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A REVIEW OF RECENT STUDIES OF IN VITRO REACTIVITY OF SURFACES OF CALCIFIED TISSUES

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A REVIEW OF RECENT STUDIES OF IN VITRO REACTIVITY OF SURFACES OF CALCIFIED TISSUES

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Much scientific data have been accumulated on the chemical organization of the organic matrix and the chemistry of the mineral phase of enamel, dentin and bone.(1-7) Relatively little information is available, however, regarding the chemical and physical characteristics of the surfaces of the components of tooth structure. Furthermore, *in vitro* techniques to modify calcified tissue surfaces under an environment approximating conditions that are likely to be encountered clinically have received only scant attention. Such studies are of utmost importance not only in gaining more basic knowledge, but also in the solution of a wide spectrum of clinical problems such as improved anti-cariogenic agents, remineralization, speedier wound healing techniques, more compatible implants and the development of efficient medical and dental tissue adhesives and biodegradable sutures.

The effectiveness of potential surface conditioning agents can be demonstrated by changes in the surface properties of the substrate. Physico-chemical methods to detect these surface modifications include scanning electron microscopy, infrared spectroscopy using multiple internal reflectance techniques, as well as measurements of (1) the effective surface area, (2) adsorption isotherms, (3) heat of immersion and heat of reaction, (4) contact angle and critical surface tension, (5) oil wettability under water, and (6) adhesionability.

The following discussion emphasizes the efforts made in various laboratories to modify tooth surfaces to obtain an adhesive bond between the restorative material and tooth structure so that the interface between the restoration and the tooth would support a mechanical load in addition to preventing the ingress of oral fluids. Potential adhesive systems are summarized in Table 1 and include both application of surface conditioning agents and various polymeric materials cured in situ. The evaluation of the resinous materials as adhesives will be discussed by other participants in this symposium. The usefulness of inorganic polymers and bioadhesives (such as barnacle cement) for clinical applications has not been demonstrated.

ADSORPTION

1. From the Gaseous Phase

Adsorption of a gas on tooth surface presents the simplest example of adhesion. Studies of the interaction of gases by Beebe and coworkers have provided considerable insight into the details of the surfaces and structures of hard tissues (8,9). Dry and Beebe have demonstrated the strong affinity of hydroxyapatite surfaces for water and the dependence of apparent surface areas on water content (9). A significant decrease in specific surface area (determined from gas adsorption measurements by the BET method) occurred when chemisorbed layers of water blocked the very narrow pores or grain boundaries of the substrate and thus effectively reduced the area accessible to nitrogen adsorption. From adsorption isotherms and calorimetric heats of adsorption of methanol on mineral surfaces, it was possible to separate the total adsorption into chemisorbed and physisorbed fractions. In the case of water, such a separation was not as clearly delineated. Relatively high heats of adsorption in the first monolayer for both methanol and water indicate a high energy of binding by hydroxyapatite and may be attributed to hydrogen bond formation involving exposed oxygen atoms at the surface.

The structures and surface properties of bone and tooth mineral have also been studied by Loebenstein, utilizing the low temperature nitrogen adsorption BET technique(10). Surface areas of powdered dentin ranged between 6.8 and 11.1 m^2/gm, while powdered and defatted bovine femur shaft revealed a surface area of only 1.47 m^2/gm. Samples of dentin and bone rendered anorganic by treatment with ethylene diamine presented specific surfaces of 130 and 114 m^2/gm, respectively. This increase in surface area was attributed to the removal of dried collagen that denied access of nitrogen molecules to the micropores of dentin and bone. The low surface area of defatted bone (1.47 m^2/gm) supports the concept of pore blockage by collagenous material.

Loebenstein developed a simple apparatus for obtaining, gravimetrically, water vapor adsorption measurements at ambient temperatures(10a). For components of teeth, typical Type II BET isotherms resulted which could be used to calculate surface areas available to water. Well-developed hysteresis loops extending over the entire range of humidities were found on comparison of the adsorption and desorption isotherms.

Results of pore size experiments indicate that some of the detailed pore-volume profile patterns for anorganic bone are shared by both hydroxyapatite and octacalcium phosphate, while others are more closely aligned with hydroxyapatite(10). The relative amplitudes of peaks and troughs for the mineral component of bone and teeth, as well as their location, along the pore diameter axis, were found to be dependent, not upon their organic matrix, but rather upon their crystallographic makeup.

To provide information regarding the mechanism and chemistry of surface forces by which tooth structure and adhesive interact, Loebenstein studied the adsorption of carbon dioxide and ammonia on tooth components(11). These two reactive gases were chosen because they should indicate the presence of acidic and basic groups, respectively, as possible bonding sites for an adhesive. The extent of the surface area available after preconditioning the solid substrate was compared for each of these gases, as well as for nitrogen. Presence of collagen in dentin exerted a profound influence on the BET area depending on the gas used.

By making measurements near the boiling point of the adsorbate, but at different temperatures, the isosteric heat of adsorption (the heat liberated per constant number of moles of gas adsorbed per gram of adsorbant) was determined. From the magnitude of the heat of adsorption, the intensity of the reaction between adsorbate and adsorbent can be estimated. The isosteric heats of adsorption of carbon dioxide and ammonia to about two statistical layers on anorganic whole tooth powders and dentin powders fell within the van der Waals physical adsorption range; that is, below 12 kcal, although in most instances they were greater than for hydrogen bonding. The isosteric heat fell off rapidly until a monolayer was formed and at higher coverage generally did not change appreciably. This work demonstrates the use of adsorption and area of coverage measurements as criteria for the selection of chemically reactive groups for incorporation in potential adhesives. Extrapolation of these findings would suggest that amino groups incorporated into a dental adhesive may provide better bonding than carboxylic acid groups.

2. From Solution

Studies of adsorption on hydroxyapatite and tooth surfaces from solution are required to determine quantitatively the effectiveness of tooth conditioning procedures and to ascertain the effect of application variables such as concentration of conditioner in the solvent, exposure time to the solution, and rinsing time. Furthermore, adsorption studies may assist in the disclosure of those materials that would serve most effectively as surface conditioners.

A number of investigators have studied adsorption from solution to tooth surfaces and hydroxyapatite in order to determine the nature and magnitude of the interfacial chemical interactions occurring between teeth and selected model organic compounds. In addition to the complex structural characteristics of enamel and dentin, the deposition and smearing of organic debris accompanying operative cutting and grinding procedures render hard tooth tissues poor substrates for such studies.

Bertolucci and Chamberlain studied interfacial reactions of synthetic and natural hydroxyapatites, as well as other calcium phosphates, using infrared attenuated total reflection (ATR) techniques(12). The organic adsorbates were applied to the finely divided solids from aqueous and non-aqueous solutions. The infrared spectra were recorded before and after adsorption. Shifts in the infrared absorption bands are indicative of the nature and degree of interaction between apatite and the organic compounds. Adsorption of citric acid and oxytetracycline (as the HCl salt) on synthetic hydroxyapatite produced shifts of between 5 to 9 cm^{-1} in the asymmetric $P{-}O^-$ stretching frequency in the vicinity of 1027 cm^{-1}. Similar shifts were not observed on exposure of commercially available hydroxyapatites, natural apatite, fluorapatite or calcium phosphate to these adsorbates. From a fundamental point of view, it is likely that complete interpretation of infrared spectra should give absolute information about the interaction with the surface and therefore of the wetting. The technique is limited at the present time mainly because it has not been investigated in detail.

The adsorption isotherms for water on bovine tooth powder and on synthetic hydroxyapatite have been determined gravimetrically by Pitt(13). Although the amount of water absorbed by the hydroxyapatite was apparently not considered, it appears that a relative humidity of about 25% is necessary to adsorb a monolayer of water on bovine tooth powder. Based on the values given in the literature(14) for the adsorption of moisture on collagen (kangaroo tail tendon), the quantity of water sorbed on tooth powder is somewhat lower than that computed on the assumption that dentin is comprised of 80% hydroxyapatite and 20% collagen by weight. This discrepancy may be the result of the partial inaccessibility of the collagen caused by its bonding to the mineral component of tooth structure. Citric acid treatment of tooth surfaces produces little change in the isotherm. Treatment of bovine tooth powder with aqueous silver nitrate decreases the total amount of moisture that is reversibly adsorbed. At high relative humidity, where multilayer adsorption is likely to occur, this treatment reduces the uptake of water to half that of untreated tooth powder. Assuming that the mineral portion of the composite was not significantly affected by the aqueous silver nitrate, adsorption of moisture on the protein must have been greatly reduced.

Schwartz and Galligan studied the equilibrium adsorption of aqueous solutions of linoleic acid and sorbic acid on hydroxyapatite having a particle size of 2 to 16 μm(15). The amount of conditioner that could be removed on exposure of the apatite substrate to fresh solvent was also determined. Adsorbed linoleic or sorbic acid could not be removed by the solvent. This behavior, as well as similarities in the adsorption isotherms at 20°C and 37°C, are characteristic of chemical adsorption or reaction rather than physical adsorption.

Patrick et al determined the adsorption isotherms of a commercial hydroxyapatite with a series of polymers dissolved in methylene chloride(16,17). A typical rubbery interliner that interacts with the physiologic substrate as well as the resin, and thus should provide a zone that would absorb stresses, was selected. The greatly increased adsorptivity, and thus the strongest interaction with the hydroxyapatite, of this partially saponified acrylonitrile-butyl acrylate copolymer as compared to the other copolymers studied was clearly indicated from the isotherms obtained. Furthermore, the adsorption isotherms for this polymer were nearly the same when human or bovine dentin or enamel were used as substrates.

Ericson and Ericson(18) reported that protein adsorption was less on fluoride-containing hydroxyapatite than on fluoride-free hydroxyapatite. Glantz and Nyquist assume that fluoride ions cause a reduction in the free surface energy of hydroxyapatite crystals immediately after topical application of fluorides(19). Mannerberg hypothesized that if the free surface energy of enamel is decreased by fluorides, the possibility for adhesion of an organic pellicle or plaque may also be decreased(20).

HEATS OF IMMERSION

The forces of attraction can vary greatly in the case of two different substances spread on the same solid even if the wetting angle is zero in both cases.(21) Greatest adhesion occurs when chemical bonds are formed and the wetting is truly complete. Enthalpies of adhesion give a direct measure of the forces of interaction and hence the "wetting" irrespective of the contact angle. Furthermore, it has been pointed out by Zettlemoyer that a study of heats of immersion will indicate if adhesion between two contacting surfaces will be maintained or disrupted by water or saliva(21). The measurement and evaluation of these heats of immersion and heats of reaction therefore facilitates the study of any modification of tooth structure necessary to achieve a chemical bond and aids in the determination of those groups that will bond effectively to the components of tooth structure in an aqueous environment.

