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Nanoporous alkoxy-derived titanium oxide coating: a reactive overlayer for functionalizing titanium surface

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Effective immobilization of bioactive substances such as adhesive proteins, synthetic peptides and growth factors on metallic substrates is required for a number of medical applications. In the present work, evidence is presented to show that an alkoxy-derived nano-porous titanium oxide coating, synthesized electrochemically on titanium in methanolic electrolytes, may act as an effective interface for functionalizing a titanium surface. It is demonstrated that nanoporous oxide coatings could facilitate fast diffusion of small organic molecules within the oxide network and form strong chemical bonds with the functional groups of these molecules at room temperature. Fourier transformed–infrared spectroscopy was used to investigate the nature of the interfacial interactions between the oxide network and a range of molecules containing COOH, OH, NH_2, C=O and phosphoric acid functional groups. The results indicate that the nanometre-sized oxide clusters within the coating may play an essential role in effective immobilization of organic molecules by providing numerous binding sites for chemisorption of these species. The surface-derivatized oxide coating may provide a solid phase for the subsequent attachment of a broad range of biochemically active molecules on the titanium surface.

1. Introduction

Titanium is known to be biocompatible and has shown impressive clinical results as a dental and orthopaedic implant material. The excellent tissue response to titanium is believed to be related to the chemical and biochemical properties of titanium oxide at the titanium surface [1]. However, the process of bone bonding and calcification at the titanium oxide–bone interface has not been fully clarified. In addition, because induction of new bone at the bone–implant interface is not always satisfactory, there is still a need for modification concerning acceleration of bone formation at the site of implantation. Rapid cell attachment and spreading are essential for early bone formation at the bone–implant interface. Cell adhesion involves several groups of molecules, including the cytoskeleton, the ECM components, and transmembrane proteins known as integrins which link the internal and external cellular milieu [2]. Cell adhesion is known to be profoundly affected by the chemical properties of the underlying substratum. Although the structural requirements of an adhesive substratum at the microscopic level are still largely unknown, it is clear that hydrophilic polar and ionic groups such as hydroxyl, carbonyl, carboxylate and sulphate groups may play an important role in cell–substratum interactions [3]. Attachment and spreading of cells are also shown to be mediated by specific Arg–Gly–Asp (RGD)-containing adhesive proteins such as fibronectin and synthetic RGD peptides [4]. In addition, in orthopaedics, a number of enzymes and growth factors have been identified which influence osteoblast and may play an important role in the healing process. All these substances must remain on the implant surface in sufficient amounts and should retain their functional characteristics in order to be able to control and activate appropriate cell responses. This often requires the properties of the implant surface to be tailored with complex organic functional groups to provide attachment points, for example for peptides and other biomolecules. The systems of self-assembled monolayers (SAMs) of alkanethiolates [5], alkyltrichlorosilanes [6], *n*-alkonic acids [7] and alkylphosphates [8] are probably the best that are currently available to accomplish the functionalization of metal surfaces required for many applications in biomaterial science. SAMs of alkanethiolates on gold and silver are generally the most useful in scientific applications [5]. However, alkanethiols do not adsorb to the surface of many metal oxides [9]. Alkylphosphonic and *n*-alkonic acids adsorb but the adsorption can be weak [10]. Most studies, however, have involved metal surfaces exposed to air prior to adsorption and thus largely refer to adsorption on native oxide overlayers. It would be expected that the properites of the oxide such as its stoichiometry, crystallinity, porosity, defect density and surface chemistry may significantly influence the

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affinity of the oxide for functional groups and the stability of adsorbed organic molecules. We have recently developed an electrochemical process for fabrication of alkoxy-derived nanoporous titanium oxide coatings (up to 40 μm thick) on titanium and titanium alloys [11–13]. The process involves direct synthesis of titanium methoxide ($Ti(OCH_3)_4$ by anodic oxidations of titanium in methanolic electrolytes and its rapid hydrolysis and conversion into titanium oxide in the presence of water. In this process, an inorganic network consisting of Ti–O–Ti bridges is formed on the titanium substrate as a result of hydrolysis and controlled polycondensation of titanium methoxide. The polycondensed oxide network is nano-porous and poorly crystallized at room temperature and contains numerous OH groups. It is envisaged that an alkoxy-derived porous oxide network may form strong chemical bonds with certain functional groups at room temperature through simple chemical reactions and thus may allow immobilization of a range of functionalized biomolecules on the oxide surface. On the other hand, because the coating is porous, immobilization of small biomolecules (e.g. peptides) within the nano-metre-sized pores of the oxide may be realized. Immobilization through entrapment in the oxide pores has the potential advantage of enhancing attachment and the stability of biomolecules and may allow various biocompatible functional coatings with novel combinations of properties to be synthesized. The successful development of such coatings, however, requires an understanding of interfacial interactions between alkoxy-derived oxide and the reactive moieties of the organic molecules. In this work, the interaction between nanoporous titanium oxide and a number of simple organic molecules containing carboxyl, amino carbonyl and hydroxyl functional groups was investigated by Fourier transformed–infrared (FT–IR) spectroscopy. These reactant included carboxylic acids, *L*-lysine and *L*-ascorbic acid. The homologous series consisting of formic, acetic and propionic acids were selected to investigate the interaction between carboxyl groups and the titanium oxide. *L*-lysine was chosen because it possesses bifunctional chemical groups (i.e. carboxyl and amino groups) and may act as a coupling agent. *L*-ascorbic acid was selected as a reactant because it possesses carbonyl and hydroxyl functionalities which may be hydrogen bonded to porous hydrated oxide. Ascorbic acid is known to serve as an important cofactor for some of the post-translational processing enzymes. Ascorbic acid has also been shown to upregulate type I and III collagen synthesis [14]. In addition, the interaction between the alkoxy-derived oxide and the phosphorus-containing groups was also investigated using phosphoric acid as a reagent.

