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Investigation of boundary conditions for biomimetic HA deposition on titanium oxide surfaces

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Abstract To improve the clinical outcome of metal implants, i.e. earlier loading and reduction of the incidence of revision surgery, better bone bonding ability is wanted. One method to achieve this is to change the surface chemistry to give a surface that facilitates bone bonding in vivo, i.e. a bioactive surface. Crystalline titanium oxide has recently been proven to be bioactive in vitro and is an interesting option to the more common hydroxylapatite (HA) coatings on implants. A materials possible in vitro bioactivity is tested through soaking in simulated body fluid and studies of possible HA formation on the surface. For bioactive materials, the formed HA layer can also be used as a coating. The aim of the current paper is to investigate some boundary conditions for HA formation on crystalline titanium oxide surfaces regarding influence from coating thickness, soaking time and soaking temperature. The influence from soaking time and temperature on the HA growth were investigated on oxidised Ti samples, (24 h at 800°C) resulting in a rutile surface structure. The oxidised samples were tested at three temperatures (4, 37 and 65°C) and four times (1 h, 1 day, 1 week and 4 weeks). The influence from titanium coating thickness on the HA growth was investigated via depositing thin films of crystalline titanium dioxide on Ti plates using a reactive magnetron sputtering process. Four different PVD runs with coating thicknesses between 19 and 74 nm were tested. The soaking temperature had an effect on the HA formation and growth on both rutile surfaces and native oxide on Ti substrates. Higher temperatures lead to easier formation of HA. It was even possible, at 65°C, to grow HA on native titanium oxide from soaking in PBS. The coating quality was better for HA formed at 65°C compared to 37°C. All PVD-coatings showed HA growth after 1 week in PBS at 37°C, thus even very thin coatings of crystalline titanium oxide coatings are bioactive.

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1 Introduction

Titanium is widely used as permanent implant in orthopaedic and dental applications. It is well known that Ti shows a stable interface towards bone (osseointegration). The good biological properties are due to beneficial properties of the native oxide (TiO_2) that form on Ti when exposed to oxygen. The native titanium oxide on Ti is amorphous and very thin, 3–7 nm [1, 2]. Amorphous titanium oxide is bioinert and is not considered to bond to bone. The lack of bone bonding leads to increased risk of implant failure. Bone bioactive materials form a stable unit with bone through a spontaneous formation of hydroxyapatite (HA) on their surface. The formed HA layer acts as a bonding layer to the bone and a bond develop.

Titanium dioxide has two bioactive crystal structures, anatase and rutile [2]. The bioactivity of anatase and rutile is due to their ability to adsorb and dissociate water, creating Ti–OH groups and a negative surface charge. Titanium's native oxide can also dissociate water but shows no bioactivity [3]. The crucial part is how well matched the interface between the organized hydroxylated surface and HA nuclei are and also the surface charge.

When observing hydroxylated $110_{anatase}$ and 0001_{HA} it is suggested that there are three important parts in matching their interfaces.

- Hydrogen bond interaction. Ti–OH groups can form hydrogen bonds with OPO_3^{-3} on 0001_{HA}, and also with OH^- on the 0001_{HA}.
- Crystal lattice matching. How the Ti–OH groups are arranged on the $110_{anatase}$ matching the 0001_{HA}.
- Stereochemical matching. The anatase OH^- arrangement surrounding a Ca^{2+} ion along the *c*-axis of HA, resulting in oriented nucleation [4].

The 101_{rutile} surface also has a lattice match with 0001_{HA} [5]. The nucleation of the crystallized species and their orientation tend to be determined more by stereochemical matching than lattice matching [6].

Anatase is speculated to have a higher bioactivity than rutile, due to a better lattice match with HA and a higher acidity, lower surface ζ-potential, caused by a larger number of hydroxyl groups on the surface [7]. The degree of surface acidity at a given pH is the value of the surface ζ-potential. The surface ζ-potential is lower for a more acidic surface.

It has been shown that deposition of HA on anatase, at pH 7.4, is faster than on rutile at the same pH, while a less negative ζ-potential will inhibit the HA nucleation [7]. Interestingly a rise in temperature and ion concentration increases the growth rate of HA [8].

