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# Evaluation the possibility of Bioactivation of Niobium and Titanium by Biomemtic Process

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## 1. Introduction

Commercially pure titanium is widely used as dental implant material because of its suitable mechanical properties and excellent biocompatibility. The latter is mainly due to its excellent corrosion behaviour in the physiological environment by a tenacious layer of passivating titanium oxides that appear on the implant's surface immediately after exposure to oxygen **(Ozawa et al 2002)[1],[2] (Aparicio et al, 2003)**.

Although the osseointegrated titanium implants are reliable alternatives for safety and long time treatment, it was found that titanium exhibited the lower corrosion tendency in media representing conditions normally found in the human mouth [3]**(Mabilleau et al, 2006)**.

At the same time Ti alloys such as (Ti6Al4V) may lead to serious danger, It was reported that aluminum(Al) and vanadium (V) were dissolved from Ti alloy (Ti6Al4V). This could be a cause for concern since Al is a growth inhibitor of bone and a possible cause of Alzheimer's disease, and V has strong cytotoxicity. It is suspected that Ti6Al4V alloy implants may have a harmful effect on the recipient organism [4]**(Matsuno et al, 2001)**

Niobium (Nb) has already been investigated in the 1980's and recent studies demonstrated the potential of Nb as an implant material .Niobium was choosen for this study as an alternative to cpti implant material due to its bioinert behavior and lower elastic modulous and moderate cost in addition to corrosion resistance.**[1][5] (Matsuno et al, 2001)(Li and Xu,2017)**

Niobium and some niobium alloys are physiologically inert and thus hypoallergenic. For this reason, niobium is found in many medical devices such as pacemakers. It is well known that, when implanted, titanium and its alloys do not bond with the bone by a chemical or biological interaction, but simply by morphological connection to the bone[6],**(Gil et al, 2002) and[7] (Verne et al, 2004)**.

Various strategies to improve osseointegration have been specifically focused on the implant surface characteristics such as surface *morphology* and *roughness*, and *chemical composition* to promote bone apposition through the acceleration of the chemical bonding between the new bone and implant [8]**(MacDonald et al, 2002) and[9] (Giavaresi et al, 2004)**.

In vitro studies have shown that surface roughness alters osteoblastic attachment, proliferation, and their differentiation and matrix production. Also it influences the cytokines and growth factors in the milieu thereby modulating the healing process[10]**(Jayaraman et al, 2004)**. Moreover, it has been shown that mechanical locking may be enhanced by an increase of the surface roughness and the stress distribution can be improved. Thus, surface topography plays a critical role in the interaction of dental implants with adjacent tissues [11]**(Peto et al, 2002),[12] (Karacsa et al, 2003) and [13] (Citeau et al, 2005)**.

The development of coating system was driven by combining the bioactivity of the ceramics with the mechanical properties of the metallic implant material **[14](Puleo et al, 1999)**.

In the last two decades, calcium phosphate coating in particular hydroxyapatite bioceramics is widely used for both orthopaedic and dental implants because of their unique advantage as they have chemical and mineral components similar to that of teeth and bone. The commercial coating technique is preferentially plasma spraying, because of their rapid and strong bonds to living bone tissues [15]**(Hamdi et al, 2001)**. Several important drawbacks such as the low cohesion of the coating, the presence of different undesired phases and a poor coating-to-substrate adhesion are associated with this method. As a result, the hydroxyapatite layer is mechanically and chemically unstable [16]**(Arias et al, 2002) and[6] (Gil et al, 2002)**.

Alternatives to the plasma spraying method have been developed to obtain films of calcium phosphate, like the *Biomimetic Method*. The biomimetic process is a physicochemical method in which a substrate is soaked in a solution that simulates the physiologic conditions, for a period of time enough to form a desirable layer of calcium phosphate on the substrate. Prior to the immersion in the SBF solution, the substrate is usually treated with an alkaline solution to generate a modified surface so that the apatite crystals could be deposited chemically [17]**(Teixeira et al, 2004)**.

The formation of this bioactive phase on the surface of metal substrate could be induced by the Ti-OH groups, which reveals negative charge to interact with calcium ions in the simulated body fluid. The amorphous calcium titanate is postulated to reveal positive charge, thereby interacting with the phosphate ions in the fluid to form the amorphous calcium phosphate, which eventually crystallized into HA-like phase[18]**(Simon et al, 2005)**.

