

# Bone Cell Attachment to Dental Implants of Different Surface Characteristics

Narong Lumbikanonda, PhD<sup>1</sup>/Rachel Sammons, PhD<sup>2</sup>

*Previous studies to compare the influence of surface characteristics of dental implants on cell behavior have used model systems to simulate the implant surface. In this study, bone cell interactions with smooth titanium, titanium dioxide-blasted, titanium plasma-sprayed, and hydroxyapatite plasma-sprayed implants, as manufactured for clinical use, were compared. Implants were exposed to neonatal rat osteoblast cells in suspension for a 20-minute period and, by means of scanning electron microscopy, attached cells were classified according to stage of attachment. Quantitative analysis showed that cells spread most quickly on the titanium plasma-sprayed implants. Fully spread cells on the smooth titanium implants were closely adherent to the surface, while on the titanium dioxide-blasted surface they showed no adaptation to surface irregularities. On the hydroxyapatite-coated implants, cells adhered closely only to smooth areas. To avoid the use of proteolytic enzymes for cell derivation, the authors developed a novel organ culture system in which the implant was contained in a nylon pocket surrounded by bone fragments, permitting cells to migrate onto the implant surface. Cultures were maintained for up to 4 weeks, allowing comparison of cell migration, proliferation, and differentiation on the implant surfaces. (INT J ORAL MAXILLOFAC IMPLANTS 2001;16:627–636)*

**Key words:** cell attachment, dental implants, hydroxyapatite, osteoblast-like cells, titanium

Dental implant surface characteristics can influence cell attachment and subsequent osseointegration in vivo.<sup>1–3</sup> Much information on the interactions between cells and materials has been gained from in vitro studies of the influence of surface characteristics on cell behavior using model systems in which the implant surface is simulated by a test material, often in the form of discs. Factors that have been investigated include surface roughness,<sup>4</sup> titanium,<sup>5</sup> plasma cleaning,<sup>6</sup> sterilization techniques,<sup>7,8</sup> and the crystallinity of hydroxyapatite surfaces.<sup>9</sup> However, model systems cannot easily simulate any effects of implant design or changes that may be made to the surface during the process of fabrication and packaging. In the present study, established techniques<sup>4,5</sup> were used to compare cell attachment

and spreading on 5 different commercially available dental implants, as manufactured for clinical use. In previous investigations, numbers of attached cells have usually been determined indirectly from counts of detached cells,<sup>4–9</sup> by using radioactively labeled cells,<sup>10</sup> or from captured images.<sup>11</sup> In the experiments described here, the actual number of attached cells was determined by counting all the cells attached to the different implants as viewed by scanning electron microscopy (SEM). This permitted quantification and comparison of the numbers of cells at different stages of attachment, as described by Rajaraman and coworkers,<sup>12</sup> following a 20-minute attachment period on the different implant surfaces.

As with the work of previous authors, the current study examined cells in suspension. Such studies have been open to criticism<sup>13</sup> because of the use of proteolytic enzymes, such as trypsin, in the process of preparation of the cell suspensions. To avoid this, some researchers have placed test materials in direct contact with, for example, rat calvarial bone, from which the cells migrate onto the test material.<sup>14</sup> In the present study this method was adapted in the development of a novel “pocket”

<sup>1</sup>Lecturer, Department of Oral Surgery, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand.

<sup>2</sup>Lecturer, University of Birmingham School of Dentistry, Birmingham, United Kingdom.

**Reprint requests:** Dr R. L. Sammons, University of Birmingham School of Dentistry, St Chad's Queensway, Birmingham B4 6NN United Kingdom. E-mail: r.l.sammons@bham.ac.uk

**Table 1 Dental Implants Used in This Study**

Implant	Shape (diameter)	Material and surface characteristics	System/manufacturer
Smooth Ti	Screw (3.5 mm)	Smooth Ti, as-machined	Astra/Astra Tech, Mölndal, Sweden (distributed by Astra Tech Ltd UK)
TiO <sub>2</sub> -blasted	Screw (3.5 mm)	TiO <sub>2</sub> -blasted	Astra/Astra Tech, Mölndal, Sweden (distributed by Astra Tech Ltd UK)
TPS (ITI)	Screw (4.0 mm)	Ti plasma-sprayed	ITI/Straumann, Waldenburg, Switzerland (distributed by Straumann Ltd UK)
TPS (IMZ)	Cylinder (3.3 mm)	Ti plasma-sprayed	IMZ/Friatec AG, Mannheim, Germany
HA-coated (IMZ)	Cylinder (3.3 mm)	HA plasma-sprayed	IMZ/Friatec AG, Mannheim, Germany

All implants were 8 mm in length.

Ti = commercially pure titanium; TiO<sub>2</sub>-blasted = titanium implant blasted with pure titanium dioxide powder; TPS = titanium plasma-sprayed; HA = hydroxyapatite.

organ culture system, in which the implant is completely surrounded by bone fragments and contained in a nylon pocket. This method more closely simulates the *in vivo* situation and permits comparison of cell migration, proliferation, and subsequent differentiation on different implants.

