From dry bonding to water-wet bonding to ethanol-wet bonding.
A review of the interactions between dentin matrix and solvated resins
using a macromodel of the hybrid layer

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ABSTRACT: Purpose: To review the use of a new resin-dentin bonding model called the macro-hybrid layer, to quantify resin uptake and matrix shrinkage during resin infiltration and solvent evaporation. A secondary purpose was to introduce the concept of ethanol-wet bonding where water-saturated acid-etched dentin is exchanged with ethanol to create ethanol-saturated dentin. Adhesive monomers seem to penetrate ethanol-saturated dentin more thoroughly than water-saturated dentin. (Am J Dent 2007;20:7-21).

CLINICAL SIGNIFICANCE: Infiltration of solvated resins in demineralized dentin can be quantitatively followed by using disks of completely demineralized dentin. The results obtained from the macro-hybrid layer can be used to predict how well adhesives can bond to dentin. They indicate ethanol-wet bonding may be superior to water-wet bonding.

Introduction

When Nakabayashi et al discovered that resin bonding to dentin involved diffusion of liquid monomers into spaces around collagen fibrils in the demineralized matrix, it provided important new insight into bonding mechanisms. This interdiffusion zone is composed of about 50% collagen matrix and 50% resin, making it a hybrid of two very different materials, therefore, the popularization of the term hybrid layer. Unfortunately, the maximum depth of hybrid layers created by etch-and-rinse adhesives is only about 5 µm, while those produced by mild to moderately aggressive self-etching adhesives are only 0.5-1.5 µm deep. The microscopic size of these interfaces makes them very difficult to study. SEM and TEM studies indicated that hybrid layers created by etch-and-rinse adhesives were often poorly infiltrated with resin and that they often collapsed during bonding procedures. However, there were no methods available for measuring the dynamics of infiltration of dentin matrices by solvated monomers or the response of the matrix to different concentrations of monomers or solvents. Too often, investigators believe that the dentin matrix is a relatively inert, stable collagen scaffold that does not interact with solvated monomers. The work described in this review reveals that the dentin matrix is very sensitive to solvents, monomers, air-drying, and other bonding procedures.

Macro-hybrid layer model

We recently developed what has become known as the macro-model of the hybrid layer by creating 0.2 mm thick (i.e. 200 µm) disks of mid-coronal dentin from human extracted third molars. These dentin disks are completely demineralized in either 0.5 M EDTA (pH 7.4) or 37% phosphoric acid, leaving a soft, demineralized dentin matrix. A thin coating of a viscous cyanoacrylate is placed on their bottom sides to glue them to the floor of aluminum wells placed beneath the contact probe of a linear variable differential transformer (LVDT) of a thermal mechanical analyzer (Model TMS-2) (Fig. 1). A weight pan on top of the LVDT permits application of known weights to the macro-hybrid layer, to permit measurements of its indentation stiffness. The extra weight is then removed so that less than 0.01 N of force is normally applied to the matrix. This is necessary to keep the probe in contact with the specimen when it shrinks. Beginning with the well filled with water, the LVDT probe is placed in contact with the demineralized specimen to measure the height of the fully expanded matrix
Fig. 2A. Shrinkage in the matrix height of demineralized dentin when dried (left, N₂), rehydrated (water), and reshrunk (N₂). From the shrunken state, a number of polar solvents were evaluated for their ability to re-expand dried, shrunken matrix. 100% HEMA was ineffective; butanol caused a slight expansion; propanol slowly expanded the matrix but ethanol, ethylene glycol and formamide were all moderately effective. Methanol was almost as effective as water at fully expanding dried matrices (from Pashley et al., with permission).

Fig. 2B. Scanning electron micrograph of human dentin that was acid-etched with 37% phosphoric acid for 15 seconds, rinsed with water and then briefly air-dried. Note the disappearance of spaces between the collagen fibrils in the top 1 µm of the demineralized zone that extends 5 µm below the surface. Although liquid monomers can easily flow down open tubules, they cannot easily penetrate through intertubular dentin. Note the crust-like appearance of the collapsed surface collagen fibrils between the white arrows. TL = tubule lumen, ID = intertubular dentin, CF = collapsed collagen fibrils.

Fig. 2C. The effects of different polar solvents on the modulus of elasticity of demineralized dentin. The ability of each solvent to compete with collagen hydrogen bonding is determined by its Hoy's solubility parameter for hydrogen bonds ($\delta_h$). Acetone, with a $\delta_h$ value of 11 (J/cm³)½, cannot prevent the spontaneous stiffening of dentin matrix that occurs when acetone removes water from water saturated matrix. Water, with a $\delta_h$ value of 40 (J/cm³)½, prevents all interpeptide H-bonding. Ethanol with a $\delta_h$ of 20 prevents some but not all interpeptide hydrogen bonding.

Fig. 2D. Schematic of dry collapsed dentin matrix collagen peptides on the left that are stiff because of interpeptide H-bonds. In water-saturated dentin matrices, water molecules cluster around those functional groups in collagen peptides that can H-bond. The $\delta_h$ value of water is so high [40 (J/cm³)½], and its concentration is so high (55 mol/L), that no interpeptide H-bonds can form. Such water-saturated matrices are completely expanded and very soft (i.e. modulus of elasticity of 1-2 MPa).}

**Interpeptide hydrogen bonds**

Our work has shown that chemical or physical dehydration of dentin causes acid-etched dentin matrices to increase their face. When this occurs, collagen fibrils will not become coated by 20-30 nm films of resin, but will remain naked. Since resin cannot infiltrate collapsed matrices, resin retention is very low (i.e. resin-dentin bond strengths are very low) and "adhesive fillings" such as Class V restorations with cavosurface margins completely in dentin may debond and exhibit microleakage and/or dentin sensitivity.
stiffness from 2 MPa to 170 MPa (Fig. 2C). This stiffening effect is rapidly reversed by water. These results were confirmed by more recent work on mouse tail collagen and non-demineralized equine dentin and cortical bone. For parameter for polar forces; hydrogen bonding forces holding collagen peptides in the collapsed, stiff state are about 15 (J/cm$^3$)$^{1/2}$. The higher the $\delta_h$ of a polar solvent above 15, the greater is the degree of expansion (from Pashley et al., 2001, with permission).

