

The Electrodeposition of Strontium-Hydroxyapatite (Sr-HA) on TiO₂ nanotubes layer fabricated by anodizing of Ti metal and Ti-6Al-4V Alloy to increase the bonding with bones

The 5th International Scientific Conference for Nanotechnology and Advanced Materials and Their Applications (ICNAMA 2015) 3-4 Nov.2015

Aqeel Faleeh Hasan

Ministry of Science and Technology

Email: aqeelhasan65@gmail.com

Dr. Abdul Husain Kudhair Ltaief

Ministry of Science and Technology

Jamal Fadhil Hamodi

Ministry of Science and Technology

Abstract

In this research the bond of strontium hydroxyapatite that electrochemical deposited on TiO₂ nanotube with biomimetically hydroxyapatite formed was in vitro investigated. The TiO₂ nanotube synthesised by anodizing titanium metal and Ti-6Al-4V alloy using special electrochemical cell prepared for this purpose, the solution that used consisted from 70% vol. glycerine, 30% vol. water and 1.5 % wt. NH₄F. Different concentrations of strontium hydroxyapatite (0,50,100)% were electrochemical deposited by using solution consist of Ca(NO₃)₂.H₂O, (NH₄)₂HPO₄, Na(NO₃)₂ and Sr(NO₃)₂ as required, then the samples were calcined at 500 °C. Then the samples coated biomimetically by immersing in concentrated simulated body fluid (SBF×5) for 30 days in order to investigate the formation of naturally hydroxyapatite on them. The samples were calcined at 500 °C in tubular furnace under air atmosphere. The optical microscope, XRD and SEM tests were achieved for each step, and FTIR test for electrochemical coated samples and biomimetically coated samples were made. The final result shows that the TiO₂ nanotube is successfully synthesized. The samples that coated with strontium hydroxyapatite (Sr 100% without calcium) achieved a good formation of naturally hydroxyapatite similar to bone composition than the others samples (that contain 50% Sr or 0% Sr).

Keywords: Electrochemical deposition, strontium hydroxyapatite, Biomimetic coating.

الترسيب الكهروكيميائي للسترونتيوم - هايدروكسيأباتيت (Sr-HA) على طبقة TiO₂ نانوتيوب المصنعة بطريقة الأنودة لمعدن التيتانيوم وسبيكة Ti-6Al-4V لزيادة الترابط مع العظام

الخلاصة

في هذا البحث تمت دراسة الترابط بين السترونتيوم هايدروكسي أباتيت المرسيب كهروكيميائياً على أكسيد التيتانيوم نانوتيوب (TiO₂ nanotubes). أكسيد التيتانيوم نانوتيوب تم تخليقه عن طريق أنودة معدن التيتانيوم وسبيكة Ti-6Al-4V باستخدام خلية كهروكيميائية خاصة أعدت لهذا الغرض،

المحلول المستخدم مؤلف من 70% نسبة حجمية من الكلسرين ، 30% نسبة حجمية من الماء و 1.5% نسبة وزنية من فلوريد الأمونيوم . تراكيز مختلفة من السترونتيوم هايدروكسي أبتايت (100،50،0) % تم ترسيبها كهروكيميائياً من محلول مؤلف من نترات الكالسيوم أحادي الماء ، ثنائي أمونيوم هايدروجين فوسفات ، نترات الصوديوم ونترات السترونتيوم حسب ما يتطلب من التراكيز ، بعدها تم كلسنة النماذج بدرجة 500 °م . تم بعدها طلاء النماذج بالتخليق الذاتي الإحيائي بالتغطيس بمحلول مركز مشبه بمحاليل الجسم (SBF×5) لمدة 30 يوماً لغرض التحقق من الهايدروكسي أبتايت المتكون طبيعياً عليها. النماذج تم كلسنتها بدرجة حرارة 500 °م في فرن أنبوبي بجو من الهواء . تم إجراء إختبارات XRD ، SEM و FTIR لكل خطوة للنماذج المرسب عليها الهايدروكسي أبتايت كهروكيميائياً وكذلك بالتخليق الذاتي الإحيائي . النتائج النهائية اظهرت التخليق الناجح لطبقة التيتانيوم نانوتيوب . النماذج المرسب عليها سترونتيوم هايدروكسي أبتايت (100% سترونتيوم وبدون كالسيوم) أحدثت تكوين جيد للهايدروكسي أبتايت المتكون طبيعياً مشابه لتكوين العظم خلافاً لبقية النماذج (الحاوية على 0% و 50% سترونتيوم) .

INTRODUCTION

Many researches aim to achieve to an increase in the ostiointegration of titanium and titanium alloys implants with bones (i.e. increasing bonding with bone). The researchers were applied many ways to this purpose, such as creating titanium oxide nanotube array on the surface of implants [1, 2 and 3]. This modification technique was done by various electrolytes and producing thick oxide layer with relative high rough surface.

All electrolytes used must contain fluoride ion in order to achieve a drilling action on the metal surface, by using applied voltage in the range of 10-40 volt and can result in a self-ordered nano-tubular structures which possess tubes with diameters between 15-200 nm [4] . There are many factors affecting the morphology of TiO₂ nanotube array beside the voltage , such as electrolyte pH ,temperature, fluoride ion concentration and the time of anodizing [5, 6 and 7]

