Operating parameters effect on physico-chemical characteristics of nanocrystallineapatite coatings electrodeposited on 316L stainless steel

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Abstract
Hydroxyapatite (HAp) was known as a bone implant material due to its biocompatibility, biodegradable, chemical stability and its compositional similarity to natural bone. In this work nanocrystalline HAp coatings were prepared on 316L stainless steel (316LSS) substrates using a potentio-dynamic method (potential scanning in the range from 0 to −1.6 V/SCE) in the presence of dissolved 3 × 10−2 M Ca(NO3)2 + 1.8 × 10−2 M NH4H2PO4 + 0.15 M NaNO3 and 6% H2O2 (w/w). We report the influence of experimental conditions such as temperature (25 °C–60 °C), scanning rate (1 mV s−1–10 mV s−1) and scanning times (1 times–7 times) on the morphology, structure and composition of the HAp coatings by FTIR, XRD and SEM analysis. The results show that the morphology and purity of the HAp coating were greatly affected by temperature, scanning rate and reaction time with rate of 5 mV s−1, reaction time of 26.67 min (corresponding 5 scanning times) and 25 °C, giving better coatings. The in vivo test results after 3 months grafting on femur of dogs of HAp/316LSS material showed that: the material did not induce any osteitis, osteomyelitis or structural abnormalities. The osteitis and osteomyelitis were not observed in microscopy images.

Keywords: 316L stainless steel (316LSS), scanning potential, electrodeposition, nanocrystallineapatite coating, in vivo
Classification numbers: 2.03, 2.05, 4.03, 5.08
1. Introduction

Austenitic 316LSS, also referred to as surgical stainless steel, is an alloy of iron, chromium, nickel, and molybdenum that exhibits relatively good strength and corrosion resistance. 316L stainless steel is therefore a common choice of material for implants that are widely used in the field of orthopedic and dental prostheses, due to significant advantages especially in terms of mechanical properties sustaining stresses occurring in the body, as well as good biocompatibility [1]. Despite lower strength per weight than titanium alloy (TA6V), 316LSS is preferred to TA6V alloy because of its low production costs. But in some cases, 316LSS is prone to corrode in body environment and corrosion products are claimed to be toxic to osteogenic cells, affecting their proliferation and differentiation stages. Moreover, some of the elements constituting the 316L alloy, such as nickel for instance are known to be allergenic. Finally, those implants cannot form strong chemical bonds with natural bony matter and eventually weaken the surrounding bone, causing failure at the bone/implant interface. To overcome all these disadvantages, dedicated research was focused on ways to increase the durability and osteointegration of 316LSS implant materials by coating approaches involving osteoconductive biomaterials such as ceramics. Among them, hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂, noted HAp) and even more notably non-stoichiometric nanocrystalline apatitic systems (biomimetic apatites, which will be denoted as ‘BAp’ in the following) are noteworthy bioactive materials. The latter have excellent properties such as a high ability to induce bone tissue growth (osteoadaptation and osteoinduction) and an intrinsic biocompatibility. After implantation, BAp can form strong chemical bonds with natural bone and promote new bone formation/development because of its similar chemical composition, (micro) structure, and crystallographic structure to bone mineral [2, 3]. In addition, BAp coating provides protection to the titanium substrate against corrosion in the biological environment, and acts as a barrier against the release of metal ions from the substrate into the surrounding environment [4]. Additionally, BAp coating can enhance bone growth across a gap of 1 mm between the implant and the peripheral bone tissue; it is also capable of limiting the formation of fibrous tissue and/or of converting a motion-induced fibrous membrane into a bony anchorage [5].

Currently, most commercial coatings are made from stoichiometric HAp powders using the plasma spray technique. However, this process has several drawbacks including high temperature treatment of the HAp precursor and exposure of the substrates to intense heat flux (both limiting the use of metastable, hydrated compounds such as biomimetic apatites), residual thermal stresses within the coatings, and the inability to coat complex lattice shapes [6, 7]. In contrast, HAp coatings have been produced implementing low temperature wet processes: immersion coating, sol-gel and electrodeposition that has recently attracted considerable attention due to both its ability to deposit on non-line-of-sight, porous or complex shapes of substrates and its availability and low cost of the equipment [6, 8–10]. Furthermore, as electrodeposited coatings are expected to be composed of biomimetic apatite they should exhibit adapted and interesting mechanical and biological properties and possibly improved bond strength as compared to HAp plasma sprayed coatings. However, the disadvantage of the electrodeposition process is related to the accumulation of H₂ bubbles at the cathode surface that could affect the coating adhesion. To limit this problem, the potential scanning method was implemented successfully in a previous work and is still explored in the present study [3].

