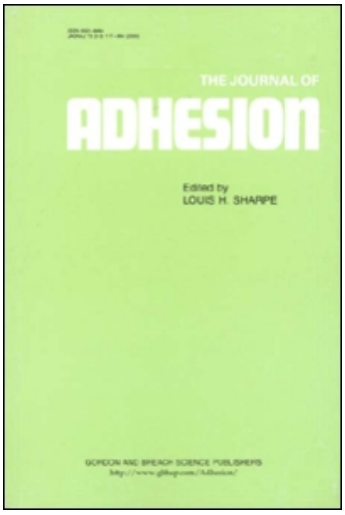


The Journal of Adhesion

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713453635

In Vitro Cell Attachment to Characterized cp Titanium Surfaces

John C. Keller[a]; Wm. J. Dougherty[a]; Gary R. Grotendorst[ab]; James P. Wightman[c]
[a] Medical University of South Carolina, Charleston, SC, U.S.A. [b] Dept. of Biology, University of South Florida, Tampa, FL, U.S.A. [c] Virginia Polytechnic Institute and State University, Blacksburg, VA, U.S.A.

To cite this Article Keller, John C. , Dougherty, Wm. J. , Grotendorst, Gary R. and Wightman, James P.(1989) 'In Vitro Cell Attachment to Characterized cp Titanium Surfaces', The Journal of Adhesion, 28: 2, 115 — 133
To link to this Article: DOI: 10.1080/00218468908030878
URL: http://dx.doi.org/10.1080/00218468908030878

J. Adhesion, 1989, Vol. 28, pp. 115–133
Reprints available directly from the publisher
Photocopying permitted by license only

Printed in the United Kingdom

In Vitro Cell Attachment to Characterized cp Titanium Surfaces†

JOHN C. KELLER‡, WM. J. DOUGHERTY and GARY R. GROTENDORST§

Medical University of South Carolina, Charleston, SC 29425, U.S.A.

JAMES P. WIGHTMAN

Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, U.S.A.

(Received March 14, 1988, in final form June 6, 1988)

An *in vitro* assay was developed to study cell attachment to modified cp titanium surfaces. Titanium surfaces were polished to different roughnesses and were modified by rinsing in either deionized water, pH 7 or pH 11 buffers or NaOH solution following acid passivation with nitric acid. Smooth acid passivated surfaces rinsed in deionized water resulted in a surface having minimal contamination which promoted optimum cell attachment. Surface characterization, including SEM (Scanning electron microscopy) and XPS (X-ray photoelectron spectroscopy) indicated that most rinses resulted in deposition of particles whose morphology and composition were dependent upon the nature of the rinse solution. Cell attachment was dramatically affected by the surfaces with altered chemistry. These results indicated that cellular attachment is dependent, in large part, on the chemistry of the implant surface. A better understanding of the role of implant surface chemistry on cell attachment and other biological processes is needed.

KEY WORDS Cell adhesion; titanium; XPS; SEM; surface pH.

INTRODUCTION

Considerable attempts have been made to understand the relationship and interactions between cells and synthetic polymer substrates.[1–7] This type of research has helped define (1) the role of certain extracellular matrix molecules in cell attachment and organization,[8–18] (2) the *in vitro* morphological changes which occur during cellular attachment[19–22] and (3) some of the important characteristics

† Presented at the Eleventh Annual Meeting of The Adhesion Society, Inc., Charleston, South Carolina, U.S.A., February 21–24, 1988.

‡ Correspondence and Current Address: Dows Institute for Dental Research, University of Iowa, College of Dentistry, Iowa City, IA 52242, U.S.A.

§ Current Address: Dept. of Biology, University of South Florida, Tampa, FL 33620, U.S.A.

of the substrate surface which are required for optimum cell and tissue interaction with biomedical polymers.[2–7]

While the mechanism of interaction between the tissues and the substrate surface are not fully elucidated, it is now apparent that these interactions are dependent, in part, upon surface properties of the substrate. Two biological phenomena which occur *in vivo* may be affected by the substrate surface chemistry. First, cell attachment is dependent in part upon the deposition of a glycoprotein-containing extracellular matrix.[8–17] It is upon this poorly understood matrix that platelets, fibroblasts and other cells and factors involved in wound healing may attach, proliferate and help orchestrate the tissue healing response. Secondly, *in vitro* studies of cell attachment on synthetic polymers have demonstrated that following initial cell attachment to the substrate, the cells reorganize their cytoplasm by flattening and spreading in order to maximize their anchorage to the substrate.[19–21] Chemical alterations[2,5–7] of the polymer surface can significantly affect these events. Based on this knowledge, it would seem likely that chemical and/or physical modifications of metallic implant surfaces may have similar effects.

Currently many metallic biomedical implants are fabricated from commercially pure (cp) Ti or Ti-6A1-4V,[23–29] but surprisingly little is known regarding the surface characteristics required for optimum cellular and tissue interaction to Ti or other biomedical metallic substrates. Because of recent claims that bone tissue is capable of directly bonding to the surface of cpTi implants,[25–28] biomedical researchers are beginning to realize that implant surface properties such as roughness, oxide composition and thickness, and the presence of contaminants, may affect overall biological success.[23–31]

The objective of this work was to begin a systematic study of the effects of some surface modifications, including roughness and chemical composition, on cell adhesion to cpTi using surface characterization techniques and an *in vitro* biological assay for cell attachment, which is an integral part of the healing reaction following placement of biomedical implants.[1,8,9,13–15]

MATERIALS AND METHODS

Specimens were prepared from cp Ti.† A chemical analysis of this lot by the manufacturer revealed the composition shown in Table I Discs (4 mm thick) were cut from 1.25 cm diameter bar stock and were hand polished through either 240 or 600 grit silicon carbide metallographic papers or 1 μm diamond paste in order to prepare surfaces with different roughnesses. The specimens were subsequently solvent cleaned in methylethyl ketone (5 min.), washed in deionized water (10 min.), acid passivated in 30% nitric acid[32] (30 min.) and given a final rinse (15 min.) in either deionized water, standard pH 7 or 11 buffers‡ or 5 M NaOH.

† Alfa Products, Danvers, MA 01923, U.S.A. (Lot D21G).
‡ Fluka Chemical Corp., Ronkonkoma, NY 11779, U.S.A.

