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Preparation of bioactive microporous titanium surface by a new two-step chemical treatment

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Microporous oxide layers allowing fast deposition of calcium phosphate layers (CPLs) were formed on commercially pure titanium (c.p.Ti) after the application of a newly developed two-step chemical treatment. The micropores were of submicrometre size. The two-step treatment was carried out by etching c.p.Ti samples with HCl and H_2SO_4 first and then treating them in boiling 0.2 N NaOH solution at 140 °C for 5 h. Conformal CPLs, about 20 μm thick, were deposited on the two-step treated c.p.Ti surface by means of a two-day immersion in an *in vitro* supersaturated calcification solution. The CPL was characterized to be mainly composed of two sublayers, i.e. an outside loose octacalcium phosphate crystal sublayer and an inside dense carbonated apatite sublayer. A scratching test indicated that the apatite sublayer was strongly bonded to the c.p.Ti substrate. Moreover, it was observed that the untreated or single-step treated c.p.Ti surfaces are not only morphologically different from one another but significantly different from the two-step treated one, in that no precipitation was observed on them up to 14 d immersion in the same calcification solution. It is indicated that the two-step chemical treatment is a simple and easily controllable method to prepare bioactive titanium surfaces and subsequently to induce the rapid precipitation of conformal and adherent CPL from *in vitro* supersaturated calcification solutions. © *1998 Chapman & Hall*

1. Introduction

Titanium and its alloys are extensively used as orthopaedic implants owing to their superior mechanical properties. However, their bioactivity is not as good as that of bioactive materials and during implantation they may fail to develop a bond with living bone and release corrosion products into the body [1–3].

To overcome these disadvantages, bioactive hydroxyapatite (HA) thin layers have been coated on titanium implant devices [4]. Plasma spraying is presently the most popular technique for this purpose. But because plasma spraying is a high-temperature and line-of-sight process, problems, such as the deteriorating effect of intense heat on substrates and the limitation in making coating on complex implant devices with internal cavities [5–7], have not yet been solved. An optimal technique for bioactive coating application has still to be developed.

In recent years, there has been increasing interest for the preparation of calcium phosphate layers on titanium implant substrates at low temperature (< 100 °C) from *in vitro* aqueous supersaturated calcification solution (SCS) in order to mimic the process of natural biological apatite formation [8–10]. This method has or might have at least the following advantages over conventional ones, such as plasma spraying: (1) conformal coatings can be produced on to complex-shaped and/or microporous implants; (2) the biomimetic calcium phosphate layer (CPL) is expected to show higher bone-bonding ability; (3) it is a simple and cost-effective way to acquire calcium phosphate coatings; (4) no adverse effect of heat on substrates.

Some exciting progress has been made so far in the CPL fabrication on titanium implants from low-temperature aqueous SCS. Abe *et al.* [8] produced bone-like apatite coatings on titanium substrates in a simulated body fluid (SBF) by introducing a plate of glass (CaO, SiO_2-based glass) as a source of nucleating agent of apatite. Campbell *et al.* [10] reported that the Ti6Al4V surface modified by self-assembled monolayers of alkyl silane could induce CPL formation in SCS. They both aimed at assisting the nucleation of Ca–P compounds by special reagents rather than improving the bioactivity of titanium implants themselves. It was also reported that identical CPLs could be directly deposited on well-polished titanium implant surfaces [9, 11]; a problem met was how to

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apply the mechanical polishing procedure to complex-shaped implants.

More recently, bioactive porous TiO_2 hydrogel layers have been prepared on titanium implant surfaces by simple chemical treatments like immersion in high-concentration alkali or H_2O_2 solution [12–15]. After being immersed in SBF, the TiO_2 hydrogel layer could directly induce the precipitation of CPL that was expected to have high adhesive strength to the treated metallic surface [15, 16]. Other researches related to modifying titanium implant surfaces by chemical treatments also have been reported [17, 18]. It looks attractive to explore further the potential of simple chemical treatments to prepare biologically useful titanium implant surfaces.

It was always foreseen that an ideal bioactive surface for bone-bonding biomaterials might be the surface with the required negative density and pore structure which could induce the rapid formation of a physiologically stable HA layer after immersing in SBF or even in real body fluid [19]. Efforts are being made in our group to search for simple chemical treatments to prepare such titanium surfaces.

