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An effective approach to activate 316L stainless steel for biomimetic coating of calcium phosphate: electrochemical pretreatment

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Abstract In this paper, an electrochemical (EC) method to activate 316L stainless steel (denoted as 316L) surface for biomimetic calcium phosphate (Ca–P) coatings was reported. After EC treatment, a gel-like Ca–P film with a thickness of 150 nm was generated on the stainless steel surface after treatment, which was composed of amorphous phase of calcium phosphate with a large number of crystal nuclei of octacalcium phosphate (OCP) inside. This Ca–P thin film is the main factor that causes Ca–P formation under biomimetic condition. The effectiveness of EC treatment was also compared with alkali heat (AH) pretreatment in producing biomimetic coating on 316L. A uniform Ca–P coating formed on EC treated samples after samples were immersed in saturated calcium solution (SCS) for several hours, while only some island-like deposits were found on the sample surface with AH treatment followed by immersion in SCS for several days. This work has explored a novel and effective pretreatment method to activate 316L implant surface, which can be expected to be applied to activate other metal implants.

Introduction

316L stainless steel (316L) has long been used as prosthetic implant in orthopedic surgery [1–3]. However, the lack of bioactivity of stainless steel limits its application in clinics. In order to achieve bioactivity for metal implants as bone substitutes, calcium phosphate coating is usually introduced onto their surfaces. The most commonly applied techniques for implant coating are plasma spraying [4–6], electrochemical deposition [7–9], electrophoretical deposition [10–12], laser-pulse deposition [13], sol–gel deposition [14–16], and biomimetic deposition [17–21]. Among them, biomimetic deposition methods are recently developed ones, which have attracted much research interest because of their flexibility to be used on different surfaces and the readiness to control the composition of coating. However, until recently, few of them were operated on stainless steel because no good pretreatment method was available. As we know, biomimetic deposition methods usually require certain surface conditions, such as roughness, surface charge, and surface energy etc. [22–26], which are critical factors for the nucleation of calcium phosphate crystal on the substrate. Substantial amount of work has been done on biomimetic deposition on titanium and its alloys [17–20, 22–25]. The most commonly used pretreatment method for titanium activation are alkali-heat (AH) treatment, acid–alkali treatment. After those

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treatments, the specimens can be immersed in saturated calcium solutions (SCS) to obtain the biomimetic calcium phosphate coatings [23, 25].

The previous work of Kim failed to apply AH treatment to bioactivate 316L [26]. Lin et al. have tried to bioactivate stainless steel by AH treatment, followed by immersion of the pretreated samples in different solutions with different Ca, P ion concentration at different temperatures. They found that, at 37 °C, 4 weeks were needed for hydroxyapatite (HA) formation in 1.5 times SBF solution, while 1 week was needed for HA formation in SBF solution at 80 °C [21]. Our previous work showed that OCP coating well formed on EC pretreated titanium after biomimetic method treatment [27]. In this study, we are exploring the possibility of applying EC method as pretreatment to 316L for biomimetic Ca–P deposition.

Materials and methods

Sample pretreatment and coating deposition

316L plates were used as substrate in this study. The specimens (10 mm × 10 mm × 1 mm) were ultrasonically cleaned by acetone, ethanol, and distilled water for 15 min each. Then, they were abraded with #600 SiC sandpaper, and washed with distilled water in an ultrasonic cleaner before being used.

