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ORIGINAL ARTICLE

Tetrahydrofuran as solvent in dental adhesives: cytotoxicity and dentin bond stability

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Abstract
Objectives The aim of this study was to investigate the cytotoxicity and 1-year dentin bond stability of solvated etch-and-rinse dental adhesives based on tetrahydrofuran (THF), acetone, or ethanol, containing water or not.
Materials and methods Seven primers were prepared using the following solvents: THF, acetone, ethanol, water, THF/water, acetone/water, and ethanol/water. Bovine dentin was used, and specimens for microtensile bond strength (μTBS) test were prepared. Specimens were tested after storage in distilled water for 24 h or 1 year. Cytotoxicity of the solvents was evaluated in 3T3/NIH mouse fibroblasts using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay after exposure for 24 h.
Results No significant differences were detected among solvents after storage for 24 h, except for the water-based group, which showed the lowest μTBS values. After storage for 1 year, the THF-based adhesive system resulted in more stable bonds. Yet, THF showed an intermediate cytotoxicity when compared with the other solvents, being less toxic than phosphate monomer and similar to 2-hydroxyethyl methacrylate.
Conclusion THF seems to be a suitable solvent for adhesive systems.
Clinical relevance THF is a promising solvent that can be used to improve dentin bond stability.

Keywords Acetone · Aging · Dental bonding · Ethanol · Tetrahydrofuran · Toxicity

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Introduction

Dental adhesive systems commonly contain solvents as vehicles [1]. In etch-and-rinse systems, the solvent improves diffusion of the resin monomers into the exposed wet collagen matrix [2]. Solvents may also aid in reexpanding collapsed collagen fibrils when bonding involves overdried dentin [2, 3]. In self-etch systems, because water is required to ionize the acidic resin monomers [4], ethanol is usually present to facilitate the removal of excess water. However, there is a general consensus that solvents need to be completely evaporated from resin-infiltrated dentin matrix, since the residual solvent may compromise the properties of the polymer [5–7].

Various methods have been proposed to improve solvent evaporation and resin impregnation into the demineralized dentin before light-curing, including rubbing action [8], prolonged air-drying, and use of warm air [9]. The residual solvent could also hypothetically reach the dental pulp through dentinal tubules and generate inflammatory responses, mainly in deep cavities [10]. In addition, poor polymerization of the adhesive system by incomplete solvent evaporation might facilitate the diffusion of other adhesive components towards the pulp [11].

Current solvated adhesives include acetone, ethanol, water, or any combination of these as solvents [1, 12]. Due to its advantageous volatile properties (e.g., high vapor pressure), tetrahydrofuran (THF) was recently reported as a feasible alternative solvent for a model adhesive system [13]. Introducing a solvent with advantageous volatile properties may improve the stability of the adhesive polymer on the hybrid layer. However, there is no report on the degradation of dentin bonds of THF-based adhesives over the course of time. In addition, little is known about the biological effects of THF.

The aim of the present study was to evaluate the influence of THF, used as an alternative solvent in the primer of an experimental etch-and-rinse adhesive system, on the resin–dentin bond strength after 24 h and 1 year of water storage. The null hypotheses tested were that (1) the solvent type and (2) the storage time would have no significant effect on the bond strength of dentin. This study also compared the cytotoxicity caused by THF with commonly used adhesive solvents.

Materials and methods

Formulation of the adhesive systems

Seven solvated experimental primers were used in this study. The solutions were formulated containing a 50% mass fraction of 2-hydroxyethyl methacrylate (HEMA), 10% of phosphate monomer [4], and 40% of solvent, as described in Table 1. A photoactivated (di)methacrylate comonomer blend based on bisphenol-A glycidyl dimethacrylate, triethylene glycol dimethacrylate, and HEMA was used as a bonding resin.

Tooth preparation and restorative procedures

The buccal enamel of 42 bovine incisors was wet ground in order to expose a flat dentin surface. The surfaces were wet polished using 600-grit SiC paper for 60 s to standardize the smear layer. Considering the remaining dentin thickness of the specimens, the interfaces were located in medium dentin.