Based on several chemical treatments reported to modify the titanium implant surface [14, 17, 18], in this work, a new two-step treatment is developed and applied on commercially pure titanium (c.p.Ti). The main idea of the two-step treatment is to etch c.p.Ti samples with HCl and H_2SO_4 to acquire a large surface area, which by immersion in boiling low-concentration alkali solution turns into a relatively thick and stable titanium surface oxide layer. This layer is expected to improve the bioactivity of titanium implants. It was quite interesting to find that a microporous titanium oxide surface layer was formed after our two-step treatment. Conformal and adherent CPLs were rapidly precipitated on our treated surfaces after soaking in an *in vitro* SCS. No precipitation was observed on any untreated, or only acid or alkali treated, sample after 2 wk in the same SCS. Scanning electron microscopy (SEM) combined with energy dispersive X-ray (EDX) analyses, X-ray diffraction (XRD) and infrared (IR) spectrophotometry were employed to investigate the modifications of the c.p.Ti surfaces by various chemical treatments and to characterize the formed CPLs.

2. Materials and methods

2.1. Sample cutting, cleaning and chemical treatment

A hundred small rectangular blocks 10 mm × 10 mm × 2 mm in size were cut as samples from a large 2 mm thick c.p.Ti plate by using a WOCO 50 sawing machine and a WOCO 997 sawing blade (Wolfgang Conrad). All the samples were ultrasonically cleaned in acetone for 15 min, in 70% alcohol solution for 20 min, and then in distilled water for 20 min. For control experiments, 20 cleaned samples were kept untreated. The remaining samples were divided into three groups which were subjected to the following various chemical treatments.

1. Etching in a mixture of 100 ml 18 wt % HCl and 100 ml 48 wt % H_2SO_4 solutions for 30 min.
2. Immersion in boiling 0.2 N NaOH solution at 140 °C for 5 h in a pressure canner.
3. A two-step chemical treatment, i.e. (1), then (2).

2.2. SCS preparation and calcification experiments

For testing the bioactivity of the various treated samples (including the untreated ones), *in vitro* experiments in a SCS were performed. The SCS was prepared with the following ion concentrations: $[Na^+] = 136.8$ mM, $[Cl^-] = 144.5$ mM, $[K^+] = 3.71$ mM, $[Ca^{2+}] = 3.10$ mM, and $[HPO_4^{2-}] = 1.86$ mM. The solution was buffered at pH 7.4 with *tris*-hydroxymethylamino-methane and 1 N HCl at room temperatnure. The $Ca^{2+}K^+$ and HPO_4^{2-} concentrations in the SCS were 80% of those in the accelerated calcification solution, which has been successfully used for the quick test of the calcification ability of some biopolymers [20]. The experiments were carried out by the following procedure. Each sample was immersed into 15 ml SCS in a polystyrene vial. The vials were sealed and put in a calibrated water-bath at 37 °C. The solutions were refreshed every day. The pH values of the solutions removed during refreshing were measured by using a PHM85 precision pH meter (Radiometer, Copenhagen). The samples were removed at regular time intervals, thoroughly rinsed with distilled water, and then dried in an oven at 50 °C.

2.3. SEM, EDX and XRD

Before and after calcification, various c.p.Ti surfaces were coated with thin gold or carbon films for the SEM (Philip SEM525, at 20 kV) morphological observations or EDX (Voyager) chemical analyses. A Philips automatic vertical X-ray powder diffractometer PW 1050 and a measuring system PW 1710 (CuK_α radiation, at 40 kV/35 mA) were used to determine the crystallographic features of the obtained CPLs. The XRD patterns are assigned with the JCPDS [21] standard cards of apatite (HA, $Ca_{10}(PO_4)_6(OH)_2$, carbonated apatite, etc.) and octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_65H_2O$).

2.4. Scratching test and IR

The CPLs deposited on c.p.Ti surfaces were scratched to varying degrees using a scalpel in order to investigate the internal structures of the CPLs and their interfacial bonding to the metallic substrates. The CPLs deposited on the new-cut side faces (derived from the cutting procedure) of several samples were removed to observe the thickness of the CPLs. The material that was peeled off was ground to powder that was analysed by a IR spectrophotometry (Perkin–Elmer 783) using the KBr tablet technique.

