
Reduced plaque accumulation on hydrocarbon thin film deposited on restorative acrylic polymers

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Abstract: The deposition of a thin polymeric film from ethylene plasma was used to modify the surface properties of acrylic teeth, commonly used in the dental practice for crown and bridge restorations. The effects of the surface modification process on the surface composition, morphology, and energetics were evaluated by electron spectroscopy for chemical analysis, atomic force microscopy, and contact angle measurement respectively. Plaque accumulation on the plasma-coated and untreated material was evaluated in *in vivo* experiments, in which the same patient received conven-

tional and plasma-coated restorations. The hydrocarbon-like surface of the plasma-coated restoration remained remarkably free from plaque, even in the absence of brushing. On the other hand, plaque accumulation was observed on the unmodified restoration. Results are discussed according to recent theories on bioadhesive phenomena. © 1997 John Wiley & Sons, Inc. *J Biomed Mater Res*, **36**, 216–222, 1997.

Key words: surface modification; plasma deposition; contact angle; surface free energy; plaque; dental polymers

INTRODUCTION

Dental plaque is a soft film that forms on the surface of the teeth, consisting mainly of bacterial cells surrounded by a polysaccharide matrix. As in many other bioadhesive phenomena, the first event in plaque genesis is the formation of a thin organic film (conditioning film), as a result of the attachment of acidic glycoproteins from the saliva.¹ This film provides a firmer attachment site for the colonization and growth of specific bacterial microcolonies.² As a result of the extensive growth of these organisms, a thick bacterial zone is formed consisting mainly of filamentous bacteria closely packed and extending out perpendicular to the surface of the tooth, embedded in an amorphous matrix.³

Plaque accumulation on teeth enhances susceptibility to teeth decay and causes several oral diseases. When plaque accumulates on prosthetic restorations, it adversely affects gingival and periodontal health. For these reasons, many studies have been devoted to understanding the basic bioadhesive phenomena involved in plaque formation. In 1969, a positive correlation was reported between the surface free energy

of a material placed in the oral cavity and its maximum plaque-retaining property.⁴ Several papers discussed the relationship between one surface free energy descriptor, the critical surface tension,⁵ and bioadhesive events in the oral cavity. Baier and coworkers studied, in a flow cell system, the effect of the critical surface tension on the initial retention of microorganisms from human saliva.⁶ They showed that surfaces of medium critical surface tension (i.e., 35–38 mJ/m²) representative of human tooth surfaces and most restorative dental materials retain the highest number of microorganisms in comparison with those of either low (20–22 mJ/m²) or high (>50 mJ/m²) critical surface tension. Moreover, they reported that the substratum surface properties dictate the bound conformation of adsorbed adhesive salivary proteins.⁷ Olsson and coworkers used surface treatment with different siloxane polymers to reduce *in vitro* bacterial adherence to ceramic surfaces.⁸ According to these findings, retention of microbial populations at interfaces might therefore be controllable by advance selection of the critical surface tension of the prosthetic material.

As to clinical studies, Jendresen and Glantz reported that both tooth surfaces and surfaces of dental materials with variations in their initial critical surface tension value arrive at a similar state of bioadhesiveness following salivary exposure,^{9,10} a result confirmed by the findings of Van Dijk and coworkers in *in vivo* studies in beagles.¹¹ In a recent study, no significant differences regarding bacterial colonization were found among titanium, hydroxyapatite, and amalgam surfaces.¹²

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The aim of this work was to look for further insights into the relationship between plaque accumulation and surface properties of dental restorative materials. The missing link between works^{6,8} which show a marked effect of the substrate surface properties on bacterial adhesion and retention *in vitro* and clinical studies^{9,12} which indicate that all materials are brought to the same free surface energy state, and thus to the same surface adhesiveness,¹⁰ is that in the latter case, no low-energy surfaces (according to Baier's definition⁶) were tested. Actually, testing involved a "medium free energy" dental polymer¹⁰ and inorganic materials whose surface properties in normal atmosphere are dictated by the outermost layer of adsorbed molecules.

In the present study, to evaluate plaque adhesion on a low-energy surface, several "resin teeth" commonly used in prosthetic dentistry for the building-up of missing teeth in crown and bridge restorations were surface-modified by deposition of a thin polymeric film from ethylene plasma. This treatment produces a hydrocarbon-like, low-energy, apolar surface.