Samples for AH treatment were soaked in 10 mol/L NaOH aqueous solution at 60 °C for 24 h, and then heated at 600 °C for 1 h. Samples for EC treatment were welded to copper wire by electric resistance welder (Odoneoweld V220 Monofase, Ellimanfredi, Italy). The weld spot and copper wire were sealed with silicone gel. Then, the

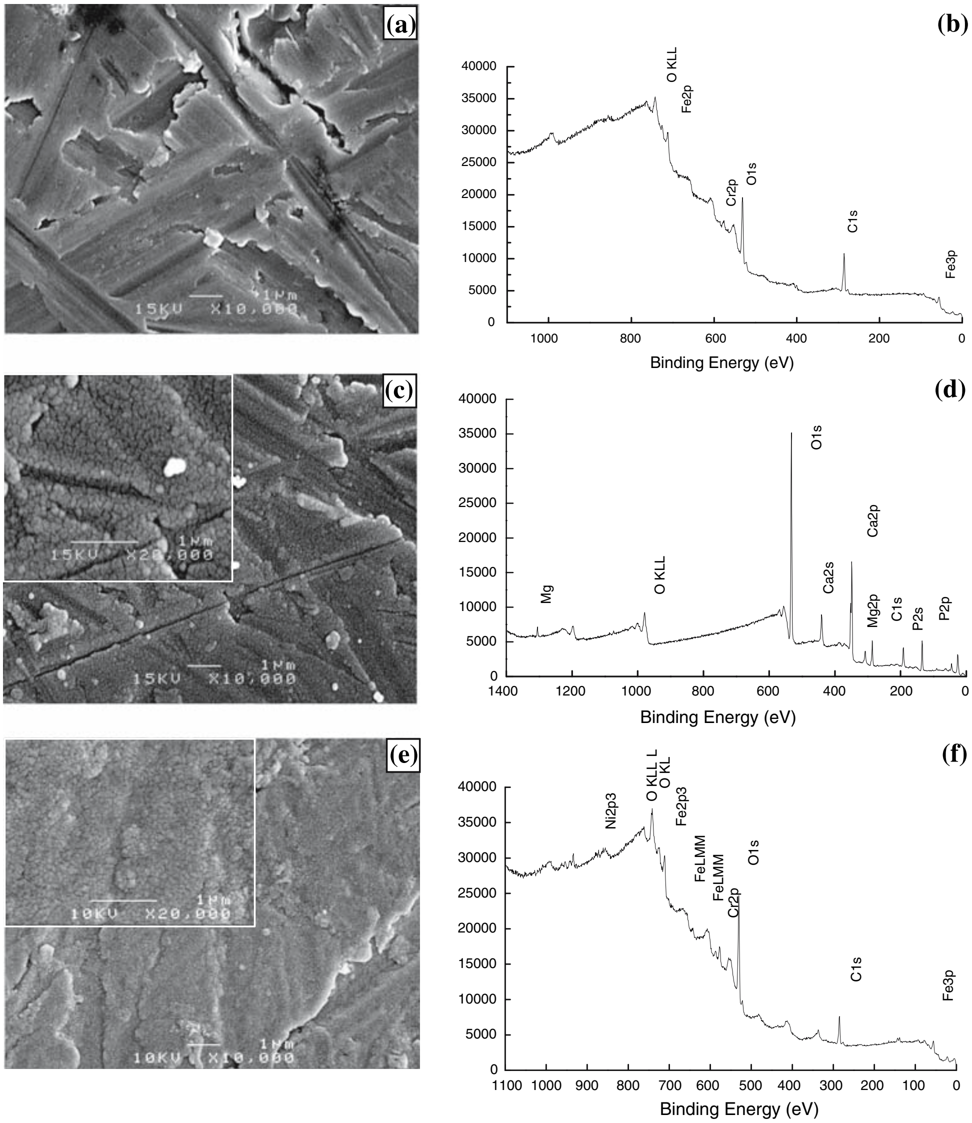


Fig. 1 The morphologies and compositions of the 316L stainless steel after EC and AH treatment. (**a**) Untreated surface of 316L; (**b**) the XPS pattern of untreated 316L; (**c**) EC treated 316L, the rectangular is the higher magnification; (**d**) the XPS pattern of EC treated 316L; (**e**) AH treated 316L, the rectangular is the higher magnification; (**f**) the XPS pattern of AH treated 316L

