EFFECTS OF DEHYDRATION ON THE MODULUS OF ELASTICITY OF DEMINERALIZED HUMAN DENTIN

EFEITOS DA DESIDRATAÇÃO NO MÓDULO DE ELASTICIDADE DA DENTINA HUMANA DESMINERALIZADA

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Dentin bars of approximately 0.7 X 0.7 X 5.0 mm were obtained from the crown of human third molars. The specimens had their ends covered with nail varnish and were subjected to demineralization in 0.5 M EDTA for 72 hours. The specimens were stressed in tension, griped by their mineralized ends. The load/displacement data was transformed to stress/strain data using the original cross-sectional area and the length of the demineralized area (gauge-length). A low strain (0-2%) and the maximum moduli were calculated for specimens tested in water (control), dehydrated in acetone and in HEMA and air-dried. All dehydration procedures caused significant stiffening of the collagen network. Air-dried specimens were the most stiffer, followed by acetone-dehydrated and HEMA-dehydrated specimens. The stiffening effect of organic solvents on acid-etched dentin, may alter the permeability of the collagen network to adhesive resins.

Uniterms: Dentin, permeability; Dentin, elasticity.

INTRODUCTION

Dentin can be regarded as a biological composite made up of a collagen fibers network, filled and reinforced by apatite mineral crystals. Most of the difficulties encountered when attempting to achieve a strong bond to this substrate may be due to the complex properties of the dentin matrix. Although dentin cannot be selectively etched as is done with enamel, acidic solutions can be used to remove the mineral component of dentin and to expose the underlying collagen network. Once the collagen matrix is exposed, resin monomers can be infiltrated into the spaces around the fibers and, upon polymerization, create micromechanical retention with the collagen fibers (NAKABAYASHI; KOJIMA; MASUHARA[8], 1982). This creates a hybrid layer composed of collagen and resin

and results in the most effective dentin bonding mechanism currently known. Since the effects of hybrid layer formation on the resultant bond strength of resins to dentin can be evaluated by simple analysis of bond strength and microleakage tests, little attention has been focused on the effects of the bonding procedures on the physical properties of the substrate. Acid etching of dentin removes its stiff mineral component which should result in significant changes in its mechanical behavior. Mineralized human dentin has been reported to have an ultimate tensile strength (UTS) of approximately 90-100 MPa and elastic modulus (E) of about 13 GPa (SANO et al.[10], 1994). Once dentin is completely demineralized, the UTS falls to approximately 30 MPa and E to 0.2 GPa (SANO et al.[10], 1994). However, some of the original mechanical properties of dentin can be recovered or even improved if the mineral phase that was removed by acid-etching is replaced by infiltrated resin (SANO et al.[11], 1995) during the hybrid layer formation. In clinical bonding, before the adhesive resin is brushed on the dentin surface, it receives several treatments, i.e., water rinsing, air-drying and resin-primers application. These are intended to improve the hybrid layer formation and consequently the bond strength. Most of the primer solutions used with the current dentin bonding systems are resin monomers mixed with organic solvents. Among those, 2-hydroxyethylmethacrylate (HEMA) is a representative of the resin monomers and acetone is a frequently used solvent. Once they are applied to acid-etched and rinsed dentin, they must replace the water content between the collagen fibers, resulting in a chemical dehydration of the collagen network. Some adhesive systems recommend air-drying the dentin after acid-etching and rinsing, resulting in a physical dehydration of the collagen network during this procedure. The dehydration of the exposed collagen fibers during the bonding procedures may induce physical changes that may alter subsequent hybrid layer formation and the resultant bond strength.

The objective of this study was to evaluate the effects of air-drying, acetone and HEMA on the modulus of elasticity of demineralized human dentin.