## MATERIALS AND METHODS

### Dental Implants

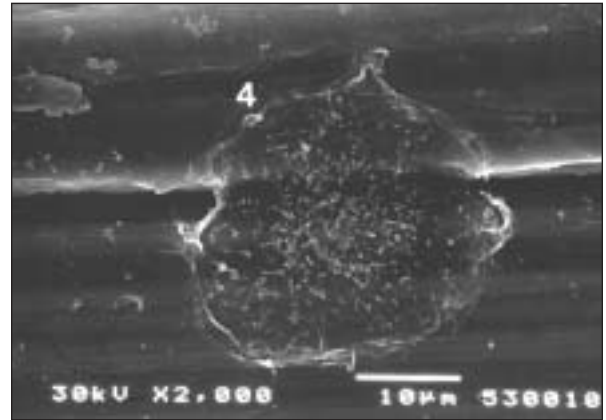
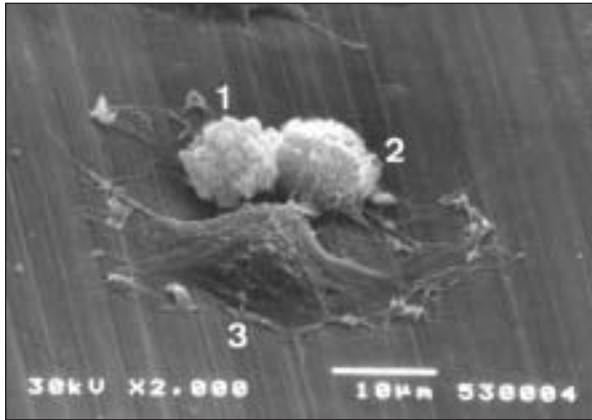
The dental implants used in this study and their designs and manufacturers are listed in Table 1.

### Preparation of Cell Suspensions

Primary calvarial cells were isolated from parietal plates of 3-day-old albino Wistar rats.<sup>13</sup> To increase the exposed surface from which bone cells could grow, following removal of periosteal tissue from both surfaces of parietal bone, the bone pieces were cut into roughly triangular pieces with sides approximately 1 mm long. The bone from 2 animals was transferred to Fitton Jackson's modification of Bigger's medium (Gibco BRL, Paisley, United Kingdom) containing 10% fetal calf serum (Northumbria Biological, Crumlington, United Kingdom); 25 mMol/L HEPES, L-glutamine (200 mMol/L, Sigma, Poole, Dorset, United Kingdom); and penicillin/streptomycin (50 units/mL, Sigma) in a 25-mL

tissue culture flask. This was incubated in 5% (v/v) carbon dioxide (CO<sub>2</sub>) at 37°C for 7 to 8 days until the cells that had migrated off the bone were confluent, with changes of medium after the first 48 hours and then every 2 to 3 days. Similarly derived populations of osteoblast-like cells have been shown *in vitro* to exhibit an osteoblastic phenotype, including expression of alkaline phosphatase activity, and synthesis of collagen type 1, osteopontin, and osteocalcin.<sup>15</sup>

Cells were detached from the flask using trypsin-EDTA (0.25%, Sigma). The enzyme was inactivated by adding 5 mL of culture medium, and the resulting cell suspension was filtered through nylon gauze (100- $\mu$ m aperture, Fisher Scientific, Loughborough, United Kingdom) to remove remaining clumps of cells and bone pieces. This filtered suspension was then centrifuged at 1,500 rpm for 5 minutes and the supernatant discarded. The pellet of packed cells was resuspended in 2 mL of the above-mentioned medium and placed for 1 hour recovery in an incubator at 37°C in 5% CO<sub>2</sub>. The cell number in the suspension was determined and adjusted to 10,000 cells/mL in the culture medium. Approximately 50,000 cells in 5 mL media were exposed to the 5 different implants contained within the same sterile 10-mL universal tube. After 20 minutes' incubation at 37°C in 5% CO<sub>2</sub>, the medium containing unattached cells was pipetted



**Figs 1a and 1b** Stages in cell spreading according to Rajaraman and associates,<sup>12</sup> as represented by cells attaching to a smooth Ti implant after 20 minutes' incubation in contact with an osteoblast-like cell suspension. Sequential changes in appearance from rounding, with a few filopodia (stage 1, left), to cells with focal cytoplasmic extensions or lamellipodia (stage 2, left), circumferential spreading (stage 3, left), and full spreading and flattening into a polygonal shape (stage 4, right).