3. Results

3.1. Surface texture development after various treatments

The morphology and composition of the c.p.Ti surfaces after various chemical treatments were different from one another. Fig. 1 shows the scanning electron

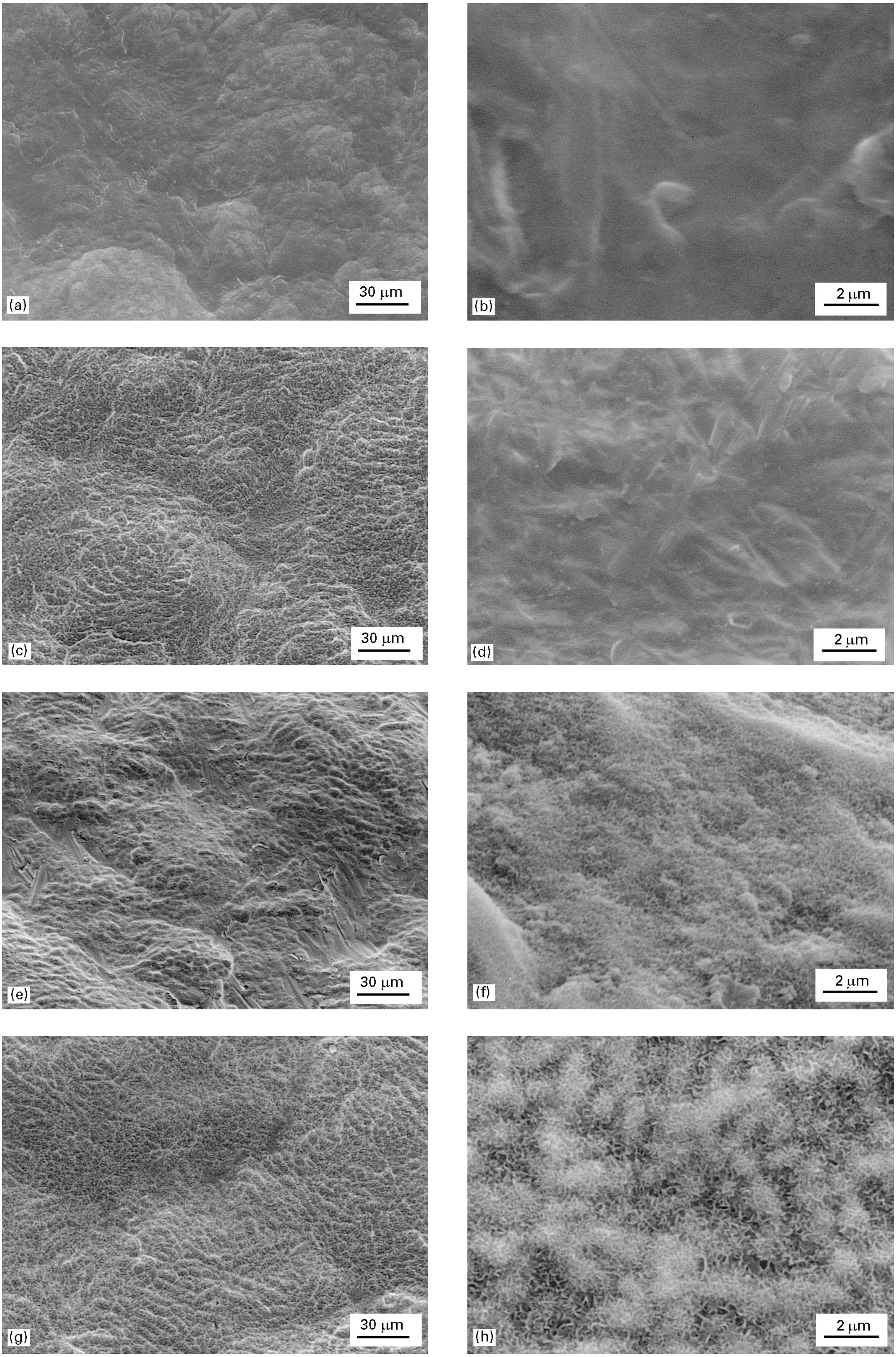

Figure 1 SEM low- and high-magnification micrographs of the c.p.Ti surfaces after various chemical treatments. (a, b) Untreated. (c, d) Etching 30 min in HCl and H_2SO_4. (e, f) Immersion 5 h in boiling NaOH solution. (g, h) Two-step treated, i.e. acid etch first and then alkali treatment.

micrographs of the differently treated surfaces. At low magnification, the untreated c.p.Ti surface ("as-received") appeared not smooth while the alkali-treated surface looked different to some extent from the acid-etched one and the two-step treated surface seemed similar to the acid-treated one. At high magnification, however, significant differences between the various treated surfaces are demonstrated. The untreated surface is dense and relatively smooth. Many micrometre-sized acid etched pits or grooves were seen after acid treatment and large amounts of nano-sized fine pits were produced on the surface by the alkali treatment. It is surprising to note the change that occurred at the surface after the two-step treatment. A completely microporous surface was formed in which the pore sizes are larger than those of the alkali etched pits but also on a submicrometre scale.

