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Bonelike apatite formation on niobium metal treated in aqueous NaOH

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The essential condition for a biomaterial to bond to the living bone is the formation of a biologically active bonelike apatite on its surface. In the present work, it has been demonstrated that chemical treatment can be used to create a calcium phosphate (CaP) surface layer, which might provide the alkali treated Nb metal with bone-bonding capability. Soaking Nb samples in 0.5 M NaOH, at 25 °C for 24 h produced a nano-porous ~ 40 nm thick amorphous sodium niobate hydrogel layer on their surface. Immersion in a simulated body fluid (SBF) lead to the deposition of an amorphous calcium phosphate layer on the alkali treated Nb. The formation of calcium phosphate is assumed to be a result of the local pH increase caused by the cathodic reaction of oxygen reduction on the finely porous surface of the alkali-treated metal. The local rise in pH increased the ionic activity product of hydroxyapatite and lead to the precipitation of CaP from SBF that was already supersaturated with respect to the apatite. The formation of a similar CaP layer upon implantation of alkali treated Nb into the human body should promote the bonding of the implant to the surrounding bone. This bone bonding capability could make Nb metal an attractive material for hard tissue replacements.

Introduction

An artificial biomaterial (metal, ceramic or polymer) implanted into a bone is typically encapsulated by a scar tissue that walls it off from the surrounding hard tissue. The presence of this capsule prevents successful osteointegration of the implant thereby compromising its long-term stability. At the same time, there are several Calcium phosphate (CaP) ceramics (e.g. hydroxyapatite (HA)) and low-SiO_2 glass compositions that can bond to a living bone and even encourage bone tissue formation [1]. These ''bioactive'' materials have found clinical use as bone fillers and bone substitutes in various non-load-bearing locations. Being intrinsically brittle, however, bioactive glasses and ceramics cannot be used in the highly loaded long bones (femur and tibia). On the other hand, metal alloys that are sufficiently strong and tough to withstand the loads sustained by long bones normally do not exhibit any bone-bonding ability. To achieve better osteointegration, surgical metals (especially titanium) are plasma sprayed with HA [2]. These HA coatings, however, suffer from poor adhesion to the metal substrate and degrade in the body environment after implantation.

Recently, it has been shown that a bioactive amorphous sodium titanate layer can be formed on a pure titanium surface by soaking this metal in alkali solutions [3–5]. Thus treated Ti spontaneously and tightly bonds to the bone via the formation of a bone-like apatite layer as in the case of bioactive glasses and ceramics. Titanium alloys such as Ti–6Al–4V respond similarly to the alkali treatment although the effect of the alloying species on the bone-bonding ability is not clear yet [6].

An important requirement of implants designed to replace or interact with bone, is a low elastic modulus matching as closely as possible that of the surrounding bone tissue [1]. In our previous research [7] it has been shown that a low modulus β-Ti–45 wt % Nb alloy $(E = 62\,\text{GPa})$ subjected to a simple electrochemical treatment in an alkali solution could exhibit bioactive behavior in a simulated body fluid (SBF), similarly to the earlier studied Ti alloys. In the present work, the possibility of bioactivating pure niobium (Nb) has been evaluated. Nb was chosen for this study since it is one of the components of the low modulus β-Ti–45 wt % Nb alloy, however, unlike titanium, bioactive behavior of Nb has never been reported. At the same time, tantalum (Ta) – a metal successfully used in medical implants and closely resembling Nb was reported to become bioactive after the alkali treatment [8]. Given the much lower cost, density and elastic modulus of Nb compared to Ta, this metal could become an attractive biomaterial, especially if made bioactive.

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TABLE I Ionic concentrations (mM) and pH of the simulated body fluid compared to those of the human blood plasma

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}	pH
SBF	142.0	5.0	1.5	2.5	148.8	4.2	1.0	0.5	6.9 ± 0.1
Blood plasma	142.0	5.0	1.5	2.5	103.8	27.0	1.0	0.5	7.20–7.40

Materials and methods

Chemical soaking treatment

Wrought Nb sheets (99.8% purity) were purchased from Performance Alloys, Ltd. The sheets were cut into plates, $15 \times 10 \times 2\,mm^3$ in size, polished and ultrasonically cleaned in ethyl alcohol. The specimens were soaked in an aqueous solution of 5 or 0.5 M NaOH at a constant temperature of either 60 °C or 25 °C for 24 h. The course of experiments that guided our way in choosing the conditions is described in Section 3. The temperature was maintained using a water bath. After the treatment, the substrates were ultrasonically cleaned in deionized water.