The advantages of this method are its simplicity and its low investment cost in relation to the plasma spray process usually used. Besides, it can be used to coat substrates with complex geometry or porous substrates since the coatings are formed in solution, and it makes it possible to include organic molecules in the film to induce and to accelerate the regeneration of living tissues[19]**(Mao et al, 1999)**.

Although the biomimetic calcium phosphate coatings have been largely studied on titanium substrate, but to the author knowledge, there is no corresponding study concerning the development of biomimetic coating on Nb metal as a screw implant and study it invitro and invivo. Accordingly in this study, calcium phosphate coating was deposited on the surface of commercially pure titanium and Nb metals by Biomimetic Approach. Invitro and invivo experiments were done to investigate the effect of this technique on the quality of the deposited layer and bone- implant interface.

## 2. Materials and Methods

### 2.1 Study design

a total of 40 plates divide according to the type of material used into(20 niobium and 20 titanium) then were subdivided into control group (not treated) and coated group(treated)

### 2.1.1 Sample preparation

20 titanium plates (cpTi) and 20 niobium plates, of (10x10x1 mm) in length, width, and thickness respectively, were obtained by cutting the main large plate into the desired small plates, These specimens have a polished mirror surface.

Plates inserted in Ultrasonic bath of ethanol for 15 min to get rid of contamination and debris , **figure (2-1)**, then in distilled water bath for 10 minutes, after that the plates left to dehydrate at room temperature [20]**(Shukur, 2014)**.

Using the ultrasonic agitation is believed to initiate chemical attack more rapidly by removing contaminants remaining after processing of the plates [21]**(Sitting et al, 1999a)**.

### 2.1.2 Biomimetic surface treatment

The surface treatment performed in this study includes coating with calcium phosphate compound (CaP) by biomimetic approach that was carried out to enhance the biocompatibility of dental implants made from cpTi and Nb since it mimics nature’s way of mineral formation in the body.

**In the first step**, the samples were etched in 36% HCl under inert atmosphere of Ar for 2h. The inert atmosphere was used in order to provide a controlled procedure and prevent excessive etching. 250 ml was used for etching the cpTi and Nb specimens

The etched samples were soaked in 10M NaOH aqueous solution. CpTi and Nb samples were soaked in a 450 ml of NaOH solution. After 24h of incubation at 60°C in oven, the plates were washed with deionized water and dried at 100°C **[22] (Jonasova et al, 2004)**.

**In the second step**, the samples were soaked in a concentrated SBF. The concentration of the salts was 5 times than that found in human blood plasma **[24](Chen et al, 2005)**. The solution was prepared by dissolving reagent-grade NaCl, KCl, NaHCO3, MgSO 4. 7 H2O, CaCl2 and KH2PO4 into deionized water using hot plate stirrer with a magnetic stirring facilities.

**Table (1)** shows the salt concentration used in this study. The concentrations were multiplied by a factor of 5 from those in standard SBF.

**Table 1:** Chemical composition of simulated body fluid

| Item | description | Quantity gm/l |
|---|---|---|
| 1 | NaCl | 8.036 |
| 2 | KCl | 0.225 |
| 3 | $CaCl_2$ | 0.293 |
| 4 | $NaHCO_3$ | 0.352 |
| 5 | $K_2HPO_4$ | 0.230 |
| 6 | $MgCl_2.6H_2O$ | 0.311 |
| 7 | $NaSO_4$ | 0.072 |

After complete dissolution, the solution was buffered at pH=7.3 with $NH_4OH$ and HCl at 37°C. Buffering the solution to near physiologic levels is difficult due to the instability of pH of the solution caused by formation of carbonates after absorption of carbon dioxide from the air **[23].**

The samples were exposed to the solution under static conditions, to allow for nucleation which is required for successful CaP deposition, in a biological thermostat at 37°C for 14 days. The samples were immersed vertically in specific prepared slot in Teflon disc as shown in **Figure 1[23] (Baker et al, 2005)**.

After exposure, they were washed in deionized water and dried before the subsequent analysis.