Calorimetric measurements of the heats of immersion of well-characterized powders of the components of human tooth structure have been used by the author and coworkers to assess the nature and degree of modification of well-characterized tooth surface produced by the presence of various ions and functional groups in an aqueous environment(22-24a).

The specific surfaces (BET) of the dentin, enamel and anorganic whole tooth powders used in these experiments were obtained from nitrogen adsorption measurements and were 10.5, 3.9, and 75 m^2/gm, respectively.

Comparison of the apparent heats of immersion values ($-\Delta H_w$) of dry tooth powders (Table 2) illustrates the reactive nature of the organic constituents when present in tooth substance(23). The specific surface of dry anorganic whole tooth tissue was approximately 7.2 times that of dry dentin. The apparent heat of immersion value for dentin, however, was higher than that of anorganic whole tooth tissue by a factor of 4.9 per gram of substrate, and by a factor of 35 on a unit area basis. The magnitude of the value obtained for powdered dentin suggests that more than simple wetting occurs when this substance is immersed in water.

Heats of wetting values of +37 cal/gm have been reported by Kanagy(25) for purified bovine corium collagen. Since the $-\Delta H_w$ values for anorganic whole tooth are low (2.06 cal/gm), it is likely that the unexpectedly large $-\Delta H_w$ values obtained for dentin were caused by the presence of collagenous material at the surface of the powder particles.

The apparent $-\Delta H_w$ values obtained for dentin greatly exceeded the $-\Delta H_w$ values for inert solids (0.007 to 0.15 cal/m^2) previously reported by other investigators for other substances(26). Therefore, the collagenous component of dentin binds water through hydrophilic groups and may further absorb, dissolve or react upon contact with water. The observed $-\Delta H_w$ values for anorganic whole tooth, however, were found to be within this range.

The observed heats of immersion of odontic powders with solutions containing disodium EDTA, in contrast to the observed heats of immersion of these powders with ammonium citrate and methacrylic acid, are more dependent upon the calcium available for chelation than upon the presence of organic matter (Tables 3-5). Examination of the apparent heats of immersion values on a unit area basis reveals that the reactions of disodium EDTA with odontic powders appear to be independent of available surface area.

The presence of NO_3^-, NO_2^-, CNS^-, $SO_4^=$, and I^- at pH 5 lowered the heat of immersion of dentin. Other anions (F^- and Cl^-) had no effect. At pH 8, however, anions did not alter the heat of immersion values of dentin (Table 6).

The depressed $-\Delta H_w$ values observed for powdered dentin upon immersion in dilute aqueous solutions containing certain anions at pH 5 suggest the direct endothermic interaction of these anions with $R\text{-}NH_3^+$ groups. An alternative mechanism may involve the preferential binding of these anions to positively charged sites on the dentinal collagen at the expense of water. Chloride and fluoride ions apparently possess a low affinity for dentinal collagen.

In vitro studies have shown a decrease in the acid solubility of tooth structure after treatment with dilute fluoride solutions(27). If an enamel or dentin surface can be modified in such a way that it will resist acid attack, it is reasonable to assume that the substance producing the modification must first react with the surface of tooth. Values observed for the heats of immersion of dentin (23.4 cal/gm), enamel (3.0 cal/gm) and anorganic whole tooth (1.09 cal/gm) with 8% stannous fluoride solution indicate that this reagent reacts mainly with the organic matter of the powdered samples of these tissues:(23) The immersion of dentin in dilute aqueous solutions of sodium fluoride yielded values similar to those observed upon the immersion of dentin in water. The marked change in the heat of immersion value obtained when dentin was immersed in stannous fluoride solution was therefore probably dependent on the presence of stannous ions.

The addition of aldehyde groups to the liquid adsorbate at pH 8 resulted in the lowering of the heat of immersion of dentin, although addition of aldehyde groups to the aqueous phase at pH 5 had only a slight effect upon the observed $-\Delta H_w$ of dentin and anorganic whole tooth powders.

Reductions in the $-\Delta H_w$ values of dentin in aqueous phenol and urea from those obtained in water suggest that these compounds react either endothermically with the dentinal protein or are bound preferentially to charged sites at the expense of water(24a). Exposure of dentin to dilute hydrogen peroxide yielded values similar to those obtained on immersion of this powder in water. The 2% aqueous potassium permanganate, a stronger oxidizing agent than 3% hydrogen peroxide solution, reacted exothermically with dentin. /This reaction is probably an exhaustive process that results in destruction of the organic matrix. Dentin also reacted exothermically with silver nitrate. The reactions, however, were erratic and prolonged and possibly were caused by a reduction of the silver nitrate at the surface. Precise $-\Delta H_w$ measurements, therefore, could not be made. For anorganic whole tooth, the apparent heat of immersion in water did not differ appreciably from the values obtained on exposure to the aqueous solutions investigated.

Thus, the organic matrix of dentin presents active sites at which bonding may occur with certain ions and functional groups under proper environmental conditions. Furthermore, the reactions which occur at the surface of dentin are similar to those exhibited by purified collagen.

The surface of dentin may be modified by treatment with nonpolar solvents(24). A decrease in the heat of immersion value of dentin also occurs on immersion in 2-40% aqueous solutions of organic solvents (ethanol, acetone, butanol, ethylene glycol), suggesting strengthening of electrostatic bonds between polar groups of the collagenous matrix of dentin and also reflects the presence of a coating of collagenous material at the surfaces of the powder particles. In absolute ethanol and hexane, dentin absorbs heat, indicating the weakening of hydrophobic bonds. Values for anorganic whole tooth in aqueous solutions of organic solvents, however, are not appreciably different from those obtained in water.

A precise explanation for the decrease in the $-\Delta H_w$ values of dentin as the solvent medium becomes more organic in nature cannot be offered. This behavior may be attributed to the strengthening of electrostatic linkages of the dentinal collagen which, in turn, could result in (1) the reduction of the number of sites accessible to water, or (2) a slight decrease in the surface area of the specimen through tightening of the structure of collagen. An endothermic interaction of the substrate with compounds such as butanol or ethylene glycol could also be a possible cause. The apparent absorption of heat which was observed when dentin was immersed in absolute ethanol and hexane could also be the result of the endothermicity of the transfer of the non-polar side chains of the collagenous matrix of dentin from the aqueous environment provided by its residual water content to an overwhelmingly hydrophobic environment(28).

Water-vapor-saturated samples of dentin powders more closely approximate the native or physiologic state of intact dentin. Therefore, expanded investigations of the heats of reaction of water-vapor-saturated tooth powders would be appropriate from a clinical point of view.

Pitt has shown that the integral heats of immersion as a function of moisture sorption are similar for both untreated bovine tooth powder and citric acid treated powder(13). The values for hydroxyapatite are considerably lower. Silver nitrate treated powder is intermediate at low amounts of presorbed moisture. Silver nitrate treatment reduces the immersion energy by a factor of two with some values approximating those of hydroxyapatite, indicating the formation of a significantly altered surface by this treatment. Analysis of the energy of immersion of bovine tooth powder indicates a significant concentration of adsorption sites having an energy in the region of 3 kcal per mole of water adsorbed.

WETTABILITY

Formation of a strong adhesive joint requires: (1) good wetting, (2) solidification of the liquid adhesive, and (3) sufficient deformability to reduce the build-up of elastic stresses in the formation of the joint(29). The wetting of biological surfaces has been reviewed by Zisman et al(30), Baier et al(31,31a) and Huntsberger(32).

Intimate interfacial contact between the adhesive and its substrate is the most important requirement for good adhesive performance. When the maximum possible number of interfacial "contacts" is achieved between a given adhesive-substrate pair, the adhesive completely wets the substrate. Electron photomicrographs of dentin and enamel show that their cut surfaces exhibit roughness with grooves of the order of a few hundred angstroms(33). This roughness is important in determining the extent of wetting. A finite time interval may be required to achieve complete wetting of the surface.

Young provided the first good description of wetting and spreading of a liquid on a solid, as well as the relation of these characteristics to the equilibrium contact angle(34). This relationship is expressed by the equation: $\gamma_{sv}o - \gamma_{sl} = \gamma_{lv}o \cos \theta$, where $\gamma_{sv}o$, γ_{sl} and $\gamma_{lv}o$ are the surface tensions at the solid-vapor, solid-liquid and liquid-vapor interface at equilibrium, respectively. The contact angle θ is the angle between solid-liquid and liquid-vapor interface. When $\theta = 0$, the liquid spreads over the surface of the solid spontaneously. On a sufficiently smooth and homogeneous solid, θ is independent of the volume of the liquid drop. Since the tendency for a given mass of liquid to spread and adhere increases as θ decreases, the contact angle is a useful inverse measure of wettability, whereas $\cos \theta$ is an obvious direct measure.

Contact angles above 0° occur primarily between liquids and low energy surfaces. For this reason, contact angle measurements do not provide differentiation for high energy surfaces. Under normal conditions, a high energy surface seldom retains its hydrophilic properties. Such a surface, because of its high free surface energy will attract and be covered by low energy substances.

If the adhesive behaves as a Newtonian liquid, the wetting rates will be directly related to the product of the liquid-vapor interfacial tension and the cosine of the advancing contact angle. Wetting rates will also be inversely proportional to the viscosities of the adhesives. Huntsberger showed a reasonable correlation between the values of $\gamma_{lv}o \cos \theta_{adv.}$ and adhesive performance(32). His data indicate that good adhesive performance can be attained with adhesives that exhibit relatively large contact angles.

The difficulty in measuring accurately small contact angles was offset by Zisman's concept of critical surface tension γ_c, the maximum surface tension of a liquid which will form a zero contact angle. Provided the molecules in the solid surface do not form hydrogen bonds or otherwise associate strongly with the liquid, a rectilinear relationship was shown to exist between the cosine of the contact angle and the surface tensions of a homologous series of organic liquids(30,35,36). The critical surface tension γ_c for wetting was defined as the intercept of the horizontal line where cos θ is equal to one with the extrapolated straight-line plot of cos θ against $\gamma_{lv}o$. Even when cos θ is plotted against $\gamma_{lv}o$ for a variety of non-homogeneous liquids, the plotted points fall close to a straight line or collect around it in a narrow rectilinear band. Although this intercept is less precisely defined than the critical surface tensions of a homologous series of liquids, it is a more useful parameter because it is solely a characteristic of the solid and a good, but not complete, criterion for predicting adhesive bonding. The γ_c values are also useful for ranking the surface energies of solids. Completely fluorinated polymers have the lowest γ_c values. Values of γ_c for a series of hydroxyl-rich surfaces ranged from 40 to 45 dyne/cm, and Nylon 66, with its many exposed amide groups, yielded the highest γ_c value (46 dyne/cm)(31).