Table 1. Hoy's solubility parameters for nonpolar vs. polar solvents.

<table>
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<tr>
<th>Structure</th>
<th>Solution</th>
<th>$\delta_d$</th>
<th>$\delta_p$</th>
<th>$\delta_h$</th>
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<tr>
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$\delta_d =$ Hoy's solubility parameter for dispersion forces; $\delta_p =$ Hoy's solubility parameter for polar forces; $\delta_h =$ Hoy's solubility parameter for hydrogen bonding forces, all in (J/cm$^3$)$^{1/2}$.

hydrogen bonds (H-bonds). Water easily re-expands (Fig. 2A) to a collapsed state, re-expanding to a stiff state (Fig. 2C). This stiffening effect is rapidly reversed by water. These results were confirmed by more recent work on mouse tail collagen and non-demineralized equine dentin and cortical bone. For parameter for polar forces; hydrogen bonding forces holding collagen peptides in the collapsed, stiff state are about 15 (J/cm$^3$)$^{1/2}$. The higher the $\delta_h$ of a polar solvent above 15, the greater is the degree of expansion (from Pashley et al., 2001, with permission).

Table 2. Hoy's solubility parameters of collagen and adhesive primers and monomers.

<table>
<thead>
<tr>
<th>Substance</th>
<th>$\delta_d$</th>
<th>$\delta_p$</th>
<th>$\delta_h$</th>
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Values are all (J/cm$^3$)$^{1/2}$. Abbreviations: HEMA - 2-hydroxyethyl methacrylate; TEGDMA - triethylene glycol dimethacrylate; Bis-GMA - 1:2 addition product of bisphenyl-A diglycidyl ether and methacrylic acid; $\delta_d =$ Hoy's solubility parameter for dispersion forces; $\delta_p =$ Hoy's solubility parameter for polar forces; $\delta_h =$ Hoy's solubility parameter for hydrogen bonding forces; $\delta_t =$ Hoy's total solubility parameter, equivalent to Hildebrand's solubility parameter.
Fig. 3A. Transmission electron micrograph of adhesive resin-bonded acid-etched dentin. R= resin tag in dentin tubule orifice. In such thin sections (ca. 90 nm), the adhesive appears transparent. Note the presence of transparent adhesive-filled spaces surrounding collagen fibrils in intertubular dentin. Magnification bar = 1 µm (from Eick et al., with permission).

Fig. 3B. Illustration of the plots that are shown in Figs. 4, 7, 8 and 9. The height of the demineralized dentin matrix is shown on the left, labeled displacement (µm). Starting at the top left with demineralized dentin saturated with water, it is fully expanded. When excess water is removed and dry nitrogen gas is blown on the dentin, it rapidly shrinks, allowing the contact probe to follow its negative displacement. This shrinkage stops when the collagen fibrils condense into a solid mass. If the collapsed, shrunken collagen was left dry, its “height” would remain constant over time (indicated by the lowest horizontal dashed line). If an ethanol-based adhesive is applied to dried, collapsed dentin, and if it can break interpeptide hydrogen bonds, the matrix will begin to expand. The rate and extent of that expansion depends upon the difference between the $\delta_h$ value for the solvated adhesive and the $\delta_h$ of the collagen matrix, which is the intrinsic tendency of the peptides to form interpeptide hydrogen bonds. When the matrix expansion has reached its steady-state height, the dry N2 gas was then turned on again to evaporate the ethanol component of the ethanol/comonomer mixture. This causes a decrease in the height of the matrix. As the matrix collapses, it extrudes some of the resin that was already taken up. The matrix height after solvent evaporation is the net adhesive resin uptake that represents the amount of adhesive that coats each collagen fibril.

Fig. 3C. Summary of dimensional changes in dried, collapsed demineralized matrix that was 200 µm thick in response to A = 100% HEMA (no expansion), B = 10% H2O/90% HEMA (slow 19% expansion), C = 25% H2O/75% HEMA (faster, 33% expansion), D = 50% H2O/50% HEMA (relatively rapid, 59% expansion).

$\delta_h$ values, since it has no ring substitutions that are polar or that can H-bond. When a chlorine is substituted on the ring, the $\delta_h$ values increase because the molecule is more polar. When the benzene ring is substituted with groups that can H-bond, the $\delta_h$ values increase. Hydroxyl groups have much higher $\delta_h$ values than do amino groups. Milller et al. calculated a Hansen interpeptide H-bonding force ($\delta_h$) of about 23.6 (J/cm$^3$)$^{1/2}$ for 30% water/70% collagen peptide mixture. We calculated a Hoy's $\delta_h$ for the same state of collagen of 22.5 (J/cm$^3$)$^{1/2}$ that is nearly the same (Table 2). This is a good estimate of water-saturated matrices but not for dried matrices (Table 2). We calculated a Hoy's $\delta_h$ value of 14.8 (J/cm$^3$)$^{1/2}$ for 0% water/100% collagen peptide. Much of the hydrophilicity of collagen is due to water, not collagen. 2-hydroxyethyl methacrylate (HEMA), a common constituent of dentin adhesives, with a $\delta_h$ value (Table 2) of 15.2 (J/cm$^3$)$^{1/2}$, apparently is too close to the $\delta_h$ of collagen-collagen H-bonding of 14.8 (J/cm$^3$)$^{1/2}$, so 100% HEMA was unable to expand the matrix (Fig. 2A). Ethanol ($\delta_h = 20.0$), methanol ($\delta_h = 24$) and water ($\delta_h = 40.4$) were successful in breaking those interpeptide hydrogen bonds, allowing the matrix to soften to the point that it can expand (Fig. 2A) rapidly with water or methanol, or more slowly with ethanol. When the degree of matrix expansion was plotted against Hoy's $\delta_h$ values, a highly significant correlation ($R^2 = 0.82$, $P < 0.005$) was found with a y intercept of about 15 (J/cm$^3$)$^{1/2}$ that is close to the presumed $\delta_h$ of collagen peptides (Fig. 2F). The problem with ethanol, methanol, or water is they are all volatile and none of them can polymerize. The presence of water in dentin during wet bonding has led to adhesives being blended to make them more hydrophilic by adding HEMA and solvating in ethanol-water mixtures. Water-HEMA mixtures can expand and infiltrate acid-etched dentin well because their $\delta_h$ values are much higher than that of dry collagen (Table 2). This prevents interpeptide H-bonding and maintains wide interfibrillar spaces that serve as the diffusion channels (Table 2) for resin infiltration. One can quantitate monomer uptake using an LVDT in contact with demineralized dentin (see schematic in Fig. 3B for interpretation of LVDT data). When varying concentrations of water were mixed with HEMA (Fig. 3B), the degree of expansion was proportional to the water concentration, not