Phosphorus is known to form organic compounds via oxygen, i.e. compounds containing C–O–P groupings. These include monofunctional molecules, and polymers with a multitude of phosphorus-containing functional groups. In each of these substances, the phosphorus moiety may provide the necessary chemical reactivity to form functional biomaterials.

2. Materials and methods

Alkoxy-derived titanium oxide coatings (~ 12 μm) were synthesized on commercially pure titanium plates ($3 \times 5\ cm^2$) which were pre-etched in hydrochloric acid (HF). These coatings were prepared at room temperature and at constant voltage (5 V) for 1 h in methanolic electrolytes according to the procedure described earlier [12, 13]. The electrolyte was made by adding 10 g analytical reagent sodium nitrate ($NaNO_3$) to 1 l of an analytical reagent methanol containing less than 1% water. As-synthesized coatings were incubated in distilled water for 24 h to remove traces of nitrate ions left in the oxide pores and subsequently dried at room temperature. The structure of the oxide coating was examined using transmission electron microscopy (TEM) and selected-area diffraction technique. Samples for TEM analysis were prepared by placing powders of the oxide removed from the substrate on carbon-coated copper grids. Although obtaining a high-resolution image is difficult, due to the electron absorption capacity of the material, the fringe regions of many particles were thin enough to obtain quality micrographs without microtoming. For the absorption experiments, oxide coatings were reacted with the organic reagants at room temperature for 24 h. Formic acid (90%), acetic acid (99.7%) and propionic acid (99%) were used as-received without further dilution. For adsorption tests involving *L*-lysine and *L*-ascorbic acid, reagent powders were dissolved in distilled water to prepare 0.15 M solutions. To avoid photodegradation of solutions, all adsorption tests were performed in dim light. After removal from the reagent solutions, the samples were rinsed repeatedly with water and dried in a stream of nitrogen. Fourer transformed–infrared (FT–IR) spectroscopy was employed to investigate the nature of interfacial interactions between the nanoporous oxide coating and the functional groups of the organic reagents.

3. Results and discussion

3.1. Formation and characterization of alkoxy-derived oxide coating

Fig. 1 shows the TEM bright-field micrograph and the selected-area electron diffraction pattern of the as-prepared alkoxy-derived oxide coating. The coating consists of nanometre-sized amorphous particles (building blocks) and nanospaces between the particles. The porous oxide network is therefore expected to provide a large surface area for the effective absorption of organic molecules. These results are consistent with the thin-film X-ray diffraction, TG and DTA results reported earlier [12, 13]. The amorphous particles are thought to have a structure similar to oxide clusters or colloidal oxide particles and may form as a result of controlled hydrolysis and polycondensation of titanium methoxide in the presence of water. The following anodic reaction is suggested to account for the synthesis of titanium methoxide at the titanium–electrolyte interface

