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Dental adhesives and strategies for displacement of water/solvents from collagen fibrils

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Abstract

Objectives—To evaluate the influence of temperature of evaporation in adhesive systems with different solvents on the apparent modulus of elasticity and mass change of macro-hybrid layers modified by proanthocyanidins (PACs).

Methods—Adhesive resin beams (A) from Single Bond Plus (SB), Excite (EX) and One Step Plus (OS) were prepared after solvent evaporation at 23°C or 40°C (n=12). Macro-hybrid layers (M) (n= 12) were prepared using demineralized dentin beams sectioned from extracted human third molars. The demineralized dentin specimens were infiltrated with each one of the three adhesive systems at 23°C or 40°C; with or without prior dentin treatment with PACs for 10 minutes. The apparent modulus of elasticity (E) and mass change (W_{mc} %) of adhesives beams and resin-infiltrated specimens were assessed in dry and wet conditions after immersion in water (24 h, 1, 3 and 6 months). The E was statistically analyzed by Tukey-Kramer test and the W_{mc} % by Kruskal Wallis, and Dunn ($\alpha=0.05$).

Results—Solvent evaporation at 40 °C resulted in higher E values for adhesive resin beams at all storage conditions, regardless of the adhesive system ($p < 0.05$). Increased Mass Loss (3 months: –0.01%; 6 months: –0.05%) was observed in One Step resin beams ($p = 0.05$). In the macro hybrid layer models the pretreatment with PACs along with solvent evaporation at 40 °C increased E and decreased the W_{mc} % (3 months: –2.5; 6 months: 2.75%) for adhesives evaluated over time ($p < 0.05$). No significant differences in Ratio (resin/dentin) were found for the macro hybrid layers ($p > 0.05$).

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Significance—Improved solvent evaporation at higher temperature, and increased collagen cross-linking induced by PACs, enhanced the mechanical properties resulting in highly stable macro-hybrid layers over 6 months storage.

Keywords

dentin; collagen cross-linkers; dental adhesive; temperature volatilization; apparent modulus of elasticity

Introduction

The clinical success and longevity of dental adhesive restorations depends on the stability of the resin-dentin interfacial components. The infiltration of resin monomers in a mineral depleted dentin matrix, results in the formation of a hybrid layer. Thus, the presence and the quality of the hybrid layer are believed to be essential for adhesion to dentin [1,2]. However, there is no evidence that water in the intra and inter-microfibrillar compartments of the collagen matrix is completely replaced by resin [3–5]. The remaining water present in dentin during resin infiltration increases the water absorption of hydrophilic resins [6], plasticize polymers, and accelerate degradation of the adhesive interface [7].

The resin co-monomers are dissolved in organic solvents that facilitate monomer diffusion within the collagen fibrils, and assist with the removal of water during solvent evaporation [8, 9]. Therefore, the residual water in the dentin [5, 10] and the organic solvents in adhesive systems [11] need to be adequately volatilized or they can interfere in the mechanical properties of the hybrid layer [1]. Clinical techniques using heat for evaporation of solvents result in higher mechanical properties [12–16] and decreases interfacial micro permeability [13, 15]. However, despite the fact that these techniques have increased the evaporation of the solvents, there are limited long-term studies evaluating such techniques. Although evaporation of excess solvents can promote a highly cross-linked resin polymer, the hydrophilic nature of the monomer would not change, thus water absorption could plasticize the polymer chains and degrade the adhesive interface at the long-term [13].

The modification of dentin by plant-derived proanthocyanidins (PACs) has the ability to modify the structure of collagen by increasing the number of inter- and intra-molecular cross-links [17] enhancing the mechanical properties of the hybrid layer [18] and making the hybrid layer resistant to degradation [19]. The enhanced mechanical properties may also be due to the type of bond between PACs and collagen, decreasing the hydrophilicity of the dentin matrix [20,21]. This could decrease the degradation of the adhesive interface caused by long-term water absorption. In this context, flexural tests of macro-hybrid layer models can provide valuable information regarding the quantification of matrix shrinkage and resin uptake, as well as mechanical properties of the hybrid layer after infiltration of the adhesive [22–25].