Soaking in simulated body fluid

In order to check the material's capability to spontaneously form a bonelike apatite layer *in vitro*, alkali treated samples were soaked in a SBF at 36.5 ± 0.5 °C, for six to 20 days. The temperature was maintained using a water bath, no stirring was used. The SBF was prepared by dissolving reagent-grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$ and Na_2SO_4 into deionized water. The final compositions and pH of the SBF (vs. human plasma) are given in Table I. Chemically treated and untreated Nb plates were suspended in the solution on a nylon string. One to five samples were soaked in 0.5 l solution. After soaking for selected time periods, the specimens were removed from the fluid, washed with deionized water and dried in air. The pH of the SBF before, after and during soaking samples was measured using a pH meter.

After the alkali treatment and SBF soaking, the samples were examined and characterized using high resolution scanning electron microscopy (HRSEM), SEM equipped with an energy dispersive electron probe X-ray analyzer (EDS), X-ray diffraction (XRD) and Auger Electron Spectroscopy (AES).

Results

When Nb specimens were soaked in 5 M NaOH for 24 h at 60 °C (conditions previously reported to produce bioactive layers on Ti metal [4, 5]), no changes were observed in the surface layer by SEM/HRSEM. When the same treatment was performed in 0.5 M NaOH, a porous surface layer with a fiber-like morphology was formed (Fig. 1) that suffered from a very poor adhesion to the substrate. Soaking Nb specimens in the 0.5 M NaOH solution for 24 h at a lower temperature of 25 °C yielded a porous surface film with a very fine morphology, Fig. 2, which strongly adhered to the Nb substrate. It can be seen that the surface structure formed is homogenous with an average pore size of around 100 nm, and it is similar to the bioactive surface films observed on Ti and its alloys [4–7].

According to the AES depth profile (Fig. 3(a)), the surface film formed on Nb after 24 h soaking in 0.5 M NaOH at 25 °C is approximately 40 nm thick, and it contains Nb, oxygen and Na. The presence of Na and O is also confirmed by the results of the EDS analysis (Fig. 4(a)). By analogy with Ti metal where the

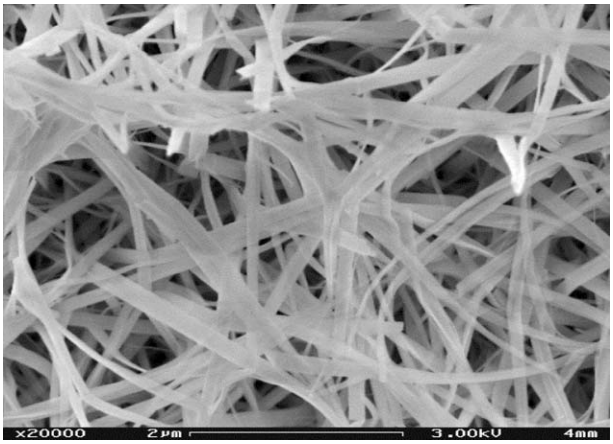
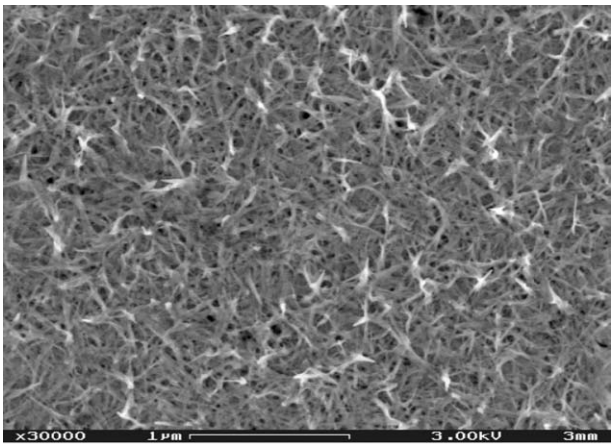


Figure 1 A porous Nb–Na–O layer on Nb surface formed by the alkali treatment in 0.5 M NaOH, at 60 °C, for 24 h.



