C for 24 h.](image)

Fig. 3. TF-XRD pattern of titanium substrate treated with 5.0 M NaOH at 60 °C for 24 h.
substrates. It can be seen that a porous network structure was formed on the surface of titanium with the NaOH treatment.

Fig. 2 shows SEM micrographs of the surfaces of NaOH pretreated titanium substrates were immersed in simulated body fluid (SBF) at 36.5 °C for regular intervals. As can be seen, after 1 week’s immersion in SBF solution, apatite nuclei formed on the surface of the pretreated titanium substrates. Then, the apatite nuclei grew and the amount of apatite increased with the extension of immersion time. In the stage of nuclei formation (Fig. 2a), there were some spherical apatite islands on the network structure. With the increase in the immersion time, islands of apatite grew, and the network structure was gradually covered by apatite, and with the further increase in the immersion time, the growing apatite islands coalesced, and the network structure was completely covered with apatite (Fig. 2b). This indicated that this network structure formed on the titanium surface by alkaline treatment could induce the nucleation and enhance the formation of apatite, which made titanium to be bioactive.

### 3.2. XRD results

Fig. 3 shows the TF-XRD pattern of the surface of titanium treated with 5.0 M NaOH solution at 60 °C for 24 h. A broad bump and small peaks at around 24, 28 and 48° were observed in the XRD pattern, indicating that the surface porous network layer, which was formed by the NaOH treatment, is an amorphous sodium titanate phase [2,4].

Fig. 4 shows the TF-XRD patterns of alkaline-treated titanium immersed in the SBF solution at 36.5 °C at regular intervals. In comparison to the

![Graph](image)

Fig. 4. TF-XRD patterns of alkaline-treated titanium immersed in the SBF solution at 36.5 °C at regular intervals: (a) 1, (b) 2, (c) 3, (d) 4, (e) 6 and (f) 8 weeks.

![Graph](image)

Fig. 5. Typical bode plot for alkaline-treated titanium immersed in the SBF solution (8 weeks).
pattern in Fig. 3, all the new peaks appeared in the patterns in Fig. 4 are ascribed to crystalline bone-like apatite, indicating that the network structure formed on the surface of titanium could induce the nucleation and growth of bone-like apatite on titanium. In the stage of nucleation (Fig. 4a), the counts of the peaks for apatite were very low, and peaks for titanium substrate were also observed. With an increase in immersion time in simulated body fluid, the counts of the peaks for apatite were getting higher, and no peaks for the titanium substrate were observed (Fig. 4b–f), indicating the growth of apatite and the surface was fully covered with apatite.

3.3. Electrochemical impedance spectroscopy

A typical EIS spectrum is shown in Fig. 5. When alkaline-treated titanium is exposed to simulated body fluid, the spectra appears very different and varies significantly with exposure time, especially for the spectra at higher frequencies, which indicates the nucleation and growth of apatite. It is interesting to note that the remarkable change in the spectrum coincided with the nucleation and growth of apatite on the pretreated titanium.

3.4. Analysis of EIS spectra

For analysis of the impedance data, a software program, ‘Equivalent Circuit,’ was used. The program used a variety of electrical circuits to numerically fit the measured impedance data. A constant phase element (CPE), $Q$, are used for the equivalent circuit in this study.

Fig. 6 shows the equivalent circuit based on a three-layer model (inner, hydrogel and apatite layers), which

![Equivalent Circuit Diagram]

