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The Investigation of Corrosion Performance and Durability of Hydroxyapatite-Coated Titanium Implants and the Effect of Antibiotic Additives

Demet YAZICI[1], Nilgün ALPAY[2], Başak DOĞRU MERT[*3]

[1]*Adana City Training and Research Hospital, Ear Nose and Throat Department, Adana*
[2]*Çukurova Üniversitesi, The Faculty of Dentistry, Adana*
[3]*Adana Alparslan Turkes Science and Technology University, Engineering Faculty, Energy Systems Engineering Department, Adana*

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Abstract

In this study, amoxicillin and potassium clavulanate were loaded as antibiotic additives to hydroxyapatite coating (L-HAP) and were used to enhance biocompatibility and corrosion resistance of titanium (Ti) *in- vitro* conditions. Coating was achieved using the Successive Ionic Layer Adsorption and Reaction (SILAR) process. Scanning electron microscopy (SEM) images, energy dispersive X-ray (EDX) analysis, atomic force microscopy (AFM) images, X-ray diffraction (XRD) analysis, and attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) were used to determine the surface morphology. The corrosion test was performed using electrochemical impedance spectroscopy (EIS) and polarization curves in artificial saliva at 310 K. Furthermore the quantum chemical parameters of amoxicillin and potassium clavulanate were investigated and associated with the adsorption ability of these molecules. Results revealed that the corrosion performance of Ti was improved by L-HAP, which had a lower anodic current density and better corrosion resistance. This situation dealt with the more durable, compact film that had been produced on the surface.

Keywords: Artificial saliva, Biocompatibility, SILAR, Titanium

Hidroksiapatit Kaplı Titanyum İmplantların Korozyon Performansı ve Dayanıklılığının ve Antibiyotik Katkı Maddelerinin Etkisinin Araştırılması

Öz

Bu çalışmada, hidroksiapatit kaplamaya (L-HAP) antibiyotik katkı maddesi olarak ilave edilen amoksisilin ve potasyum klavulanat, *in-vitro* koşullarda titanyumun (Ti) biyouyumluluğunu ve korozyon direncini arttırmak için kullanılmıştır. Kaplama, Ardışık İyonik Katman Adsorpsiyon ve Reaksiyon (SILAR) yöntemi ile gerçekleştirildi. Yüzey morfolojisi, taramalı elektron mikroskobu (SEM), atomik kuvvet mikroskobu (AFM), enerji dağıtıcı X-ışını (EDX) analizi, X-ışını kırınımı (XRD) analizi, azaltılmış toplam yansıma-Fourier dönüşümlü kızılötesi spektroskopisi (ATR- FTIR) ile belirlendi. Korozyon testleri, elektrokimyasal impedans spektroskopisi (EIS) ve polarizasyon eğrileri yardımıyla 310 K'de yapay tükürük çözeltisinde elde edildi. Ayrıca amoksisilin ve potasyum klavulanatın kuantum kimyasal parametreleri araştırıldı ve bu moleküllerin adsorpsiyon yeteneği ile ilişkilendirildi. Sonuçlar, Ti'nin korozyon performansının, daha düşük anodik akım yoğunluğuna ve daha iyi korozyon direncine sahip olan L-HAP ile iyileştirildiğini ortaya koydu. Bu durum, yüzeyde üretilen daha dayanıklı, kompakt film ile ilişkilendirildi.

Anahtar Kelimeler: Yapay tükürük, Biyouyumluluk, SILAR, Titanyum

[*]Sorumlu (Corresponding author) yazar: Başak DOĞRU MERT, *bdogrumert@atu.edu.tr*