The other way to increase ostiointegration is by coating the implants with hydroxyapatite [8] ,that because the main composition of the bone was consisted from hydroxyapatite. Hydroxyapatite (HA) is one of the main ceramics employed in clinical applications for bone and dental regeneration. It offers excellent biocompatibility and ability to bond with bone tissues. One of its main structural characteristics is its ability to accept a great variety of isomorphous substitutions while retaining the hexagonal space group P6₃/m. Divalent cations , such as Zn, Fe, Cu, Mg, Ni, Cr, Mn, Co, Sr, Pb and Cd, and anions such as F⁻, Cl⁻, CO₃⁻² and VO₄⁻³ may substitute for Ca⁺² , OH⁻ and (PO₄)⁻³ respectively in the HA structure. These substitutions modify its thermal stability and biological performance. From all the cation strontium has grate interest because it shares the same physiological pathway as calcium and can be deposited into the mineral structure of the bone. As a trace element in bone, strontium has been reported to exert a beneficial effect on osteoblastic activity. Various in vitro and in vivo studies reported the beneficial effects of strontium ions (Sr⁺²) as a potential bioactive element to enhance bone healing. Strontium has a positive effect on osteoblast-related gene expression such as osteonectin, and the alkaline phosphates (ALP) activity of mesenchymal stem cells (MSCs). Also it can inhibit the differentiation of osteoclasts [9, 10 and 11]. Thus it enhances matrix deposition, inhibits bone absorbing activity of osteoclasts, and, ultimately, encourages rapid new bone formation. Consequently, strontium is thought to be effective in enhancing the bioactivity and biocompatibility of some biomaterials

.In this research we improve the bonding with bone by synthesis a TiO₂ nanotube array by anodizing titanium metal and Ti-6Al4V alloy using a solution of 70% vol. glycerin 30% vol. water and 1.5%wt. NH₄F with applied voltage 20 volt in special electrochemical cell prepared for this process,then we coated the nanotube layer with strontium hydroxyapatite containig strontium of (0,50,100 %) by electrodeposition from aqueous solution contains Ca(NO₃)₂.H₂O 8gm/l ,Sr(NO₃)₂ 7gm/l or as required ,(NH₄)₂HPO₄ 3.5 gm/l and Na(NO₃)₂ 8gm/l,then we investigate the naturally biomimetic formation of hydroxyapatite by immersing in concentrated simulated body fluid (SBF×5) for one month ,and investigate the morphology of the coated layer and naturally formed layer by XRD test, optical microscope ,SEM and FTIR .

Materials and Methods:

TiO₂ nanotube preparing:

The samples of titanium metal and Ti-6Al-4V alloy disc with 20 mm diameter and 3 mm thickness were grinded ,then the samples were rinsed with distilled water ,etched by HNO₃:HF:H₂O solution (3:1:6 % vol. respectively) for 5 minutes to remove oxide layer, rinsed with dist. water , cleaned with acetone ultrasonically for (20 min.) twice , and cleaned ultrasonically with dist. water for (20 min.) one time .The solution of anodizing and TiO₂ nanotube synthesis were prepared by mix 3 gm of NH₄F to 60 ml of dist. water and 140 ml of glycerin ,mixed for 20 min. The sample was fixed inside electrochemical cell prepared specially for anodizing as shown in fig (1) and used as anode , platinum disc (30 mm diameter) was used as cathode , the potential wre applied at 20 volt for 1 hr, the coated samples was heat treated in tubular furnace at 500 °C for 1hr. ,then the samples was cleaned by dist. water in ultrasonic bath for 20 min. one time .Then the morphology of TiO₂ nanotubes was investigated by XRD,SEM .

Electrodeposition of Apatite:

Three solutions of electrodeposition of calcium hydroxyapatite , strontium – calcium hydroxyapatite 50 % and strontium hydroxyapatite was prepared by mixing : a- 7.53 gm/l of Ca(NO₃)₂.H₂O , 3.560 gm/l of (NH₄)₂HPO₄ and 8.5 gm/l Na(NO₃)₂ in dist water as calcium hydroxyapatite souldion b- 3.72gm/l of Ca(NO₃)₂.H₂O , 3.72 gm/l of Sr(NO₃)₂, 3.560 gm/l of (NH₄)₂HPO₄ and 8.5 gm/l Na(NO₃)₂ in dist water as streontium-calcium hydroxyapatite 50-50 % solution. c- 7.53 gm/l of Sr(NO₃)₂ , 3.560 gm/l of (NH₄)₂HPO₄ and 8.5 gm/l Na(NO₃)₂ in dist water as strontium hydroxyapatite solution.The pH adjusted at 5.5 by using NH₄OH or diluted HNO₃ as required, then the sample was fixed in the same electrochemical cell that mentioned before and was used as a cathode , Titanium disc with 20 mm diameter and 4 mm thickness was used as an anode in all experiments .The distance between the two electrode was 4 cm. The potential was applied at 12 volt for 1 hour with gently stirring .The coated samples were thermally treated in tubular furnace at 500 °C for 1hr. The morphology of TiO₂ nanotubes was investigated by XRD, SEM, FTIR and optical microscope images.

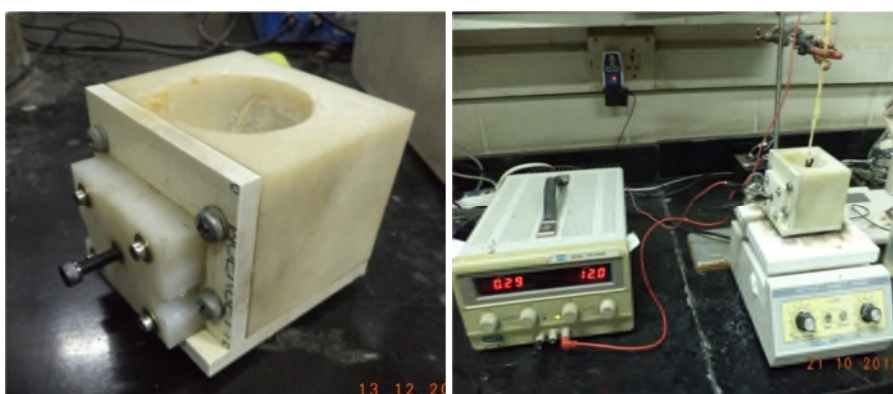
Biomimetic Treating:

Titanium and Ti-6Al-4V samples were immersed in simulated body fluid concentrated five times (SBF×5) for one month to investigate the bond with hydroxyapatite naturally formed from SBF solution ,table (1) shows the SBF

chemical composition. After immersing, the morphology of the samples was investigated by XRD, SEM, FTIR and optical microscope images.