When envisioning the preparation of HAp coatings by electrochemical methods, several factors have to be considered, including solution composition, pH, deposition temperature, applied potential or current density. These operating parameters indeed affect the purity, crystallinity, stoichiometry, morphology and mechanical strength of the resulting coatings [3, 11–15].

*In vitro* bioactivity of HAp coating was evaluated by immersing in the simulated body fluids (SBF) [2, 3]. Validity of the material can be best observed under *in vivo* conditions after its implantation in an organism. These results showed that after 14 d implanted in femur bone of dog, the HAp coating had higher bone apposition ratios than those exhibited by bare Ti alloy and resulted in bone-like tissue with the characteristic templating of self-assembled collagen fibrils by HAp platelets [6].

In this context, this study focuses more specifically on how the electrochemical experimental conditions may affect the phase composition, stoichiometry, and morphology of the coatings. We discuss the electrodeposition of calcium phosphates on 316L stainless steel substrates under different conditions, especially in terms of temperature, scanning rate and electrodeposition time. The apatite coatings are characterized by several complementary techniques including: x-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR).

2. Materials and methods

2.1. Materials and surface preparation

Electrodeposition was carried out in this study on commercial 316LSS disks (Goodfellow France, diameter 16 mm
(±0.5 mm), thickness 2 mm, working area ~2 cm²) used as working electrodes. The substrates were subjected to a mechanical surface treatment before deposition: degreasing by immersion in ethanol and acetone followed by rinsing with deionized water; polishing with P600 up to P1200 SiC grit papers; and rinsing in tap water and in deionized water. All these immersions were made at room temperature, without stirring, and the samples were dried with compressed air between each treatment. The average roughness of the substrates after the surface preparation was measured around 21 µm ± 2 µm. Prior to the deposition tests, a silicone resin was used to cover the non-working surfaces.

2.2. Electrodeposition of calcium phosphate coating

All chemicals were purchased from Merck with an analytical reagent grade and used without any further purification. The electrolyte containing $3 \times 10^{-2}$ M Ca(NO₃)₂ and $1.8 \times 10^{-2}$ M NH₄H₂PO₄ was formulated so that the Ca/P molar ratio was kept constant: the value of ca. 1.67, equal to that in the stoichiometric composition of hydroxyapatite was selected here. 0.15 M NaNО₃ was also added in order to increase the ionic strength of the electrolyte and for potentially exploiting the electrochemical reduction of NO₃⁻ ions which contributes to generate OH⁻ [11, 16]. The initial pH of the electrolyte was set to 4.5. Hydrogen peroxide (H₂O₂) with a concentration of 6% (w/w) was also added to the electrolyte so as to decrease acidity and to provide an alternative electrochemical source of hydroxide ions at the substrate (cathode) surface following the equations [12, 17]:

\[
\begin{align*}
\text{H}_2\text{O}_2 + 2e^- + 2\text{H}^+ &\rightarrow 2\text{H}_2\text{O}, \\
\text{H}_2\text{O}_2 + 2e^- &\rightarrow 2\text{OH}^-.
\end{align*}
\]

Indeed, an increase in the amount of OH⁻ ions formed at the vicinity of the cathode can favor the chemical reactions leading to the formation of calcium phosphates. Therefore, the
formation of $H_2$ is expected to be minimized, and the adhesion of the coating should be certainly improved [18, 19].

The electrodeposition was carried out on Solatron 1279 (Ammeter, USA) with scanning potentials ranging from 0 to $-1.6$ V/SCE, scanning rates from 1 to 10 mV s$^{-1}$ with scanning times from 1 to 7 times. The reaction temperature was accurately controlled by a regulated (thermostat model NNT-2400, Eyel) water bath in the range 25 to 60 °C. A Pt grid was used as counter electrode (anode) and a saturated calomel electrode was used as reference electrode. The electrodes were cleaned before each experiment using distilled water. After deposition, the specimens were rinsed with distilled water to remove residual electrolyte, then dried at room temperature for 24 h for further analysis.

