Microtopography and clinical adhesiveness of an acid etched tooth surface

An *in-vivo* study

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The clinical adhesiveness as expressed by the critical surface tension and roughness of an acid etched tooth surface was studied as a function of time; from time of acid «conditioning» and at selected time intervals up to seven days.

The critical surface tensions of the tooth surface were calculated from clinical contact angle measurements as were values for change in roughness. SEM micrographs of the surfaces were obtained for visual comparisons.

The results indicate that the surface of the tooth returns to its orginal state of adhesiveness and roughness in a relatively short period of time as a consequence of the adsorption of a biofilm, the acquired pellicle.

Key-words: Pellicle; adhesiveness; clinical; roughness

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The clinical adhesiveness of tooth surfaces has been studied by several investigators using a variety of approaches and methods: (1, 2, 4, 5, 6, 7, 9, 10 & 14). There is general agreement between the aforementioned authors and many others that the surface of the tooth is covered with a biofilm, commonly called pellicle. Investigators who have studied this pellicle in detail include: Meckel (13), Leach & Saxton (11), Mayhall (12), Sönju & Rölla (18), and Rölla (15).

In spite of the many studies and the contributions from the body of work cited above, several questions still remain about the microtopography or roughness of the tooth surface. Its role in the clinical adhesiveness of the tooth surface is unkown. In this era of acid conditioning or etching of the tooth surface for clinical restorative and preventive procedures, questions arise concerning subsequent event to these altered surfaces.

It has been shown that acid treat-

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ment of tooth enamel surfaces produces changes in two well defined ways (16). Firstly, a shallow layer of enamel is removed along with the adsorbed film or pellicle. Secondly, after removal of this shallow layer of enamel the remaining surface is rendered porous by the acid solution. Silverstone, Saxton, Dogon & Fejerskov (17) reported that the exposure to phosphoric acid produced three basic etching patterns on the surface. All three patterns were found to occur on a single tooth surface. Gwinnett (8) reported that both 50 percent phosphoric acid and 50 percent citric acid produce a gross loss of approximately five µm and create or enhance existing enamel surface porosity for an additional 20–25 µm in depth.

The purpose of this investigation was to study the microtopography and clinical adhesiveness of the tooth surface immediately after acid conditioning and thereafter, for selected periods up to one week in a clinical setting. Contact angle determinations were used to calculate, first, the critical surface tensions (γ_c) of the tooth surface as a measure of its bioadhesiveness, and secondly, the relative surface roughness.

Wenzel (20) found that both small and large irregularities of a solid surface had influence on contact angle values. He described his findings with a simple equation, where he expressed the roughness factor (R) of the surface as the ratio of the true area of the solid (R^{1}) to the area of the ideally plane solid (R°). This ratio he found to be the same as that between the cosines of the measured contact angles (θ) for a certain liquid on the rough ($\cos \theta^{1}$) and the ideally plane surface ($\cos \theta^{1}$):

$$R = \frac{R^{1\prime}}{R^{\circ}} = \frac{\cos \theta'^{1}}{\cos \theta^{\circ}}$$

Scanning electron microscopic (SEM) studies were also planned to further demonstrate the changes in the microtopography.

MATERIALS AND METHODS

One female 54 years of age was chosen as test person. She considered herself to be in good general health, and was found to be in good dental health upon clinical examination.

Baseline measurements of the clinical adhesiveness were recorded for the normal tooth surface using the test liquid and methods described in detail by Glantz, Jendresen & Baier (7).

The maxillary anterior teeth were then isolated with a well placed rubber dam, and the surface of the right central incisor, free of any obvious debris, was acid etched or «conditioned» with a 40 percent aqueous solution of ortho-phosphoric acid for one minute. The etched surface was rinsed with copious amounts of water for 30 seconds and then dried with a stream of filtered compressed air for another 30 seconds. The result produced was a surface that appeared dull and chalky white.

While the tooth surface was still isolated from the oral environment by the rubber dam, the test liquids mentioned above were again applied to the newly created surface.

Following the application of the test liquid series, the isolated tooth surface was again acid treated, rinsed and dried as previously described. At this time the rubber dam was carefully removed. Then, the test person was instructed to lick the etched surface once and allow the placement of first a polar liquid (glycerol) microdroplet and second a non-polar liquid (methylene iodide) droplet to the surface. The resultant contact angles were recorded. 30 seconds and 15 minutes later, during which time the test person was allowed to close her mouth, a second set of contact angle measurements were made using the same two liquids.