Table (1) The composition of simulated body fluid (SBF) and concentrated simulated body fluid (SBF×5) (A. HELEBRANT, L. JONASOVA, L. SANDA, 2002, Ceramics vol. 46 (1), P:(9-14).

| ITEM | SBF gm/l | SBF×5 gm/l |
|--|----------|------------|
| NaCl | 8.035 | 40.175 |
| NaHCO ₃ | 0.355 | 1.775 |
| KCl | 0.225 | 1.125 |
| K ₂ HPO ₄ .3H ₂ O | 0.231 | 1.155 |
| MgCl ₂ | 0.311 | 1.555 |
| CaCl ₂ | 0.292 | 1.46 |
| Na(SO ₄) ₂ | 0.072 | 0.36 |



(A)

(B)

Figure (1) (a) The electrochemical cell that designed and used for TiO₂ nanotube synthesis (b) the electrochemical cell under operation.

Results and discussion

TiO₂ nanotube formation:

After anodizing titanium and Ti-6Al-4V alloy we obtained a successful nanotube array, and figs (2 and 3) show the SEM images for nanotube obtained on metal and alloy respectively, we see obviously the TiO₂ nanotube that has approximately 100 nm diameter, and fig (4) shows the XRD patterns for both metal and alloy, we see the titanium oxide peak compared with (PDF 21-1272) at 2 θ = 25.281,44.049 and 62.288, and there is no big difference between TiO₂ nanotube obtained on Ti or Ti-6Al-4V alloy

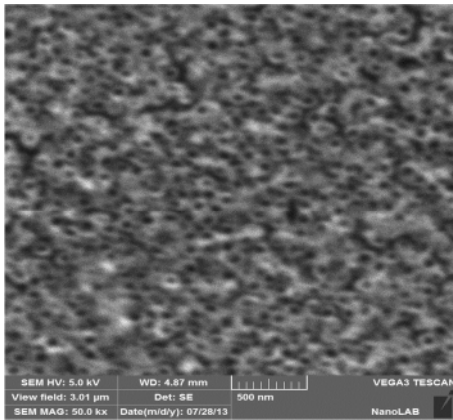


Figure (2) nanotube formed on Ti Metal.

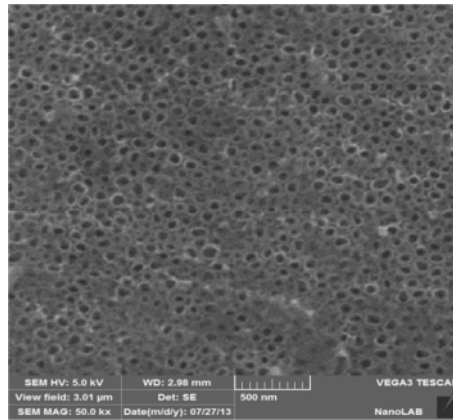


Figure (3) nanotube formed on Ti-6Al-4V alloy.

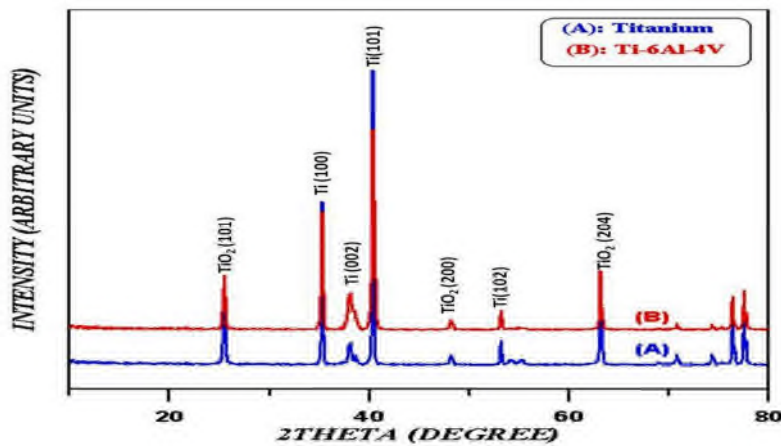


Figure (4) XRD for titanium metal and Ti-6Al-4V alloy after anodizing.

Electrodeposition of Apatite:

After electrodeposition of three types of apatite, we observe that strontium hydroxyapatite has a thicker layer from strontium – calcium hydroxyapatite and calcium hydroxyapatite, fig (5) shows the camera images for the samples of metal and alloy after coating by electrodeposition.



(a) HA



(b) HA



(c) Sr-CaHA 50%



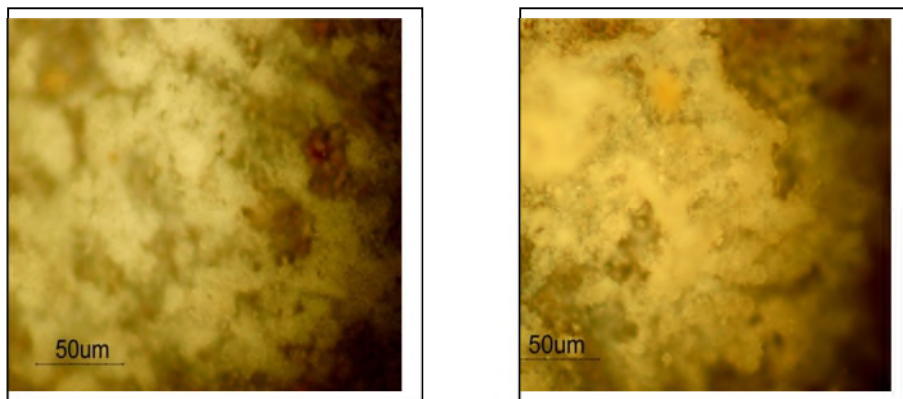
(d) Sr-CaHA 50%

(e) SrHA

(f) SrHA

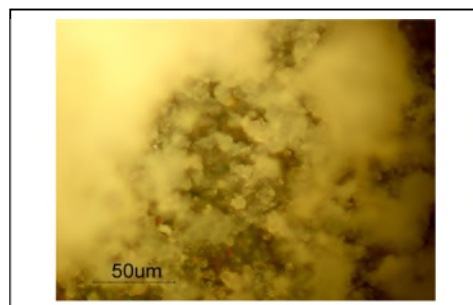
Figure(5) The samples after coating : (a,c,e) coated on titanium metal and (b,d,f) coated on Ti-6Al-4V alloy.