The teeth were randomly assigned into seven experimental groups, according to the primer to be used. The dentin was etched with 35% phosphoric acid for 15 s and rinsed with distilled water for 30 s, followed by the removal of excess surface moisture. A single coat of one of the solvated primers was applied to the dentin with slight agitation for 30 s using a microbrush. Air-stream was used to aid solvent evaporation for 10 s at a 10-cm distance. One coat of the bonding resin was then applied and light activated for 20 s using a LED unit (Radii; SDI, Bayswater, Victoria, Australia) with 1,200 mW/cm^2 irradiance. Resin composite restorations were incrementally built up on the surfaces (Charisma; Heraeus Kulzer, Hanau, Germany).

Table 1 Composition of the experimental primers used in the study

Solvent	Reagents (% mass fractions)					
	HEMA	PM	THF	Acetone	Ethanol	Water
THF	50	10	40	0	0	0
Acetone	50	10	0	40	0	0
Ethanol	50	10	0	0	40	0
Water	50	10	0	0	0	40
THF/water	50	10	20	0	0	20
Acetone/water	50	10	0	20	0	20
Ethanol/water	50	10	0	0	20	20

HEMA 2-hydroxyethyl methacrylate, *PM* phosphate monomer, *THF* tetrahydrofuran

Microtensile testing and failure analysis

After storage in distilled water at 37°C for 24 h, the restored teeth were longitudinally sectioned in both the mesiodistal and incisocervical directions across the bonded interface to obtain beam-shaped specimens with a cross-sectional area of 0.5 mm^2. Six specimens were produced from each tooth and were randomly assigned to be tested immediately or after storage in distilled water containing 0.4% sodium azide, at 37°C. The storage medium was not changed, and its pH was monitored monthly.

The bond strength tested was conducted on a mechanical testing machine (DL500; EMIC, São José dos Pinhais, Paraná, Brazil) at a crosshead speed of 1 mm/min. Bond strength values were calculated in megapascal. Data were statistically analyzed using a two-way ANOVA (solvent type × storage time) followed by a multiple-comparison Tukey's post hoc test ($P<0.05$). Beam-shaped specimens were used as the experimental unit.

Fractured specimens were evaluated under light microscopy at a×500 magnification to determine the failure type. Eighteen specimens were evaluated per group. The predominant failure mode was classified as adhesive, mixed, cohesive within resin, or cohesive within dentin. Prematurely debonded specimens (failure during specimen sectioning or water storage) were also registered, but not included in the averages or statistical analysis.

Cytotoxicity assay

Dulbecco's Modified Eagle Medium (DMEM) was supplemented with 10% fetal bovine serum, 2% L-glutamine, penicillin (100 U mL^{-1}), and streptomycin (100 mg mL^{-1}) (GIBCO-BRL, Grand Island, NY, USA). Mouse fibroblasts of the 3 T3/NIH-immortalized cell line were maintained

as a stock culture in DMEM and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air until subconfluency. Five groups were determined to compare the cytotoxicity of THF with solvents commonly used in dentin adhesive formulations (acetone, ethanol, HEMA, and phosphate monomer). The solvents were diluted serially with the culture medium (1:8,000, 1:4,000, 1:2,000, and 1:1,000) and filtered.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to assess cell metabolic function by mitochondrial dehydrogenase activity. Mouse fibroblasts 3T3/NIH (2×10^4/well) were maintained in DMEM in 96-well plates for 24 h. Cytotoxicity produced by different dilutions of solvents was assessed in a 24-h cell exposure time. After removing the test product, cells were washed with phosphate-buffered saline (PBS). Two hundred microliter of medium with 20 μL of MTT solution (5 mg of MTT mL^{-1} PBS) was added 2×10^4 to each cell well. After 5 h of incubation at 37°C in the dark, the blue formazan precipitate was extracted from the mitochondria using 200 μL/well dimethyl sulfoxide on a shaker at 150 rpm for 5 min. The absorption at 540 nm was determined with a spectrophotometer (Thermo Fisher Scientific, Rockford, IL, USA). Each group had eight replicates, and the experiment was repeated three times. The cell viability data (absorbance) were statistically analyzed by a one-way ANOVA followed by a multiple-comparison Tukey's post hoc test ($P<0.05$) for each dilution.

Results

Microtensile bond strength and failure analysis

The statistical analysis showed that the factors "solvent type" and "storage time" were both significant; their interaction was also significant ($P<0.001$). At 24 h, no significant differences were detected among the primers, except for the material with pure water as the solvent, which presented significantly lower bond strength. After storage for 1 year, the primers with solvent THF, acetone, or THF/water presented the significantly highest bond strengths. However, the only group having similar bond strength between the times 24 h and 1 year was the THF-based primer without water combination. Significantly lower bond strength was observed for the water-based primers compared to its water-free versions after storage for 1 year (Table 2).