samples' surfaces were electrochemically processed in an electrochemical cell with the samples served as the cathode, platinum plate as anode and Hg_2SO_4/Hg electrode as reference electrode (E° = 0.6158 V). The electrolyte solution with supersaturated calcium and phosphorus ions was developed by Habibovic et al. [22], which was made by dissolving NaCl (40.00 g), $CaCl_2$ (1.39 g), $MgCl_2 \cdot 2H_2O$ (1.52 g), $NaHCO_3$ (1.06 g), $Na_2HPO_4 \cdot 2H_2O$ (0.89 g) and 1 mol/L HCl 40 ml in 1 L deionized water. Tris-hydroxymethyl aminomethane (TRIS) was used to adjust the pH value to 6 to maintain the chemical stability of the electrolyte. The treatment of samples was conducted with PGP201 potentiostat/galvanostat (Radiometer, Denmark) at a constant potential of –1.7 V at room temperature for about 10 min until the samples' surfaces showed blue or purple in color. Then, the treated samples were washed with deionized water and dried at 40 °C before further examination and coating deposition. After pretreatment, samples were immersed in SCS solution for biomimetic deposition for different time periods. SCS solution was composed of NaCl (8.00 g), $CaCl_2$ (0.42 g), $Na_2HPO_4 \cdot 2H_2O$ (0.27 g) in a liter of deionized waterand was buffered to pH 7.4 with TRIS and HCl at 37 °C.

Characterization

Composition and chemistry of EC and AH treated 316L surfaces were examined by X-ray photoelectron spectroscopy (XPS, Physical Electronics 5600 multi-technique

Table 1 Chemical compositions of untreated 316L surface and AH treated 316L surface (atomic concentration %)

	C1s	O1s	Cr2p	Fe2p	Ni2p
AHT treated 316L	31.40	49.72	4.79	12.42	1.68
Untreated 316L	49.80	37.68	1.35	10.43	0.75

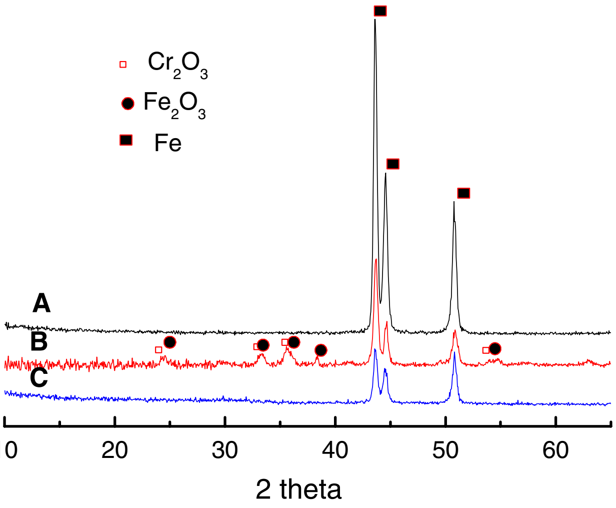


Fig. 2 The XRD pattern of pretreated and untreated 316L stainless steel. A: Untreated 316L; B: AH treated 316L; C: EC treated 316L