TABLE I
Composition of cpTi

Element	Maximum %
Ti	99.7
Fe	0.19
O	0.117
N	0.008
C	0.01
H	0.078
Residuals	<0.10

The ingredients of the pH 7 and pH 11 buffers were potassium dihydrogen phosphate and disodium hydrogen phosphate, and glycine, sodium hydroxide and sodium chloride, respectively. Following the final rinse in one of the above solutions, the specimens were allowed to air dry prior to any surface characterization procedures or cell attachment assays. These final rinse regimens were used in order to alter intentionally the final oxide chemistry. Specimens were not sterilized prior to the *in vitro* assays, but were used immediately after preparation. Specimens which were rinsed in deionized water closely approximated those surfaces which are currently imparted on many, but not all, biomedical implants.[23–32]

Surface characterization

1) *Scanning electron microscopy* Following the previously described methods, selected specimens were mounted onto aluminum stubs, vacuum deposition coated with Au–Pd and examined using scanning electron microscopy. These evaluations were performed to study the effects of varying surface treatments on resulting surface morphology. The surfaces were examined using a JEOL JSM 35C SEM† with an electron beam voltage of 20–25 kV.

2) *Surface pH* Methodology was modified from previous investigations to determine the relative acidity-basicity of the prepared surfaces.[33,34] A series of indicator dyes was used to identify visually the color changes associated with the pH ranges of the surfaces prepared by the above treatment. Standard tissue culture plates used for the cell attachment assays (see below) were also studied by this method and served as a control surface. A syringe was used to place 0.3 ml of dye onto the specimens. This volume was enough to cover the entire surface of each specimen. Color changes generally occurred within a few minutes; however, final visual color changes associated with the surface pH were observed 15 minutes later. Table II shows the range of pH's measured by visual color identification. An example of how this assay works is as follows: on a given

† J.E.O.L. Ltd., Tokyo, Japan

TABLE II
Indicator dyes and pH ranges

Dye	pH
Bromophenol blue	3.0 (yellow) – 4.6 (blue)
Bromocresol purple	5.2 (yellow) – 6.8 (blue)
Bromothymol blue	6.0 (yellow) – 7.6 (blue)
Orange 1	7.6 (orange) – 8.9 (violet)
Thymol blue	8.0 (yellow) – 9.6 (blue)

surface of unknown pH if bromophenol blue turns blue (pH > 4.6) and bromocresol purple turns yellow (pH < 5.2), the pH of the surface would be in the range of pH 4.6–5.2.

3) *X-ray photoelectron spectroscopy (XPS)* Chemical analyses of the prepared surfaces were performed using a Perkin–Elmer 5300 X-ray photoelectron spectrometer† employing a Mg anode with a $K\alpha_{1,2}$ radiation energy of 1253.6 eV. The identity of various elements present on the surface as a result of the described treatments were qualitatively evaluated by wide scan spectra. Narrow scan spectra were subsequently taken to establish the binding energy and photopeak area for semi-quantitative analysis of the elements. The binding energies for all photopeaks were corrected taking the carbon 1s photopeak at 285.0 eV. The atomic percent of each element on the surface was calculated from the photopeak area. The surface composition was expressed as ratios of the calculated atomic percentages.

Cell attachment assays

In these preliminary studies, human fibroblasts obtained from foreskin explants[35–37] were used as our cell model to investigate the effects of surface modifications of cp Ti on cell attachment. All culture media and solutions were utilized in sterile fashion. These cell cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% CO_2 atmosphere. Cells were retrieved from culture plates when confluent by exposure to a trypsin/EDTA (1 mg/ml each) solution for 3–5 minutes. Retrieved cells were counted using a Coulter counter.‡ A quantity of 5×10^4 cells in DMEM/10% sera were then added to tissue culture plates containing the treated Ti implant specimens at the bottom of each well. The surfaces were incubated with the cells for 15, 30 or 60 minutes. At the appropriate times, the media containing unattached cells was removed by careful pipetting. The surfaces were washed twice with buffered salt solution (phosphate buffered saline, pH 7.3) to remove loosely attached cells. The number of unattached cells was quantified and the percentage of attached cells was calculated by subtraction from the original known cell population. At

† Perkin–Elmer Corp., Instruments Div., Norwalk, CT 06856, U.S.A.
‡ Coulter Electronics, Hialeah, FL 33012-0145, U.S.A.

least five specimens were tested per surface conditions. Standard tissue culture plates,† which had been treated to enhance cell attachment, were used as a control substrate for cell attachment.[2,38]

Selected specimens were chosen for SEM evaluation following the cell attachment assays in order to study changes in cellular morphology during the attachment process. Surfaces with attached cells were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 hours and post fixed in cold 2% OsO_4 for 2 hours.

The specimens were then dehydrated in an ascending series of ethanols to 100% and critical point dried using Peldri II.‡ Dried specimens were vacuum deposition coated with Au–Pd prior to examination in the SEM at 10 kV. Stereomicroscopic observations were not routinely performed. The figures shown in the Results section are typical of the patterns of cell attachment and spreading for a given condition.

RESULTS

Surface characterization

Scanning electron microscopy Scanning electron microscopy (SEM) was used to evaluate qualitatively the morphology of the surface following rough polishing through 600 grit metallographic papers, washing, and acid passivation with a final rinse in deionized water. A regular pattern of defects created by polishing was observed (Figure 1a). Small flake-like areas approximately 10–20 μm in size were present along the polish lines. Higher magnification of these surfaces revealed that the flakes were often associated with deeper penetrations or defects on the specimen surface (Figure 1b). These flakes may represent a smear surface layer following polishing; however, analyses of these surfaces failed to detect Si (see XPS results below).

The surface of a specimen which had been treated as described and given a final rinse in pH 7 buffer is shown in Figure 2. Several types of particles were visible on the surface. Long flake-like particles, approximately 10–20 μm in diameter and >50 μm in length, were associated with the polish lines. Smaller, irregular shaped particles were also observed on the surface with no apparent regular distribution. The composition of either particle type was not determined. In addition to the particulate deposition, the depth of the polish lines suggests that the surface may have been etched in some manner by the buffer rinse.

Particulate deposition was also seen with specimens which had been given a final rinse in pH 11 buffer (Figure 3). These particles were approximately 10 μm in diameter. The composition of these particles was not determined.

† Corning Glass Works, Corning, NY 14831, U.S.A.
‡ Ted Pella, Inc., Tustin, CA 92681, U.S.A.