From fig (6) which represent the optical images of titanium coated samples, we see the difference in thickness clearly between strontium hydroxyapatite and other two types of apatite.



(a) CaHA

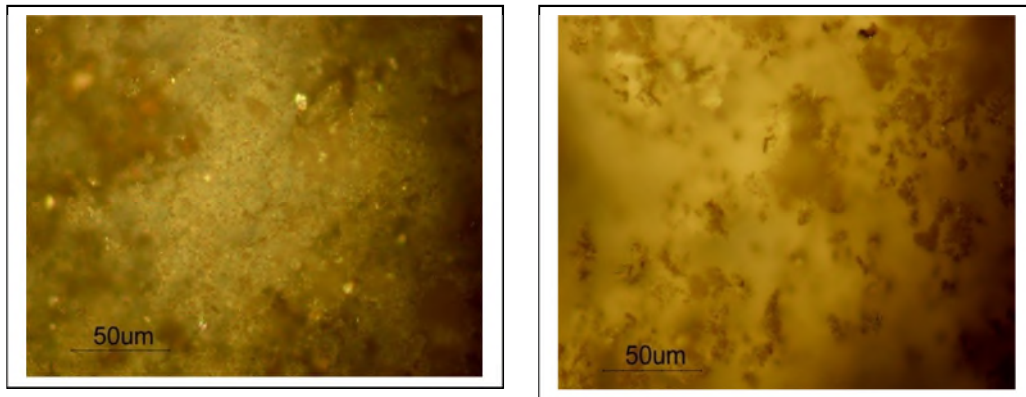
(b) Sr-CaHA 50%



(c) SrHA

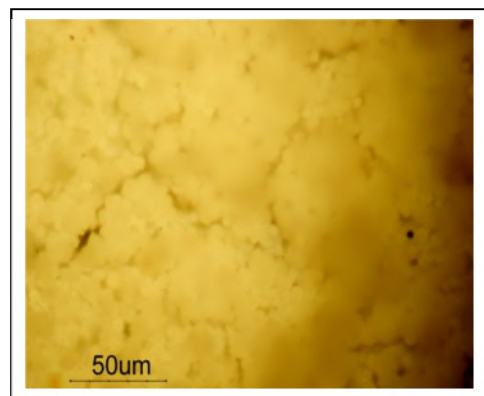
Figure (6) optical microscope images for Ti metal coated with apatite after anodizing .

And from fig(7) which represents the optical images for Ti-6Al-4V coated samples ,we see the difference in thickness clearly between strontium hydroxyapatite and other two types of apatite also.



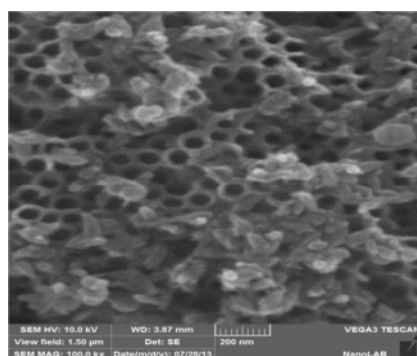
(a) Ca HA

(b) Sr-CaHA 50%

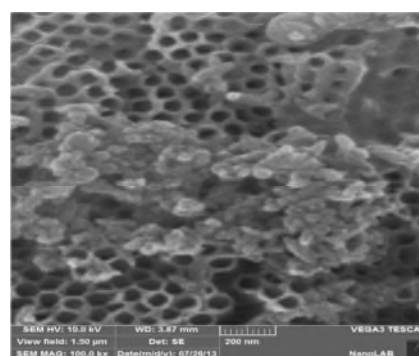


(c) SrHA

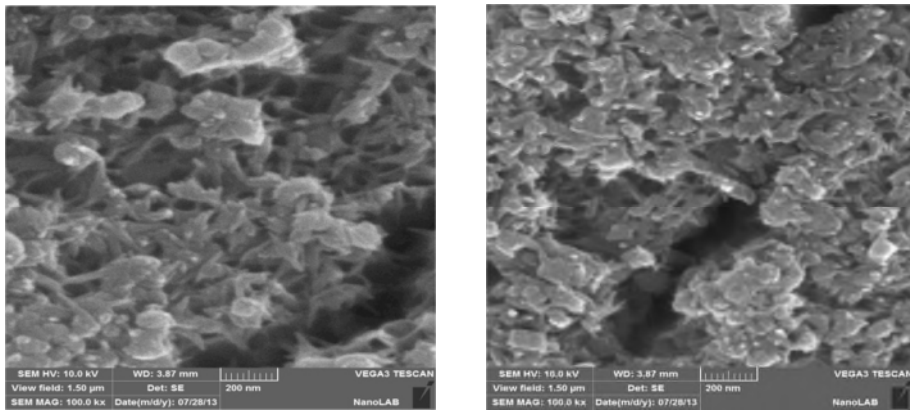
Figure (7) optical microscope images for Ti-6Al-4V coated with apatite after anodizing.