Fig. 4A. Rate of expansion of dried, collapsed, demineralized dentin by 35% HEMA in 65% water, methanol, ethanol or propanol. Note that 35% HEMA/65% propanol produced no expansion; 35% HEMA/65% ethanol produced slow, partial expansion; 35% HEMA/65% methanol produced rapid, partial expansion; 35% HEMA/65% water produced rapid, complete expansion. However, after reaching an expansion plateau, when the solvents were evaporated, the HEMA/water mixture lost most of its expansion while HEMA/methanol lost the least matrix height. Loss of matrix height indicates loss of resin. The net uptake of resin is the difference between the uptake of solvated resin during the infiltration phase, and the loss of matrix height during the evaporation phase of bonding. Apparently, evaporation of solvents creates stresses on the matrix that can cause matrix collapse under some circumstances (from Eddleston et al, with permission).

Fig. 4B. The relationship between the percent expansion of dried, demineralized dentin and the Hoy's solubility parameter for hydrogen bonding ($\delta_h$) of water and HEMA/alcohol primers. Note that 35% HEMA/propanol ($\delta_h = 16.2$) could not expand the matrix, while HEMA/ethanol ($\delta_h = 18.3$) and HEMA/methanol ($\delta_h = 20.9$) could partially expand the matrix (from Eddleston et al, with permission).

Fig. 4C. Plot of matrix shrinkage upon solvent evaporation versus the pre-evaporation stiffness of the matrix. Water and HEMA/water mixtures prevented interpeptide H-bonding, making the matrix too compliant to resist shrinkage. HEMA/ethanol and HEMA/methanol could not prevent all interpeptide H-bonds from forming, allowing a stiffness of the matrix of 12-13 MPa. This reduced the matrix shrinkage during solvent evaporation to only 16.18% (from Eddleston et al, with permission).

Fig. 4D. The stiffness of dentin matrices just prior to solvent evaporation is compared to the Hoy's solubility parameter for hydrogen bonding ($\delta_h$) of water and 35% HEMA/65% alcohol mixtures. Note that the $\delta_h$ values for primers needs to be about 20 (J/cm$^3$)$^{1/2}$ to develop significant matrix stiffness (from Eddleston et al, with permission).

that even 50% H$_2$O/50% HEMA could not break all interpeptide H-bonds (Fig. 3C, Table 2). Those solvents that can expand the collapsed matrix have solubility parameters for hydrogen bonding ($\delta_h$)$_{28}$ greater than 16 (J/cm$^3$)$^{1/2}$. The higher the $\delta_h$ value above 14.8 (J/cm$^3$)$^{1/2}$, the more rapid the expansion and the greater the extent of expansion. Cohesive energy densities can be ranked by calculating their Hoy's solubility parameters (Table 2, $\delta_u$, $\delta_p$, $\delta_h$ and $\delta_t$)$_{9,10}$ The influence of $\delta_h$ will be discussed later when miscibility issues are covered in fully expanded matrices. Most monomers used in adhesive dentistry have $\delta_h$ values below those of dried dentin (Table 2), calculated to be 14.8 (J/cm$^3$)$^{1/2}$. Thus, in their neat form, such resins cannot expand dried, acid-etched dentin. This is why dry bonding to acid-etched dentin seldom gave shear bond strengths over 5 MPa.$^{33,34}$ Wet bonding$^{15,16}$ expands the dentin matrix maximally because water has a very high $\delta_h$ value of 40 (J/cm$^3$)$^{1/2}$ that breaks all interpeptide H-bonds (Fig. 2D) in the matrix.$^{7,10,11}$ However, not all adhesive monomers are soluble in water. Dimethacrylates, like Bis-GMA, are not water-soluble and can undergo phase changes in water-saturated dentin.$^{10,35}$ This is why most commercial dentin adhesives also contain HEMA, which is an excellent, relatively hydrophilic, nonvolatile, polymerizable solvent for dimethacrylates.

Can water/HEMA mixtures serve as expandable primers? When increasing concentrations of water (i.e. 0, 10, 25, 50 vol%) were mixed with HEMA, their ability to expand dried collapsed dentin increased (Fig. 3C) in proportion to their water concentration,$^7$ giving expansions of 0, 18.7 ± 6.0%, 32.5 ± 7.8% and 58.9 ± 13.4% (Fig. 3C), with 100% water causing 100% expansion. The Hoy's $\delta_h$ values for those water/HEMA mixtures were 15.2, 17.7, 21.5 and 27.8 (J/cm$^3$)$^{1/2}$, indicating the HEMA concentration. Thus, HEMA-containing primers are effective not because of the HEMA, but because of polar solvents used to solvate HEMA. Pure HEMA was unable to expand the matrix at all (Figs. 2A, 3C).

Hybrid layer review
We also investigated the expansion of dried matrices by 35% HEMA solvated in 65% water, ethanol, methanol or propanol (Fig. 4A). HEMA/propanol, with a $\delta_h$ of 16.2 (J/cm$^2$)$^{1/2}$, was unable to expand the matrix at all. HEMA-ethanol, at a $\delta_h$ of 18.3 expanded it slowly but incompletely. HEMA/methanol ($\delta_h$= 20.9) expanded it faster but incompletely. HEMA/water expanded it completely ($\delta_h$ = 31.6).

A plot of the expansion of dried matrices vs. $\delta_h$ of the primers (Fig. 4B) gave a highly significant correlation ($R^2 = 0.90$, $P< 0.025$). Although HEMA/water gave almost complete expansion, the matrices shrank 86% when the water was evaporated, leaving very little HEMA in the matrix (Fig. 4B). Similar, but less dramatic shrinkages occurred when ethanol or methanol was evaporated from the infiltrated HEMA mixtures (Fig. 4B).