The distribution of failure modes is presented in Fig. 1. After 24 h of storage, the predominance of cohesive failures within dentin was observed for water-free primers, whereas the group with pure water as the solvent showed no failure of this type. At 1 year, three and four prematurely debonded specimens were observed for the water and ethanol/water-based primers, respectively.

Cytotoxicity assay

The results for the mitochondrial reducing activity after cell contact with serial dilution of solvents for 24 h are presented in Fig. 2. All solvents showed some degree of cytotoxicity against fibroblast 3 T3 cells. In general, the same pattern of cytotoxicity was shown to all tested concentrations (1:8,000, 1:4,000, 1:2,000, and 1:1,000). Acetone and ethanol showed the lowest cytotoxic effect; THF and HEMA had an intermediate cytotoxicity, while phosphate monomer showed the highest cytotoxic effect.

Discussion

While it is generally agreed that moisture is necessary for proper dentin bonding, the optimal amount of surface wetness may differ depending on the solvent used in each system [14, 15]. Decreased bond strengths in solvated systems may be attributed to incomplete solvent evaporation negatively affecting the mechanical properties of the polymer, therefore interfering with the bonding [5, 16]. Thus, the presence of both residual solvent and dentin water is a reported factor responsible for the degradation of resin–dentin interfaces over the course of time [17]. With regard to the solvent type, some of the most important characteristics are the solvent boiling temperature, vapor pressure, and hydrogen bonding capacity [1]. The first two properties are related to the evaporation properties of the solvent, while the ability to form hydrogen bonds is necessary to reexpand the collapsed dried collagen matrix [2, 3]. Acetone (boiling temperature 56°C, vapor pressure 184 mmHg at 20°C), ethanol (boiling temperature 78°C, vapor pressure 44.6°C mmHg at 20°C), and water (boiling temperature 100°C, vapor pressure 23.8 mmHg at 20%C) are the most commonly used solvents in dental adhesives [1].

Fontes et al. [13] evaluated the performance of THF as a solvent in an experimental etch-and-rinse system. The authors

Table 2 Means (±S.D.) for bond strength, MPa

Solvent	24 h	1 year
THF	55.3 (13.2)A, a	46.2 (14.8) A, a
Acetone	56.0 (14.3)A, a	42.6 (14.6)B, ab
Ethanol	61.1 (11.4)A, a	29.6 (16.4)B, bc
Water	42.0 (12.1)A, b	13.2 (11.3)B, c
THF/water	59.3 (16.6)A, a	38.1 (16.4)B, ab
Acetone/water	56.5 (19.6)A, a	23.0 (10.3)B, c
Ethanol/water	58.5 (14.0)A, a	15.9 (8.2)B, c

Distinct capital letters in the same line indicate differences between 24 h and 1 year. Distinct lowercase letters in the same row indicate differences for solvent type ($P<0.05$). Eighteen specimens per group were tested in each evaluation period