To obtain strong adhesion, it is advantageous to use an adhesive with a surface tension slightly less than the critical surface tension of the adherent. Thus, a value for γ_c of 30 dyne/cm would permit the organic liquids used in most common liquid adhesive compositions to spread readily over the surface without void formation or excessive trapping of air bubbles.

Because of the highly localized nature of the forces between each solid surface and the molecules of the organic liquid, and also between the molecules of each liquid, a monolayer of adsorbed molecules is usually sufficient to give the high energy surface the same wettability properties as the low-energy solid having the same surface constitution. Thus, the value of the critical surface tension of solids is dependent upon the nature and packing density of the outermost or exposed atoms and functional groups at the surface. To obtain maximum adhesive strength, the adhesive-adherent interface must be kept free from exposure to low-energy organic films. In this way, the number and size of interfacial voids is kept to a minimum. Adsorbed water greatly decreases the surface energy(37,38). The adsorption of water molecules on clean high-energy surfaces results in the formation of only a fraction of a monolayer, yet, as low as at 0.6% relative humidity, this fraction drastically lowers the critical surface tension of wetting. The increased amount of water adsorbed at 95% relative humidity forms a film comprising little more than a condensed monolayer and further lowers γ_c to a value somewhat above that of bulk water. Thus, after exposure to a humid atmosphere, the surface energy of any clean, smooth, high-energy hydrophilic surface (glass, sapphire, metal, or metal oxide) or low-energy surface such as keratin, depends mainly on the concentration of water or other contaminants adsorbed on the surface. The chemical nature of the underlying hydrophilic substrate has little effect on wetting and spreading properties.

Schwartz and Galligan used water insoluble liquids to displace water from prepared tooth surfaces(15). The wetting characteristics of freshly ground tooth surfaces depended on the method of preparation. Air and alcohol-dried surfaces were not readily wetted by organic liquids. Soap or detergent washing "conditioned" tooth surfaces so that they were wetted under water by organic liquids of widely ranging polarities. Alcohol washing or mild abrasive cleaning removed the conditioned layer. Tooth surfaces can, however, be more durably conditioned by substances containing free carboxylic acid groups. Critical surface tension values of tooth sections that had been allowed to dry were influenced by the relative humidity during the drying period.

The contact angles between various liquids and human enamel, dentin and whale dentin are given in Table 7(39). The liquids were chosen because their different specific free-surface energies made them useful for determining the critical surface tensions of low-energy solids(40,41). The results indicated that untreated teeth have low-energy surfaces consisting of organic membranes. These surfaces are apparently derived from the organic constituents of teeth since synthetic hydroxyapatite is hydrophilic and, therefore, does not participate in the formation of the low-energy surfaces. The mechanism of the formation of these low-energy surfaces can be explained on the assumption that at least some parts of the organic phases of enamel and dentin are mobile and exist in the form of a gel(42).

The observation of Uy and Chang that the contact angle increases during the first hour after grinding and varies with relative humidity(43) could not be confirmed by Glantz(39). It is likely that traces of organic matter were leached out from the embedded teeth used by Uy and Chang. This may have modified both the wetting characteristics of the surface, as well as the adhesive properties.

The calculated mean critical surface tensions at 23°C for human dentin, whale dentin and enamel show that these substances have roughly the same specific free-surface energies (Table 7)(39). Uy and Chang, however, gave values for γ_c at 37°C of 31.5, 24-29, and 39.5 dyne/cm, respectively, for human enamel, bovine enamel and dentin(43). The fairly large discrepancy in the critical surface tensions for enamel reported in these two studies may be the result of differences in surface preparation procedures and points out the difficulty in obtaining reproducible results, even under relatively well-controlled conditions. Comparison of these values with those of γ_c in Table 7 suggests that enamel and dentin have surface energies similar to polyethylene and poly(methyl methacrylate), respectively. Thus, a non-hydrogen bonding adhesive applied to enamel should wet and spread on this low-energy surface.

On the conventional $\cos\theta$ versus γ_{lv}^{o} plot, there are distinct differences between the location of the contact angles for methylene iodide and 1-bromonaphthalene and those of the other liquids used(39). The surface tension of the former two liquids is made up almost entirely of van der Waal's dispersion forces, whereas those of the other test liquids also contain polar forces and hydrogen bonds(44). The value of the contact angle is determined by the magnitude of the interacting forces of both the liquid and the solid phases. The position of the cosines of the contact angles of the tw liquid groups in the $\cos\theta$ vs. γ_{lv}^{o} plot indicates that the free-surface energies of both enamel and dentin are partially built up of polar forces and/or hydrogen bonds. The values for the total work of adhesion and the work of adhesion resulting from the interaction of non-dispersion forces (Table 8) also bear out this point. The total work of adhesion W_A was calculated from the Dupré equation $W_A = \gamma_{lv}o(1 + \cos\theta)$. The work of adhesion resulting from interaction of non-dispersion forces W_A^P was obtained from the following equation:

$$W_A^P = \gamma_{lv}o\ (1 + \cos\theta) - (\gamma_{lv}^d o + \gamma_{sv}^d o)$$

where $\gamma_{lv}^d o$ and $\gamma_{sv}^d o$ are respectively the free-surface energies (or free-surface tensions) at the liquid-saturated vapor and solid-saturated vapor interface resulting from van der Waals dispersion forces. From contact angle measurements on enamel and dentin surface, the van der Waals dispersion forces were calculated to be about 43 dyne/cm(39). Among the test liquids used, water appeared to have the greatest work of adhesion from interactions with the non-dispersion forces. The linear relation between the work of adhesion and the surface tensions of the liquids suggests that many attractive forces interacting within the liquids also interact with enamel and dentin surfaces on adhesion.

By studying the influence of stannous fluoride solutions on EDTA decalcified human dentin, and on hydroxyapatite, the observed decrease in wettability could be attributed to the organic component of dentin.

Fluorides of tin, silver and nickel changed the wetting characteristics of enamel and dentin. Chromium, copper, zinc and sodium fluoride did not appreciably lower the surface free energy. Glantz assumed from these results that the resistance of fluoridized enamel surfaces to acids may be partially dependent on the formation of fluorocarbon derivatives which are produced on the enamel and dentin on catalytic reaction with fluorine.

Measurement of the contact angles demonstrated that the wettability of hard tissues is affected by lactic acid (Table 9)(39). The effect varied in degree with the concentration of the acid solution, the exposure time and the type of hard tissue. Human enamel surfaces could be made hydrophilic (water spreads when applied) by 0.02% lactic acid treatment within five hours. Similar treatment, however, did not render the surfaces of human or whale dentin hydrophilic. Those tooth surfaces which were not rendered hydrophilic by the treatment, reverted to their original wettability status when the attack by lactic acid had ceased.

Table 9 indicates that pretreatment of tooth surfaces with stannous fluoride delayed the effect of lactic acid on the wettability of enamel and dentin. Presumably, the gradual solubilization of the low-energy fluoride containing surface layer is required before significant changes in wettability characteristics occur.

Tooth surfaces can also be made hydrophilic by treatment with 85% phosphoric acid(45). These surfaces give essentially zero contact angles with both water and epoxy resins. The tooth surfaces could be restored to their original hydrophobic condition by polishing with dental pumice.

Snyder et al. measured contact angles of a methyl methacrylate-poly(methyl methacrylate) system (without initiator and accelerator) before and after treatment of tooth surfaces with 50% phosphoric acid(46). Contact angles were determined in a nitrogen atmosphere saturated with methyl methacrylate vapor to prevent evaporation of the monomer from the liquid drop. Phosphoric acid treatment drastically lowered the observed contact angles.

The contact angles rose steadily on untreated surfaces as the percentage of polymer in the mix was increased. This was to be expected since the surface tension of methyl methacrylate monomer (28 to 29 dyne/cm at 20°C) was considerably lower than the critical surface tension of wetting for poly(methyl methacrylate) (γ_c = 39 dyne/cm). After phosphoric acid treatment, the contact angles for the monomer-polymer mixes were nearly the same, suggesting that there was little difference in the surface wetting ability of the adhesive studied. Since the phosphoric acid treatment drastically improved bond strengths in a closely related system, it was clearly demonstrated that surface wetting was an important factor in improving adhesion.

Immersion of dentin in aqueous silver nitrate solution and subsequent reduction of the silver nitrate by light is accompanied by a distinct change in the wettability of the dentin(13,47). The contact angle of water is increased from 13° for freshly polished dentin to 46° after silver nitrate treatment. The critical surface tension of dentin, which may be as high as 49 dyne/cm, is correspondingly decreased to 33 dyne/cm. This transformation in hydrophilic character is reflected by a greatly reduced water vapor adsorption on silver nitrate treated powder. The surface treatment, which presumably makes the surface less polar by occupying polar adsorption sites, results in increased adhesion. The resulting bond strength depends on the duration of the treatment, concentration of the reagent and exposure of the dentin surface to light prior to bond formation. This treatment, however, must be considered solely as a prototype means of obtaining adhesion to dentin. Dark unaesthetic staining resulting from the inclusion of silver salts within the dentinal tubules precludes the use of this procedure in clinical dentistry.

SURFACE TREATMENTS

1. Etching

Chemical treatment of teeth to provide a modified surface offers an attractive approach for the improvement of adhesion between tooth structure and dental restorative materials. In 1955, Buonocore reported that treatment of enamel with 85% phosphoric acid increased adhesion to acrylic filling materials(48). Since then, Buonocore and coworkers(49,50,51,51a), Gwinnett(52,53), Ellison and Fookson(54), Lee, Cupples, Swartz and Schubert(55), and Eden, Craig and Peyton(56) have demonstrated improved bonding by pretreatment of tooth surfaces with acids and sequestering agents prior to placement of the restorative. The acids used in these studies included phosphoric-, hydrochloric-, and citric acid and the sodium salts of ethylene diamine tetraacetic acid (EDTA).

Brauer et al. have determined the relative efficiency of simple surface treatments of enamel using a variety of polyfunctional acids and chelating agents to obtain bonding between tooth surfaces and commercially available acrylic resins(57,58).