Therefore, this study evaluated the association of tissue biomodification and temperatures for solvent evaporation to minimize the effects of water from the dentin substrate and excess of solvents from adhesives systems on the modulus of elasticity and mass change of macro hybrid layers over a 6 month storage period. The null hypothesis tested was that the

biomodification of dentin matrix associated to warm temperature of solvent evaporation (40°C) would not affect the mass change and modulus of elasticity of macro hybrid layers created by different adhesives systems.

2 Materials and methods

2.1 Adhesive systems

Commercially available etch-and-rinse adhesive systems with different solvents were selected: Single Bond Plus [SB-ethanol and water based], 3M/ESPE, St. Paul, MN, USA, One Step Plus [OS- acetone based], Bisco, Schaumburg, IL, USA and Excite F [EX- ethanol based], Ivoclar Vivadent AG, Bendererstrasse, Schaan, Liechtenstein. The composition of the adhesive systems is described in Table 1.

2.2 Adhesive resin beam preparation

One mL of each adhesive system was dispensed in a circular-plate and their initial mass was measured using an analytical balance (XP 504DR, Mettler Toledo Inc., Columbus, OH, USA). Each circular plate with the adhesive system was repeatedly weighed after 15, 30, 45 and 60 min until weight was stabilized. To keep traces of solvent in the adhesive, the time of volatilization was set at 30 min (based on pilot studies). The adhesive systems were kept in the dark for 30 min in an oven at 40 ± 1 °C or at room temperature (23 ± 1 °C). After time elapsed, the adhesives were placed in polyvinyl siloxane molds (0.28 mm $\times$ 1.5 mm $\times$ 7.0 mm) and were immediately light-cured for 60 seconds on each side. Photo-activation was performed using a halogen-based unit (Optilux 501, Kerr Corp., Orange, CA, USA) with light intensity of 800 mW/cm². Then, all adhesive resin beams (n=12 per group) with final dimensions 0.28 mm thickness $\times$ 1.5 mm width $\times$ 7.0 mm length were hand-polished with 600-grit silicon carbide paper with a silicone oil emulsion (Sigma-Aldrich, St. Louis, MO, USA) [26].

2.3 Macro hybrid layer model

2.3.1 Dentin specimen preparation—Twenty-five extracted human third molars were obtained under a protocol approved by the Institutional Review Board Committee from Piracicaba Dental School- University of Campinas (protocol 068/2014). Teeth were stored in thymol solution 0.1%, at 4 °C and cleaned with periodontal curettes to remove organic and inorganic debris. Afterwards, the root and crown enamel were removed from the teeth, and dentin disks were obtained from mid-coronal dentin using a diamond, wafering blade (Buehler-Series 15LC Diamond, Buehler, Lake Bluff, IL), under constant water irrigation. Serial sections in the mesio-distal orientation were performed, resulting in dentin beams with 0.28 mm thickness (n = 6 per tooth). Thus, 144 beams were obtained (n = 12 per group) with final dimensions 0.28 mm thickness $\times$ 1.5 mm width $\times$ 7.0 mm length and a dimple was made at the margin of the beam for all measurements to be performed on the same surface [18].

2.3.2 Dentin beams demineralization—The dentin beams were immersed in 10% phosphoric acid solution (Ricca Chemical Company, Arlington, TX, USA) for 5 hours to

fully demineralize the dentin specimen [20]. For all groups, demineralized dentin specimens were rinsed 5 times with distilled water.

2.3.3 Dentin matrix biomodification treatment—The dentin beams were randomly divided into twelve groups ($n = 12$) according to dentin treatment: distilled water (control) or 6.5% PACs in water [95% proanthocyanidin-rich *Vitis vinifera* (MegaNatural™ gold grape seed extract, California, USA)] [20]. The pH of the biomodification solution was adjusted to 7.2 and the demineralized dentin beams were immersed into the respective solutions for 10 min and rinsed.