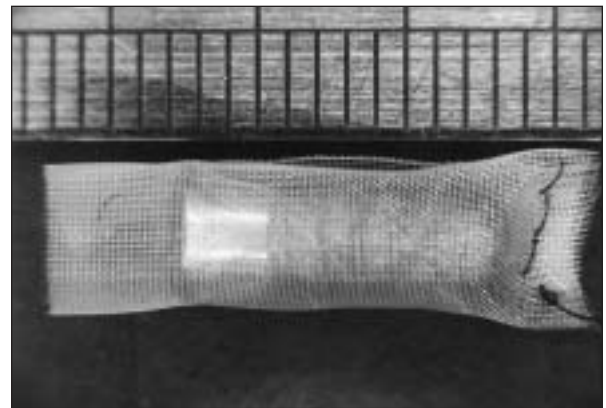
off, and the implants were rinsed 3 times with phosphate-buffered saline (PBS) to remove any unattached cells. Samples were fixed for 1 hour in 2.5% glutaraldehyde (EM Grade, Agar Scientific, Stansted, United Kingdom) in 0.1 mMol/L sodium cacodylate buffer pH 7.3 dehydrated in ethanol and critical point-dried from liquid CO<sub>2</sub> using a Polaron critical point-drier (Agar Scientific). Samples were coated with gold using a Denton Desk II sputter coater (Microfield Scientific Ltd, Kingston Bagpuize, Oxfordshire, United Kingdom) for 60 seconds and viewed using a JEOL 53000LV SEM (JEOL Ltd, Welwyn Garden City, United Kingdom) operating in secondary electron imaging mode with a beam current of 20 to 30 kV and a working distance of 13 to 14 mm.

### Scanning Electron Microscopic Evaluation of Cell Attachment and Spreading

At 20 minutes, all the attached cells were identified and counted by scanning the root-like portion or coated surface of each implant. The distribution of cells was not consistent over the entire surface on each implant because of differences in implant design and surface topography, the setting and position of the implants in the container, and the gravitation of the cells. All cells present were counted and their morphology recorded.

### Cell Attachment Assays

The numbers of cells attached to implant surfaces were determined from 3 replicate experiments (3 implants of each type in total). Cells were classified according to the 4 stages of attachment described by Rajaraman and coworkers<sup>12</sup> (Figs 1a and 1b). In this classification, the first stage of attachment is charac-



**Fig 2** Nylon pocket with inserted implant before packing with bone fragments. The markings on the ruler are 1 mm apart.

terized by rounded cells with a few filopodia, which progress to cells with focal cytoplasmic extensions or lamellipodia (stage 2), circumferential spreading (stage 3), and full spreading and flattening into a polygonal shape (stage 4). The numbers of cells at each stage were expressed as a percentage of the total and examined by 1-way analysis of variance (ANOVA). *P* values < .05 were considered significant.

### "Pocket" Cultures

Cylindric nylon mesh pockets, 4 to 5 mm in diameter and 15 mm in length, were made from nylon gauze (100- $\mu$ m aperture, Fisher Scientific), sewn together with suture thread, and sterilized by autoclaving at 1.1 Bar, 120°C for 20 minutes. A dental implant was placed into each pocket, with the parietal bone fragments from 4 animals (approximately 30 fragments) packed around and in direct contact with the surface of the implant (Fig 2). The pockets

**Table 2 Total Numbers of Cells Present on Each of the Implants**

Experiment	Smooth titanium	TiO <sub>2</sub> -blasted	TPS (ITI)	TPS (IMZ)	HA
1	579	633	759	648	28
2	104	104	166	107	108
3	257	285	336	310	260

As evaluated after 20 minutes' exposure to suspension of osteoblast-like cells in 3 separate experiments. In each experiment, 1 implant of each type was placed in the same container.

were then transferred individually to 25-mL flasks containing cell culture medium (as above), which were placed upright in the incubator. Cultures were maintained at 37°C in 5% (v/v) CO<sub>2</sub>. The medium was changed after the first 48 hours and then every 2 to 3 days. After 1, 2, or 4 weeks, pockets were rinsed twice in sterile PBS and fixed and dehydrated as above. The nylon pocket was then cut open. Bone pieces mostly remained attached to the nylon and were easily separated from the implant, which was then mounted for SEM.

## RESULTS

### Assessment of Cell Numbers at Different Stages of Attachment on Implant Surfaces

Three identical experiments were carried out on different days, with 1 implant of each type in the same culture vessel per experiment. Actual numbers of cells counted on the implants in each experiment are shown in Table 2. As can be seen, similar numbers of cells attached to each of the implants except for the hydroxyapatite- (HA) coated implant in experiment 1, to which very few cells had attached. To minimize the effect of variations in cell numbers between experiments, they were treated separately and, for each implant, the numbers of cells at each stage of attachment were expressed as a percentage of the total cell number in that experiment. The means and standard deviations were then calculated. Results are shown in Figs 3a to 3e.

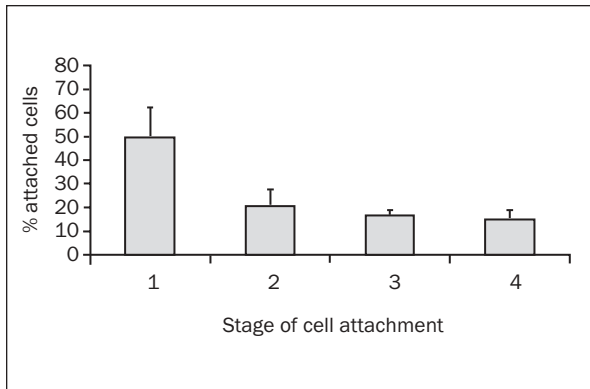
Cells representing all 4 stages of attachment were observed on all 5 kinds of implants. Two distinct patterns of cell behavior were observed: the first was seen in the HA-coated (IMZ, Interpore International, Irvine, CA), titanium dioxide- (TiO<sub>2</sub>) blasted (Astra, Astra Tech, Mölndal, Sweden), and smooth Ti implants (Astra), in which the highest