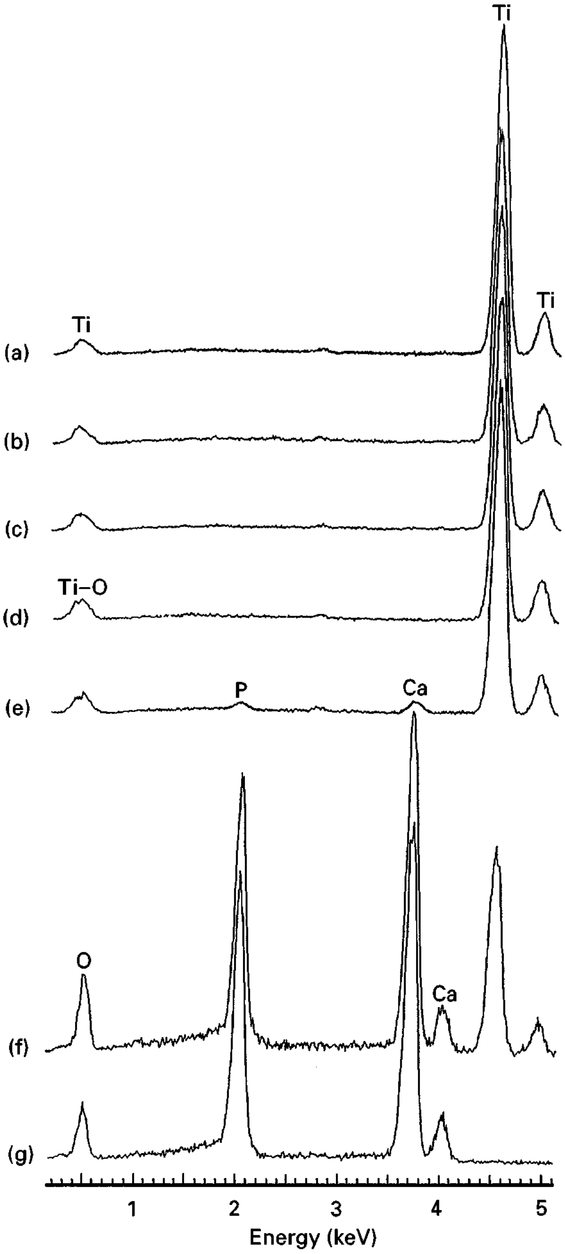

Figure 2 EDX spectra of various c.p.Ti surfaces. (a) Untreated. (b) Etching 30 min in HCl and H_2SO_4. (c) Immersion 5 h in boiling NaOH solution. (d) Two-step treated, i.e. (b) and then (c). (e–g) Heavily, lightly and unscratched CPL deposited on the two-step treated surfaces, respectively.

The EDX spectra of the c.p.Ti surface layers after various treatments were shown in Fig. 2a–d. The differences between the spectra in the energy range of 0.35–0.55 keV are visible. Although the overlap of several titanium peaks (0.395–0.519 keV) and OK_α peak (0.523 keV) in this range makes it difficult to analyse the oxygen content quantitatively, the order of oxygen concentrations in the various surfaces can be estimated as two-step > alkali > control > acid treated. The oxygen shoulder is clearly seen in the spectrum of the two-step treated sample. It is indicated that the microporous surface of the two-step treated samples is mainly made of titanium oxide of unknown composition.

It was observed that the morphology and compositon of the c.p.Ti new-cut side faces after various treatments were similar to the "as-received" surfaces, except for the existence of cut scratches.