Note that the matrix height after solvent evaporation represents the height of the hybrid layers. Hybrid layers can have different heights depending on the net amount of resin retained after solvent evaporation.

Matrix shrinkage and resin retention

The large matrix shrinkage seen in 35% HEMA/65% water infiltrated matrices following water evaporation (Fig. 4C) was due to the ability of water to completely block interpeptide H-bonding thereby softening the matrix. Apparently, small shrinkage forces develop during solvent evaporation. If the matrix is too soft, it cannot resist these forces and collapses, thereby squeezing out the unpolymerized monomers before they can be polymerized. The stiffness of the infiltrated HEMA/alcohol infiltrated matrices was higher$^{10}$ (Fig. 4D) than that of HEMA/water. There was a significant inverse correlation between matrix stiffness and the $\delta_h$ of the infiltrated mixture. Matrix shrinkage that occurred during solvent evaporation was highest when the stiffness was lowest$^{10}$ (Figs. 4C,D). Finally, the higher the $\delta_h$ of the HEMA/solvent mixtures, the greater was the matrix shrinkage, because the matrices were so compliant (Fig. 4D). If the matrix had been pre-stiffened to about 12-13 MPa by allowing partial interpeptide H-bonding to develop, then less matrix shrinkage would have occurred during solvent evaporation. These results were obtained with constant HEMA/solvent concentrations (i.e. 35% HEMA/65% solvent).

In the macro-model of the hybrid layer, the amount of shrinkage of the matrix following air-drying, represents the cumulative shrinkage of a stack of several hundred 60-70 nm diameter collagen fibrils (Table 3) surrounded by 20-30 nm wide interfibrillar spaces. If resin-dentin hybrid layers are to be retentive (i.e. to serve as anchors for resin composite restorations), then resins must be able to diffuse through open interfibrillar channels to the depth of the etch (Fig. 2E). This process is called the infiltration phase of dentin bonding. The use of a contact probe of an LVDT provides quantitative infor-
Fig. 6. Plot of microtensile bond strength versus the width of interfibrillar spaces between collagen fibrils in human dentin primed with 35% HEMA in ethanol, methanol, propanol or water. Significantly lower bond strengths were obtained using 35% HEMA/65% propanol because the interfibrillar spaces (that serve as resin diffusion pathways during infiltration) fell from 30 down to 10 nm wide. The highest bond strengths were obtained using 35% HEMA in 65% ethanol (from Carvalho et al, with permission).

Fig. 7A. Shrinkage of water-saturated demineralized dentin matrix in response to 100% acetone, 100% HEMA, Prime & Bond 2.0, One-Step or Single Bond. All treatments caused a 20-25% shrinkage of the matrix due to water loss from the matrix into the excess solutions. Insert shows schematic of how intertubular dentin could shrink by loss of interfibrillar spaces rather than any change in fibril diameter.

Fig. 7B. Expansion of dried demineralized dentin matrix by various ethanol/HEMA concentrations, with values in (J/cm^2) given in parentheses. Note that 30% ethanol/70% HEMA at a G of 16.7 could not expand the matrix, but that 50% ethanol/50% HEMA at a G of 17.6 could slowly expand the matrix.

Fig. 7C. Expansion of dried demineralized dentin matrix by various methanol/HEMA concentrations, in the same manner as Fig. 7B. Here, all concentrations of methanol/HEMA expanded the matrix, even 30% methanol/70% HEMA (δ = 17.9). Both monomers and solvents are very important in expanding dried matrices.

Tensile bond strength of resins to dentin varies directly with the width of interfibrillar spaces (Table 3). In a study comparing 35% HEMA/65% solvent primers, the highest bond strengths were achieved with 35% HEMA/65% ethanol, rather than water or methanol or propanol (Table 3). During air-drying of 35% HEMA/65% propanol, the interfibrillar spaces become much smaller (Table 3) as did the collagen fibril diameter. As described above, the loss of matrix height is due to the development of new weak associations (polar and hydrogen-bonding forces) between collagen peptides that cause 20-30-fold increases in matrix stiffness. It is important that the matrix has a critical amount of stiffness after comonomer infiltration (ca. 12-15 MPa) to be able to avoid collapse during solvent evaporation. When 35% HEMA was solvated in 65% water, the matrix expanded well and took up much of the resin mixture during infiltration of the matrix (Fig. 4A). However, when the water was evaporated, the matrix collapsed because the HEMA/water mixture had not allowed any interpeptide H-bonding and the matrix was so compliant (stiffness of 1-2 MPa) that surface tension forces at the air-matrix interface pulled the matrix down and extruded the HEMA before it could be polymerized. In the Carvalho et al study, the bonded specimens were immersed overnight in silver nitrate. Silver nitrate diffuses into any water-filled portions of the hybrid layer that were not well-infiltrated with resin. Figures 5B, D, F and H show silver nanoleakage in hybrid layer primers with 35% HEMA in water, ethanol, methanol and propanol, respectively. The least nanoleakage was seen using 35% HEMA in ethanol followed by 35% HEMA in water, methanol and propanol. A mixture of 35% HEMA in 65% ethanol produced more net resin infiltration, because it permitted some interpeptide H-bonding within collagen. When the ethanol was evaporated, there was much less matrix collapse, allowing more HEMA to remain in the matrix where it protects collagen fibrils and strengthens the hybrid layer (Figs. 5C, 5D). When bond strength was plotted against interfibrillar space (the volume
occupied by infiltrated resin), an excellent correlation was obtained ($R^2 = 0.83$, Fig. 6).