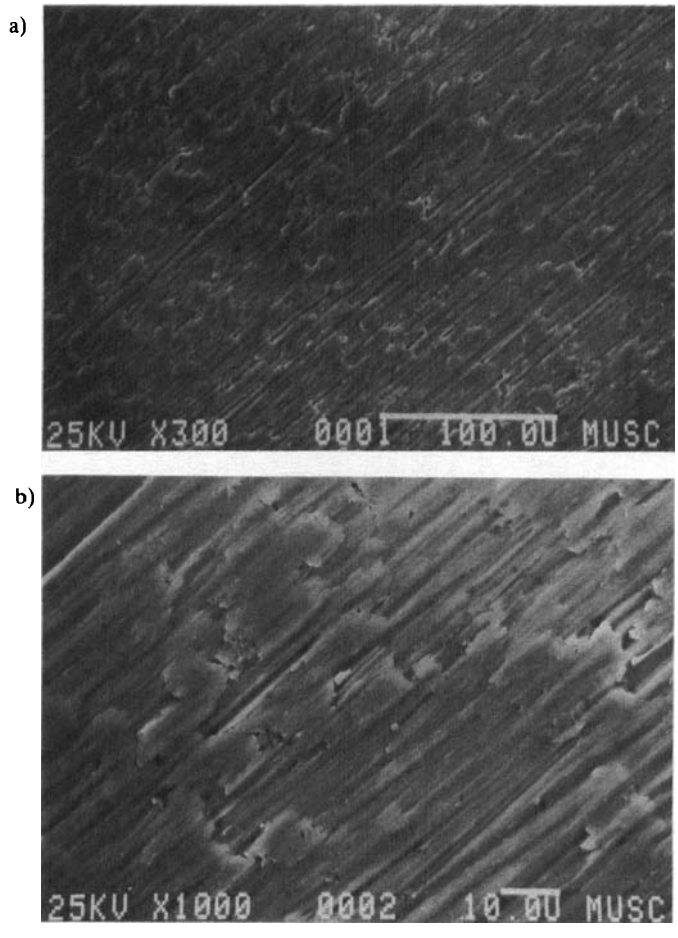


FIGURE 1 a) Micrograph of cpTi surface (600 grit polish, acid passivated, deionized water rinsed), 300×. b) Higher magnification (1000×) demonstrating defects created by polishing.

Surfaces which had been given a final rinse in 5M NaOH demonstrated prominent particulate deposition (Figure 4). The morphology was varied and at least three distinct forms were observed. Flattened, irregular-shaped particles, 10–50 μm in length, were seen in close proximity to the Ti surface. More circular particles with a brighter image were a 20–30 μm in diameter, but appeared to be raised off the Ti surface. At higher magnification, smaller particles (<10 μm) were seen. Although chemical analysis of the particles was not performed, the varied morphological forms of the particles suggests that the particles had different compositions.

Indicator dye experiments A series of indicator dyes was utilized to measure the relative acidity-basicity (pH) of the prepared cpTi surfaces and the tissue culture plate polystyrene. The results of these experiments are shown in Table III.

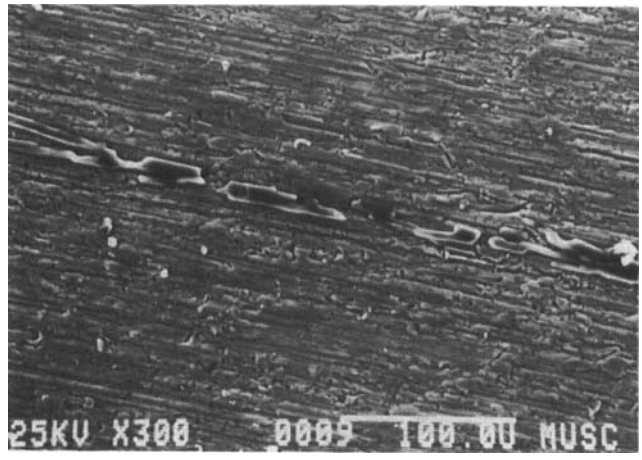


FIGURE 2 Micrograph of cpTi surface (600 grit polish, acid passivated, pH 7 buffer rinse), 300×. Long flake particles and smaller, irregular shaped patterns are demonstrated.

The data indicated that the resulting surface pH was dramatically affected by the composition of the final rinse solution. While deionized water rinsed specimens were clearly acidic, rinsing acid-passivated surfaces in neutral or basic solutions resulted in marked changes in surface pH. Both standard buffer solutions produced neutral surfaces, while the sodium hydroxide solution produced a basic surface. It is interesting to note that the surface pH of treated tissue culture plastic was acidic, in the same range as acid-passivated specimens rinsed in deionized water.

XPS of prepared surfaces X-ray photoelectron spectroscopy was performed on cp Ti surfaces prepared as described. The atomic percent ratios for different

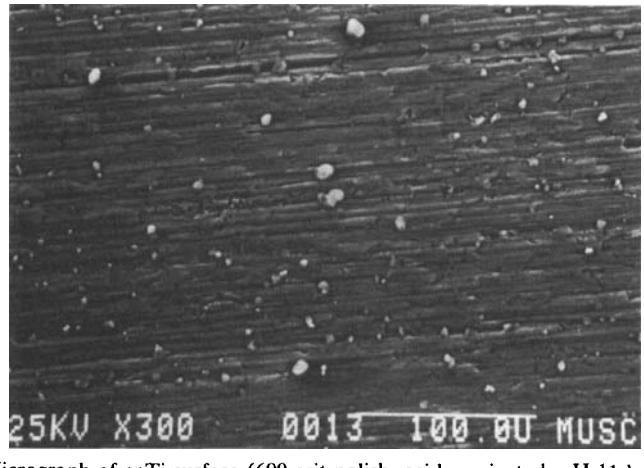


FIGURE 3 Micrograph of cpTi surface (600 grit polish, acid passivated, pH 11 buffer rinse), 300×. Small particles are demonstrated.

