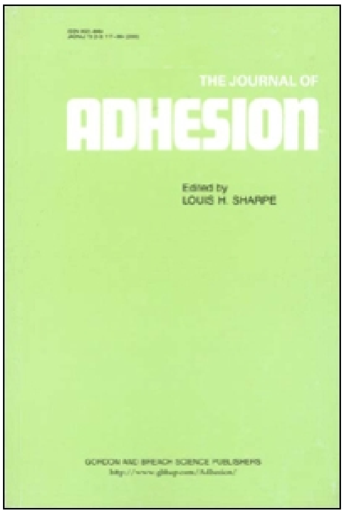

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Surface Characteristics of Hydroxyapatite and Adhesive Bonding. I. Surface Characterization†

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Adhesion to hydroxyapatite (HA), the mineral phase of calcified tissues, is important in cellular attachment and bonding of synthetic adhesive materials. The surface preparation of HA was found to affect the adhesion greatly. A pure synthetic HA was studied to examine the effect of commonly-used preparative techniques on surface morphology and activity. Surface grinding and polishing and organic acid etching gave rough surfaces that were hydrophobic. Etching with HCl or HNO_3 and firing at 800°C gave hydrophilic surfaces. ISS, SIMS and ESCA studies showed various degrees of organic and inorganic contamination. Plasma cleaning removed hydrophobic contaminants but inorganic contamination from the plasma vessel increased as time of exposure was increased. It was evident that current preparative procedures give highly variable surfaces that would be expected to influence adhesion of both monomer–polymer adhesive systems and cells.

KEY WORDS Adhesion; dentistry; morphology; preparative techniques; surface characterization; synthetic hydroxyapatite.

INTRODUCTION

Hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, is the principal inorganic (mineral) phase of calcified tissues. The outer enamel layer of teeth

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is almost 98% HA and the inner dentin and bone both contain about 60% HA. These tissues consist of a mass of small crystallites bonded together by a protein aqueous gel.[1] Adhesion between proteins and the HA is obviously important to structural integrity; protein binding is also important in relation to cellular attachment of the soft tissues and to the attachment of bacteria as in the instance of bacterial plaque accumulation on teeth. In addition to the adhesion of *in vivo* substances to HA, there is considerable interest in bonding extra-corporeal materials to the mineral phase of teeth and bones for reconstructive surgery and dentistry.

In dentistry, extensive use is made of acid etching of enamel to bond polymerizable coating and filling materials;[2] recently, so-called "dentin-bonding agents" have been developed that are of interest also as systems that have potential for adhesion to bone in surgical reconstruction.[3] These latter materials are monomer systems, based on polycarboxylic acid or polyphosphate or phosphonate derivatives,[3–5] which are polymerized after application through free radical initiation by a peroxide-amine system or by visible light-activated diketones.[6] Their use may be preceded by an acid etch treatment procedure. In principle, bonding occurs through chemsorption and complex formation of functional groups to the Ca in the HA.[3] A second dental adhesive system is based on aqueous polyacrylic acid cements.[5,7,8] These zinc polyacrylate and glass ionomer cements show substantial bonding *in vivo* via the polyacrylate anion interaction with the Ca^{++} of the enamel and dentin. The latter mechanism has been established by a variety of investigations.[8–12] Little work has been done on the mechanism of attachment of the polymerizable monomer systems, however, although numerous studies of bond strength have been carried out.[3,13–15]

The mineral phase of bone and teeth is a defect HA containing numerous other ions, notably F.[1] However, synthetic hydroxyapatite produced by sintering the precipitated material under controlled conditions[16] has well-defined reproducible properties[16,17] and has been shown to be similar to enamel in properties such as fluoride uptake,[18] bacterial plaque attachment[19] and bone adherence.[20] The majority of the existent commercial and experimental dentin adhesive systems have been formulated on the basis of specific reactivity towards HA[3,4] and should, therefore, display bonding to both the synthetic and natural substrates.

The purpose of the present work was to study factors affecting the potential bonding of representative adhesive systems to synthetic HA to establish a more basic approach to improved materials. In these experiments the attachment of various reactive monomers and primers to a synthetic HA has been investigated.

Preliminary work showed that the adsorption and stability of attachment of primers to HA was greatly affected by the surface preparation. Thus, a necessary approach to the problem was first to characterize the HA surface better and this is the subject of the present report.

MATERIALS AND METHODS

(A) Hydroxyapatite

A commercial pure sintered hydroxyapatite† was used in the present work. The preparation and properties of the basic material have been described elsewhere.[16,17] Sintered sheets of the material were cut into plates 10 × 10 × 2 mm. The experimental surface was ground with a 400 and then a 600 grit silicon carbide slurry under water cooling. The plates were finally cleaned ultrasonically using acetone/methanol and, finally, distilled water. This was the as-received state. A 1 mm diam. hole was drilled in one corner of each plate and two similar holes 1 mm deep in the non-test face to facilitate handling the plate without contamination. The prepared plates were suspended in test solutlions by a 316L stainless steel wire loop and handled only at the edges with PTFE coated forceps.

When required, the experimental surface was reground (Lunn Major Grinder, Struers, Denmark) manually to 600 grit SiC paper with water cooling and a virgin paper surface for each movement. Polishing was carried out using a Minimet Polisher (Buehler Inc.) and three successive series of diamond polishing pastes: 6, 1 and 0.25 micrometres (Grades M.6, H.1 and H.1/4, Micro Metallurgical Ltd.). After grinding and polishing, the plates were cleaned ultrasonically in 0.5% aqueous Pluronic 31R4, distilled water, methanol and distilled water.

Certain plates were suspended in a Harrick Plasma Cleaner

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† Durapatite^{R}—Sterling-Winthrop Research Institute.

(Harrick Scientific Corp., Ossining, N.Y.) and cleaned in air at a pressure of 0.3 to 0.5 Torr for a period of 5–15 minutes using a power setting of 7–10. The plasma cleaner itself was purged with creation of a plasma several times before use.