$$Ti + 4CH_3OH \rightarrow Ti(OCH_3)_4 + 4H^+ + 4e^- \quad (1)$$

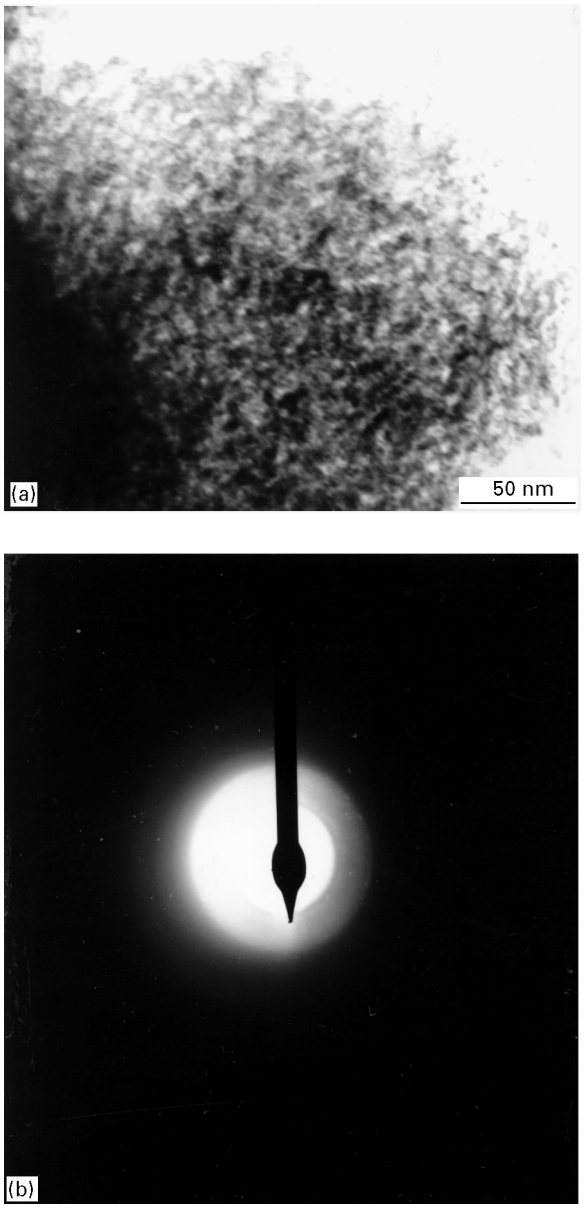


Figure 1 (a) Transmission electron micrograph of the as-synthesized alkoxy-derived titanium oxide coating and (b) the selected area electron diffraction pattern of the same specimen.

It is speculated that during the coating process, nanometre sized amorphous particles consisting of Ti–O–Ti bridges are formed as a result of hydrolysis and polycondensation of titanium methoxide. The terminating bonds of this oxide polymer may contain OH and probably OCH_3 groups. It is known that the polycondensate materials from metal alkoxide can never reach 100% oxide since this would require an infinite polymer with no terminal bonds [15]. The concentrations of OH and OCH_3 groups, however, would be expected to depend on the hydrolysis conditions. A hydrolytic polycondensation equation which would take into account this variability of the oxide content and the polymeric nature of the condensate can be written as

$$n\mathrm{Ti(OCH_3)_4} + \frac{(4n + x - y)}{2}\mathrm{H_2O} \rightarrow \mathrm{Ti}_n\mathrm{O}_{[2n-(x+y)/2]}(\mathrm{OH})_x(\mathrm{OCH_3})_y + (4n - y)\mathrm{CH_3OH} \quad (2)$$

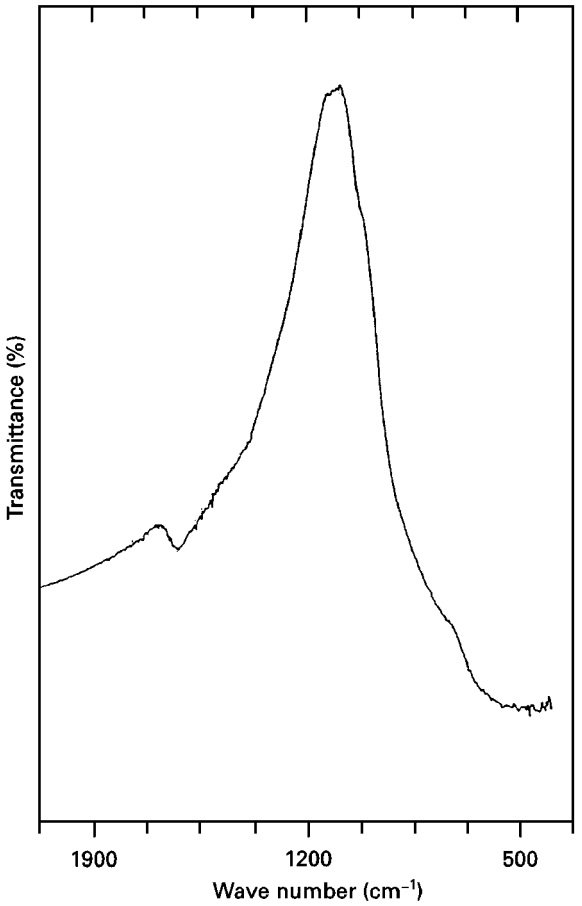


Figure 2 FT–IR spectrum of the as-synthesized alkoxy-derived titanium oxide coating.

Previous XPS studies [12] have indeed shown the presence of hydroxyl and carbon residue in the oxide structure but the carbon species were found to be predominantly located on the oxide surface. The FT–IR spectrum of the powdered oxide is shown in Fig. 2. The absorption band at $\sim 1620\,\mathrm{cm}^{-1}$ is attributed to the bending motion of coordinated hydroxyls in the oxide matrix, and confirms the previous XPS results. However, no significant absorption band associated with $-OCH_3$ groups was detected by the FT–IR analysis. It is known that the rate and the extent of hydrolysis of alkoxides depends on the availability of water and the size of the alkyl group in the alkoxide. The rate of hydrolysis of titanium methoxide in the presence of water is relatively fast compared with the higher alkoxides [15]. Thus, the apparent absence of the $-OCH_3$ absorption bands in Fig. 2 may indicate complete hydrolysis of titanium methoxide under the present experimental conditions. However, as we shall see in the following sections, the as-synthesized oxide is not thermodynamically stable and readily forms strong chemical bonds with the functional groups of various organic molecules.