Using the macromodel of the hybrid layer, Nakajima et al. showed that application of solvated commercial bonding agents to matrices saturated with water caused a matrix shrinkage (Fig. 7A) between 23–28%, depending on the product, as the ethanol or acetone solvents removed much of the water in the matrix, allowing spontaneous development of interpeptide H-bonding that resulted in progressive matrix shrinkage. Matrix shrinkage involves a decrease in the width of interfibrillar spaces (Fig. 7A, insert). As peptides come closer together they can develop new H-bonds if the resin-solvent mixture $\delta_h$ value approaches that of collagen peptides ($\delta_h = 14.8$). Acetone, with a $\delta_h$ of only 11 ($J/cm^3)^{1/2}$ tends to make solvated comonomer blends relatively hydrophobic (Table 2). Water is miscible with acetone, so water in wet interfibrillar spaces diffuses into the acetone-solvated resins. It is likely that the final $\delta_h$ of the mixed fluids in the interfibrillar spaces is between 11-15 ($J/cm^3)^{1/2}$ because the volume of the applied solvated adhesive is so much larger than the original volume of water in those spaces. That low $\delta_h$ (i.e. 11-15) value would allow some interpeptide H-bonding to develop. However, as the acetone rapidly evaporates, the concentration of the comonomers may rise to molar concentrations. If high concentrations of solvated comonomers are applied to water-saturated matrices, they may actually prevent interpeptide H-bonding. That is, as the monomers diffuse around and into collagen fibrils, they may physically block adjacent peptides from developing interpeptide H-bonds. If 80% acetone/20% comonomer blends are applied to water-saturated matrices, the matrices may shrink much more than if 27% acetone/73% comonomer was added.37

In the Nakajima et al. study, the additional shrinkage associated with solvent evaporation was not measured. After resin-infiltration, these specimens were removed from the device, the solvent evaporated and the resins cured. After 24 hours storage in water at 37°C, the ultimate tensile strength (UTS) of the macromodel of the hybrid layer (i.e. resin-infiltrated matrices) was measured and found to vary between 30-42 MPa.8 Thus, resin infiltration of demineralized dentin significantly strengthened (i.e. more than doubled) the tensile strength of the matrix which had a UTS of only 16.4 MPa. Our original measurements of the UTS of resin-infiltrated dentin matrices of >100 MPa were too high because they were done on relatively dry specimens, before the plasticizing effects of water were understood. The prolonged use of chemical dehydration and hours of resin infiltration are not clinically relevant but have been shown to create perfect hybrid layers.20

### Experimental adhesives

Since manufacturers do not disclose the compositions of their adhesive systems, we could not calculate their Hoy's solubility parameters. To make correlations between changes in matrix height during monomer infiltration, we had to create our own chemically-defined primers. To test the influence of monomer and solvent concentration on the expansion of nitrogen-dried matrices, HEMA was mixed with ethanol at different concentrations. The degree of expansion was proportional to the $\delta_h$ of the mixture. A mix of 30% ethanol/70% HEMA ($\delta_h = 16.7$) was unable to expand dried, collapsed matrix (Hoy's $\delta_h = 14.8$) (Fig. 7B), while 50% HEMA/50% ethanol ($\delta_h = 17.6$) slowly expanded the matrix. Thirty percent HEMA/70% ethanol ($\delta_h = 18.6$) expanded the matrix moderately quickly but 10% HEMA/90% ethanol ($\delta_h = 19.5$) expanded it very rapidly (Fig. 7B). However, when the ethanol was evaporated, the matrix shrank in proportion to the alcohol concentration in the infiltrated matrix. By simply replacing ethanol with methanol, although the degree of expansion was no higher, the net resin uptake (i.e. that remaining after solvent evaporation) was higher (Fig. 7C) and the 70% HEMA/30% methanol was able to expand the matrix, albeit slowly. The relative net expansion that is attributed to resin uptake from each HEMA/solvent blend may be identified from these displacement-time curves (Figs. 7B, 7C) by subtracting the displacement after solvent evaporation from the baseline displacement obtained after drying of the water-saturated demineralized dentin matrix. Thus, for a particular solvent series, the amount of net expansion of the resin-infiltrated dentin matrix

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### Table 4. Composition of neat experimental resins 1-5 and Hoy's solubility parameters for the solvated comonomers and for demineralized dentin (collagen).

<table>
<thead>
<tr>
<th>Resin #</th>
<th>Resin composition</th>
<th>Hoy's solubility parameters ($J/cm^3)^{1/2}$</th>
<th>$\delta_d$</th>
<th>$\delta_h$</th>
<th>$\delta_t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70 wt% E-BisADM; 28.75% TEGDMA; 1.0% EDMAB; 0.25% CQ</td>
<td>15.0 10.3 6.6 19.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>70% BisGMA; 28.75% TEGDMA; 1.0% EDMAB; 0.25% CQ</td>
<td>15.9 12.4 6.5 21.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>70% BisGMA; 14.4% HEMA; 1.0% EDMAB; 0.25% CQ</td>
<td>15.6 13.0 8.5 22.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>40% BisGMA; 30% TCDM; 28.75% TEGDMA; 1.0% EDMAB; 0.25% CQ</td>
<td>16.5 12.9 7.0 22.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>40% BisGMA; 30% BisMP; 28.75% HEMA; 1.0% EDMAB; 0.25% CQ</td>
<td>15.1 13.5 11.1 23.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Collagen 30% water, 70% peptides: 11.8 15.3 22.5 30.1
Collagen 30% ethanol, 70% peptides: 12.0 12.5 18.1 25.1
Collagen 0% water, 100% peptides: 11.7 12.1 14.8 22.5
Ethanol: 12.6 11.2 20.0 26.1
Water: 12.2 22.8 40.4 48.0

All Hoy's solubility parameters were calculated using commercially available software (Computer Chemistry Consultancy <www.compchemconsult.com>). $\delta_d$ - Hoy's solubility parameter for dispersive forces; $\delta_h$ - Hoy's solubility parameter for hydrogen bonding forces; $\delta_t$ - Hoy's solubility parameter for the total cohesive forces, that is equivalent to Hildebrand's solubility parameter: $\delta_t = \sqrt{\delta_d^2 + \delta_h^2 + \delta_t^2}$.