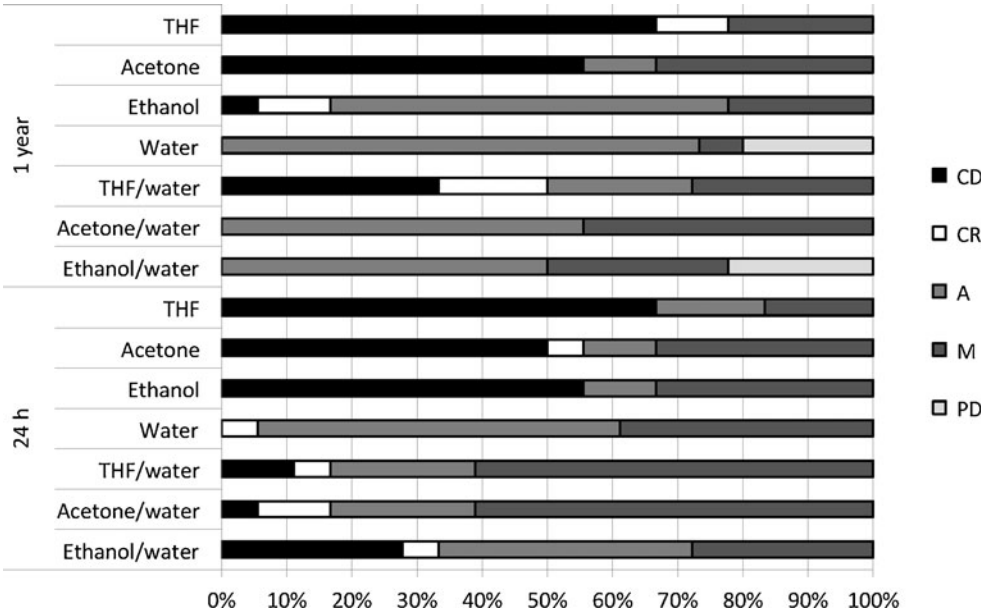

Fig. 1 Distribution of failure modes among groups (*CD*, cohesive failure within dentin; *CR*, cohesive failure within resin; *A*, adhesive failure; *M*, mixed failure; *PD*, prematurely debonded specimen). Eighteen specimens were tested per group in each evaluation period

showed that acetone, THF, and THF/water-based primers maintained bond strength on dentin over a 6-month aging period. In the present long-term study, however, the only group that maintained the initial bond strength was the THF-based primer, without water combination. The stability of the bonded interface could be attributed to favorable physical properties of THF (boiling temperature 65–67°C, vapor pressure 143 mmHg at 20°C). The high volatility implies that this solvent is easily eliminated from adhesive solutions after application on the tooth. Moreover, THF may act as a chelating agent, potentially providing additional chemical bonding to the tooth structure [18]. Considering the failure analysis, the current results showed a high incidence of cohesive failures within dentin for the specimens bonded using THF-based primers, suggesting a greater degradation resistance of the bonded interfaces.