3.2. Interaction of aliphatic carboxylic acids with the alkoxy-derived titanium oxide

The FT–IR spectra of titanium oxide after reacting with various carboxylic acids are shown in Fig. 3. The resultant IR spectra indicate that the most

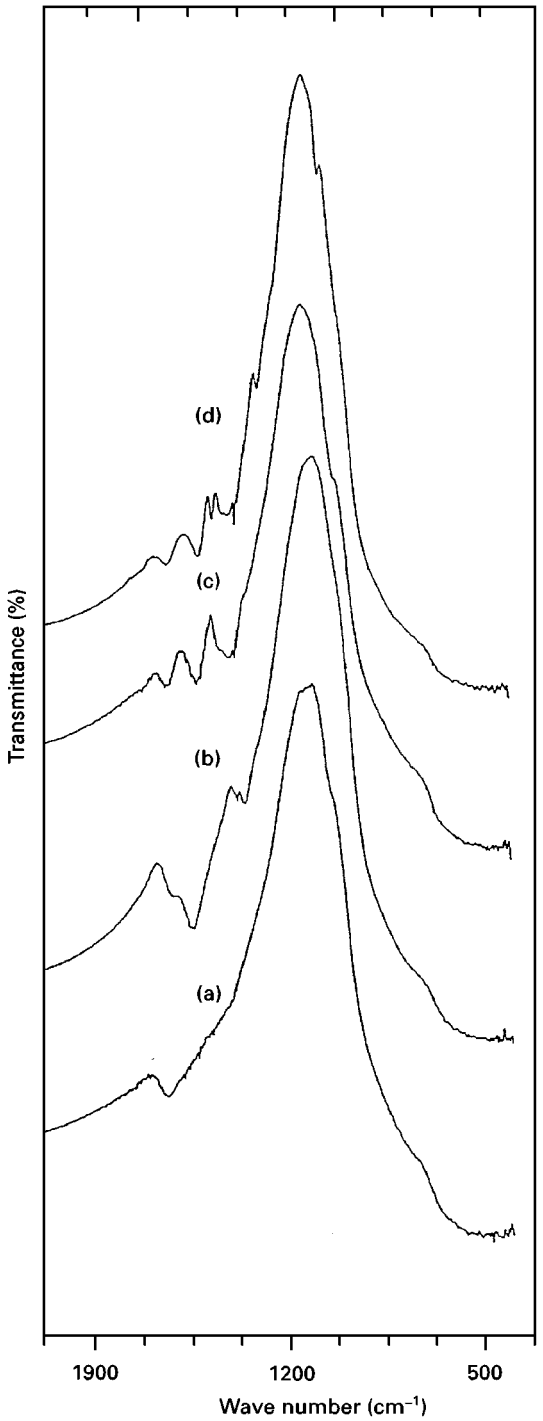


Figure 3 FT–IR spectra of the alkoxy-derived oxide coating (a), before and after reacting with (b) formic acid (c) acetic acid, and (d) propionic acid for 24 h.

pronounced changes take place in the frequency range of $\sim$1350–1800 cm^{-1}. All carboxylic acids yielded adsorbed species with a common band at $\sim$1545 cm^{-1}. This band is attributed to the metal carboxylate, RCOOM asymmetric stretching absorbance [16]. The original carboxylate acids do not possess this band in their infrared spectra. Other peaks at $\sim$1350–1450 cm^{-1} can be associated with the combination of symmetrical vibration of metal carboxylate and bending vibration of hydrocarbon groups which are not involved in the reaction. The band at $\sim$1545 cm^{-1} persisted after repeated rinsing, which suggests that metal carboxylates are indeed chemically bonded to titanium oxide. No peaks from free carboxylic acids are detected in the FT–IR spectra of the oxide. These functional groups usually exhibit a sharp strong peak around 1700 cm^{-1} which is caused by the carbon–oxygen double bond stretching absorbance of the free carboxylic acids. It appears, therefore, that any free acids are washed away and only chemically bonded species remained adsorbed within the oxide matrix. The irreversible adsorption of carboxylic acids may involve deprotonation and formation of $RCOO^-$ anions which may directly react with the hydrated amorphous particles (building blocks) of the oxide network. It is known that the surface hydroxyl groups of titania in solutions tend to be polarized and electrically charged (17). The hydroxyls of titania are protonated and positively charged below its isoelectric point (IEP) of pH 6.2

$$\text{Ti–OH} + \text{H}^+ \rightarrow \text{TiOH}_2^+ \qquad (3)$$

At acidic pH values less than 6.2, $RCOO^-$ species can be adsorbed on the positively charged titanium oxide particles. The adsorption of $RCOO^-$ anions on these particles may result in the formation of strong chemical bonds between surface titanium atoms and $RCOO^-$ anions

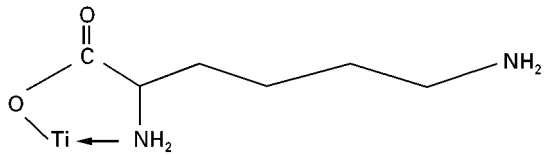
$$\text{O–Ti–OH} + \text{H}^+ + \text{RCOO}^- \rightarrow \text{O–Ti–O–C(=O)–R} + \text{H}_2\text{O} = \text{O–Ti–OH}_2 \qquad (4)$$