**Figure 1:** Plates stands vertically in SBF

### 2.1.3 Heat treatment

Sintering of the coated specimens was carried out for densification using Carbolite furnace**.** The treatment is done under inert gas (argon), to prevent oxidation of the specimen. . The coated specimens were sintered at 400 °C for one hour **[24](Ghasak et al, 2015).**

### 2.1.4 X-Ray phase analysis

Phase analysis was employed on samples before and after coating with different materials. Phase analysis was studied using 3121 powders X-ray Diffractometer using Cu Kα radiation .The 2 angles were swept from 20- 60° in step of one degree. The peak indexing was carried out based on the JCPDS (joint committee on powder diffraction standards). SEM supported by EDX (energy dispersive X-ray analysis) technique was performed to determine the composition and concentration of the layer that was formed on the surface.

## 3. Results

### 3.1 Nanosurface feature [Morphological analysis (SEM)]

SEM images of cpTi surface after SBF immersion is showed on **(Figure2).** SEM of the coating obtained as in **(Figures 2a&b)** revealed that the surfaces of the cpTi specimen were wholly and homogeneously coated with a fine-structured layerSome distinct cracks were seen in the coating, these had probably formed during the drying process. The coating was found to consist of globules in the range of 1–2 µm **(Figure 2c).**in this case also The examination of an individual globule at further higher magnification **(figure 2d)** exhibited nanometric nuclei.

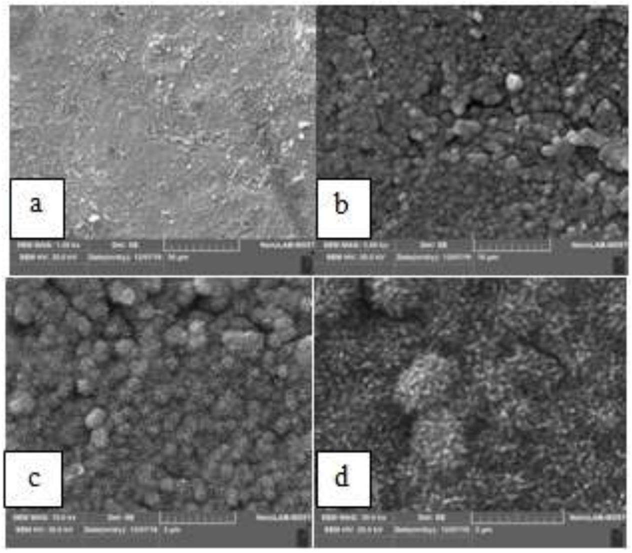

**Figure 2:** SEM micrographs cpTi specimen at (a &b) lower magnification, (c&d) higher magnification after immersion in 5XSBF

While the SEM ,After soaking the Nb plate in SBF for two weeks, showed a layer of coating with different shapes and grains in lower magnification **(fig. 3 a&b)**then the higher magnification **(fig. 3 b&d)** revealed a large and small crystals in different aggregations forming a tree like or spindle like arrangements

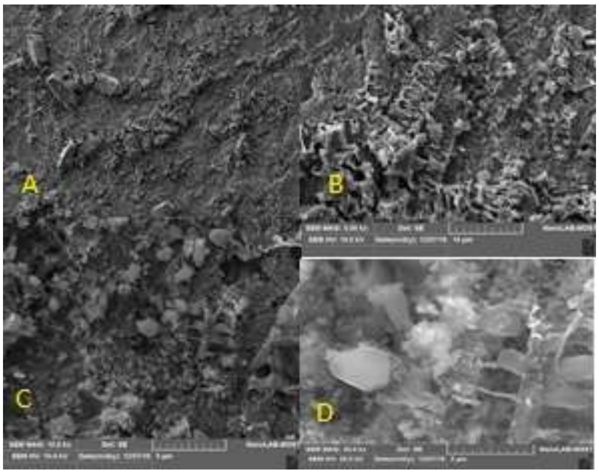

**Figure 3:** SEM micrographs Nb specimen at (a&b) lower magnification, (c&d) higher magnification after immersion in 5XSBF

### 3.2 Elemental Analysis (EDX)

The corresponding EDX patterns of Ti substrate immersed in SBF for two weeks are shown in **(Figure 4)**,whichindicating the appearance of transition energies of the calcium element CaKα and CaKβ at 3.68 and 4.03 KeV respectively. The phosphorustransition energies PKα and PKβ 2.02 and 2.13 KeV and the energies of matrix element TiKα and TiKβ 4.51 KeV and 4.92 KeV are shown in the pattern. This means the layer formed on the surface are comprised of calcium and phosphoruselements. The appearance of other elements like Na, Mg and Cl are precipitate in the layer from the salts contains in the SBF solution.