60 minutes after the first exposure to the oral environment and saliva, the acid etched labial tooth surface was again evaluated using the complete series of test liquids in the manner previously described (7).

These same evaluations were repeated at two hours, 72 hours, and at seven days following the conditioning of the tooth surface. Contact angles were obtained at each of these time intervals and the critical surface tensions, according to Zisman (21), were calculated using the equations and graphic modifications described by Baier (1). Studies on some possible sources of error in the method have previously been reported by Glantz et al (7).

Approximately 90 days following the first acid treatment of the test person's tooth for contact angle determinations, a second identical surface treatment was performed in order to study the microtopography on the labial surface of the same tooth.

A baseline impression was obtained to provide a replica of the natural untreated labial tooth surface. The impression material used was a vinyl polysiloxane, light bodied type President (Coltène Inc., Altstaetten).

Following the phosphoric acid application on the isolated tooth for one minute, rinsing for 30 seconds and drying the surface with filtered compressed air for 30 seconds, the second elastomeric impression was obtained. Additional impressions were obtained at one and 24 hours. The surfaces of the impressions were then prepared for viewing in a scanning electron microscope (SEM).

Standard techniques were used. The impressions were coated with goldpalladium by cold sputtering using a Denten Vacuum model «Desk 1». The

Table 1. *Cosines of recorded angles of contact between certain test liquids (γ_{LV}) and a human enamel surface before and after etching with phosphoric acid, and calculated values for the changes in surface roughness from the pre-acid etched surface roughness (R_Δ)*

	LIQUIDS							
	Water		Glycerol		Thiodiglycol		Methylene iodide	
	$\gamma_{LV} = 72.4$		$\gamma_{LV} = 63.7$		$\gamma_{LV} = 53.3$		$\gamma_{LV} = 51.7$	
SURFACE	cos θ	R_Δ	cos θ	R_Δ	cos θ	R_Δ	cos θ	R_Δ
Normal (R_n)	0.6428		0.6551		0.8988		0.5299	
Directly post Acid Etch	1.0000*	-	1.0000*	-	1.0000*	-	1.0000*	-
1 Hr. post	0.7414	1.15	0.6819	1.04	0.8988	1.00	0.6293	1.19
2 Hr. post	0.7414	1.15	0.7880	1.20	0.8910	0.99	0.6293	1.19
72 Hr. post	0.7771	1.21	0.6018	0.92	0.8988	1.00	0.6691	1.26
7 Days post	0.7431	1.16	0.9646	1.06	0.8090	0.90	0.5878	1.11

$$R_\Delta = \frac{R_f}{R_n} = \frac{\cos\theta_f}{\cos\theta_n} = \frac{\text{cos θ of the contact angles of the newly formed film surface}}{\text{cos θ of the original normal tooth surface preacid etch}}$$

when $R_\Delta = 1.00$: The new film surface has the same roughness as the original preacid etched surface

*: Spreading liquid

Table 2. *Calculated critical surface tensions (γ_c) and slopes of best line fit (S) for a human enamel surface before and after etching with phosphoric acid*

Condition of Surface	γ_c (dynes/cm)	S (cm/dyne)
Normal Tooth	28.6	- 0.010
Directly post acid etch	> 72.4	-
1 Hr. post acid etch	34.4	- 0.009
2 Hr. post acid etch	30.1	- 0.007
72 Hr. post acid etch	27.7	- 0.007
7 Days post acid etch	29.7	- 0.008

specimens were coated for one minute producing a light coat of approximately 20 nm. The speciments were coated a second time in exactly the same fashion achieving a final coat of approximately 40 nm thick.

A Joel JXA - 35 scanning electron microscope was used with a beam of 7 KV at magnifications of 10X, 30X, 90X, 200X, and 500X.

RESULTS

The cosines of the five recorded complete sets of angles of contact between the tooth surface and the seven different test liquids are given in Table 1.

Because of time constraints only two contact angles were recorded 5 and 30 seconds as well as 15 minutes after the exposure of the acid etched surface to saliva. Both the angles measured were comparatively low after 5 seconds of saliva exposure (10° for glycerol and 48° for methylene iodide). For the polar glycerol the angle increased to 30° at 30 seconds and to 46° after 15 minutes. The angles measured for the non-polar methylene iodide remained fairly constant during this same time period and then increased as the film appeared to become well formed.