2.3.4 Dehydration of the Dentin beams—Due to the increased thickness of the macro hybrid layers, as compared to the hybrid layer clinically created [22], a slow dehydration and infiltration process is necessary to assure that the resin infiltrates throughout the demineralized dentin beams. The dehydration process was performed with increasing concentrations of ethanol [27] or acetone, according to the solvent present in each adhesive (SB and EX – ethanol; OS – acetone), starting from 50%, 70%, 80%, 90% immersion for 30 min, and two immersions in 100% for 30 min each [27].

2.3.5 Evaporation temperature and resin infiltration—The dehydration process was followed by immersion of dentin beams in 50% solvent in the darkness (according to the solvent present in each adhesive)/ 50% adhesive system solution at room temperature for 30 min, and two immersions in 100% adhesive system for 30 min each. For the third immersion in 100% adhesive system, the beams were kept in the dark for 30 min at room temperature (23 ± 1 °C) or in an oven (Isotemp, Fisher Scientific, Pittsburgh, PA, USA) at 40 ± 1 °C to enable solvent evaporation. The macro-hybrid layers are thicker than hybrid layers created in clinical procedures [22], therefore, a passive volatilization of solvents was done (without the use of air jet) for 30 min but with the vial (containing the infiltrated beam) semi open to allow evaporation of solvents.

The resin-infiltrated dentin beams were placed between two mylar strips and immediately light cured as described for adhesive resin beams. The excess of adhesive around the dentin beams was removed using a scalpel blade and the margins and surface were hand-polished using 600-grit silicon carbide grinding paper with silicone oil emulsion (Sigma-Aldrich, St. Louis, MO, USA) [26].

2.4 Mass change evaluation

Resin-infiltrated specimens were weighed after demineralization of the dentin beams (M_0), immediately following resin-infiltration (M_1), and after specimens were kept in distilled water for 24 h (M_2), 1 month, 3 and 6 months.

Dry mass (M_0 and M_1) was assessed after specimens were individually stored in a desiccator containing anhydrous calcium sulfate at 37 °C for 24 h. The specimens were, then, immersed in distilled water [28] for 24 h, 1 month, 3 months and 6 months in order to assess M_2 , M_3 , M_4 , M_5 , respectively. The excess water was wiped from the specimen surface using absorbent paper and then, they were immediately weighed [26] on an analytical balance (XP 504DR, Mettler Toledo Inc., Columbus, OH, USA).

The resin/dentin matrix ratio (R) was assessed using the formula [22]: $R (\%) = 100 - ((M_0 \times 100) / M_1)$, where M_0 is the dentin matrix mass- after demineralization and M_1 is the dentin beam after the resin-infiltration. In addition, the percent mass change ($W_{mc} \%$): was calculated according to the percentage mass gain of the adhesive resin beam or the resin-infiltrated dentin beam after immersion in 24 h of water storage using the formula [28]: $W_{mc} (\%) = (M_2 - M_1) / M_1$

2.5 Apparent modulus of elasticity– 3- point bending flexural method

The modulus of elasticity (E) of adhesive resin beams and resin-infiltrated dentin beams was assessed using the non-destructive three-point bending flexural test at a 2% strain [22], in the EZ Graph machine (Shimadzu, Kyoto, Japan) with 1 N load cell at 0.5 mm/min crosshead speed. The modulus of elasticity was obtained after 24 h storage in dry condition, and after immersion in distilled water for 24 h, 1 month, 3 months and 6 months. E was expressed in MPa (Mega Pascal) and calculated according to a previous study [29].

3 Statistical Analysis

3.1 Apparent Modulus of Elasticity - E

The mixed models analysis for repeated measures was applied by PROC MIXED of SAS statistical program (SAS Institute Inc., Cary, NC, USA, Release 9.2, 2010). Multiple comparisons were performed by Tukey-Kramer test ($\alpha = 0.05$).