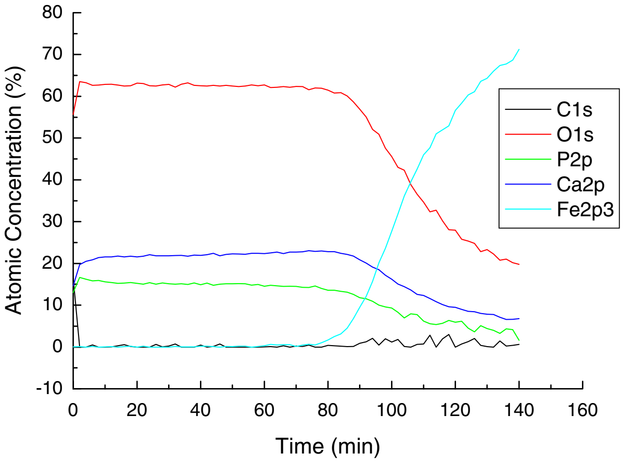
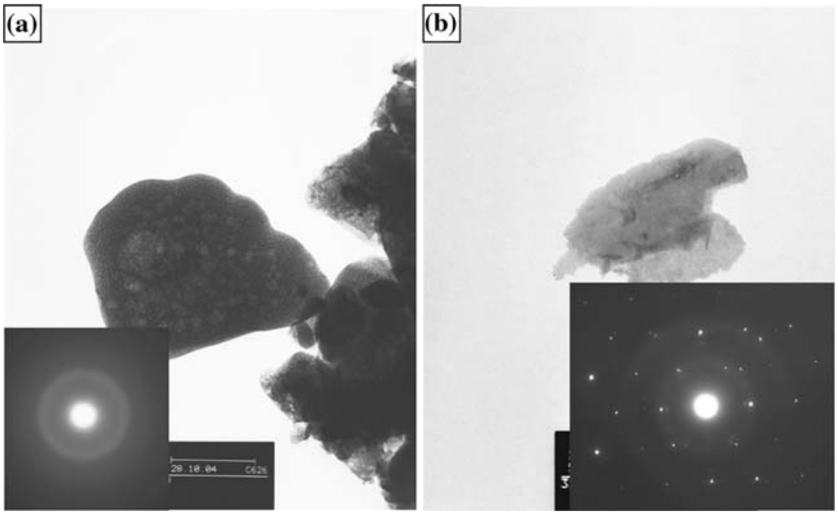


Fig. 3 The depth profile of the EC modified 316L stainless steel

Fig. 4 TEM of the EC pretreated 316L surface. (**a**) A representative of the amorphous phase; (**b**) A representative of the crystal phase, the pattern is OCP with B = [215]



system). The XPS was equipped with a standard Al Ka excitation source ($h\nu = 1486.6$ eV). The analysis area was elliptical with a short axis of 800 µm. Samples were positioned at the electron take-off angle of 45° with respect to the analyzer, which worked at a pass energy of 187.85 eV for survey spectra. The depth profile was conducted by Ar ion bombardment, and the acceleration voltage was 4 kV. Surface morphology was then examined using SEM (JEOL 6300F). TEM (Philips CM20 and JEOL 2010) was used to examine the structure of the pretreated surface layer and its coating. The TEM samples of thin film were extracted from EC pretreated surfaces by scratching, and the samples of Ca–P coatings were obtained by ultrasonic separation in ethanol.

Results and discussion

Pretreatment of 316L by EC and AH methods

The morphology and composition of 316L after EC and AH treatment were analyzed with SEM, XPS, and XRD.

