Organic surface chemistry on titanium surfaces via thin film deposition

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Abstract: In order to develop a synthetic strategy for the fine tuning of the interfacial properties of titanium-based implants and implant parts, a thin polymeric film was deposited from ethylene plasma on the surfaces of Ti foils. The intended aim was to further modify the adherent, delamination-resistant organic coating using the techniques of surface modification of polymers to direct interfacial interactions at the metal foil–biological phase interface. In particular, air-plasma treatment and Ce(IV)-induced hydroxyethylmethacrylate grafting, two typical reactions of biomedical polymers surface chemistry, were used to improve cell adhesion or to impart cell resistance to the plasma-coated Ti. Results indicate that a plasma-deposited thin polymeric film effectively can act as a viable substrate for further surface chemical modifications and allow the application of a huge background of surface-modification polymers to metallic devices. © 1997 John Wiley & Sons, Inc., *J Biomed Mater Res*, **37,** 198–206, 1997.

Key words: titanium; plasma deposition; plasma treatment; surface modification; surface-free energy; cell adhesion

INTRODUCTION

Titanium has been widely used in prosthetic dentistry since the discovery by Branemark and colleagues of the favorable interaction of titanium surfaces with bone tissue.[1] Since then many studies have been devoted to Ti surfaces and their interactions with bone tissue and cells.[2–5]

The construction of implant-based prosthethes actually results in the formation of several different kinds of interfaces between the material and the biological phases. In particular,[6] when fixtures have been installed, protective metal caps are screw connected to the exposed terminals of the fixtures, a precaution that prevents bone from forming in their internal threaded parts. Returned sutured soft tissue then is positioned to completely cover the installed fixtures. After the healing period, transmucosal titanium abutment cylinders are connected to the bone-embedded fixture. Abutment cylinders face partly the soft tissue and partly emerged in the oral cavity (Fig. 1).

It is clear that Ti fixtures and implant parts need to withstand, in addition to osteointegration, several interface-related challenges. In particular, bone growth on protective metal caps sometimes occurs during the healing period. The newly formed bone must be surgically eliminated before removal of metal caps, a practice that sometimes triggers a small but definite osteoclastic activity. Then bacterial adhesion and plaque accumulation in the oral cavity may be a concern, especially if coupled with a poor sealing between Ti and the soft tissue in the transmucosal portion of the abutment cylinders. Penetration of bacteria along the transmucosal pathway results in the development of periimplantitis.

While the current high success rate of dental implants indicates that materials, surgery, and postsurgery treatments are satisfactory, a small but significant percentage of failures, fixtures loosening, and development of peri-implant infections remains. The thesis of this paper and of the ongoing work in our lab is that at least some of these failures and infections could be eliminated by the proper surface engineering of Ti implant parts. Long and accurate laboratory work and clinical practice have shown that the surface properties of Ti are optimal for osteointegration. Surface modification techniques could be used to tailor the surface chemistry of Ti implant parts to optimize their interfacial properties in critical, nonbone-contacting areas.

Modifications of both surface morphology[7–11] and surface composition have been shown to affect the cell response on Ti surfaces. With regard to surface chemistry, surface cleaning and sterilization, the nature of the surface oxide layer, and nitridation treat-

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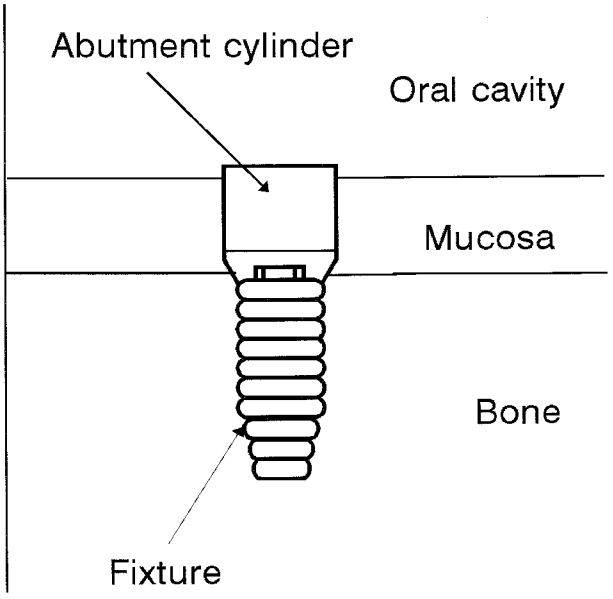

Figure 1. Interfaces between biological phases and Ti in dental implants.

ments are the chemical variables most frequently discussed.[2,12–14]

Surface modification of organic materials to direct interfacial interactions with cells and bacteria undoubtedly presents many more possibilities for manipulation of the details of the surface chemistry than does modification of inorganic surfaces.[15] In principle, a polymer coating on an inorganic substrate could provide an organic surface on which further chemistry could be performed. Actual application of this simple principle to biomaterials is limited by poor coating / substrate adhesion, coating delamination in wet physiologic environments, abrasive wear, uncertain surface chemistry of additive-containing polymeric coatings, and lack of suitable reactive groups on the surface.