3.2. Calcification

The CPL was found to be deposited only on the two-step treated samples after immersion in the SCS. No precipitation was observed on any untreated, acid-treated and alkali-treated samples within the two-week period. The precipitation rates of the CPLs on the two-step treated samples were very high. After 2 d in the SCS, it could be seen by the naked eye that all the surfaces and side faces of the samples were covered by white coatings. The pH values of the solutions dropped to, on average 7.30 and 7.20 at the first and second day, respectively. On the contrary, when no precipitation took place, the pH values remained within the range of 7.4 ± 0.05.

3.3. SEM morphological features of the CPL and scratching tests

Figs 3 and 4 show the SEM morphological features of the CPL formed on the two-step treated samples after 2 d in the SCS. Fig. 3a shows that conformal CPLs were precipitated on both the surface and side faces of the sample. In the high-magnification micrograph of the surface (Fig. 2b), it is seen that the outside part of the CPL is loose and consists of small crystal plates. The thickness of the crystal plates is below a micrometre and their large surface sizes are around several micrometres. The crystal plates were shown to be preferentially grown with their large surfaces nearly vertical to the sample surface. The CPL thickness on the surface was noticed to be about 20 μm, as shown in Fig. 3c. The tight interfacial bonding between the CPL and the metallic surface is demonstrated in this figure.

The SEM morphologies of lightly and heavily scratched zones of the CPL are different from that of unscratched zones, as shown in Fig. 4. It is observed that the CPL deposited on the two-step treated c.p.Ti surface is composed of two sublayers, i.e. an outside loose crystal sublayer and an inside dense sublayer. The growth of the outside crystals from the inside sublayer is shown in Fig. 4b. The thickness of this inside part was estimated to be more than 10 μm. In the scratching tests, the outside part of the CPL could be easily removed by lightly scratching, while it was