Abbreviations: BisGMA = 2,2-bis(4-(2-hydroxy-3-methacryloyloxypropoxy))-phenyl propane; TEGDMA = triethylene glycol dimethacrylate; EDMAB = 2-ethyl dimethyl-4-aminobenzoate; CQ = camphorquinone; E-BisADM = ethoxylated Bisphenol A diglycidyl methacrylate; HEMA = 2-hydroxyethyl methacrylate; TCDM = dichydroxyethylmethacrylate ester of 5-(2,5-dioxotetrahydrofurfuryl)-3-methyl-3-cyclohexane-1,2'-dicarboxylic acid; BisMP = Bis[2-(methacryloyloxy)ethyl] phosphonate.
Expansion of dried demineralized dentin matrix by various ethanol/experimental resin 4 (see Table 4 for composition) mixtures. Application of 100% resin 4 (with no solvent) was ineffective at causing any expansion. The numbers in parentheses are the Hoy’s solubility parameters for hydrogen bonding forces, $\delta_h$ in (J/cm$^3$)$^{1/2}$. The higher the $\delta_h$ values, the faster and higher the matrix expanded.

When 100% ethanol was used to replace water in water-expanded demineralized dentin, there was a slight shrinkage as water was removed and some interpeptide H-bonds formed. This was followed by a slight expansion of the matrix height as ethanol broke some of the newly formed interpeptide H-bonds. When various concentrations of ethanol/resin 4 mixtures were applied to ethanol-saturated matrix, there was no further change in matrix height during infiltration. When ethanol was evaporated, the matrices shrank in proportion to ethanol content. Note that 100% resin 4 did not shrink because it infiltrated the ethanol-saturated matrix. Apparently, ethanol in the matrix diffused into the solvent-free neat resin during infiltration, as the comonomers are not volatile.

Net resin uptake is proportional to the solvent concentration employed in the HEMA/solvent blend (see Fig. 3B). Methanol, having a higher $\delta_h$ value than ethanol (24 vs. 20, Table 2) increased the $\delta_h$ of all HEMA/methanol mixtures so that all of their $\delta_h$ values were above that of the dried matrix (Hoy's $\delta_h$ = 14.8 (J/cm$^3$)$^{1/2}$). This demonstrates that the $\delta_h$ of monomer/solvents can be altered by changing either the monomer or the solvent or both.

While the HEMA/solvent series of experiments improved our understanding of the effect of $\delta_h$ of monomer/solvent blends on re-expansion and subsequent shrinkage of demineralized dentin matrices, commercially available adhesives contain not only HEMA, as the latter is employed as a co-solvent for water-immiscible resin monomers, but other monomers including dimethacrylates. Thus, a series of more complex, resin comonomer mixtures were blended to create experimental hydrophobic, intermediate or hydrophilic solvated adhesives (Table 4). Experimental resins 1 and 2 represent non-solvated hydrophobic adhesives that are used in the final step of three-step etch-and-rinse adhesives or the adhesive applied over two-step self-etching primers. Resin 3 is representative of the hydrophilic primers that are commonly employed in the second step of three-step etch-and-rinse adhesives. Resins 4 and 5 are acidic comonomers due to the presence of carboxylic acid or phosphoric acid methacrylate derivatives, respectively, and are representative of typical single-bottle etch-and-rinse adhesives containing resin monomers with these acidic functional groups. They are very hydrophilic (Table 4) relative to resins 1 and 2 that are more hydrophobic. When these ethanol-solvated resins were applied to dried collapsed matrices, they expanded the matrix in proportion to their $\delta_h$ values (Fig. 8A). Even when 70–90% ethanol concentrations are used to solvate adhesive monomers, the rate of matrix expansion is slow (i.e. the time from initial application of the ethanol-solvated resin blend to the time when maximum expansion is achieved) and the extent of net expansion after evaporating the ethanol is only 35–40% (Fig. 8A). These realities limit the application of solvated adhesives to dried demineralized dentin. This is why dry bonding to acid-etched and water-rinsed dentin is no longer recommended by manufacturers.

However, if ethanol-solvated adhesives with relatively low $\delta_h$ values are applied to water-saturated, already expanded matrices, the physical presence of resins in expanded matrices prevents most new H-bonds from forming during the resin infiltration phase. However, when the solvent of solvated resins is evaporated, the resin-infiltration matrices collapsed in proportion to their ethanol content (Fig. 8B).

There is little correlation between net resin uptake and the...
When the demineralized dentin matrix was saturated with water, there was no change in the height of the matrix that remained fully expanded. After waiting 30 minutes for monomer infiltration, the excess resin 4 was removed by aspiration and dry N₂ gas was blown over the surface. This led to a rapid 90% shrinkage of the matrix as residual water was evaporated from the matrix. This result indicates that the comonomers of resin 4 were unable to remove water from the matrix because they did not infiltrate the interfibrillar spaces. When the matrix was saturated with ethanol, resin 4 diffused into the matrix as ethanol diffused out of the matrix. When dry N₂ gas was blown on the matrix, there was little shrinkage because the interfibrillar spaces were full of resin. In control experiments, resin-free matrices saturated with ethanol collapsed when the ethanol was evaporated because ethanol is volatile and there was no resin in the interfibrillar spaces to prevent interpeptide H-bonding from developing that pulled the matrix down.

Ethanol wet bonding

Because the macromodel of the hybrid layer is 200 µm thick (i.e., about x40 thicker than typical etch-and-rinse hybrid layer), the processes of infiltration versus solvent evaporation occur relatively slowly, thereby permitting careful study. For instance, we have found that the higher the solvent concentration in monomer blends, the greater the matrix shrinkage upon evaporation of that solvent. An unexpected observation was that it is possible to infiltrate neat comonomer hydrophilic resins into dentin moistened with ethanol, and that there is little shrinkage of the monomer-infiltrated matrix when nitrogen gas was directed on these surfaces (compare dentin moist with water in Fig. 8B and dentin moist with ethanol in Fig. 8C). The implications of this are that if ethanol is used to replace rinse-water from acid-etched matrices, one may infiltrate relatively hydrophobic neat Bis-GMA/TEGDMA resins into demineralized dentin to create hydrophobic hybrid layers. In other words, one is no longer limited to using hydrophilic resins for dentin bonding. Some believe that dentin adhesives have become far too hydrophilic. These resins absorb far too much water, which lowers their mechanical properties. By using hydrophobic resins applied to ethanol-wet dentin, we can lower water sorption five-fold. This should result in more durable resin-dentin bonds.