2.3. Characterizations of the coatings

The phase purity and crystallinity of the calcium phosphate coatings deposited on 316LSS were analyzed by x-ray diffraction (Bruker D8 and Bruker D5005 equipments, Cu-K$\alpha$ radiation ($\lambda = 1.54056 \text{ Å}$), with the following parameters:

- Step angle of 0.030°, scanning rate of 0.042 85° s$^{-1}$, and $2\theta$ in a range of 10–60°.

The coating microstructure/morphology was characterized by FESEM using Hitachi S-4800 equipment. Fourier transform infrared (FTIR) spectra were recorded in the range of 4000–400 cm$^{-1}$, with a resolution of 4 cm$^{-1}$ with a Nicolet 5700 spectrometer, using the KBr pellet technique. The spectra were the sum of 64 scans. All measurements were performed at room temperature.

2.4. Test in vivo

2.4.1. Surgery. HAp/316LSS samples were implanted into femur of dog for 3 months, each weighing about 10kg. Animal protocols followed were those specifically approved by the institutional animal care at the 103 hospital, Vietnam Military Medical University.

2.4.2. Hematological indices analysis method. Hematological indices were analyzed by using Swelab Alpha Hematology analyzers, airline Swelab, Sweden.
Three months after surgery, the dog was killed by injection t/m Ketamine with high-dose (2 mg kg\(^{-1}\)). Removing 316LSS material then sawing bone close to HAp area and bones were preserved in 10% formalin solution for 5–7 d.

The bone was removed calcium, paraffin casting by special molds and cut slices about 5 mm on mower Microtom Reichert (Austria) and placed onto a glass slide as a template. Then it was removed paraffin before microscopic image analysis.

**Figure 6.** SEM images of HAp formed on 316LSS in solution: \(3 \times 10^{-2} \text{ M Ca(NO}_3\text{)}_2 + 1.8 \times 10^{-2} \text{ M NH}_4\text{H}_2\text{PO}_4 + 0.15 \text{ M NaNO}_3 + 6\% \text{ H}_2\text{O}_2 \text{ (w/w)}, \text{pH} = 4.5, 25^\circ \text{C} \) at various scanning rate: (a) 1 mV s\(^{-1}\), (b) 2 mV s\(^{-1}\), (c) 3 mV s\(^{-1}\), (d) 4 mV s\(^{-1}\), (e) 5 mV s\(^{-1}\), (f) 6 mV s\(^{-1}\), (g) 7 mV s\(^{-1}\) and (h) 10 mV s\(^{-1}\).
Microscope images were analyzed on an optical microscope Olympus IX 70 (Japan) that is mounted the camera and micro-eyepiece with the magnification to 800 times.

3. Results and discussion

This study focuses on the effects of various deposition conditions on the characteristics of the coatings, with the objective to deposit nanocrystalline apatites. The following sections address successively the influence of the main experimental settings.

3.1. Effect of deposition temperature

The deposition temperature is an important factor that could affect the morphology, the phase structure and the crystallinity of the obtained coatings. To investigate the role of temperature in the calcium phosphate coating formation process, the electrochemical deposition was performed at scanning potentials ranging from 0 to $-1.6 \, \text{V/SCE}$ for a series of temperatures, i.e. 25 $^\circ \text{C}$, 37 $^\circ \text{C}$, 50 $^\circ \text{C}$ and 60 $^\circ \text{C}$, during 5 scanning times with a scanning rate of 5 mV s$^{-1}$. The achieved coatings were then characterized by FTIR, XRD and SEM.

The cathodic polarization curves of 316LSS in the electrolytic solution containing dissolved Ca(NO$_3$)$_2$, NH$_4$H$_2$PO$_4$, NaNO$_3$ and 6% H$_2$O$_2$ (w/w), pH = 4.5, 25 $^\circ \text{C}$ at various scanning rate: 1 mV s$^{-1}$, 2 mV s$^{-1}$, 3 mV s$^{-1}$, 4 mV s$^{-1}$, 5 mV s$^{-1}$, 6 mV s$^{-1}$, 7 mV s$^{-1}$ and 10 mV s$^{-1}$.