3.2 Mass Change - $W_{mc} (\%)$

The mass change's exploratory data analysis indicated that the data did not meet the assumptions of parametric analysis. Therefore, the mass change percentage was analyzed using Kruskal Wallis ($\alpha = 0.05$).

3.3 Resin-Dentin Matrix Ratio

After exploratory data analysis for the Resin/dentin matrix ratio, 3-way ANOVA was carried out using the SAS statistical program ($\alpha = 0.05$).

3.4 Pearson Correlation

The effects of percent mass change on modulus of elasticity were evaluated with the Pearson correlation test ($\alpha = 0.05$)

4 Results

4.1 Adhesive Resin beams

The E mean values and standard deviations from three etch-and-rinse adhesive beams at 40° C and 23° C and different storage conditions are presented in Table 2. Solvent evaporation at 40° C resulted in higher modulus values for OS, EX and SB in all storage conditions ($p < 0.05$). EX and SB presented higher modulus of elasticity than OS in the different experimental conditions after 1 month of water storage, regardless of the temperature evaluated ($p < 0.05$). When the storage conditions were compared for OS, the lowest E was observed after 3 months of immersion in distilled water, regardless of the temperature of

volatilization ($p < 0.05$). All adhesives evaluated in this study showed a decrease in modulus after 24 h water storage. However, for EX and SB, E increased and no statistically significant difference were observed between the storage conditions of 24 h Dry and 6 months of water storage, regardless of temperatures of volatilization ($p > 0.05$).

The storage times tested did not significantly affect the mass change of EX and SB adhesive resin beams (Fig 1A and 1B). In contrast, OS presented higher W_{mc} (%) than SB and EX after 3 months and 6 months of water storage ($p < 0.05$). There was no difference between the temperatures of solvent evaporation, regardless of the adhesive and storage conditions.

4.2 Resin-infiltrated dentin beams

In the control group, solvent volatilization at 40°C resulted in increased E values for the macro hybrid layer models produced by OS and EX when tested in dry conditions ($p < 0.05$). The SB resin infiltrated dentin beams presented increased E values at 24 h of water storage (Table 3). The combination of both strategies, pre-treatment of the dentin with PACs extract and increased temperature of volatilization for the solvents present in adhesive systems had the highest modulus of elasticity when compared with the control groups for all adhesives, from the third until the sixth month of aging in water ($p < 0.05$). However, this effect was not seen when demineralized dentin was treated with PACs and infiltrated by the adhesives that had the solvent volatilized at room temperature. The poor durability of resin-infiltrated dentin beams seemed to be eliminated when both strategies (PACs and heating) were associated ($p < 0.05$). The dentin infiltrated by acetone-based adhesive (OS) showed greater decrease in E values when the samples did not receive treatment with grape seed extract ($p < 0.05$). All adhesives evaluated in this study have shown a decrease in E after 24 h of water storage ($p < 0.05$). However, EX and SB groups immersed in water remained stable overtime when the demineralized dentin was previously treated with PACs.

Macro hybrid layers treated with PACs and volatilized at 40°C presented statistically less mass change (% Mass loss/ gain) (Fig 2B- $p < 0.05$). Similar to what happened in the groups volatilized at 23°C (Fig 2A and 2C) for the W_{mc} (%), the dentin infiltrated by OS and volatilized at 40 °C (Fig 2D) presented a greater W_{mc} (%- Mass loss- $p < 0.05$) than those infiltrated by EX and SB after 6 months of water storage. However, this difference was not observed ($p > 0.05$) when the dentin was treated with PACs and volatilized at 40°C (Fig 2B). Mass change (loss or gain) of the resin- infiltrated dentin beams varied from 13.8% mass gain and -8.3% mass loss. The lowest mass change values (values between -2.5 and 2.75%) were observed in the dentin beams which were treated with PACs and infiltrated by adhesive volatilized at 40 ° C (Fig 2B). Increased Mass Loss (23 °C: - 8.33%_Fig 2C; 40 ° C: -4.68%_Fig 2D) was observed in the dentin beams that were not treated with PACs.