Deposition of polymeric films from plasma allows one to bypass several of the just quoted shortcomings of polymer coatings on inorganic surfaces.[16,17] In particular, it allows the production of an adherent, pinhole, and additive-free surface layer resistant to hydrolysis and delamination in humid environments.[17] Several reports discuss the use of plasma deposition for organic-chemistry based surface modification of inorganic materials. Marchant et al. described the surface modification of glass, silicon, and aluminum using a glow discharge surface-modification technique.[18] A thin film polymerized from hexane provided an adherent protective coating for the substrate material and covalent bonding sites for an outer hydrophilic layer of plasma polymerized N-vinyl-2-pirrolidone. Seeger and co-workers grafted, by γ-irradiation, hydrophilic polymers on an organic surface layer deposited from Argon / Hexane plasma on stainless steel.[19]

The aim of the present work is to demonstrate that the methods of surface modification of polymers can be used to manipulate the interaction between cells and Ti surfaces. For this to be done, a thin (<50 nm) hydrocarbon-like polymeric organic layer is deposited on the Ti surface from ethylene plasma. Confirming previous *in vitro* findings of Baier and co-workers,[20] we recently have shown that this kind of surface modification greatly reduces plaque accumulation on a prosthetic dental polymer *in vivo*.[21] The thin, delamination-resistant, pinhole-free polymeric coating is the starting point for further surface modifications in response to two classic reactions of surface chemistry of medical plastics: air-plasma treatment is used to promote cell adhesion on the hydrocarbon-like organic coating, and Ce(IV)-induced hydroxyethylmethacrylate (HEMA) polymerization and grafting are used to prevent cell adhesion. A scheme of the surface modification strategy adopted is shown in Figure 2.

The effect of the surface modification treatments on Ti surface chemistry and energetics is evaluated by electron spectroscopy for chemical analysis (ESCA) and contact angle measurement while adhesion and growth of a continuous fibroblast cell line (L 929) is used to assess the effect of surface modification on the material–cell interaction. This cell line is of no direct clinical relevance for the intended applications of the surface modification processes discussed. Nonetheless it can give reliable and reproducible information on the key point of this study, i.e., the evaluation of the *in vitro* cell-adhesive or cell-repellent properties of modified Ti surfaces.

MATERIALS AND METHODS

Experiments were performed on titanium foils 2.5 cm wide and 0.2 mm thick (99.7%). Samples were polished through 600-grit SiC metallographic papers. After being polished the specimens were solvent cleaned in methylethylketone for 5 min, washed in ultrapure water for 20 min, acid passivated in 30% nitric acid for 30 min, according to the ASTM procedure,[22] and rinsed again in ultrapure water for 20 min.

Surface modification

Deposition of ethylene plasma

Plasma deposition was performed in a capacitively coupled parallel-plate reactor with the samples located on the water-cooled grounded electrode. Both the reactor and electrodes are made of stainless steel. The reactor volume is about 3 dm^3 and the distance between the electrodes 10 cm.

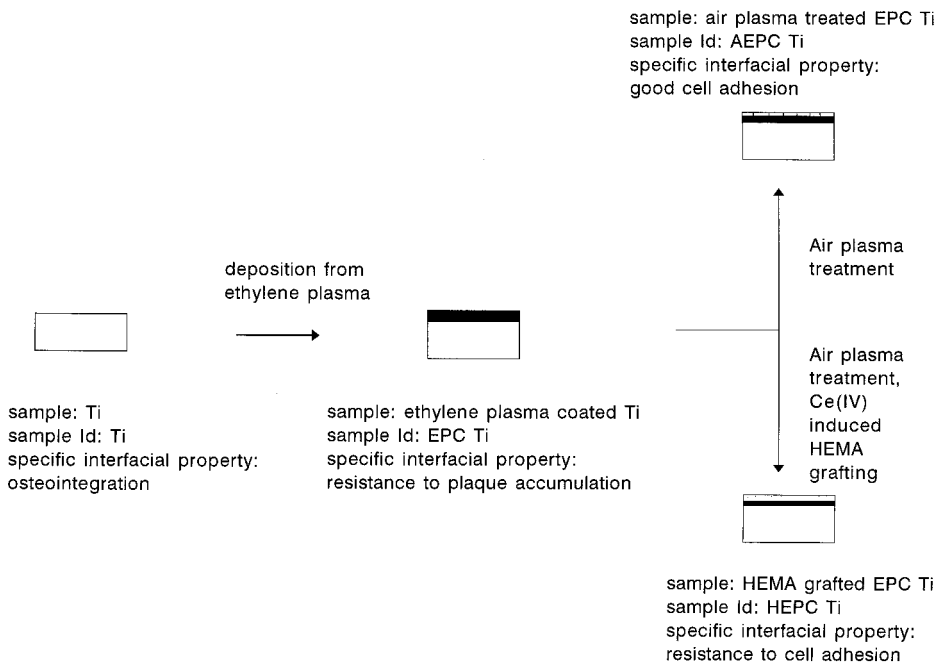

Figure 2. Schematic of the surface modifications performed.

The flow rate was controlled by a MKS mass flow controller. The monomer pressure inside the chamber before the onset of the discharge was 15 Pa. Based on previous experience, a discharge power of 40 W, a flow rate of 40 sccm (standard cubic centimetres per min), and a deposition time of 1.5 min were used. When the plasma was turned off, the ethylene flow was maintained for 30 s to quench active radicals.

These experimental conditions yield a polymeric film of about 40 nm thickness, as detected by a quartz crystal microbalance (Intellemterics).

Air plasma treatment

Several samples coated by deposition of ethylene plasma were subjected to a further air-plasma treatment in the same reactor (described above) using a discharge power of 30 W, a flow rate of 20 sccm, and a treatment time of 15 s. After treatment, samples were stored overnight in an oven at 60°C to avoid artefacts due to aging.[15]

HEMA grafting

HEMA grafting on Ti samples coated by deposition of ethylene plasma was performed by air-plasma treating the samples, as described before, and, immediately after treatment, putting the samples in a 10% HEMA solution in water containing 0.2 weight % of Ammonium Cerium Nitrate, 98.5% pure. Both reagents were purchased from Aldrich. The reaction was carried on for 2 h at room temperature. Samples were extracted overnight in ethanol.