1. INTRODUCTION

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$ – HAP) and titanium (Ti) are well-known and commonly used biomaterials due to high biocompatibility and physical properties [1-9]. Especially in the bone reconstructive surgery and prosthetic treatments, surgeons prefer these materials because after surgical operation, Ca^{2+} and PO_4^{3-} ions may released and absorbed to gain repair of tissues. Furthermore titanium exhibits high corrosion resistance and it has crucial effect for human health. The corrosion happens when metals and its compounds react with their environment chemically or electrochemically and get degraded and damaged by forming oxides, hydroxyoxides and other compounds. More critically, these corrosion products have the ability to permeate tissues, causing damage to human cells. The fluids in human body consist of water, soluble oxygen, proteins and various ions like chloride, hydroxide and etc. For this reason, the human body is an extremely corrosive environment for the metals used as biomaterials [10,11]. This corrosive environment reduces the strength of metals. One of the solutions for preventing corrosion of these metals is coating these materials with corrosion-resistant coatings as hydroxyapatite [12-17]. Therefore several scientists have been studied in this field and very impressive applications were presented. Wang et al. developed an organ-like MXene-Ti_3C_2 material that was used to immobilize hemoglobin and design & create a mediator-free biosensor with an oxidized surface. [1]. Electrochemical deposition of hydroxyapatite on a pure Ti substrate was used to measure corrosion resistance in a simulated body fluid (Hank's balanced salt solution). The findings revealed that it is a biomaterial that is reasonably appropriate for bone implantation [2]. The Ag deposited HAP coatings were produced and applied on anodized Ti, due to enhance corrosion resistance of material [3]. Mirzaee et al. [3] declared that the sample with content of Ag (0.05 molar ratio) had high corrosion resistance and further showed good antimicrobial efficiency (almost 99% reduction in viable cells). In the study of Coskun et al. [4] bio-metallic CoCrMo alloys were coated with hydroxyapatite at various pH and corrosion performance of the layers was determined. Results showed that pH influenced the properties of layers (chemical composition and surface morphology) and at high pH, HAP coating exhibit more homogenous structures and high corrosion resistance [4]. Gopi et al. claimed that carbon nanotubes (CNT) enhance strength and toughness of HAP. The addition of 1% CNT to HAP enhanced the coating's corrosion resistance and biomechanical properties [18]. Usinskas et al. [19] applied calcium titanate sub-layers and produced HAP coating on Ti with the help of sol-gel method further they obtained pre-heating some samples. According to the results, the morphology of the HAP thin films was not effected by surface alteration of the Ti substrate, but, contact angle measurements revealed that raising the number of HAP layers from 20 to 30 resulted in hydrophilic activity. Consequently many researches [15-31] showed that the biocompatibility and corrosion properties of HAP coating on Ti biomaterials could enhanced but the main phenomenon was “accelerating healing process in treatment”. For this purpose antibiotic additives (amoxicillin and potassium clavulanate) were used while preparing HAP coatings. Because of the ease of the technique, the SILAR procedure was used for application. Then surface morphology and corrosion properties were clarified with SEM, EDX, AFM, XRD analysis and electrochemical techniques.

2. MATERIAL AND METHOD

2.1. The Production of L-HAP Films

The titanium (Ti) sheet (it is known that Ti is preferred in dentistry applications) with a thickness of 0.25 mm (99.7% purity), was purchased from Sigma–Aldrich. This Ti sheet was cut 1x1 cm. The amoxicillin (Figure 1a) and potassium clavulanate (Figure 1b) were purchased from Sigma–Aldrich. $CaCl_2$ and $Na_3PO_4.12H_2O$ were purchased from Merck. The Ti was cleaned using 0.1 M HCl, acetone and double distilled water for 1 min. each in ultrasonic bath. Then HAP films were deposited as mentioned below: Ti sheets (1x1 cm) were immersed into 0.1 M $CaCl_2$ and kept there for 30 s. Then immediately immersed into distilled water for 30 s and then immersed into 1 mg/mL amoxicillin + 1 mg/mL potassium clavulanate + 0.05 M $Na_3PO_4.12H_2O$ and kept there for another 30 s

after being taken out from the bath. This process is called to be one SILAR cycle. Due to get a reasonably thick film (almost 10 µm), the cycle has to be repeated 50 times at room temperature

2.2. Characterization of L-HAP Films

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were used to study the surface morphology of electrodes. A Carl Zeiss Evo 40 SEM was used to obtain the SEM images. The Park SYSTEMS instrument was used to obtain the AFM images. The chemical composition was determined by energy dispersive X-ray spectrometer (EDX) which is part of SEM device. ATR-FTIR spectroscopy was used to evaluate the chemical composition of L-HAP. The X-ray diffraction (XRD) pattern was recorded on a Bruker AXS D8 with CuKα radiation (λ = 0.15406 nm) in the 2θ range of 10–90°.