There are different methods available to form crystalline TiO_2 on a surface [2, 9]; methods that change the surface structure, e.g. oxidation, and methods that deposits a coating on the surface, e.g., sol–gel or physical vapour deposition. There are some obvious drawbacks with using methods that change the surface structure since they include a heat treatment step, which reduces the mechanical properties of the base metal. Therefore, mainly the low temperature coating methods are of interest for biomedical applications. The method to test surfaces for bone bioactivity through soaking in simulated body fluid (SBF) or phosphate buffered saline (PBS) is well established [2]. The same method can also be used as a low temperature coating method for HA coatings [10, 11]. The HA layer grows with soaking time and can, depending on the surface pre-treatment method, reach some 10–50 μm.

The aim of this paper is to investigate the limits for HA formation on titanium oxide surfaces as coupled to soaking time and temperature as well as titanium oxide coating thickness.

2 Materials and methods

Two sets of experiments were conducted:

1. Immersion of oxidised surfaces in PBS and studying the possible HA formation as function of soaking time and temperature.
2. Immersion of sputter coated Ti plates in PBS with varying coating thickness with fixed immersion time and temperature.

2.1 Materials

Crystalline surfaces of titanium oxide were manufactured through two routes:

1. Pure titanium sheet metal pieces, 20 × 20 × 0.5 mm, were ultrasonically washed in acetone, ethanol and deionised water for 5 min, respectively, and then blow dried with pressured air. They were then put in a furnace for 36 h, starting at room temperature and heated to 800°C in air at the rate of 5°C per minute during the first 2 h 40 min. After 24 h at 800°C the heating was turned off, letting the titanium pieces cool slowly for about 10 h.
2. To achieve thin films of crystalline titanium dioxide a reactive magnetron sputtering process was used. A titanium target was mounted in a physical vapour deposition (PVD) chamber (Balzers 640R) with oxygen as reactive gas. The substrates were cleansed before deposition in an alkaline cleaning agent

Table 1 Deposition sequence

		Total pressure (mbar)	P_{Ar} (mbar)	Time (min)	Arc current (A)	Substrate voltage (V)
1	Pump	2×10^{-5}	0	~60	0	0
2	Heat	3×10^{-3}	3×10^{-3}	45	180	0
3	Etch	1.6×10^{-3}	1.6×10^{-3}	5	140	−200
4	Coat	4.5×10^{-3}	3.0×10^{-3}	5, 10, 15 or 20 min	150	−20
	Magnetron effect 1.5 kW		O_2 partial pressure 1.5×10^{-3} mbar			
5	Pump	2×10^{-5}	0	–	0	0
6	Cool	1×10^{2}	0	20	0	0
7	Pump	2×10^{-5}	0	–	0	0

Table 2 Ion concentrations in Dulbecco's PBS (10^{-3} M)

Ion	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HPO_4^{2-}
Blood plasma	142.0	5.0	1.5	2.5	103.0	1.0
PBS	145.0	4.2	0.49	0.91	143	9.6

(UPON, pH 11.6) in an 60°C ultrasonic water bath for 5 min and then rinsed in deionised water, followed by 5 min of ultrasonic bath of ethanol. The substrates were carefully dried with compressed air and then mounted on the substrate holders. Four different PVD runs were made with the coating times: for 5, 10, 15 and 20 min. The titanium oxide deposition was performed in seven steps according to Table 1. At each run six titanium substrates were PVD processed. A reference sample of silicon 100 mm wafer was sputtered for 20 min with the same parameters as for the titanium substrates. This reference was later used for measuring and estimating the thickness of the sputtered layers on the titanium substrates.

2.2 Bioactivity testing

All oxidised and PVD samples were cleansed and prepared the same way before tested for in vitro bioactivity (i.e. HA growth). They were ultrasonically washed in acetone, ethanol and deionised water for 5 min each and blow dried with compressed air. Then the samples where soaked in 40 ml of preheated (37°C) Dulbecco's Phosphate Buffered Saline (Sigma Aldrich, PBS), see Table 2. The PBS was changed every week to new preheated PBS to maintain the right test temperature and concentration. To end a test, the samples were rinsed with deionised water and air dried slowly at room temperature.