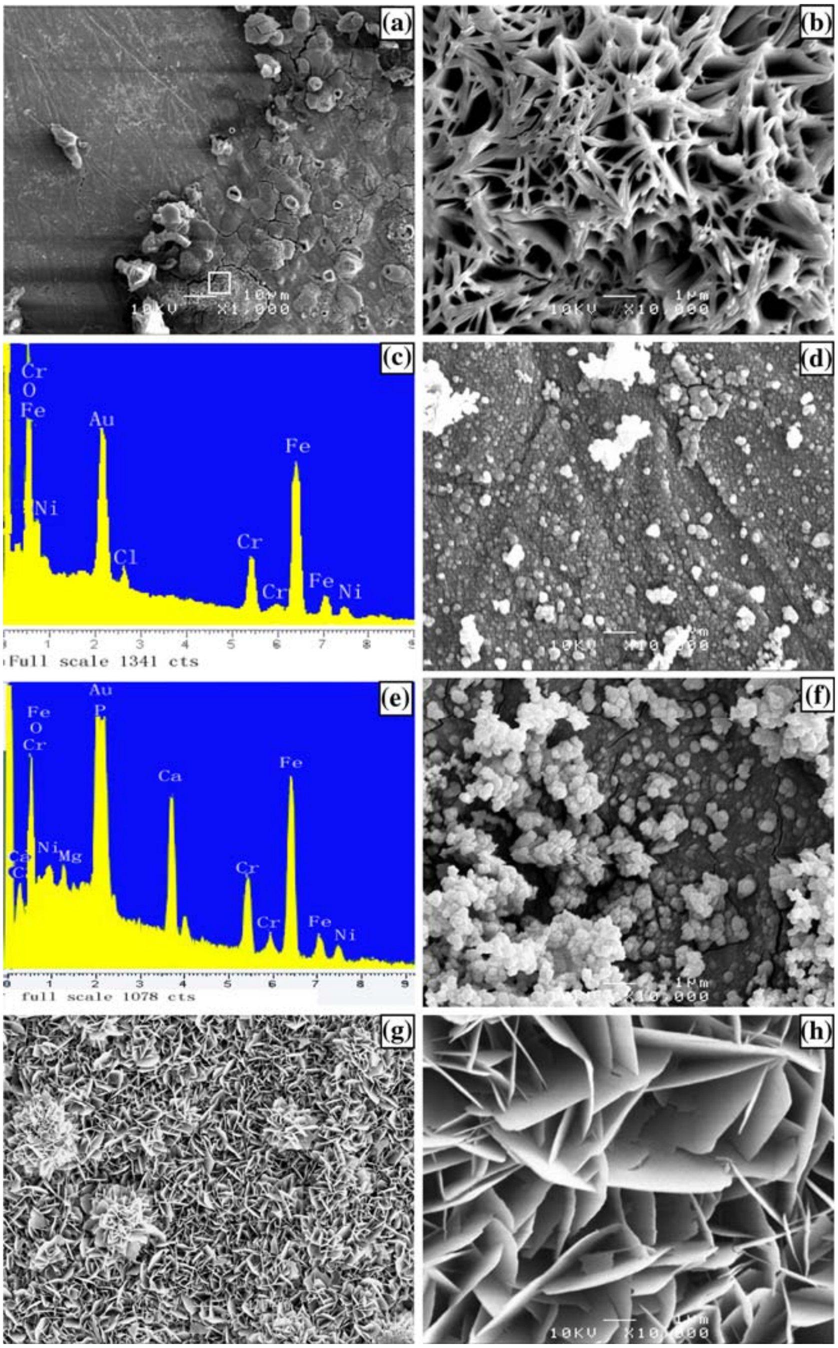


Fig. 5 Morphology of AH and EC treated 316L after immersion in SCS solution. (**a**) AH pretreated 316L after immersion for 1 day; (**b**) the magnification of the rectangular area of **a**; (**c**) EDX of the area of **b**; (**d**) the higher magnification of the **a**; (**e**) the EDX of the **d**; (**f**) AH pretreated 316L after immersion for 3 days. (**g**) EC pretreated 316L after immersion for 12 h; (**h**) higher magnification of **g**

As shown in Fig. 1. After EC treatment, the surface was covered with a compact layer of tiny particles (shown in Fig. 1c). The XPS profile showed that the layer was calcium phosphate by nature (Fig. 1d). However, after AH treatment, the treated surface was also covered by tiny grains (Fig. 1e) and the composition of the grains was mainly the titanium oxide (Fig. 1f). From Table 1, we could find that there was a great increase of the O atoms as well as Cr and Ni after AH treatment. From the XRD patterns of differently pretreated surfaces in Fig. 2, the peaks of iron oxide and chromium oxide on AH pretreated surfaces were found and no other phase was found on the XRD pattern of EC treated samples besides Ti. The reason is that the film formed on 316L surface by EC treatment is too thin to be detected by XRD even the angle of incidence is as small as 0.5 degree. From the XRD pattern of AH treated 316L (Fig. 2B), some oxide peaks of the substrate were found, which coincided with the XPS results. Lin et al. [21] has reported that there was Na_4CrO_4 formation after AH treatment of 316L. However, in our study, except for some peaks of oxide (shown in Fig. 2), no peak of Na_4CrO_4 in XRD pattern and Na in XPS spectrum was observed. The XPS depth profile (Fig. 3) of EC treated 316L showed that the thickness of the film was approximately 150 nm. The ratio of Ca/P was around 1.4 and the ratio of O/P was around 4, which showed the characteristics of the amorphous calcium phosphate [28].