(b) Reagents

Solvents, inorganic acids and other compounds were purified grades, in most cases meeting "reagent grade" ACS standards. Polyacrylic acid (PAA) of average molecular weight 20,000 was prepared by us using the method of Smith.[21] The material was dialyzed and the test solutions filtered through a polycarbonate membrane with 5 μm pores (Nuclepore Corp.). Distilled water was obtained from our own still; for all of the contact angle experiments "Omnisolv" distilled water (BDH Chemicals, Toronto) was used. Deuterium oxide was a gift from D. W. Priddle, Department of Chemistry, University of Toronto. Sources of other materials were: disodium ethylenediaminetetraacetate (EDTA)—Fisher Scientific #S-311; lactic acid—J. T. Baker #0194 (88% aqueous solution containing lactic acid and its anhydride); Pluronic 31R4—BASF Wyandotte (block copolymer of ethylene oxide and propylene oxide).

(c) Procedures

1. *Etching*—Etching of HA surfaces was carried out by suspending the prepared plates in aqueous solutions of one of the appropriate reagents as follows: (a) 0.5% solutions of hydrochloric, nitric, and lactic acids and 1% EDTA for 10 min (b) 0.5% EDTA for 2 min and (c) 0.5% PAA for 20 min. The plates were then washed with copious amounts of water or methanol and air dried in a glass chamber.

2. *Firing*—Certain test plates were fired in air in an electic furnace up to 800° over a 2 hr period followed by cooling over 1 hr to 300°C before storage in a glass chamber.

3. *Surface energy from measurements of contact angles*—The advancing contact angle of water on test surfaces was determined

using a sessile drop method. An 0.8 μL drop of water was pipetted on to the surface and the contact angle measured immediately using a lens with protractor eyepiece. Four determinations at different locations were made and the mean calculated. For very hydrophilic surfaces, accurate determination was not attempted for angles below 10°. The surface tension of water was determined by measuring the force exerted by the liquid when in contact with a platinum plate attached to a microbalance. The plate was cleaned in a methanol flame just before use. The energy of the surface was calculated by the method of Neumann *et al.*[22] from the surface tension and mean advancing contact angle using a computer programme furnished by Professor A. W. Neumann, Department of Mechanical Engineering, University of Toronto.

4. *Surface analysis*—SIMS and ISS spectra of the prepared HA surfaces were obtained using a 3M-Kratos Model 535 BX combined ISS/SIMS spectrometer with the assistance of Dr. G. R. Sparrow (Advanced R and D Inc.) A surface area of about 6 mm^2 was examined for SIMS and about 2 mm^2 for ISS. The positive ions beams used were He or Ar with a beam energy of 2 keV or, more rarely, 4 keV.

ESCA spectra were obtained using a McPherson ESCA 36 instrument and Mg K-alpha X-radiation. (Surface Science Western, University of Western Ontario). Additional ESCA spectra were obtained using a Surface Science Labs SSX-100 spectrometer with the assistance of Dr. N. S. McIntyre (Surface Science Western, University of Western Ontario) and Dr. B. Ratner (National ESCA Center for Biological Materials, University of Washington).

SEM and EDX observations were carried out using an ISI-60 scanning electron microscope fitted with a Princeton Gamma Tech energy dispersive analysis system.

RESULTS

(a) Morphological findings

Typical ground surfaces of a HA plate as-received and after resurfacing with 600 SiC grit are shown in Figures 1 and 2 respectively. These features were little altered by firing.

FIGURE 1 Ground surface of as-received HA plate (SEM, ×1500).

Etching by the acids resulted in significant dissolution of the surface layer. HCl treatment led to separation of segments of a surface film which remained insoluble. Almost all the film separated in 10 min under the chosen conditions. Repetition of this procedure on a treated plate resulted in no further separation of a film or particles. HNO_3 etching did not cause separation of intact film segments but small white particles did separate from the surface during the first few minutes of etching. SEM observations showed that these mineral acids gave pitted surfaces (Figures 3, 4, 5). The small craters (about 0.5 μm) may be due to the removal of individual

FIGURE 2 Surface of HA plate ground with 600 SiC grit and ultrasonically cleaned with methanol and water (SEM, ×1000).

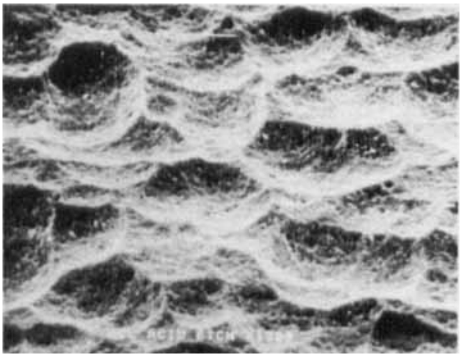

FIGURE 3 HA plate etched with 0.5% HCl for 10 min, washed in water (SEM, ×1500).

HA crystallites whereas the large craters of 7–20 μm diam. may be due to loss of aggregates. Washing with methanol to remove HCl after etching (Figure 4) did not result in any insoluble surface precipitation as contrasted to water rinsing (Figure 3).

Etching with the organic acids resulted in quite different microstructures. Lactic acid gave a generalized rough, porous appearance with some cavitation (Figure 6) whereas PAA showed localized pitting (Figure 7). The EDTA-treated surface resulted in a roughened appearance with some cavitation, though less so than the lactic acid (Figure 8).

FIGURE 4 HA plate etched with 0.5 HCl for 10 min, washed with methanol (SEM ×1500).

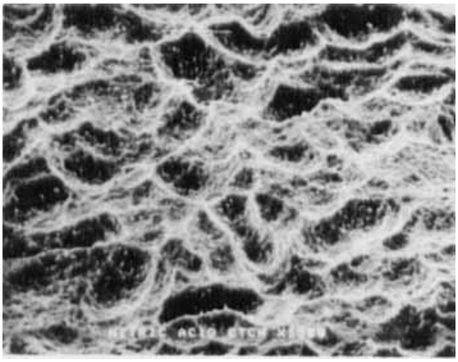

FIGURE 5 HA plate etched with 0.5% HNO_3 for 10 min, washed with methanol (SEM ×1500).