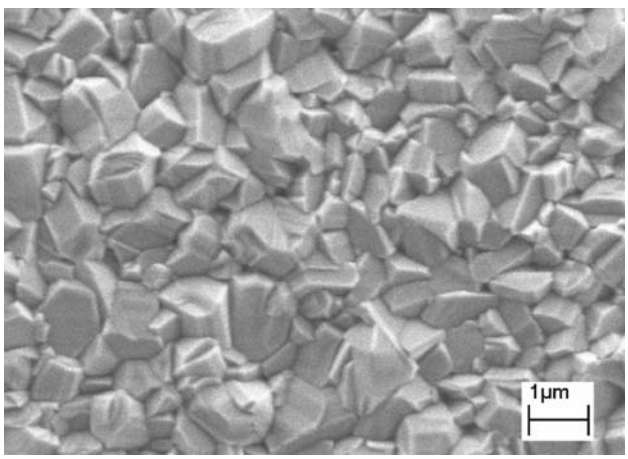

Fig. 1 SEM image of an oxidised titanium sample with a rutile surface. The crystal size is approximately 0.3–1 µm

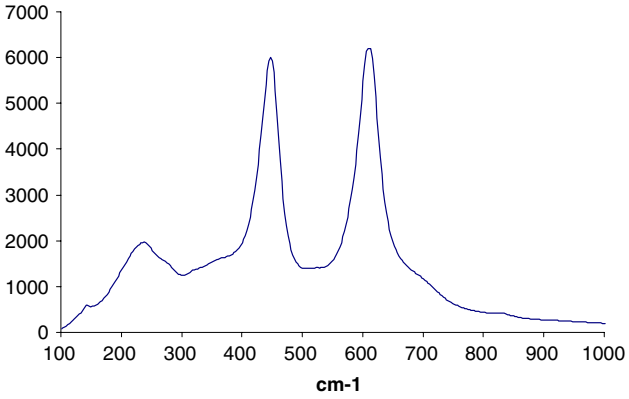

Fig. 2 Raman spectrum of furnace sample, showing characteristics of rutile

Table 3 HA growth for temperature versus time test matrix on oxidised rutile surfaces

	1 h	1 day	1 week	4 weeks
4°C furnace	–	–	–	–
37°C furnace	–	HA	HA	HA
65°C furnace	–	HA	HA	HA

Table 4 HA growth for temperature versus time test matrix on reference samples with native titanium oxide

	1 h	1 day	1 week	4 weeks
4°C reference	–	–	–	–
37°C reference	–	–	–	–
65°C reference	–	–	HA	HA

2.3 Temperature during and time for PBS immersion

Oxidised samples were tested in a matrix with respect to temperature versus time, three temperatures (4, 37 and 65°C) and four times (1 h, 1 day, 1 week and 4 weeks). Two samples of oxidised titanium and one untreated used as reference, were tested at each temperature and time.

2.4 PVD coating thickness (deposition process time)

The samples from the four PVD times were soaked in PBS at 37°C, for 1 week. Five samples were tested for each coating thickness.

2.4.1 Surface analysis

X-ray diffraction (XRD) analysis was performed on a Siemens D5000 diffractometer with parallel beam geometry.