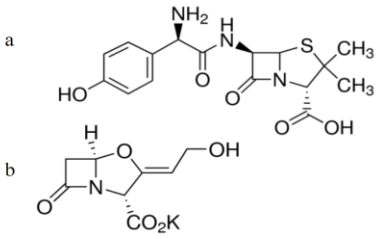

Figure 1. The molecular structure of amoxicillin (a) and potassium clavulanate (b)

2.3. Electrochemical Measurements

The corrosion tests were performed at 310 K, which was body temperature, open to the atmosphere, using a CHI 604D A.C. electrochemical analyzer (R0633). The counter electrode was a platinum sheet with a surface area of 2 cm^2 and the reference electrode was an Ag/AgCl (3 M KCl) electrode. The corrosion performance of electrodes were investigated in artificial saliva. The composition of artificial saliva was given below [11]: 0.4 g/L NaCl, 0.4 g/L KCl, 0.6 g/L $CaCl_2$, 0.54 g/L NaH_2PO_4 and 1 g/L urea (pH: 6.6). The EIS (electrochemical impedance spectroscopy) experiments were conducted in the frequency range with high limit of 100 kHz and low limit of 0.01 Hz. The amplitude was 0.005 V. The polarization curves were potentiodynamically obtained between 1.30 and 1.75 V with a scan rate of 1 mV s^{-1}.

3. RESULTS AND DISCUSSION

In Figure 2a, the SEM micrograph of L-HAP coated Ti surface is demonstrated. As seen in Fig. 2a, the electrode surface is nodular and narrow porous areas are seen between nodules. Usinskas et al. [19] present nodular views for HAP on Ti without initial pre-heating. According to Gopi et al. [25], the presence of pores on the HAP composite coating is advantageous for the initiation of bone formation by providing an excess of sites for osseous tissue growth. Rafieerad et al. [29] claimed that porous and nodular morphology enhances osteoblast adhesion, proliferation and accelerates the healing process and promotes bone mineralization. Therefore this new L-HAP coating should be suitable for surgical operations. Due to figure out contribution of antibiotics to HAP layer EDX analysis is present in Figure 2b. %16.8 C is proved that amoxicillin and potassium clavulanate loaded in HAP [25].

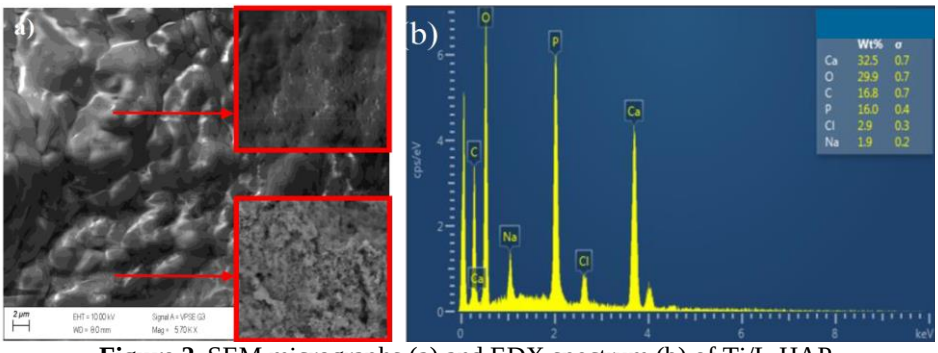

Figure 2. SEM micrographs (a) and EDX spectrum (b) of Ti/L-HAP

In Figure 3, the AFM results of L-HAP coated Ti surface is presented. 2D image (Figure 3a) correlated the SEM micrograph. The nodular structures and blackberry type shapes are seen in Figure 3b. According to section analysis (Figure 3c), the average surface porosity of L-HAP is almost 240 nm. The adhesion of coating increase with increasing roughness and porosity [32] therefore L-HAP coating may increase osteoblast adhesion.