Such an interaction may lead to the formation of the carboxylated oxide clusters $(RCOO)_y Ti_nO_{[2n-(x+y)/2]}(OH)_x$ within the porous coating. The small sizes of the oxide particles within the coating are thought to be important for the formation of strong chemical bonds with carboxylic acids. These particles have large surface to volume ratio and therefore may be capable of binding ligands at the surface with more than one bond per surface titanium atom. Thus, unlike the air-formed films previously used in the adsorption studies, alkoxy-derived oxide is highly reactive and thus may form strong chemical bonds with various organic substances containing COOH functional groups. On the basis of these results, it is envisaged that stable monolayers of *n*-alkonic acids $(CH_3)(CH_2)_mCOOH$, $m = 2$–18 with highly orientated alkyl tails may be obtained on these oxide coatings. The strong chemical affinity of COOH groups for the alkoxy-derived titanium oxide as evinced from FT–IR results, also offers the potential to immobilize various hydrophilic polar and ionic functional groups into the porous titanium oxide by using functionalized carboxylic acids. The reaction of the functionalized carboxylic acids with the alkoxy-derived oxide would be expected to result in the formation of coatings consisting of organofunctional oxide clusters containing (OOC–X–A) moieties were the functional group A is linked via the carboxylate group and some hydrocarbon spacer X to the oxide. The functionalized carboxylic acids can also be specially designed to act as

interface coupling agents to promote a durable and strong bond between titanium oxide and biomolecules such as peptides and proteins.

3.3. Adsorption of L-lysine and L-ascorbic acid

The IR spectrum of unreacted lysine and lysine-impregnated titanium oxide coatings are shown in Figs 4 and 5, respectively. The characteristic C=O stretching vibration of lysine appears at 1736 cm^{-1}. The N–H bending vibration of lysine is located at approximately 1620 cm^{-1} while the N–H stretching modes are located at 2350–2750 cm^{-1}. As seen in Fig. 5, the absorption band at 1736 cm^{-1} associated with the C=O stretching frequency of lysine disappeared upon absorption of lysine on the oxide. On the other hand, a new peak at $\sim$1560 cm^{-1} appears, which can be attributed to the metal carboxylate band as a result of the formation of the lysinate complex $(H_2N(CH_2)_4)CH(NH_2)COO_y\ Ti_nO_{[2n-(x+y)/2]}((OH)_x]$. The FT–IR results, however, do not give clear evidence for the coordination mode of amino groups of lysine. It is possible that the α-amino groups of lysine be engaged in coordination to the titanium atom in a thermodynamically stable five-membered ring as shown below. Thus, the dangling NH_2 group could be available for further chemical reactions



The X-ray structure analysis of ($Ti(OEt)_3$–glycinate) has also shown that α-amino groups participate in binding the carboxylate ligand to the metal and improve stability of carboxylate derivative by formation of a five-membered chelate ring [18].

The adsorptive behaviour of *L*-ascorbic acid on titanium oxide was found to be quite different from that of carboxylic acids. Fig. 6 shows the FT–IR spectra of the unreacted ascorbic acid and ascorbic acid-loaded titanium oxide coating. The absorption spectrum of powdered ascorbic acid shows a pronounced band at a frequency of 1735 cm^{-1}. This band is due to the stretching vibration of carbonyl groups (C=O) of ascorbic acid. As is evident from this figure, the C=O band disappeared upon adsorption of ascorbic acid on the hydrated oxide. However, the IR spectrum of ascorbic acid-loaded titanium oxide does not show the metal carboxylate band as observed for the other organic acids, and therefore other mechanisms for the interaction must be sought. One possibility is the formation of hydrogen bonds between ascorbic acid and hydrated titanium oxide. Indeed the IR spectrum of ascorbic acid-loaded oxide in the range of 1500–1800 cm^{-1} clearly indicates that hydrogen bonding between C=O groups and the polar hydroxyls of the oxide may play an important role in the interaction between ascorbic acid and the oxide coating. It is seen that while the C=O band disappeared,

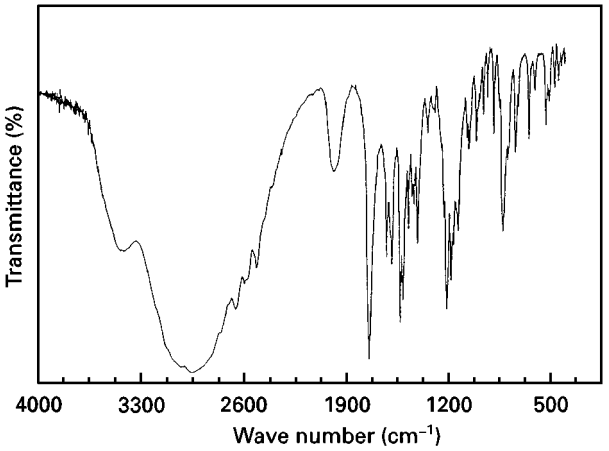


Figure 4 FT–IR spectrum of the unreacted *L*-lysine powder.