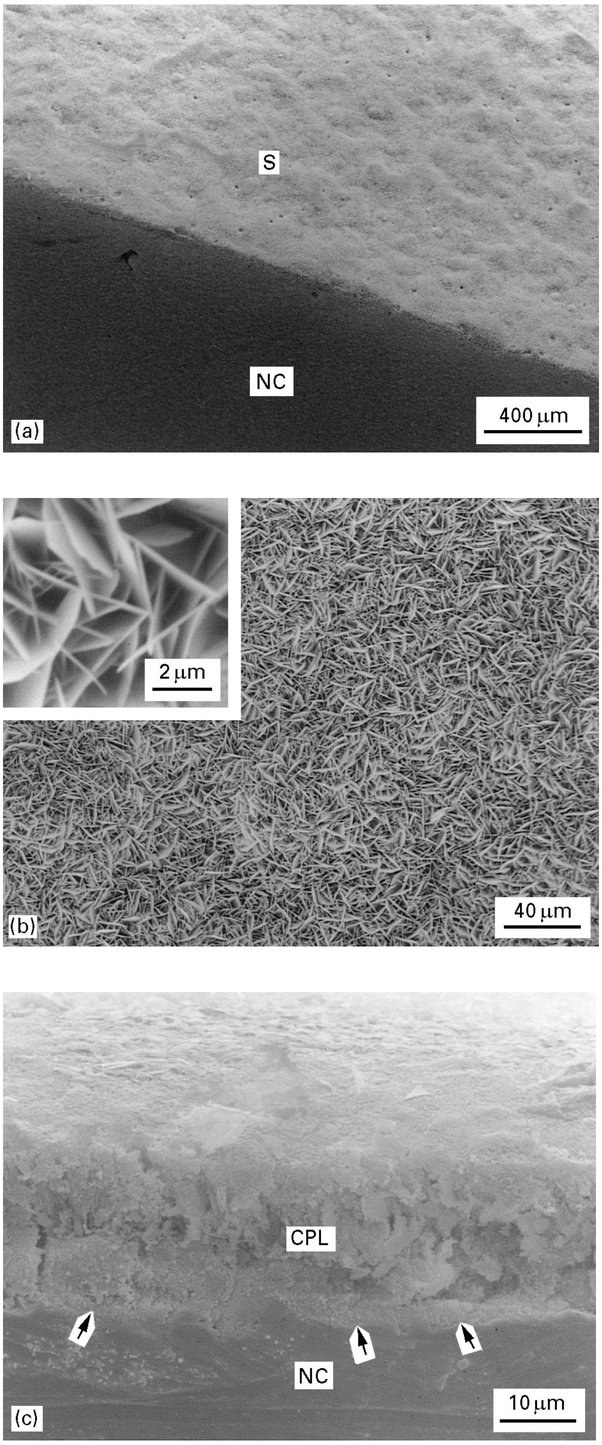

Figure 3 Scanning electron micrographs of the CPL deposited on two-step treated c.p.Ti surfaces after 2 d in the SCS. (a) Conformal CPL covered both surface (S) and new-cut side faces (NC). (b) Small crystals constituting the CPL outside part. An enlarged view of the crystals is shown in the top left of the figure. (c) The thickness of the CPL is seen about 20 μm after the deposition on the c.p.Ti new-cut side face (NC) was removed away. Arrows indicate the interface of the surface CPL/c.p.Ti substrate.

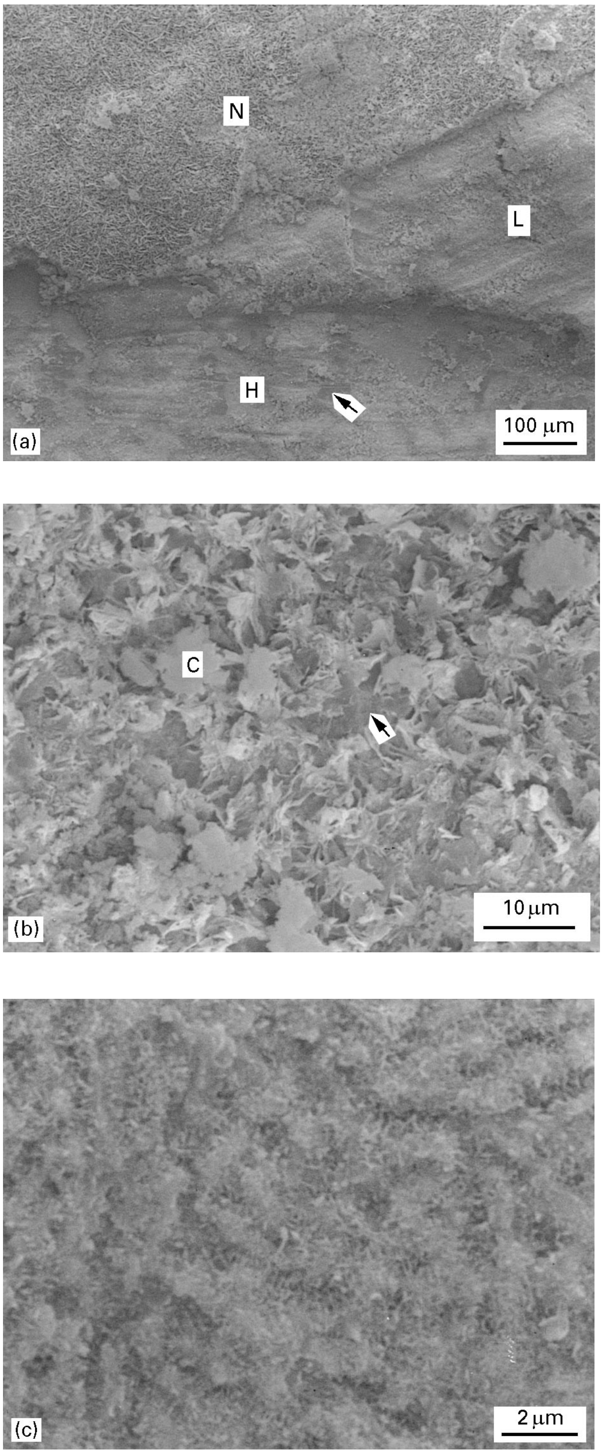

Figure 4 (a) Scanning electron micrographs of three different CPL zones on two-step treated c.p.Ti surface, i.e. not (N), lightly (L) and heavily (H) scratched. (b) Enlargement of L zone of (a). Some crystals (C) of the CPL outside sublayer were seen to have grown from the dense inside sublayer as indicated by the arrow. (c) A high-magnification micrograph taken from the area arrowed in zone H of (a).

difficult to peel the inside part off. The CPLs were found to be bonded to the metallic surface so tightly that they could not be completely removed from the interface, even by very heavy scratching. The heavily scratched zone shown in Fig. 4c was morphologically similar to the original two-step treated surface although most of the originally empty micropores were filled with Ca–P substance as indicated by EDX analysis (Fig. 2e). Most probably this calcium phosphate was precipitated in the nucleation stage or in the early stages of growth.

