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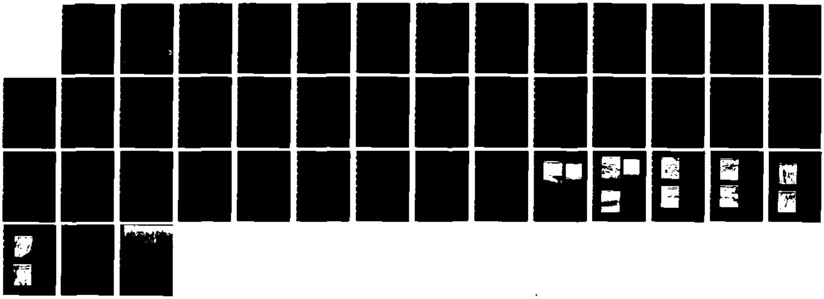

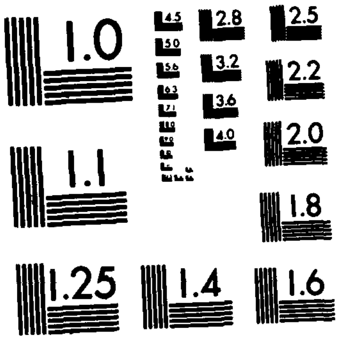
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Report Number 8

Annual Report

Feasibility Study on a Process for Electroless Metal Deposition In Pits and Fissures of Teeth for Use in Preventive Dentistry

Thomas J. O'Keefe

August 1981

Supported by

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Further studies were undertaken to test the feasibility of using metal films, deposited by electroless or chemical means, for use in the military dental program.

The research effort during the past year was concentrated in the following areas: Testing alternate etching agents, evaluating the effects of pretreatment on the silver deposit, testing alternate additives and optimizing the plating conditions on dentin.

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INTRODUCTION TO ANNUAL REPORT 1981

During the past year research emphasis has been on refining and optimizing the existing silver delivery system and initiating or intensifying efforts to evaluate the use of the chemically deposited silver layer as a liner for amalgam fillings. The results of the work to date are very encouraging, with some very important advances being made in a number of different areas. In particular, it has been found that citric acid may be a suitable substitute for phosphoric acid, particularly on dentin. The use of a stannous fluoride treatment after etching also has given good results. Not only does the adherence of amalgam seem to increase, but the SnF_2 seems to minimize the formation of layers of silver salts (thought to be Ag_3PO_4) found when the silver solutions were used first in the plating sequence. This may prove to be very beneficial in that it should minimize the amount of silver that might ultimately leach and be transferred into tissue. The latter could cause undesirable and unacceptable histological effects.

A number of different organic additives were evaluated, and again the results are encouraging. Cysteine appears to perform adequately on dentin, thus replacing the thiourea which posed possible toxicity problems. Equally important was the realization that we might be able to estimate the efficacy by noting the chemical structure of the compound, the presence of an active sulfur site as well as one other functional group. Even though the behavior of the additives is not exactly known, it has at least advanced to the point where technical estimates are possible. The additives have, to date, been evaluated by physical testing methods (shear stress, wear resistance, etc) which seemed appropriate in order to obtain the necessary data to allow the project to advance and provide a system which has a good chance of success in human use. There is now a strong possibility that even greater improvements may be possible by finding accelerators for silver deposition or suitable combinations of additives.

There seems to be little doubt that the chemically deposited silver film is in intimate contact with the tooth structure and has excellent adherence. The film offers the opportunity of acting as an intermediate substrate in conjunction with amalgam fillings which should greatly improve microleakage and adherence. Certainly the properties will require improvement as indicated by in vivo testing, but the basic system seems to now be adequate for a next level of evaluation.

I. ETCHING AGENTS

The influence of several etching agents on hydroxyapatite (HAp) was investigated; for instance phosphoric acid (H_3PO_4), citric acid ($C_{10}H_{18}O$), ethylene diamine tetraacetic acid (EDTA), and sodium hypochlorite (NaOCl) were used. Research by Gustafson[1] shows that phosphoric and citric acid etch the center of the inorganic HAp prisms while EDTA etches the periphery of the prisms and NaOCl etches the organic material between the HAp crystals.[1] Ethylene diamine also has a potential as an etchant as it removes organics (particularly proteins) from the substrate[2] and will be investigated later. The above mentioned information is taken from morphological studies conducted on enamel[1,2] and the etching agents may not have exactly the same influence on dentin and ivory. Ivory is the main material used in this investigation, but all three substrates are used in studying the physical properties of interest. To date wear resistance, electrical conductivity, and shear stress of amalgam have been used.

A. Phosphoric Acid

42.5% H_3PO_4 has been used in this process for etching for several years and earlier screening tests have shown that optimal etching time is 1 min. Scanning electron microscopy (SEM) studies show a fairly deep penetration of the acid that leaves a residual, amorphous layer on the substrate surface; see Fig. 1a. The thickness of the layer is dependent on the time of etching; after 1 min. it is 3 µm. The amorphous layer is believed to be organic, because the energy dispersive x-ray analysis done in connection with SEM studies does not show any sizeable quantities of either Ca or P; see Fig. 1b. This kind of x-ray analysis is capable of detecting elements of atomic number 11 and higher. The amorphous layer seems porous because Ag^+ penetrates through it and reacts with HAp to form Ag_3PO_4 underneath the etched layer. The penetration occurs when $AgNO_3$ solution is applied for 1 min. on the etched sample surface. Ag_3PO_4 is reduced by $FeSO_4$ and the reduction is complete on the surface, but deeper in the bulk it is incomplete. The reason for the depth dependent reducing power of $FeSO_4$ is not known, but it is believed that the reduction on the surface is fast and a metallic silver layer or other chemical layer covers the surface, blinding the underlying Ag_3PO_4 from being reduced.

When ivory is etched and plated, several distinct layers are observed in the cross section micrograph; see Fig. 2a. Layer 1 contains Ag; layer 2 is amorphous underneath the surface layer but towards the bulk of the sample it is fibrous and the composition is P:Ag:Ca = 1:5:1. Layer 3 looks like ivory but contains P:Ag:Ca = 2:1:3; see Fig. 2b.

A piece of ivory was etched with 42.5% H_3PO_4 for 20 min. and prereduced. Fig. 3 shows that the amorphous layer is extensive and completely structureless. The composition of the layers are layer 1, P:Ag:Ca = 1:3:1; layer 2, P:Ag:Ca = 1:4:1; layer 3, P:Ag:Ca = 1:2:1; and layer 4 = 1:5:1. The P:Ca ratio in the ivory is 1:2; the phosphoric acid etch seems to leach out Ca. Layer 4 in Fig. 3 appears grainy and it is high in Ag; it is believed that this layer contains Ag_3PO_4, which was formed during the prereduction step. The P:Ag ratio for Ag_3PO_4 powder is 1:6. The phases present in the amorphous layer with high Ag and P concentrations are not known but some may be in the form of Ag_3PO_4.