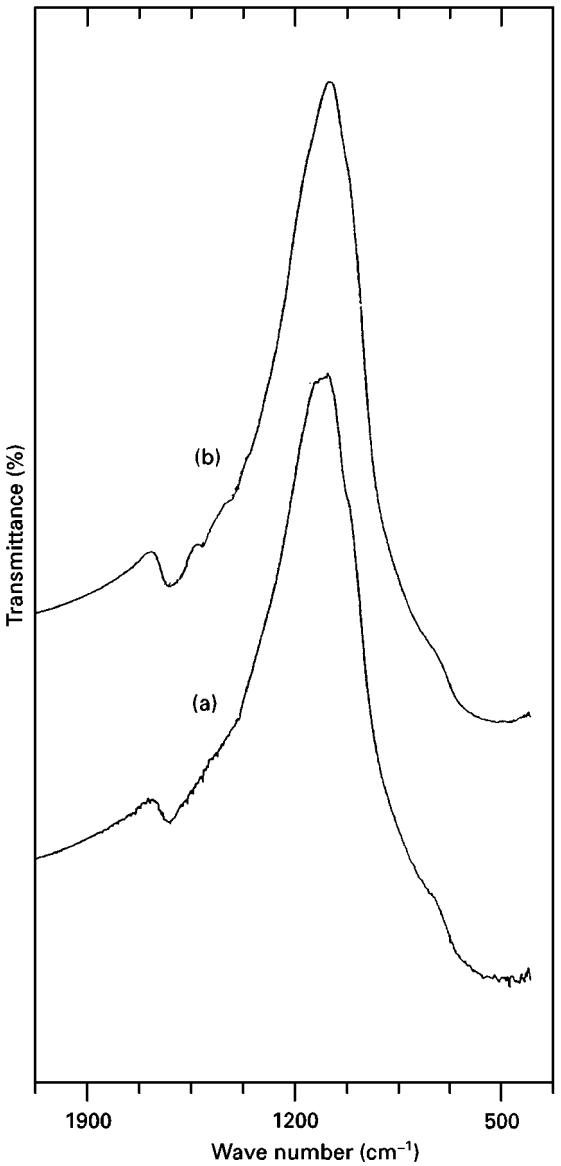
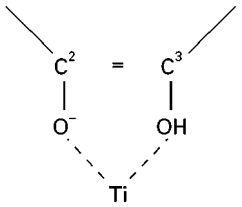


Figure 5 FT–IR spectra of the alkoxy-derived oxide coating (a), before, and (b) after reacting with *L*-lysine for 24 h.

the bending frequency of the OH groups at 1620 cm^{-1} shifted to a lower frequency. These changes in absorption bands of C=O and OH groups can be interpreted in terms of the formation of hydrogen bond

C=O–H_2O. The shift of the OH band is probably due to the lowering of the H–O–H bond order as a result of hydrogen bond formation through the carbonyl oxygen. The appearance of the band at 1170 cm^{-1} may also be related to the coupling between in-plane OH bending and C=O stretching of carbonyl groups. On the other hand, ascorbic acid may also be linked through its hydroxyl functionalities to the hydrated titanium oxide. It is known that ascorbic anion possesses the ability to form complexes with metallic cations through ionization of OH groups on the C-2 site [19]



Thus the appearance of the absorption band at $\sim$1113 cm^{-1} may be attributed to bands such as C–O … Ti. However, hydrogen bonding seems to be the predominant force in the case of *L*-ascorbic acid. Fig. 7 shows that when ascorbic acid-loaded oxide is exposed to pure water, ascorbic acid can be replaced with water and slowly leaches out from the porous oxide. The reversible adsorption of ascorbic acid may offer the opportunity for the controlled delivery of this substance and its derivatives at the bone–implant interface by adjusting the pore sizes of the oxide network. Further developments, such as the use of functionalized ascorbic acid molecules possessing hydrophobic moieties such as hydrocarbon chains, may further reduce the desorption rate of ascorbic acid. Larger loadings may also be obtained by precipitating phosphate-modified ascorbic salts into the pores of the oxide. Previous work [13] has shown that phosphate compounds such as brushite and hyroxyapatite can be incorporated into the oxide network by the precipitation method.

3.4. Immobilization of phosphoric acid functional groups into nano-porous titanium oxide

Fig. 8 shows the FT–IR spectrum of alkoxy-derived oxide after reacting with concentrated phosphoric acid for 24 h. The strong absorption band appearing at 1035 cm^{-1} is attributed to the anti-symmetric P=O stretching (v3) of the adsorbed phosphate groups. This band persisted after repeated rinsing which suggests that phosphate groups are indeed forming strong chemical bonds with the alkoxy-derived oxide. According to Stutman *et al.* [20], the anti-symmetric P–O stretching absorption of free phosphate anion appears at 1082 cm^{-1}. Therefore, the shift of the phosphate band observed in the present study can be interpreted in terms of formation of a chemical bond between the adsorbed phosphate groups and the nanoporous oxide