Further work to identify the phase of this film was conducted through TEM As shown in Fig. 4. Many amorphous gel-like grains of calcium phosphate randomly distributed on the surface, among which a few crystals formed. Although the crystals were not well-developed and not well-organized, we could index the diffractions in Fig. 4 as those of OCP $(\bar{2}40)$ and $(42\bar{2})$.

The biomimetic coating on differently pretreated 316L

After immersed in SCS for 12 h, EC pretreated 316L plates were covered by evenly distributed flake-like plate layer. However, only some island-like deposits were observed on the surface of AH pretreated 316L after 1 day's SCS immersion. With time increased, the number of islands on AH pretreated samples did not increase, but the thickness and area of the islands increased (shown in Fig. 5). Figure 5a showed the edge of an island. At the very edge of the island, there were some net-like structures that consisted of needle-like crystals. EDX spectrum (Fig. 5c) showed that its chemical composition was not calcium phosphate but chlorides. However, in other place of the island, the surface was covered by small grains (Fig. 5d) with the chemical composition of mainly calcium phosphate. After longer immersion, the islands grew thicker and the needle-like edge was replaced by grains with a larger size than that found after 1 day immersion. The XRD pattern of EC treated 316L after immersion for 12 h was shown in Fig. 6. Obviously, there was OCP in the coating. However, there was no such peak on AH-treated 316L after immersion in SCS even for 7 days. TEM analysis was conducted to identify the compositions of substances on AH pretreated samples after SCS immersion.

Large number of crystal flakes was observed on EC pretreated samples after immersion in SCS for 12 h and their morphology was shown in Fig. 7a. The diffraction of the crystal was indexed as OCP, B = [110]. However, different situations in AH pretreated samples after immersion in SCS for 7 days were found. There were some small crystals (shown in Fig. 7b) and the diffraction of them showed that they were OCP with zone axis B = [110]. However, no diffraction were observed from those gel-like grains. They were amorphous and EDX result showed that they were complexes of the substrate. As shown in Fig. 7c and d, the substances were gels of oxides of iron and chromium (in Fig. 7c), and gels with chemical composition of Ni, Fe, Ca, and O (in Fig. 7d). Such kind of gel came from AH pretreated substrate, and a lot of amorphous calcium phosphate clusters were also found. As we know, crystal growth begins after nucleation. As EC-treatment provided nuclei, crystals could form on them quickly. However, under the same saturated solution, AH-treated 316L could not support many calcium phosphate crystals to grow. We can conclude that AH-treatment is not effective to endow 316L with bioactivity to form calcium phosphate.

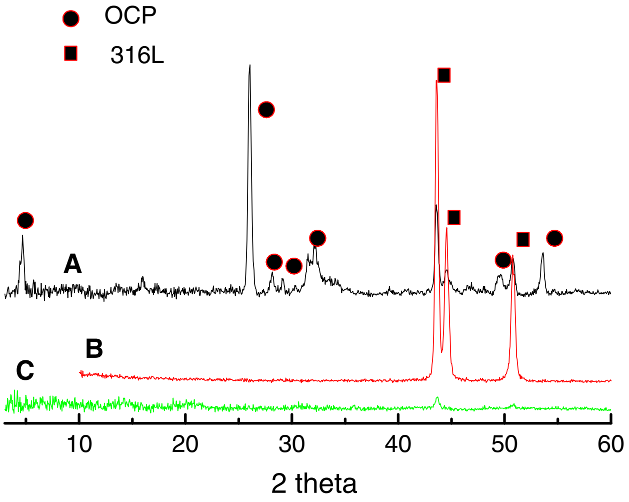


Fig. 6 The XRD pattern of AH and EC pretreated 316L after immersion in SCS. A: EC pretreated 316L after immersion in SCS for 12 h; B: untreated 316L; C: AH pretreated 316L after immersion in SCS for 7 days