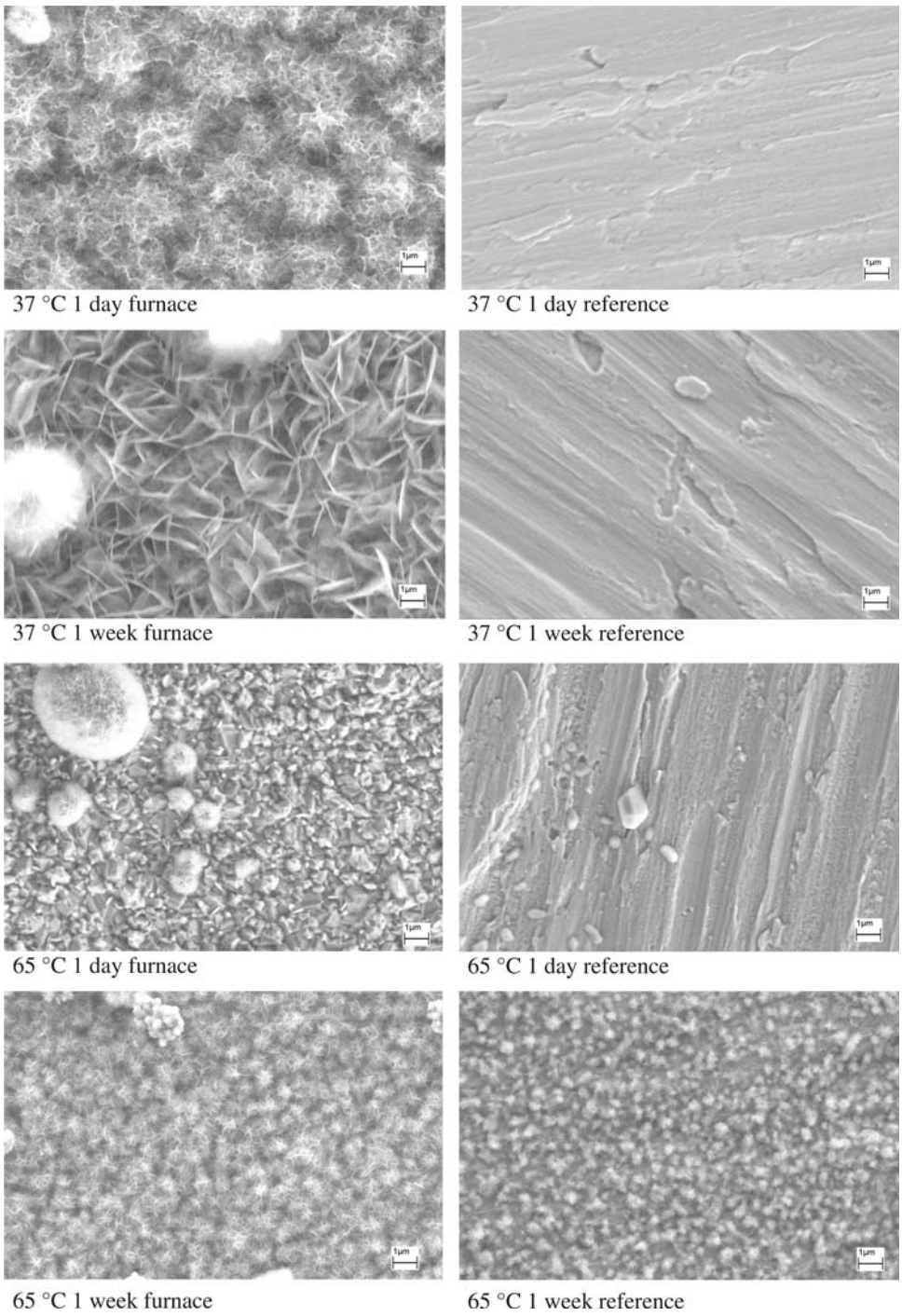

Fig. 3 SEM of HA formation at 37°C and 65°C on furnace treated samples and untreated references. None of the reference samples showed any HA growth except the 65°C immersed for 1 week

Detector scan with small incident angles ($\theta = 1°$) for the incoming X-ray was applied to specifically focus on the surface of the samples. The detector scanned continuously with a 2θ angle from 24° to 58° with the step size 0.1 and 10 s/step. The results were analysed and compared to JCPDF reference files[1] using the software EVA.

Scanning electron microscopy (SEM) studies were performed in a LEO 1550 with Field Emission Gun to analyse the samples surface. No actions were taken to avoid charge accumulation. Instead, a low acceleration voltage of 5 kV was used.