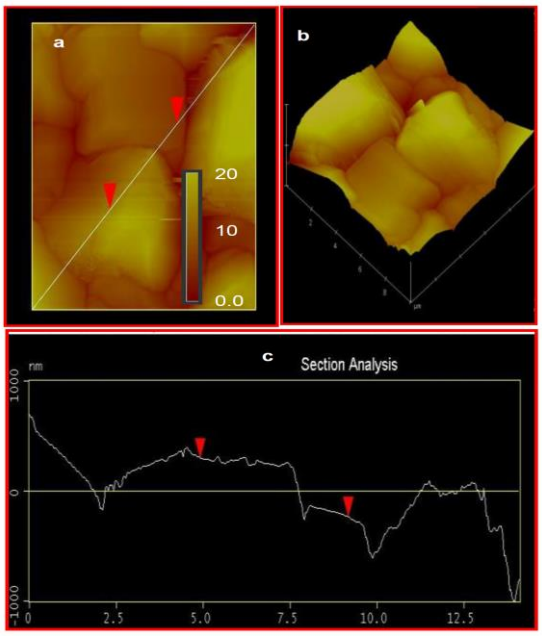

Figure 3. 2D (a), 3D (b) AFM images, and sectional analysis (c) of Ti/L-HAP (scan size: 10µmx10µm, scan rate: 1.001 Hz)

The ATR-FTIR spectrum of L-HAP is presented in Figure 4, PO_4^{3-} group of HAP is seen at 560.08 cm^{-1}, wagging mode of NH_2 of amoxicillin and υ_4 mode of PO_4^{3-} are obtained at 600 and 601.81 cm^{-1}. Jankovic et al. [17] presented almost same results for HAP and declared that vibrational bands at 601 and 560 cm^{-1}, corresponding to the υ_4 mode of PO_4^{3-} group. Indira et al. [31] enounced that υ_3 mode of PO_4^{3-} group is seen at 1030 cm^{-1} and in this study it is detected at 1021.10 cm^{-1}. Secondary amine peak of potassium clavulanate and amoxicillin is seen almost 1412.82 cm^{-1} and streching of N-H and O-H is obtained at 3378.86 cm^{-1}. Furthermore, υ aromatic (C-H) is seen almost 1100.50 cm^{-1} and bending mode of OH is obtained at 1650 cm^{-1}. The υ_3 and υ_4 mode of PO_4^{3-} group and O-H bond of carboxylic acid group is seen at 3735.07 cm^{-1} [33-35]. Consequently, Figure 4 proved that amoxicillin and potassium clavulanate loaded to hydroxyapatite coating.