Surface characterization

Contact angle measurement

Contact angle measurement was performed using the following liquids: H_2O (doubly distilled), CH_2I_2 (99%, Aldrich), Dimethylsulfoxide (DMSO, >99.5%, Fluka).

The contact angle of test liquids on the samples' surfaces was measured by the sessile drop technique[15] using a Kruss, G 23 contact angle goniometer (Kruss, Hamburg, Germany). Keeping the capillary pipette of the microsyringe immersed in the probe fluid during the whole measurement, advancing and receding angles were measured by increasing or decreasing the drop volume while moving the three phase boundary. To avoid cross contamination of liquids, a dedicated microsyringe was used for each liquid.

Surface composition

Surface composition was evaluated by electron spectroscopy for chemical analysis (ESCA) using a Perkin Elmer PHI 5500 ESCA system. The instrument is equipped with a monochromatic X-ray source (Al Kα anode) operating at 14 kV and 250 W. The diameter of the analyzed spot is 400 μm. The base pressure was 10^{-8} Pa. Peak deconvolution and quantification of the elements was accomplished using the software and sensitivity factors supplied by the manufacturer. The angle between the electron analyzer and the sample surface was 45°C. In high-resolution spectra, all bind-

ing energies were referenced by setting the CHx peak maximum in the resolved C_{1s} spectra to 285.0 eV.

Cell adhesion and growth

Cell adhesion and growth was evaluated using the continuous mouse fibroblasts cell line L-929. Experimental cell culture medium (BIOCHROM KG, Berlin) consisted of minimum Eagle's medium without L-glutamine, 10% fetal bovine serum, streptomycin (100 μg / L), penicillin (100 U / mL), and 2 mmoles / L L-glutamine in a 250 mL plastic culture flask (Corning™). Cells were cultured at 37°C in a humidified incubator equilibrated with 5% CO_2. Fibroblasts were harvested prior to confluence by means of a sterile trypsin-EDTA solution (0.05 trypsin, 0.02 EDTA in normal phosphate buffered saline, pH 7.4) from the culture flasks, resuspended in the experimental cell culture medium, and diluted to 1×10^5 cells / mL. Five mL of the cell suspension were seeded into polystyrene petri dishes (5 cm diameter; Corning™) containing the Ti and surface-modified Ti samples. The dishes previously had been coated with 10% poly(hydroxyethylmethacrylate) in order to ensure that the fibroblasts would grow on only the Ti foils and not on the culture well. Experiments were performed in triplicate.

Cell observation

At the designated times, cell growth on the samples quickly was observed with a metallographic microscope (Nikon Optiphot) without any fixation procedure and submerged in their culture medium. In order to enhance the contrast, micrographs were taken after staining with a 0.1% solution of toluidine blue in PBS.

Cell counts

Cell proliferation on Ti and surface-modified Ti samples was determined after 48 h. At this time, cells on the samples were gently rinsed with PBS and removed from the growth surface by incubation with 1 mL of a sterile trypsin-EDTA solution in PBS for 2 min. An aliquot of the cell suspension then was counted with a hemocitomer. Experiments were performed in triplicate. The data were analyzed using an analysis of variance and a *post hoc* LSD test.

RESULTS

Surface composition

The surface composition of the samples, as detected by ESCA analysis, and the results of the C1s peak curve fitting are shown in Table I. Surface analysis of Ti samples yields the typical results observed on Ti surfaces: beside Ti and oxygen, contamination from surface adsorption of airborne carbon-containing compounds and from several other elements is detected. From a quantitative point of view, the overall surface composition agrees with published findings.[23] Peak shape analysis of the Ti peak, not shown, is dominated by the Ti(IV) component of TiO_2 (b.e. 459 eV), with a small but definite signal from metallic Ti (b.e. 454 eV). This observation suggests that the surface oxide layer is thinner than the XPS sampling depth, which published data indicate to be a thickness of about 4 nm for nitric acid-passivated Ti.[24]

Ethylene plasma-coated (EPC) Ti shows the typical composition of hydrocarbon surfaces, and, in addition to carbon, only a very small amount of oxygen is detected. The C1s peak is nearly perfectly symmetrical, with only a very small and difficult to accurately quantitate contribution at higher binding energy. These results confirm that a hydrocarbon film, whose thickness is greater than the XPS sampling depth, homogeneously coats the metallic substrate. ESCA analysis of air-plasma treated EPC samples (AEPC) shows the expected increase in oxygen concentration and a marked broadening of the C1s peak due to the introduction in the surface of single and multiple carbon–oxygen bonding. Finally, HEMA-grafted EPC samples (HEPC) show a surface composition close to the theoretical value expected from the atomic ratio found in poly(HEMA) (PHEMA). The C1s peak shape agrees with the PHEMA molecular structure.

TABLE I
Surface Composition (% at.) and C1s Peak-Shape Analysis of Untreated and Surface-Treated Ti

	Surface Composition			% Occupied Area			
Sample	O	C	Ti	C−C, C−H	C−O	O−C−O, C=O	O−C=O
Ti	43.6	38.3	13.9[a]	82.0	10.0	7.1	1.9
EPC Ti	0.9	99.1		≈100			
AEPC Ti	16.1	83.9		63.0	25.9	7.4	3.7
HEPC Ti	30.2	69.8		54.5	34.1		11.4

[a] = also detected, less than 1% each: N, Na, Ca.