Plaque adhesion and retention were evaluated *in vivo* experiments in which the same patient received bilateral restorations: a three-element restoration made from a metal framework and conventional acrylic teeth is placed on one side of the lower dental arch. A similar prosthesis, ethylene-plasma-coated (EPC), as described in Materials and Methods, is placed on the other side of the dental arch. This experimental scheme allows one to bypass the inherent variability of the composition and properties of saliva, which vary from individual to individual and within the same individual, as a consequence of physiologic and emotional factors.¹³

For this study, deposition from plasma was chosen among other surface-modification techniques for the following reasons: (a) it allows one to produce an adherent, pinhole-free surface layer resistant to hydrolysis and delamination in the humid oral environment, and (b) the conformal nature of the coatings deposited from plasma allows one to modify surface chemistry without affecting surface morphology.^{14,15} The latter feature plays an obviously important role in bacterial adhesion and retention.¹⁶ As shown by the results of surface morphology analysis described in the relevant section, acrylic teeth have a peculiar surface morphology that likely plays a role in adsorption and adhesion from biological fluids, and which could be easily altered by surface-modification processes.

The surface composition, morphology, and energetics of the untreated and the plasma-modified material were evaluated by electron spectroscopy for chemical analysis (ESCA), atomic force microscopy (AFM), and contact angle measurements, respectively. Rather than the old (if glorious) critical surface tension, the more modern Lewis acid-base approach^{17,19} was used to dis-

uss the relationship between plaque accumulation and surface energetics.

MATERIALS AND METHODS

Materials

Conventional acrylic teeth (Detry; Dentsply) were used in the prosthetic work. Although their exact formulation is proprietary, it is known that they are made by a highly crosslinked acrylic polymer. This is confirmed by the infrared spectrum shown in Figure 1, which was obtained after digestion of a tooth in methylene chloride, drying of the resulting suspension, and preparation of a KBr pellet. The spectrum is dominated by the typical adsorption of the carbonyl group of the acrylate ester at 1735 cm^{-1} .

Deposition from 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 were made of stainless steel. The reactor volume was about 3 dm^3 , and the distance between the electrodes, 10 cm.

Flow rate was controlled by an 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, flow rate of 40 standard cubic centimeters per minute (sccm), and deposition time of 1.5 min were used. When plasma was turned off, the ethylene flow was maintained for 30 s, to quench active radicals.

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

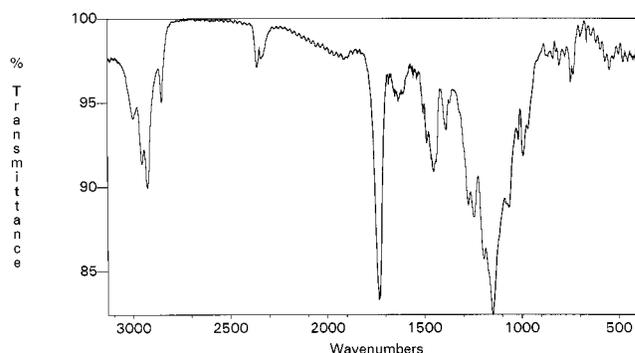


Figure 1. Infrared spectrum of the commercial resin teeth used in this study.

Surface characterization

Contact angle measurement

Contact angle measurements were performed for the untreated and surface-modified teeth. The following liquids were used: H₂O (doubly distilled), CH₂I₂ (99%; Aldrich), and dimethylsulfoxide (DMSO) (>99.5%; Fluka).

The contact angle of test liquids on the samples surface was measured by the sessile drop technique¹⁹ using a Kruss G 23 contact angle goniometer (Kruss, Hamburg, Germany). Advancing and receding angles were measured by increasing or decreasing the drop volume until the three-phase boundary was moved, keeping the capillary pipette of the microsyringe immersed in the probe fluid during the whole measurement. To avoid cross-contamination of liquids, a dedicated microsyringe was used for each liquid.

The peculiar shape of the samples posed a problem for the accuracy of the measurements, because it was difficult to find a reasonably flat area big enough to receive the liquid drop without adverse edge effects on the drop shape. For this reason, the labial surface of incisors was used for contact angle measurements.