Microscope images were analyzed on an optical microscope Olympus IX 70 (Japan) that is mounted the camera and micro-eyepiece with the magnification to 800 times.

Stage 1: reduction of H$_2$O$_2$ (equations (1) and (2)) and oxygen (equation (3)) (a long plateau extending from $-0.4 \, \text{V to } -1\text{V}$)

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-. \tag{3}$$

Stage 2: reduction of H$_2$O (equation (4)) or H$^+$ (equation (5) for acidic pH) ($-1.0 \, \text{to } -1.6 \, \text{V}$)

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^-, \tag{4}$$

$$2H^+ + 2e^- \rightarrow H_2. \tag{5}$$

As the solution is becoming basic, H$_3$PO$_4$ initially present because of the acidic pH of the solution (pH ~ 5) are changing into HPO$_4^{2-}$ ions for pH between 7.2 and 11.8 and then for pH over 11.8 PO$_4^{3-}$ ions are the majority, which is favorable to produce stoichiometric apatite.

At temperatures over 50 $^\circ \text{C}$, calcium phosphate powders appeared in the bulk solution of the electrochemical cell. At these temperatures, the mobility of the ionic species in the electrolyte and the reaction rate to form apatite increased noticeably, as evidenced by the clear rise in current density. Therefore, the rapidly formed precipitated apatite crystals did not have enough time to first well adhere to the substrate and then to the growing coating. As the adhesion of apatite grains on top of the others was poor, strikethrough particles were released in the solution.

The temperature has not only an effect on the reaction rate as well as on the diffusion rate of ions which can promote the synthesis of calcium phosphate crystals either as a film on the surface or bulk precipitates but it can also limit their solubility. Finally, the rise of temperature can lower hydrogen bubble attachment on the substrate surface, making the growing apatite films less damaged and more adherent. In summary, by adjusting the temperature, the whole deposition process,

![Figure 7. XRD patterns of HAp formed on 316LSS in solution: $3 \times 10^{-2} \, \text{M Ca(NO}_3\text{)}_2 + 1.8 \times 10^{-2} \, \text{M NH}_4\text{H}_2\text{PO}_4 + 0.15 \, \text{M NaNO}_3 + 6\% \, \text{H}_2\text{O}_2 (w/w), \, \text{pH} = 4.5, \, 25 \, \text{°C at various scanning rate: 1 mV s}^{-1}, \, 2 \, \text{mV s}^{-1}, \, 3 \, \text{mV s}^{-1}, \, 4 \, \text{mV s}^{-1}, \, 5 \, \text{mV s}^{-1}, \, 6 \, \text{mV s}^{-1}, \, 7 \, \text{mV s}^{-1}$ and 10 mV s$^{-1}$.

![Figure 8. FTIR spectra of HAp coatings deposited in solution: $3 \times 10^{-2} \, \text{M Ca(NO}_3\text{)}_2 + 1.8 \times 10^{-2} \, \text{M NH}_4\text{H}_2\text{PO}_4 + 0.15 \, \text{M NaNO}_3 + 6\% \, \text{H}_2\text{O}_2 (w/w), \, \text{pH} = 4.5, \, 25 \, \text{°C at various scanning rate: 1 mV s}^{-1}, \, 2 \, \text{mV s}^{-1}, \, 3 \, \text{mV s}^{-1}, \, 4 \, \text{mV s}^{-1}, \, 5 \, \text{mV s}^{-1}, \, 6 \, \text{mV s}^{-1}, \, 7 \, \text{mV s}^{-1}$ and 10 mV s$^{-1}$.](image-url)
i.e. mass and thickness of apatite coatings can be controlled as already noticed [3, 20].

The effect of the electrodeposition temperature on the morphology of the calcium phosphate coatings was displayed in figure 2. At all temperatures, coating surface morphology seems to be constituted of fine needles and plates organized in a coral-like structure covered by flaky agglomerates of various sizes which are well adapted for cell proliferation [21]. The network of fine needles and plates covers the entire substrate surface but some cracks are present at deposition temperature of 37 °C, 50 °C and 60 °C (figure 2). At 60 °C, the crack is larger and a part of the coating is detached (figure 2(d)).