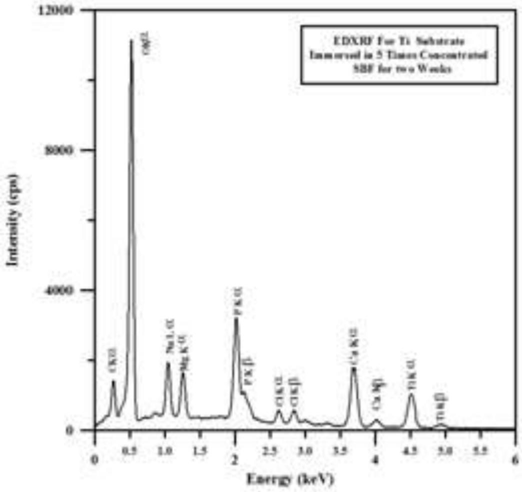

**Figure 4:** EDX of cpTibiomimemtically treated by immersion in SBF for two weeks

The pattern clearly show that the Ca/P layer covered well the Ti surface as the transition energies intensity of Ca and p are much higher than that of Ti. **Table (2)** shows the elemental concentration of the layer formed on the Ti substrate taken from two different spots .The Ca/P ratio varied between 1.83 and 2.14.

**Table 2:** Elemental Analysis of the layer formed on Ti biomimemtically

| Spectrum Label | O | Na | Mg | P | Cl | K | Ca | Ti | Fe | Ni | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Spectrum 3 | 45.02 | 3.99 | 3.09 | 8.96 | 2.32 | 0.55 | 16.45 | 18.48 | 0.68 | 0.44 | 100 |
| Spectrum 4 | 39.88 | 2.75 | 2.55 | 7.04 | 2.05 | 0.40 | 15.08 | 30.24 | - | - | 100 |

EDX patterns of Nb substrate immersed in SBF for two weeks are shown in **(Figure5),** whichare showing the transition energies of the calcium element CaKα and CaKβ at 3.70 and 4.01 KeV respectively. The phosphorustransition energy PKα are 2.02 KeV and the energy of matrix element NbKα 2.17KeV are shown in the pattern. This means the layer formed on the surface are comprised of calcium and phosphoruselements. The appearance of other elements like Na, Mg and Cl are precipitate in the layer from the salts contains in the SBF solution.

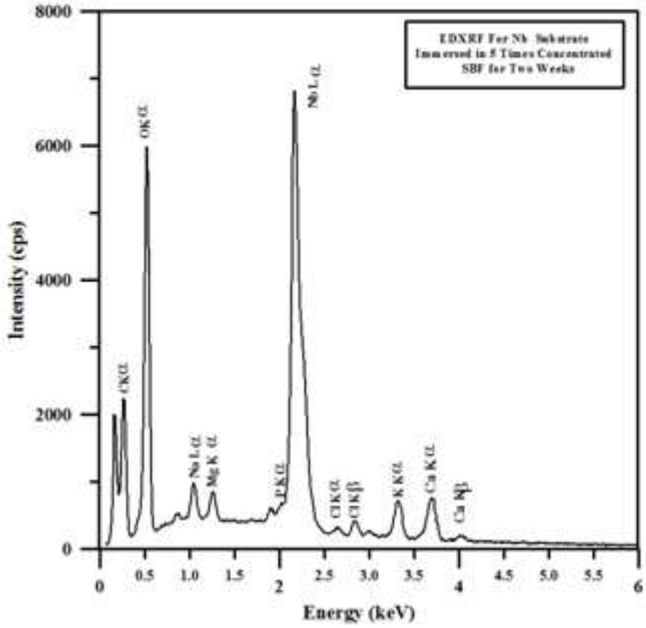

**Figure 5:** EDX of Nbbiomimemtically treated by immersion in SBF for two weeks

The pattern clearly show that the Ca/P layer covered well the Ti surface as the transition energies intensity of Ca and p are much higher than that of Ti. **Table (3)** shows the elemental concentration of the layer formed on the Nb substrate taken from two different spots. The Ca/P ratio varied between 1.77 and 1.67.