Raman spectroscopy (RS) was conducted using a Renishaw Micro-Raman system equipped with a green laser of wavelength 514.5 nm and a notch filter for suppression of the incident laser light

[1] Reference files: Hydroxylapatite 72–1243, Titanium oxide anatase 21–1272, Titanium oxide rutile 21–1276, Titanium 44–1294, NaCl Halite 05–0628

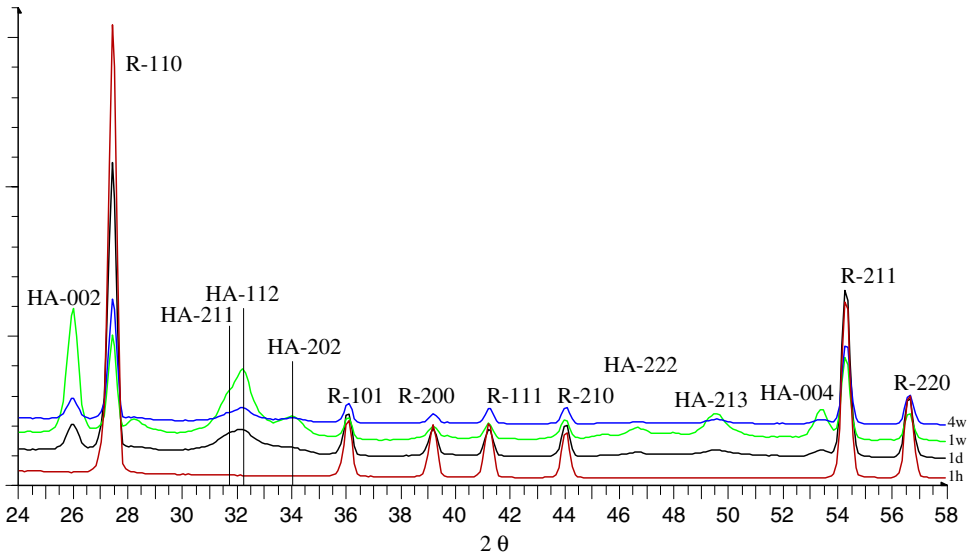

Fig. 4 XRD of PBS 37°C, oxidised sample. The 4 week sample has unexpectedly lower intensity of hydroxyapatite, probably due to poor adhesion and flaking. HA = hydroxylapatite, R = rutile

3 Results

3.1 Time and temperature testing on oxidised surfaces

SEM analysis of the oxidised samples showed polycrystalline rutile, with a size distribution of approximately 0.3–1 μm in diameter, see Fig. 1. Raman spectroscopy of the oxidised sample, see Fig. 2, showed the characteristic pattern of rutile [12].

The results from the HA growth testing are schematically presented in Table 3 for the oxidised samples and in Table 4 for the reference samples. PBS soaking at 4°C did not result in any detectable HA regardless of time or surface. The oxidised samples showed HA growth after 1 day of soaking in PBS at 37°C and 65°C but not at 4°C. When comparing the HA growth on the 37°C 1 day surface and the 65°C 1 day surface, they were different, see Fig. 3. On the 37°C there was a covering HA coating and on the 65°C there were "snowballs or sea urchins", spread on the surface. The 37°C samples had overall larger crystals than the 65°C samples. All samples soaked for 1 day or more at 37°C showed clear peaks from crystalline HA in diffraction, see Fig. 4.

3.2 Influence of coating layer thickness on the in vitro bioactivity

The PVD produced titanium oxide coatings all showed anatase phase composition but only with weak peaks (at ~25.3°[2]), see Fig. 5. The four other peaks come from titanium. On two of the titanium peaks there were a broadening towards the left, probably due to overlapping from a TiO_x phase. Using Raman spectroscopy, it could be showed that all coatings were identified as nano-crystalline anatase, see Fig. 6 [13]. The thickness of the titanium dioxide coating deposited during 20 min on the reference 100 silicon wafer was 74 nm, see Fig. 7. The thicknesses for the other three sputtering times were calculated from this measurement to 56 nm for 15 min, 37 nm for 10 min and 19 nm for 5 min.