MATERIALS AND METHODS

Extracted human third molars stored at 4°C in saline containing 0.2% sodium azide were used in this study. The teeth had their cusps ground down on 240-grit SiC paper under water rinsing to create a flat surface (Buehler, Lake Bluff, IL). They were mounted upside down on plexiglas cylinders with sticky wax and the roots were cut off perpendicular to the long axis of the tooth, at the dentin-cementum junction, with a diamond blade (Isomet, Buehler, Lake Bluff, IL, USA) under water irrigation. The pulpal side of the resulting crown segment was ground flat until the pulp horns were no longer visible. By mounting the plexiglas cylinder in the Isomet saw in such a manner that the flat dentin surface was perpendicular to the blade, several parallel cuts, approximately 0.7 mm apart and 1.5 mm deep, were done along the dentin surface. Next the plexiglas cylinder was remounted in the original position (used when the roots were cut off) and two additional cuts, 0.7 mm apart, were performed perpendicular to the long axis of the tooth, starting from the flat dentin surface. In this manner, several dentin bars of approximately 0.7 X 0.7 X 5.0 mm could be obtained from a single tooth crown (Figure 1).

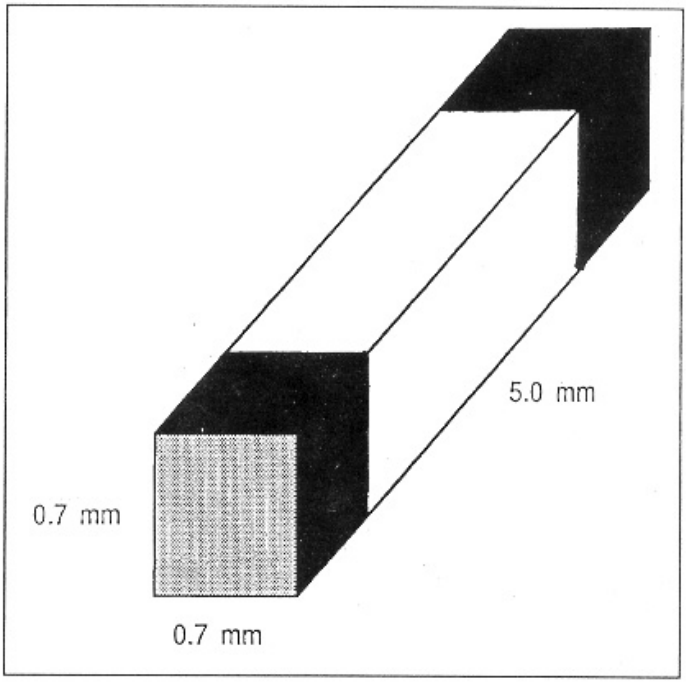

FIGURE 1- Schematic view of the shape of the dentin specimens used. The ends were covered with nail varnish and the middle (2.0 mm) area was exposed to the demineralizing solution

The dentin bars had their ends covered with nail varnish leaving approximately 2.0 mm of dentin exposed in the middle. They were then immersed individually in 20 ml of 0.5 M EDTA containing 0.05 mM phenylethyl sulphonyl fluoride (pH 7.0) for 72 hours to demineralize. The specimens were removed from the EDTA solution and

washed in distilled water for 2 hours. The excess varnish covering the demineralized areas was carefully removed and the length, width and thickness of the demineralized area was measured with a digital caliper (Sylvae Ultra-Cal II, Fowler, Co., Inc., Newton, MA,) under a light microscope to avoid compression of the softened surfaces. This was done while the specimens were immersed in water to avoid premature dehydration of the specimens. The length of the demineralized zone was carefully measured and was used as the gauge length. The width and thickness of each specimen was recorded to the nearest 0.01 mm. The specimens were then randomly divided into groups according to the following dehydration procedures:

Dehydration procedures

Acetone dehydrated specimens were subjected to three sequential immersions in 20 ml of fresh 100% acetone of 15 minutes each. HEMA dehydrated specimens were subjected to the same regimen but 100% HEMA was used instead of acetone. Air-dried specimens were removed from the water and allowed to air-dry at room temperature and RH (22°C and 44%, respectively) for 30 minutes, immediately before testing. Specimens that were not dehydrated were tested as the control group.