4.3 Resin-dentin matrix Ratio

No significant differences in resin/dentin ratio were found for the demineralized dentin beams immediately following resin-infiltration, regardless of adhesive systems ($p > 0.05$; Table 4), temperature of volatilization ($p > 0.05$), and dentin matrix treatments ($p > 0.05$; Table 4).

4.4 Pearson Correlation

A negative correlation was observed between the W_{mc} (%) and E values ($r = -0.2047$, $p < 0.001$) for the macro resin-infiltrated dentin. In contrast, no significant correlation was observed between W_{mc} (%) and E ($r = 0.0512$, $p = 0.3326$) for adhesive resin. The mass change affected the modulus of elasticity of the macro hybrid layers model.

Discussion

The association of dentin biomodification and warm temperature of volatilization increased the mechanical properties of the macro hybrid layer when compared to the control groups. Moreover, when associated with an evaporation temperature of 40°C, PACs also increased stability as verified by the mass change of the macro hybrid layers exposed to water degradation. Therefore, the null hypothesis of the present study was rejected.

The adhesive resin beams volatilized at 40 °C showed higher modulus of elasticity compared to those volatilized at 23 °C for all tested storage conditions (Table 2). However, the same was not observed in resin-infiltrated dentin control groups, except when associated to PACs pre-treatment of the dentin matrix, as shown in Table 3. These findings suggest that, the degradation of the dentin matrix, and not only the resin monomers degradation, was crucial for the stability of the macro hybrid layer when subjected to warm solvent evaporation temperature.

In contrast to what was seen in the modulus of elasticity (Table 2), there was no difference in the mass change of the adhesive resin beams between the temperatures (Fig 1). This was confirmed by the no significant correlation between mass change and modulus of elasticity for the adhesive resin beams. In addition, it was shown that the pre-treatment of dentin with PACs associated with 40°C solvent evaporation reduced the mass changes (Fig 2B) and a negative correlation was noted with the mechanical properties (Table 3).

Water sorption within resin-dentin interfaces and elution of unpolymerizable monomers are the most common causes of degradation at the adhesive interface [30–35]. Inadequate evaporation of solvent and residual water hinders the interaction of monomers during the polymerization process [9, 36, 37]. Thus, one can assume that at 40 °C the evaporation of solvents improved, resulting in higher cross-linked polymers [13] immediately after polymerization. Moreover, the pre-treatment of the dentin with PACs appeared to decrease the damaging effect of water on resin-infiltrated dentin. In contrast, the resin/dentin matrix ratio (%) showed no statistically significant differences in the macro hybrid layer composition regardless of the adhesive composition, temperature of volatilization, and dentin matrix biomodification (Table 4).

The macro hybrid layer model is thicker than the hybrid layer created clinically [22]. To overcome this difference and ensure that the specimen is effectively infiltrated by the adhesive system, longer infiltration time is necessary. However, despite this limitation, the use of a macro hybrid layer reflects what occurs in a conventional hybrid layer but without variations in resin infiltration [38]. Thus, considering that, all macro hybrid layers were composed of a similar ratio of resin/dentin matrix (Table 4); the positive effect of warm

temperature and PACs had no direct relation to the resin infiltration rate but rather improved resin mechanical properties.

The heat absorbed by adhesives during the solvent evaporation process may have increased the polymer cohesion in the macro hybrid layers by decreasing spaces between polymer channels [13, 15] immediately after polymerization. In a clinical situation, the fluid infiltration increases the permeability of the hybrid layer [15], resulting in solubilization of the residual and retained solvent molecules [39] compromising the long-term volume and modulus of elasticity of the material [40].