All PVD-processed samples showed HA growth after 1 week of soaking, see Figs. 8 and 9. Interestingly, the surface that was under the montage gear during the coating process and thus was not covered with anatase showed no HA growth. The morphology of the HA coating was similar on the surfaces with coatings thicker than 20 nm, whereas the surface with the thinnest coating (19 nm) had smaller and less defined HA crystals, see Fig. 9.

4 Discussion

4.1 Effect from soaking temperature and time on HA growth

It is interesting that the shape of the HA crystals differed at different soaking temperatures. Smaller crystals in small groups at high temperature and large crystals evenly scattered on the surface at moderate temperature. The lower temperature, 4°C, gave no HA in spite of decreased solubility of ions in the solution. Minerals have in general higher solubility at higher temperature, which should lead to lower growth rate of HA. But the crystal growth rate increase with temperature due to increase ion mobility [8]. In the soaking experiment at 65°C, many small crystals were observed. The nucleation started later in 65°C than in

[2] Reference file: NaCl Halite 05–0628

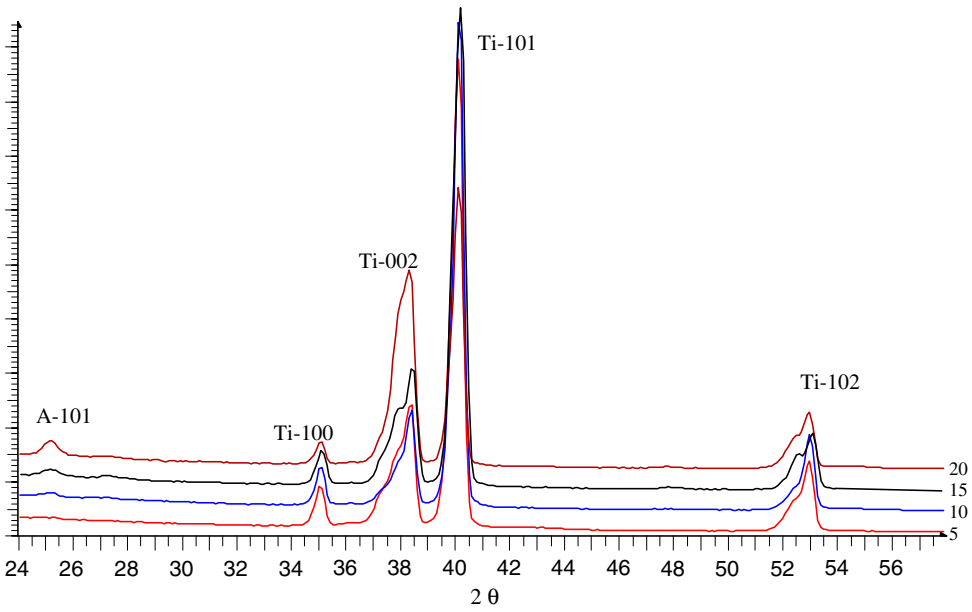

Fig. 5 XRD of PVD samples. The broadening towards the left of the Ti-002 and Ti-102 peaks is probably due to overlapping with some TiO_x peaks. Ti = Titanium and A = Anatase