The modulus of elasticity of the experimental and control groups were obtained by stressing the specimens in tension at 0.6 mm/sec in a Vitrodyne Testing Machine (Vitrodyne V 1000, Chatillon, NC). The specimens were firmly griped in the machine by the mineralized ends leaving the demineralized area free (gauge length). The chemically dehydrated specimens and the control specimens (water) were stressed while fully immersed in their respective solvents. The air-dried specimens were removed from the water, gently blot-dried with filter paper and mounted in the testing machine. The griped ends were gently tightened and the specimens were allowed to air-dry for 30 minutes. Then, the grips were loosened to permit shrinkage stresses to be relieved and the grips were firmly tightened again before testing. The load/displacement data was simultaneously collected in a computer coupled to the testing machine. The stress was then calculated in MPa by dividing the load by the original cross-sectional area and the strain calculated based on each individual gauge length and expressed as percent strain. Stress-strain curves were plotted for each specimen and a low-strain (0-2%) modulus and the highest modulus calculated. Significant changes in the moduli were tested by ANOVA and Student-Newman-Keuls test.

RESULTS

The effects of dehydration on the modulus of elasticity of demineralized dentin specimens as compared to the water-immersed control specimens are presented in Table 1. At low-strain (0-2%), immersion in 100% HEMA increased the apparent modulus of elasticity of demineralized dentin about 10-fold above that measured in water. Acetone caused demineralized dentin to become 20-fold stiffer than was seen in water, and 30 minutes of air-drying increased the apparent modulus of demineralized dentin approximately 45-fold when compared to the control, water-immersed group. The maximum (i.e. high strain) modulus observed with the control group was significantly higher than its low-strain apparent modulus (Table I, $p < 0.05$). The maximum moduli of acetone- and HEMA-dehydrated specimens were not significantly higher than their respective low-strain moduli (Table I). The maximum modulus of air-dried specimens was statistically significant higher than the respective low-strain modulus. Figure 2 shows representative stress/strain curves calculated and plotted for each one of the four groups. More significant changes in the modulus of elasticity of demineralized dentin occurred at the very low strain magnitudes. All the dehydration procedures caused significant stiffening of the collagen fibers.

DISCUSSION

The main finding in this study was that demineralized dentin becomes stiffer when dehydrated by either physically by air-drying or chemically by immersion in water-miscible organic solvents. In clinical bonding with some of the current dentin bonding systems, dentin is acid-etched, rinsed and then dried with air blasts. This has been shown to cause a collapse of the exposed collagen network that may interfere with the infiltration of the resin monomers to form a hybrid layer (SUGISAKI [12], 1991; PASHLEY et al.[9], 1993). When demineralized dentin is air-dried, the water that replaced the mineral content that was removed by acid-etching, quickly evaporates. During evaporation, the surface tension between air and water generates forces that act to collapse the collagen fibers down to a residual volume of approximately 30% of its original volume (CARVALHO et al.[1], 1995). This shrinkage is mainly a result of the loss of the unbound water surrounding the collagen fibers. It is not likely that air-drying at room temperatures would be able to remove the bound water within the collagen fibrils. Therefore, the shrinkage that is

observed is probably due to the spaces around the collagen fibers becoming smaller rather than a decrease in the diameter of the individual fibers. Air-drying caused a dramatic increase in the modulus of elasticity of our specimens, particularly within the low strain range (Figure 2). This stiffening effect occurs when dentin collagen is in a collapsed state which lowers the permeability of the matrix to adhesive resin monomers. Accordingly, many current dentin bonding systems recommend the so called "wet bonding" technique (KANCA [5], 1992). In this case, the dentin surface is not air-dried after acid-etching. It is kept moist and a primer solution, which usually contains organic solvents, is applied in an attempt to "chase" and replace the water around the collagen fibers. This chemical dehydration is accomplished by water diffusion into the water-free, water-miscible solvents applied to the demineralized dentin. As the concentration of the organic solvents increases around the collagen fibers, the water around them diffuses out as the solvents diffuse in, rendering the collagen fibers surrounded by the organic