Surface composition

Surface composition was evaluated by ESCA using a Perkin Elmer PHI 5500 ESCA system. The instrument is equipped with a monochromatic X-ray source (Al K α anode), operating at 14 kV and 250 W. The diameter of the analyzed spot is 400 μ m. The base pressure was 10⁻⁸ Pa. Peak deconvolution and quantification of the elements were accomplished using the software and sensitivity factors supplied by the manufacturer. The angle between the electron analyzer and sample surface was 45°C. In high-resolution spectra, all binding energies were referenced by setting the CHx peak maximum in the resolved C_{1s} spectra to 285.0 eV.

Surface morphology

A Nanoscope III AFM (Digital Instruments) was used for evaluation of the surface morphology. Samples were imaged using an Si₃N₄ cantilever with a spring constant of about 0.12 N/m and a 125 \times 125- μ m scanner (J scanner). The "Height" data type mode was used; that is, data correspond to the change in the piezo height needed to keep the cantilever deflection constant.

The nominal contact force was calculated from the cantilever spring constant and the force calibration graph (accessible through the instrument control software). A 20-nN contact force between the cantilever tip and the sample surface was measured.

Dental restoration and evaluation of plaque accumulation

Experiments were performed in triplicate on volunteers who were thoroughly informed about the study details. Dental restoration was performed as follows: Two titanium implants (3i, West Palm Beach, FL) were placed in specular position on each side of the lower dental arch. The work was completed by placing transmucosal titanium abutment cylinders (3i) on the fixtures, onto which a metal framework was applied. The latter was made according to the normal dental technician practice, using the lost wax technique and a silver-based crown and bridge alloy (Platincast; Nobil Metal, Villafranca d'Asti, Italy). After the usual postoperative healing period, the work was completed by screwing acrylic teeth onto the metal framework. Conventional acrylic teeth were placed on the right side of the dental arch, and EPC teeth on the left.

Plaque accumulation was evaluated by direct observation, in the dentist cabinet, at fixed time intervals.

RESULTS

Surface composition

The surface compositions of the untreated and EPC teeth, detected by ESCA analysis, and the results of C_{1s} peak curve-fitting are shown in Table I. The surface of the untreated material contained carbon, oxygen, and a small amount of nitrogen. The latter could be due to some additive (initiators or accelerators) or the presence of urethane acrylates, frequently used in the formulation of dental polymers. The C_{1s} peak showed the typical features of (meth)acrylate polymers, with a marked contribution to the peak shape of the carboxylic O—C=O component (290 eV). Note also that the components of inorganic pigments and/or filler which are normally present in plastic teeth formulation were not detected by ESCA analysis. As their surface free energy is higher than that of the polymeric matrix, it is likely that the uppermost layers are actually built up by the comparatively lower surface free energy organic matrix.

Ethylene plasma-coated teeth show the typical composition of hydrocarbon surfaces; besides carbon, only a very small amount of oxygen is detected. The C_{1s} peak shape is nearly perfectly symmetrical, with a contribution that is very small and difficult to quantitate accurately at higher binding energy. Oxygen is usually 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

TABLE I
Surface Composition (% atm) and C_{1s} Peak Shape Analysis of Untreated and EPC Acrylic Teeth
(H Is Not Detected in ESCA Analysis)

Sample	Surface Composition (% atm)			% Occupied Area		
	O	C	N	C—C, C—H	C—O, C—N	O—C=O
Untreated	21.1	77.7	1.2	65.4	14.2	19.4
EPC	0.9	99.1		≈100		

the atmosphere after treatment. These results confirm that a hydrocarbon film whose thickness is higher than the X-ray photoelectron spectroscopic sampling depth homogeneously coats the acrylic substrate.

Surface morphology

The surface morphology of untreated and plasma-coated teeth as detected by AFM analysis is shown in Figure 2. The surface of the untreated tooth [Fig. 2(a)] shows a rough morphology, at least at this level of

vertical resolution, with a mean roughness of some tens of nanometers (as evaluated by the AFM image analysis software). Some comparatively major pores (whose depth is about 150 nm) are also observed. Figure 2(b) shows the surface morphology of the EPC tooth. No significant difference is observed when the two images are compared. Both the general shape of the surface features and the roughness dimension are unaffected by the surface-modification treatment, confirming the conformal nature of plasma-deposited polymeric coatings.