When the demineralized dentin matrix was saturated with water, application of different concentrations of resin 4 (Table 4) in ethanol produced variable amounts of matrix shrinkage (Fig. 8B) during infiltration that were proportional to their ethanol concentration. Similarly, during subsequent solvent evaporation (Fig. 8B), further matrix shrinkage occurred that was proportional to the ethanol concentration of the mixture.

The exceptions were the neat resins. When neat resin 4 was applied to water-saturated demineralized dentin, there was no change in matrix height during what should have been the monomer infiltration period (Fig. 8B). After removing excess neat resin 4 from the well, the presumed neat monomer-infiltrated matrix was subjected to a steady stream of dry N₂ gas, a procedure that has been shown to evaporate any volatile solvent including water. This led to a very large shrinkage of the matrix to a level almost as much as the original evaporation of water that is part of every experimental protocol (Fig. 8B). The net result was very little net resin uptake. Apparently, the non-agitated neat resin 4 simply layered on top of the water-saturated dentin matrix as if it were not miscible with water. After allowing sufficient time for monomer infiltration, and after removing excess neat resin, dry N₂ gas was able to evaporate the residual water and caused almost complete matrix shrinkage (Fig. 8B).

Solubility parameter theory has been used to predict the miscibility of two different solutions by comparing their δ values. It predicts that if there is less than 5 (J/cm⁻³)½ between the solubility parameter for the total cohesive energy (δₜ) of a solution and a second solution or a substrate, that the solution will wet the substrate and cause it to swell enough to permit entry of the solution. In Table 4, the Hoy’s δₜ values for neat resin 4 and the water-saturated collagen matrix are listed as

<table>
<thead>
<tr>
<th>Dentin treatment</th>
<th>Surface condition</th>
<th>SD (n) in MPa</th>
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</thead>
<tbody>
<tr>
<td>320 grit SiC paper</td>
<td>Dry</td>
<td>11.7 ± 4.8 (10)</td>
</tr>
<tr>
<td>Etched with 10% H₃PO₄, 30 seconds</td>
<td>Wet</td>
<td>24.3 ± 5.2 (10)</td>
</tr>
<tr>
<td>Etched with 32% H₃PO₄, 20 seconds</td>
<td>Dry</td>
<td>20.7 ± 10.8 (10)</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n) in MPa.
22.1 and 30.1 (J/cm³)½, respectively. The difference between their δ values of 8.0 (J/cm³)½ suggests that the two are not miscible. Indeed, adding 1 ml of neat resin 4 to 1 ml of water produces a milky mixture indicating phase changes and monomer precipitation (specifically BisGMA) (Fig. 8E). In contrast, the Hoy's δ values for resin 4 versus ethanol-saturated collagen are 22.1 and 25.1 (J/cm³)½, respectively (Table 4). The 3.0 (J/cm³)½ difference in δ indicates that they should be compatible and miscible. This was confirmed when 1 ml of neat resin 4 was added to 1 ml of 100% ethanol (Fig. 8E). There were no phase changes. This is another example of how Hoy's solubility parameter theory can be used to explain results obtained using the macromodel of the hybrid layer.

When ethanol was applied in excess to water-saturated dentin matrix (Fig. 8C), there was a small (ca. 18%) matrix shrinkage as water diffused from the matrix into the overlying 100% ethanol, followed by a plateau in matrix height. When neat resin 4 was applied to ethanol-saturated dentin matrix for 30 minutes to allow monomer infiltration, there was no further change in matrix height. After removal of excess neat resin 4, dry N₂ gas was blown on the neat resin 4 infiltrated matrix to determine if it would shrink as it did for the ethanol-saturated matrix. This did not occur, indicating that neat resin 4 diffused into the ethanol-saturated interfacial spaces and ethanol must have diffused out into the excess overlying neat resin 4. When dry N₂ gas was blown on the monomer-infiltrated matrix, there was very little shrinkage because none of the monomers in resin 4 are volatile and apparently all of the ethanol was gone. In control experiments, after exchanging ethanol for water in the matrix, instead of applying neat resin 4 to the ethanol-saturated matrix, the ethanol was evaporated by blowing dry N₂ on the matrix. This led to a rapid shrinkage of matrix indicating that ethanol-saturation of the matrix does not stiffen the matrix so much that it cannot shrink when ethanol is evaporated (Fig. 8D). Although neat resin 4 was used as an example, this behavior was seen in all experimental resins 1-5. Thus, the macromodel of the hybrid layer may be used to quantify net resin uptake even when using neat resins.

In the early 1990's, Kanca14-16 demonstrated that wet-bonding with water gave higher bond strengths (Table 5) than dry bonding. The work of Gwinnett22 showed that the rationale for the water wet-bonding technique was attributed to water-induced expansion of shrunken, dried matrices. We now know that rapid, spontaneous development of interpeptide H-bonding actively pulls dried matrices down about 50% (see Fig. 3C) and stiffens them in the collapsed state so that resins cannot infiltrate their surface.46 Water, with a Hoy's δh value of 40 (J/cm³)½ is able to break all interpeptide H-bonds (Hoy's δh = 14.8 for dry collagen, Table 2). However, water is not a solvent for collagen, which remains insoluble in body fluids because the δh for water, 48 (J/cm³)½ is far removed from that of dry collagen, with a δh = 22.5 (J/cm³)½ (Table 2). To solvate collagen, one must use a solvent with a δh close to 22.5 (J/cm³)½. Acetic acid, with a δh of 26.5 (J/cm³)½ is used to solubilize collagen in biochemical studies. Water does not solubilize collagen, but plasticizes it by breaking interpeptide hydrogen bonds that open up spaces between collagen fibrils for resin infiltration. When bonding to expanded matrices, monomer infiltration is more rapid because the interfacial spaces are as wide as possible. The disadvantage of wet-bonding is that the matrix is very compliant (i.e. too soft) and can easily shrink when the solvent is evaporated.