**Table 3:** Elemental Analysis of the layer formed on Nbbiomimemtically

| Spectrum Label | O | Na | Mg | Si | Cl | K | Ca | P | Co | Ni | Nb | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1) Spectrum 1 | 24.34 | 1.38 | 0.97 | 0.03 | 0.74 | 4.82 | 6.65 | 3.75 | 0.34 | 0.00 | 56.98 | 100.00 |
| 2) Spectrum 2 | 7.30 | 0.38 | 0.17 | 0.23 | 0.52 | 0.35 | 7.56 | 4.52 | 0.03 | 0.10 | 79.30 | 100.00 |

## 3.3 Phase Analysis (X-Ray Diffraction XRD)

### 3.3.1 XRD of cpTi

XRD patterns of cpTi specimens before and after immersion for two weeks in simulated body fluid SBF (biomimetic treatment) are shown in **Figure (6).** The XRD results were indexed according to the data reported by the International Center for Diffraction Data (ICDD) for the hexagonal Ti (JCPDS-ICDD file # 44-1294) and the hexagonal $Ca_5(PO_4)_3(OH)$ (JCPDS-ICDD file # 09-0432).

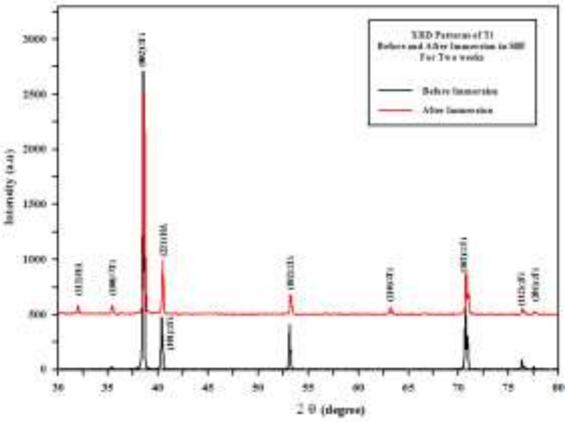

**Figure 6:** X-ray diffraction pattern of Ti substrates before and after immersion in SBF for two weeks.

The lattice of α-Ti phase is expanded in the treated region and the peak positions are shifted to higher Bragg angles. The pattern shows a systematic shifting of most peaks of α-Ti toward upper 2θ side as can be shown in **figure (7)**. Such a shift in the Ti peak position indicating the formation of new compound on the surface and it can be seen clearly. From **Figure (6)** the formation of (112) HA at 2θ position 32.192 and the shift of (121) αTi from its position at 2θ 40.370 to for (211) HA at 2θ 40.452. The (112) and (211) peaks are corresponding to the crystalline hydroxyapatite ($P6_3/m$) belonging to the hexagonal symmetry.