FIGURE 6 HA plate etched with 0.5% lactic acid for 10 min, (SEM, ×1500).

FIGURE 7

FIGURE 8 HA plate etched with 0.5% EDTA for 2 min (SEM ×1500).

(b) Surface energy changes

Table I shows that an as-received HA plate had a hydrophobic surface with the low surface energy of 34 ergs/cm^2 indicating organic contamination during preparation. Samples of these plates were then subjected to a variety of surface preparation methods, as listed in Table I, including grinding, polishing, ultrasonic cleaning, immersion in boiling water and heating to 190°C in air or in vacuum. The resulting surface energies (Table I) were quite variable. Some surfaces had even lower energies than the untreated plates but most had higher energies, *i.e.*, were more hydrophilic. Removal of the (presumed) organic contamination was variable and dependent on the original plate as shown by entries 5 and 6 where energies of 57 and 72 ergs/cm^2 respectively were obtained using the same procedure on different plates. Polishing procedures produced low energies (entries 10–12) though exposure to boiling water improved the situation. In contrast, plasma cleaning (entry 13) gave a completely hydrophilic surface with a contact angle of less than 10° and a surface energy greater than 72 ergs/cm^2.

Removal of organic contaminants by firing the plates at 800°C for 3–5 hours gave surface energies equal to or greater than 72 ergs/cm^2 for 4 plates tested (Table II). Surface energies of plates etched by the various agents are also given in Table II. Only HCl and HNO_3 produced markedly hydrophilic surfaces (Table II; entries 2–4). Washing the HCl-etched plates with water gave variable results

TABLE I
Surface energies of hydroxyapatite plates after various cleaning procedures

Entry #	Surface treatment	Surface energy (ergs/cm^2)	Advancing contact angle
1	None	34	84 ± 1°
2	Immersed in boiling water for 2 hours	44	68 ± 0°
3	Heated at 190° for 2 hours at atmospheric pressure	38	87 ± 2°
4	Heated at 190° for 2 hours in a vacuum of ~1 mm	26	97 ± 3°
5	a. Ground with 600 grit SiC paper; b. cleaned in ultrasonic bath.	57	47 ± 3°
6	As above, different plate	72	15 ± 2°
7	a. Ground with 600 grit SiC paper, b. cleaned in ultrasonic bath, c. immersed in boiling water for 2 hours	63	36 ± 5°
8	a. Ground with 600 grit SiC paper b. cleaned in ultrasonic bath, c. heated at 190° for 2 hours at atmospheric pressure	51	57 ± 2°
9	a. Ground with 600 grit SiC paper, b. cleaned in ultrasonic bath, c. heated at 190° for 2 hours in a vacuum of ~1 mm	34	84 ± 4°
10	a. Polished with 6μ, 1μ, and finally $\frac{1}{4}\mu$ diamond paste, b. cleaned ultrasonically.	33	86 ± 2°
11	a. Polished with 6μ, 1μ, and finally $\frac{1}{4}\mu$ dimond paste, b. cleaned ultrasonically, c. immersed in boiling water for 2 hours	61	40 ± 0°
12	a. Polished with 6μ, 1μ, and finally $\frac{1}{4}\mu$ diamond paste, b. cleaned ultrasonicaly, c. heated at 190° for 2 hours at atmospheric pressure.	43	71 ± 2°
13	Plasma cleaned.	>72	<10°

perhaps because of localized slow drying and absorption of atmospheric contaminants. Washing with methanol was much more consistent and the hydrophilicity did not change on exposure to boiling water (entry 3). The organic agents gave much lower and comparable energies (Table II; entries 5–8) suggesting residual organic materials.

TABLE II
Surface energy of hydroxyapatite plates cleaned by firing at 800°C or by etching with acids

Entry #	Surface treatment	Surface energy ergs/cm^2
1	Fired at 800°C	≥72
2	HCl etch (0.5% for 10 min; methanol wash)	≥72
3	a. HCl etch, as above, b. immersion in boiling water for 2 hours	72
4	Nitric acid etch (0.5% for 10 min; methanol wash)	~72
5	Lactic acid etch (0.5% for 10 min; water wash)	46 ± 4
6	EDTA etch (0·5% for 2 min)	37 ± 1
7	EDTA etch (1% for 10 min)	45 ± 2
8	PAA etch (0.5% for 10 min; water wash)	47 ± 4

(c) Surface spectroscopy

Selected plates subjected to the various "cleaning" procedures were examined using ISS, SIMS and ESCA as noted previously. Figure 9 shows the ISS spectrum of an as-received plate. Curve A is the sum of many scans taken during the first 16 secs. of sputtering and represents the outer atomic layers whereas curve B resulted from

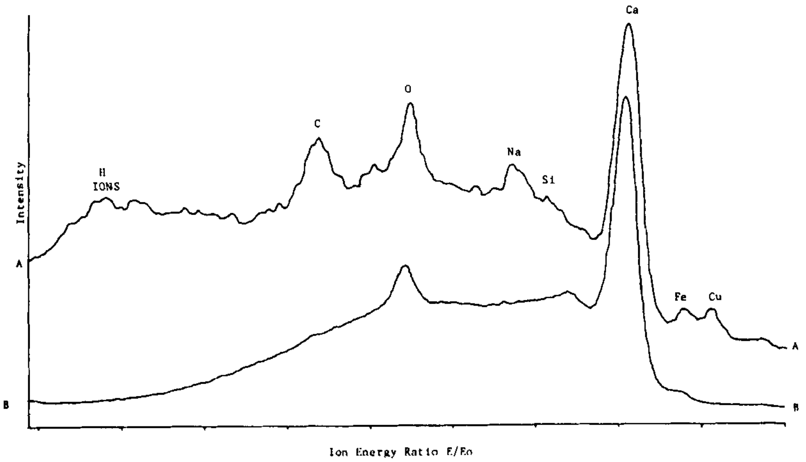

FIGURE 9 ISS spectra of as-received HA plate (A) and after 128 secs of sputtering (B).