(a) Ti coated with CaHA



(b) Ti-6Al-4V coated with CaHA



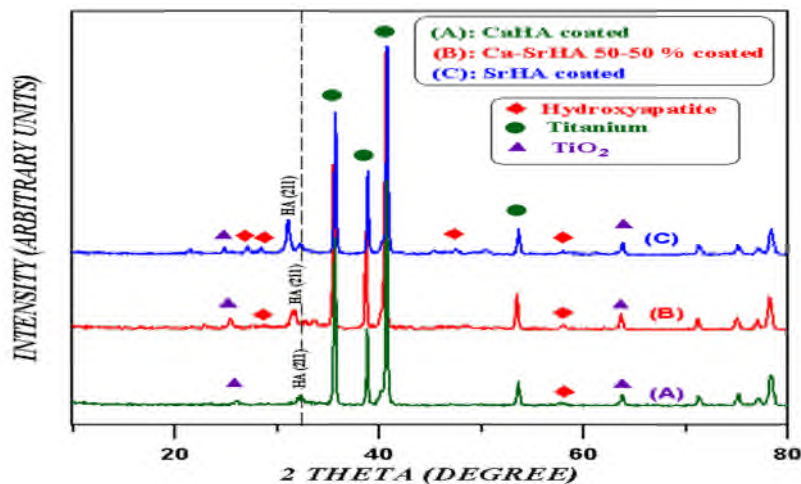
(c) Ti coated with SrHA

(d) Ti-6Al-4V coated with SrHA

Figure (8) SEM for titanium and Ti-6Al-4V coated with CaHA and SrHA.

To understand the difference in morphology between calcium hydroxyapatite and strontium apatite : fig (8) shows the SEM images for metal and alloy that coated with the two type of apatite

Fig (9,10) shows the XRD patterns for titanium and Ti-6Al-4V coated with different type of apatite ,we see the shift in the peek at (h,l,k) (211) from $2\theta=31.8^\circ$ to 30.6° ,comparing with ICDD (PDF 09-0432) for calcium hydroxyapatite and ICDD (PDF 33-1348) for strontium hydroxyapatite, that shifting in the peek occurred because strontium ion substitute the calcium ions in the hydroxyapatite crystal and convert from calcium hydroxyapatite to strontium hydroxyapatite by increasing the strontium concentration in the electrolyte that used in the electrochemical deposition process.



Figure(9) XRD test for titanium coated with different apatite .

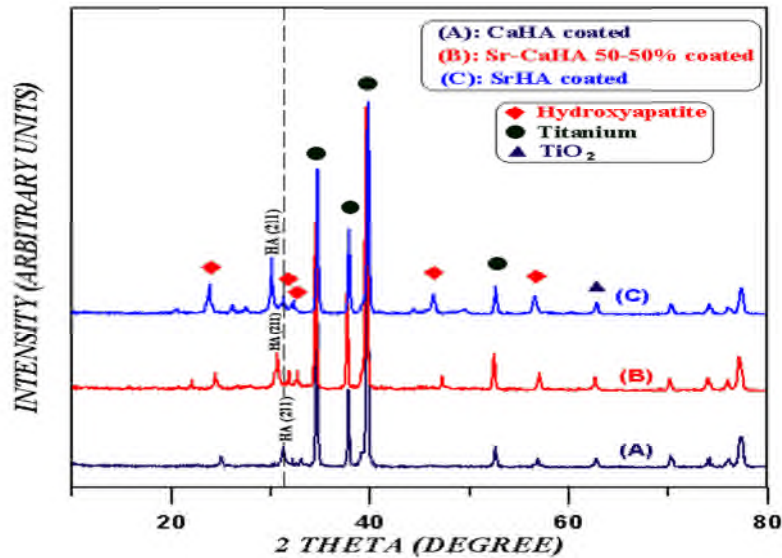


Figure (10) XRD test for Ti-6Al-4V coated with different apatite.

This conversion from calcium hydroxyapatite to strontium hydroxyapatite increases the lattice parameters of apatite ,so the lattice volume increases , that because the difference in ion volume for each calcium and strontium (Ca^{+2} ionic radius = 0.100 nm ; Sr^{+2} ionic radius = 0.118 nm) , table (2) shows the change in lattice parameters for apatite deposited on titanium metal, the parameters calculated from Bragg s low and from hexagonal shape lattice parameters relation as shown in eq.(1) and eq.(2) [12] respectively:

$$n\lambda = 2d \sin \theta \quad \dots(1)$$

$$\frac{1}{d^2} = \frac{4}{3} \left(\frac{h^2 + hk + k^2}{a^2} \right) + \frac{l^2}{c^2} \quad \dots (2)$$

Where $\lambda = 1.5402 \text{ \AA}$

θ = diffraction angle

h, k, l = miller indices

a, c = hexagonal shape distances

d = distance between parallel indices

$$\text{Lattice volume for hexagonal shape} = 0.866 \times a^2 \times c$$

Table (2) Lattice parameter for Apatite deposited on Titanium.

| Lattice parameters | a Å | c Å | Lattice volume (Å ³) |
|--------------------|-------|-------|----------------------------------|
| CaHA | 9.161 | 6.906 | 502.0 |
| Sr-CaHA 50-50 % | 9.450 | 7.012 | 542.29 |
| SrHA | 9.938 | 7.168 | 612.4 |
| CaHA Standard | 9.418 | 6.884 | 528.8 |
| SrHA Standard | 9.766 | 7.276 | 600.9 |

The particle size of deposited apatite on titanium which calculated from shirrer equation display that for CaHA = 51.88 nm , CaSrHA=125.87 nm and for SrHA= 332.7 nm ,we see obviously that the particle size also increase when the strontium ion substitute the calcium ion.