The formation of a thin film by EC modification

The cathode was under constant potential and the reaction on cathodes was conducted continuously. However, the electric current decreased gradually to reach a stable state, as shown in Fig. 8. As electrolyte diffusion, there was a gradient distribution of OH^- from the cathode surface to the solution, and at the vicinity of cathode, the conditions were satisfied for the formation of calcium phosphate. Thus, a thin layer of calcium phosphate was formed on the cathode. We can deduce the process as the following: At the beginning, the hydroxyl accumulated on the surface of cathode; then, supersaturation of calcium phosphate was satisfied, which was the driving force to form calcium phosphate; after that, the quick formation of the calcium phosphate occurred because of the quick reaction of the electrode, which resulted in the amorphous calcium phosphate film [27]. The difference of EC pretreatment from the commonly used electrochemical deposition of calcium phosphate is that the electrolyte is maintained at a high concentration. Therefore, the reaction rate maintains at a high level, and the time needed for surface modification is short. If long period was applied, precipitation in the solution will occur and the precipitates will not adhere to the substrate.

The comparison of AH-treatment and EC-treatment

Lin et al. [21] claimed that a calcium phosphate layer would form on the surface of 316L when 316L was immersed for 4 weeks in 1.5 times SBF solution at 37 °C after AH treatment. The mechanism of the formation of