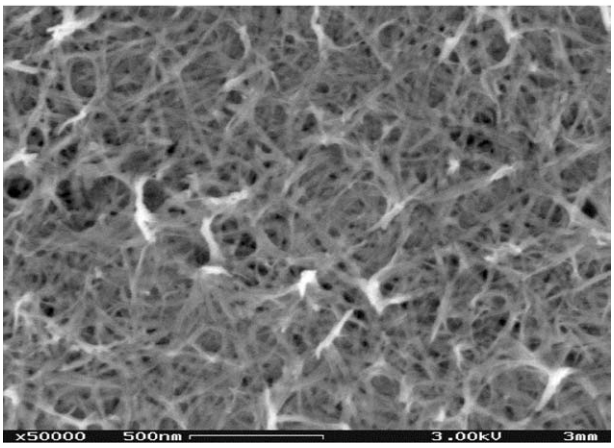


Figure 2 A nano-porous Nb–Na–O layer on Nb surface formed by the alkali treatment in 0.5 M NaOH at 25 °C for 24 h.

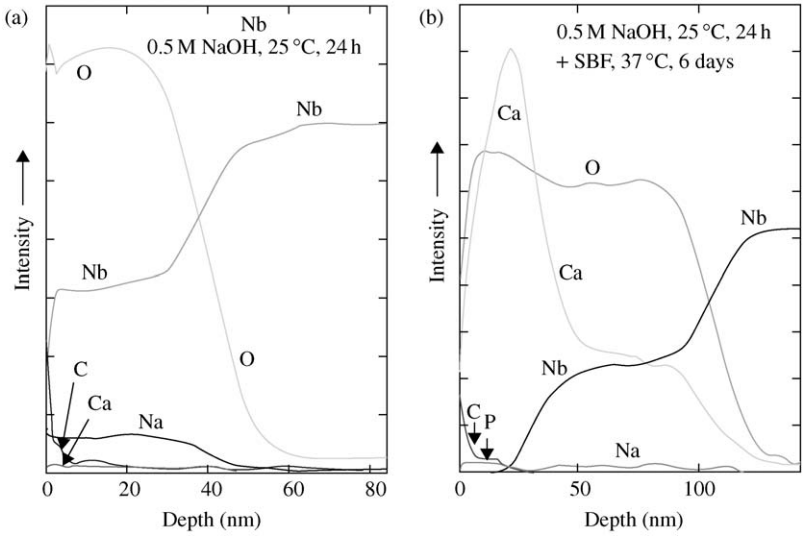


Figure 3 AES depth profile taken from the surface of the alkali treated Nb specimen (0.5 M NaOH, 25 °C, 24 h) (a), and subsequent soaking in SBF for six days (b). The depth scale is relative to the sputtering rate of Ta_2O_5. The relative signal intensity does not necessarily reflect the relative element content.

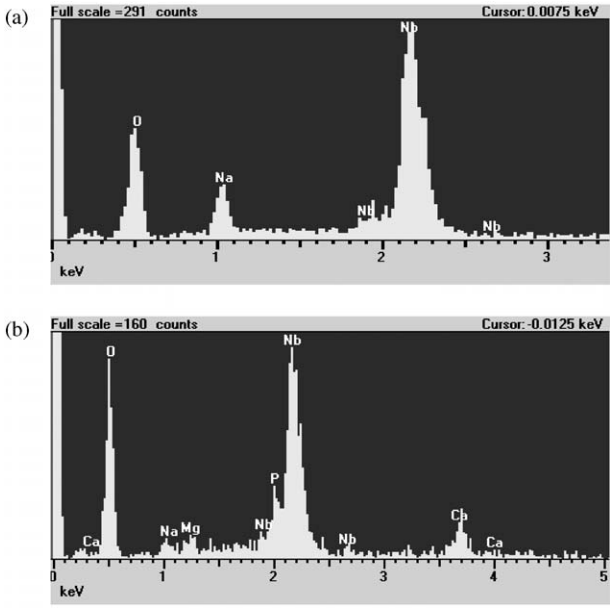


Figure 4 EDS spectrum of Nb surface after the alkali treatment (0.5 M NaOH, 25 °C, 24 h) (a), and subsequent soaking in SBF for six days (b).