The adhesive infiltration in the dentin matrix occurs by replacing water by solvated liquid monomers [3] within the dentin matrix, composed of type I collagen fibrils with glycosaminoglycan (GAGs), proteoglycans and phosphorylated proteins [41–43]. It is known that, even though the warm temperature may significantly increase the evaporation of the solvents, resulting in a more cross-linked polymer, subsequent water absorption could continue to degrade the adhesive interface over time [13]. Clearly since the nature of the monomer could not be changed by heating [15], there is a need to use a technique that acts directly on the replacement of unbound water from the dentin matrix to minimize the effects of long-term degradation.

The permeability of the hybrid layer [7] has been associated with the propulsion actuation of GAGs as sponges, binding and organizing water molecules among the demineralized collagen fibrils [41]. Bedran-Russo *et al.*, 2012 [20] presented evidences of interactions between PACs and proteoglycans and also, an inverse relationship between concentration of PACs and collagen solubilization. Dentin treated with grape seed extract showed decreased GAG content. Thus, the authors speculated that the decrease in GAGs might have positively influenced the mechanical properties by reducing the effects of degradation of the dentin matrix and changing the hydration rates.

PACs can act as dentin matrix stabilizers [44], resulting in a stronger hybrid layer [45]. It is likely that GAGs removal from the dentin matrix, after treatment with PACs improved resin infiltration resulting in a stable macro hybrid layer against water absorption over time. Thus, decreasing the amount of GAGs, a stronger and more stable dentin matrix reduced the ability to bind water, thus impairing breakdown of the highly cross-linked adhesive polymers promoted by evaporation at 40 °C.

Among the three adhesive systems tested, the modulus of elasticity as well as the mass change of OS were more significantly affected by the storage conditions than those of SB and EX for both adhesive resin beams (Table 2 and Fig 1) and resin- infiltrated dentin beams (Table 3 and Figs 2A, 2C, 2D). A higher susceptibility of OS to degradation in water can be explained by its more hydrophilic composition. OS presents polar/hydrophilic functional groups in BPDm (carboxylic group) and high concentration of HEMA, a polymerizable solvent with hydrophilic nature that helps resin infiltration within collagen brils [46] and results in a linear polymeric chain that acts as a hydrogel in its composition [47–49], making OS more prone to water sorption [50].

Moreover, OS contains acetone as solvent, which has a higher vapor pressure than the ethanol/water (present in both EX and SB), and is shown to interfere with solvent evaporation and the polymerization process [51]. Allied to this, studies speculate that, after volatilizing of the acetone-based adhesives, a sufficient amount of residual solvent may remain during the polymerization process, leading to porosity in the adhesive layer [52]. This finding could explain OS behavior under the storage conditions of this study.

As mentioned earlier, the remaining water/solvent associated with the hydrophilic nature of the adhesives could increase water absorption [6] after water storage. However, as PACs appeared to remove GAGs from dentin matrix [20] reducing the presence of bound water in the dentin matrix, even the dentin infiltrated by OS, might be less affected by water storage (Fig 2B and Table 3). This can be seen by the difference in the apparent modulus of elasticity between the treated group (PACs rich extract associated with 40°C solvent evaporation) and the control group for OS after 3 months of water immersion (Table 3).

The mass change process was initiated immediately after immersion of the beams in distilled water (24h wet), and increased with time (Fig 1 and 2). In a broader view, after 3 months of water storage, the percentage of mass gain of the adhesive resin beams and resin-infiltrated dentin beams produced by EX and SB decreased, progressing to stability (percentage values near zero). Weight loss was observed in OS groups after 6 months of water storage. It is important to consider that in the macro hybrid layers and the adhesive resin beams, all external surfaces were exposed to water. The high surface area exposed makes them more susceptible to the effects of degradation than if they had been sandwiched between resin composite and mineralized dentin.