B. Citric Acid

Citric acid is a weak, organic acid; the pH of a 50% solution with water is 1.3. This acid etches ivory in a similar way as H_3PO_4 but it is not as destructive to the HAp structure. After 1 min. of etching, prereduction and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$, a layer structure can be observed in sample cross section; see Fig. 4. Layer 1 is metallic Ag; layer 2 is the amorphous film created by etching and now has an atomic ratio of P:Ag:Ca = 1:5:1; layer 3 is fibrous with the composition P:Ag:Ca = 1:5:0. Underneath these layers is the starting, bulk ivory.

One visible difference between H_3PO_4 and citric acid etched samples was observed during the first $FeSO_4$ application in the prereduction step. H_3PO_4 etched samples turned greyish dark while the citric acid etched samples turned brownish dark.

Prolonged etching with citric acid, 20 min., was tried on one sample and resulted in an extensive amorphous layer, about 2/3 of the thickness obtained after 20 min. H_3PO_4 etching; see Fig. 5. The sample was also prereduced so a Ag_3PO_4 layer (layer 4) was present underneath the amorphous layer. The atomic ratios of the layers are: layer 1, P:Ag:Ca = 1:1:0; layer 2, P:Ag:Ca = 1:3:0; layer 3, P:Ag:Ca = 1:4:0; and layer 4, P:Ag:Ca = 1:5:0. Twenty min. of citric acid etch seems to remove all the Ca to at least 17 μm depth.

C. EDTA

Ethylene diamine tetraacetic acid, EDTA, is a weak acid that etches the peripheral part of the inorganic HAp crystals. A saturated, 20% EDTA solution was used for etching ivory for 1 min. followed by prereduction and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$. Some layering was observed in a micrograph of the cross section; see Fig. 6. The surface was covered with a metallic silver layer (= layer 1) and underneath it was only a continuous fibrous layer but no amorphous layer due to etching was present. There are, however, some differences in darkness. A light layer rich in Ag and P with low Ca concentration is believed to be Ag_3PO_4. Layer 1 contains only Ag, layer 2 has the atomic ratio of P:Ag:Ca = 3:10:1, and layer 3 P:Ag:Ca = 1:1:1.

The penetration depth of Ag^+ is small compared to those observed for H_3PO_4 and citric acid etched samples. A higher EDTA concentration would hardly increase the penetration depth because the 20% solution is already saturated. By prolonging the etching time an increase might be obtained but that would prolong the total plating time which is undesirable for practical use. The shear stress values for EDTA etched samples were low, which may be due to poor penetration properties of the acid.

D. Sodium Hypochlorite

NaOCl is an alkali that etches the organic material between the HAp crystals. The pH of a 50% NaOCl solution is 9.4. A piece of ivory was etched for 1 min. with 50% NaOCl followed by prereduction and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$. An amorphous layer was not found, but several fibrous layers were present besides the metallic Ag layer on the surface (= layer 1); see Fig. 7. Layer 2 contains P:Ag:Ca = 2:6:1 and layer 3 P:Ag:Ca = 1:5:1 and above the HAp structure is layer 4, brighter in color and with the composition P:Ag:Ca = 1:3:1. The Ag penetration was deeper than after EDTA etch and about the same as after citric acid etch.

E. Chemical Properties

The etching properties of the chemical reagents used are important to the behavior of the deposited Ag layer. Besides providing for mechanical keying the structure may be dissolved or altered sufficiently to allow greater penetration of the Ag solution. The pH seems to be important for the etching, maybe by breaking chemical bonds in HAp allowing the substrate to reprecipitate and react with Ag^+ to form Ag_3PO_4. H_3PO_4 caused the most extensive etching, indicated by the thickness of the amorphous layer formed. Citric acid was only slightly less effective than H_3PO_4 and gave a very similar microstructure. EDTA is a weak etching agent with very limited ability to penetrate to any depth. NaOCl seems satisfactory on ivory but was the poorest of all the reagents for dentin.

Ag_3PO_4 is formed after H_3PO_4 etching as well as after the other etches, which indicates that the PO_4^{3-} in Ag_3PO_4 is not only from H_3PO_4 but from HAp. If the sample is unetched it will still turn yellow as soon as Ag^+ solution is applied, bright yellow when the pH is low (2-3) and light yellow at higher pH (5-6) of the Ag^+ solution.

An amorphous layer allows penetration of Ag^+ resulting in the formation of a Ag_3PO_4 layer. The influence of the presence of the amorphous layer on the physical properties is not yet known, but it is desirable to ascertain this as soon as possible.

F. Physical Properties

The physical properties, for instance resistance, wear resistance, and shear stress, of etched and electroless plated ivory and dentin were evaluated for the different etching agents. A prolonged 5 min. etching time was made on ivory to get a pronounced effect of the etching agents. The results are presented in Table I. H_3PO_4 provides the highest shear stress and a medium wear resistance. The citric acid etched sample had a lower shear stress but a higher wear resistance than the H_3PO_4 etched sample. NaOCl etched ivory gave a relatively high shear stress but had the poorest wear resistance. EDTA was not satisfactory. Table II presents the results obtained after 1 min. of etching and prereduction plus 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$ on dentin. H_3PO_4 and citric acid show the highest shear stress while EDTA and NaOCl are poor. SnF_2 pretreatment improved the deposit after H_3PO_4 etching and would probably have done the same after citric acid etching. The low shear stress values are due to the peeling of the silver layer from dentin. The peeling took place on all the samples but the one with SnF_2 pretreatment. The peeling took place after the samples had been aged 24 hours in water at 37°C and the tape was removed from the surface. After the Ag layer had peeled off the darkened dentin was visible.

II. PRETREATMENT STUDIES

Pretreatment is made after the tooth (enamel or dentin) or piece of ivory is etched and before the first silver application is made. Tin (II) fluoride (SnF_2) has been used extensively as a topical fluoride solution to form fluoroapatite (FAp), where the -OH in hydroxyapatite (HAp) is exchanged for F^-.[2] P. Gron[2] investigated the reactions of a 10% SnF_2 solution with enamel. According to his results, SnF_2 penetrates 20 μm during a 30 sec. application time and it reacts with the organic material in the enamel forming a tenacious film. In the same publication it was mentioned that low pH and low F^- concentration favors the formation of FAp, while high pH and high concentration favors CaF_2. CaF_2 is a salt soluble in saliva and it is believed not to supply F^- to HAp and consequently is undesirable.

The SnF_2 solutions used in this investigation were 0.5 to 50 gpl SnF_2 in demineralized water and the pH ranged from 2 to 4. At pH 4, the SnF_2 solubility is lower than at natural pH, which is 2.9-3.2 depending on the concentration; the higher the concentration the lower the pH. The application time varied from 1 to 3 minutes. The influence of the mentioned variables were studied in a screening test and the results given in Tables III and IV. The results of the screening test show that a 2 min. residence time for the SnF_2 is better than 1 min. residence time for improving shear stress. A large variation in shear stress values occurred because the fine cut Caulk alloy pellets used before for amalgamation were not available. Instead fine cut Caulk non-zinc alloy pellets were used without knowing the optimum trituration time for use in our process.