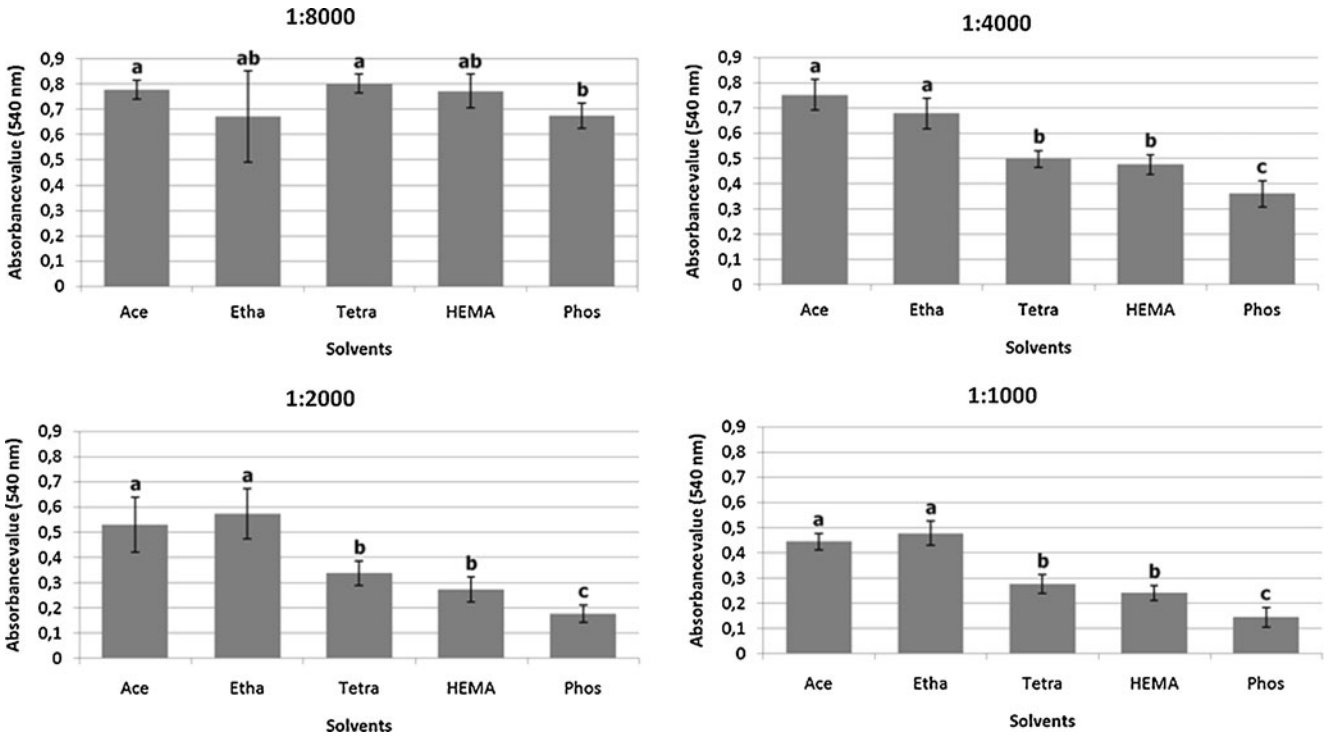

Fig. 2 Absorbance values (mean ± SD) of the solvents compared with each other for each dilution used (1:8,000, 1:4,000, 1:2,000, and 1:1,000 (*v*/*v*)). Different *lowercase letters* indicate significant differences ($P<0.05$). *Ace* = acetone, *Etha* = ethanol, *Tetra* = tetrahydrofuran, *HEMA* = 2-hydroxyethyl methacrylate, and *Phos* = phosphate monomer

It is known that water is a strong polar solvent that can usually be added to resin monomers in dental adhesives systems [1]. However, the present findings indicate that the role of water in the composition of etch-and-rinse adhesives should be reevaluated because water-containing primers presented significantly lower bond strengths compared to their water-free versions, especially after storage for 1 year. Ethanol and water can form hydrogen bonds with resin monomers [19], resulting in increased retention of these solvents. In addition, their high boiling temperature and low vapor pressure imply that ethanol and water are difficult to remove even by thorough air-drying [6]. These features may explain why significantly lower bond strengths were obtained for ethanol and water-based adhesives after 1 year of storage. Moreover, all the premature failures were observed for water and ethanol/water-based primers, indicating that it may affect the mechanical stability of the resin–dentin bonds.

There are no reports in the literature on the cytotoxic effects of solvents used in the composition of dental adhesives, since these studies are focused on the evaluation of methacrylate monomers. Cytotoxicity assays have been considered a useful tool for initial screening of the toxic effects of dental materials [20–22]. Control of the physicochemical and physiological environment, reduction in animal experimentation, and reproducibility are some advantages of cytotoxicity assays [20–22]. However, the major limitation is the difficulty in mimicking the in vivo conditions [20–22]. HEMA is related to the reduction in cell viability of primary cultures in concentrations of 1 and 3 mmol/L [23]. This study also demonstrated a reduction of procollagen alpha1 type I protein and an overexpression of tenascin protein [23]. Another study demonstrates that even in low concentrations, HEMA can interfere on procollagen synthesis and mRNA expression [24]. Despite these negative characteristics, HEMA continues to be used in the formulation of adhesive systems, and its use is not related to problems for the patient, since the material is not used directly on the exposed dental pulp or in very deep cavities without prior protection. On the other hand, THF is a solvent widely used in chemistry, but only recently, it has been proposed for the composition of dental adhesives [13]. THF is recognized as a weak toxin, with approximate acute LD50s in the range of 2 to 3 g/kg, 8 to 20 mg/L, and 800 mg/kg following oral, inhalation, and intravenous routes, respectively [25]. Taking into account that THF is used in the manufacture of food packaging, with direct contact with food that is ingested, a great concern exists about the effects of THF in chronically exposed humans. In this context, some in vivo and in vitro studies have evaluated the mutagenic and carcinogenic effects of THF, showing nontoxic effects [25–27]. When compared with the other solvents tested, THF showed an intermediate cytotoxic effect. Consequently, THF seems to perform acceptably with respect to cytotoxicity.

The null hypotheses tested were rejected, since it was demonstrated that both the solvent type and storage time significantly affected the bond strengths. Although most studies have suggested some modifications of standard clinical protocols to optimize dentin bonding and reduce the aging effects [17], the present study proposes the incorporation of an alternative solvent in the composition of etch-and-rinse systems. The development of new formulations is a promising method for a significant improvement of the bonding performance. In spite of the favorable mechanical and biological performance of THF-based primers, this topic still requires further investigation.

Conclusion

THF-based adhesive systems presented increased stability of the bonded interfaces over a 1-year water storage period. The presence of water in dental adhesives significantly compromised the long-term longevity of the bonds. With respect to cytotoxicity, THF showed promising in vitro results that should be confirmed with in vivo studies.

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Conflict of interest The authors declare that they have no conflict of interest.

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