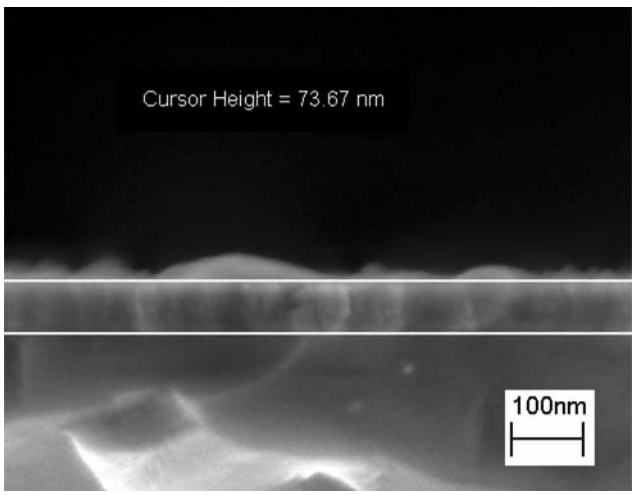

Fig. 6 SEM image of PVD sample 20 min, TiO_2 on silicon wafer

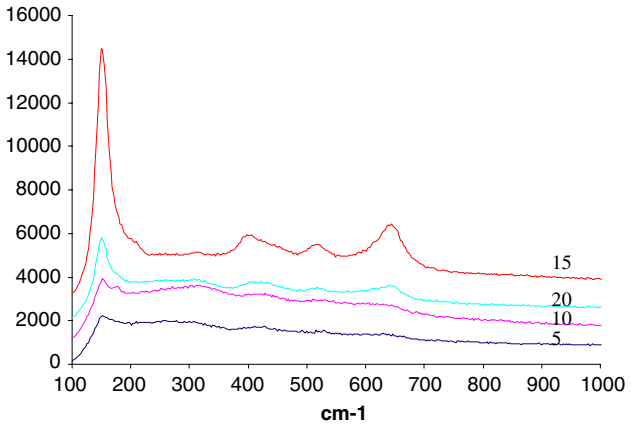

Fig. 7 Raman spectroscopy of PVD samples. The intensities of the curves have been shifted for better overview. A total of 1000 counts have been added to 10, 2000–20 and 3000–15. Note that 15 have higher intensity and better defined peaks

37°C PBS, concluded from comparing the SEM pictures of the morphology. This indicates that the formation and growth of HA is temperature sensitive with a maximum at a temperature close to 37°C.

The result that HA can grow on a native titanium oxide at elevated temperature was not observed in the literature search done within this work. Possibly it is the first observation of its kind. How the formation regime is connected to the temperature and why HA can grow on native titanium dioxide with raised temperature, requires further understanding of the mechanisms of HA growth. One possible explanation is that the surface potential changes with temperature, leading to a lower surface potential at higher temperature [14].

Comparing the adhesion of the deposited HA, all 37°C samples flake when inserted in the SEM vacuum after 1 week or more. There is no flaking of the 65°C samples, any mater of time. Since it was not possible to measure the thicknesses of the HA this eventual difference can not be neglected. Except from the possibility of thinner HA depositions at higher temperature (and thus higher adhesion) there is a possibility that an increased temperature could lead to stronger bonding due to that higher movement of the ions at higher temperature leading to nucleation at sites that have high lattice match between the crystals and therefore have better adhesion.

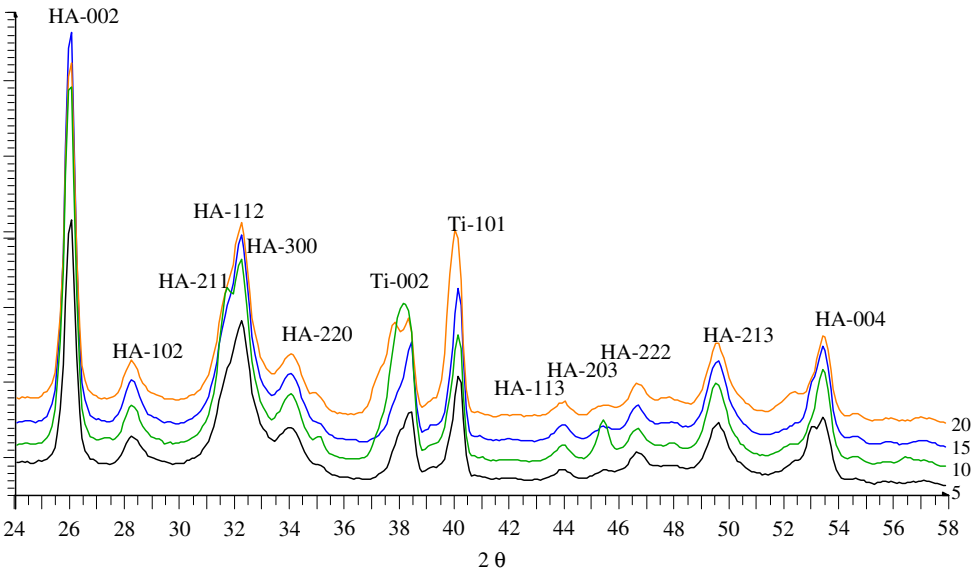

Fig. 8 XRD of hydroxyapatite on PVD samples. The XRD pattern for all the samples is in unity, showing strong diffraction from hydroxyapatite. HA = hydroxylapatite, Ti = Titanium