128 secs of sputtering when about 1 nm would have been removed. Figure 10 shows a set of sequential ISS spectra giving a depth profile of the plate. The successive curves show the spectra after the indicated interval of sputtering.

SIMS spectra of the as-received plates are given in Figures 11–13. In the positive spectrum shown in Figure 11, curve A corresponds to the ions collected during the first 40 seconds of sputtering, and Curve B corresponds to the ions collected during a later 40-second period from the 80th second of sputtering to the 120th second of sputtering. Barium does not show up in the spectra in Figure 11, which were obtained with a helium ion beam, but it does apear in the spectrum in Figure 12, which was determined using a beam of argon ions. Figure 13 shows the negative $^3He^+$ SIMS spectrum. Since negative SIMS is sensitive to electronegative elements such as carbon, nitrogen, oxygen, and the halides, the prominence of m/e 16 (oxygen) from the hydroxyapatite and m/e 12 and 13 (C and CH from the organic surface layer) is expected.

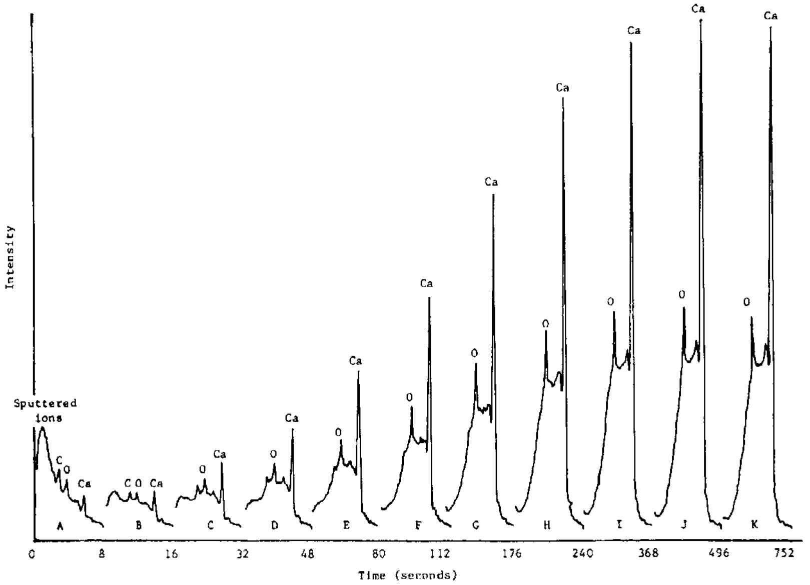

FIGURE 10 Sequential ISS spectra of as-received HA plate showing changes in major surface peaks with time of sputtering.

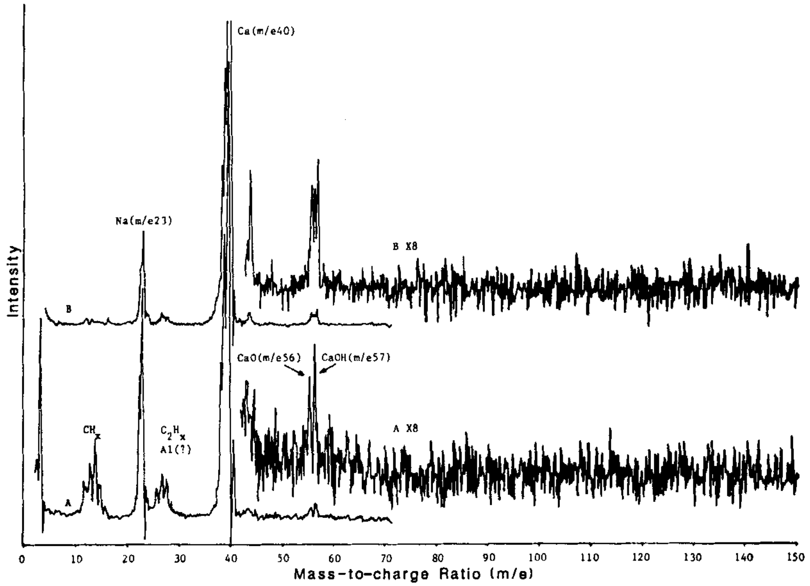

FIGURE 11 Positive SIMS spectra (He$^+$: 2 KeV) of as-received HA plate, showing initial state (A) and after 120 secs of sputtering (B).

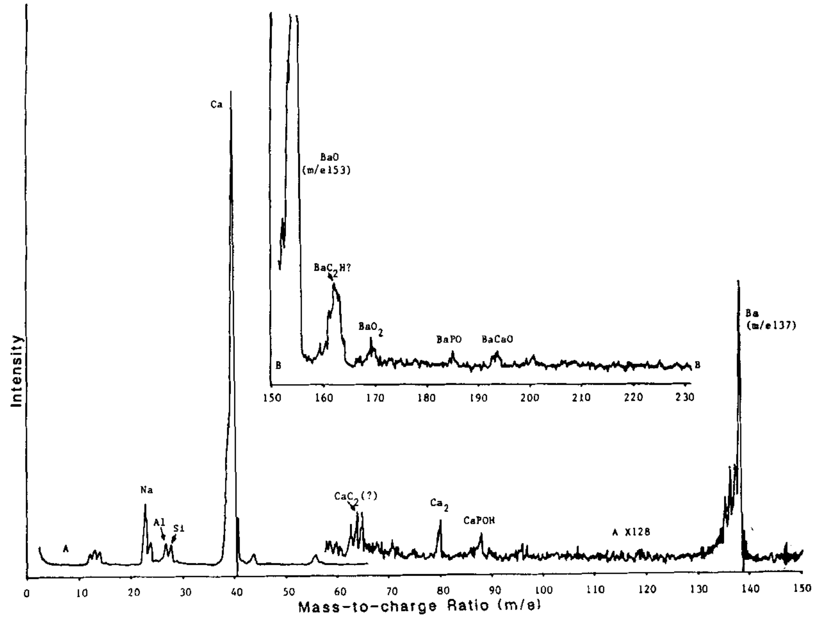

FIGURE 12 Positive SIMS spectra (40 Ar$^+$: 2 KeV) showing presence of Ba (compare Figure 11).