Contact angles and surface energetics

The measured contact angles and surface free energy components, calculated according to the Good-Van Oss-Chaudhry approach to Lewis acid-base interfacial interactions,^{18,19} are shown in Table II. The untreated acrylic is mildly hydrophobic, with an advancing water contact angle similar to that measured on poly(methylmethacrylate). EPC teeth are much more hydrophobic, and the measured advancing water contact angle is now close to that observed on genuinely hydrocarbon surfaces such as that of poly(ethylene). Contact angle hysteresis is small, confirming the homogeneity of these surfaces as already suggested by the ESCA data.

Surface energetics calculation shows that the surface of the untreated tooth is basically an electron donor (Lewis base) monopole; according to published data on acrylate polymers, poly(methylmethacrylate) is a prototype basic polymer.^{17,18} On the other hand, EPC teeth are substantially apolar surfaces, with a very small Lewis base character.

In vivo plaque accumulation

A typical result of the *in vivo* plaque accumulation experiment is shown in Figure 3. These observations were performed after 2 weeks. In this experiment the patient was asked to avoid brushing teeth on both sides of the restoration. Figure 3(a) shows that plaque accumulates on the untreated acrylic teeth, while the EPC teeth [Fig. 3(b)] remain remarkably free from plaque and maintain a lustrous and clean appearance. The same results, with even slightly less plaque accumulation on untreated teeth, were observed when the

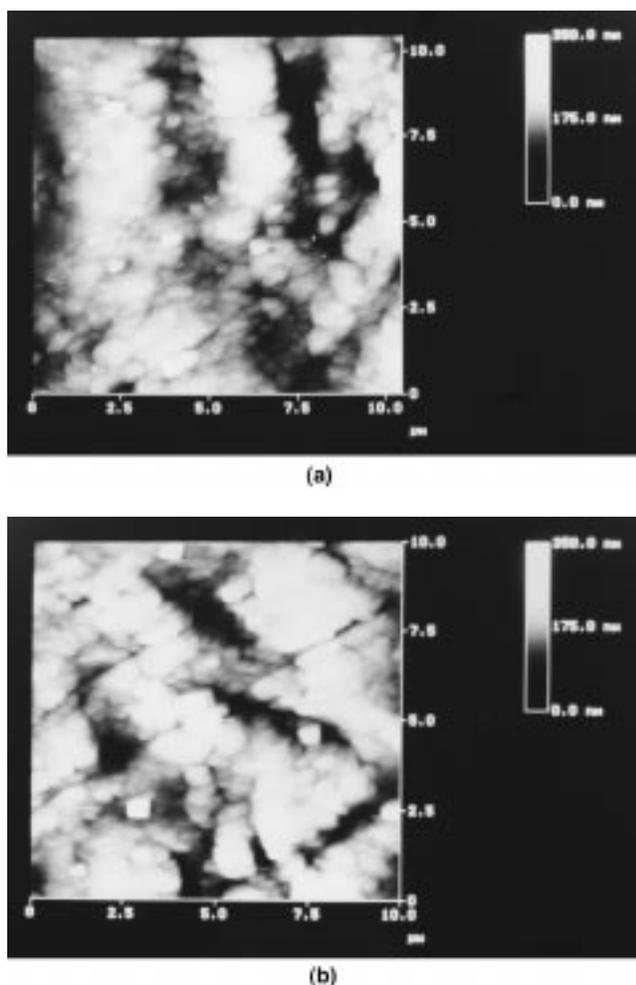


Figure 2. AFM image of the surface morphology of (a) untreated and (b) EPC acrylic teeth.

TABLE II
Measured Contact Angles (deg) and Calculated Surface Free Energy and Surface Free Energy Components (mJ/m²) of Untreated and EPC Acrylic Teeth*

Sample	Contact Angle [†]			Surface Free Energy Components			
	$\theta_{\text{H}_2\text{O}}$ Adv-Rec	θ_{DMSO} Adv	$\theta_{\text{CH}_2\text{I}_2}$ Adv	γ^{T}	γ^{LW}	γ^+	γ^-
Untreated	69 ± 5-45 ± 7	38 + 6	46 ± 7	36.5	36.5	0	16.2
EPC	95 ± 4-80 ± 5	49 + 5	48 + 6	35.4	35.4	0	1.0

* γ^{T} = Total surface free 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. The input values of the surface free energy of the liquids were taken from Ref. 22 and are as follows: H₂O, γ^{LW} = 21.8, γ^+ = 25.5, γ^- = 25.5. DMSO, γ^{LW} = 36.0, γ^+ = 0.5, γ^- = 32. CH₂I₂, γ^{LW} = 50.8, γ^+ = 0, γ^- = 0.