The influence of the pH was not detectable in the range from 2.0 to 4.0. The residence time, concentration, and pH of the SnF_2 solutions seem to have little influence on the physical properties of the electroless plated Ag layer. There is, however, a significant difference between samples that are pretreated with SnF_2 or not pretreated at all. SnF_2 definitely improves the physical properties; see Table V.

III. SnF_2 - CHEMICAL PROPERTIES

Without SnF_2 pretreatment, the sample surface turns yellow as soon as Ag^+ solution is applied, indicating Ag_3PO_4 is formed. Formation of Ag_3PO_4 is dependent on the pH of the Ag^+ solution; the lower the pH the brighter yellow and more concentrated is the Ag_3PO_4 layer. With SnF_2 pretreatment the surface does not turn yellow when Ag^+ is applied; it turns very dark instead. The dark color indicates that Ag^+ is reduced to Ag(s) by the Sn^{2+} ions that have penetrated into the surface of the substrate when SnF_2 was applied. One possibility is that Sn^{2+} has replaced some Ca^{2+}, which could occur because Sn^{2+} and Ca^{2+} have the same electrical charge and the ionic radii are 1.12 Å and 0.99 Å, respectively. The small difference in ionic radius allows exchange of Ca^{2+} to Sn^{2+} theoretically. Another possibility is that Sn^{2+} follows F^- into the HAp structure without being chemically bound to the HAp or FAp. Sn^{2+} is not easily removable, because an etched and SnF_2 pretreated sample was rinsed ultrasonically in water without decreasing its reducing power. Even though the exact form or species of Sn^{2+} present is not known, it does seem to be available as a reducing agent for the Ag^+, and additional research may be desirable to optimize its use. Ag_3PO_4 is not formed in a significant amount after SnF_2 pretreatment because SEM micrographs show the absence of a typical Ag_3PO_4 layer. Sn^{2+} reacts with Ag^+ and a dense Ag layer is formed on the surface, which might cause blinding to subsurface penetration. When SnF_2 is used, the rate of reduction with $FeSO_4$ solution is faster and the surface becomes metallic looking after 3-5 $AgNO_3$-$FeSO_4$-$AgNO_3$ repetitions.

IV. PREOPTIMIZATION OF CYSTEINE

Thiourea is used successfully as an additive in $FeSO_4$. Other additives have been tried, and the most promising is cysteine [$HSCH_2CH(NH_2)CO_2H$], a sulfur containing amino acid. $FeSO_4$ with cysteine forms a denser Ag deposit than with thiourea, and it looks metallic after 3-4 Ag^+-Fe^{2+}-Ag^+ repetitions. Reduction of Ag^+ with $FeSO_4$ + cysteine is rapid and the surface darkens uniformly in the beginning, but the final thickness of the Ag deposit seems less than when thiourea is used.

The influence of $AgNO_3$ and additive concentration on physical properties was studied in a preoptimization test. The pH of the $AgNO_3$ solution might play an important role and will be studied later. The plating procedure on ivory was: 1 min. etch using 42.5% H_3PO_4, 2 min. pretreatment with 50 gpl SnF_2, 1 min. 100-200 gpl $AgNO_3$, 1 min. saturated $FeSO_4$ + 0.2 to 1.0 gpl cysteine, followed by 9 repetitions of $AgNO_3$-$FeSO_4$ (plus desired concentration of additive)-$AgNO_3$. The samples were thoroughly rinsed between every treatment. For comparison, one piece of ivory was plated using thiourea as the additive instead of cysteine. The results are presented in Table VI. The highest wear resistance was obtained with thiourea but the best shear stress values were obtained with cysteine. An interesting observation is that a high $AgNO_3$ concentration needs a high additive concentration. When the $AgNO_3$ and cysteine concentrations are high, the Ag deposit is shiny and metallic and when the concentrations are low, the Ag deposit is grey and similar to those obtained with thiourea. There seems to be an optimum ratio for $AgNO_3$ and additive concentrations. Further optimization studies are needed before a final decision is made about using thiourea or cysteine.

V. OPTIMIZATION OF PLATING CONDITIONS

Tests comparing H_3PO_4 to citric acid, SnF_2 pretreatment to no pretreatment and thiourea to cysteine were done on dentin, enamel and ivory. Shear stress tests were made on dentin and wear resistance tests on enamel and ivory; electrical resistance measurements were done on all samples. A good shear stress value is very important, while wear resistance has no practical meaning on dentin. Plating conditions that give good shear stress values do not necessarily result in good wear resistance.

The results from the above mentioned tests are presented in Table VII. One can see that citric acid is better than H_3PO_4 for shear stress on dentin but worse for wear resistance on enamel. Citric acid is obviously too weak an etching agent on enamel, because the surface is still glossy after 1 min. of etching, while it is dull after H_3PO_4 etch. A yellow Ag_3PO_4 layer is not found when $AgNO_3$ is applied after citric acid etch, but the enamel turns bright yellow when H_3PO_4 is used. $HC\ell$ and HNO_3 (50% bottle concentration) were used as etchants for comparison to check Ag_3PO_4 formation after $AgNO_3$ application. The enamel turned yellow although not as bright as when H_3PO_4 was used. This proves that $AgNO_3$ reacts with hydroxy apatite in enamel and not only with phosphate residue from H_3PO_4 etch.

SnF_2 pretreatment improves the overall results compared to no pretreatment, see Table VII. There is one exception: when SnF_2 is used in combination with cysteine as an additive in $FeSO_4$ on dentin, then there is no significant difference between pretreatment and no pretreatment.

Cysteine has been considered a potential additive instead of thiourea. From Table VII one can see that cysteine gives a better shear stress value than thiourea, although thiourea is better on enamel and ivory. Cysteine gives a dark and uniform layer on the first or second repetition and a shiny metallic layer after 10 repetitions. Thiourea gives a chalky deposit and the rate of reduction seems slow and inhomogeneous during the first few repetitions but it improves after three or four repetitions.

A test comparing AgF to $AgNO_3$ and to $AgNO_3$+ NaF was done on dentin. At the same time, a comparison between cysteine and thiourea was done, the results are presented in Table VIII. The fluoride containing solutions give better shear stress values than when only $AgNO_3$ is used and the cysteine additive is superior to thiourea. Fluoride solutions have been shown to be better on enamel and ivory in previous work. From the above results, cysteine seems better than thiourea and citric acid is also preferred to H_3PO_4 on dentin.

A statistical screening test was done to optimize the plating conditions on dentin. The lower and the upper limits of the variables are presented in Table IX and the results in Table X. The electrical resistance main effect estimates give an indication of the continuity and thickness of the Ag layer: the lower the resistance, the better is the layer. The influence of the variables on the resistance is highly significant, as can be seen from the confidence level in Table X. The longer etching time and the higher SnF_2 concentration are preferred as well as the lower $AgNO_4$ pH, $FeSO_4$ pH and cysteine concentration.