Table 1

<table>
<thead>
<tr>
<th>Time</th>
<th>1 h</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
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<tr>
<td>$R_a$ (Ω)</td>
<td>78</td>
<td>167</td>
<td>199</td>
<td>211</td>
<td>346</td>
<td>356</td>
<td>418</td>
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<tr>
<td>$C_a$ (F)</td>
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<td>$3.6 \times 10^{-4}$</td>
<td>$2.3 \times 10^{-4}$</td>
<td>$1.1 \times 10^{-4}$</td>
<td>$2.9 \times 10^{-4}$</td>
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</tr>
<tr>
<td>$R_p$ (Ω)</td>
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<td>$2.0 \times 10^3$</td>
<td>$1.2 \times 10^3$</td>
<td>$5.6 \times 10^4$</td>
<td>$3.8 \times 10^5$</td>
<td>$1.1 \times 10^5$</td>
<td>$6.8 \times 10^2$</td>
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<tr>
<td>$C_p$ (F)</td>
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<td>$1.7 \times 10^{-4}$</td>
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<td>$1.6 \times 10^{-4}$</td>
<td>$5.7 \times 10^{-4}$</td>
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<td>$R_b$ (Ω)</td>
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<td>$9.3 \times 10^7$</td>
<td>$2.7 \times 10^8$</td>
<td>$1.9 \times 10^7$</td>
<td>$1.3 \times 10^7$</td>
<td>$5.4 \times 10^7$</td>
<td>$2.8 \times 10^8$</td>
</tr>
<tr>
<td>$C_b$ (F)</td>
<td>$3.1 \times 10^{-5}$</td>
<td>$2.2 \times 10^{-5}$</td>
<td>$2.8 \times 10^{-5}$</td>
<td>$2.7 \times 10^{-5}$</td>
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<td>$3.4 \times 10^{-5}$</td>
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can be satisfactorily used for fitting the spectra obtained from alkaline-treated titanium immersed in simulated body fluid at different periods of time. The fitting results are listed in Table 1. As can be seen that $R_a$ (apatite layer resistance) continuously increases with the exposure time. At the apatite nucleation stage (from 1 h to 1 week), even though apatite could not be clearly seen under scanning electron microscopy (SEM) observation, the increase in $R_a$ indicated apatite nucleation. And the growth of apatite corresponded to the continuous increase of $R_a$.

4. Discussion

A broad bump in the TF-XRD pattern of titanium treated with NaOH solution at 60 °C for 24 h suggests that the network structure on the surface is an amorphous sodium titanate hydrogel. When the treated titanium discs immersed in simulated body fluid solution at 36.5 °C, this hydrogel layer could induce the nucleation and growth of apatite on the surface. After about 1-week nucleation time, islands of apatite were seen on the surface of pretreated titanium under SEM observation, and weak peaks for apatite with peaks for titanium substrate appeared in the TF-XRD pattern. With an increase in immersion time in simulated body fluid, islands of apatite were seen to grow and coalesce on pretreated titanium under SEM observation, and strong peaks of apatite with no peaks for titanium substrates appeared in the TF-XRD patterns.

Electrochemical impedance microscopy analysis has been shown to be a useful method for investigating the nucleation and growth of bone-like apatite on pretreated titanium. Based on the EIS spectra, a three-layer (inner, hydrogel and apatite layers) model was used to interpret the obtained spectra. The results coincided with those obtained from SEM and TF-XRD very well. At the apatite nucleation stage, even though no apatite nuclei formed on the surface (apatite could not be clearly seen under SEM observation), the increase of resistance in the outmost surface of pretreated titanium discs indicated apatite nucleation. With an increase in immersion time in simulated body fluid, islands of apatite were seen to grow and coalesce on pretreated titanium under SEM. The continuous increase of the resistance of apatite layer indicated the growth of apatite on the pretreated titanium. In comparison to the results obtained from other methods such as SEM, TF-XRD, XPS, etc., the growth of apatite are quantitative. Therefore, EIS can be used to quantify apatite growth on pretreated titanium.

5. Conclusions

Bone-like apatite formed on the surface of titanium pretreated with NaOH solution after the pretreated titanium discs had been immersed in the simulated body fluid solution. Electrochemical impedance spectroscopy (EIS) has been shown to be a useful method for investigating the nucleation and growth of bone-like apatite on pretreated titanium. At the apatite nucleation stage, even though apatite could not be clearly seen under scanning electron microscopy (SEM), the increase in electrical resistance in the outmost surface of pretreated titanium discs indicated apatite nucleation. With an increase in immersion time in simulated body fluid, islands of apatite were seen to grow and coalesce on pretreated titanium under SEM. The growth of apatite corresponded to the increase in electrical resistance of the surface layer. EIS can be used to quantify apatite growth on pretreated titanium.

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