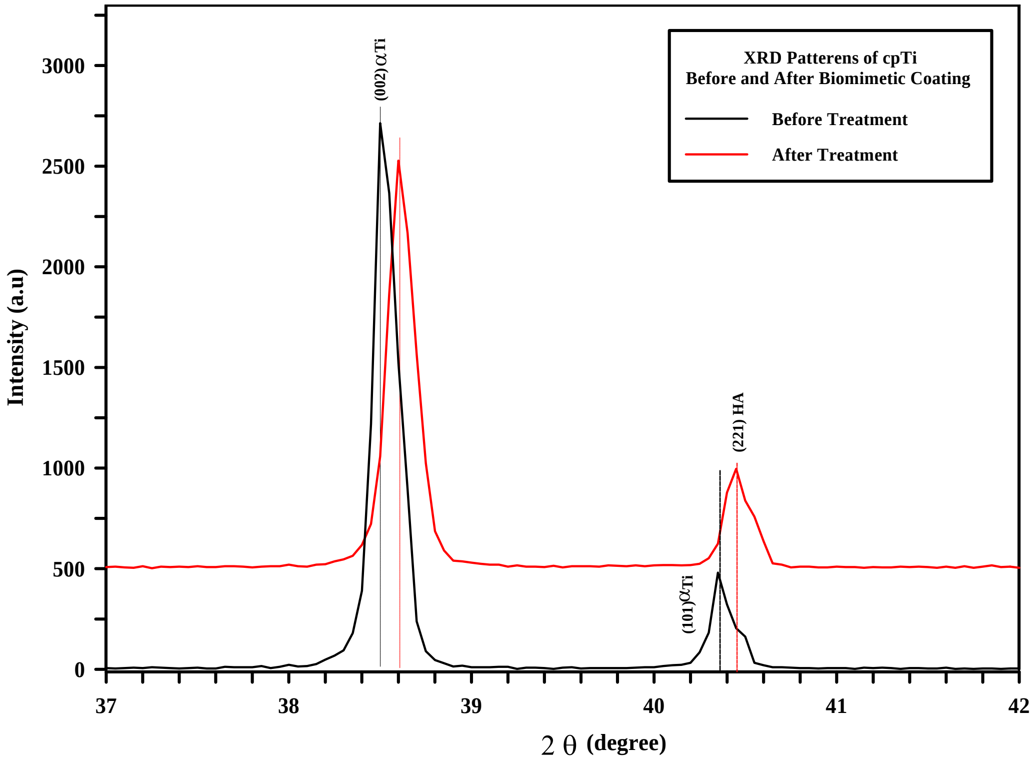

**Figure 7:** X-ray diffraction pattern of cpTi substrates before and after immersion in 5XSBF for two weeks showing the shift in the peak position

### 3.1.2 XRD of Nb

**Figure (8)** illustrate the structural changes in the surface of Nb substrate biomimemtically treated in SBF. The XRD patterns show a dramatic shift of the α-Nb peaks toward upper 2θ side. The HA formation after the immersion of Nb substrate for two weeks in SBF can be seen from strong peaks of (322) HA at 2θ 55.879 and (512) at 2θ 69.699.

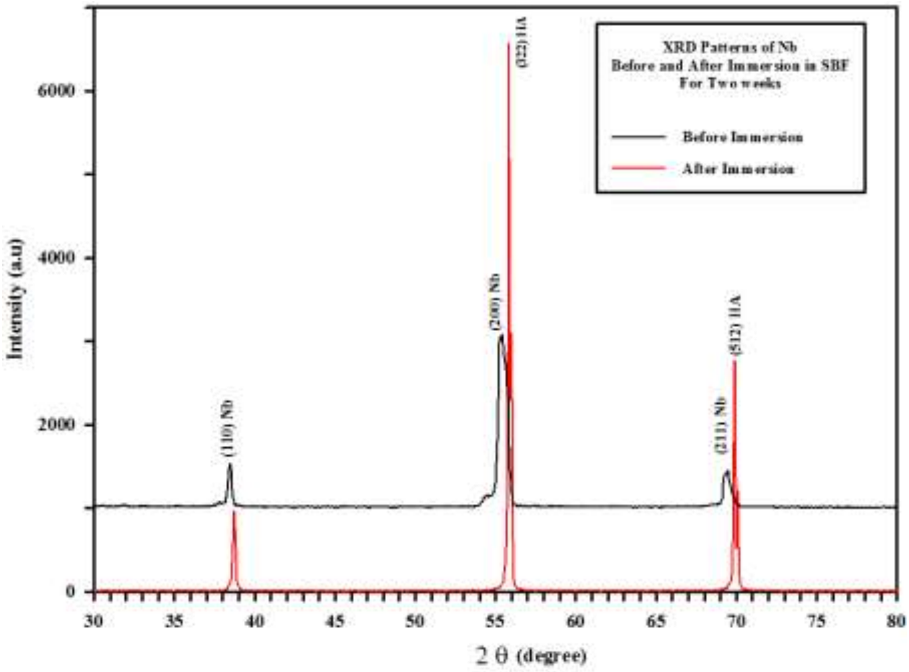

**Figure 9:** X-ray diffraction pattern of Nb substrates before and after immersion in 5XSBF for two weeks

## 4. Discussion

Many studies have focused on surface characteristics and chemical composition as a way to provide improved osseointegration process**. [25](Mendonça et al , 2009).** One reason is the fact that the physical, chemical and biochemical properties of the implant surface control the performance-relevant processes such as protein adsorption, cell-surface interaction and cell-tissue development at the interface between the body and the biomaterial **[26](Textor et al, 2001)**.