Figure 9. SEM images of Ca–P formed on 316LSS in solution: $3 \times 10^{-2}$ M Ca(NO$_3$)$_2$ + $1.8 \times 10^{-2}$ M NH$_4$H$_2$PO$_4$ + 0.15 M NaNO$_3$ + 6% H$_2$O$_2$ (w/w), pH = 4.5, 25 °C, 5 mV s$^{-1}$ with various times: (a) 1, (b) 2, (c) 3, (d) 4, (e) 5, (f) 6 and (g) 7 scanning times.
This is entirely consistent with the observed phenomena during the electrodeposition process.

The XRD patterns of the electrodeposited coatings at different temperatures are shown in figure 3. It is clearly evidenced that all coatings exhibit the same crystallographic structure and are poorly crystallized. These coatings patterns have peaks that can be attributed to a poorly crystallized hydroxyapatite phase at 2θ ≈ 26° and 32° corresponding to (002) and (2 1 1) planes [22]. The other peaks at 2θ ≈ 44°, 45° and 51° were attributed to 316LSS substrate and its oxides [3, 23]. The peak observation related to 316LSS substrate in all these XRD patterns implies that the coatings are quite thin.

FTIR spectra of coatings were recorded in wavenumber range 4000–400 cm\(^{-1}\) to investigate the effect of temperature (figure 4). The spectra all indicated that coating material is a moderately-crystallized calcium phosphate apatite [24]. In particular the asymmetric stretching vibration of P–O bond was characterized by a band located at 1040 cm\(^{-1}\) (\(\nu_3\)(PO\(_4\)) mode). The \(\nu_4\)(PO\(_4\)) asymmetric O–P–O bending mode was also noticeable (by way of two maximum around 611 cm\(^{-1}\) and 564 cm\(^{-1}\)). Absorption at 3572 cm\(^{-1}\) and 632 cm\(^{-1}\) usually assigned to the O–H stretching vibration in hydroxyapatite were not clearly distinguished on these spectra. This can be explained by the nonstoichiometry of the apatite phase, with a deficiency in calcium and hydroxide groups. Water bands were visible by a large band in the range 3000–3600 cm\(^{-1}\) (O–H stretching from water molecules) and by the H–O–H bending band at 1646 cm\(^{-1}\). The association of water molecules with apatite is not surprising, especially for nonstoichiometric apatite which exhibit a hydrated layer on the surface of their constitutive crystals, the extent of which is dependent on synthesis conditions [25]. IR spectra also indicate the presence of some hydroxides which are available and have time to react) that leads to the apatite formation. During the electrodeposition process, it was also observed that apatite powder forming in the solution around 25 °C for the different scanning rates, \(E_{\text{corr}}\) and \(i_{\text{corr}}\) have been extracted and are shown in figure 5. For a fixed number of scanning times and whatever this number (from 1 to 5), \(E_{\text{corr}}\) and \(i_{\text{corr}}\) are decreasing while the scan rate increases from 1 to 10 mV s\(^{-1}\). This trend could be related to the reaction time that is increased with the scan rate which favors both the 316LSS corrosion because of the negative potentials applied during scanning and the precipitation reaction (more OH\(^{-}\) are available and have time to react) that leads to the apatite formation. During the electrodeposition process, it was also observed that apatite powder forming in the solution around the working electrode is promoted by the decrease of the scanning rate, which is consistent with the working duration.

Observations of the synthesized apatite coatings were performed by SEM (figure 6). All coatings which were electrodeposited at the scanning rate of 1, 2, 3, 4, 5, 6, 7 and 10 mV s\(^{-1}\) have coarse granular agglomerates on their surfaces with relatively high surface roughness [27]. Beside, all coatings have a coral-like structure but they are organized by different HAp crystals. HAp crystals are needle or rod shape with scanning rate of 1, 2, 3, 4, and 5 mV s\(^{-1}\) (figures 6(a)–(e)). The scanning rate increases of 6 or 7 mV s\(^{-1}\), it is plate shape (figures 6(f) and (g)) and its shape changes spherical with scanning rate of 10 mV s\(^{-1}\) (figure 6(h)).