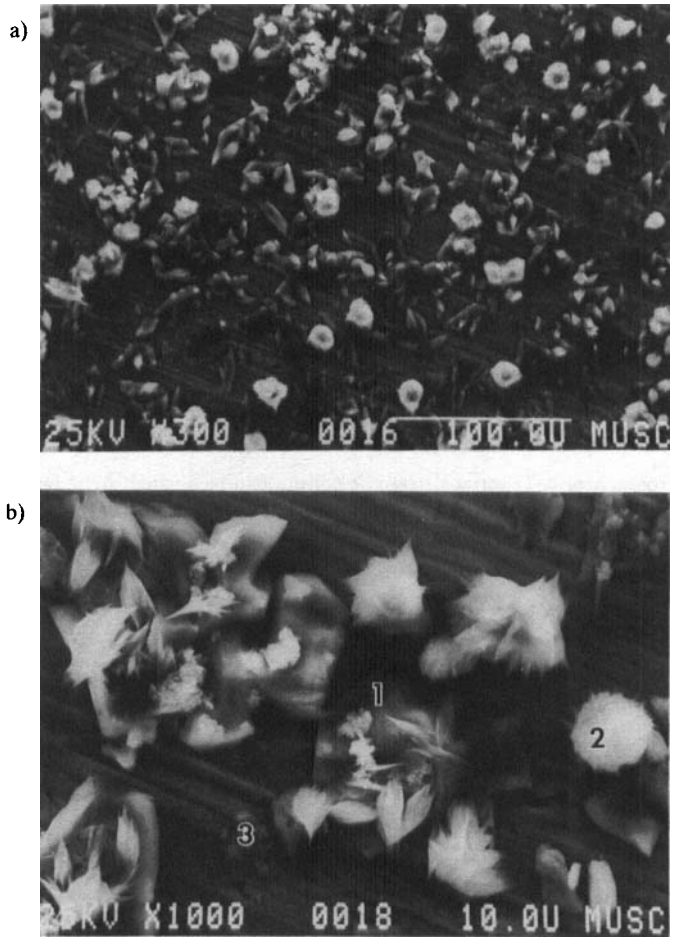


FIGURE 4 a) Micrograph of cpTi surface, (600 grit polish, acid passivated, NaOH rinse), 300×. Three distinct particle morphologies were demonstrated. b) Higher magnification (1000×) of 4a demonstrating flattened, irregular particles (1), circular particles (2), and smaller particles (3).

TABLE III
Ranges of surface pH measured by indicator dyes

Final Rinse	Surface pH
Deionized water	3.0 – 4.6
pH 7 buffer	6.8 – 7.5
pH 11 buffer	6.8 – 7.5
5M NaOH	>8.0 – 9.0
Tissue culture plate	3.0 – 4.6

TABLE IV
Chemical Analyses (XPS) of cpTi Surfaces

Final Rinse	C/Ti	O/Ti	Na/Ti	N/Ti	Cl/Ti	K/Ti	P/Ti
Deionized Water	1.5	3.1	0.28	—	—	—	—
pH 7 buffer	21.0	90.0	24.0	—	—	5.9	20.0
pH 11 buffer	44.0	24.0	8.2	5.5	0.78	—	—
5 M NaOH	17.0	30.0	21.0	—	1.3	—	—

surface preparations are shown in Table IV. Note that Si was not detected on any surface utilized in this study.

Carbon was present on the surface of all specimens and probably results, in part, from compounds adsorbed from the air. The data presented here indicated that the composition of the Ti oxide following the different described surface treatments was dependent upon the nature of the final rinse.

The deionized water rinse resulted in the least contaminated surface. In addition to C, only a trace amount of Na was detected. The O/Ti ratio (3.1) was closest to the theoretical value of 2.0 for TiO_2, expected for cpTi covered with a surface oxide layer. The pH 7, pH 11 and NaOH rinses resulted in a significant increase in the C/Ti (17.0–44.0) and 0/Ti (24.0 – 90.0) ratios as well as in the detection of surface contaminants such as K, P, Na, N and Cl.

Cell attachment assays To begin to understand the effect of surface modifications (chemical and physical) on basic biological reactions to cpTi substrates, *in vitro* assays to quantify cell attachment were performed using human foreskin fibroblasts.[35–37] Standard tissue culture plates, which had a commercial treatment

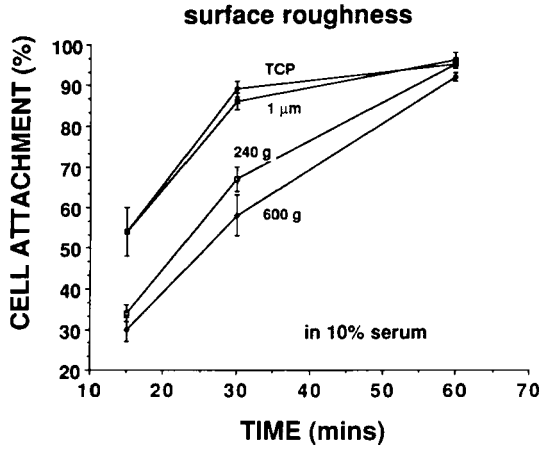


FIGURE 5 Graph of % cell attachment *vs* time for cpTi surfaces, acid passivated and rinsed in deionized water.

to enhance cell adhesion, were used as a positive control substrate for cell attachment. Cell attachment assays were performed for 15, 30 and 60 minutes as a function of surface roughness for specimens rinsed in deionized water. These data are shown in Figure 5. It is readily apparent that for cp Ti surfaces which were acid passivated and given a final rinse in deionized water, maximum cell attachment at all times was obtained with the smoothest surface (1 μm diamond paste). The percent cell attachment for the 1 μm surface closely approximated the tissue culture plate control. SEM of the retrieved surfaces indicated that for each of the three roughnesses studied the cells typically had a ruffled, rounded appearance when exposed to the surface for 15 minutes (Figure 6a,b,c). Only the cells on the roughest surface (240 grit) demonstrated cytoplasmic extensions