Bond strengths of dental resins to tooth structure, as evaluated in the tensile test of Lee, Swartz and Culp(59), were determined after pretreatment of yearling calf anterior teeth specimens with more than 40 different chelating or acidic substances. To minimize variations in results, the following procedure was employed. Resin-mounted teeth which had been stored in water were: (1) patted dry with tissue, (2) swabbed with a solution for a specified time, (3) rinsed with water, and (4) the pretreatment repeated after a specified time to obtain reproducible results. The curing cycle of the resin to be bonded to the tooth surface was rigidly controlled. Coefficients of variation of the bond strengths after 24-hour water exposure were of the order of 20 to 40%--an order of magnitude generally associated with values obtained in adhesion testing of non-homogeneous surfaces. Representative results are given in Table 10. Bonding was not obtained without pretreatment.

In these initial studies, Brauer employed a 5% aqueous tetrahydrofuran-2,3,4,5-tetracarboxylic dianhydride (TTAD) pretreatment. This compound on hydrolysis yields a polycarboxylic acid that may also react as a chelating agent. Bonding was not obtained without the treatment, whereas a single treatment gave a tensile adhesion of 21 kg/cm^2 and a repetition of this treatment applied in approximately 3 minutes raised the bond strength to 28 kg/cm^2. If the second application is delayed, e.g., as much as an hour, a significant increase in bond strength occurs (e.g., 50 kg/cm^2). Further delay in the second application of this acid did not increase the tensile adhesion, nor did further applications of the acid. A 5% solution of TTAD appeared to give optimum results. A longer application of the solution (for 30 seconds, instead of 10 seconds), mechanical roughening of the surface or cleaning the enamel in an ultrasonic bath did not further increase adhesion. Enamel pretreatment with TTAD neutralized to pH 7 did not result in measurable adhesion. A 5% solution of tetrahydrofuran-2,3,4,5-tetracarboxylic acid (TAA) gave a 64 kg/cm^2 tensile strength value even if the second pretreatment took place after 3 minutes. Thus, the repeated application of the free acid within a short period of time promotes improved adhesion, as compared to the anhydride treatment. This result may be caused by the time required to hydrolyze the anhydride to the free acid, which is the effective adhesion promoter.

To correlate chemical structure of the acid with bonding efficiency, the effect of the treatment of bovine enamel surfaces with simple acids, as well as higher molecular weight acids and chelating agents, containing polyfunctional groups on adhesive strength was studied (Table 10). The 50% phosphoric, formic or citric acid treatments did not yield improved adhesion compared to 5% TTA solutions. Five percent citric or phosphoric acid solutions gave inferior adhesion values. Lactic acid treatment yielded very promising results. A 20% lactic acid treatment gave a bond strength of 61 kg/cm^2, which was similar to the value obtained for a 42.5% solution of the same acid. Addition of 1% methacrylic acid to the monomer to obtain a more polar polymer, or treatment with a wetting agent before the etch to possibly increase the rate of ingress of acid into the enamel, actually decreased adhesion. Use of 50% phosphoric acid containing 7% zinc oxide gave a significantly lower bond strength than the 20% lactic acid. A solution of a carboxylated polyester, which improved adhesion of coatings to metal and plastic substrates because of the presence of a large number of free acid groups(60), when applied to the enamel surface after the lactic acid treatment, gave only a low tensile adhesion value.

By substitution of ethanol for water, 5% ethanolic solutions of water insoluble compounds were obtained. The lack of adhesion found when enamel surfaces were treated with 5% ethanolic solutions of various acids is caused by the decreased dissociation of the acid in ethanol. The lower concentration of hydrogen ions reduces the etching effect of the acid on the enamel surface. Because of the insolubility of many organic acids in water, selection of acids with the required structural differences to pinpoint structure-adhesive properties relationships was possible only on a very limited scale.

A number of aliphatic acids, besides lactic acid, such as citric-, dihydroxytartaric-, dihydroxymaleic- and 1,3,5-pentanetricarboxylic-acid, gave adequate adhesion. Some cyclic and aromatic compounds also improved adhesion. With both the *cis*,*cis*,*cis*,*cis*-1,2,3,4-cyclopentanetetracarboxylic dianhydride or the corresponding acid, significant adhesion was obtained. No correlation was found between the number of carboxylic acid groups attached to the benzene ring and adhesive properties. Thus, treatment with 5% aqueous 1,2,4-benzenetricarboxylic acid induced bonding; the pentacarboxylic acid treatment resulted in a bond strength of 56 kg/cm^2, but saturated solutions of ($<5\%$) 1,2,4,5-benzenetetracarboxylic acid and benzenehexacarboxylic acid were ineffective for this purpose.

While only nominal bonding was obtained on treating the enamel surface with a saturated EDTA solution, a somewhat stronger adhesive bond was developed on application of disodium EDTA. Treatment with a 5% solution of tetrasodium EDTA in 0.001 N NaOH, which should more readily chelate calcium present in the tooth enamel, did not produce evidence of bonding. However, some chelating agents such as N-(2-hydroxyethyl) ethylenediamine triacetic acid and phosphonomethyliminodiacetic acid promoted bonding probably by the reaction of the carboxylic acid groups with calcium.

The improved adhesion gained as a result of acid treated enamel surfaces was not restricted to resins containing amine accelerators.

Similar results were observed with resins cured with other accelerators and with a representative composite resin. For these restoratives, bonding was not obtained when untreated enamel surfaces were used.

For the enamel surfaces treated with 5% TTAD, 5% TTA, and 5% or 20% lactic acid, the bond strength to the resin was not reduced after storage in an aqueous environment for periods as long as 90 days.

Most of the 5% acid solutions gave pH values in the 1.3 to 2.3 range. Especially interesting are the pH values of 20% lactic and 5% tetrahydrofurantetracarboxylic acid which were 1.5 and 1.4, respectively.

A fairly large number of water soluble acids, especially those containing polyfunctional groups, induced bonding to enamel surfaces. Perhaps most significant were the results obtained after treatment with 20% lactic acid, an acid which apparently has not been used by previous investigators to increase adhesion. Treatment of enamel with an aqueous solution of the acid resulted in adhesion values greater than were obtained with a 50% phosphoric acid solution containing 7% zinc oxide. The latter solution had a considerably more acidic pH. This solution has been used clinically by Buonocore as an efficient pretreatment for pits and fissure sealants without any apparent deleterious effect on the tissues(51). Thus, it is likely that the less acidic lactic acid treatment can be tolerated by the oral tissues and may actually be more desirable for this application. It must also be realized that the total amount of acid applied to the enamel is probably less than 0.1 ml and under clinical conditions is partially neutralized within a few seconds on contact with the enamel surface.

It is unfortunate that the effect of the application of the solutions of many of the effective adhesion promoters to dental tissues is unknown.

Pretreatment with chelating agents such as ethylene diamine tetraacetic acid is reported to improve adhesion(52,61). Treatment of the tooth surface with the tetrasodium salt of this acid in 0.001 M NaOH, a highly effective chelating agent for calcium, did not produce evidence of bonding. Thus, it appears that adhesion to enamel is not promoted markedly by application of an efficient chelating agent for calcium.

Acid treatment removes surface debris, etches enamel and makes its surface more wettable. The improved adhesion is not caused by strong chemical bonding of the resin to the modified enamel surface. Mechanical retention plays a most important role in adherence of resins to treated enamel surfaces. The penetration of uncured resin into the enamel pores improves retention. Etching creates an increased surface area and opens up pores into which the resin can flow. Since acid treatment makes the surface more wettable, the extent of penetration will depend greatly on the number of pores into which the resin can flow. Greater porosity allows an easier ingress of uncured resin and therefore better mechanical interlocking of the hardened resin with the enamel phase.

Scanning electron micrographs (SEM) of enamel before and after acid treatment prove beyond doubt that this treatment leads to preferential dissolution of portions of the enamel surface and to exposure of the enamel rods(54,55,62-67). A typical SEM photograph of bovine enamel surface before (bottom, Fig. 1), and after (top, Fig. 1), two 30-second applications of a 5% tetrahydrofuran-2,3,4,5-tetracarboxylic dianhydride solution shows clearly the removal of debris and the exposure of the rod structure of the enamel(62). This acid treatment also removes the debris and exposes the tubular structure of dentin. Similar results have been obtained with human tooth tissue(54). The removal of loose debris remaining in the cavity preparation with a 50% solution of citric acid appears to be complete within a 3-minute period(63). Similar treatment with water leaves the surface substantially unchanged.

Hoffman et al found that etching of sound human enamel for 15 minutes with 0.2M acetate buffer, pH 4, causes demineralization of prism cores, whereas treatment with 0.12M ethylenediamine tetraacetic acid (EDTA) neutralized to pH 7.9 attacks their peripheral boundaries(65). EDTA action on enamel appears to follow a specific selective pattern, which starts at the peripheral borders of the prisms and progresses centrally, so that at first only portions of the peripheral prism boundaries are delineated(66). With prolonged action there is more complete prism isolation until only prismatic cores remain after 24 hours. Furthermore, surface coatings seem to diminish with progressive loss of mineral. Topical stannous fluoride treated enamel inhibited surface alterations when subjected to acetate buffer(68). This supports the concept that fluoride protection involves a reduction in enamel solubility.

Wei used the electron microprobe to analyze the calcium and phosphorous concentration of sound, acetic acid-potassium acetate (pH 5.5) acid-etched and remineralized human enamel(67). Acid etching of enamel produced a decrease in calcium and phosphorous concentration with a rise in the Ca:P ratio. Under the experimental conditions, the mineral loss was mainly confined to the first 9 to 12 μm from the surface.

2. Enzymes

The abrasive or etching pretreatment of dentin prior to the application of dental restoratives leaves the protein component of tooth structure substantially untouched. This predominantly collagenous surface appears to interfere with the adhesion of the inorganic constituent of dentin to organic adhesives.

To provide a completely inorganic substrate, dissolution of the collagenous matrix of dentin by enzymatic treatment has been suggested(54,64,69). Collagenase, which is capable of attack on native collagen at or near physiological pH, was employed(64,69,70). This enzyme is stable in "tris" buffer containing calcium chloride and hydrolyzes collagen to peptides. The collagenase was effective in reducing the collagen level on the surface of acid-etched dentin(70). A thirty-minute immersion time in the 5% collagenase solution at pH 7 was required. The enzyme treatment, however, did not improve adhesion to bovine dentin. Lee et al used bovine pancreas trypsin, a proteolytic enzyme that preferentially catalyzes the hydrolysis of peptide bonds formed between the carboxyl group of arginine or lysine and the amino group of any other amino acid in denatured non-native collagen(64,69). The trypsin solution was found quite effective in removing surface debris, as judged from photomicrographs. Subsequent etching of the inorganic substrate with citric acid and then treating with silane primer was attempted. The application of a 10% trypsin solution, however, had only a slight effect on adhesion.