3.4. Structure and composition of the CPL

The XRD pattern of the CPL formed on the two-step treated sample after 2 d is shown in Fig. 5a. Some

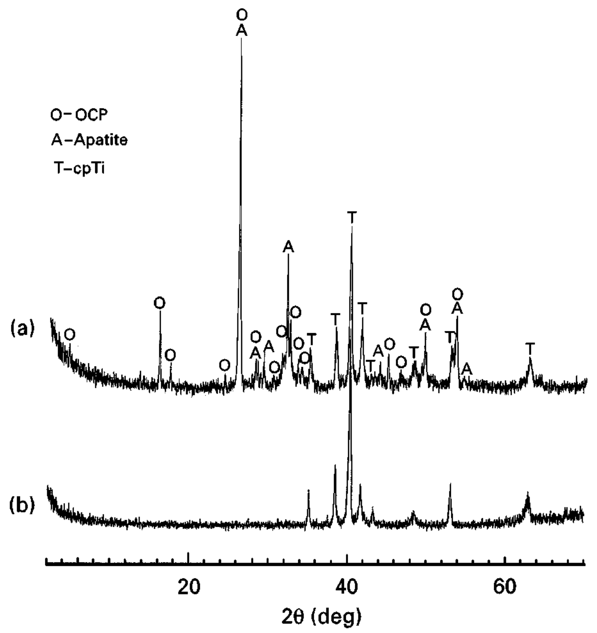

Figure 5 The XRD patterns of "as-received" c.p.Ti and the CPL deposited on the two-step treated c.p.Ti surface after 2 d in the SCS. o, OCP; A, apatite; T, c.p.Ti.

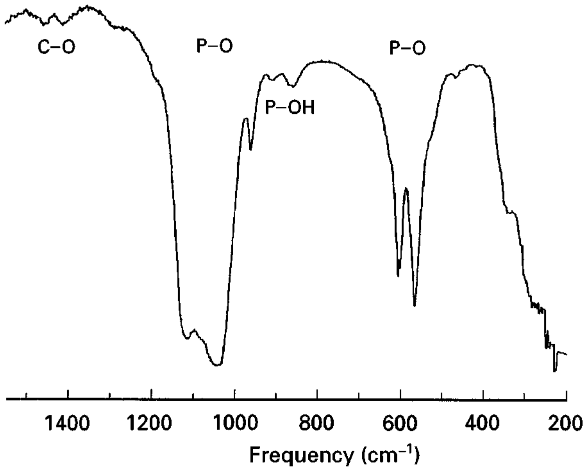

Figure 6 The IR spectrum of the CPL deposited on the two-step treated c.p.Ti surface after 2 d in the SCS.

peaks were from the titanium background as appeared by comparison with the c.p.Ti control pattern (Fig. 5b). Compared with the standard cards of JCPDS [21], the other peaks in Fig. 5a could be indexed as apatite and/or OCP. The crystallographic structures of apatite and OCP are almost similar and some peaks of them appear at about the same positions. The (002) preferential orientation of apatite or OCP at 25.9° is clearly seen in the pattern of the CPL.

Fig. 6 is the IR absorption spectrum of the CPL powder scratched from the samples. According to the previously reported results of OCP and apatite [22], the main absorption bands in the spectra could be assigned to be C–O of the CO_3 group at 1470 and 1425 cm^{-1}, to P–O of the PO_4 group at 1115, 1042, 970, 610 and 570 cm^{-1} and to P–OH of the HPO_4 group at 920 and 870 cm^{-1}. No obvious O–H absorption band of the OH group is seen in the spectrum. From XRD results, it can be determined that the PO_4 groups are from both apatite and OCP and that the HPO_4 group is from OCP. The CO_3 group probably existed in the apatite phase and arose from CO_2 dissolution in the calcifying solution. The apatite phase detected by XRD may thus be carbonated apatite rather than HA.

The Ca/P atomic ratios of the heavily, lightly and unscratched zones of the CPL were calculated from the EDX spectra (Fig. 2e–g) to be 1.85, 1.61 and 1.54, respectively. The ratio of the lightly scratched zone is close to that of HA (1.67). The EDX point analyses indicated that the atomic ratio of Ca/P of the crystal plates in the CPL outside sublayer was on average 1.34, which is almost consistent with that of OCP (1.33). It is inferred from the results of XRD, IR and EDX that the CPL is a mixture phase of OCP and carbonated apatite. The OCP phase is present in the outside loose crystal sublayer and carbonated apatite is the main phase of the dense inside sublayer.