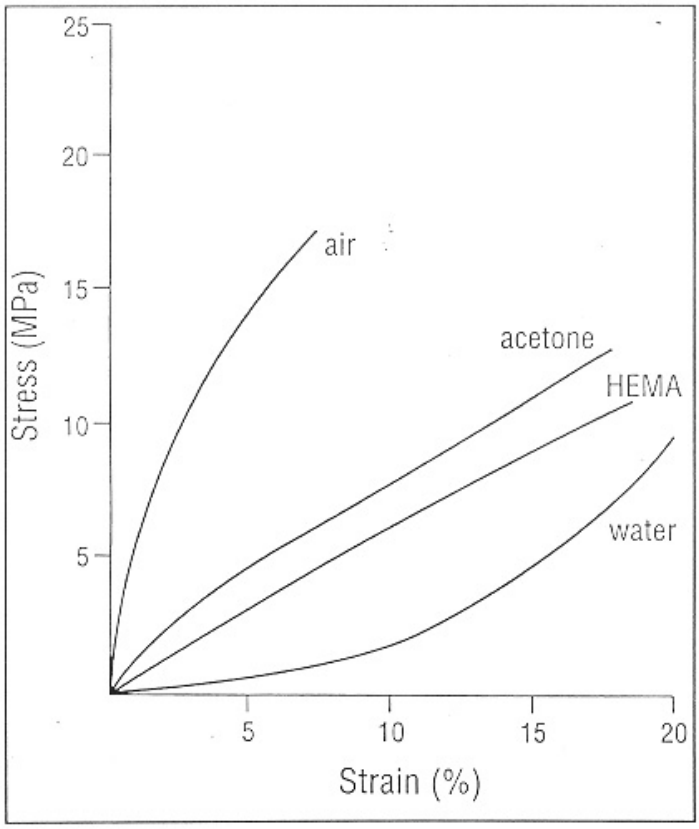

FIGURE 2- Representative stress/strain curve of the control, water-immersed group as compared to the dehydrated groups. Note that the most significant changes in modulus occurred at low-strain (0-2%)

solvent. In this study, we demonstrated that acetone-dehydration of demineralized dentin specimens caused stiffening of the collagen matrix (Figure 2). If acetone-dehydrated specimens were then allowed to air-dry, the stiffening collagen and the much lower surface tension of acetone with air would permit acetone evaporation without causing the collagen network to collapse (CARVALHO et al.[2], 1995). The demineralized dentin collagen is thus stiffened in an expanded spongy-state. Ethanol is also commonly used as solvent in dentin bonding systems. It has been recently shown to increase the elastic modulus of demineralized dentin matrix in a manner similar to that of acetone (MACIEL et al.[7], 1995). Resin monomers such as HEMA are usually components of the adhesive-primer solutions. They are infiltrated into the demineralized dentin concomitantly with the organic solvents. Later, after evaporation of the organic solvents, the adhesive resin monomers polymerize and reinforce the demineralized zone forming the hybrid layer (NAKABAYASHI; KOJIMA; MASUHARA[8], 1982; SANO et al.[11], 1995). In this study, we studied the effects of 100% HEMA alone on the mechanical properties of demineralized dentin matrix. Dehydration of our specimens by substitution of water by HEMA also caused an stiffening effect on the collagen fibers (Figure 2). The stiffening effect was also caused by the removal of water, similar to what was observed with acetone. These results confirm previous work employing the cantilever technique for measuring modulus of demineralized dentin specimens (MACIEL et al.[7], 1995).

Calculations of the modulus of elasticity require information of the gauge length and cross-sectional area of the specimens. Our gauge-lengths were individually measured as the length of the demineralized area after scrapping off the nail varnish. The original, demineralized water-filled cross-sectional area was used in the calculations. Although the cross-sectional area of the air-dried specimens was much smaller (due to shrinkage) than those chemically dehydrated, the area occupied by collagen fibers was the same. The difference resides on the fact that chemically-dehydrated specimens were stiffened in an expanded state and air-dried specimens stiffened in a shrunken state. The amount of collagen is the same for all the specimens and it is the collagen that is responsible for the modulus of elasticity that was measured. Nevertheless, corrected calculations can be done. For example, if air-dried collagen had shrunk to 30% of the original measured volume, the values obtained for stress utilizing the original cross sectional area could be corrected by multiplying the value by 3.3. This corrects the stress to that portion of the cross-sectional area occupied by collagen. The corrected

values of stress would be approximately 3-fold higher as would the corrected modulus of elasticity which is based on the stress.