Ellison and Fookson employed a 1.5% papain or 1% alcalase solution at pH 7 which were applied for 5, 10 and 20 minutes to bovine dentin surfaces(54). Scanning electron micrographs indicated that the five-minute papain treatment had a negligible effect, while the twenty-minute treatment produced more extensive changes. The enzymatic treatments under certain conditions enhanced adhesion of bovine dentin to methacrylate resin using a titania filled urethane adhesive, but the improvements were not as significant as those obtained by acid etching and subsequent silane pretreatment.

3. Coupling Agents

Coupling agents are multi-functional molecules that promote adhesion of the solid substrate to the restorative. These adhesion promoters function by adsorbing on and altering the surface of the mineralized tissue to facilitate interaction with the restorative by either chemical or physical processes. The portion of the molecule which is not directly responsible for its adsorption presents a surface which can be more easily wetted by the restorative. An adhesion promoter is ideally adsorbed as a monomolecular layer and will prevent contamination of the solid by organic substances. It should be inert chemically, should have a low surface energy and should form a hydrophobic barrier film to avoid the accumulation of water which could prevent efficient wetting by an uncured liquid restorative.(31,71,71a) Since its small size, high-surface mobility and short relaxation time makes permeation of organic coatings by water a constant threat to the integrity and longevity of the adhesive joint, the outer surface of the coupling agent film should be more hydrophobic then hydrophilic. The reacted film of the coupling agent should be as coherent and impermeable to water as possible, have a minimum water solubility and have optimum resistance to hydrolysis.

The choice of the group of the coupling agent to be adsorbed to the solid surface will depend on the nature of that solid and on whether or not chemical interaction with the adherent surface can provide high resistance to water degradation of the joint. Molecules which should give optimum results, with an outermost surface specifically designed to have good wetting by most liquid adhesives, are reported to have a p-chlorophenyl and mono- or polycarboxyl groups; the two portions being connected by a sufficiently long aliphatic chain which serves as a hydrophobic spacer(72). The carboxylic acid groups should be particularly effective in promoting adsorption and adhesion to cationic surfaces such as hydroxyapatite.

Perhaps the earliest application of coupling agents in dentistry is the use of glycerophosphoric acid dimethacrylate as a component of a cavity seal liquid(49,73,74). Application of this compound modifies dentin as shown by staining of the dentin layers adjacent to the adhesive(73,74). The compound, when incorporated into methyl methacrylate autopolymerizing resin, adheres to some degree to tooth surfaces(49) and is used in a commercial cavity liner. Bowen has synthesized a number of potential coupling agents(61,75-77). An adduct of N-phenylglycine and glycidyl methacrylate produced a statistically significant increase in adhesive strength after short-term water exposure of dentin or enamel joined to methacrylate resins. The adhesion was enhanced after pretreatment of the tooth surface with dilute acid, base or a 10% solution of EDTA. Similarly, Masuhara and co-workers have synthesized chelating agents that are capable of bonding to calcium and contain polymerizable methacrylate groups (78-80). Among these, 2-methacryloyloxy ethyl phthalate, N-methacryloyl-N'-dicarboxymethyldiaminobenzene and 2-hydroxyethyl methacrylate improved adhesion to enamel. The resulting bond strength was rapidly weakened in the presence of water. However, a tributyl borane initiated methacrylate polymer adhered well to enamel after the surface had been pretreated with a phosphoric acid solution and a silanizing agent (78). Methacrylato chromic chloride (Volan L) and a saturated solution of phosphonomethyliminodiacetic acid also improved adhesion of enamel to acrylic resins(81).

Bonding was not obtained with <u>trans</u>-isoeugenol, 4-hydroxy-3-methoxystyrene, 4-hydroxy-3-nitrostyrene and its methacrylate ester, 4-hydroxy-3,5-dinitrostyrene, allylphosphonic acid and its hydroxyethyl methacrylate ester and N-allylimino-N,N-dimethylene diphosphoric acid. Thus, many ligands that chelate calcium do not bond strongly to enamel.

Organo-functional silanes appear to improve adhesion of tooth substrate to dental restoratives. Lee (55,64,82) used silanes such as glycidoxypropyltrimethoxysilane hydrolyzed in acetic acid as adhesion promoting primers to improve rigid or flexible liners formulated from epoxy resins, epoxy-polyurethane blends or catalyzed polyurethane resins. As shown in Figure 2, silanes (such as γ-aminopropyltriethoxysilane) theoretically should be capable of performing two functions: 1. react with water adsorbed on the surface of the dentin, and 2. react with the inorganic constituents of the dentin and the resin to provide a chemical link between the tooth and restorative. On incorporation into a polyurethane of 1% (based on total resin solids) of N-beta-(aminoethyl)-gamma-aminopropyltrimethoxysilane, the silane reacts instantaneously with the isocyanate(63).

Major improvement in bond strength does not result from the use of silanes prior to placing a polyurethane liner(54). When other coupling agents were incorporated into the polyurethane, however, improved adhesion was obtained. The coupling agents used were 2-hydroxyethyl-, 2-hydroxypropyl- and 2-*t*-butylaminoethyl methacrylate and 1,3-di(allyloxy)-2-ethyl-2-(hydroxymethyl)propane. Optimum adhesion was obtained when the bovine enamel test surfaces were treated for one minute with 50% formic acid, rinsed and air-dried before application of the thin polyurethane liner (formulated from a titanium dioxide filled polymethylene polyphenyl isocyanate-castor oil resin) and an acrylic rod. To insure that the acrylic moiety of the coupler would bond to the rod, its surface was wetted with a drop of monomer prior to assembling the joint. Best results, with bond strength up to 900 kg/cm^2, were obtained with 2 to 5% concentration of the coupling agent. Similar values resulted when the coupling agent was added to the monomer used to wet the acrylic rod instead of adding it to the polyurethane formulation. In this test, the hydroxyethyl methacrylate coupler was especially effective.

The use of a partially saponified acrylonitrile-butyl acrylate copolymer rubbery liner resulted in some improvement in restoration shear strength(17). This value was enhanced by the addition of a silane (γ-aminopropyltriethoxysilane) to the liner. Silane treated specimens exhibited improved moisture resistance and provided some protection when the joint was subjected to vigorous thermocycling. The synergistic effect of the silane and the rubbery polymer indicated pronounced interaction of the silane with the physiologic substrate, as well as interaction of the silane and the polymer.

4. Surface Grafting

During the past few years, significant progress has been made in the chemical modification of proteinaceous materials with the aim of improving the physico-chemical properties in the resulting products. The grafting of organic monomers to collagen, dentin or bone (so that after polymerization of the monomer the proteinaceous surface is bonded chemically to the polymer chains) offers an attractive technique of improving surface characteristics. Employment of the proper monomer in the grafting procedure may make it possible to vary widely or even control the surface properties of the resulting substrates.

Rao and coworkers have described experimental conditions for grafting methyl methacrylate, acrylonitrile and acrylamide to insoluble collagen(83,84). Collagen powder was reacted with monomer in water or dimethylformamide in the presence of ceric ammonium nitrate. The reaction presumably involves the hydroxy groups in the side chains of hydroxyproline, hydroxylysine, serine and threonine residues.

$$\mathrm{Ce}^{IV} + \mathrm{R\ CH_2OH} \rightleftharpoons \text{Ceric alcohol complex} \longrightarrow \mathrm{Ce}^{III} + \mathrm{H}^+ + \mathrm{R\ \dot{C}HOH}$$

Mino and Kaizerman have shown that the ceric ion-alcohol complex reacts reversibly with alcohols and glycols(85). Dissociation of this complex is the rate-determining step. Thus, the hydroxyl group in the collagen molecule is the reducing agent. If the reaction is carried out in the presence of a vinyl monomer, the free radical formed on the carbon atom attached to the hydroxyl group of the polymeric backbone acts as the initiator for the polymerization of the graft side chain.

Brauer and Termini reacted purified steer hide collagen powders or collagen films with over twenty monomers in water in the presence of 1 to 2 x 10^{-3} M ceric ammonium nitrate in 1N nitric acid(86,87). Maximum grafting was attained after soaking collagen in 2% wetting agent, deaerating the solution and allowing the reaction to proceed for 3 hours. Product yields were highly dependent on the monomer used. However, with reactive monomers such as methyl methacrylate or glycidyl methacrylate, an increase in weight of over 15% (based on the original weight of collagen after extraction of the homopolymer with suitable solvent) was obtained even after a 15-minute reaction time. Generally, the lower molecular weight acrylates and methacrylates gave higher yields (over 100% increase in the original weight of collagen) than the higher homologs (Tables 11 and 12). The yield of the graft polymer is greatly reduced if the reaction is conducted in air. A marked increase in graft polymer yield is obtained using a 2% concentration of wetting agent. Among these dioctyl sodium sulfosuccinate, an anionic agent, proved most successful. Probably the hydrophobic portion of the wetting agent interacts with the hydrophobic portion of the collagen. Possibly partial unfolding of the collagen structure occurs with an accompanying increase in the number of reactive sites. Reduced yields of graft polymer on employing higher molecular weight monomers may have been caused by steric hindrance of the more bulky monomer, the decreased solubility of the monomer in the reaction mixture, as well as the slower rate of diffusion of monomer into the collagen.

Methacrylate monomers containing glycidyl, hydroxyethyl, dimethylamino or _tert_-butylamino groups could be grafted to collagen.

These functional groups may provide additional centers for further surface modification. Dimethacrylates such as ethylene- or 1,3-butylene dimethacrylate, can be grafted to collagen in excellent yield. The polymers may be crosslinked and would therefore be likely to decrease the solubility and increase chemical resistance of the resulting products. Grafting of collagen to monomers such as styrene, acrylonitrile and vinylpyrrolidone is also possible.

Grafting yields vary, but are likely to depend on the solvent system employed. Amount of homopolymer formed, as determined by weight loss on extraction with a suitable solvent such as acetone or ethylene dichloride for poly(methyl methacrylate), was generally less than 5%. Only the butylene dimethacrylate graft product had a large weight loss (22.1% on extraction with chloroform), which may be caused by the presence of unreacted monomer adsorbed on the reaction product. Especially promising are grafts with glycidyl methacrylate. Infrared absorption curves of the product indicate that the glycidyl group is not affected during polymerization. Since this group can be converted to the highly reactive hydrophilic hydroxyl group using mild alkaline hydrolysis, a whole new field of investigation is opened up.