numbers of cells were seen at stage 1 of attachment, with fewer cells at later stages of attachment; and the second was seen in the 2 titanium plasma-sprayed (TPS) implants (ITI, Straumann, Waldenburg, Switzerland; and IMZ), in which there were approximately equal numbers of cells at each of the stages. Statistical analysis of the normalized results showed that there were no significant differences between the percentages of cells at stages 2, 3, and 4 on the HA-coated, TiO<sub>2</sub>-blasted, and smooth Ti implants ( $P$  values = .29, .46, and .42, respectively), but the numbers of cells at these stages were significantly lower than the numbers at stage 1 in each of these cases ( $P$  = .001, .0005, and .002, respectively). In contrast, there were no statistical differences between numbers of cells at any of the stages on either of the TPS implants ( $P$  = .8 [ITI] and .1 [IMZ]) or between these 2 implants when the numbers of cells at each stage were pooled ( $P$  = 1). This suggests that the TPS surface favors cell spreading, whereas on the other implant surfaces, spreading may occur relatively more slowly.

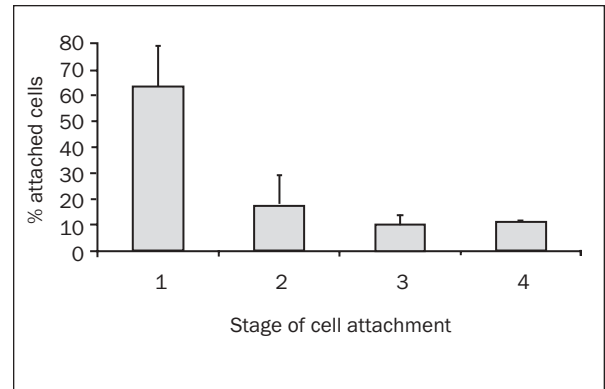
Comparison of the percentages of cells at stage 1 on each of the implants showed there to be no significant differences between the smooth Ti and the 2 TPS implants ( $P$  = .14), or between these 3 and the HA-coated implants ( $P$  = .19). However, there was a significantly higher percentage of cells at stage 1 on the TiO<sub>2</sub>-blasted implant compared with the other titanium implants when percentages of cells at stage 1 on these were pooled ( $P$  = .02).

These data suggest that of all the implants, the TiO<sub>2</sub>-blasted implant surface was the least conducive to cell spreading, although the HA-coated implants also had relatively higher numbers of cells at stage 1 compared with later stages. However, caution should be exercised when interpreting all these results, because sample numbers were small in all cases.

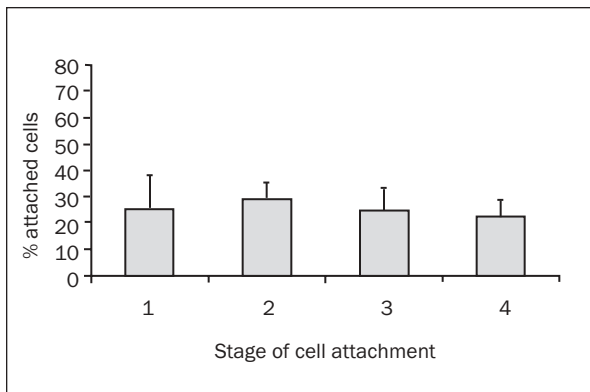
**Figs 3a to 3e** Stages in the progression of attachment and spreading on different implant surfaces, evaluated after 20 minutes' incubation in an osteoblast-like cell suspension. The graphs show the mean number of attached cells at each stage of attachment, expressed as a % of the total number of attached cells. Error bars represent the standard deviations of the mean (n = 3).



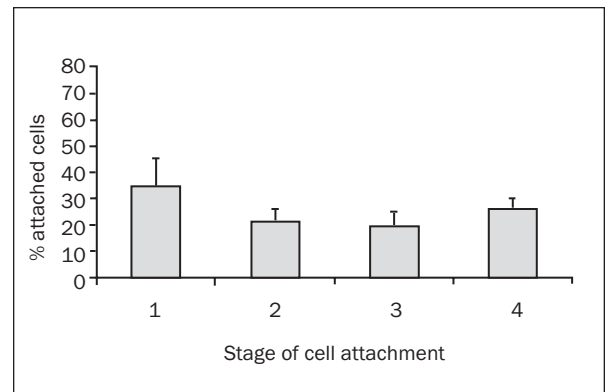
**Fig 3a** Smooth Ti implants.



**Fig 3b** Titanium dioxide-blasted implants.



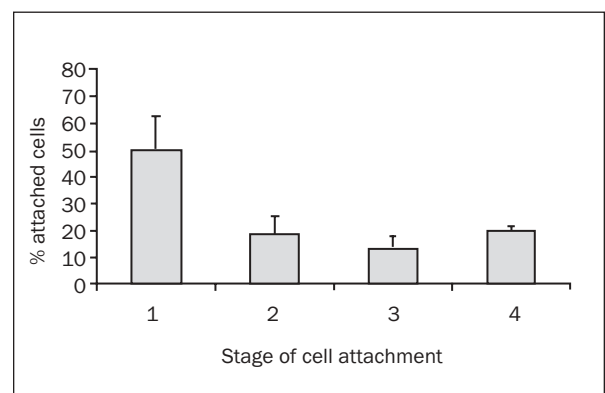
**Fig 3c** Titanium plasma-sprayed implants (ITI).