In this study the use of the Wenzel equation (I) was extended to show the relative change in roughness (R_Δ) of the surface as the ratio of the true area of the biofilm (R_f) to the area of the original untreated tooth surface, (R_n) in relation to the contact angles measured on these changing surfaces. Eick, Good & Neuman (3), have shown this to be true in the equilibrium thermodynamic state. Therefore, the relationship given below should be understood as an approximation. Hence, from the beginning of film formation on the acid etched surfaces, for periods up to seven days, the relative change in roughness was calculated according to the following equation:

$$R_\Delta = \frac{R_f}{R_n} = \frac{\cos \theta^f}{\cos \theta^n}$$

where θ^f denotes the contact angle on the newly formed film surface and θ^n that on the original untreated surface.

The results of these calculations are given in Table 1.

A value for (R_Δ) of 1.00 would indicate that the surface microtopography had returned to the same state that existed prior to acid etching of the tooth surface. On a liquid by liquid basis, the values for (R_Δ) at seven days, suggest the surface of the tooth nearly returned to its original state of roughness. This was confirmed by the test person's subjective assessment of the tooth's surface roughness at the seven day evaluation in both experiments.

The clinical adhesiveness of the tooth surface is shown in Table 2 as exhibited by its calculated critical surface tension (γ_c) and the slope of the best straight line fit (determined by the method of least squares) through a plot

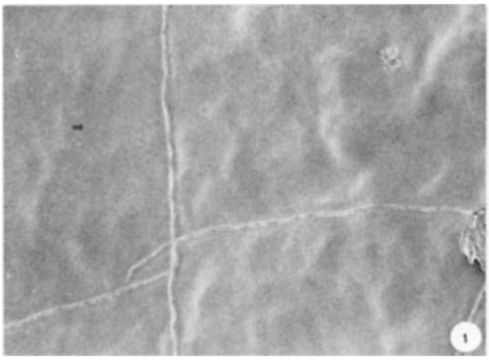

Fig. 1. A scanning electron micrograph from an enamel surface prior to acid etching or «conditioning» with 40 percent phosphoric acid. Original magnification is 500X. Cracks on the surface were created during the microscopic examination.

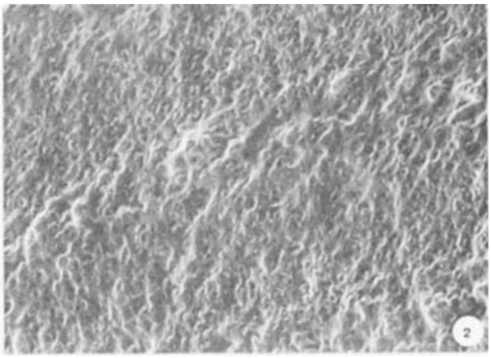

Fig. 2. Surface of the same tooth as in Fig. 1 immediately following acid etch for one minute, rinsing 30 seconds, and drying for 30 seconds. Original magnification is 500X.

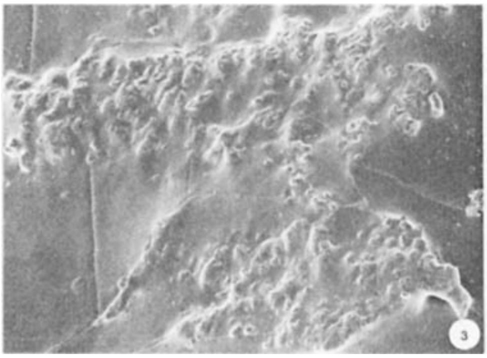

Fig. 3. Surface of the same tooth as in Figs. 1 & 2 at five minutes after acid etch. Orginal magnification is 500X.

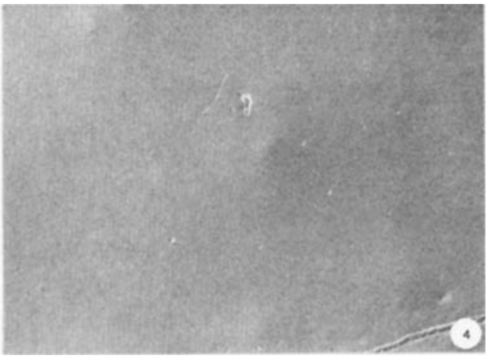

Fig. 4. Surface of the same tooth as in Figs. 1 – 3 at one hour after acid etch. Original magnification is 500X.

of the cosines of the contact angles from the test liquids against the liquid/vapor surface tension of the liquids.