Appearance of the products often varied considerably from the original collagen powder. The grafted materials consisted of powders after reaction with some monomers, but formed loose mats and even tough films with others.

The large variety of monomers (acrylates, methacrylates, methacrylic acid, styrene, vinyl acetate and vinylpyrrolidone, and others) graftable to collagen indicates that surfaces with the desired degree of hydrophilic-hydrophobic balance to fit specific applications can be obtained. Monomers were also grafted to powdered bone that had been presoaked in 2% dioctyl sodium sulfosuccinate for one hour(87). On grafting glycidyl methacrylate, dimethylaminoethyl methacrylate, 1,3-butylene dimethacrylate or isobutyl methacrylate to powdered bone, an increase in weight of 60%, 27%, 19% and 11%, respectively, was obtained after acetone and chloroform extraction. Infrared spectra show the presence of the oxirane ring of the glycidyl group (899 cm^{-1} and 843 cm^{-1}), as well as a C=O stretching band at 1730 cm^{-1}, a C=O stretching band at 1270 cm^{-1}, and an absorption band at 747 cm^{-1}, characteristic of the methacrylate group indicating the glycidyl methacrylate was grafted to bone. The presence of 1,3-butylene dimethacrylate and dimethylaminoethyl methacrylate could also be detected by this technique. Grafting to bone surface is possible within one hour reaction time, as indicated by a slight increase in weight. However, a number of monomers such as (methyl methacrylate, ethyl acrylate) do not appear to react with bone.

Methyl methacrylate was also grafted to dentin(86). An 8% weight increase was obtained after removal of the homopolymer by acetone extraction, whereas a 21% weight reduction was noted for powder treated in the absence of monomer. Presence of the poly(methyl methacrylate) grafts was established from the $-COOCH_3$ absorption at 1720 cm^{-1}. No appreciable amounts of polymer grafts or increase in weight could be detected after reacting the dentin with ethyl methacrylate, isobutyl methacrylate, glycidyl methacrylate, dimethylaminoethyl methacrylate, vinyl acetate or 1,3-butylene dimethacrylate. These results seem to indicate that the dentin surface is not as susceptible to grafting as the surface of powdered bone.

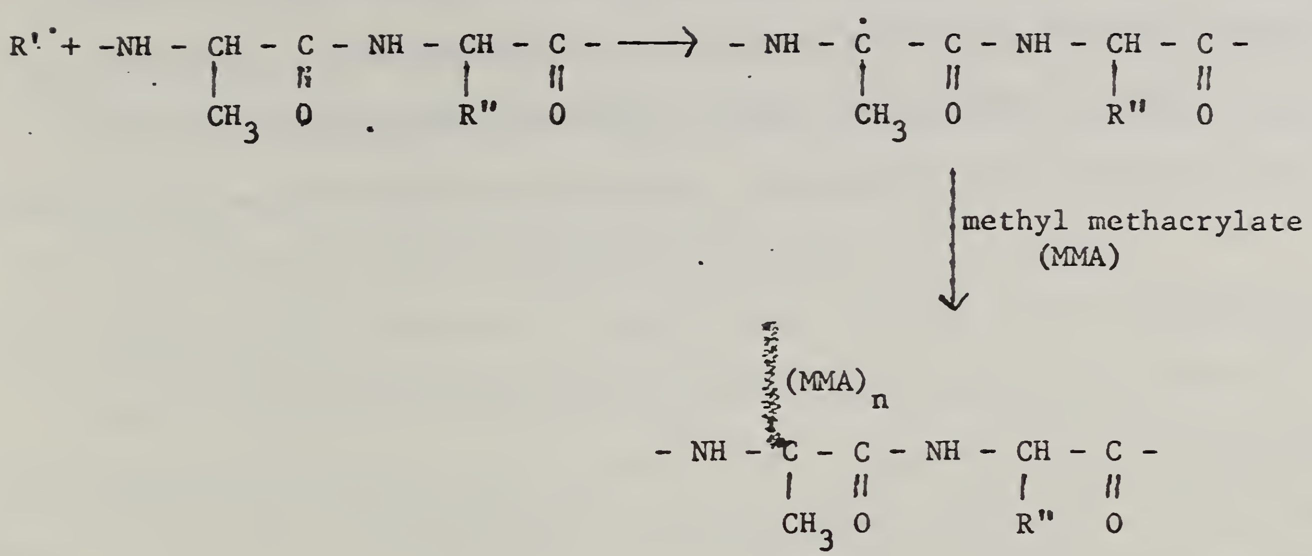
Experiments with calfskin indicate a less reactive surface for grafting than is available in collagen powder(87). The epidermal layer of the calfskin being keratinous in nature, may provide a much less reactive surface for this purpose. Thus, the relative grafting efficiency of monomers to the solid substrate decreases in the following order:

Powdered collagen > collagen film > powdered bone > epidermal calfskin > powdered dentin.

Although this grafting procedure is conducted in an acid environment at a pH around 2.2 that may not be tolerated by living tissues, suitable in vivo techniques for modifying tooth and bone surfaces may eventually be developed. Characteristics that could presumably be improved by such treatment are better adhesive properties of tooth surfaces to restorative materials, greater resistance of teeth to caries formation, and increased resistance of bone and skin to fungal and bacterial diseases.

Grafting to collagen, other proteinaceous materials and polypeptides has also been accomplished through polymerization of methyl methacrylate with tri-n-butyl borane(78,88-90). The following mechanism has been suggested:

$$R_3B \xrightarrow{O_2} R_2BOOR \xrightarrow{2R_3'B} 2R'^{\bullet} + R_2BOBR_2' + \text{other products.}$$



$$R'^{\bullet} + -NH-\underset{CH_3}{\underset{|}{CH}}-\underset{O}{\underset{\|}{C}}-NH-\underset{R''}{\underset{|}{CH}}-\underset{O}{\underset{\|}{C}}- \longrightarrow -NH-\underset{CH_3}{\underset{|}{\overset{\bullet}{C}}}-\underset{O}{\underset{\|}{C}}-NH-\underset{R''}{\underset{|}{CH}}-\underset{O}{\underset{\|}{C}}-$$

$$\big\downarrow \text{methyl methacrylate (MMA)}$$

$$-NH-\underset{CH_3}{\underset{|}{\overset{(MMA)_n}{C}}}-\underset{O}{\underset{\|}{C}}-NH-\underset{R''}{\underset{|}{CH}}-\underset{O}{\underset{\|}{C}}-$$

Tri-n-butyl borane is not suitable for use as a dental material because it is very unstable in air. Complexes of tributyl borane with ammonia or primary and secondary amines are, however, easily reactivated by the action of isocyanates, acid chlorides and sulfonyl chloride (91). The complex of tributyl borane with ethylene diamine is stable and can be reactivated with p-toluenesulfonyl chloride. It reduces the curing time at room temperature to half that obtained with tri-n-butyl borane and does not affect adversely other properties of the cured resin. Bond formation of the borane-cured resin to dentin appears to be enhanced by the presence of moisture and bond strength is retained rather well after water immersion. An experimental restorative that exhibits a somewhat variable degree of adhesiveness to dentin has been evaluated.(92,93) Lenz and Viohl (92) reported a setting time of 12 minutes for this material which was too long for clinical use. Adhesion was not adequate in 20% of the cases.

SUMMARY

Studies of the reactivity and modification of surfaces of calcified tissues offer an attractive approach for obtaining useful information which may be applied toward reduced dental caries and improved adhesion between tooth structure and restoratives. Investigation of the interaction of gases on such surfaces provides considerable insight into the structural details of surfaces of hard tissues. Effectiveness of potential conditioning agents can be demonstrated from alterations of the surface properties of teeth or bone as indicated by changes in: critical surface tension, oil-wettability, adsorption isotherms and adhesionability. Measurement and evaluation of heats of immersion and heats of reaction are useful for observing modifications and determining those groups that bond to tooth structure in aqueous environments.

Dissolution of the collagenous matrix of dentin by enzymatic treatment enhances adhesion under certain conditions, but the improvements are not as significant as those obtained by acid etching.

Coupling agents comprising multifunctional molecules promote adhesion of the solid substrate to restoratives. These adhesion promoters function by adsorbing on and altering the surface of the mineralized tissue to facilitate interaction with the restorative by either chemical or physical processes. Coupling agents, especially organo-functional silanes, improve adhesion of tooth substrate to dental restoratives.

Application of dilute polyfunctional acids removes debris, etches enamel and increases surface wettability. Etching creates an increased surface area and opens pores into which resin can flow. Excellent adhesion is obtained after hardening because of mechanical interlocking of resin at the enamel-resin interface.

Long side chains have been grafted to collagenous surfaces (collagen powder or film, bone). Grafting monomers containing additional functional groups that are potential reactive centers for further modification is feasible. Surfaces with the desired degree of hydrophil-lipophil balance to suit specific applications can be prepared.

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Table 1

Potential Adhesive Systems

A. Surface Treatments
   1. Etching agents
   2. Enzymes
   3. Coupling agents
   4. Surface grafting

B. Polymers
   1. Acrylics
   2. Zinc polyacrylate
   3. Alkyl cyanoacrylate
   4. Epoxy resins
   5. Polyurethanes
   6. Inorganic polymers
   7. Bioadhesives

Table 2

APPARENT HEATS OF IMMERSION OF COMPONENTS OF TOOTH STRUCTURE AND SYNTHETIC FLUORAPATITE[a]

Powder	Samples Conditioned	Apparent Heats of Immersic	
		$-\Delta H_w$ Average (calories/gm)	Average (calories/ sq meter)
Dentin (400 mesh)	Anhydrous $CaSO_4$	-7.71	-0.734
Dentin (400 mesh)	10^{-1} mm Hg	-10.29	-0.980
Dentin (200 mesh)	10^{-1} mm Hg	-10.00	-0.952
Dentin (400 mesh)	10^{-3} mm Hg	-12.11	-1.15
Dentin (400 mesh)	Saturation with H_2O vapor	-0.31	-0.029
Anorganic dentin (400 mesh)	Saturation with H_2O vapor	-0.995	-0.009
Enamel (400 mesh)	10^{-1} mm Hg	+0.58[b]	+0.148[b]
Enamel (400 mesh)	10^{-3} mm Hg	+0.64[b]	+0.164[b]
Enamel (400 mesh)	Saturation with H_2O vapor	+0.07[b]	+0.02 [b]
Anorganic whole tooth (120 mesh)	10^{-1} mm Hg	-2.06	-0.028
Anorganic whole tooth (120 mesh)	10^{-3} mm Hg	-1.85	-0.024
Anorganic whole tooth (120 mesh)	Saturation with H_2O vapor	-0.66	-0.009

[a] Reprinted (23) p. 296)

[b] The small endothermic values obtained for enamel may be artifacts induced because of lack of sufficient surface area.