For Ti-6Al-4V also the lattice parameters increase with the increasing of strontium ion substitution as shown in table (3)

Table (3) Lattice parameter for Apatite deposited on Ti-6Al-4 alloy.

| Lattice parameters | a Å | c Å | Lattice volume (Å ³) |
|--------------------|-------|-------|----------------------------------|
| CaHA | 9.207 | 6.895 | 506.39 |
| Sr-CaHA 50-50 % | 9.421 | 6.99 | 537.28 |
| SrHA | 9.965 | 7.152 | 615.05 |
| CaHA Standard | 9.418 | 6.884 | 528.8 |
| SrHA Standard | 9.766 | 7.276 | 600.9 |

The particle size of deposited apatite on Ti-6Al-4V which calculated as before display that for CaHA = 45.7 nm , and for CaSrHA=166.5 nm and for SrHA= 541.59 nm ,we see obviously that the particle size also increases when the strontium ions substitute the calcium ions in hydroxyapatite crystal as we see in titanium before.

Biomimetic coating:

After immersing in (SBF×5) for one month ,we see a clear layer of CaHA coated the samples of titanium and Ti-6Al-4V that coted with different type of apatite briviously as shown in fig (11) of camera images for all samples and from fig (12) which shows the optical microscope images for all samples , and we see obviously the thick layer of hydroxyapatite that formed on the samples and specially that coated with SrHA before , this indicates clearly that SrHA will motivate the bone to build on SrHA quickly and strongly bond.

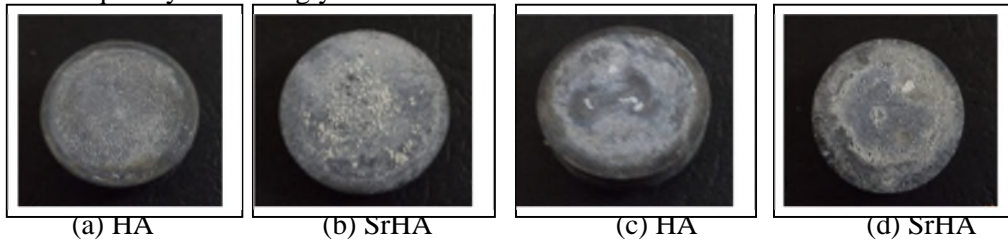


Figure (11) camera images for biomimetic coated samples (a,b)titanium samples coated with HA,SrHA before biomimetic, (c,d) Ti-6Al-4V samples coated with HA,SrHA before biomimetic.

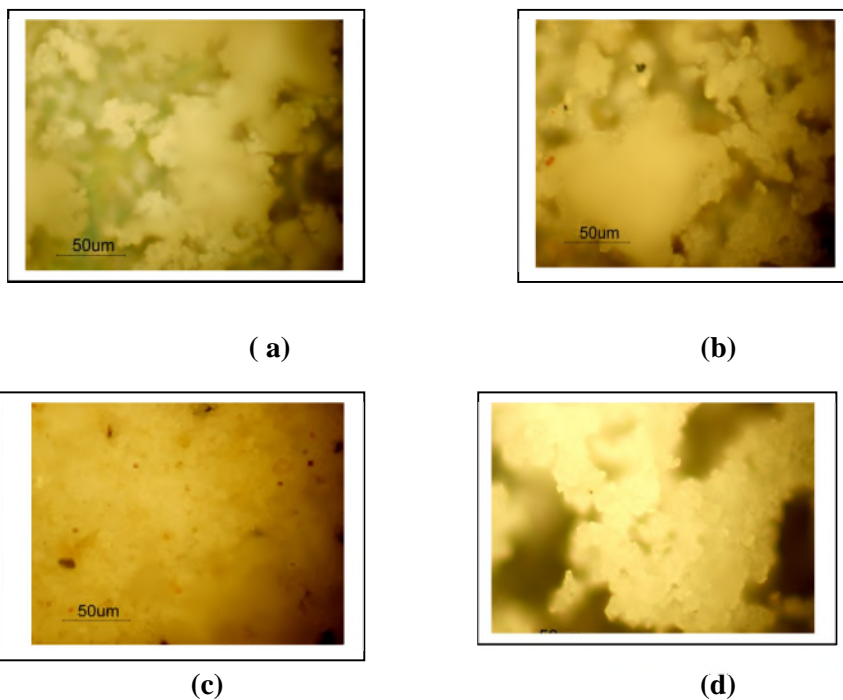
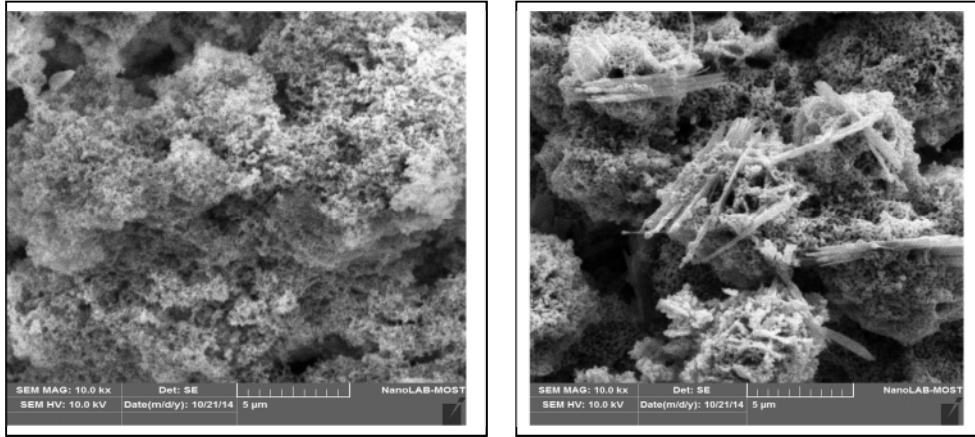


Figure (12) optical microscope images for biomimetic coated samples (a,b)titanium samples coated with HA,SrHA before biomimetic, (c,d) Ti-6Al-4V samples coated with HA,SrHA before biomimetic.