From the data presented in Tables 1 and 2 it is evident that the tooth surface quickly returned to a surface energy state that is close to what it was before the acid treatment.

The results from the scanning electron micrographs confirm the above data by illustrating, as shown in Figs. 1–5, that the formation of a biofilm does indeed occur in a short time. At one hour the surface is entirely covered by the biofilm or pellicle, completely

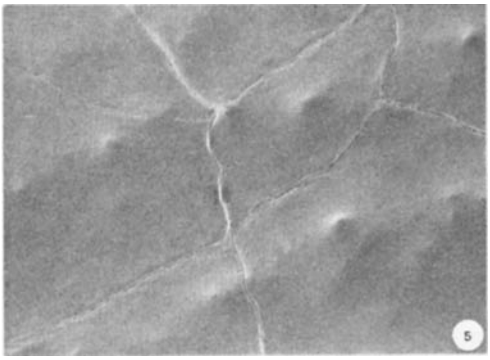

Fig. 5. Surface of the same tooth as in Figs. 1 – 4 at 24 hours after acid etch. Original magnification is 500X.

masking the underlying microtopography or roughness resulting from the acid treatment.

DISCUSSION

The methods used to determine tooth adhesiveness have previously been discussed by Glantz et al (7) and Jendresen & Glantz (9). In this study a methodological difference from these previous studies consisted of not showing separate critical surface tensions for the polar and non-polar groups of test liquids. The main reason for this was that too few liquids were found with angles of contact above 0°. Therefore, in order to increase the precision of the determinations only total critical surface tension values are given.

The surface texture or microtopography of a solid surface may have an overwhelming effect on the wetting characteristics of that solid surface. Thus, increased roughness of a solid will increase its wettability if the liquid in contact with a plane surface of the same solid has a contact angle of less than 90°. The converse is true, when the liquid used forms an angle of more than 90° with the plane surface. Under such conditions an increase in roughness will decrease wetting. Most organic liquids, however, form contact angles below 90° when applied to clean plane surfaces.

The roughness of the tooth surface could therefore be expected to increase the wetting of that surface by the oral fluids, which are mainly organic in nature.

It was observed in this study that spontaneous spreading of the test liquids occurred immediately after the surface was acid etched. This could be explained both as a result of the change in surface roughness and to the creation of a high energy surface, by exposing parts of the mineral phase of the tooth structure. Subsequently, when the test person was allowed to lick the high energy surface, the saliva immediately wet the surface and the formation of the biofilm or pellicle began. The two contact angles measured on the surface at that time (5 seconds) were relatively low but increased as the film appeared to become well formed. The gradual increase in the observed values for θ correlate well with the visual evidence of early film formation and decreased surface roughness as evidenced by the scanning electron micrographs, figs. 3 and 4. At this stage it is difficult to determine if the values for θ are due to energy components of the surface or due to the microtopography.

Considering the tooth surface after exposure to the oral environment at one hour and thereafter, there seems to be a definite effort to return to the surface energy state and microtopography that existed prior to the acid treatment.

If the $\frac{\cos \theta^{r}}{\cos \theta^{n}}$ – relationships are examined for the separate test liquids, it is, however, evident that water was a test liquid, with which no changes with time could be recorded for the surface roughness of the film covered etched enamel. There could be several explanations to this finding. One is based on the fact that out of the test liquids used, water has by far the smallest molecular size. Thus, as has been demonstrated by Glantz (5) water is capable of recording much smaller surface irregularities than the rest of the liquids. Another possible explanation is that even seven days after etching there could be an increased water content in the film, perhaps as a consequence of an ongoing maturation process. In that case, after acid etching the recorded angles would be more of the receding type.

The observed rate of film acquisition is in good agreement with Baier &

Glantz (2) and Sönju & Rölla (18). The apparent thickening of the film, as indicated by the calculated decrease in surface roughness with time, supports the ellipsometric findings reported by Baier & Glantz (2).

The findings from this study, when compared with the findings of others conducted under quite different circumstances, point to an organized and specific biological mechanism designed to quickly protect and maintain the hard, mineral tooth surface, by acquiring a relatively low state of surface free energy through the adsorption of a biological film, the acquired pellicle.

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