Table 3 (23)

APPARENT HEATS OF WETTING AND HEATS OF REACTION OF DENTIN (400 MESH) IN AQUEOUS SOLUTION

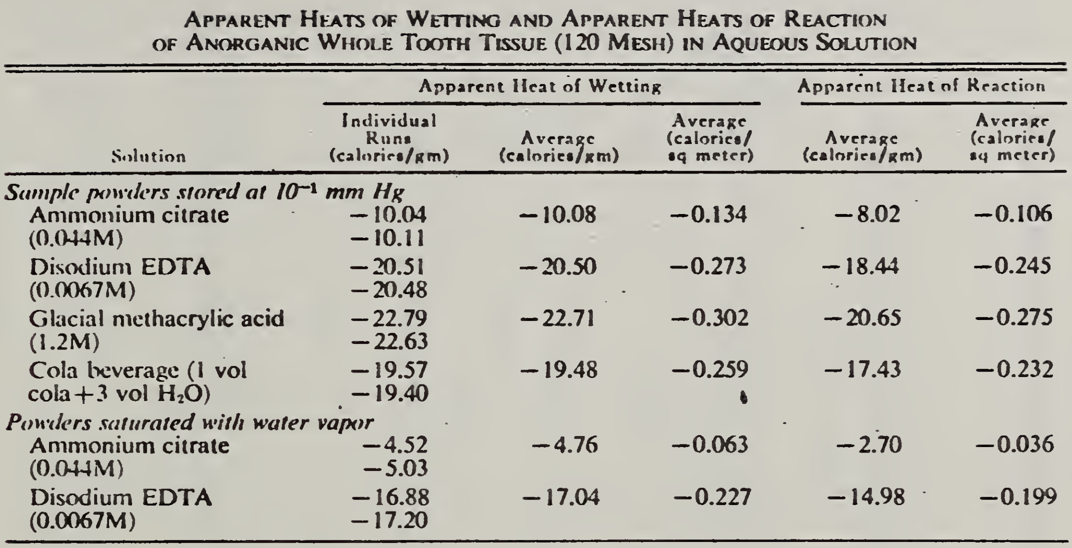
Solution	Apparent Heat of Wetting			Apparent Heat of Reaction	
	Individual Runs (calories/gm)	Average (calories/gm)	Average (calories/sq meter)	Average (calories/gm)	Average (calories/sq meter)
Sample powders stored at 10^{-1} mm Hg					
Ammonium citrate (0.044M)	−45.52 −46.15 −45.27	−45.65	−4.43	−35.35	−3.37
Disodium EDTA (0.0067M)	−25.99 −25.86	−25.92	−2.46	−15.64	−1.49
Malonic acid (0.02M)	−56.54 −55.48	−56.01	−5.33	−45.72	−4.35
Ascorbic acid (0.56M)	−39.37 −39.60	−39.48	−3.76	−29.20	−2.78
Citric acid (0.047M)	−42.40 −42.75	−42.58	−4.05	−32.29	−3.07
Glacial methacrylic acid (1.2M)	−66.29 −66.06	−66.18	−6.30	−55.89	−5.32
Methyl methacrylate (0.094M)	−9.87 −9.78	−9.82	−0.945	negligible	negligible
Cola beverage (1 vol cola + 3 vol H_2O)	−17.91 −18.00	−17.96	−1.70	−7.66	−0.729
Powders saturated with water vapor					
Ammonium citrate (0.044M)	−16.25 −15.83	−16.04	−1.52	−15.73	−1.49
Disodium EDTA (0.0067M)	−8.01 −7.62	−7.82	−0.743	−7.50	−0.714

Table 4 (23)

APPARENT HEATS OF WETTING AND APPARENT HEATS OF REACTION OF ENAMEL (400 MESH) IN AQUEOUS SOLUTION

Solution	Apparent Heat of Wetting			Apparent Heat of Reaction	
	Individual Runs (calories/gm)	Average (calories/gm)	Average (calories/sq meter)	Average (calories/gm)	Average (calories/sq meter)
Sample powders stored at 10^{-1} mm Hg					
Ammonium citrate (0.044M)	−10.51 −10.47	−10.49	−2.68	−11.07	−2.83
Disodium EDTA (0.0067M)	−17.52 −17.95	−17.74	−4.54	−18.32	−4.69
Glacial methacrylic acid (1.2M)	−19.63 −19.10	−19.36	−4.96	−19.95	−5.11
Methyl methacrylate (0.094M)	+0.86 +0.56	+0.71	+0.18	negligible	negligible
Cola beverage (1 vol cola + 3 vol H_2O)	−16.78 −16.45	−16.62	−4.26	−17.20	−4.41
Powders saturated with water vapor					
Ammonium citrate (0.044M)	−2.49 −2.29	−2.39	−0.61	−2.46	−0.63
Disodium EDTA (0.0067M)	−8.91 −8.90	−8.90	−2.28	−8.98	−2.30

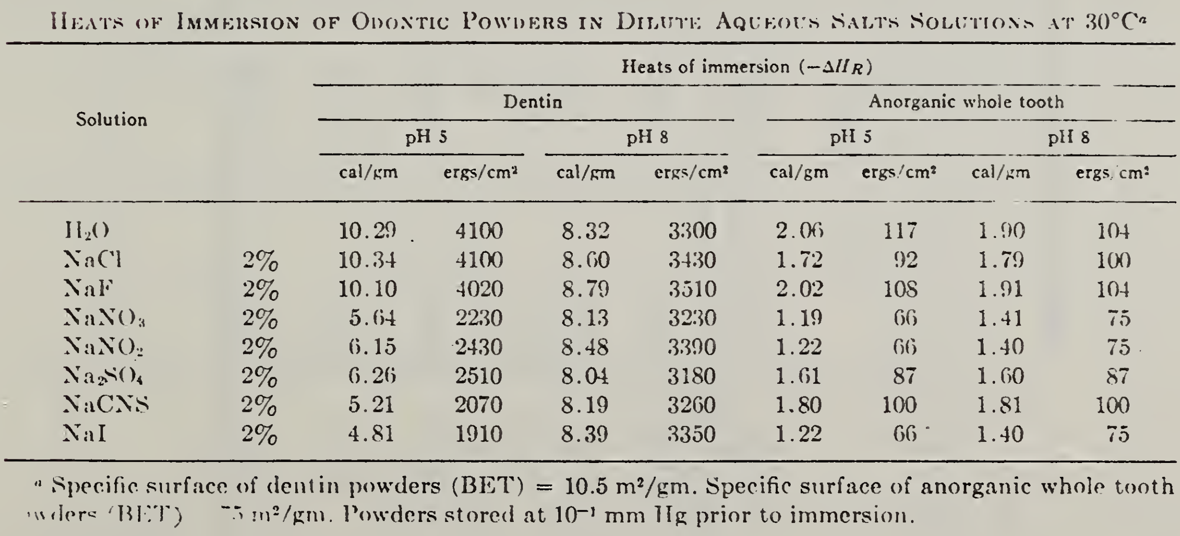
Table 5 (23)



APPARENT HEATS OF WETTING AND APPARENT HEATS OF REACTION OF ANORGANIC WHOLE TOOTH TISSUE (120 MESH) IN AQUEOUS SOLUTION

Solution	Apparent Heat of Wetting: Individual Runs (calories/gm)	Apparent Heat of Wetting: Average (calories/gm)	Apparent Heat of Wetting: Average (calories/sq meter)	Apparent Heat of Reaction: Average (calories/gm)	Apparent Heat of Reaction: Average (calories/sq meter)
Sample powders stored at 10^{-1} mm Hg					
Ammonium citrate (0.044M)	−10.04 −10.11	−10.08	−0.134	−8.02	−0.106
Disodium EDTA (0.0067M)	−20.51 −20.48	−20.50	−0.273	−18.44	−0.245
Glacial methacrylic acid (1.2M)	−22.79 −22.63	−22.71	−0.302	−20.65	−0.275
Cola beverage (1 vol cola+3 vol H_2O)	−19.57 −19.40	−19.48	−0.259	−17.43	−0.232
Powders saturated with water vapor					
Ammonium citrate (0.044M)	−4.52 −5.03	−4.76	−0.063	−2.70	−0.036
Disodium EDTA (0.0067M)	−16.88 −17.20	−17.04	−0.227	−14.98	−0.199

Table 6 (24)



HEATS OF IMMERSION OF ODONTIC POWDERS IN DILUTE AQUEOUS SALTS SOLUTIONS AT 30°C[a]

Solution		Dentin pH 5 cal/gm	Dentin pH 5 ergs/cm²	Dentin pH 8 cal/gm	Dentin pH 8 ergs/cm²	Anorganic whole tooth pH 5 cal/gm	Anorganic whole tooth pH 5 ergs/cm²	Anorganic whole tooth pH 8 cal/gm	Anorganic whole tooth pH 8 ergs/cm²
H_2O		10.29	4100	8.32	3300	2.06	117	1.90	104
NaCl	2%	10.34	4100	8.60	3430	1.72	92	1.79	100
NaF	2%	10.10	4020	8.79	3510	2.02	108	1.91	104
$NaNO_3$	2%	5.64	2230	8.13	3230	1.19	66	1.41	75
$NaNO_2$	2%	6.15	2430	8.48	3390	1.22	66	1.40	75
Na_2SO_4	2%	6.26	2510	8.04	3180	1.61	87	1.60	87
NaCNS	2%	5.21	2070	8.19	3260	1.80	100	1.81	100
NaI	2%	4.81	1910	8.39	3350	1.22	66	1.40	75

Heats of immersion ($-\Delta H_R$)

[a] Specific surface of dentin powders (BET) = 10.5 m²/gm. Specific surface of anorganic whole tooth powders (BET) = 75 m²/gm. Powders stored at 10^{-1} mm Hg prior to immersion.