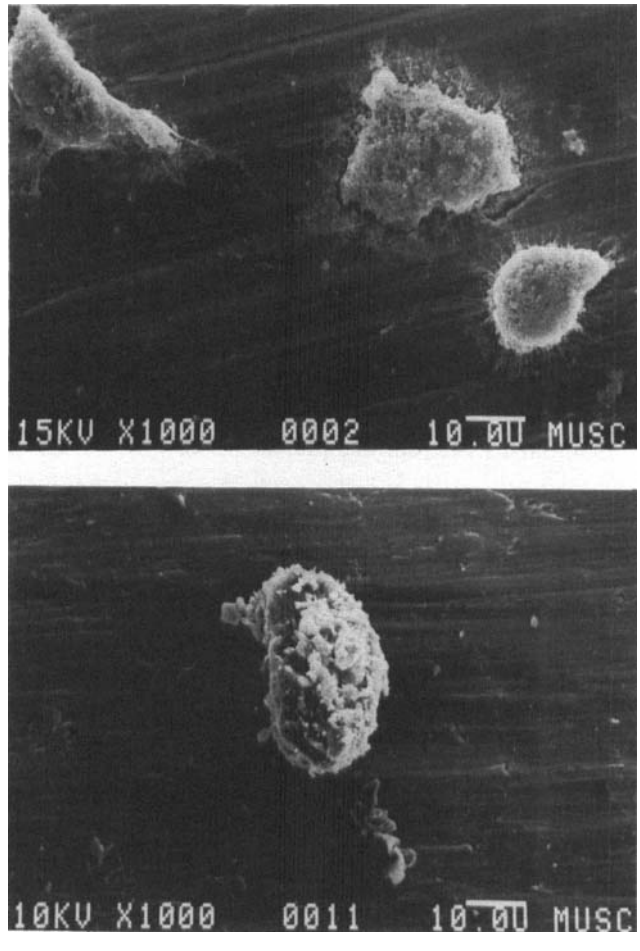


FIGURE 6 Micrographs of cpTi surfaces following 15 minute cell attachment assays (acid passivated, deionized water rinse), 1000×. a) 240 grit surface demonstrating cells with some cytoplasmic extensions. b) 600 grit surface demonstrating few cells with cytoplasmic extensions. c) 1 μm surface demonstrating cells with some cytoplasmic extensions.

c)

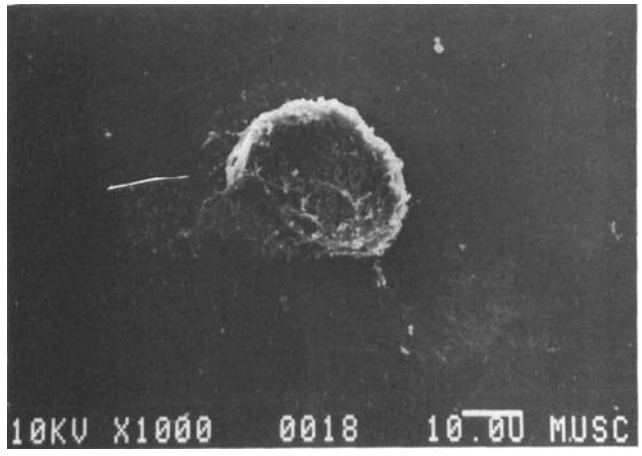


FIGURE 6 (*continued*)

which were indicative of cell spreading. By 60 minutes, however, the cells on all surfaces had undergone considerable flattening and spreading (Figure 7a,b,c).

Additional cell attachment assays were performed on 1 μm, acid-passivated surfaces which had been subjected to a final rinse of either pH 7 or pH 11 buffers or 5 M NaOH. The percentage of cell attachment for these specimens are shown in Figure 8. The data for the water-rinsed specimens are also shown for comparison purposes. It is readily apparent that cell attachment at 15 minutes was significantly inhibited when the surfaces were rinsed in the buffers or NaOH. Increases in cell attachment were then observed at longer time periods; however,

a)

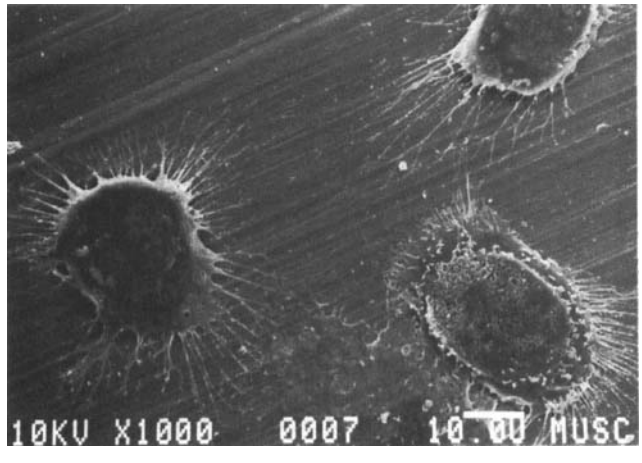
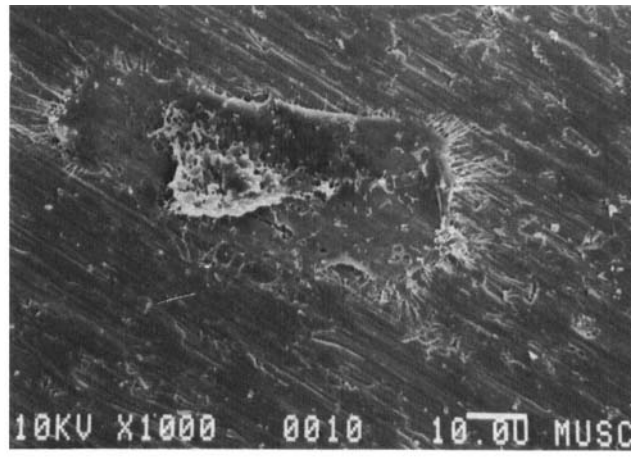


FIGURE 7 Micrographs of cpTi surfaces following 60 minute cell attachment assays (acid passivated, deionized water rinse), 1000×. All figures demonstrate cells which have flattened and spread *via* cytoplasmic extensions. a) 240 grit surface b) 600 grit surface c) 1 μm surface

b)



c)

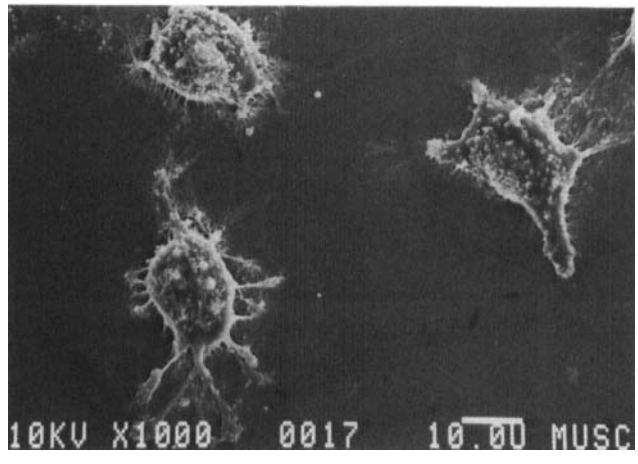


FIGURE 7 (*continued*)

none of the three surfaces permitted cell attachment rates which were comparable to the water rinsed surface.