### 4.1 Pretreatment methods before immersion

Acid etching of cpTi and Nb alloy in HCl under inert atmosphere was performed in this study as an alternative pretreatment to the other traditional biomimetic treatments to obtain a uniform initial metal surface before alkali treatment and provide a favorable environment for ion exchange on the metal substrate when immersed in alkali solution and SBF.[27](al-mudarris 2006)

**Kumta et al in 2005** revealed that by increasing the NaOH concentration, it renders the synthesized CaP as stoichiometric and more stable under invariant physiological pH conditions. As a result, 10M NaOH solution was used in this study for soaking the samples for 24 hour.[28]

It was noticed that during the alkali treatment, the surface-passive oxide layer partially dissolves into alkaline solution because of the corrosive attack of hydroxyl groups. Furthermore, it has been observed that the surface morphology appeared more randomly oriented.as shown in studies of **Gil et al in 2002** [6].

This method was also successfully performed in this study on Nb and on cpTi as confirmed by SEM and EDX after immersion in SBF. This is agreed with the study of **Gross and Berndt, 2002**, which indicated that the apatite precipitation could be induced on the surface of other materials.[29]

### 4.1.2 Biomimetic coating method

various research suggests the application of various biomimetic coatings that can be beneficial for implant therapy success [30]**(Preshaw, 2015)**and [31]**(Kulkarni et al, 2014)**. This study employed an HA surface coating on the samples because of its proven osteoconductive properties [32]**(Lee et al,2014).** The biomimetic approach which exploits the advantageous consequences of using SBF solution to coat HA onto titanium and niobium surfaces was used. An HA layer on titanium surfaces using SBF is capable of achieving crystallinity and morphology similar to those of a bone-like apatite [34]**(Gandolfi et al, 2015)**. A favourable aspect of this study coincides with the proposition that increased crystallinity of HA coatings appears to slow resorption of HA[35]**(Sorensen et al,2015)**.

The type of CaP and the crystal size formed are largely dependent on the conditions of synthesis, i.e., presence of seeds, the degree of supersaturation, ionic strength, the immersion time, solution pH, temperature, etc. all are known to affect the course of synthesis of CaPbioceramics from aqueous solution **(Tas,2000) and (Macaskie et al, 2005)**.

In this study the results, obtained from using a 5 times concentrated SBF, are in consistence with the investigation of[24]**Chen et al in 2005** that the use of more than one time concentration will accelerate the formation of CaP with resultant similar properties.

The quantity of magnesium and carbonate, both inhibitors of crystal growth, may be adjusted for optimal attachment of coating and formation of a uniform, strong and wear resistant layer as concluded by **Gross and Berndt in 2002 and Layrolle et al in 2005**. According to these results the concentration of magnesium and carbonate used in this study was 10 and 50 mmol/L, respectively to control the size and crystallinity of the deposited layer. Sodium chloride, and other salts were added to maintain the ionic strength of the coating solution as previously indicated by **Layrolle et al in 2005**.

Regarding the temperature of the solution that was used in the immersion of specimens for different time periods was 37ºC to simulate the degree of body temperature and provide for better surface coating quality as shown in the results of **Backer et al in 2005**.

The pH range from 7.3-7.4 was also used in the present study to simulate the same condition of apatite formation inside the body as has been proven by **Macaskie et al in 2005** that the form of phosphate ion released intothe solution is pH-dependent, and the type of calcium phosphate precipitated would be dependent on the pH value. The major forms of which in aqueous solution would be H3PO4 and H2PO4− below pH 4.6, H2PO4− and HPO4−2 from pH 4.6 to 9.7 and HPO4−2 and PO4−3 for pH values above 9.7. In addition the precipitated crystal shape changed from a plate-like morphology to flaks by increasing the pH from 7.2 to 7.4 in accordance with the study of **Gross and Berndt in 2002**. Furthermore **Wang et al in 2004** showed that all coatings could be seen by eyes dissolving quickly and completely into acidic SBF.