The calcium phosphate phases deposited at various scanning rates were analyzed by XRD (figure 7). Depending on the XRD device implemented, dicalcium phosphate dihydrate (CaHPO\(_4\)·2H\(_2\)O, DCPD or brushite) phase is also detected thanks to two peaks at 2θ ≈ 12° and 29° corresponding to (0 2 0) and (1 4 1) planes [22], because of the orientation of the main axis of the crystals parallel to the substrate surface. It could be hypothesized that DCPD is the first CaP compound that is precipitating on the surface because HPO\(_4^{2-}\) ions are available due to local pH conditions. But at neutral and

![Figure 10. XRD patterns of HAp formed on 316LSS in solution: 3 × 10\(^{-2}\) M Ca(NO\(_3\))\(_2\) + 1.8 × 10\(^{-2}\) M NH\(_4\)H\(_2\)PO\(_4\) + 0.15 M NaNO\(_3\) + 6% H\(_2\)O\(_2\) (w/w), pH = 4.5, 25 °C, 5 mV s\(^{-1}\) with various times: 1, 2, 3, 4, 5, 6 and 7 scanning times.](image-url)
basic pH, DCPD is also exhibiting a high solubility (where \( K_{sp} \) is solubility product constant with \( -\log K_{sp} = 6.59 \) at 25 °C) in contrast to HAp (\( -\log K_{sp} = 116.8 \) at 25 °C) what is therefore constituting a driving force for HAp precipitation. Poorly crystallized apatite was the major phase with DCPD as minor structure of the coating deposited at the scanning rate of 1 mV s\(^{-1}\). The peaks intensity attributed to substrate at \( 2\theta \approx 45^\circ \) and 51° were very low. Hence, a quite thick covering Ca-P coating was deposited at this rate. The peaks intensities of DCPD and those related to the substrate compared to the one of HAp increased with the scan rate (patterns recorded at 5 and 6 mV s\(^{-1}\) are discarded because arising from a different XRD device). This result indicates that the coating is probably becoming thinner with the scan rate increases and therefore this emphasizes the response of the surface phases (especially those from the substrate). Besides, it has to be noted that DCPD crystals could be highly oriented, which further emphasizes the dependency between HAp and DCPD XRD responses.

The FTIR spectra of HAp coatings synthesized at different scanning rates presented in figure 8 have similar shapes to the ones previously shown. They exhibit bands that can specifically be attributed to poorly crystallized non stoichiometric apatite. No distinguishable evolution of the chemical structure was noticed with the scan rate.

### 3.3. Influence of scanning times number

The reaction time is also an important parameter that could affect drastically the deposited coatings. By varying the number of scanning times from 1 to 5 and the scanning rate from 1 to 10 mV s\(^{-1}\) while keeping constant the scanning potential range from 0 to –1.6 V/SCE, the overall coating duration was varied between 160 s and 8000 s. It is clearly evidenced in figure 5 that after 3 scanning times whatever the scanning rate \( E_{corr} \) and \( k_{corr} \) are stabilizing. This could suggest that a covering coating was synthesized that protects the metallic substrate from corrosion. Theapatite coatings were then characterized by SEM and XRD.

Figure 9 shows the surface morphology of electodeposited Ca–P coatings at 25 °C with a scanning rate of 5 mV s\(^{-1}\) at different time as revealed by SEM. The apatite crystals have spherical shape with the small size and uniformity after 1 scanning times (figure 9(a)) and the large size and heterogeneity after 2 scanning times (figure 9(b)). With 3, 4 and 5 scanning times, HAp is fine needles organized in a coral-like structure (figures 9(c)–(e)). If the scanning times increase to 6 or 7 scans, HAp will have plate-like shape organized in a coral-like structure (figures 9(f) and (g)).