SEM of the retrieved surfaces with attached cells indicated that, in general, the nature of the final rinse solution also affected the ability of the cells to spread and flatten (Figures 9a, b, c; 10a, b, c). At 15 minutes the cells were typically rounded and demonstrated only slight ruffling. Cells on the pH 7 and pH 11 buffer-rinsed surfaces also demonstrated slight cytoplasmic extensions, which appeared to develop somewhat with time by 60 minutes (Figures 9a, b; 10a, b). The particle morphology which was present on surfaces rinsed in pH 7 or pH 11 buffer (Figures 2, 3) was not present following the cell attachment assay. In contrast to

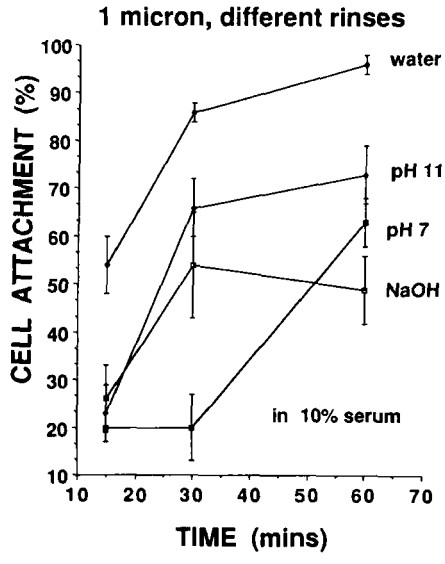


FIGURE 8 Graph of % cell attachment *vs* time for 1 μm cpTi surfaces; acid passivated, rinsed in buffers or NaOH.

the above surfaces, the ability of the cells to flatten and spread on the NaOH rinsed surface appeared to be hindered, even at 60 minutes (Figures 9c, 10c). Particulate debris was also present, but these particles had a much different morphology from those which were present on the surface immediately following the NaOH rinse (Figure 4).

a)

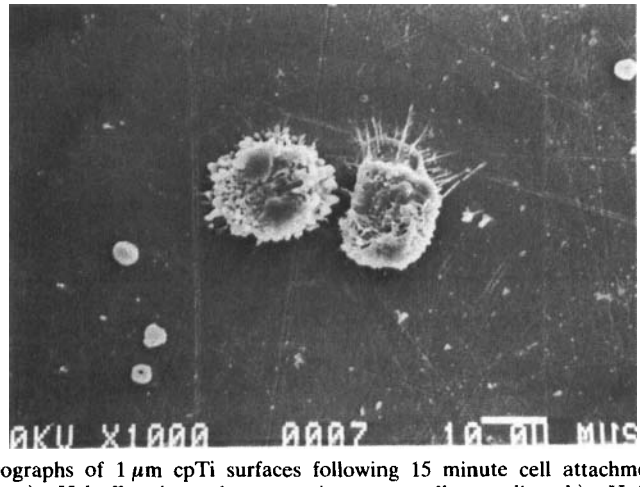
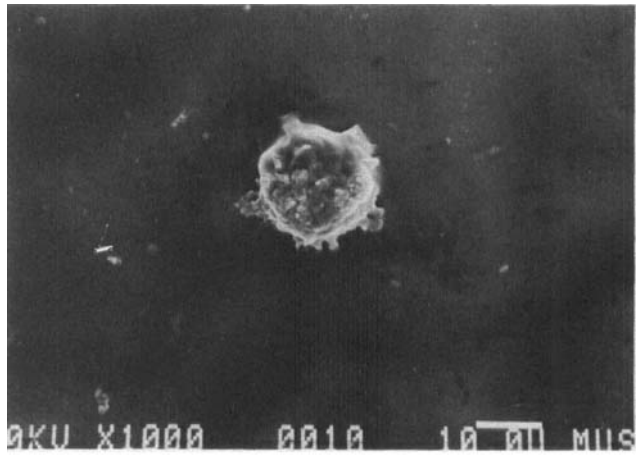


FIGURE 9 Micrographs of 1 μm cpTi surfaces following 15 minute cell attachment assay (acid passivated) 1000×. a) pH buffer rinse, demonstrating some cell spreading. b) pH 11 buffer rinse, demonstrating little cell spreading. c) NaOH rinse, demonstrating little cell spreading.

b)



c)

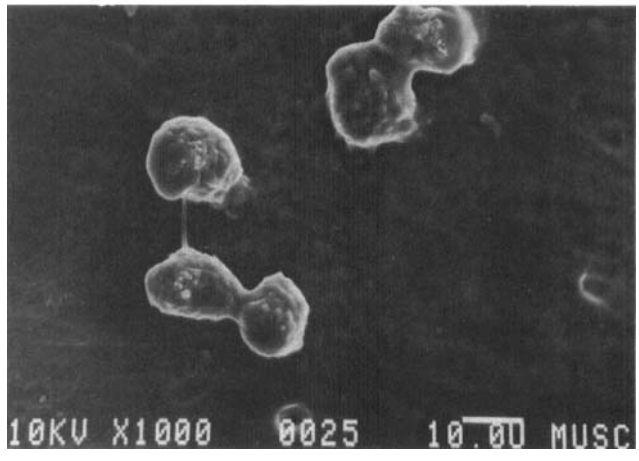


FIGURE 9 (*continued*)

DISCUSSION

Characterization of cpTi surfaces by SEM, XPS and the indicator dyes indicated that the overall surface morphology, and chemical compositions, were modified by the pH 7, pH 11, and 5 M NaOH rinses, compared to the deionized water rinse. Modified titanium surfaces have been reported following certain chemical treatments, including NaOH anodization.[33,34] However, there have not been any systematic studies relating how modifications on Ti implant surfaces affect biological processes such as cell attachment. Particulate deposition of variable morphology was observed on all surfaces that were rinsed in either the buffers or NaOH. XPS characterization confirmed these observations and indicated that not only were the surfaces contaminated with Na, N, K, P and Cl, but also that the

C/Ti and O/Ti ratios were dramatically increased. The increased C/Ti ratio may have been in part due to C-containing contaminants from the atmosphere; however, the data suggest that some C species as well as O, N, K, P, and Cl containing species were introduced as contaminants on the oxide surface following rinsing. The present of these elements correlated well with the specific constituents of the final rinses. For example, the highest C/Ti ratio for the pH 11 buffer is due to adsorbed glycine since N was also observed for this sample. Studies are currently underway to determine at what specific steps the particles are formed.