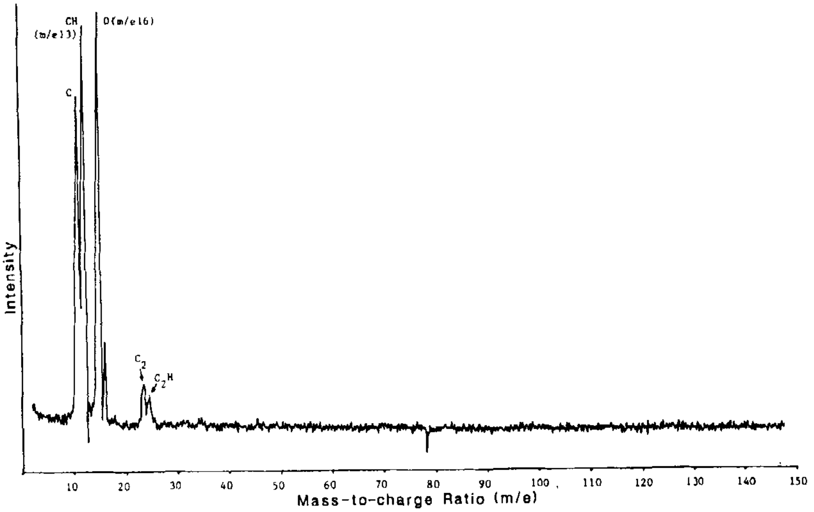

FIGURE 13 Negative SIMS (He: 2 KeV) of as-received HA plate after sputtering for 80 secs.

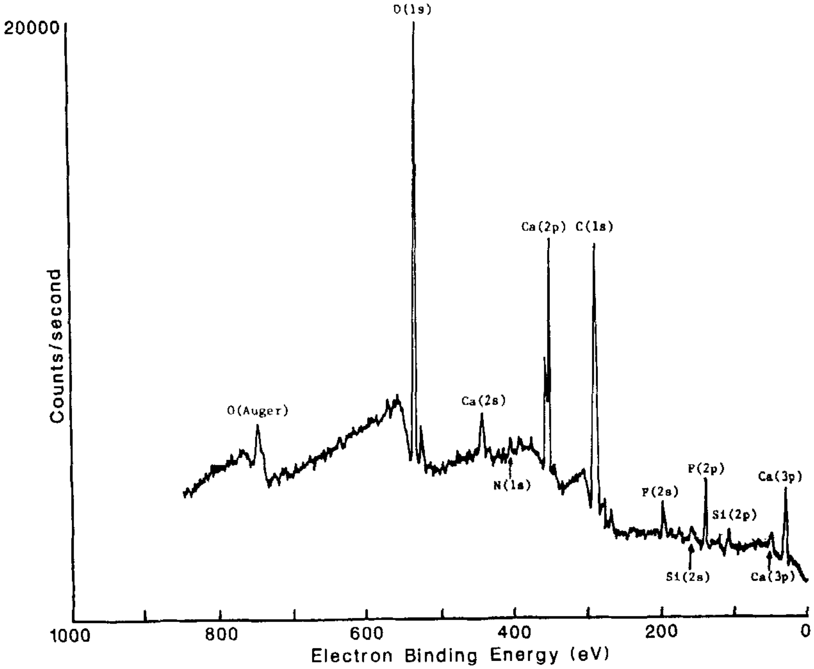

FIGURE 14 ESCA spectrum of as-received HA plate.

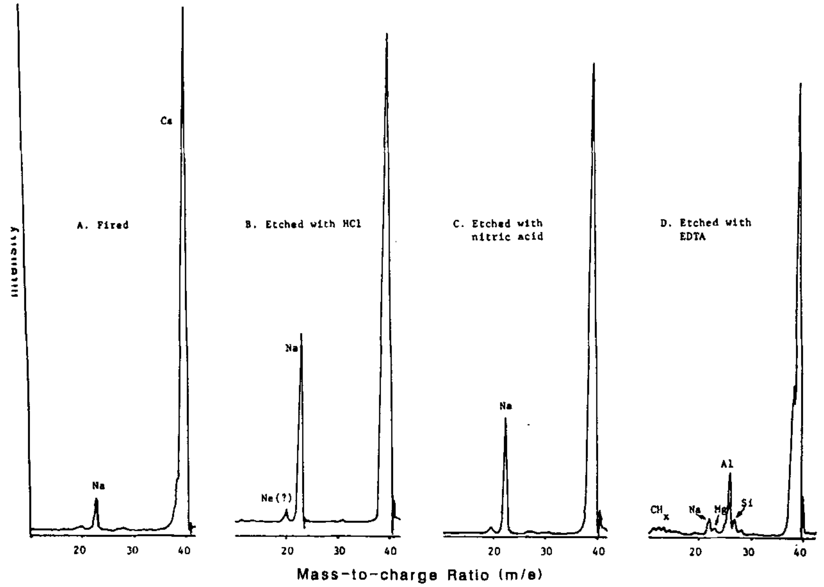

FIGURE 15 Sectional positive SIMS (He$^+$: 2 KeV) spectra for HA surfaces exposed to various firing and etching procedures.