TABLE II
Measured Contact Angles (deg) and Calculated Surface-Free Energy and Surface-Free Energy Components[a] (mJ/m^2) of Untreated- and Surface-Treated Ti

Sample	Contact Angle[b]			Surface-Free Energy Components			
	θ_{H_2O} adv-rec	θ_{DMSO} adv	$\theta_{CH_2I_2}$ adv	γ^T	γ^{LW}	γ^+	γ^-
Ti	52 ± 5 − 0	23 ± 6	26 ± 7	—	—	—	—
EPC Ti	92 ± 4 − 80 ± 5	46 ± 5	47 ± 6	36.12	35.93	0.01	1.54
AEPC Ti	56 ± 4 − 10 ± 5	22 ± 5	38 ± 6	41.62	40.60	0.01	25.94
HEPC Ti	52 ± 4 − 0	32 ± 5	45 ± 6	37.01	37.01	0.00	34.33

[a] γ^T = total surface energy; γ^{LW} = Lifshitz–van der Waals component of the surface-free energy; γ^+ = Lewis acid component of the surface-free energy; γ^- = Lewis base component of the surface-free energy.
[b] The input values of the surface-free energy of the liquids were taken from ref. 26 and are as follows: H_2O, γ^{LW} = 21.8, γ^+ = 25.5, γ^- = 25.5. DMSO, γ^{LW} = 36.0, γ^+ = 0.5, γ^- = 32; CH_2I_2, γ^{LW} = 50.8, γ^+ = 0, γ^- = 0.

Contact angle measurement

Results of contact angle measurement and of calculation of surface free energy components, according to the Lewis acid-base approach,[25,26,15] are shown in Table II. Contact angles measured on high-energy surfaces (such as those of metals) reflect the details of the surface layer of adsorbed contaminants and are more indicative of the cleaning routine used or of the level of environmental contamination than of the true nature of the sample surface.[27,15] Here they are reported more to indicate the starting point of the following surface modification steps rather than to speculate on the nature of the Ti surface. Accordingly, no surface free energy components were calculated in this case. Contact angle analysis on EPC surfaces shows that plasma deposition produces a marked increase of the water contact angle. Contact angle hysteresis is rather small, suggesting, in agreement with ESCA findings, that this treatment produces a homogeneous hydrocarbon coating on Ti surfaces. The hydrophobic nature of EPC is clearly reflected in the calculated values of the surface-free energy components, which indicate on overwhelmingly apolar surface with a very small Lewis base component. Both AEPC and HEPC surfaces are more hydrophilic than EPC, as shown by the decrease of the water contact angle and, when it comes to surface free energy, by the increase of the Lewis base component of the surface free energy. Apparently, HEPC surfaces are more basic than AEPC ones.

Cell adhesion and growth

Typical results of cell adhesion experiments are shown in Figures 3 (a–d). These photographs indicate the cell behavior on the test surfaces after 2 h of cell culturing. Clearly, cells maintain a rounded morphology and do not spread on EPC [Fig. 3(b)] and HEPC Ti [Fig. 3(d)], while cell spreading is evident on both Ti [Fig. 3(a)] and AEPC Ti [Fig. 3(c)]. In particular, in the latter case several cells are already completely flat while spreading on the pure Ti surface seems to lag behind.

The indications of short-term cell adhesion experiments are confirmed by the observation of cell growth after 48 h of cell culturing [Fig. 4 (a–d)]. EPC [Fig. 4(b)] and HEPC [Fig. 4(d)] Ti surfaces remain free from spreaded cells while cells form a confluent layer on AEPC Ti [Fig. 4(c)]. The pure Ti surface obviously supports cell adhesion and growth, but cells are not confluent yet [Fig. 4(a)]. Note that in every case the surface morphology of the substrate is unaffected by the modification processes, at least within the limits of optical microscopy.

The qualitative results shown in Figure 4 are confirmed by the quantitative cell counting results, shown in Table III. The number of cells found on the AEPC Ti surfaces is significantly higher than that of cells on the pure Ti surface. Obviously, both are much higher than cells counted on EPC and HEPC surfaces.

DISCUSSION

Previous studies demonstrated classic surface chemistry for polymeric biomaterials: cells do not attach and grow on apolar, hydrophobic, hydrocarbon-like surfaces. Plasticware for cell culturing, the so-called tissue culture plastic, is indeed plasma treated (or subjected to other kinds of surface oxidation) to promote cell adhesion and growth.[28] Then heavily hydrated, hydrogel-like surfaces, such as those of PHEMA, do not support cell adhesion.[29] Actually, coating from PHEMA / ethanol solution is a practice widely used in cell culturing to inhibit cell adhesion to tissue culture plasticware.