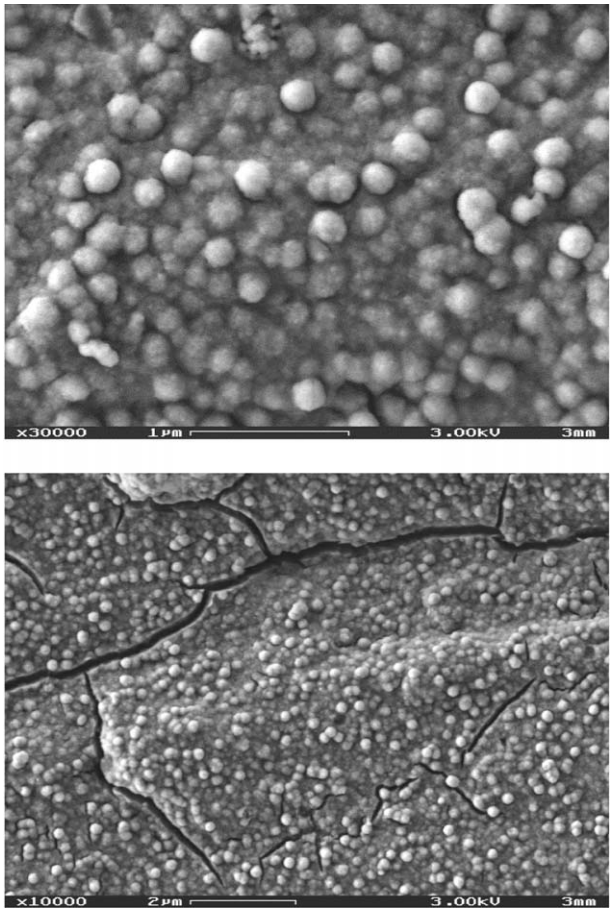


Figure 5 A calcium-phosphate layer formed on the surface of the alkali treated (0.5 M NaOH, 25 °C, 24 h) Nb specimen after soaking in SBF for six days.

formation of a porous sodium titanate hydrogel was observed after soaking in NaOH [4, 5], we assume that the porous layer formed on our Nb samples is a sodium niobate hydrogel.

After the soaking of NaOH-treated Nb samples in SBF for six days, a continuous layer of spherical grains was formed on their surface (Fig. 5). Morphologically, the layer in Fig. 5 resembles the CaP layer observed on the surface of bioactivated Ti–Nb alloy after soaking in SBF (Fig. 6). The formation of CaP layers with a similar morphology was reported for titanium and several Ti alloys [4–7]. The cracks in the calcium phosphate layer in Fig. 5(b) must have formed as a result of contraction of the porous hydrated layer when the sample was dried after soaking in SBF – a phenomenon previously observed on alkali-treated Ti [9].

EDS spectrum in Fig. 4(b) suggests that the layer formed on Nb specimen after soaking in SBF is also a calcium phosphate since it contains appreciable amounts of Ca, P and O. Phosphorus could be identified with certainty by EDS although the peak of P overlaps with that of Nb (from the underlying substrate). It can also be seen that very few Na was left on the surface after soaking in SBF. XRD analysis of SBF-soaked Nb specimens did not detect any peaks that could be

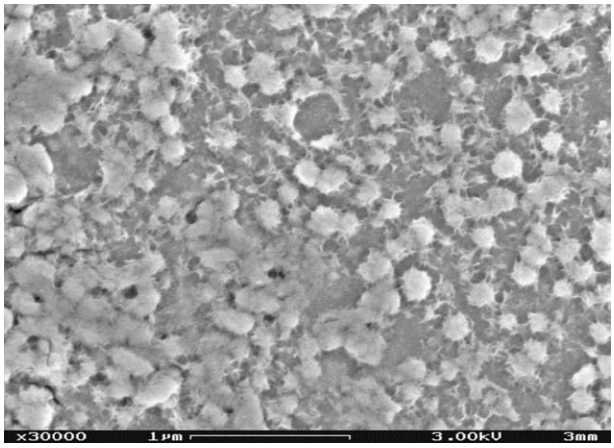


Figure 6 A calcium-phosphate layer formed on the surface of electrochemically alkali treated (0.5 V, 5 M NaOH, 60 °C, 30 min) Ti–45Nb specimen after soaking in SBF (buffered with TRIS) for 20 days.