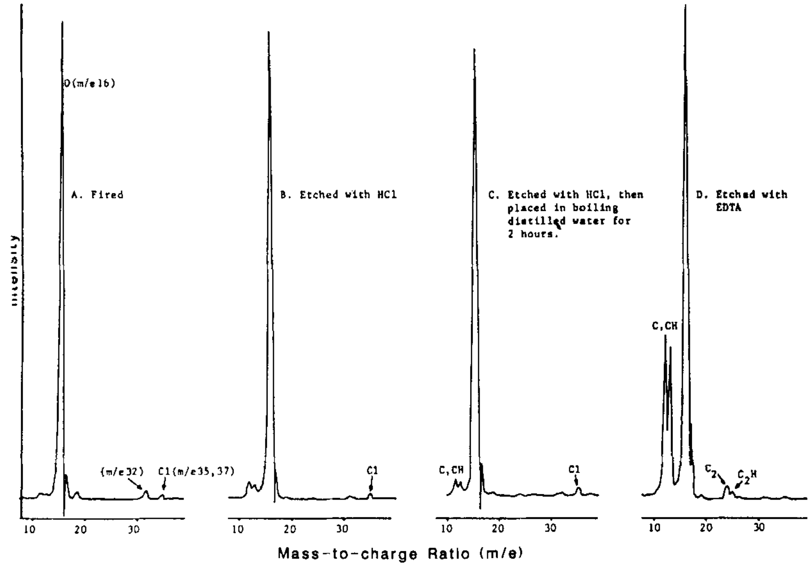

FIGURE 16 Negative SIMS (He$^+$: 2 KeV) sectional spectra of HA surfaces exposed to various firing and etching procedures.

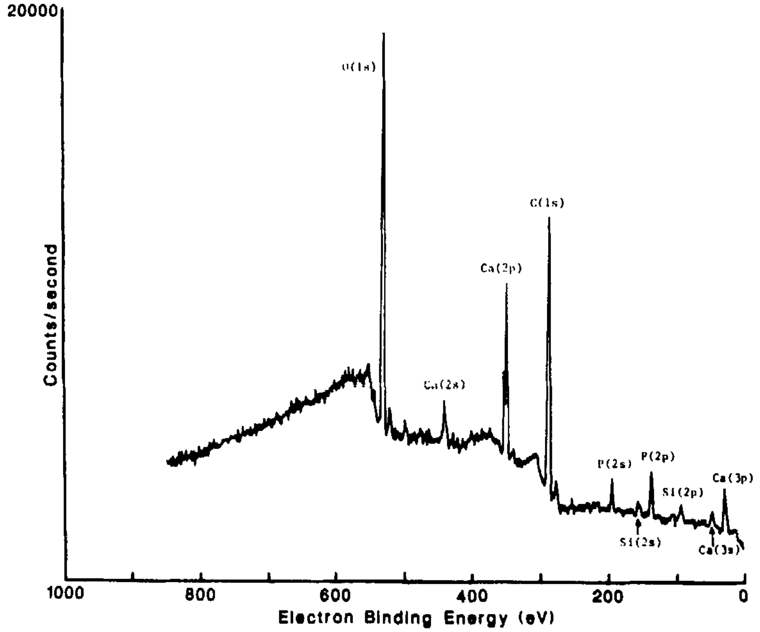

FIGURE 17 ESCA spectrum of HA plate etched with lactic acid.

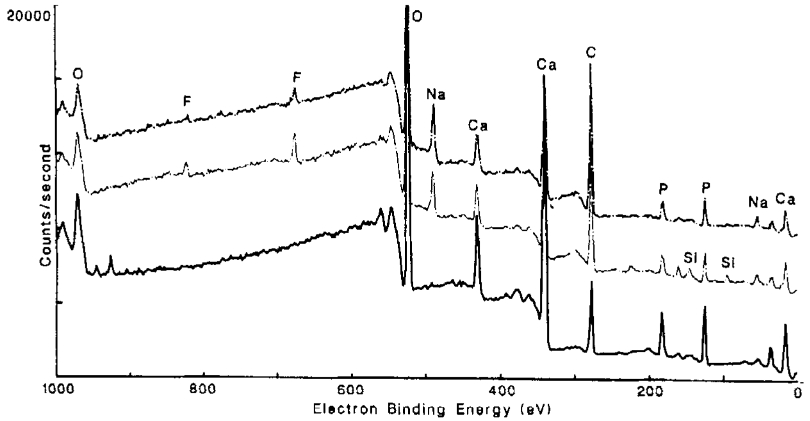

FIGURE 18 ESCA spectra of HA plates subjected to argon plasma for 1 min (bottom), 5 min (top), 15 min (middle).

Figure 14 shows an ESCA spectrum of the as-received surface.

Figure 15 shows positive $^3He^+$ SIMS spectra of fired, HCl-etched, nitric acid-etched, and EDTA-etched plates.

Figure 16 shows negative SIMS spectra for the fired, HCl-etched, HCl-etched followed by immersion in boiling water, and EDTA-etched plates.

The ESCA spectrum of a lactic acid-etched late is shown in Figure 17.

Figure 18 shows the ESCA spectrum of a plasma-cleaned plate that was subjected to various cleaning periods in an argon atmosphere as opposed to air.

DISCUSSION

Bonding to hydroxyapatite both *in vivo* and *in vitro* on enamel, dentin and bone as well as synthetic HA normally involves machined, ground, polished or etched surfaces[2–6,11,13–15,18–20] which vary in their adherence and biocompatibility. The extent of the influence of residual cutting debris (on teeth, the 'smear' layer[23]) and contaminants, both inherent and acquired, on the adhesion is uncertain as are the techniques for removal to effect durable bonding.[3,23] The surfaces examined in the present work are thus reasonable models of those currently used in adhesion investigations.

As might be expected, the morphological and spectral data show the presence of a variety of inorganic and organic contaminants. Figures 1 and 2 illustrate that the roughened debris-laden surface of the as-received surface was converted to a well-defined abraded surface after grinding and ultrasonic cleaning. This surface was less hydrophobic than the original (Table I, entry 1) both as supplied and after exposure to boiling in water or heating at 190°C (Table I, entries 2–4). One ultrasonically cleaned-ground specimen was hydrophilic with a contact angle of 15° but washing, drying and polishing procedures applied to this surface generally resulted in greater hydrophobicity (entries 7–12) through, probably, adsorbed adventitious organic contaminants. Boiling water treatment showed some tendency to improve wetting but only plasma cleaning of the hydrophobic surfaces resulted in easily wettable surfaces with contact angles of 10° or less and a surface energy greater than 72 ergs/cm^2 (Table I, entry 13), thus supporting the hypothesis that stripping off surface organic films with little change in morphology resulted in a hydrophilic surface.