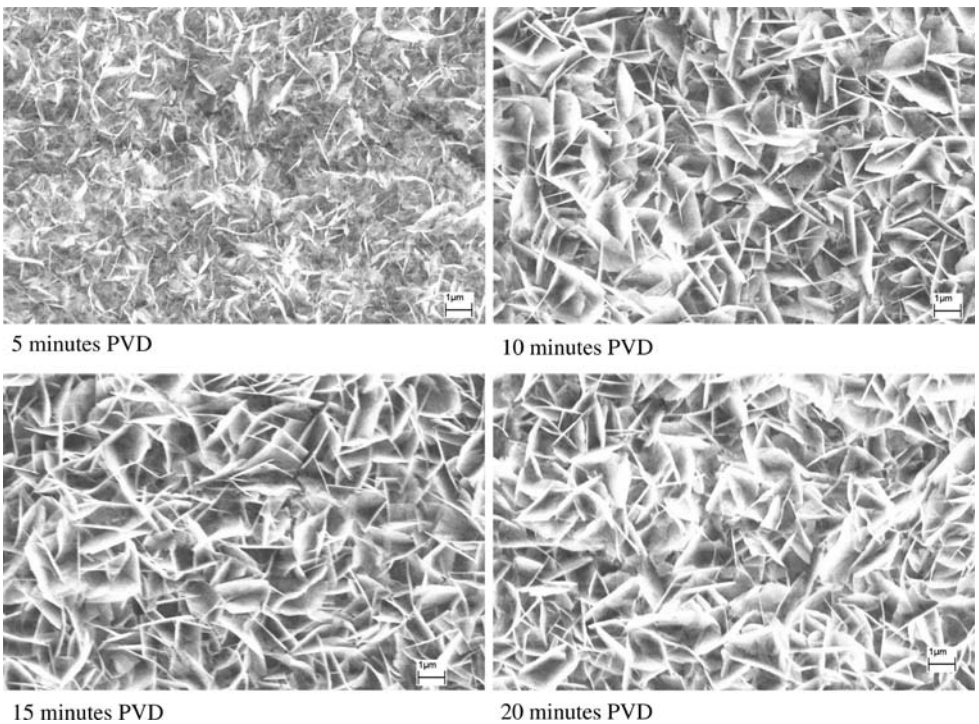

Fig. 9 SEM of hydroxyapatite on PVD samples. Only the 5 min sample showed a difference in the growth of hydroxyapatite after immersion in PBS, at 37°C for 1 week, with smaller and less defined crystals

4.2 Effect from titanium oxide PVD coating thickness on HA growth

It is reasonably to assume that in vitro bioactivity (at fixed soaking conditions) is mostly dependent on the surface chemistry. In this paper, the boundary condition for when a surface turns from inert to active as function of layer thickness was sought for. Even though the thinnest coating (5 min of deposition time with a calculated thickness of 19 nm) had a different appearance of the HA layer compared to the other coatings, all coatings were active. This is interesting from a surface modification point of view; it could well be enough with very thin coatings to obtain the desired biological effect. One obvious benefit with thin coatings is the higher adhesion compared to thicker coatings. Thick coatings have higher internal stresses leading to higher probability of coating delamination.

5 Conclusions

The soaking temperature had an effect on the HA formation and growth on both rutile surfaces and native oxide on Ti substrates. Higher temperatures lead to easier formation of HA. It was even possible, at 65°C, to grow HA on native titanium oxide from soaking in PBS. The coating quality was better for HA formed at 65°C compared to 37°C.

All the thin PVD-coatings gave HA growth after 1 week soaking in PBS at 37°C, thus even very thin crystalline titanium oxide coatings are bioactive.

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