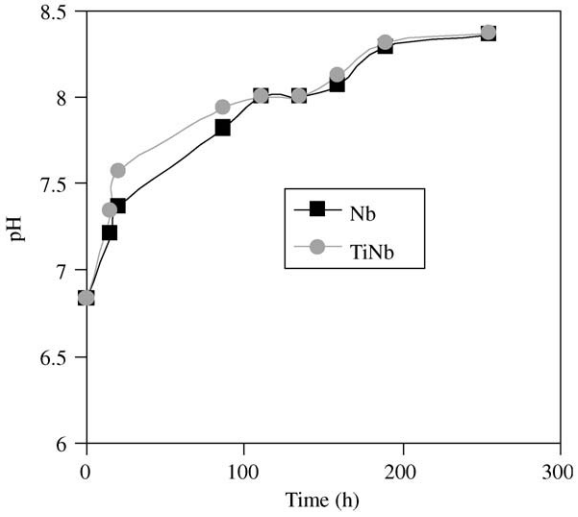


Figure 7 pH increase of unbuffered SBF as a function of soaking time for the alkali treated Nb (0.5 M NaOH, 25 °C, 24 h) and electrochemically alkali treated Ti-45Nb alloy (0.5 V, 5 M NaOH, 60 °C, 30 min).

attributed to CaP compounds, indicating that the layer is very thin or amorphous.

AES depth profile in Fig. 3(b) confirms that a thin layer containing Ca, P and O and practically no Nb (presumably CaP) is present on the surface of SBF-soaked Nb. The depth scale does not reflect the real thickness of the CaP layer since the depth profile is relative to the sputtering rate of Ta_2O_5. The decrease in the amount of Na is also in agreement with the EDS results in Fig. 4(b). Below the CaP layer, an intermediate zone of Ca, Nb and O is observed in the AES profile in Fig. 3(b) – presumably a calcium niobate layer. A similar calcium titanate layer was reported to form on alkali treated Ti as a precursor to the deposition of calcium phosphate [10]. The gradual change in Ca concentration could suggest the occurrence of chemical bonds between the coatings and the metallic substrate. It should be noted, however, that the interpretation of the AES profile is not unambiguous since the precipitation of CaP inside the pores of the oxide layer could also result in a diffuse depth profile.

The pH of the SBF was found to increase gradually during the soaking of alkali treated Nb, Fig. 7, indicating the increasing concentration of OH^- ions. A similar increase of pH was observed during the soaking of electrochemically alkali treated Ti–45Nb alloy samples in an unbuffered SBF (Fig. 7).

Discussion

The results obtained suggest that when Nb metal is soaked in NaOH solution, a sodium niobate hydrogel layer with very fine porosity is formed on its surface, similar to the previously reported porous sodium titanate and sodium niobate titanate hydrogel layers formed during different alkali treatments of Ti and Ti–Nb alloys, respectively [4–7]. When the NaOH treated Nb is immersed in a simulated body fluid, a calcium phosphate compound is formed on its surface. Such behavior of the NaOH treated Nb is considered an indication of bioactivity and is, again, similar to the bioactive behavior of the alkali treated Ti and Ti–Nb alloys [4–7].

The observed pH increase during the soaking of alkali-treated Nb and Ti–Nb alloy in SBF, Fig. 7, is in agreement with the previous studies of alkali-treated Ti and its alloys [4–6]. It is believed the pH raise caused by local events on the surface of alkali-treated metals decreases the solubility of HA by increasing its ionic activity product according to the following equilibrium in SBF [4]:

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

As a result, HA starts to precipitate on the metal surface. Once the apatite nuclei have been formed, they spontaneously grow by consuming the calcium and phosphate ions from the surrounding fluid, because SBF is already supersaturated with respect to the apatite.