In the present experiments, this classic scheme of tuning cell adhesion, typical of polymeric biomaterials, was applied to a metallic ''device,'' i.e., a titanium foil whose surface was rendered ''organic'' by deposition of a thin polymeric film from ethylene plasma. The

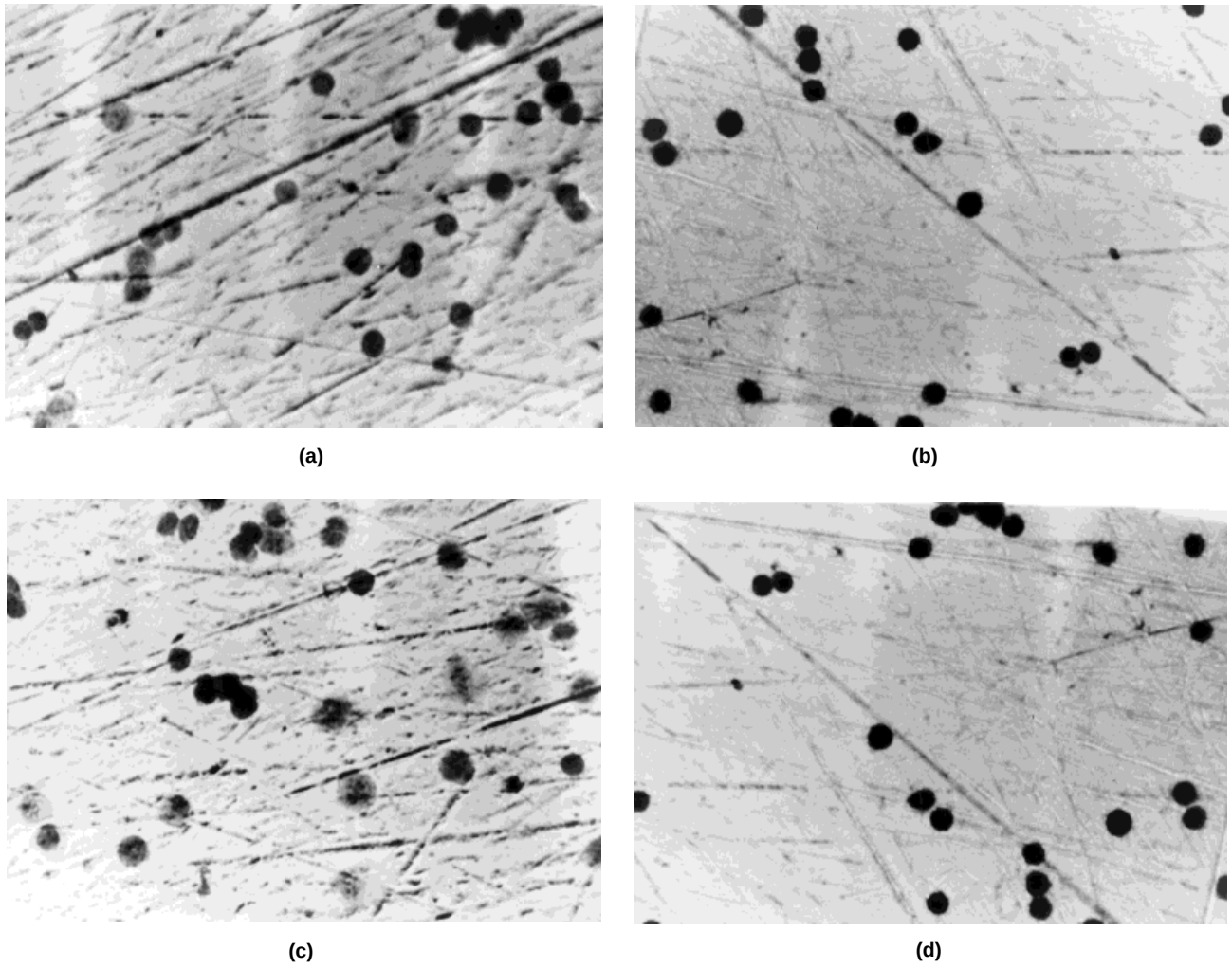

Figure 3. Optical microscope image, after 2 h of culturing, of L 929 fibroblasts on: (a) Ti, (b) EPC Ti, (c) AEPC Ti, (d) HEPC Ti. Original magnification 200×.

results, shown in Figures 3 and 4, convincingly indicate that the thin polymeric film effectively acts as a substrate on which the methods of surface modification of polymers can be used to direct the cell behavior on the metal surface.

Concerning the different steps of surface modification used, it must be noted that Marchant and co-workers reported cell adhesion and growth on a polymeric film deposited from hexane plasma onto glass.[30] According to their results, fibroblasts on plasma-polymerized hexane grow at a rate similar to that observed on conventional tissue culture polystyrene (TCPS). The disagreement between the present data, which show that films polymerized from hydrocarbon plasma are very poor substrates for cell adhesion and growth, and the data of Marchant and co-workers easily can be understood by comparing the reported surface compositions, as detected by ESCA analysis. In the present case, the polymeric film contains less than 1% oxygen while in the quoted work, about 6% oxygen and 2% nitrogen were detected together with a much lower water contact angle than that measured in our study. Oxygen usually is incorporated in hydrocarbon films deposited from plasma because of a side reaction between active radicals and residual oxygen molecules in the reactor atmosphere, or by the reaction of long-lived radicals and atmospheric oxygen when the samples are exposed to the atmosphere after treatment.[16] Reaction with leaking air or post-treatment reactions with the atmosphere also are indicated by the incorporation of nitrogen. In the present experiments much care was taken to avoid side reactions with oxygen (see the Materials and Methods section), and both ESCA and contact angle data indicate that a very homogeneous, hydrocarbon-like surface was obtained. Cells behave accordingly. It must be noted that in order for the EPC surfaces to act as an effective plaque-resistant substrate, as discussed in the Introduction, they should contain as few as possible Lewis base (or, in the old terminology, polar) surface sites.[21]