From the confidence level for the shear stress values, it appears that the variables are insignificant. The exact reason is not known. One possible explanation is that the lower and upper limits are too close to each other to give sufficiently varied results or otherwise, the shear stress is independent of these variables.

VI. MICROSTRUCTURE OF DENTIN IN CROSS SECTION

The microstructure of ivory in cross section has been studied several times before but only a limited amount of work has been done on Ag plated dentin in cross section. The influence of different etching agents and of the deposition following manner: the enamel on the cusp was ground away on a diamond disc, the tooth was cold mounted and then cut parallel to the cusp. Both sides of the tooth slices were polished c.. a 600 grit emery paper and then they were treated in the desired way. Only one slice, approximately 3 mm thick, per tooth was obtained.

Figure 8a shows untreated, polished dentin in cross section. Some of the tubuli openings seem filled (or clogged) with debris, mostly perhaps as a result of the polishing. The P:Ca ratio for untreated dentin is 1:2, obtained by x-ray energy dispersive analysis. Penetration of the solutions into tubuli was observed after dentin had been etched with H_3PO_4 and $AgNO_3$ had been applied for 1 min: P:Ag:Ca = 3:5:6, see Fig. 8b. The walls of the tubuli look amorphous and some of these walls are filled with an Ag containing deposit. From the x-ray energy dispersive analyses it is impossible to tell if the Ag is reduced by organic material in the dentin, or if it is a salt Ag_3PO_4, which could be the product of a reaction between $AgNO_3$ and hydroxy apatite (or residue of the H_3PO_4 etch).

The sample in Fig. 8C was etched 1 min. with 50% citric acid and $AgNO_3$ was applied for 1 min: P:Ag:Ca - 3:1:7. The fracture surface looks "cleaner" (an amorphous layer is not present), perhaps because citric acid is a much weaker etching agent than H_3PO_4. A comparison between the chemical composition of the samples in Fig. 8b and 8c shows that the H_3PO_4 etched sample contains more Ag and less Ca than the citric acid etched sample, which also has been the case with ivory, H_3PO_4 seems to leach out Ca.

The sample in Fig. 8d is etched with citric acid and plated with $AgNO_3$ and $FeSO_4$ containing 1 gpl cysteine, prereduction plus 9 repetitions. The metallic Ag layer on the surface is 5 μm thick and its structure is dense. Penetration of Ag into the substrate can be observed because at 15 μm depth, the P:Ag:Ca ratio is 3:1:6. The penetration has most likely taken place along the tubuli: a light deposit can be observed in the middle of the lower edge of Fig. 8d. This deposit is not analyzed but similar deposits in other samples have shown the presence of Ag. Any kind of layering of salt films, as observed in ivory, is not found in dentin cross sections.

The optimization of the plating conditions on dentin is not completed, but the results so far are very promising. It is a great advantage to be able to use the less harsh citric acid rather than H_3PO_4, and cysteine, a naturally occurring amino acid in the body, instead of thiourea. It is important that the chemicals used on dentin are non-toxic due to the fact that the risk of penetration into the blood stream and tissues is higher than from enamel.

The SnF_2 pretreatment still needs to be investigated and the optimum $AgNO_3$ concentration during the first repetitions may not be the same as later in the procedure.

There are many different brands of amalgam pellets on the market, with different chemical composition and grain size. The chemical composition may influence the adherence of the amalgam to the Ag plated tooth by forming different compounds at the Ag-amalgam interphase. Several chemically different (presence or absence of Sn, Cu, etc.) brands should be tried, because there may be an optimum composition.

Another area of interest is the use of organic sealants in combination with electroless plating of Ag, as well as organic fillings instead of amalgam fillings.

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TABLE I

INFLUENCE OF ETCHING AGENTS ON Ag DEPOSIT ON IVORY

Plating Conditions: 5 min. etch, 1 min. 200 gpl $AgNO_3$, pH 2.8, 1 min. saturated $FeSO_4$ + 1 gpl thiourea and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$. Four shear stress tests and two wear resistance tests were done on one sample.

	Resistance (Ω)		Wear Resistance	Shear Stress
	5X	10X	2 min/10 g	(psi)
42.5% H_3PO_4	5.6	0.35	3.0	831 ± 395
50% NaOCl	6.0	0.4	2.0	769 ± 233
50% Citric Acid	4.0	0.23	3.5	662 ± 456
20% EDTA	1.85	0.32	3.0	415 ± 219

TABLE II

INFLUENCE OF ETCHING AGENTS ON Ag DEPOSIT ON DENTIN

Plating Conditions: 1 min. etch, 1 min. 200 gpl $AgNO_3$ pH 2.75, 1 min. saturated $FeSO_4$ + 1 gpl thiourea and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$.

		Resistance (Ω)		Shear Stress (psi)	
	Tooth #	5X	10X		
42.5% H_3PO_4	I	0.6	0.086	162	550
	V	1.1	0.13	938	
42.5% H_3PO_4	II	0.36	0.11	997	1076
3 min. 50 gpl SnF_2 pH 2.6	III	0.3	0.09	1154	
50% NaOCl	III	4-100	0.26	377	226
	IV	40.000	3.5	0	
50% Citric acid	IV	2.8	0.18	423	541
	II	1.2	0.12	659	
20% EDTA	V	0.95	0.23	138	154
	I	11.6	0.21	181	

The Ag layer peeled off from all samples but the one treated with SnF_2.

TABLE III

Statistical screening test for pretreatment on ivory. Plating conditions: 1 min. 42.5% H_3PO_4 etch, SnF_2 pretreatment, 1 min. $AgNO_3$ pH 2.75, 1 min. $FeSO_4$ + 1 gpl thiourea pH 2 and 9 repetitions of $AgNO_3$-$FeSO_4$ + thiourea-$AgNO_3$.

Variables	Limits Low	Limits High	Center Point
x_1 = SnF_2 residence time	1 min.	3 min.	2 min.
x_2 = SnF_2 concentration	0.5 gpl	50 gpl	25 gpl
x_3 = SnF_2 pH	2	4	3
x_4 = $AgNO_3$ concentration	100 gpl	200 gpl	150 gpl
x_5 = $FeSO_4$ concentration	saturated-100 gpl	saturated	saturated-50 gpl
x_6 = dummy	--	--	--
x_7 = dummy	--	--	--

TABLE IV

SUMMARY OF STATISTICAL SCREENING TEST FOR PRETREATMENT ON IVORY

Responses		Resistance (Ω)		Wear Resistance, $\frac{2\text{ min}}{10\text{ g}}$		Shear Stress*	
Variables		Main Effect Estimate	Confidence Level (%)	Main Effect Estimate	Confidence Level (%)	Main Effect Estimate (psi)	Confidence Level (%)
x_1	SnF_2 residence time	-0.09	36	0.06	0	244	96
x_2	SnF_2 concentration	-0.25	71	1.13	50	53	48
x_3	SnF_2 pH	0.04	17	0.13	0	104	72
x_4	$AgNO_3$ concentration	0.06	25	0.38	18	162	86
x_5	$FeSO_4$ concentration	-0.25	71	1.13	50	65	55
x_6	Dummy	0.09	36	0.63	32	46	43
x_7	Dummy	-0.23	68	1.88	73	72	59

*Fine cut Caulk non-zinc alloy pellets were used, because fine cut Caulk alloy pellets were not available. The optimum trituration time for the new pellets was not known, resulting in a big variation from one shear test to the other.