**Fig 3d** Titanium plasma-sprayed implants (IMZ).

### Morphology of Fully Spread Cells on Implant Surfaces

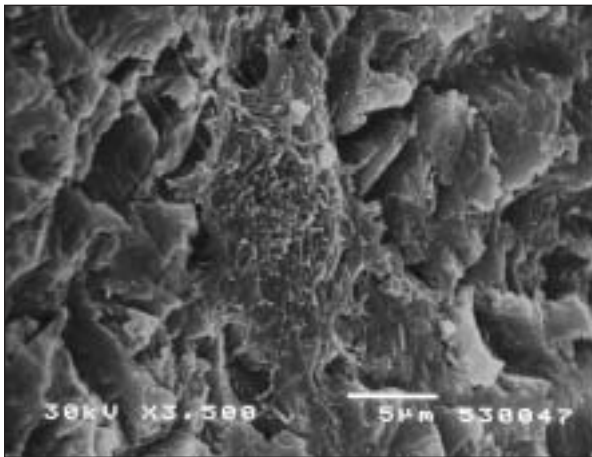
The morphology of cells at stage 4 was compared on each implant surface. Although variable, cells at this stage were identified by extensive flattening and spreading of the cell cytoplasm, with broad cytoplasmic extensions. In some cases, the bulge of the cell nucleus was visible. On the smooth Ti implants, cells were polygonal or circular in shape, with complete cytoplasmic extension of the cell body on the substrate surface. The cell nuclei were not usually visible. Except in the case of deep surface ridges, the cell body formed such intimate contact with the implant surface that the underlying topography of the surface was visible beneath the cell (Fig 1b), and the edges of the spreading cells were often so thin that they became indistinguishable from the substrate surface. On the TiO<sub>2</sub>-blasted surface, cells were more irregularly shaped, and adaptation to



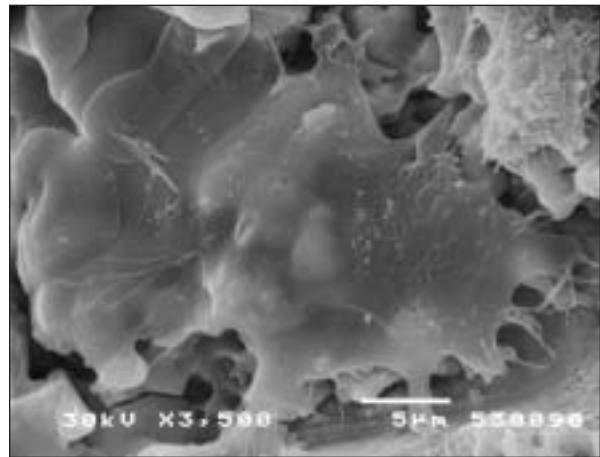
**Fig 3e** Hydroxyapatite plasma-sprayed implants (IMZ).



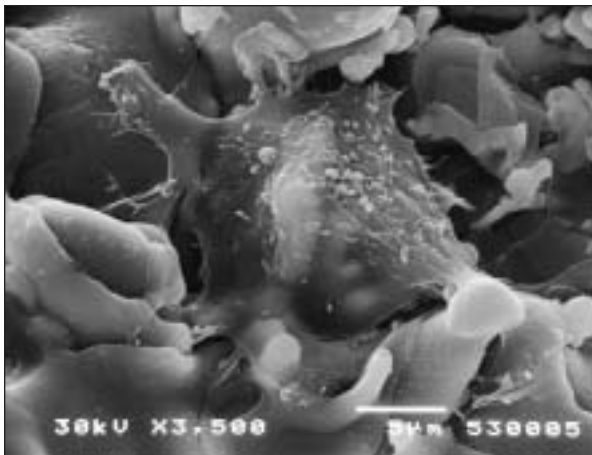
**Figs 4a to 4d** Cell morphology on different implant surfaces. Representative images of cells at stage 4 (fully spread) on each of the implants tested. A cell at stage 4 on smooth titanium is shown in Fig 1b.



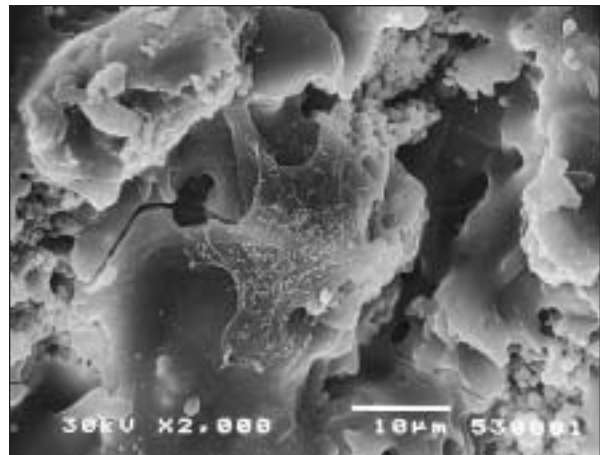
**Fig 4a** Titanium dioxide-blasted implant.