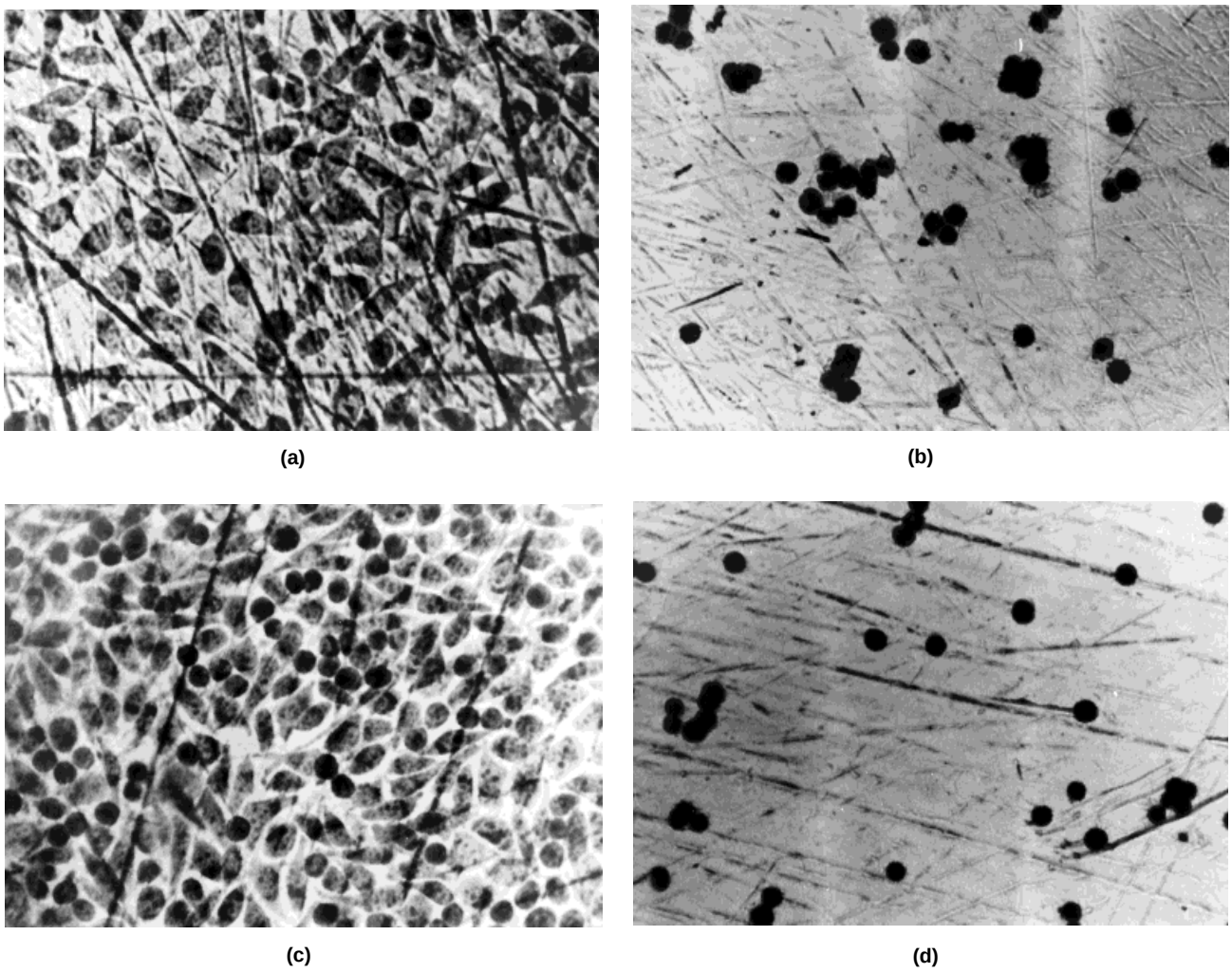

Figure 4. Optical microscope image, after 48 h of culturing, of L 929 fibroblasts on: (a) Ti, (b) EPC Ti, (c) AEPC Ti, (d) HEPC Ti. Original magnification 200×.

Oxygen-containing organic functionalities are introduced on the EPC surface by the air-plasma treatment, as shown by ESCA analysis and contact angle measurement / surface free energy calculations. Steele and co-workers recently discussed the effect of plasma oxidation of polystyrene on adsorption and molecular potency of cytoadhesive proteins such as fibronectin and vitronectin.[31] The present results indicate that a similar effect is produced by air-plasma treatment of hydrocarbon-based, plasma-deposited polymers.

TABLE III
Results of Cell Counting on Untreated and Surface-Treated Ti after 48 h of Cell Culturing[a]

Sample	Number of Attached Cells (cells / $cm^2 \times 10^4$)
Ti	8.9 ± 0.8
EPC Ti	1.8 ± 0.8
AEPC Ti	14.0 ± 0.7
HEPC Ti	1.3 ± 0.9

[a]Difference between cell numbers on EPC and HEPC Ti is not statistically significant. All other differences are statistically significant ($p < 0.01$).

The AEPC surface is a better substrate for L-929 fibroblast adhesion and growth than is nitric acid-passivated Ti. Keller and co-workers found that there were no significant differences in the percent of cell attachment between a TCPS control and acid-passivated Ti surfaces.[12] These different results can be accounted for both by the different cell line (L-929 vs. human fibroblasts) and by the different nature, albeit stemming in both cases from a plasma-treated hydrocarbon polymer, of TCPS and AEPC surfaces.