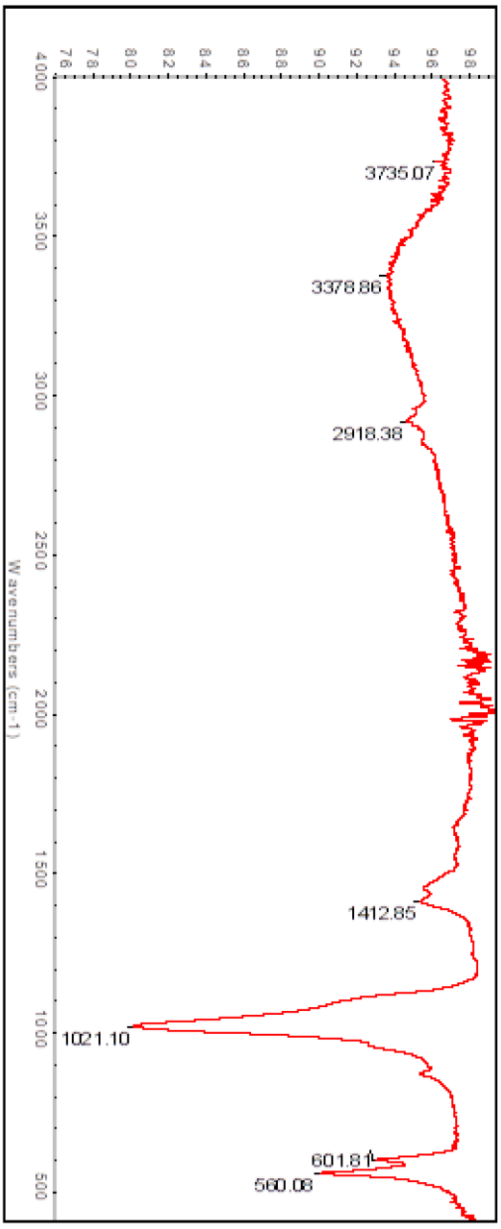

Figure 4. FTIR spectra of L-HAP

The XRD pattern of Ti/L-HAP is given in Figure 5. The peaks at 32.18°, 32.58° and 46.78° are assigned to (112), (300) and (222) planes of HAP, respectively. The peaks at 36.24°, 39.32° and 52.96° are assigned to (002), (101) and (102) phases of Ti, respectively [17,18,33,36,37]. High intensity of peaks reveals that L-HAP is obtained on Ti and results correlates the ATR-FTIR analysis.

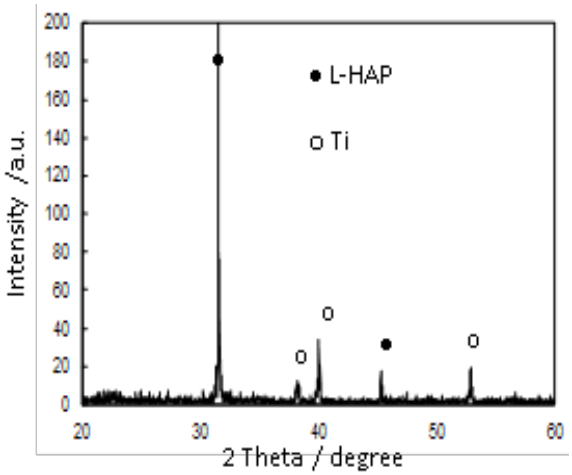

Figure 5. XRD pattern of Ti/L-HAP

The Nyquist plots of Ti and Ti/L-HAP, after 2 h immersion time in artificial saliva at 310 K, are presented in Figure 6. The semi-circular shape with a diameter of $1.2x10^4$ / Ω cm^2 is seen for Ti which is attributed to charge transfer controlled corrosion reaction [38,39]. The maximum phase angle is 70.3° (Figure 7). The EIS result of electrode has significantly changed by L-HAP coating. In Figure 5, for Ti/L-HAP, non-closed curve is seen. In the high frequency region (at 100 Hz), the resistance is almost 126 Ω cm^2 and this region is attributed to corrosion process occurring within pores of L-HAP layer. The resistant and phase angle at 0.01 Hz are almost $1.8x10^4$/Ω cm^2 and 75.4° (Figure 7), respectively and this region is attributed to the L-HAP film resistance. According to EIS results of these electrodes, L-HAP has protective effect against corrosion in artificial saliva at 310 K. Due to further insight, the polarization curves are presented in Figure 8.