These documented physical and chemical modifications of the oxide surface

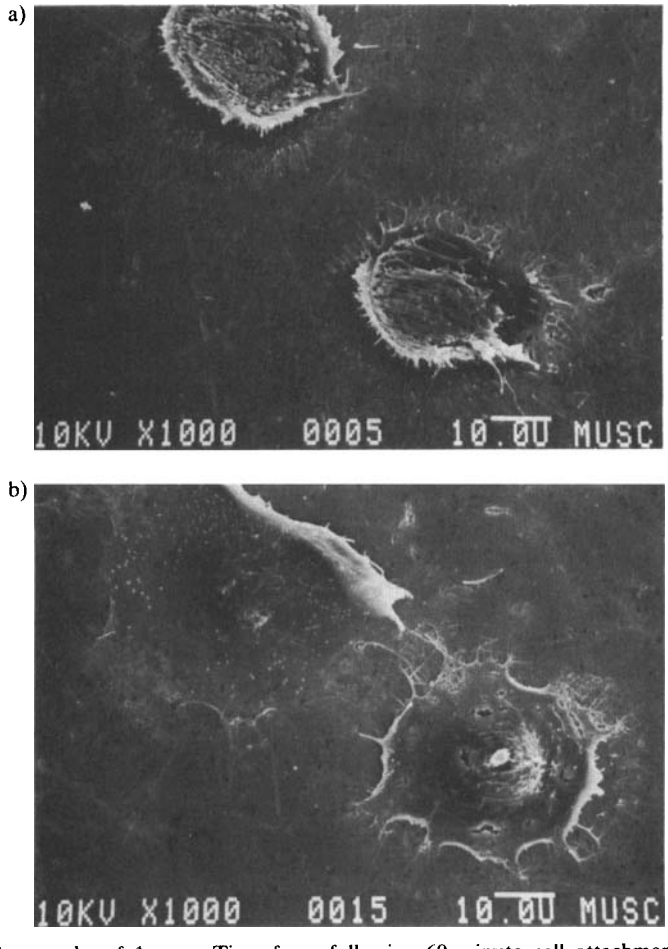


FIGURE 10 Micrographs of 1 μm cpTi surfaces following 60 minute cell attachment assay (acid passivated), 1000×. a) pH 7 buffer rinse, demonstrating some cell spreading. b) pH 11 buffer rinse, demonstrating cell flattening. c) NaOH rinse, demonstrating some cell flattening but little spreading. Particulate debris is present on surface.

c)

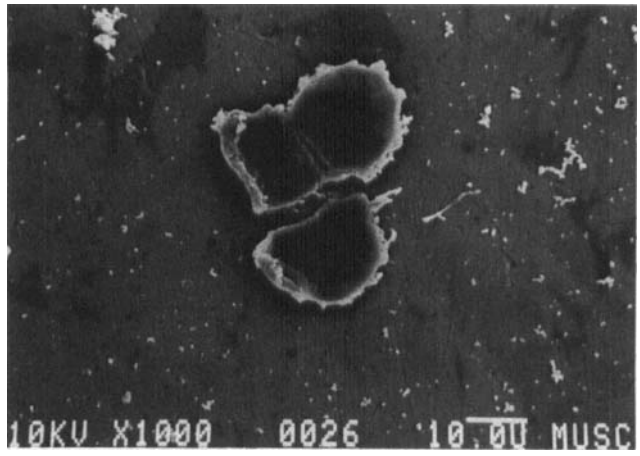


FIGURE 10 (*continued*)

may have important biological ramifications, as our investigation into *in vitro* cell attachment has demonstrated. The modifications in surface morphology and chemistry induced by high pH rinses substantially inhibited cellular attachment as measured by this assay. In this study, the surfaces were tested following the final rinse in deionized water, pH7, 11 buffers or NaOH and air drying. Characterization of the surfaces was not performed following an additional deionized water rinse although additional but as yet undetermined alterations may occur on the surfaces. Studies of this type would help answer whether the contaminants were actually part of the oxide or only accumulation of contaminating debris on the surface. The SEM data suggest the latter.

Initial experiments were designed to investigate cellular attachment as a function of surface roughness on acid-passivated surfaces rinsed in deionized water. Initial cell attachment rates (15 minutes) indicated that optimum attachment was obtained with the smoothest surface (1 μm). However, by 60 minutes, all surface roughnesses produced cell attachment rates which were comparable. This may indicate that initially the cells can recognize, or can attach to, the smoothest surface but, given time, the cells can settle onto a rougher, more irregular surface. This phenomenon may be, in part, related to the deposition of certain attachment factors from the cell media onto the cpTi surface.[8,9] The cell culture medium, Dulbecco's modified Eagle medium, used in this work was supplemented with 10% fetal calf serum containing attachment factors, most notably fibronectin.[10–17] The effects of surface roughness on extracellular matrix-mediated cell attachment may be distinguished in future work by using serum-free media for the assays.

A second series of experiments were designed to investigate cellular attachment as a function of altered surface chemistry induced by the different rinses. Not only were the short term attachment rates on high pH rinsed surfaces lower than the attachment rates on deionized water rinsed specimens, but the longer term

attachment rates and the ability of the cells to spread on the surface were affected as well. Since the attachment of cells is dependent in part upon the deposition of an extracellular matrix, it is conceivable that the surface modifications produced by the final rinses interfered with the deposition of the required extracellular matrix. This hypothesis is currently under investigation. The cells and media may have combined to overcome this "interference layer" of particles in that some increases in cell attachment were observed by 60 minutes. This may have been accomplished by the dissolution of the particles by the media, since the particle morphology seen on freshly-prepared surfaces was not generally observed following either short (15 minute) or long (60 minute) cell attachment assays. In addition, the cells may have required additional time to seek out uncontaminated areas of the surface prior to participating in the events of attachment. Cell spreading assays may provide additional information on the ability of the cells to adapt to these surfaces.

In this work, while all culture media and solutions were kept and used in sterile fashion, the Ti surfaces were not specifically sterilized prior to performing the cell attachment assays. This step was intentionally not performed here to avoid further potential changes in the surface of the Ti specimens. Changes in the oxide chemistry have been observed in other studies following sterilization.[29,31] Characterization of sterilized cpTi surfaces including XPS, pH studies and *in vitro* cell attachment assays is the topic of a current investigation of our research group.

Regardless of the mechanism of attachment, it is not known if the cell attachment rates would improve with increased time or, more importantly, if these modified surfaces would inhibit or alter subsequent tissue reactions if present in an *in vivo* situation.