Air- or oxygen-plasma treatments are commonly used, in addition to surface oxidation, to clean surfaces by etching away organic contaminants or films.[16,32] In general, ablation and etching always occur during oxidative plasma treatment of polymers. In the present case the rate of etching of the plasma-deposited films was not measured. However, ESCA data show that the thickness of the oxidized organic film is still higher

than the XPS sampling depth. This is reasonable considering the very short treatment time used for the air-plasma treatment.

The use of Ce(IV) to graft acrylate monomers to surfaces containing hydroxyl groups, or to synthetic organic surfaces plasma- or corona-treated to introduce oxygen-containing functionalities, has been widely exploited.[15,33] From a strict surface chemistry point of view, it must be noted that no direct evidence exists that the polymerized acrylate is, indeed, covalently linked (grafted) to the substrate surface. A strong physical interaction or an entanglement between the plasma-treated EPC surface and the growing PHEMA chains could explain the observed results as well. Xue and Wilkie recently discussed chemical grafting versus physical interaction in vinyl monomers-poly(ethylene-terephthalate) systems.[34] Many of their observations can be extended to other monomer-substrate systems, including the present one. From a practical point of view, it is important to note that the PHEMA surface coating successfully withstands extraction in ethanol and effectively acts as a cell-resistant surface structure. No difference was observed, as shown in Figures 3 and 4 and in Table III, with respect to the resistance to fibroblast colonization of the hydrophobic EPC and of hydrophilic HEPC surfaces. While in the present *in vitro* experiments the two surface modification strategies yield similar results and can be considered, as far as cell adhesion is concerned, equivalent, they likely will produce different tissue response in *in vivo* trials. Hydrophobic and hydrophilic surfaces produce a completely different pattern of protein adsorption, and these differences will likely show up in more complicated biological environments.

Coming back to Figure 1 and the goal of Ti implant parts endowed with finely tuned, interface-specific properties, it is important to remark that all the surface modifications reported stem from the modification of a common precursor, i.e., the hydrocarbon film deposited from ethylene plasma. From the point of view of the fabrication of the device, this is an important observation because existing and well-established masking and patterning technology could be used to produce Ti implant parts with variable and engineered surface properties.[35,36]

CONCLUSIONS

In conclusion, the present results show that the methods of surface modification of polymers can be used effectively to direct the cell–material interactions on Ti surfaces. Deposition from ethylene plasma produces a thin, homogeneous, adherent film of reliable surface chemistry, which, besides being endowed with increased resistance to plaque[21] and cell [Figs. 3(b) and 4(b)] adhesion, can be used as the starting point for further surface modification treatments and reactions. This hydrocarbon-like film effectively acts as an amplifier of the possibility of chemical manipulations of the Ti surface and opens the way to the fine tuning, *via* the well-developed methods of surface chemistry of organic materials, of the interactions between Ti, or, more generally, between metal-based biomedical devices and biological phases.

References

1. R. Adell, U. Leckholm, B. Rockler, and P. I. Branemark, ''A 15-year study of osseointegrated implants in the treatment of edentulous jaw,'' *Int. J. Oral Surg.*, **10,** 387–416 (1981).
2. T. Albrektsson, ''The response of bone to titanium implants,'' *CRC Crit. Rev. Biocomp.*, **1,** 53–84 (1984).
3. H. A. Hansson, T. Albrektsson, and P. I. Branemark, ''Structural aspects of the interface between tissue and titanium implants,'' *J. Prosthet. Dent.*, **50,** 108–111 (1983).
4. B. Kasemo, ''Biocompatibility of titanium implants: Surface science aspects,'' *J. Prosthet. Dent.*, **49,** 832–837 (1983).
5. B. Lowenberg, A. Shiga, P. Exbrayat, and J. E. Davies, ''Titanium and titanium alloy thin film sputtered flexible substrata: A novel *in vitro* bone cell culture system,'' *Transactions of the 4th World Biomaterials Congress*, Berlin, 1992, p. 571.
6. G. E. White, *Osseointegrated Dental Technology*, Quintessence Publishing Co. Ltd., London, 1993.
7. B. Chehroudi, T. R. L. Gould, and D. M. Brunette, ''Effects of a grooved titanium-coated implant surface on epithelial cell behavior, *in vitro* and *in vivo*,'' *J. Biomed. Mater. Res.*, **23,** 1067–1085 (1989).
8. B. Chehroudi, T. R. L. Gould, and D. M. Brunette, ''Titanium-coated micromachined grooves of different dimensions affect epithelial and connective tissue cells differently *in vivo*,'' *J. Biomed. Mater. Res.*, **24,** 1203–1219 (1990).
9. D. M. Brunette, ''The effects of implant surface topography on the behavior of cells,'' *Int. J. Oral Maxillofac. Impl.*, **3,** 231–246 (1988).
10. K. Bowers, J. Keller, B. Randolph, D. Wich, and C. Michaels, ''Optimization of the surface micromorphology for enhanced osteoblast responses *in vitro*,'' *Int. J. Oral Maxillofac. Impl.*, **7,** 302–310 (1992).
11. J. Y. Martin, Z. Schwartz, T. W. Hummert, D. M. Schraub, J. Simpson, J. Lankford, Jr., D. D. Dean, D. L. Cochran, and B. D. Boyan, ''Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63),'' *J. Biomed. Mater. Res.*, **29,** 389–401 (1995).
12. J. C. Keller, R. A. Draughn, J. P. Wightman, W. J. Dougherty, and S. D. Meletiou, ''Characterization of sterilized cp titanium implant surfaces,'' *Int. J. Oral Maxillofac. Impl.*, **5,** 360–367 (1990).
13. K. M. Swart, J. C. Keller, J. P. Wightman, R. A. Draughn, C. M. Stanford, and C. M. Michaels, ''Short-term plasma-cleaning treatments enhance *in vitro* osteoblast attachment to titanium,'' *J. Oral Implant.*, **18,** 130–137 (1992).