These data illustrate the difficulty of avoiding surface contamination when customarily-used grinding or polishing procedures are employed. Arends *et al.*[24] have recently published work on the surface tensions of tooth enamel and related materials including this synthetic HA (Durapatite). The specimens "were polished on chemically pure cotton in a slurry of Al_2O_3 powder with distilled water" before measurement. They found the contact angle of distilled water on the polished Durapatite surface to be 42 ± 16° (standard deviation for six measurements). In a separate study,[25] the same investigators found that the contact angle of distilled water on polished enamel, after removal of 100–200 microns by grinding, was 47 ± 9° (standard deviation for 29 patients). They concluded that "Durapatite is not a reliable substitute for human enamel as far as contact angle measurements are concerned". Our results suggest that their HA surface was contaminated. It also seems probable that their measurements and calculations on enamel and the other minerals studied are erroneous because of surface contamination. To test this hypothesis, an experiment was carried out on polished enamel surfaces to determine the contact angle before and after plasma cleaning. One human incisor and one bovine incisor tooth, both of which had been stored in water following extraction, were first ground on the buccal side with 600 grit SiC paper (Carbimet, Buehler, Inc.) with water cooling until roughly 0.4 mm had been removed at the centre of the flat surface created. The flat surface was then polished with a slurry of 1 micron aluminum oxide powder (Linde C Polishing Powder) in distilled water on a cotton cloth (Metcloth, Buehler, Inc.) and was finally washed with a stream of distilled water and dried in air. The contact angle with distilled water of the ground and polished human enamel surface was 44 ± 2° (mean and mean deviation of two drops), and that of the bovine enamel surface was 33 ± 1° (mean and mean deviation of 4 drops). The teeth were then plasma cleaned, and the contact angles of the ground and polished surfaces were re-determined. In both cases the contact angle was less than 10°.

Removal of organic surface contaminants by firing seemed to be a simple alternative to plasma cleaning. Firing at 800°C for 3.5 hours gave surface energies equal to or greater than 72 ergs/cm^2 (Table II). However, firing has the disadvantage of not removing inorganic contaminants and the possibility of changing surface composition through diffusion or contamination from other materials in the furnace. Acid etching resulted in removal of the original surface though with morphological changes. The etchants reveal the microstructure (Figures 3, 4, 5) as shown previously by Jarcho *et al.*[16] but only hydrochloric and nitric acids produced markedly hydrophilic surfaces (Table II). Flooding the etched surface with methanol after HCl etching rather than distilled water produced hydrophilic surfaces more consistently, perhaps because of more rapid drying in vacuo. The hydrophobicity showed little change after treatment with boiling water for 2 hours (Table II, entry 3). Treatment with the organic agents resulted in more hydrophobic surfaces (though the concentrations used were not optimized) even though significant roughening occurred (Figures 6–8). These results suggested residual adsorbed contaminants.

Some light was thrown on these observations by the surface spectra. The ISS spectra from the as-received plates (Figure 9) demonstrate a high level of surface organic contamination in the outermost layers (curve A) whereas after about 1 nm of the surface had been removed by sputtering the C peak almost disappeared as was also the case for Na, Si, Fe and Cu. It should be emphasized that the different elements have different sensitivities and the relative peak heights do not correspond to relative concentrations. Under the operating conditions the sensitivity of C is about 1/15 that of Ca so that on a normalized basis the C peak would be about 15 times higher. The sequential ISS depth profile (Fig. 10), after various sputtering intervals up to a total of 752 seconds, demonstrates the gradual disappearance of surface impurities and changes in the Ca/O ratio up to about 300 secs *i.e.* after about 2.5 nm had been removed.

In the positive SIMS spectra for the as-received surface (Fig. 11), the presence of the organic material on the surface is shown by the C, CH, CH_2, CH_3 cluster at mass-to-charge (*m*/*e*) ratios of 12, 13, 14 and 15. The cluster of peaks around *m*/*e* 27 may be due to aluminum and silicon (*m*/*e* 28) as well as C_2H_x. Positive SIMS is especially sensitive to alkali metals and the strong sodium peak (*m*/*e* 23) does not necessarily indicate that it is present on the surface in high concentration. In curve B the organic and sodium peaks are less prominent relative to Ca (*m*/*e* 40) after the longer sputtering interval. The argon spectrum (Figure 12) reveals the presence of Ba and more prominent peaks for Al and Si. The increased attenuation in curve B shows that most of the ion clusters contain Ba. The negative SIMS spectrum in Fig. 13 shows prominent organic C peaks; minor F (*m*/*e* 19) and chlorine (*m*/*e* 35 and 37) peaks were detected (see Figure 16).

An ESCA spectrum of the as-received surface (Figure 14), which inherently relates to information from a greater depth, showed a prominent C peak indicating an organic coating though it was relatively thin, perhaps a few monolayers. Manual deconvolution of the expanded C1s peak suggested some of the organic layer contained carboxylic groups. The most prominent major impurity was silicon; it constituted about 2% of the outer 3–4 nm to which the instrument was sensitive.

It is of interest to compare the major inorganic surface contaminants of the as-received HA plates as shown in the foregoing spectra with the impurities found in the bulk of the material. The principal surface impurities were Na, Ba, Al, Si, Fe, F and Cl. Semi-quantitative spectrographic analysis of the same batch of material supplied by the manufacturer indicated that strontium was the main impurity; boron, silicon, aluminum, manganese, iron, magnesium, and chromium were also detected but at levels less than 0.01%. The preparative procedures for the plates have clearly resulted in a differential variation in surface elemental distribution as well as adventitious contamination. This was illustrated by further ISS and SIMS spectra that were obtained for a plate that had been ground to 600 grit and then subjected to several ultrasonic cleaning cycles. The spectra showed that an organic coating was present but much thinner, or perhaps patchy, in accordance with the lower hydrophobicity (Table I, entry 5) as compared with the as-received material. Na, Ba, F, Cl and Fe levels were also reduced but Cu and As (or Sr) had been increased.