**Fig 4b** Titanium plasma-sprayed implant (ITI).



**Fig 4c** Titanium plasma-sprayed implant (IMZ).



**Fig 4d** Hydroxyapatite plasma-sprayed implant.

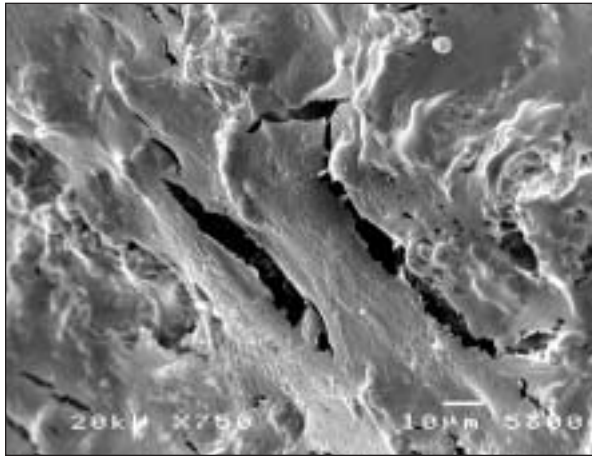
surface irregularities was not observed. The flattening and spreading cells spanned across pits and pores, mostly contacting prominent features of the surface (Fig 4a). In contrast to this, cells on the TPS implants (ITI and IMZ) were more intimately attached to the surface, with more areas of contact between lamellipodia and the surface. The cell bodies spanned pores, grooves, and pits, although some adaptation to the irregularities of the underlying surface was commonly observed (Figs 4b and 4c). The surface of the HA plasma-sprayed implant was heterogeneous, consisting of areas with a smooth, rounded profile, like that of the TPS surface, and areas made up of clusters of small grains (approx-

mately 1  $\mu\text{m}$  diameter) of incompletely melted or unmelted particles. Cells attached to all areas, but most of those on the granulated surface were at stages 1 or 2, while on the smoother areas the cells were most frequently at more advanced stages of spreading and exhibited similar morphology to cells on the TPS surfaces (Fig 4d).

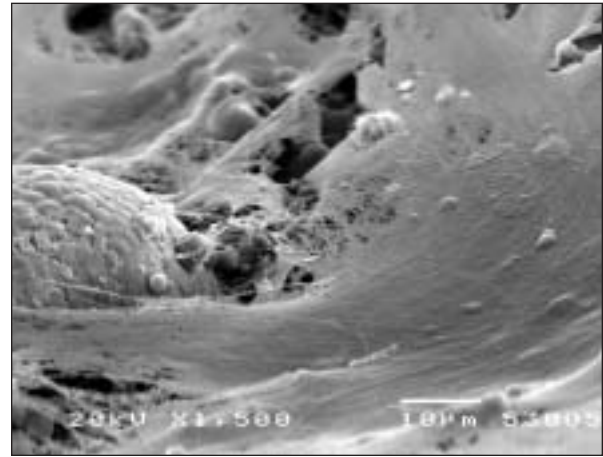
### Pocket Cultures

Two each of the TPS (ITI), TPS (IMZ), and HA (IMZ) implants were placed in separate nylon pockets surrounded by bone fragments and incubated for 1 or 2 weeks. A third TPS (ITI) implant was placed in culture for 4 weeks. Representative electron

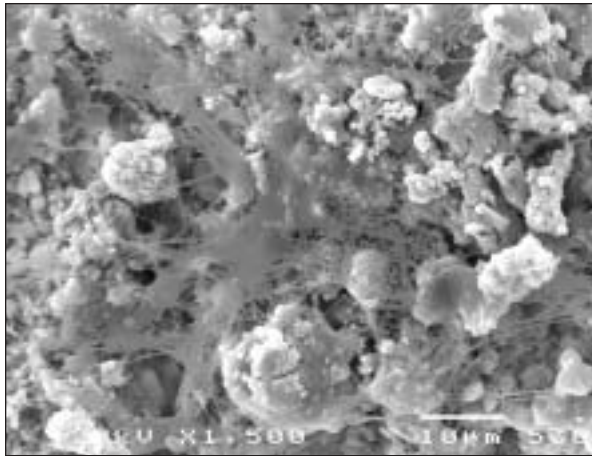
**Figs 5a to 5d** Pocket cultures: Representative electron micrographs showing morphology of cells that have migrated from bone fragments onto the implant surface. Bars = 10  $\mu$ m.