| Time after surgery \ (months) | Hematological indices | One month after surgery (1) | Two months after surgery (2) | Three months after surgery (3) | \( p_{1-3}, p_{2-3} \) |
|---------------------------|------------------------|----------------------------|-----------------------------|-------------------------------|-------------------|
| Red blood cell (RBC) (Tg l\(^{-1}\)) | 5.57 ± 0.73            | 5.69 ± 0.56               | 5.78 ± 0.65                 | >0.05                         |
| Hg (g dl\(^{-1}\))         | 15.67 ± 0.64           | 15.75 ± 0.82              | 16.12 ± 0.79                | >0.05                         |
| White blood cell (WBC) (Gg l\(^{-1}\)) | 9.46 ± 2.23         | 9.24 ± 2.14               | 9.47 ± 2.49                 | >0.05                         |
| Platelet (Gg l\(^{-1}\))   | 264.36 ± 64.27         | 247.36 ± 72.27            | 258.27 ± 81.34              | >0.05                         |

| Hematological indices | Count (Gg l\(^{-1}\)) | Percentage (%) |
|----------------------|----------------------|----------------|
| Neutrophils          | 5.67 ± 1.42          | 58.43 ± 5.57 |
| Eosinophils          | 0.76 ± 0.14          | 7.85 ± 1.03  |
| Basophils            | 0.03 ± 0.006         | 0.31 ± 0.02  |
| Monocytes            | 0.72 ± 0.16          | 7.42 ± 1.72  |
| Lymphocytes          | 2.52 ± 0.42          | 25.97 ± 3.69 |

The XRD results are presented in figure 10 and indicate that after 2 scanning times, the peaks attributed to 316LSS substrate oxides are clearly identified as well as the main phase of the coating is DCPD and almost no HAp characteristic peaks are evidenced. After 4 scanning times, the HAp peaks intensity increases while the substrate peaks intensity decreases. DCPD is still present in the coating. As previously noticed, DCPD and HAp crystallographic structures are both produced in these operating conditions. DCPD seems to be localized on the top surface but this result should be confirmed by further analysis. It could favor the bioactivity of the coating because of high solubility.

### 3.4. Test in vivo

#### 3.4.1. Hematological parameter

Table 1 showed that at three months after surgery, the data of red blood cells, white blood cells, platelet counts and hemoglobin concentration were similar at one and two months after surgery. Thus, HAp/316LSS materials did not induce infection or affect to hematopoietic function after existed three months in dog’s femur. These indices were normal range and be consistent with some previous authors [28, 29].

Table 2 shows the count and percentage of WBC at three months after surgery similar at two months after surgery and they are equal to normal indices in healthy animal without surgery.

The data in normal range of neutrophils, monocytes, eosinophils and lymphocytes presented that HAp/316LSS materials did not induce chronic infection after three months existed in femur. Further, it did not stimulate allergic and toxicity reactions to the body, presented by no change in eosinophils and basophils. These results clearly demonstrate the biological compatibility of materials in the animal body for long periods.

#### 3.4.2. Microscope image in HAp/316LSS area after three months of surgery

HAp/316LSS is implanted and exist in dog’s femur with long time of 3 months. It can cause the existence of inflammatory cells around its area in femur bone,
such as neutrophil, macrophage, monocyte, eosinophil, basophil and periosteum often reacts such as thickness, roughness, flaking shelling in inflammatory area [30, 31]. Microscope images in figure 11 showed smooth periosteum and no periosteal inflammatory reaction. There is bone formation reaction, many osteoblasts, collagen fibers and thick-wall blood vessels around material area. It is similar to the process that occurs on bone healing normally, without complications. These results indicated, HAp/316LSS material had good biological compatibility with natural bone [32, 33].

4. Conclusion

Nanocrystallized non stoichiometric apatite coatings have been successfully electrodeposited on 316LSS surface by scanning potential method in a wide range of parameters. The effect of experimental conditions (temperature, potential scanning rate, scanning times number) was investigated. It was shown that temperature affects more the reaction yield than the coating structure that is mainly apatite. The electrochemical operating parameters such as the scanning rate and the scanning times number that both control the coating duration time have also an influence on the yield but apatite and DCPD are both always present in the coatings. The DCPD presence and localization have still to be fully elucidated but the scanning potential method is well adapted to produce biomimetic apatite coating with controlled thickness on 316LSS substrates. The study results of electrodeposited and in vivo test contribute to the selection of appropriate conditions to electrodeposit HAp coating on metallic substrates, with a highly reactive crystal structure of calcium phosphate that is suitable for bone implant applications.

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