[†]Differences between CH₂I₂ contact angles are not statistically significant. All other differences are statistically significant ($p < 0.01$).

patient was asked to use normal oral hygiene practice on the uncoated teeth and avoid brushing the EPC teeth. The total follow-up period was 2 months. No plaque accumulation was observed on EPC teeth in this period in all self-rinsed areas. Only minor plaque accumulation in areas not rinsed by oral fluids, due to mechanical entrapment, occurred. On the other hand, marked plaque accumulation, depending on the details and accuracy of the patient's toothbrushing, was always observed on untreated restorations.

DISCUSSION

Interfacial interactions at the molecular level between surfaces of biomedical materials and the components of biological phases determine the results of bioadhesive events. In the present study, we evaluated the propensity of *in vivo* plaque accumulation on a typical polymeric dental material (i.e., an acrylic polymer) and on the same material surface-modified by the deposition of a thin hydrocarbon-like film from ethylene plasma. Results clearly show that modification of the molecular characteristics of the surface of the restorative material affects the interaction between the latter and the oral environment. In full agreement with previous *in vitro* findings,^{6,8} apolar hydrocarbon surfaces appear to be definitely less retentive of oral microorganisms than surfaces containing "polar" (according to the old terminology) or Lewis acid-base (using a more modern definition) sites. Previous *in vivo* studies have shown that when materials with widely different surface chemistries are placed in the mouths of human subjects, they are brought to the same surface state by a mechanism of surface film adsorption, and thus to the same surface adhesiveness.¹⁰ Accordingly, when different materials were tested, no effect on the early colonization pattern *in vivo* was observed.¹² The range of materials tested was limited by the fact that none of the presently existing materials with bioadhesive properties²⁰ has mechanical characteristics enabling it to be tested under the experimental conditions⁹ required for this kind of study. In the present work, surface modification of a common dental restorative polymer allowed us to overcome this difficulty. In particular, deposition from ethylene plasma produced a thin hydrocarbon film on acrylic teeth. The surface properties of the coated material, as shown in Figure 3, direct the process of surface film adsorption in such a way as to obtain a markedly decreased plaque accumulation. The effectiveness of the plaque-accumulation-reducing effect for the whole follow-up time confirms the strong adhesion of films deposited from plasma onto polymeric substrates even in wet and aggressive environments.

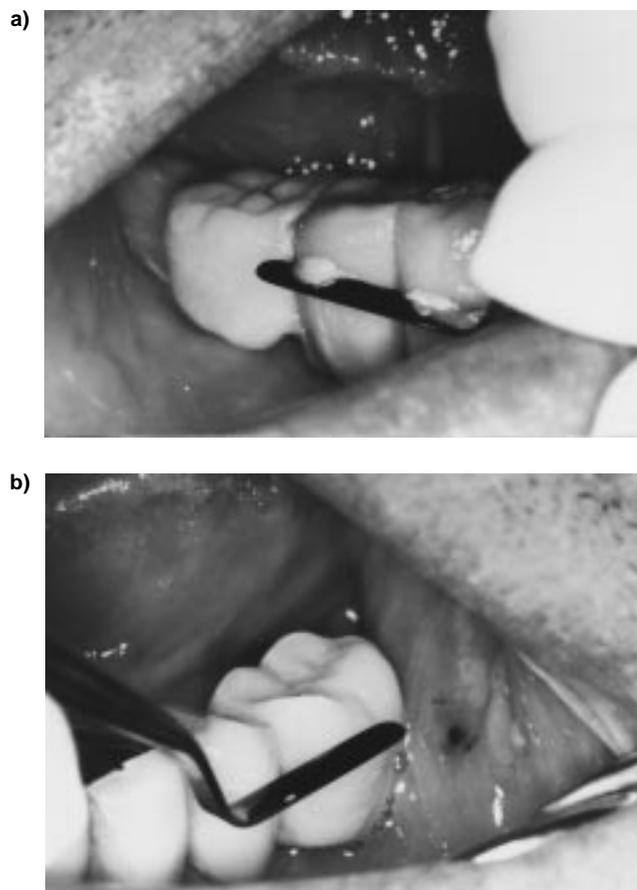


Figure 3. Plaque accumulation on (a) untreated and (b) EPC acrylic teeth after 2 weeks in the mouth.