The increase of pH on the surface of alkali-treated Ti and its alloys has been previously explained in Refs. [4–6] by ion exchange between the sodium–titanate layer and the SBF. It has been suggested that Na^+ ions are released into SBF and replaced by H_3O^+ from the solution – a process leading to the local pH increase. The EDS and AES results of our work, Figs. 3 and 4 also indicate that Na^+ ions leave the sodium niobate layer when alkali-treated Nb samples are immersed into SBF. At the same time, the depth profile in Fig. 3(b) suggests that Na^+ could be replaced by ions other than H_3O^+, for example, Ca^{2+}, in which case the ion exchange would not affect the pH. The exchange between sodium ions from the surface oxide layer and Ca ions from SBF prior to CaP deposition on alkali treated Ti was also observed in [11]. We assume that the local pH increase on the alkali-treated metal surface is related to the morphology of the surface layer rather than to its chemical composition. When a metal is immersed into an O_2-containing electrolyte solution (e.g. SBF), its surface becomes a site of the cathodic reaction of oxygen reduction:

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$$

A local pH increase caused by this reaction might be negligible for smooth surfaces, however it should be much more pronounced for the finely porous surfaces of

bioactivated metals having a very large surface area. It is this local raise in pH that leads to the deposition of HA.

The formation of the CaP layer is in accordance with a previous study of bone crystal growth mechanisms, in which crystallites were analyzed at different development stages, and a four-stage development model was proposed [12]. The first step is held to be ionic adsorption onto the substrate, leading to the nucleation of several nanometer-size particles. Then, the stable critical nuclei grow by further ion deposition. The last stage observed is lateral fusion of the crystals.

The CaP layer formed on alkali treated Nb is assumed to be amorphous, since no crystalline compounds were detected by XRD. A similar assumption was made in our previous work [7] regarding the CaP layer formed on electrochemically alkali treated Ti–45Nb alloy. Amorphous calcium phosphate (ACP) was previously shown to consist of characteristic spherical or near spherical bodies with a wide range of sizes (approximately 70–150 nm), some of the larger ones being clusters of three or more fused spherules [13]. The morphology of the CaP layer in Fig. 5 closely matches this description.

The formation of amorphous CaP is not surprising considering the processes by which organisms form minerals in nature. There is ample evidence that an unstable amorphous calcium phosphate is the first to be deposited in the course of formation and maturation of bone mineral. It has been concluded that only a portion of this amorphous precursor phase transforms into a poorly crystallized bone apatite, while the remainder persists as a separate amorphous phase even in the mature bone [14].

Parameters which lead to an amorphous phase and subsist in our experiments are high supersaturation and a large unit cell, and so HA, which has a large unit cell, is expected to form amorphous precipitates in SBF. In addition, it has been previously shown [14] that when HA is precipitated from a highly supersaturated solution of calcium and phosphate, an unstable calcium phosphate precursor phase is observed.

The unstable amorphous phase can be stabilized by a number of chemical species in solution, for example, Mg^{2+} ion. Mg enters the prenuclei structures preventing further Ca HA development by creating a structural mismatch [14]. The EDS spectrum in Fig. 4(b) indicates that the CaP layer formed on our samples also contains some Mg.

Conclusions

The essential condition for a biomaterial to bond to the living bone is the formation of a biologically active bonelike apatite on its surface. It has been demonstrated *in vitro* that chemical treatment can be used to create a CaP surface layer, which might provide the Nb metal soaked in 0.5 M NaOH, at 25 °C for 24 h with bone-bonding capability. The treatment produced ~ 40 nm thick amorphous nano-porous bioactive sodium niobate hydrogel surface layer that further induced the deposition of a CaP layer during soaking in a SBF.

The formation of calcium phosphate is assumed to be a result of the local pH increase caused by the cathodic reaction of oxygen reduction on the finely porous surface of the alkali-treated metal. The local rise in pH increases the ionic activity product of HA and leads to the precipitation of CaP from SBF that is already supersaturated with respect to the apatite. The formed CaP layer seems to be amorphous, similar to the first mineral deposited during the formation and maturation of the living bone. The formation of a similar CaP layer upon implantation of alkali treated Nb into the human body should promote the bonding of the implant to the surrounding bone. This bone bonding capability could make Nb metal an attractive material for hard tissue replacements.

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