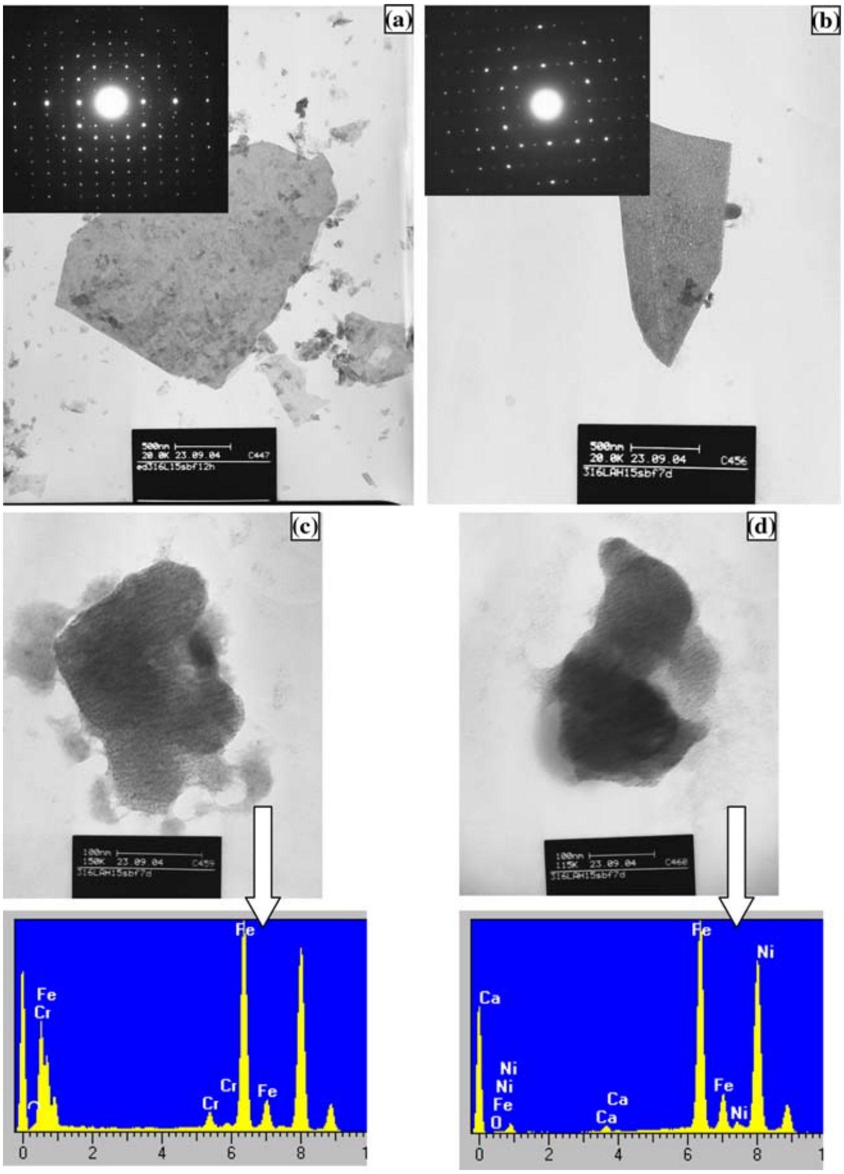


Fig. 7 TEM pictures of EC and AH treated 316L after immersion in SCS. (**a**) EC pretreated 316L after immersion in SCS for 12 h, B = [110]; (**b**) AH pretreated 316L after immersion in SCS for 7 days, B = [110]; (**c**) other phase in AH pretreated 316L in SCS for 7 days; (**d**) other phase in AH pretreated 316L in SCS for 7 days

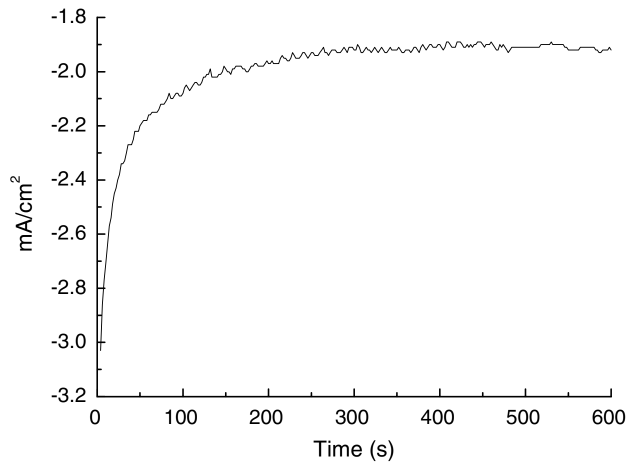


Fig. 8 The representative curve of cathodic modification

calcium phosphate was the formation of the Na_2CrO_4 on the surface and ions exchange when immersed in SBF solution. The mechanism of calcium phosphate formation on AH pretreated 316L revealed in our study is different from Lin's hypothesis, which may be described as follows: after immersion in the SCS solution, some of the chloride will deposit on the oxide layer because chloride is easy to be polarized to form complexes. The corrosion by chloride was the first step to form calcium phosphate. The morphology of chloride is needle-like, and then calcium phosphate deposit on it. When chloride replaced some of the oxygen, the charge on the surface changed. As a consequence, calcium and phosphate ions were adsorbed on the surface to form a new layer. The mechanism of calcium phosphate formation on EC pretreated 316L is different. After EC pretreatment, a film of calcium phosphate formed on the stainless steel surface. When EC treated specimens were immersed in SCS solution, the films formed in EC treatment was beneficial to a quick OCP layer growth. Because EC modified stainless steel surface was rich in calcium phosphate and nucleation sites, the nuclei grew to form calcium phosphate layer under saturated solution. The composition of the layer is OCP, which is mainly controlled by thermodynamic conditions [29].

Conclusions

This work demonstrated that EC treatmented was an effective way to pretreat 316L stainless steel for biomimetic coating deposition. EC treatment generates an amorphous calcium phosphate film with thickness of about 150 nm on the stainless steel surface. The film, containing a large number of OCP nuclei, is the main factor that causes biomimetic Ca–P coating formation.

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