TABLE V

Average shear stress and wear resistance values on ivory, enamel, and dentin. The samples are plated with 200 gpl $AgNO_3$, saturated $FeSO_4$ + 1 gpl additive (thiourea or cysteine), 10 repetitions.

	No Pretreatment		SnF_2 Pretreatment	
	Shear Stress (psi)	Wear Resistance 10 g/2 min.	Shear Stress (psi)	Wear Resistance 10 g/2 min.
Ivory	(18)* 437 ± 171	(12) 3.6 ± 1.8	(33) 746 ± 259	(20) 6.0 ± 2.5
Enamel	-	(6) 4.6 ± 1.0	_	(6) 6.0 ± 2.0
Dentin	(6) 546 ± 426	-	(6) 1076 ± 266	-

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* Numbers in parentheses are the approximate number of tests.

TABLE VI

Preoptimization of cysteine as an additive in $FeSO_4$. Plating conditions: 1 min. 42.5% H_3PO_4, 2 min. 50 gpl SnF_2, 1 min. $AgNO_3$ pH 2.75, 1 min. saturated $FeSO_4$ + cysteine pH 2 and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$. Substrate: ivory.

	Resistance (Ω) 5X	Resistance (Ω) 10X	Wear Resistance 2 min/10 g (2 tests)	Shear Stress* (psi), 4 tests
200 gpl $AgNO_3$ $FeSO_4$ + 1 gpl thiourea	0.9	0.025	8.5	569 ± 478
200 gpl $AgNO_3$ $FeSO_4$ + 1 gpl cysteine	1.9	0.96	5	719 ± 378
100 gpl $AgNO_3$ $FeSO_4$ + 0.2 gpl cysteine	0.9	0.19	6	656 ± 341
100 gpl $AgNO_3$ $FeSO_4$ + 1 gpl cysteine	3.6	1.7	0	746 ± 160
200 gpl $AgNO_3$ $FeSO_4$ + 0.2 gpl cysteine	0.5	0.25	2	514 ± 414

*Fine cut Caulk non-zinc alloy pellets were used, because fine cut Caulk alloy pellets that were used before were not available. The optimum trituration time for the new pellets was not known.

TABLE VII

The samples were treated in the following manner: 1 min. 42.5% H_3PO_4 or 1 min. 50% citric acid, 3 min 50 gpl SnF_2 or no. 1 min. 200 gpl $AgNO_3$ pH 2.85, 1 min. $FeSO_4$ + 1 gpl thiourea or 1 gpl cysteine. 9 repetitions of $AgNO_3$ - $FeSO_4$ + additive - $AgNO_3$. Numbers in parentheses are the amounts of tests.

	Shear stress (psi) Dentin	Wear resistance Ivory	Wear resistance Enamel*
H_3PO_4 etch	728 ± 421 (13)	5.58 ± 2.57 (12)	5.08 ± 2.39 (12)
Citric acid etch	1080 ± 467 (15)	7.00 ± 3.28 (12)	0 ± 0 (12)
SnF_2 pretreatment	1003 ± 380 (12)	8.42 ± 0.90 (12)	6.00 ± 1.26 (6)
no pretreatment	852 ± 536 (16)	4.17 ± 2.79 (12)	4.17 ± 2.99 (6)
Thiourea additive	789 ± 351 (11)	6.67 ± 2.74 (12)	5.83 ± 2.86 (6)
cysteine additive	1105 ± 601 (11)	5.92 ± 3.26 (12)	4.33 ± 1.75 (6)
SnF_2 - Thiourea †	951 ± 312 (6)	8.00 ± 1.10 (6)	7.00 ± 0 (3)
SnF_2 - Cysteine	1055 ± 463 (6)	8.83 ± 0.41 (6)	5.00 ± 1.00 (3)

[*] The enamel samples etched with citric acid are excluded from the average numbers in all cases but the comparison between H_3PO_4 and citric acid

[†] The samples were preheated with SnF_2 and the $FeSO_4$ solution contained either thiourea or cysteine.

Note: Samples were aged in H_2O at 37°C for 24 h.

TABLE VIII

The influence of fluoride in the Ag solution on shear stress values, when thiourea or cysteine are used as additives. The dentin was treated in the following manner: 1 min 50% citric acid, 1 min Ag solution, 1 min $FeSO_4$ + 0.5 gpl thiourea or 1 gpl cysteine and 9 repetitions of Ag^+ - $FeSO_4$ + additive Ag^+. The numbers in parentheses indicate the amount of tests.

	Shear stress (psi)	
	0.5 gpl thiourea	1 gpl cysteine
200 gpl $AgNo_3$ pH 2.8	629 ± 371 (9)	728 ± 423 (9)
200 gpl $AgNo_3$ + 50 gpl NaF pH 5.0	1058 ± 461 (9)	1320 ± 678 (9)
100 gpl AgF pH 5.0	1039 ± 447 (9)	1512 ± 610 (9)

Note: Samples were aged in H_2O at 37°C for 24 h.

TABLE IX

The variables and limits for a screening test designed for optimization of plating conditions on dentin.

	Variable	Lower limit	Higher limit	Center point
X_1	Etching time (min)	0.5	1.5	1.0
X_2	SnF concentration (gpl)	1	50	25
X_3	pH of $AgNO_3$	2.5	3.5	3.0
X_4	Cysteine concentration (gpl)	0.5	1.5	1.0
X_5	pH of $FeSO_4$	2.0	2.8	2.4
X_6	Dummy	-	-	-
X_7	Dummy	-	-	-

Etching agent: 50% citric acid

$AgNO_3$ concentration: 200 gpl

Cysteine was added to saturated $FeSO_4$ solution

TABLE X

Summary of the statistical screening test made to optimize plating conditions.

	Variables	Resistance (Ω) main effect estimate	Confidence level (%)	Shear stress (psi)* Main effect estimate	Confidence level (%)
X_1	Etching time	-0.063	>99	302	62
X_2	SnF_2 concentration	-0.108	>99	82	22
X_3	$AgNO_3$ pH	0.083	>99	-91	26
X_4	Cysteine concentration	0.603	>99	39	10
X_5	$FeSO_4$ pH	0.098	>99	-127	32
X_6	Dummy	-0.005	48	-449	76
X_7	Dummy	0.008	66	88	16

*Fine cut caulk non-zinc alloy pellets. 10 sec. trituration time.