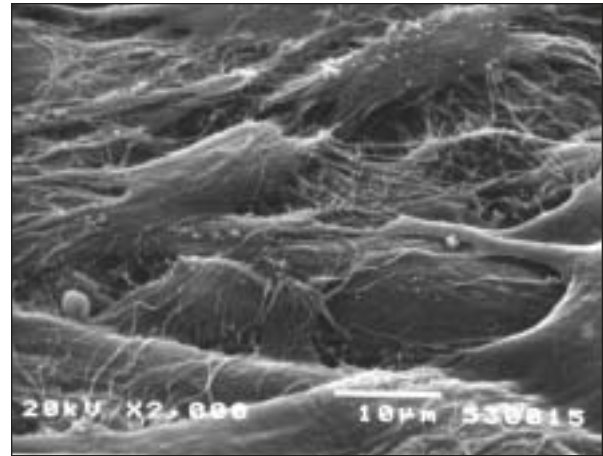
**Fig 5a** Titanium plasma-sprayed after 2 weeks (ITI).



**Fig 5b** Titanium plasma-sprayed after 2 weeks (IMZ).



**Fig 5c** Hydroxyapatite plasma-sprayed after 2 weeks.



**Fig 5d** Titanium plasma-sprayed after 4 weeks (ITI).

micrographs of the cultures are shown in Figs 5a to 5d. On the TPS (ITI and IMZ) implants, cells spanned across the rough surface of the implants, contacting prominent surface features and forming a loose lattice. The cells had smooth dorsal surfaces and were anchored to the implant surface and to adjacent cells. By 2 weeks some of the cells had formed a multiple-cell layer, through which the implant surface protruded in prominent areas. On the HA-coated implant, the cells were sparse and connected by many fine processes to each other and to the surface. Multicellular cell layers were not seen even after 2 weeks. The instability of the surface was apparent, with granular lumps of material

appearing to detach from the surface. Only 1 TPS (ITI) implant was available to be placed in culture for 4 weeks. After this period, a multilayered cell sheet had formed and extracellular matrix extended over and between the cells of different layers.

## DISCUSSION

The cell attachment assay used in this study was similar to the technique described by previous workers to compare initial cellular responses to implants of different surface characteristics.<sup>4-9</sup> Previous workers<sup>8</sup> have used fibroblasts, while others

used osteoblast-like cells.<sup>4-6,8,9</sup> However, it is apparent from the literature that osteoblast-like cells and other anchorage-dependent cells, such as fibroblasts, show similar morphologic behavior in attachment studies.<sup>11,16,17</sup> Sequential morphologic changes of osteoblast-like cells as a function of time and detailed descriptions of cellular morphology have been reported in several previous studies,<sup>5,16,18,19</sup> although cells at different stages of attachment, as defined by Rajaraman and associates,<sup>12</sup> were not quantified. In this study, cells at stage 4, though all were spread and flattened with broad lamellipodia, as is characteristic of this stage, showed markedly different adaptations to the different implant surfaces, as discussed below.

In previous studies, percentages of attached cells were estimated indirectly from counts of unattached cells<sup>4-9</sup>; in this study, attachment was assayed directly by counting all attached cells on the entire root-like surface of each implant imaged by SEM. All the different implants were present in the same culture vessel to minimize effects of implant orientation and cell gravitation within the 20-minute incubation period, and cells were assumed to adhere and spread according to their affinity for each implant surface.

Results of the cell attachment assay suggested that the TPS surfaces were most conducive to cell spreading. On the other 3 implant surfaces, cells had not spread as extensively after the 20-minute incubation period. On the TiO<sub>2</sub>-blasted implant surface, which is rough with angular pits of various sizes, the spreading cells exhibited irregular shapes, with gaps or spaces where there was no area of contact for the cytoplasmic extensions, possibly because of the steep angle of the adjacent surface, small pits, or sharp edges. Cells spanned over the surface, creating spaces or gaps underneath. Similar osteoblast-like cell morphology, with gaps beneath the spread cells, has been observed in cells attached to rough titanium surfaces blasted by alumina of 600-grit size.<sup>4,5</sup> It is possible that the sharp angularity of this type of surface hinders anchorage of cell processes on adjacent regions and thus slows down the process of attachment. Other factors known to influence the depth of the extracellular space between the ventral surface of the cell and the substratum include positive or negative surface charge<sup>19</sup> and surface free energy,<sup>20</sup> both of which affect the adsorption of serum proteins, to which the cells adhere.<sup>19</sup>

On the HA-coated surface, it was observed that there were differences in cell behavior depending on the topography of the surface, which differed from area to area. In areas of flat, smooth profiles, resembling that of the TPS implant surfaces, the cells

spread to stages 3 and 4. In contrast, cells at these advanced stages were not observed on granular surfaces. Similar differences in the morphology of cells in different areas of HA ceramic have been reported.<sup>21-23</sup> It is well known that the crystallinity and chemical composition of plasma-sprayed HA coatings differs in different areas and that granular areas may be unstable.<sup>24-26</sup> It is possible that loss of particulate material from granular areas interferes with the formation of stable focal plaques. Previous workers have noted cracking and dissolution of HA coatings in physiologic conditions<sup>27</sup> and relatively low levels of cell attachment to HA surfaces.<sup>28</sup> Chang and associates<sup>9</sup> pretreated HA surfaces in acidic conditions (at pH 5.2) to simulate an initial host inflammatory response and promote dissolution of susceptible areas. Higher levels of cell attachment were noted on the pretreated surfaces than on untreated controls. Apart from differences in stability, the surface energy of granular regions may differ from that of smooth, flat areas. Whatever the reasons for the differences in cell behavior on the different types of surfaces, the numbers of spread cells on HA-coated implants could be dependent on the relative proportions of smooth and granular areas, and this may differ from one individual implant to another.