What renders the surfaces of EPC teeth more resistant to plaque accumulation than surfaces of conventional acrylic teeth? Surface properties generally recognized to play a role in bioadhesive interactions are morphology, surface charge, and surface chemistry.²¹ In the present study, as shown in Figure 2, surface morphology of acrylic and EPC teeth is identical, so that it cannot be invoked to account for the observed behavior.

Surface charge or ζ potential were not measured in this study because the geometry and nature of the samples make it difficult to obtain reliable values. Thus, it is impossible to conclude from the present data if and how much the observed results are affected by surface electrical properties. According to Baier, however, in ionic physiologic circumstances, the events of biological adhesion are almost entirely beyond the practical influence of electrical properties.²¹

Surface chemistry, as measured by the ESCA data of Table I and reflected in the surface energy results of Table II, indicate marked differences between the two surfaces. In particular, the conventional tooth surface bears a marked Lewis base character, because of the electron lone pairs on the oxygen atoms of the ester carboxyl groups. On the other hand, the EPC tooth is practically an apolar surface. It should be noted that according to the approach used in the present work for the calculation of the surface free energy from contact angle data, the total surface free energy of the two materials is very similar. The more hydrophobic nature of the EPC surface is shown qualitatively by the high contact angle with water, whose value is typical of hydrocarbon surfaces, and quantitatively by the negligibly small value of the Lewis acid-base parameters. Both calculated γ^T values fall within the Baier's bioadhesive range. The definition and boundaries of the latter, however, are based on critical surface tension measurements. When the surface energetics of EPC teeth is measured according to the critical surface tension approach (data not shown), the obtained value places this surface within the range of Baier's low-energy, bioadhesive surfaces, as expected from the general relationships existing between surface chemistry and critical surface tension.⁵ We prefer to avoid the use of critical surface tension, because recent theoretical developments of contact angle-surface energetics theories suggest that more complete approaches should be used.¹⁸ However, it is important to appreciate how the choice of the surface free energy descriptor(s) affects not only the quantitative values of the surface energy, but also the physical explanation of the observed phenomenon: According to the critical surface tension approach, the observed behavior is accounted for by the low energy of the hydrocarbon surface. According to the Lewis acid-base approach, the most obvious explanation of the reduced plaque accumulation is the lack of Lewis base sites on the EPC tooth surface.

The first event in plaque formation, as discussed in the Introduction, is the attachment of acidic glycoproteins from the saliva.¹ This unspecific (i.e., not controlled by specific ligand-receptor interactions) event is then followed by specific adhesive interactions between bacteria and adsorbed proteins. The present results confirm the strong correlation between the initial surface properties of a material and its plaque accumulation capacity, which is apparently different from the relations expected on the basis of their relative hydrophobicity alone.⁶ It is interesting to note that other kinds of specific adsorbed proteins-suspended cells bioadhesive interactions also benefit from the presence of surface Lewis acid-base sites. Steele and coworkers showed²² that both the adsorbed amount and the molecular potency of cell-adhesive proteins such as vitronectin and fibronectin are higher on tissue-culture grade polystyrene that bears Lewis base sites²³ than on the practically apolar untreated polystyrene. Apparently, electron donor sites on the biomaterial surface play a pivotal role in directing the process of adsorption of adhesive proteins from biological fluids toward the formation of a protein layer highly effective in the resulting specific-adhesion step.

CONCLUSIONS

The deposition of a thin hydrocarbon film on conventional acrylic teeth results in a surface-modified restorative material endowed with a reduced propensity to plaque accumulation. This result confirms previous *in vitro* findings on the effect of the surface free energy, as dictated by surface composition, on the adsorption of oral adhesive proteins and retention of oral microorganisms.

Surface free energy analysis of the untreated and EPC teeth, according to the Lewis acid-base approach to interfacial energetics, suggests that the main difference between the two tested surfaces is the lack of Lewis base sites on the EPC teeth. As already reported in the case of cell adhesion on culture plastics,²² surfaces bearing Lewis sites seem to be more effective than apolar surfaces in the building-up of adsorbed adhesive-protein layers that maintain a proper molecular configuration for the resulting specific-adhesion steps.

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