From fig (13) which shows the SEM for Ti-6Al-4V which immersed in SBF solution that there is a difference in the morphology between hydroxyaptite that

formed on the sample that briviously coated with CaHA by electrodepositon than that briviously coated with SrHA by electrodeposition ,and both were different from that obtained before biomimetic coating in fig (7) .



(a) (b)
Figure(13) SEM for Ti-6Al-4V after immersing in (SBF×5) for 30 days (a) primarily coated by electrodeposition with CaHA (b) primarily coated by electrodeposition with SrHA .

From figs (14 and 15) that shown XRD for titanium and Ti-6Al-4V that biomimetic coated with CaHA after electrodepositon coating with different hydroxyapatite (CaHA,Sr-CaHA 50% and SrHA) , we obviously seen that the hydroxyapatite formed clearly on the samples that coated with SrHA more than that coated with CaHA ,and we observe carfully that there is two peaks at $(h,k,l) = 211$, first one is due to strontium hydroxyapatite that coated by electrodeposition and the other for CaHA that formed by biomimetic formation in (SBF×5) for titanium and Ti-6Al-4V samples

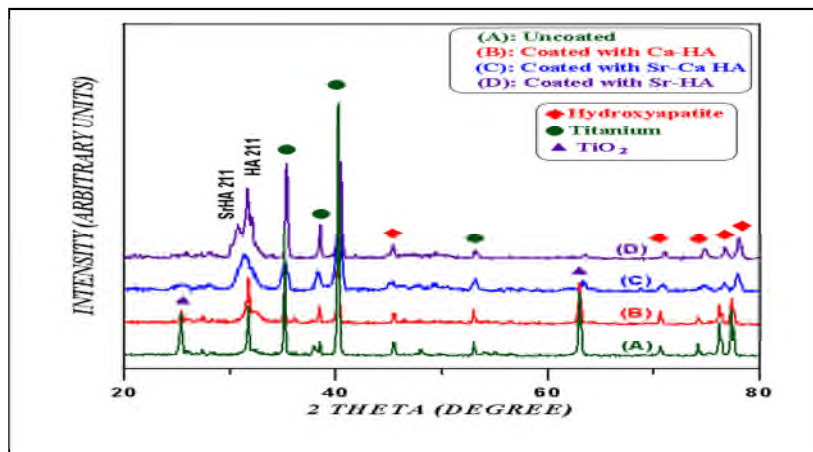


Figure (14) XRD pattern for titanium coated by biomimetic after coated by electrodeposition with different concentration of strontium hydroxyapatite.

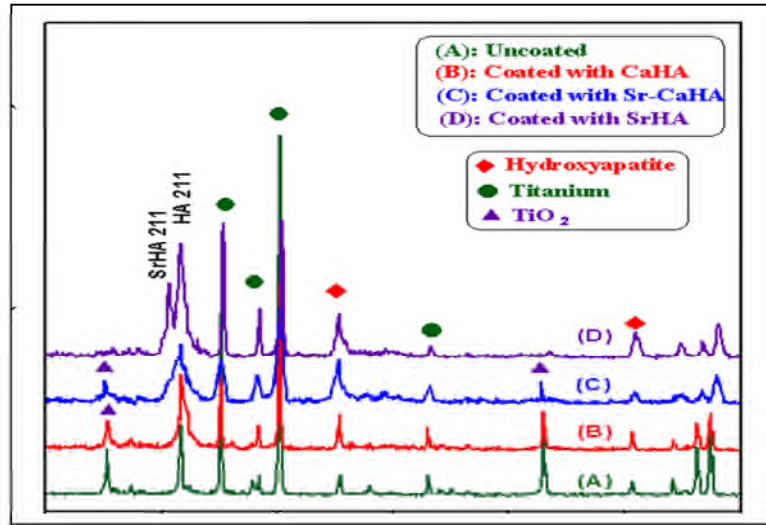


Figure (15) XRD pattern for Ti-6A-4V coated by biomimetic after coated by electrodeposition with different concentration of strontium hydroxyapatite.

From tables (4 and 5) we see the difference in lattice parameters for naturally hydroxyapatite formed on the samples of titanium and Ti-6Al-4V that coated with strontium hydroxyapatite briviouslyely.

Table (4) Lattice parameters of biomimetic coated hydroxyapatite on titanium coated with SrHA.

| Lattice parameters | a Å | c Å | Lattice volume (Å ³) |
|--------------------|-------|-------|----------------------------------|
| CaHA | 9.411 | 6.878 | 527.55 |
| Sr-CaHA 50-50 % | 9.565 | 6.875 | 544.71 |
| SrHA | 9.731 | 7.389 | 605.94 |
| CaHA Standard | 9.418 | 6.884 | 528.8 |
| SrHA Standard | 9.766 | 7.276 | 600.9 |

Table (5) Lattice parameters of biomimetic coated hydroxyapatite on Ti-6Al-4V coated with SrHA.

| Lattice parameters | a Å | c Å | Lattice volume (Å ³) |
|--------------------|-------|-------|----------------------------------|
| CaHA | 9.424 | 6.898 | 526.7 |
| Sr-CaHA 50-50 % | 9.565 | 6.936 | 549.55 |
| SrHA | 9.601 | 7.397 | 582.63 |
| CaHA Standard | 9.418 | 6.884 | 528.8 |
| SrHA Standard | 9.766 | 7.276 | 600.9 |

We see that the lattice parameters of naturally formed hydroxyapatite also affected by the presence of strontium in the previous coated layer on the surface of titanium and Ti-6Al-4V , that's mean there are different in crystal shape of biomimetic hydroxyapatite formed naturally depending on the primary layer coating if it. Calcium hydroxyapatite or strontium hydroxyapatite ,and that matches to the result obtained before from SEM in fig (13). From figs (16 and 17) which represent the FTIR for biomimetic coating layer of the samples we see the change in wave number of OH that exist in the lattice of hydroxyapatite when the samples coated with SrHA and when the hydroxyapatite formed from simulated body fluid ,this change is due to substitution of strontium instead of calcium in the hydroxyapatite that coated by electrodeposition ,while when the hydroxyapatite was formed biomimetically we see that OH band wave number return to closely the origin position with little shift effect ,and also we see that the CO₃ band was appeared because that SBF solution contained a large amount of sodium carbonate that substitute the PO₄⁻³ in hydroxyapatite formula to form carbonated hydroxyapatite which is the similar form of bone composition , so that PO₄ peaks disappear and CO₃ peak was appeared in the FTIR pattern of biomimetically coated samples .