On the smooth Ti implants, the majority of cells were at stages 1 and 2 and relatively few had spread to stages 3 and 4. This may have resulted from the surface lacking prominent features where gravitating cells can lodge and filopodia can gain anchorage, although a similar total number of cells were present on all the Ti implants. The results imply that cells spread relatively more slowly on smooth Ti than on the TPS surfaces. However, on the smooth Ti implants, the cells eventually formed very close contact with the surface, adapting to the underlying topography. Similarly, intimate cell-to-surface contact has been noted on positively charged resin beads,<sup>20</sup> an acid-etched surface, and on 60- and 120-grit polished surfaces of Ti implants,<sup>4</sup> as well as on a polished alumina surface.<sup>18</sup>

Despite the differences in implant design and surface roughness, similar numbers of cells attached to each of the 4 titanium implants. This contrasts with observations of Bowers and coworkers,<sup>4</sup> who reported that significantly higher numbers of cells attached to irregular, sandblasted surfaces than to regular surfaces. Although cells were capable of spreading on each of the implant surfaces examined in the present study, the TPS implants appeared to offer surfaces that promoted the most rapid cell spreading compared to other types of implants, as indicated by the relatively higher proportion of cells at stage 4 after 20 minutes. On the other Ti and



HA-coated implants, the majority of cells were at stage 1. The observations in this study, in common with others using model systems, suggest that initial cell attachment and spreading is affected predominantly by surface physical characteristics, such as stability and the overall topography of the surface. However, other factors known to influence cell attachment could also affect the cell responses. These include surface free energy,<sup>19,20</sup> wettability,<sup>29,30</sup> and zeta potential,<sup>30</sup> which in turn influence the adsorption of serum proteins to which the cells attach. These surface properties could vary from one batch of implants to another and from area to area on the same implant. Each of these properties may also be influenced by cleaning, sterilization, and packaging methods, as has already been shown.<sup>7,8</sup> In the case of plasma-sprayed, HA-coated implants, in which the coating is inherently variable, variation between individual implants and batches is especially important. Serious limitations of this study were the small sample size employed and limitation to 1 particular batch of implants of each type (due to cost restraints). Nevertheless, the method gave reproducible results in the 3 experiments and showed consistent differences in the cellular responses to the different implant surfaces. It is therefore recommended that a study of a larger sample be carried out to enable firmer conclusions to be drawn.

A possible inherent disadvantage of this method, as with previous studies, was the use of enzyme-derived cells, which can alter cell adherence properties.<sup>13</sup> To avoid this problem and to look at later stages in cell behavior, the pocket organ culture method was devised, in which implants were placed in direct contact with surrounding bone, from which cells could migrate onto the implant surface. This was an adaptation of the method of Matsuda and Davies.<sup>14</sup> The organ culture unit creates a 3-dimensional micro-environment, in which the implant is completely surrounded by bone fragments, allowing cells to migrate onto the surface of the implant essentially as they would do in an *in vivo* situation. The nylon mesh provides good stabilization of the tissue and test materials while allowing free access of culture medium into the pocket and diffusion of cell products away from the cells.

No differences were noted in cell numbers or morphology on the 2 different designs of TPS implants (screw versus cylinder), which potentially could have influenced bone contact with the implant surface. It is possible that differences would have been visible at earlier or later stages of culture. There appeared to be fewer cells on the HA-coated implants, which, as previously discussed, could possibly reflect a lack of stability of the HA-coated surface.

The pocket culture system provides the opportunity for the development of a 3-dimensional matrix, which is reported to be a prerequisite for bone formation *in vitro*,<sup>31,32</sup> and, as such, may be advantageous over 2-dimensional culture systems in which a test material is not completely surrounded by bone. Because of limited numbers of implants, it was possible to maintain only 1 implant in culture for 4 weeks. After this time, a multilayered cell covering had formed and an abundant, fibrillar collagen-like extracellular matrix was visible. Mineralization was not investigated in this study, but this culture system could clearly be used to monitor bone formation on different implant surfaces.

While this technique is not a substitute for *in vivo* experimentation because of the absence of complex reactions, such as the body's response to trauma, inflammatory reactions, and the healing processes, it provides a closer 3-dimensional simulation to the *in vivo* situation than culture systems in which the implant is placed onto a monolayer of cells or surrounded by an enzyme or chemically derived cell suspension. It is applicable to a variety of implant designs and cells.

## CONCLUSIONS

The methods developed and described here permit comparison of cell attachment and behavior on commercial dental implants as obtained from the manufacturer. The results of the initial cell attachment study suggest that the TPS surface promotes the most rapid cell spreading and indicate the value of examining early stages in cell attachment to detect the effects of different surfaces on initial cell attachment. The results showed some consistencies with those obtained using test materials with similar surfaces in suggesting that initial cell attachment and spreading are affected predominantly by surface physical characteristics such as stability and the overall topography of the surface. The "pocket" culture method developed here is a potentially useful method for investigating bone cell migration and subsequent behavior on different implant surfaces.

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