4. Discussion

4.1. Modification mechanism of the c.p.Ti surface during the two-step treatment

Acid immersion at the first step was helpful to increase the surface area by producing many acid etched pits or grooves as expected. The alkali treatment at the second step may have two concurrent effects on the acid-etched surface. One is the formation of a microporous surface layer and the other is the formation of more titanium oxide in this microporous layer. Each step of the treatment is crucial in acquiring the microporous titanium oxide surface which was not produced by either acid or alkali treatment only.

4.2. Bioactivity of the obtained surface

Nanosized spaces of organic matrix with special negative distribution have been reported to be necessary for the nucleation of biological minerals, such as apatite [23]. Therefore, creating a negatively charged microporous metallic surface might also be an effective way to induce apatite nucleation from a SCS as suggested by de Groot *et al.* [19]. The microporous titanium oxide surface prepared by the two-step treatment met the above requirement, because titanium oxide (usually in TiO_2 form) surface has been generally accepted to be negatively charged by OH^- in aqueous solution. The submicrometre-sized pores probably are very important to Ca–P nucleation, because without them the bioactivity of the titanium oxide layer formed naturally or by alkali treatment was insufficient to induce Ca–P precipitation from the SCS.

4.3. The formation mechanism of conformal and adherent CPL

As the distribution of micropores over the surface was uniform, this could ensure that the Ca–P nucleation occurred everywhere and conformal (i.e. tightly following the underlying texture) CPLs were precipitated.

TABLE I Various methods to prepare biomimetic CPL from *in vitro* SCS on titanium implants

Methods	Polishing procedure	Temperature (max.) (°C)	Reagents for treatments
(i) Assisting Ca–P nucleation by Glass G [8]			
	Diamond paste to 3–4 μm	37	No
(ii) Inducing Ca–P nucleation by self-assembled monolayers [10]			
	No	< 100	Alkyl silane
(iii) Polishing to certain roughness			
1. Leitão *et al.* [9]	SiC papers, diamond paste	37	No
2. Li and Ducheyne [11]	Al_2O_3 paste to 0.05 μm	37	No
(iv) Fabricating bioactive TiO_2 hydrogel layer on titanium implants [12–15].			
1. Miyaji *et al.* [12–14]	Diamond paste to #400	600	10 M NaOH or KOH
2. Osaka *et al.* [15]	Yes	37	30% H_2O_2, $TaCl_5$ or $SnCl_2$
(v) Preparing bioactive microporous titanium surface by two-step chemical treatment			
Present work	No	140	HCl, H_2SO_4, 0.2 N NaOH

As far as the adhesiveness of the CPL to c.p.Ti is concerned, two aspects are important. Firstly, the nuclei initially induced from the SCS are most probably chemically trapped in the metallic oxide layer. Secondly, as shown in the micrographs of scratched zones (Fig. 4c), the early nucleation occurred within the micropores of the treated surface, so that mechanical entanglements also contribute to the adhesion.

4.4. A simple and easily controlled way to prepare a bioactive surface and produce CPL

Several techniques recently reported for the preparation of the biomimetic CPL on titanium implants by *in vitro* immersion in low-temperature SCS are listed in Table I. Compared with the other techniques, the two-step chemical treatment is simple and easily controlled and has the following advantages: (1) a polishing procedure is unnecessary; (2) no special reagents are applied; (3) no high-temperature treatments are necessary. The simple two-step treatment ensured that the results obtained in this work were quite reliable and reproducible. It has been confirmed that the two-step treatment was also effective to prepare bioactive microporous Ti6Al4V surfaces. The related results will be published in a subsequent paper.

5. Conclusion and future directions

A bioactive microporous titanium oxide surface was formed by a newly developed two-step chemical treatment. The two-step treatment is a simple and easily controlled chemical method resulting in a titanium surface that can induce rapid formation of conformal and adhering calcium phosphate layers from *in vitro* SCS.

Two directions are suggested for the future research. One is searching for the optimum condition of the two-step chemical treatment to prepare bioactive titanium surface, such as concentration and the treating time. The other is choosing a more suitable SCS for producing the CPL with good mechanical properties on the bioactive titanium surface.

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