Note: Samples were aged in H_2O at 37°C for 24 h.

TABLE XI

Additive	Structural formula	Toxicity
Thiourea	H_2NCSNH_2	436 mg/kg intraperitoneal - rat LD 50*
Cysteine	$HSCH_2CH(NH_2)CO_2H$	no toxicity data found
Thioacetamide	CH_3CSNH_2	200 mg/kg oral - rat LDLo**
Thioureidopropionic acid	$NH_2CSNHCH_2CH_2CO_2H$	not found
Dithiocarbamylpropionic acid	$NH_2CSSCH_2CH_2CO_2H$	not found
Thiophene	C_4H_4S	100 mg/kg intraperitoneal - mouse LDLo
Thiosalicylic acid	$HSC_6H_4CO_2H$	50 mg/kg intraperitoneal - mouse LDLo
Thiolactic acid	$CH_3CH(SH)CO_2H$	50 mg/kg oral - rat LDLo
Mercaptomethylimidazole	$C_4H_6N_2S$	500 mg/kg intraperitoneal - rat LDLo
2,5-Dimercapto-1,3,4-thiadiazole	$C_2H_2N_2S_3$	500 mg/kg intraperitoneal - rat LDLo
2-Mercapto acetic acid	$HSCH_2CO_2H$	100 mg/kg intraperitoneal - mouse LDLo
3-Mercaptopropionic acid	$HSCH_2CH_2CO_2H$	10 mg/kg intraperitoneal - mouse LDLo
2-Mercaptosuccinic acid	$HO_2CCH_2CH(SH)CO_2H$	800 mg/kg oral - rat LD50
2-Mercaptoethanol	$HSCH_2CH_2OH$	200 mg/kg intraperitoneal - mouse LDLo

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LD 50 - Lethal dose 50: At this given concentration 50% of the test animals died.
LDLo - Lowest published lethal dose.

TABLE XII

Ivory was treated: 1 min 42.5% H_3PO_4, 1 min 200 gpl $AgNO_3$ pH 2.85, 1 min $FeSO_4$ + 8 gpl additive and 9 repetitions of $AgNO_3$ - $FeSO_4$ + additive - $AgNO_3$.

Additive	Resistance 10X (Ω)			Wear resistance 2 min/10 g		
	1.0 gpl	0.1 gpl	0.01 gpl	1.0 gpl	0.1 gpl	0.01 gpl
Thiourea	0.21	0.10	0.47	7	6	5
Thioacetamide	0.23	1.4	1.0	6	1	4
Thioureidopropionic acid	2.8-1.75	0.25	0.52	1	8	4
Dithiocarbamylpropionic acid	1.2	0.29	0.4	1	6	7
Thiophene	$200x10^3$	14-40	0.5	1	2	8
Thiosalicylic acid	$2x10^6$	400-35000	50	0	1	1
Thiolactic acid	$1x10^6$	12	0.16	0	1	7
Mercaptomethylimidazole	1.4	9	1.44	0	1	3
Dimercaptothiadiazole	1.2	1.0	1.2	1	2	4
Mercaptoacetic acid	1500	0.4	3.2	1	3	3
Mercaptosuccinic acid	$1x10^6$	$2x10^6$	$10x10^6$	1	0	1
Mercaptopropionic acid	$0.6x10^6$	1.45	0.41	1	4	3
Mercaptoethanol	0.14	0.17	1.0	7	8	3
No additive		1.0			4	

TABLE XIII

The dentin samples were prepared in the following manner: 1 min 50% citric acid, 1 min 200 gpl $AgNO_3$ pH 2.85, 1 min. saturated $FeSO_4$ + Xgpl additive, 9 repetitions of $AgNO_3$ - $FeSO_4$ + additive - $AgNO_3$.

Additive	Resistance 10 (Ω)	Shear stress (psi)
1.0 gpl Cysteine	0.39	579 ± 202
1.0 gpl Thioacetamide	0.17	472 ± 469
0.1 gpl Thioureidopropionic acid	0.15	562 ± 196
0.1 gpl Dithiocarbamylpropionic acid	0.20	654 ± 550
0.01 gpl Thiophene	0.16	0
0.01 gpl Thiolactic acid	0.52	295 ± 363
0.01 gpl Mercaptomethylimidazole	0.10	631 ± 317
0.01 gpl 2,5-Dimercapto-1,3,4-thiadiazole	0.55	1144 ± 754
0.1 gpl Mercaptoacetic acid	0.65	410 ± 300
0.1 gpl Mercaptopropionic acid	0.24	759 ± 477
0.1 ml/l Mercaptoethanol	0.20	369 ± 488

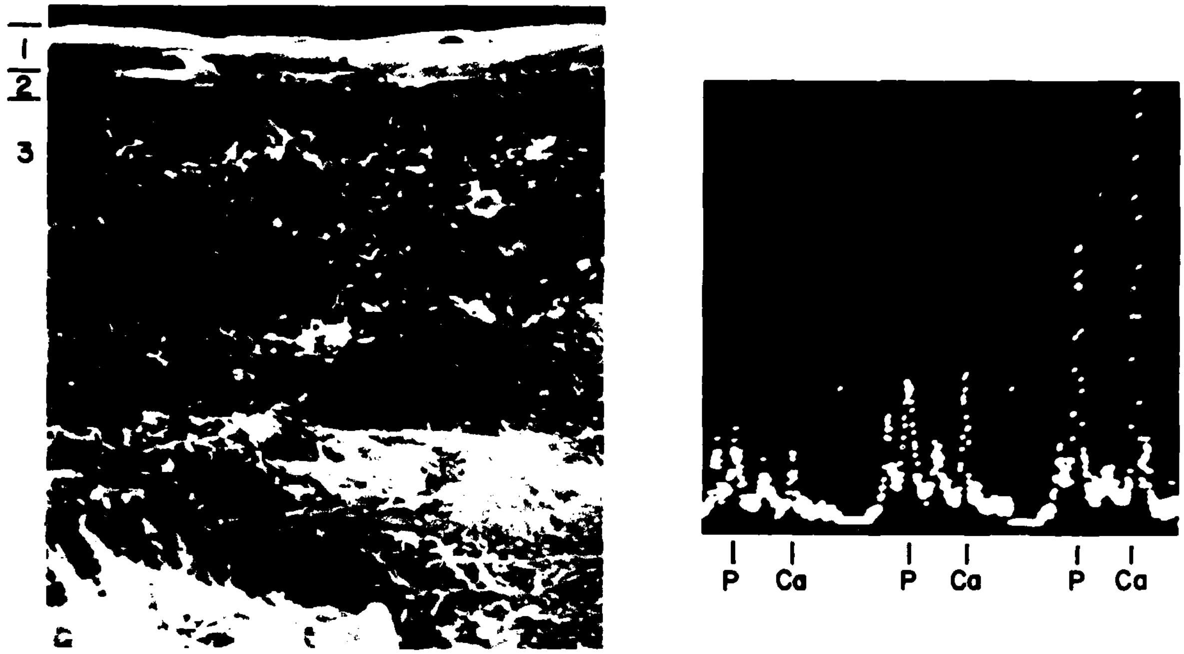

Figure 1. a) Cross section of ivory etched 1 min. with 42.5% H_3PO_4. Magnification 3000x. The layers are marked with arabic numbers and the atomic composition of each layer is presented in Figure 1b.