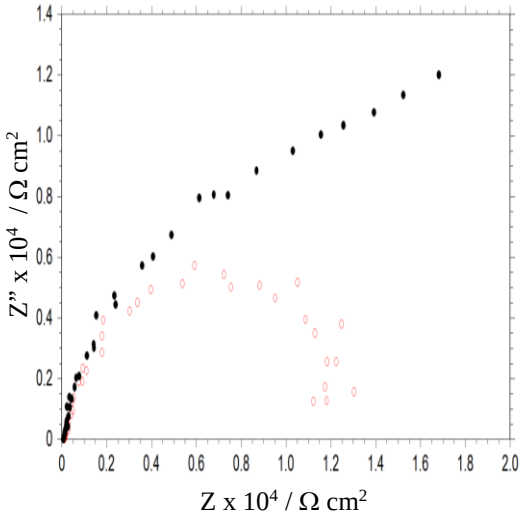

Figure 6. The Nyquist plots of Ti (o) and Ti/L-HAP (•) after 2 h immersion time in artificial saliva at 310 K

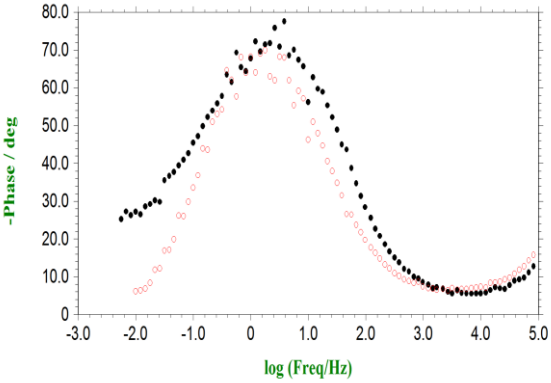

Figure 7. The log freq-phase deg. plots of Ti (○) and Ti/L-HAP (•) after 2 h immersion time in artificial saliva at 310 K

In Figure 8, the corrosion potentials (Ecorr) of Ti and Ti/L-HAP are 1.43 and 1.64 V (vs. Ag/AgCl), respectively. The nobler (more positive) Ecorr reflects high corrosion protection [40]. Furthermore lower cathodic and anodic current density are seen for Ti/L-HAP and it correlates with EIS measurements. As Radha said that [41], the electroless hydroxyapatite coating technique is most effective and economical way of coating metal . Consequently, results show that L-HAP protect Ti against corrosion effectively.

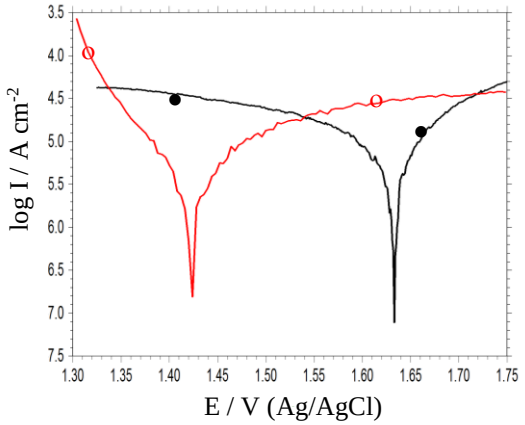

Figure 8. The polarization curves of Ti (○) and Ti/L-HAP (•) after 2 h immersion time in artificial saliva at 310 K

4. CONCLUSION

The antibiotic loaded hydroxyapatite coating (L-HAP) was produced by Successive Ionic Layer Adsorption and Reaction (SILAR) method and corrosion performance was investigated *in-vitro* conditions. The ATR-FTIR, EDX and XRD results showed that, HAP coating was produced as a $Ca_{10}(PO_4)_6(OH)_2$ chemical form and amoxicillin and potassium clavulanate penetrate the interior of HAP. The scanning electron microscopy (SEM) and atomic force microscopy (AFM) results exhibited rough surface, which was beneficial for osteoblast adhesion, proliferation. The electrochemical measurements supported that L-HAP is a convenient coating against corrosion in artificial saliva at body temperature. Especially the E_{corr} of Ti/L-HAP was denoted a high level of corrosion resistance with the value of 1.64 V but the E_{corr} of bare sample was 1.43 V. For Ti/L-HAP, the resistance which was determined with the help of electrochemical impedance spectroscopy, at 0.01 Hz was $1.8x10^4/\Omega$ cm^2, indicated high corrosion resistance.

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