These effects are apparent in the sectional positive SIMS spectra (Figure 15) for the HA surfaces subjected to the various firing and etching procedures. After firing, minimal C contamination was present but Na is prominent in this and in the hydrophilic mineral acid-etched surfaces. The EDTA-etched surface showed a cluster of peaks around *m/e* 13 due to organic contamination in addition to a relatively high Al and low Si and Na peaks corresponding to the moderately hydrophobic surface (Table II). The negative SIMS spectra (Fig. 16) confirm only a minimal level of organic contamination on the fired and HCl etched surfaces as compared with the as-received material (Figure 13). It is interesting to note the effect of immersion in boiling distilled water. Residual adsorbed material from the EDTA is again prominent.

An ESCA spectrum for a lactic acid-etched HA plate that was also moderately hydrophobic (Table II) is shown in Figure 17. Comparison with the spectrum of the as-received plate (Figure 14) indicates a similar level of organic contaminants. Since EDTA and polyacrylic acid are both known to adsorb to HA, it may be assumed that this is a layer of adsorbed lactic acid. However, this was ruled out on the basis of the shape of the expanded C1s peak which did not indicate any carboxylic C content.

Variation in exposure time and the carrier gas in the plasma cleaning process also produced significant variations in surface ion concentration.

ISS spectra of the reground plates subjected to an air plasma for 15 minutes showed very little carbon but prominent peaks for F, Si, Fe, Cu and Ba. The presence of these elements was confirmed by positive SIMS using both $3He^+$ and $40\,Ar^+$. These spectra showed also the presence of B and Sr. This was similar to the earlier findings but the Si and F were particularly prominent and appeared to increase with longer times. Corresponding plates were subjected to an argon plasma for 1, 5, 15 and 30 minutes. The data suggested that C removal was reduced compared to the air plasma but that Na, F and Si became increasingly prominent with time. This is evident in the comparative spectra for 1, 5 and 15 minutes shown in Figure 18. Thus, additional inorganic contamination, presumably from the walls of the plasma vessel, was being acquired with time. This spectral data confirms the findings of Baier *et al.*[26,27] that a maximum time of exposure of 3–5 min is desirable for air or argon plasma cleaning. Further investigation is needed to establish optimum plasma cleaning conditions for HA.

The present data show that the surface of HA resulting from commonly-used adhesion preparative procedures is highly variable and dependent on the precise technique. These morphological and chemical variations would be expected to influence adhesion both of monomer-polymer systems and cells. While this variability may be overcome by the use of reactive adhesive systems for clinical application, a defined preparative approach is desirable. Thus, further studies are indicated of the present procedures and others such as UV-ozone[28] to establish reproducible surfaces for *in vitro* investigation and a foundation for clinical techniques.

References

1. A. R. Ten Cate, *Oral Histology* 2nd Ed. (CV Mosby, St. Louis, 1985).
2. S. J. Gwinnett and D. C. Smith, in *Biocompatibility of Dental Materials,* D. C. Smith and D. F. Williams, Eds. (CRC Press, Boca Raton, 1982), Chap. 2, Vol. II, pp. 15–49.
3. E. Asmussen and E. C. Munksgaard, in *Posterior Composite Resin Dental Restorative Materials,* G. Vanherle and D. C. Smith, Eds. (3M Co., St. Paul, 1985). Section IV, pp. 217–230.
4. N. Nakabayashi, *Crit. Rev. Biocompat.* **1,** 25 (1985).
5. D. C. Smith, *Oper. Dent.* **7,** 14 (1982).
6. I. E. Ruyter, in *Posterior Composite Dental Restorative Materials* (CRC Press, Boca Raton, 1985), Sec. II, pp 109–135.
7. D. C. Smith, *Brit. Dent. J.* **125,** 381 (1968).
8. A. D. Wilson, *Chem. Soc. Rev.* **7,** 265 (1978).
9. W. J. Peters, R. W. Jackson and D. C. Smith, *J. Biomed. Mater. Res.* **8,** 53 (1974).
10. D. R. Beech, *Arch. Oral Biol.* **17,** 907 (1972).
11. S. Crisp, H. J. Prosser and A. D. Wilson, *J. Mater. Sci.* **11,** 36 (1976).
12. T. Nasu, *J. Biomed. Mater. Res.* **20,** 347 (1986).
13. R. L. Bowen, *Int. Dent. J.* **35,** 155 (1985).
14. N. Nakabayashi, *Int. Dent. J.* **35,** 145 (1985).
15. K. F. Leinfelder, *et al., J. Ala. Dent. Ass.* **70,** 15 (1986).
16. M. Jarcho, *et al., J. Mater. Sci.* **11,** 2027 (1976).
17. M. B. Thomas, *et al., J. Mater. Sci.* **15,** 891 (1980).
18. M. Jarcho, *et al., J. Dent. Res.* **57,** 917 (1978).
19. M. Jarcho, J. R. O'Connor and D. A. Paris, *J. Dent. Res.* **65,** 151 (1977).
20. M. Jarcho, *et al., J. Bioeng.* **1,** 79 (1977).
21. D. C. Smith, U.S. Pat. 3,605,605 (1972).
22. A. W. Neumann, *et al., J. Coll. Interfac. Sci.* **49,** 291 (1974).
23. W. R. Cotton, *Oper. Dent.* **9,** Suppl. 3 (1984).
24. H. P. de Jong, *et al., Surface and Colloid Phenomena in the Oral Cavity; Methodological Aspects,* (R. M. Frank, Ed. IRL Press, London, 1982), p. 106.
25. H. P. de Jong, *et al., J. Dent. Res.* **61,** 11 (1982).
26. R. E. Baier and V. A. DePalma, Calspan Corporation Report no. 176, 1982.
27. R. E. Baier, *et al., J. Biomed. Mater. Res.* **18,** 337, (1984).
28. J. R. Vig, in *Surface Contamination: Genesis, Detection and Control,* Vol. 1, K. L. Mittel, Ed. (Plenum Press, New York, 1979), pp. 235–254.