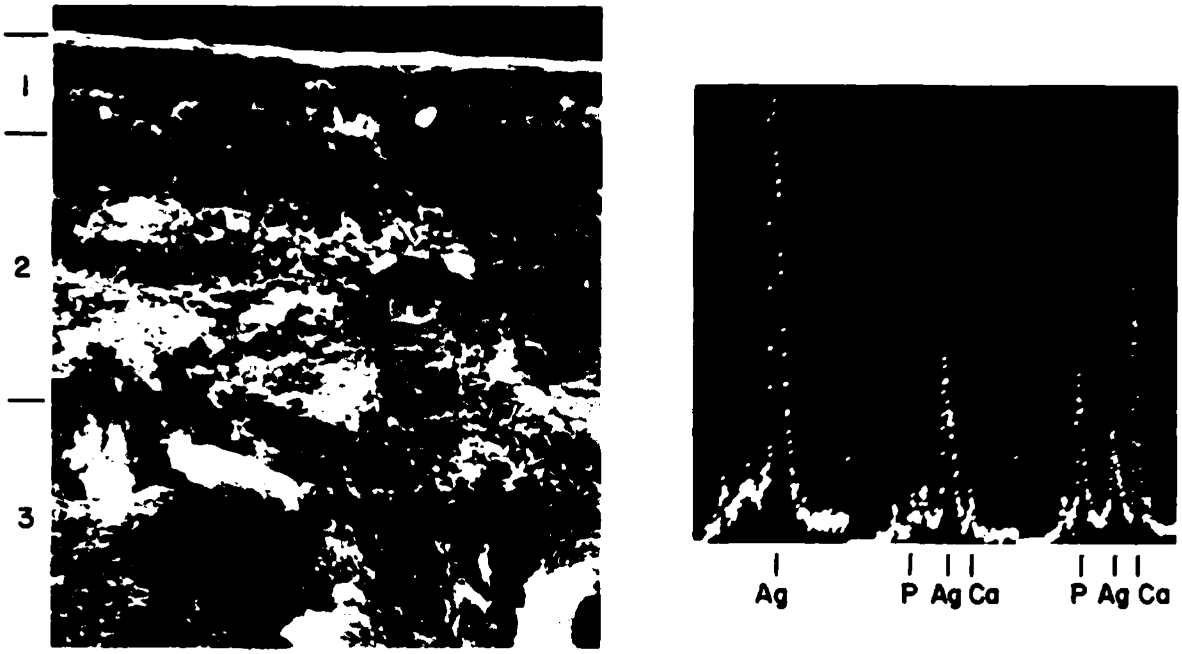

Figure 2. a) Cross section of ivory etched 1 min. with 42.4% H_3PO_4 followed by prereduction and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$. Magnification 3000x. The layers are marked with arabic numbers and the composition of each layer is presented in Figure 2b.

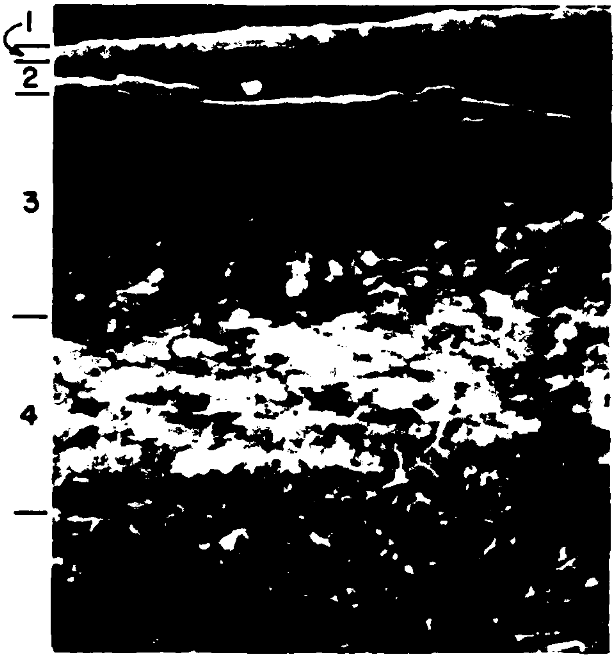

Figure 3. Cross section of ivory etched 20 min. with 42.5% H_3PO_4 followed by prereduction. The atomic composition of the layers is: layer 1, P:Ag:Ca = 1:3:1; layer 2, P:Ag:Ca = 1:4:1; layer 3, P:Ag:Ca = 1:2:1; and layer 4, P:Ag:Ca = 1:5:1. Magnification 3000x.

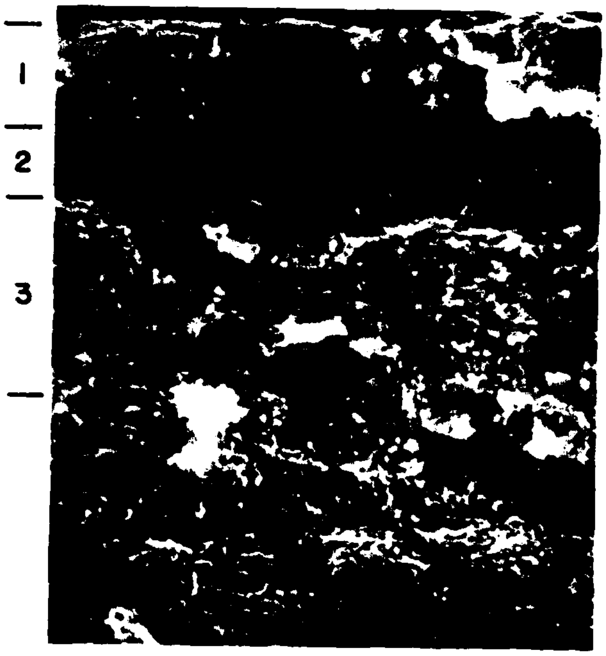

Figure 4. Cross section of ivory etched 1 min. with 50% citric acid followed by prereduction and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$. The atomic composition of the layers is: layer 1, Ag only; layer 2, P:Ag:Ca = 1:5:1, layer 3, P:Ag:Ca = 1:5:0. Magnification 3000x.