In this study, the evaporation of solvent at 40 °C may have resulted in greater cohesion among polymer chains, and lower water channels in the adhesive interface could be obtained immediately after polymerization [13]. However, over time, the dentin biomodification with PACs was crucial to assist with the displacement of water from the dentin matrix, decreasing water sorption of the hybrid layer. The implications of the association of these treatments on the effectiveness of the bonding interface in actual restorations, remains to be determined.

Conclusions

- Higher temperature of volatilization associated with the pre-treatment of the dentin substrate with proanthocyanidins can increase the apparent modulus of elasticity and minimize mass loss of macro hybrid layers; the procedures can potentially enhance the stability of dentin-resin bonds over time.
- Overall, One Step, by its more hydrophilic nature, showed the lowest values of apparent modulus of elasticity and greater mass change compared to Single Bond and Excite in the different conditions of this study.

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Figure 1. Percent mass change (Wmc %) obtained from different adhesive resin beams according to the experimental conditions

The first measurement was in the baseline condition (24h dry) followed by the measurements in the wet condition after the immersion of the adhesive resin beam in water. Increased Mass Loss (3 months: -0,01%; 6 months: -0,05%) was observed in the adhesive resin beams produced by One Step ($p = 0,05$).

Figure 2. Mass change (Wmc %) obtained from different macro hybrid layers groups according to the experimental conditions

Measurements taken at 1 month, 3 months and 6 months immersion in storage media. The symbols “α” (Single Bond), “β” (One Step) and “μ” (Excite) are representatives of each significantly difference between 23°C and 40°C evaporation temperatures in the same treatment, adhesive and storage condition ($p \leq 0.05$). * Depicts statistically significantly differences between Control (D) and PACs (C) in the same temperature and storage condition ($p \leq 0.05$). PACs: proanthocyanidins; 23°C and 40°C are evaporation temperature.

Table 1

Compositions of adhesive systems tested.

Adhesive Systems	Composition	Batch number
Excite F (Ivoclar Vivadent)	15% HEMA, dimethacrylates, Phosphonic acid acrylate, 25% Bis GMA highly dispersed silicone dioxide, initiators, stabilizers, potassium fluoride, 20% ethanol	R78651
Adper Single Bond Plus (3M ESPE)	25–35% ethyl alcohol 10–20% BIS-GMA 10–20% silane treated silica 5–15% HEMA 5–10% copolymer of acrylic and itaconic acids 5–10% glycerol 1,3-dimethacrylate <5% water 1–5% UDMA	N446453
One Step Plus (Bisco)	15–40% BPDM 10–40% HEMA 40–70% acetone photoinitiators	1200007653

Table 2

Stiffness (MPa) changes in different adhesive resin beams over time vs. solvent evaporation temperature.

Solvent evaporation (°C)	Storage Condition	Adhesive		
		One Step	Single Bond	Excite
40	24 h Dry	*660.9 (44.69)Aa	*600.6 (38.69)Aa	*570.7 (39.71)Aa
	24 h Wet	*484.2 (50.04)Ab	*496.9 (46.46)Ab	*517.8 (25.68)Ab
	1 month	*467.4 (44.15)Bb	*557.0 (58.79)Aa	*580.6 (39.41)Aa
	3 month	*362.6 (61.16)Bc	*556.7 (45.53)Aa	*574.7 (32.76)Aa
	6 month	*390.4 (50.00)Bc	*561.8 (49.87)Aa	*570.0 (36.58)Aa
	24 h Dry	691.8 (29.96)Aa	696.1 (23.95)Aa	676.4 (30.64)Aa
	24 hWet	522.1 (51.62)Bb	617.2 (48.30)Ac	600.1 (66.31)Ab
	1 month	498.3 (35.76)Bb	628.1 (55.22)Abc	642.9 (30.35)Aab
	3 month	387.9 (48.04)Bc	666.9 (29.99)Aab	619.2 (38.97)Ab
	6 month	428.6 (37.00)Bc	683.2 (35.38)Aa	643.2 (39.51)Aab

Different uppercase letters represent significant ($p < 0.05$) differences in the same horizontal row. Different lowercase letters represent significant differences between groups in vertical columns. Asterisks (*) represent significant differences among solvent evaporation temperatures.