TABLE 7

Advancing Contact Angles Using Plane Tooth Surfaces (39)

Liquid	γ_{lv^o}	Contact Angle, θ in Degrees							
		Human Enamel		Human Dentin				Whale Dentin	
		Untreated	8%SnF$_2$	Untreated	EDTA Decalcified	8%SnF$_2$	EDTA Decalcified 8%SnF$_2$	Untreated	8%SnF$_2$
	dyne /cm								
Water	72.6	40.0	90.9	45.3	45.1		91.1	45.5	91.6
Glycerol	63.4	45.6	77.3	44.6	44.5	76.9	77.4	44.5	76.9
Formamide	58.2	37.6	64.2	37.6	38.1	64.3	63.2	38.0	65.0
Thiodiglycol	54.0	27.6	59.9	33.6	32.8	59.2	57.1	35.0	59.6
Methylene iodide	50.8	38.1	47.2	36.7	38.2	48.2	47.0	37.5	48.3
1-Bromonaphthalene	44.6	16.1	32.7	16.8	17.8	31.9	32.5	17.7	32.8
Critical Surface Tension Range (dyne/cm)		40.0-55.6	37.5-40.7	40.3-51.1	35.4-46.4	37.7-39.8	37.7-40.7	40.3-50.9	37.8-39.8

TABLE 8

The Work of Adhesion between Pure Liquids and Plane Tooth Surfaces (39)

Liquid	γ_{lv^o}	$\gamma^d_{lv^o}$[a]	Human Enamel		Human Dentin		Whale Dentin	
				[d] γ_{sv^o} = 41.4 dyne/cm		γ_{sv^o} = 41.7 dyne/cm		γ_{sv^o}=41.4 dyne/cm
			W_A[b]	W_A^P[c]	W_A	W_A^P	W_A	W_A^P
	dyne/cm	dyne/cm	J/cm^2	J/cm^2	J/cm^2	J/cm^2	J/cm^2	J/cm^2
Water	72.6	21.8[e]	128.2 x 10^{-7}	65.0 x 10^{-7}	123.8 x 10^{-7}	58.3 x 10^{-7}	123.5 x 10^{-7}	60.3x10^{-7}
Glycerol	63.4	37.0[e]	107.8	29.4	108.6	29.9	108.6	30.2
Formamide	58.2	39.5[e]	104.3	24.0	104.3	23.1	104.1	23.2
Thio-diglycol	54.0	38.5	101.9	22.0	99.0	18.8	98.2	18.3
Methylene iodide	50.8	--	90.8	--	91.5	--	91.1	--
1-Bromo-naphthalene	44.6	--	87.4	--	87.3	--	87.1	--

[a] $\gamma^d_{lv^o}$ = Free surface energy (or surface tension) at the liquid-saturated vapor interface resulting from van der Waals dispersion forces.

[b] W_A = Total reversible work of adhesion.

[c] W_A^P = Reversible work of adhesion due to interaction between non-dispersion forces.

[d] $\gamma^d_{sv^o}$ = Free surface energy (or surface tension) at the solid-saturated vapor interface resulting from van der Waals dispersion forces.

[e] Data taken from ref. 44 .

TABLE 9

Contact Angles between Water and Plane Tooth Surfaces Treated with 0.02% Lactic Acid (39)

Length of Acid Treatment	Contact Angle θ in Degrees				
	Human Enamel	Pretreated with 8% SnF_2	Human Dentin	Pretreated with 8% SnF_2	Whale Dentin
hr.					
0.5	25.2	72.6	47.5	72.2	56.9
1	19.5	69.7	42.5	70.0	49.2
2.5	14.7	--	40.7	--	48.2
4	8.5	--	39.5	--	48.7
5	Spreads	--	38.0	--	50.1
5.5	--	69.5	--	69.3	--
8	Spreads	--	35.4	--	52.6
11	"	--	34.4	--	54.0
24	"	--	41.8	--	52.5
25	--	69.7	--	68.7	--
44	Spreads	--	47.0	--	49.1
83	--	23.3	--	60.5	--
85	Spreads	--	49.7	--	49.3
119	--	20.8	--	60.2	--
225	--	11.1	--	49.2	--
271	--	Spreads		--	--

Table 10

EFFECT OF SURFACE PRETREATMENT ON ADHESION OF ACRYLIC RESTORATIVES TO BOVINE ENAMEL (58)

Surfaces treated twice for 10 seconds.

Treatment	Bond Strength* Mean kg/cm^2	Bond Strength* S.D. kg/cm^2
None	0	0
5% Phosphoric acid	22	22
50% Phosphoric acid	49	27
50% Formic	57	26
20% Citric acid	48	
5% Succinic acid	20	13
5% Lactic acid	39	21
20% Lactic acid	61	21
20% Lactic acid	63†	21
42.5% Lactic acid	58	12
100% Methacrylic acid	0	0
5% Dihydroxymaleic acid	43	21
5% Tartaric acid	33	13
5% Dihydroxytartaric acid	66	18
5% 1,2,3-Propanetricarboxylic acid	29	17
5% 1,3,5-Pentanetricarboxylic acid	51	26
5% *cis*, *cis*, *cis*, *cis*-1,2,3,4-cyclopentanetetracarboxylic dianhydride	60	10
5% 1,2,4-Benzenetricarboxylic acid	36	30
5% Benzenepentacarboxylic acid	56	23
5% Benzenehexacarboxylic acid	0	0
5% Tetrahydrofuran-2-3,4,5-tetracarboxylic acid (TTA)	64	21
5% Tetrahydrofuran-2,3,4,5-tetracarboxylic acid	42‡	18
Sat. sol (<5%) EDTA	7	6
5% Na$_2$ EDTA	21	15
5% Na$_4$ EDTA in 0.001N NaOH	0	0
5% Methacrylate Chromic chloride	24	11

* Mean value of at least 5 specimens stored in water for 24 hours at 37°

† Storage time 43 days

‡ Storage time 30 days

Table 11

Grafting to Steer Hide Collagen

Reaction Time: 3 hours

Monomer	Increase in Weight*	Appearance of Product
	%	
Ethyl acrylate	82	powder
Butyl acrylate	11	powder
2-Ethylhexyl acrylate	14	powder
Methyl methacrylate	81-103	powder
Ethyl methacrylate	75	powder
Isobutyl methacrylate	87	loose mat
Lauryl methacrylate	6	loose mat
Ethylene dimethacrylate	41	loose mat
1,3-Butylene dimethacrylate	58	loose mat
Hydroxyethyl methacrylate	26	powder
Glycidyl methacrylate	92	powder
t-Butylaminoethyl methacrylate	3	loose mat
Dimethylamionoethyl methacrylate	16	tough film
Vinyl acetate	21	film

* After extraction of homopolymer

Table 12

Grafting to Steer Hide Collagen

Reaction Time: 3 hours

	Increase in Weight*
	%
Styrene	14
Acrylonitrile	2
Chloroacrylonitrile	49
Vinylpyrrolidone	12
Vinylpyridine	9

* After extraction of homopolymer with appropriate solvent

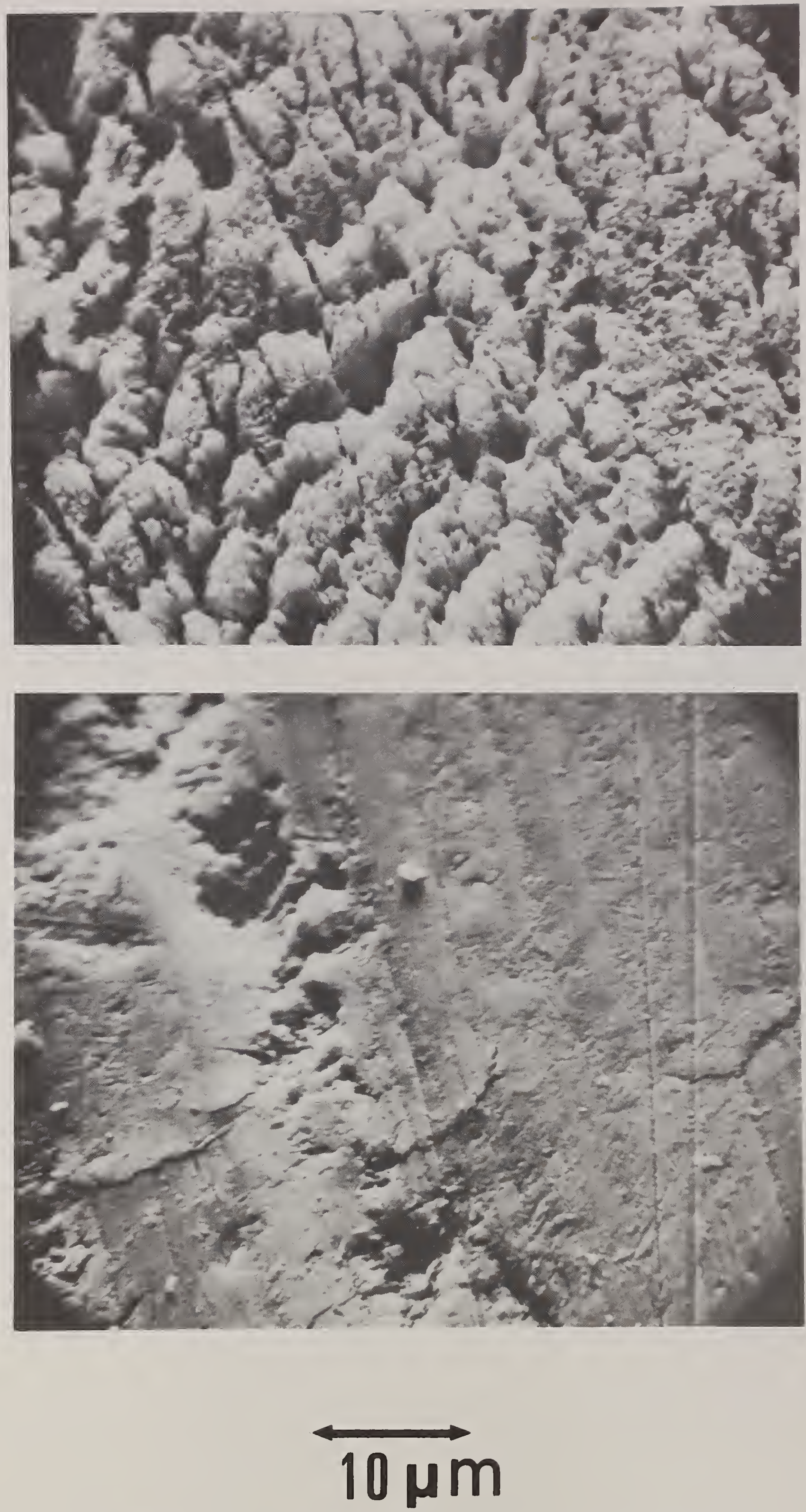


Fig. 1: Scanning electron microscope photograph of bovine enamel surface. Bottom: before acid treatment; top, after two 30-second applications of 5% aqueous tetrahydrofuran -2,3,4,5-tetracarboxylic dianhydride. (105).