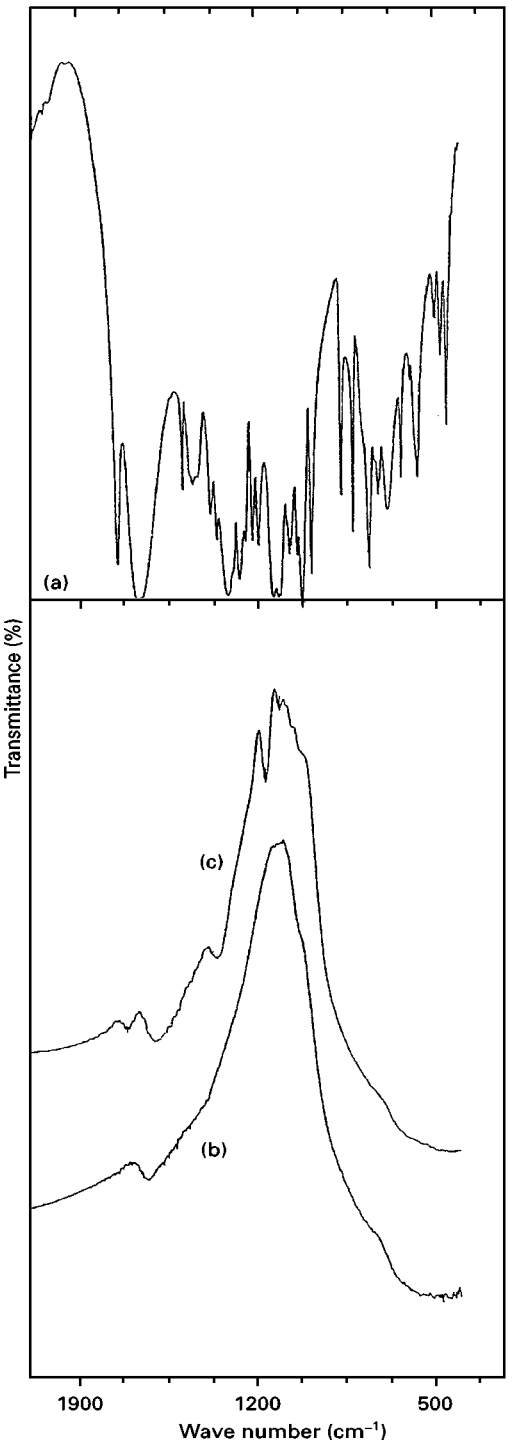


Figure 6 FT–IR spectra of (a) the unreacted *L*-ascorbic acid, and alkoxy-derived oxide coating (b) before and (c) after reacting with ascorbic acid for 24 h.

coating

$$\begin{array}{c} \mathrm{PO_4H_2} \\ | \\ \mathrm{Ti{-}OH + H^+ + H_2PO_4^- \rightarrow O{-}Ti{-}OH_2{=}O{-}Ti{-}PO_4} \\ \mathrm{H_2 + H_2O} \end{array} \quad (5)$$

The formation of this band would result in distortion of the equilibrium atomic positions of the phosphate groups. Such changes in the structural milieu surrounding these ions may, in turn, result in the band shift to the lower frequencies. As is evident from Fig. 8,