Table 3

Stiffness (MPa) differences between macro hybrid layers according to experimental conditions.

Treatment	Temperature	Storage Condition	Adhesive		
			One Step	Single Bond	Excite
PACs	23	24 h Dry	733.1 (59.70)Aa	715.5 (48.55)Aa	695.6 (79.36)Aa
		24 h Wet	478.6 (49.96)Ab	497.6 (50.65)Ab	514.5 (62.44)Ab
		1 month	413.1 (56.50)Abc	461.4 (29.69)ABb	482.4 (56.39)Ab
		3 month	361.2 (43.01)Bc	506.9 (38.85)Ab	490.9 (74.97)Ab
		6 month	346.2 (45.17)Bc	500.8 (36.47)Ab	481.9 (59.60)Ab
	40	24 h Dry	792.6 (52.79)Aa	778.5 (42.75)Aa	782.6 (51.84)Aa
		24 h Wet	538.2 (35.33)Ab	564.5 (56.51)Ab	559.9 (40.50)Ab
		1 month	479.4 (65.41)Bbc	537.5 (49.61)ABb	568.7 (49.41)Ab
		3 month	444.6 (67.96)Bc	600.7 (69.65)Ab	596.9 (71.57)Ab
		6 month	435.2 (52.69)Bc	600.6 (46.18)Ab	587.0 (57.93)Ab
Control	23	24 h Dry	*722.4 (65.38)Aa	*667.3 (46.14)Aa	*710.4 (41.86)Aa
		24 h Wet	521.2 (38.18)Ab	*412.7 (61.00)Bb	537.0 (59.44)Ab
		1 month	385.7 (96.35)Bc	375.1 (68.55)Bb	492.4 (51.77)Ab
		3 month	254.1 (57.18)Bd	359.5 (57.63)Ab	407.0 (76.81)Ac
		6 month	234.9 (59.39)Bd	394.4 (41.77)Ab	382.2 (95.15)Ac
	40	24 h Dry	857.5 (58.45)Aa	798.0 (65.66)Aa	816.0 (63.36)Aa
		24 h Wet	598.6 (59.57)Ab	536.4 (78.25)Bb	624.3 (71.02)Ab
		1 month	418.8 (98.55)Bc	471.3 (90.99)Bbc	599.7 (77.90)Ab
		3 month	282.8 (70.77)Bd	453.1 (96.21)Ac	456.4 (87.76)Ac
		6 month	234.8 (78.56)Bd	431.7 (97.95)Ac	445.7 (76.90)Ac

Groups identified by different uppercase letters are significantly different ($p < 0.05$) in groups in the same horizontal row; groups identified with different lowercase letters represent significant differences ($p < 0.05$) in the same vertical column. Asterisks (*) represent significant differences ($p < 0.05$) from the 40°C evaporation values in the same treatment and storage condition. Delta () represent significant differences ($p < 0.05$) from the Control treatment values in the same temperature and storage condition.

Table 4

Resin/dentin matrix ratio (%) obtained from different macro hybrid layers according to experimental conditions.

Treatment	Adhesive	Temperature (°C)	
		23	40
PACs	One step	67.9/ 32.1 (3.5)	70.4/ 29.6 (3.5)
	Single bond	67.6/ 32.4 (4.6)	68.7/ 31.3 (5.8)
	Excite	66.7/ 33.3 (3.3)	68.4 / 31.6 (3.6)
Control	One step	69.2/ 30.8 (3.8)	69.5/ 30.5 (3.6)
	Single bond	68.0/ 32.0 (4.1)	68.8/ 31.2 (4.5)
	Excite	68.0/ 32.0 (4.3)	67.2/ 32.8 (4.4)

There was no significant difference between the different experimental conditions: Treatment ($p = 0.8098$), Adhesive ($p=0.1483$) and Temperature ($p = 0.1790$).