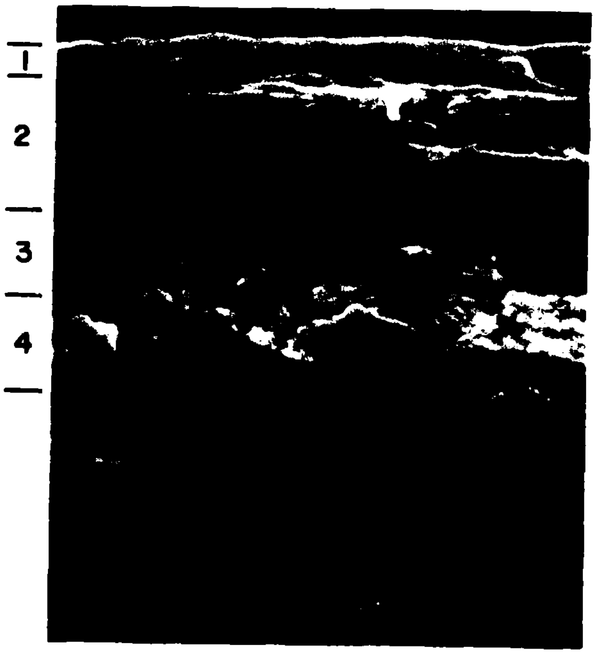

Figure 5. Cross section of ivory etched 20 min. with 50% citric acid followed by prereduction. The atomic composition of the layers is: layer 1, P:Ag:Ca = 1:1:0; layer 2, P:Ag:Ca = 1:3:0; layer 3, P:Ag:Ca = 1:4:0; and layer 4, P:Ag:Ca = 1:5:0. Magnification 3000x.

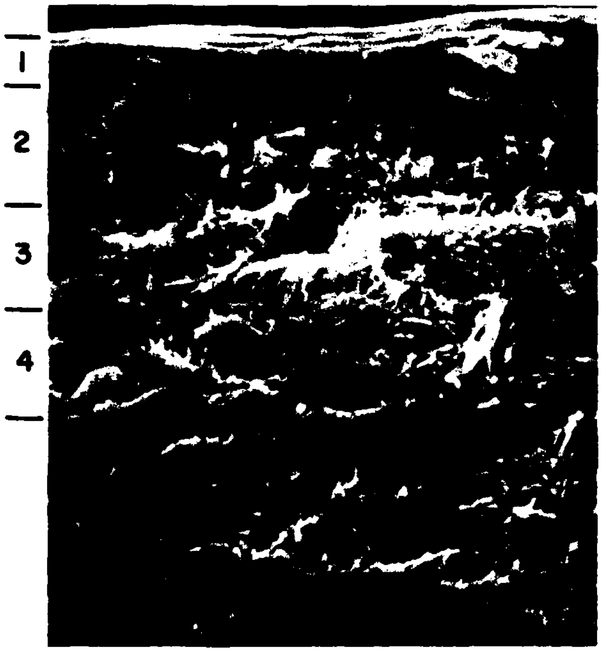

Figure 6. Cross section of ivory etched 1 min. with 20% EDTA followed by prereduction and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$. The atomic composition of the layers is: layer 1, Ag only; layer 2, P:Ag:Ca = 3:10:1; and layer 3, P:Ag:Ca = 1:1:1. Magnification 3000x.

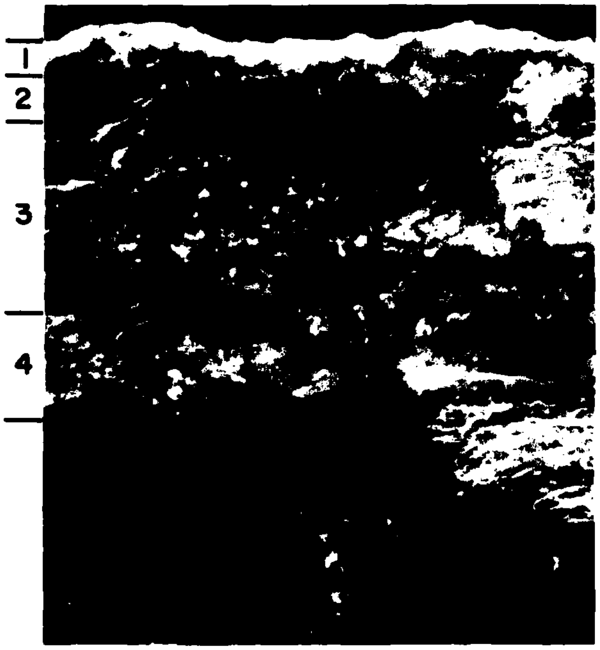

Figure 7. Cross section of ivory etched 1 min. with 50% NaOCl followed by prereduction and 9 repetitions of $AgNO_3$-$FeSO_4$-$AgNO_3$. The atomic composition of the layers is: layer 1, Ag only; layer 2, P:Ag:Ca = 2:6:1; layer 3, P:Ag:Ca = 1:5:1; and layer 4, P:Ag:Ca = 1:3:1. Magnification 3000x.

Figure 8a. Crossection of dentin, ground on diamond wheel and polished on 600 grit emery paper, no chemical treatment, P:Ca ratio is 1:2. Magnification 3000X.

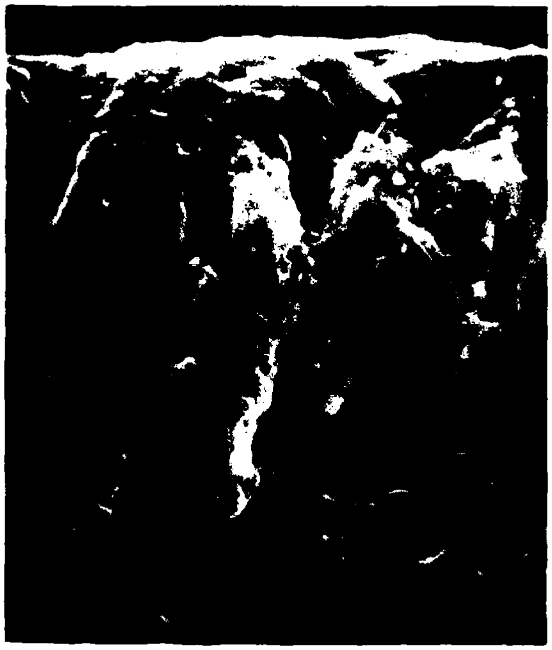

Figure 8b. Crossection of dentin, ground, polished and chemically treated 1 min with 42.5% H_3PO_4 and 1 min with 200g pl $AgNO_3$. Magnification 3000X.

Figure 8c. Crossection of dentin, ground, polished and chemically treated 1 min with 50% citric acid and 1 min with 200 gpl $AgNO_3$. Magnification 3000X.

Figure 8d. Crossection of dentin, ground, polished and chemically treated 1 min with 50% citric acid, 1 min with 200 gpl $AgNO_3$, 1 min with $FeSO_4$ + 1 gpl cysteine plus 9 repetitions of $AgNO_3$ - $FeSO_4$ + cysteine - $AgNO_3$. Magnification 3000X.

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