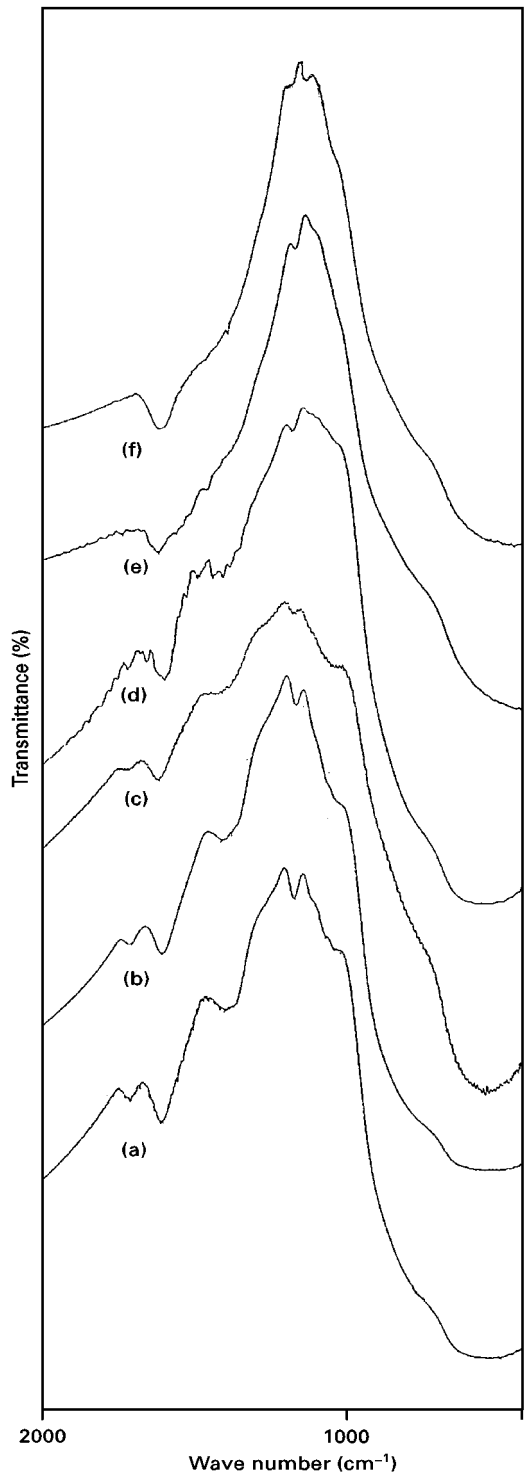


Figure 7 FT–IR spectra of ascorbic acid-loaded alkoxy-derived oxide coating after exposure to distilled water at room temperature for various time periods: (a) 0 h, (b) 0.5 h, (c) 1 h, (d) 2 h, (e) 1 d and (f) 7 d.

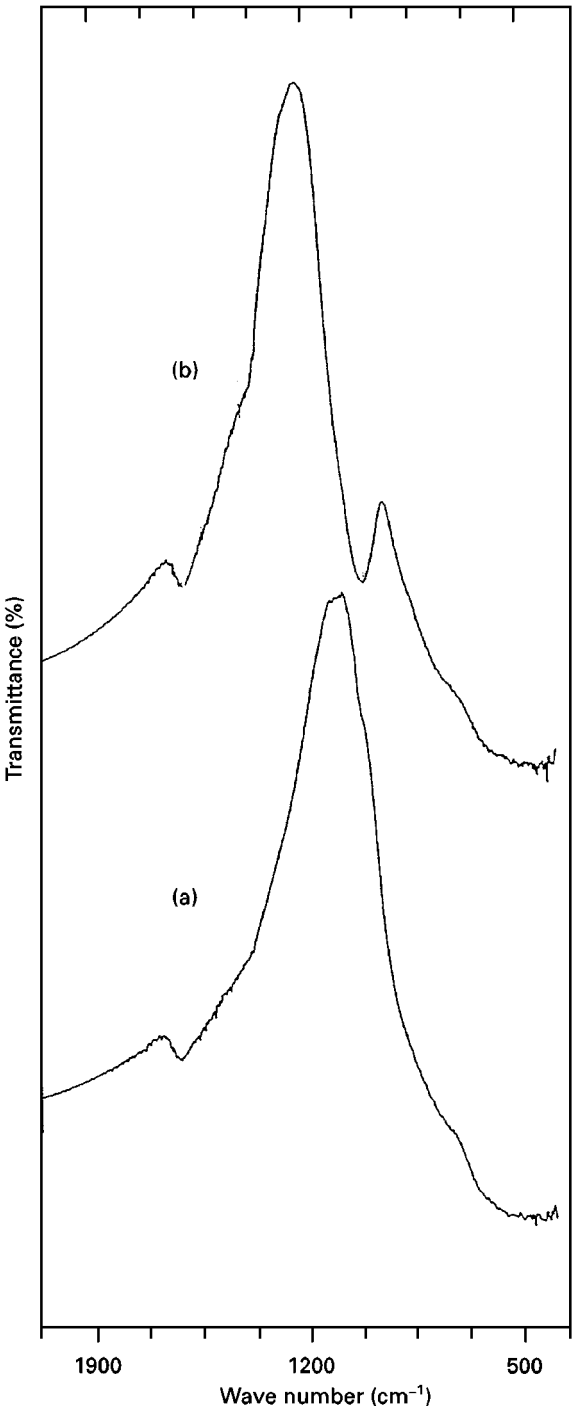


Figure 8 FT–IR spectra of the alkoxy-derived oxide coating (a) before and (b) after reacting with concentrated phosphoric acid for 24 h at room temperature.

a substantial number of phosphate groups is adsorbed on the oxide. This clearly demonstrates that phosphate groups are not exclusively located on the coating surface and that a large number of phosphate groups is indeed incorporated within the porous oxide network during the phosphoric acid treatment. The porous oxide network apparently not only facilitates fast diffusion of phosphate ions but also provides a large surface area which is required for the effective adsorption of these species. Similar results are also expected between the nanoporous oxide and the organic derivatives of phosphoric acid. A number of such compounds have been developed in recent years for applications in clinical dentistry as dentine bonding agents. A recent review of these developments is given by Nicholson and Singh [21]. Similar derivatives of phosphoric acid may be designed specifically as interface coupling agents for the immobilization of proteins, peptides and enzymes on nano-porous titanium oxide-coatings.

4. Conclusion

The results in this work have shown that the nanoporous alkoxy-derived titanium oxide coating may act as

an effective interface for immobilizing a range of functionalized organic molecules and thus may provide an attractive means for functionalizing the titanium surface. The enhanced immobilization of organic molecules is achieved through the formation of strong chemical bonds between functional groups of organic molecules and the oxide clusters within the coating and by virtue of the small pore sizes of the oxide network. The use of nano-porous titanium oxide coatings for functionalizing a titanium surface has a potential advantage over the use of adsorption or covalent bonding on non-porous surfaces, in that adsorbed molecules on such surfaces are easily removed and covalently attached molecules may be prone to chemical degradation of the anchoring bonds in the long term.

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