14. K. D. Chesmel and R. S. Tuan, ''Phenotypic behavior of human bone cells on plasma-treated Ti surfaces,'' *Trans. 20th Ann. Meeting Soc. Biomater.,* **17,** 299 (1994).

15. F. Garbassi, M. Morra, and E. Occhiello, *Polymer Surfaces, from Physics to Technology,* Wiley, Chichester, 1994.

16. H. Yasuda, *Plasma Polymerization,* Academic Press, Inc., Orlando, Florida, 1985.

17. B. D. Ratner, A. Chilkoti, and G. P. Lopez, ''Plasma deposition and treatment for biomaterial applications'' in *Plasma Deposition, Treatment and Etching of Polymers,* R. d'Agostino (ed.), Academic Press, San Diego, 1990, pp. 463–516.

18. R. E. Marchant, S. D. Johnson, B. H. Schneider, M. P. Agger, and J. M. Anderson, ''A hydrophilic plasma polymerized film composite with potential application as an interface for biomaterials,'' *J. Biomed. Mater. Res.,* **24,** 1521–1537 (1990).

19. J. M. Seeger, N. Klingman, E. Biagtan, D. Amery, P. Martin, C. Widenhouse, and E. P. Goldberg, ''Hydrophilic modification of stainless steel stents,'' *Trans. 20th Ann. Meeting Soc. Biomater.,* **17,** 343 (1994).

20. C. E. Christersson, R. G. Dunford, P. O. Glantz, and R. E. Baier, ''Effect of critical surface tension on retention of oral microorganisms,'' *Scand. J. Dent. Res.,* **97,** 247–256 (1989).

21. M. Bellanda, C. Cassinelli, and M. Morra, ''Reduced plaque accumulation on hydrocarbon thin film deposited on restorative acrylic polymers,'' *J. Biomed. Mater. Res.,* **36,** 216–222 (1997).

22. *Annual Book of ASTM Standards,* Section 13, F86-84, American Society for Testing and Materials, Philadelphia, 1990.

23. A. Wennerberg, T. Albrektsson, and J. Lausmaa, ''Torque and hystomorphometric evaluation of c.p. titanium screws blasted with 25- and 75- μm-sized particles of Al_2O_3,'' *J. Biomed. Mater. Res.,* **30,** 251–260 (1996).

24. B. Kasemo and J. Lausmaa, ''Surface science aspects of inorganic biomaterials,'' *CRC Crit. Rev. Biocompat.,* **2,** 335–380 (1986).

25. R. J. Good and C. J. van Oss, ''The modern theory of contact angles and the hydrogen bond components of surface energies,'' in *Modern Approach to Wettability: Theory and Applications,* M. E. Schrader and G. Loeb (eds.), Plenum Press, New York, 1991, pp. 1–27.

26. C. J. van Oss, *Interfacial Forces in Aqueous Media,* Marcel Dekker, Inc., New York, 1994, p. 176.

27. S. Wu, *Polymer Interface and Adhesion,* Marcel Dekker, Inc., New York, 1982.

28. C. F. Amstein and P. A. Hartman, ''Adaption of plastic surfaces for tissue culture by glow discharge,'' *J. Clin. Microbiol.,* **2,** 46–54 (1975).

29. N. G. Maroudas, ''Chemical and mechanical requirements for fibroblast adhesion,'' *Nature,* **244,** 353–354 (1973).

30. S. D. Johnson, J. M. Anderson, and R. E. Marchant, ''Biocompatibility studies on plasma-polymerized interface materials encompassing both hydrophobic and hydrophilic surfaces,'' *J. Biomed. Mater. Res.,* **26,** 915–935 (1992).

31. J. C. Steele, B. A. Dalton, G. Johnson, and P. A. Underwood, ''Polystyrene chemistry affects vitronectin activity: An explanation for cell attachment to tissue culture polystyrene but not to unmodified polystyrene,'' *J. Biomed. Mater. Res.,* **27,** 927–940 (1993).

32. R. d'Agostino, ed., *Plasma Deposition, Treatment and Etching of Polymers,* Academic Press, Inc., San Diego, 1990.

33. J. K. Yun, K. DeFife, E. Colton, S. Stack, A. Azeez, L. Cahalan, M. Verhoeven, P. Cahalan, and J. M. Anderson, ''Human monocyte / macrophage adhesion and cytokine production on surface-modified poly (tetrafluoroethylene / hexafluoropropylene) polymers with and without protein preadsorption,'' *J. Biomed. Mater. Res.,* **29,** 257–268 (1995).

34. T. J. Xue and C. A. Wilkie, ''Grafting onto poly (ethyleneterephthalate)?,'' *Polym. Prep.,* **36,** 251–252 (1995).

35. J. P. Ranieri, R. Bellamkonda, J. Jacob, T. G. Vargo, J. A. Gardella, and P. Aebischer, ''Selective neuronal cell attachment to a covalently-patterned monoamine on fluorinated ethylene propylene films,'' *J. Biomed. Mater. Res.,* **27,** 917–925 (1993).

36. B. D. Ratner, ''New ideas in biomaterials science—A path to engineered biomaterials,'' *J. Biomed. Mater. Res.,* **27,** 837–850 (1993).