We have previously studied the effects of organic solvents, resin monomers and aldehydes on the modulus of elasticity of demineralized dentin, by using a cantilever technique (MACIEL et al.[7], 1995). The tensile technique employed in this study offers advantage over the cantilever technique regarding measurement of air-dried specimens. The tensile technique is less sensitive to the dimensional changes that occur during shrinkage of the specimens. Conversely, the cantilever technique offers the advantage of being a non-destructive technique. This allowed repeated measurements of the effects of the treatments on the same specimens but at relatively low strains (0-2%). Comparisons of the effects of the various treatments on the modulus of demineralized dentin matrix at low strains (0-2%) were very good between our study and the previous one (MACIEL et al.[7], 1995). In our study, the specimens were subjected to only one solution during testing. MACIEL et al.[7] (1995), performed repeated measurements of apparent moduli on the same specimens which had been sequentially exposed to water, ethanol and HEMA. This may have caused structural changes in the collagen network. Our study reflects the changes in the modulus of demineralized dentin matrix which had occurred after 30 minutes of exposure to air, acetone or HEMA without the confounding effects of previous treatments. If the specimens had been subjected to longer periods of dehydration, the moduli would have increased even more (MACIEL et al.[7], 1995).

Glutaraldehyde is a well-known cross-linking agent of collagen fibers. It fixes and stiffens collagen by reacting with free amino groups of arginine and lysine side chains (GAGE[3], 1989). This fixative effect increases the modulus of elasticity of the collagen matrix of dentin and has been shown to be irreversible in the presence of water (MACIEL et al.[7] 1995). The reversibility of the stiffening effects promoted by organic solvents and resin monomers when collagen is immersed in water indicates that the ability of dehydration to fix or stiffen the collagen matrix is due to water removal *per se* rather than an effect of the agents themselves. In this study, the highest modulus of elasticity was measured after 30 min. of air-drying alone, in the absence of any solvent or monomer. Presumably, the removal of water from between the collagen fibers and eventually from between the collagen fibrils (KASTELIC; GALESKI; BAER [6], 1978) may increase the friction between them, resulting in increased stiffness to strain. Additionally, the absence of water within the collagen fibrils may permit hydrogen bonds to form between collagen molecules that were previously bonded with the water molecules. The collagen molecules within dentin matrix fibers is highly packed. The shrinkage that occurs when the dentin matrix is allowed to air-dry may bring the fibers closer together and thereby impairing intermolecular slippage during straining. When demineralized dentin is in the expanded state, there may be less friction between neighboring collagen fibers than occurs in the collapsed state. This could be classified as inter-fiber friction. However, in either configuration, the ability of collagen fibrils and their molecular substructures to elongate under stress may be similar at any given water content. This could be classified as intra-fiber friction. This could explain why the modulus of elasticity of acetone dehydrated specimens increased to a lower extent than was found with air-dried specimens (figure 2). Acetone- and HEMA-dehydrated specimens do not shrink to the same extent as air-dried specimens (CARVALHO et al.[1,2], 1995). The lower increase in the modulus of elasticity of the HEMA-dehydrated specimens as compared to acetone-dehydrated specimens is probably because HEMA acts as an interfiber lubricant, and facilitates the elongation of adjacent fibers. Acetone and alcohol have been shown to stiffen rat tail tendon while glycerol and ethylene glycol did not (KASTELIC; GALESKI; BAER[6], 1978).

The clinical importance of our findings resides in the fact that during dentin bonding, the treatments imposed to the top 3-5 μm of demineralized dentin cause significant changes in the physical properties of the collagen network that may alter the ability of resins to infiltrated into the substrate. If, after acid-etching and rinsing, the surface is air-dried, the collagen network will stiffen in a collapsed state, thus interfering with resin infiltration and limiting the depth of the hybrid layer. If nonaqueous acetone- or ethanol-containing adhesive resins are then applied, the collagen network will stiffen even more, becoming less permeable for resin infiltration. There is evidence that 100% solution of resin monomers and organic solvents can not re-expand shrunken demineralized dentin (CARVALHO et al.[1], 1995). If, however, the dried shrunken surface is rewetted with water or aqueous mixtures adhesive resins are applied to it, re-expansion may occur and resin permeability is re-established (GWINNETT[4], 1994). Although the effects of wetting or rewetting dentin surfaces with water have resulted in improved bond strengths (KANCA[5], 1992; GWINNETT[4], 1994), the critical amount of water that must be present in the primers to cause the same re-expansion effect is unknown. The ability of water to re-expand shrunken,