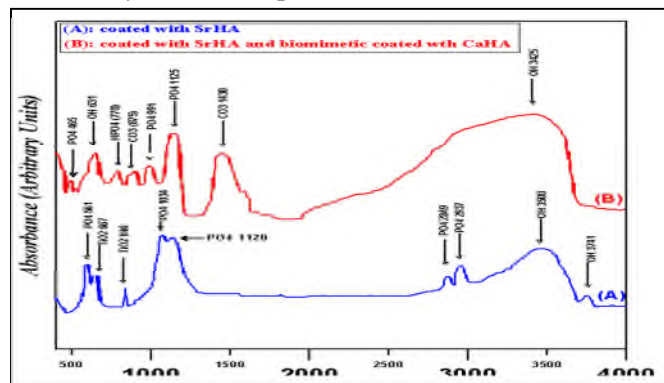


Figure (16) FTIR pattern for titanium samples (A) coated by electrodeposition with SrHA (B) biomimetically coated with HA after coated with SrHA.

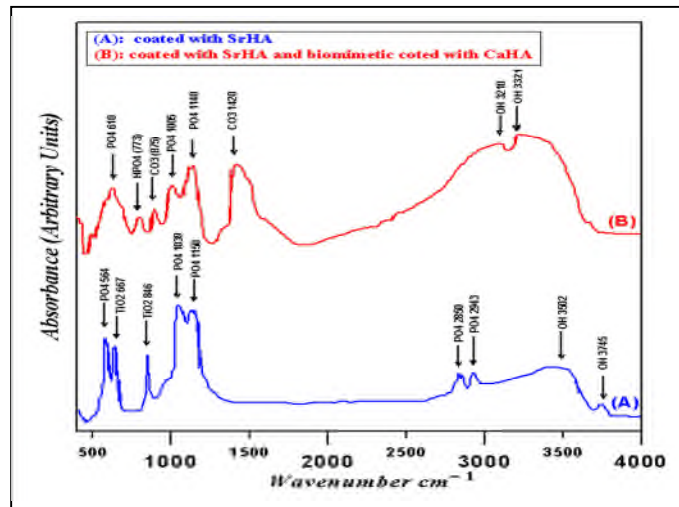


Figure (17) FTIR pattern for titanium samples (A) coated by electrodeposition with SrHA (B) biomimetically coated with HA after coated with SrHA.

Conclusions

From previous results we conclude that when the implants of titanium and Ti-6Al-4V coated with strontium hydroxyapatite after synthesis the TiO₂ nanotube give a good formation of hydroxyapatite in an environment similar to that in human body (SBF solution), and the formed hydroxyapatite was similar to bone composition, that means the coating will make a good bond with bone.

References

- [1] Ahmed El Ruby Mohamed and Sohrab Rohani, "Synthesis of Titania Nanotube array by Anodization", AIDIC conference series, vol 9.2009.
- [2] Diana Portan "Manufacturing and Characterization of TiO₂ Nanotube on Pure Titanium Surfaces for Advanced Biomedical Applications", U.P.B. Sci. Bull., vol. 73, Iss2, 2011.
- [3] Christine Jeanette Frandsen, "An Extensive Analysis of Modified Nanotube Surfaces for Next-Generation Orthopedic implants", Thesis, University of California, San Diego, 2012.
- [4] Jan Macak, "Growth of Anodic Self-Organized Titanium Oxide Nanotube Layers", Thesis, University of Erlangen-Nurnberg-Germany, 2008.
- [5] Sirmala Sreekantan, "Influence of Electrolyte pH on TiO₂ Nanotube Formation by Ti Anodization" Journal of Alloys and Compounds, vol. 485, p:478-483, 2009.
- [6] Henek Moravec and Katrina Chotova, Brno, "Chemical and Heat Treatment of Titania Nanostructures", Czech Republic, EU, Vol.10, p16-18, 2013.
- [7] Byung-Gwan Lee, Jin-Wook Choi, "Formation Behavior of Anodic TiO₂ Nanotubes in Fluoride Containing Electrolytes", Trans. Nonferrous Met. Soc. China, vol 19, p:842-845, 2009.
- [8] Wang Yue-Qin, "HA Coating on Titanium With Nanotubular Anodized TiO₂ Intermediate Layer via Electrochemical Deposition", Trans. Nonferrous Met. Soc. China, vol.18, p:631-635, 2008.

- [9] Guo-Xin Ni and Zhi-Peng, "The Effect of Strontium Incorporation in Hydroxyapatite on Osteoblasts in vitro" ,J. Mater.Med. ,vol 22 ,961-967,2011.
- [10] Dan-Li Fu and Qiao-hong Jiang,"Florescence Microscope Anaysis of Bone Osseointegration of Strontium Substituted Hydroxyapatite", Biomed. & Biotech. Vol.13 p:364-371,2012.
- [11] Jennifer H.Shepherd and David V. Shepherd "Substituted Hydroxyapatites for Bone Repair ", J.Mter. Sci. :Mater. Med., vol. 23,p:2335-2347,2012.
- [12] M.F.C. Ladd, R.A. Palmer, " Structure Determination by X-ray Crystallography" ,3rd ed., Plenum Press, New York, 1993.