Since the CaP precipitates with this process depend on the saturation of the solution with calcium and phosphate, the ratio of sample geometrical surface area to soaking solution volume S/V was 0.005 cm-1 used in this study depending upon the size of the medical device and the number of these devices that are to be placed together inside the vessel. This is agreed with **Saiz et al in2002,** who found that the formation of apatite is dependent on the amount of SBF used in the test. However in the study of **Manero et al in 2002** each cpTi plate of 10-10-0.7 mm in size was soaked independently in 40 ml of SBF.

The surface appearance after different periods of immersion in the SBF solution showed different steps of the nucleation and growth of the CaP layer. First, a few CaP nuclei appeared after 1 day. Once the first nuclei had appeared, growth of the CaP layer was very rapid. This confirmed the results of **Manero et al in 2002** that the stage that controls the kinetics of the process is precisely the nucleation stage. As the immersion time was progressed, these films exhibited a partially amorphous nature and became more homogenously coated the surface. This is agreed with **Oliveira et al in 2002 and Backer et al,2005**.

It can be supposed that the weight of the soaked samples should increase with time indicating the formation of a surface layer. However, the increase in the weight reached its maximum value after 6 days and began to decrease that almost to zero after 12 days of immersion. This is in agreement to the results obtained by **Jonasave et al in 2004**, when they showed that after 20 days of immersion of cpTi in 1.5 SBF, the increase in weight was less than that obtained after 14 days.

Generally, biomimetic coating processes are performed for about seven to fourteen days **(Oscar et al, 2015)**.

It was also noticed that during immersions, a milky white precipitate formed in solution. This is because of the supersaturation of the solution used. Itwas also determined that this so-called homogenous deposition (in-solution deposition) was a direct result of the surface conditions of the substrata as stated by **Backer et al in 2005**.

The results gained supposed that when Ti andNb plates immersed in NaOH liquid , sodium titanate and sodium niobate hydrogel layers formed on its surface respectively, this is similar to a previous study results in which porous sodium titanate and sodium niobatetitanate hydrogel layers formed when Ti-Nb alloy plates treated with NaOH solution **(Rosenberg et al,2003) and (kokubo et al 2004).**

After that plates soaked in asimulated body fluid (SBF ) for 14 days then calcium phosphate compounds attached to the surface such behaviour is called bioactivation of niobium and titanium and its like the bioactivation reported previously of alkali treated Ti and Ti-Nb alloy (kokubo et al 2004) and (Rosenberg et al,2003)

The CaP compounds precipitation on the alkali treated metal plates may be attributed to the morphology of the surface layer of the plates since immersion of treated plates into an electrolyte solution (like SBF) making its surface as cathodic reaction of oxygen reduction:

$$O2 + 2H2O + 4e^- \longrightarrow 4OH^-$$

This reaction lead to a local increase in pH at the surface which is more pronounced at the porous surface of the plates that may decrease the solubility of the HA byincreasing its ionic activity product according to the following equilibrium in SBF:

$$10Ca^{2+}+6PO_4^{3-}+2OH^- \longleftrightarrow Ca_{10}(PO4)_6(OH)_2$$

So HA start to precipitate on the surface of the metal forming nuclei of apatite which then grow by absorbing more $Ca^{2+}$ and $PO_4^{3-}$ from SBF that is supersaturated with respect to the apatite.**(Godley et al, 2004)**

The SEM micrographs for Ti and Nb supported the formation of coating layer on the plates after SBF immersion and the shapes of the particles were different between Ti and Nb, and this may be attributed to the difference in surface layer composition as this is supported by EDX result due to difference in surface roughness and hardness between the two metals.

The EDX analysis indicated a continuous calcium and phosphorus deposition on the surface. The prominent peaks corresponding to the deposited layer accompanied with a gradual decrease of titanium and niobium peaks intensity, which revealed the gradual thickening of the coated layer.

The XRD data showed peaks indicating the presence of crystalline HA phases as well. It can be concluded from the results that it was possible in this study to obtain a bioactive thin and relatively uniform coatings of HA on both pure titanium and pure niobium surfaces and thus niobium may be used as a good alternative to titanium in implantology.

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