stiffened collagen is dependent on its ability to plasticize the collagen and reduce its modulus. We have previously demonstrated that the stiffening effect of acetone, ethanol and HEMA is rapidly eliminated (within 2 min. in our large specimens) upon immersion in water (MACIEL et al.[7], 1995). Ideally, the dentin collagen network should be made stiffer in fully expanded state. This would preserve the open spaces around the fibers which are the channels for resin diffusion. The rationale for the use of water mixtures of solvents and monomers in the primer formulations is that the water may be replaced and, as the solvents and resin monomers diffuse in, stiffen the collagen fibers in expanded state. However, the stiffening of the collagen network caused by organic solvents is reversible. If water is available from dentinal fluid, gingival crevicular fluid, saliva, etc., the increased modulus of elasticity of the collagen fibers may fall rapidly to very low values (ca. 5-10 Mpa) thereby allowing surface tension forces to collapse the network (GWINNETT[4], 1994).

The effects of dehydration on demineralized dentin are remarkable. Physical and chemical dehydration caused significant increases in the modulus of elasticity of dentin collagen matrix (see Table 1 and Figure 2). This was also observed with rat tail tendon collagen. Kato et al. (1989) reported an increase in modulus from 533 MPa for the wet state to 2357 MPa for dry tendon collagen. They dried their specimens much longer than was done in our experiments. Their dry collagen moduli (2357 Mpa or 2.4 Gpa) is in the same order of magnitude of the moduli of the adhesive resins used in bonding. If adhesive resin can infiltrate the collagen network in a stiffened spongy-state and completely envelop the collagen fibers in the demineralized zone, the plasticizing effect of water may be avoided. This would contribute significantly to increase the modulus of elasticity of bonding interfaces. Conversely, if water infiltrates unprotected (i.e. unenveloped) collagen fibers, the modulus will decrease dramatically and the benefits of the stiffening effect would only be useful during the resin-infiltration step. SANO et al.[11] (1995) demonstrated that the ultimate tensile strength of demineralized, resin-infiltrated dentin could be raised to values that were similar or even higher than those measured before demineralization. In their study, however, the specimens were not subjected to further water immersion after resin infiltration. That might have had reduced the properties of collagen fibers if they had been left exposed due to incomplete resin-infiltration. Further research is needed to clarify these issues and to explore the potential for strengthening the interface between adhesive resins and dentin.

TABLE 1- Increases in modulus of elasticity of demineralized human dentin following dehydration. Mean (S.D.). All values are in MPa

	STRAIN %			
Condition (N)	0 - 2 %			Max. Strain
Water (12)	7.7 (2.7)	<<	*	>> 58.4 (16.7)
HEMA (12)	85.3 (62.6)			103.2 (59.7)
Acetone (12)	139.3 (42.6)			174.5 (47.4)
Air (12)	296.8 (91.3)	<<	*	>> 362.0 (49.1)

* Statistically significant ($p < 0.05$). All treatments were statistically significant differents for both 0-2% and maximum strains ($p < 0.05$).

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RESUMO

Barras retangulares de dentina foram obtidas da coroa de terceiros molares humanos e desmineralizadas em sua porção central por 72 horas em 0.5 M de EDTA. Alguns espécimes foram desidratados em acetona, outros em HEMA e outros expostos ao ar. Os espécimes foram testados em tração e os dados de esforço e deformação calculados em função das dimensões originais da área transversal e comprimento da área desmineralizada. O módulo de elasticidade foi calculado para os espécimes desidratados e comparado com espécimes testados em água. Todos os métodos de desidratação causaram um aumento significante no módulo de elasticidade da dentina

desmineralizada. Os espécimes secos em ar sofreram o maior aumento de módulo, seguidos dos espécimes desidratados em acetona e, finalmente, espécimes desidratados em HEMA. A mudança das propriedades mecânicas da dentina desmineralizada, causada pelos solventes orgânicos tais como acetona e HEMA, pode alterar a permeabilidade da área condicionada aos agentes adesivos. Isso pode influenciar a qualidade do processo adesivo à dentina.

UNITERMOS: Permeabilidade da dentina; Elasticidade da dentina.

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