Wet-bonding has the advantage that rinse water can prevent any interpeptide H-bonds from forming, allowing the matrix to be fully expanded with relatively wide interfacial spaces to provide maximal monomer uptake during infiltration of solvated comonomers. However, the monomers must be relatively soluble in water. The simplest way to assure that solubility is to solvate them in at least 50% ethanol or acetone. But, the more solvent in comonomer mixtures, the more the matrix shrinks when the solvent is evaporated (Figs. 7B, 8C). Conversely, the more concentrated the monomer, the less the matrix shrinks when the solvent is evaporated, resulting in more net monomer uptake (Fig. 8A), except for the neat resins applied to water-saturated matrices. Thus, net resin uptake is the difference between resin uptake during infiltration of solvated comonomers, and matrix shrinkage during solvent evaporation. It is worth repeating that the height or thickness of the hybrid layer depends upon the net resin uptake. We speculate that resin-dentin bonds may be more durable when net resin uptake is maximized because it not only provides more resin to covalently couple to resin composites, it also envelops the collagen fibrils within the hybrid layer with a coating of polymerized resin, protecting them from hydrolytic attack.

Clinical relevance

What is the evidence that the results obtained with the macromodel of the hybrid layer has any clinical relevance? The observation that macrohybrid layers saturated with ethanol permit good infiltration of hydrophobic resin 4 (Fig. 8C) encouraged us to measure microtensile bond strengths of 50% ethanol/50% hydrophobic vs. hydrophilic experimental resins into acid-etched dentin that was saturated with either water or ethanol (Table 6). With the exception of resins 1 and 2, which

<table>
<thead>
<tr>
<th>Table 6. Microtensile bond strength of experimental resins to acid-etched dentin wet with water, ethanol or dry.</th>
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</thead>
<tbody>
<tr>
<td>50 wt% ethanol-solvated resins</td>
</tr>
<tr>
<td>Resin 1 (Table 4)</td>
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<tr>
<td>Resin 2 (Table 4)</td>
</tr>
<tr>
<td>Resin 3 (Table 4)</td>
</tr>
<tr>
<td>Resin 4 (Table 4)</td>
</tr>
<tr>
<td>Resin 5 (Table 4)</td>
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</tbody>
</table>

Values are least squares means ± SEM, n = 10-16 per group. Within any vertical column, values identified by different lower case letters are significantly different (P<0.05) by Holm-Sidak test. Within any horizontal row, values identified by different uppercase letters are significantly different (P<0.05) by Holm-Sidak test.
gave low bond strengths to water-saturated dentin, there was no statistically significant difference between the bond strengths of experimental resins 2-5 to ethanol-saturated dentin, even though resin 2 is essentially an ethanol-solvated pit-and-fissure sealant (i.e. hydrophobic Bis-GMA/TEGDMA). Thus, the dimensional changes seen in the macromodel of the hybrid layer are very predictive of how the resins would behave when they are used for actual dentin bonding.

These 50% ethanol/50% resin mixtures were applied in two layers as is typically done using etch-and-rinse, two-step adhesives. There was a highly significant positive correlation (Fig. 9A) between their microtensile bond strength and the Hoy's $\delta_t$ values of the resins bonded to water-saturated dentin ($R^2 = 0.80, P < 0.025$). The lowest bond strengths were obtained using resins 1 and 2, the most hydrophobic resin blends, and the highest bond strengths were obtained using resin 5, the most hydrophilic resin blend. Even higher bond strengths were obtained when these same resins were bonded to ethanol-saturated dentin (Fig. 9B, $R^2 = 0.80, P < 0.05$). This indicates that wet-bonding with 100% ethanol may be even better than wet-bonding with water. Using water or ethanol-saturated matrices, resin uptake was directly proportional to the $\delta_t$ (Fig. 9B) of the solvated resins instead of $\delta_t$. Although the expansion of dried demineralized dentin matrices correlated best with the Hoy's solubility parameter for hydrogen bonding, when solvated comonomer mixtures are applied to already expanded matrices, the resulting bond strengths correlated better to $\delta_t$ (Hoy's solubility parameter for total cohesive forces). The correlation between the $\delta_t$ values of the experimental resins and the width of interfibrillar spaces needs to be tested, along with the correlation between microtensile bond strengths and the widths of interfibrillar spaces. Clearly, the macromodel of the hybrid layer is a useful tool for predicting how solvated resins interact with dentin matrices. The model predicted the superiority of wet over dry-bonding (Table 6), and the utility of wet-bonding with ethanol instead of water.

The concept of ethanol wet-bonding is not as far-fetched as it seems, as the idea has been used for more than half a century by electron microscopists for embedding comparatively hydrophobic epoxy resins into hydrophilic soft tissues. In tissue embedding, the water in the hydrated tissues is gradually replaced over many hours by stepwise immersion of the specimen in an ascending series of these solvents. This is fol-
dentin and enamel. These steps are crucial in ensuring the durability and long-term success of restorative procedures. The mechanical interlock provided by the hybrid layer enhances the bond strength and prevents microleakage. 

Some critical variables that affect the bond strength include the application of primers, the type of adhesive system, and the curing method. The properties of the resin and the primers, such as their hydrophilicity, also play a significant role. For instance,mando

12. Sugizaki J. The effects of various primers on dentin adhesion of resin com-

11. E. G. & G Inc., Wellesley, MA, USA.

10. Parkell, Farmingdale, NY, USA.

9. L. D. Caulk, Milford, DE, USA.

8. Bisco, Schaumberg, IL, USA.

7. 3M ESPE, St. Paul, MN, USA.

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References


12. Sugizaki J. The effects of various primers on dentin adhesion of resin com-

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**Articles Accepted for Publication**

- The effects of two monofunctional diluent monomers and two photoinitiator systems on the properties of UDMA-based composites. *N.D. Richards & J.M. Antonucci*
- Micromorphology and surface roughness of sound and demineralized enamel and dentin bleached with a 10% carbamide peroxide bleaching agent. *R.T. Basting, A.L. Rodrigues Jr. & M. Campos Serra*
- Fracture strength of restored premolars. *G.B. Camacho, M. Gonçalves, T. Nonaka & A.B. Osório*
- Retention of three post systems. *J. Peters, G. Zyman, E. Kogan, S. Kuttler & F. Garcia-Godoy*
- Spectrophotometric and visual shade measurements of human teeth using three shade guides. *G. Fani, A. Vichi & C.L. Davidson*
- Degradation of thermo-mechanically loaded adhesive Class V restorations after 18 months water storage. *T. Bortolotto, M. Ferrari, F. Tay & I. Krejci*