It is interesting to note the similarity in rates of cell attachment between the 1 μm, deionized water rinsed cpTi surfaces and the tissue culture plate substrate at all three periods. This would suggest that the surface conditions required for cell attachment are similar on both materials. One important parameter for attachment may be the acid-base character or the nature of the charge on implant surfaces.[5,22,34] The pH of these two surfaces, as determined by the indicator dye technique, were identical (pH $3.0 - 4.6$), which suggests that certain chemical aspects of these surfaces were similar. The chemical nature of the surfaces produced by the high pH rinses, as indicated by SEM, surface pH and XPS techniques reduced the rates of cellular attachment. This suggests that a chemical specificity may be required for optimum rates of attachment on biomedical materials. The high rate of cell attachment for both the treated polystyrene and water rinsed Ti surfaces may be related to the concentration of surface hydroxyl groups produced by the deionized water rinse on the oxide of cpTi[34] and the glow discharge treatment imparted on tissue culture polystyrene.[6,7] Chemical methods have been used to block the hydroxyl groups on polystyrene, resulting in decreased cell attachment.[7] Similar experiments, designed for cpTi surfaces, may further elucidate the mechanism of extracellular matrix deposition and cell attachment on implant surfaces and may lead to advances in implant surface preparation protocols.

Another interesting biological question is whether these or other surface modifications would affect bacterial or other microbial colonization. A number of recent investigations have raised this question.[39,40] While the cpTi specimens used here were not sterilized prior to the cell attachment assays, there is no reason to suspect that any of the specimens were contaminated with micro-organisms.

It should be emphasized that the surface modification procedures studied here are not being promoted as potential treatment protocols for implant surfaces, but rather they present a simple methodology for systematically altering the implant surfaces and studying resulting *in vitro* cell attachment. The work presented here may have important ramifications concerning current implants which have been shown to undergo changes in oxide chemistry following sterilization and handling.[29,31]

SUMMARY

An *in vitro* cell attachment assay and surface characterization techniques were utilized to study the attachment of human fibroblasts to modified cpTi surfaces. Following acid passivation, surfaces rinsed in deionized water produced relatively uncontaminated surfaces which promoted high rates of cell attachment. Final rinses in either standard buffer or sodium hydroxide solutions resulted in contaminated, particle laden surfaces which hindered optimum cell attachment. It is readily apparent from this work that the surface chemistry of implant materials plays an important role in the biological events of wound healing.

Acknowledgements

The authors wish to thank Drs. Robert Draughn, Jennifer Filbey and Frank Young for their comments and ideas regarding this work. We also thank Ms. Betty Davis for preparing the manuscript. This work was supported by Institutional Research Funds of the Medical University of South Carolina and a grant from the American Academy of Implant Dentistry.

References

1. E. A. Vogler and R. W. Bussian, *J. Biomed. Mater. Res.* **21,** 1197 (1987).
2. W. S. Ramsey *et al.*, *Tissue Culture Assoc. Inc.* **20,** 802 (1984).
3. K. D. Thomas *et al.*, *Biological and Biomechanical Performance of Biomaterials* (Elsevier Science Publishers, Amsterdam, 1986), pp. 379–383.
4. M. J. Lydon *et al.*, *Biomaterials* **6,** 396 (1985).
5. N. G. Maroudas, *J. Theoret. Biol.* **49,** 4 (1975).
6. F. Grinnell *et al.*, *Arch. Biochem. Biophys.* **153,** 193 (1972).
7. A. S. G. Curtis *et al.*, *J. Cell Biol.* **97,** 1500 (1983).
8. L. A. Culp, Curr. *Top. Memb. Trans.* **11,** 327 (1978).
9. F. Grinnell, *Int. Rev. Cytol.* **53,** 65 (1978).
10. F. Grinnell and M. K. Feld, *Cell* **17,** 117 (1979).
11. J. R. Couchman *et al.*, *J. Cell Bio.* **96,** 177 (1983).
12. A. S. G. Curtis and J. V. Forrester, *J. Cell Sci.* **71,** 17 (1984).
13. E. Ruoslahti *et al.*, *Cell Res.* **1,** 95 (1981).
14. H. K. Kleinman *et al.*, *J. Cell Biol.* **88,** 473 (1981).

15. R. J. Klebe *et al., J. Cell Phys.* **109,** 481 (1981).
16. D. Gospodarowicz *et al., J. Supramol. St.* **13,** 339 (1980).
17. R. Fridman *et al., Exp. Cell Res.* **157,** 181 (1985).
18. R. E. Baier, *J. Adhesion* **20,** 171 (1986).
19. S. Mai and A. E. Chung, *Exp. Cell Res.* **152,** 500 (1984).
20. F. Grinnell and B. Geiger, *Exp. Cell Res.* **162,** 449 (1986).
21. P. Knox and S. Griffiths, *J. Cell Sci.* **55,** 301 (1982).
22. J. E. Davies *et al., Biomaterials* **7,** 231 (1986).
23. G. R. Parr, *J. Prosth. Dent.* **54,** 410 (1985).
24. B. Kasemo, *J. Prosth. Dent.* **49,** 832 (1983).
25. T. Albrektsson *et al., Biomaterials* **6,** 97 (1985).
26. T. Albrektsson *et al., Ann. Biomed. Eng.* **11,** 1 (1983).
27. P. I. Branemark *et al., Biomaterials* **4,** 25 (1983).
28. P. I. Branemark, *J. Prosth. Dent.* **50,** 399 (1983).
29. J. Lausmaa *et al., Biomaterials* **5,** 23 (1985).
30. R. E. Baier *et al., J. Biomed. Mater. Res.* **18,** 337 (1984).
31. R. E. Baier *et al., Biomaterials* **3,** 241 (1982).
32. Annual Book of ASTM Standards, Section 13, F86-76, "Surface Preparation and Marking of Metallic Surgical Implants" (ASTM Publications, Philadelphia, 1983) pp. 15–17.
33. J. A. Filbey and J. P. Wightman, *J. Adhesion* **20,** (1987).
34. J. G. Mason *et al.*, J. Adhesion **11,** 315 (1981).
35. G. R. Grotendorst *et al., Proc. Nat. Acad. Sci, USA* **78,** 3669 (1981).
36. G. R. Grotendorst *et al., J. Cell Phys.* **113,** 261 (1982A).
37. G. R. Grotendorst, *Cell* **36,** 279 (1984).
38. C. F. Amstein and P. A. Hartman, *J. Clin. Micro.* **2,** 26 (1975).
39. K. Merrit *et al.*, Trans. Soc. *Biomaterials* **XI,** 444 (1988).
40. A. Gristina, *Science* **237,** 1588 (1987).