Original Article

Comparative study of different cytotoxicity of bonding systems with different dentin thickness on L929 cell line: An experimental study

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ABSTRACT

Background: Dentin-bonding agents should have good biocompatibility as they come into close and prolonged contact with vital dentin. The present study aimed to evaluate and compare the cytotoxicity of total etch and self-etch dentin bonding systems with two different dentin thickness on L929 cell line.

Materials and Methods: In this *in vitro* study 80 Class I cavities were prepared on the occlusal surfaces. The teeth were randomly divided to two groups of 40 each based on two RDT 0.5 mm and 1.5 mm. Samples were further subdivided into four subgroups of 10. Group 1: Adper Scotch bond Multi-Purpose (SBMP), Group 2: Adper Single Bond Plus (SBP), Group 3: Adper Scotch bond SE (SSE) and Group 4: Adper Easy One (EO). Group 1 and 2 were total-etch and Group 3 and 4 were self-etch. The cavities were sealed after applying of dentin bonding. Then crowns were immersed in culture medium for 24 hours and the cytotoxicity of resultant toxic extraction was measured with 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in 4 serial dilutions (neat, 1/2 , 1/10 , 1/100). Data were analyzed by Two-way ANOVA and *t*-test.

Results: For all the dilutions, cytotoxicity was significantly higher with 0.5 mm remaining dentin thickness (RDT) relative to 1.5 mm RDT ($P \leq 0.05$). In neat dilution for both RDTs, cytotoxicity was different among all the four dentin bondings. Expression in decreasing order of cytotoxicity was SBP > SBMP > SSE > EO for both RDTs in neat dilution ($P < 0.05$). For all the dilutions, cytotoxicity was significantly lower for self-etch bonding systems in comparison with total-etch bonding systems ($P < 0.05$).

Conclusion: In the present study, lower cytotoxicity was found with an increase in the dilution of toxic extract and also cytotoxicity decreased with an increased dentin thickness. The adhesive systems had degrees of cytotoxic effects on cultured L929 compared to the control, except for the EO group.

Key Words: Cytotoxicity, Dentin adhesive system, L929 cell line, Dentin thickness

INTRODUCTION

The increased request for esthetics and minimally invasive tooth restorations resulted in the rapid development of adhesive dentistry. Such advances lead to the production of different types of dental adhesives (DAs) classified according to the bonding mechanism

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and clinical application of etch-and-rinse and self-etch adhesives.[1] The 37% phosphoric acid and subsequent adhesive, provided in two bottles (i.e., primer plus adhesive), were applied for the conventional two-step system. The two-step self-etch system consisted of one bottle containing acid monomers and acid primers and other bottle with balanced concentrations of hydrophilic and hydrophobic monomers. The acidic primer with the adhesive resin and elf-etch systems in one bottle, known as "all in one", were used for single-stage adhesives and two-step self-etch systems.[2]

The 2-hydroxyethyl methacrylate (HEMA) and triethylene glycol dimethacrylate (TEGDMA) as the components of adhesives and restorative resins can diffuse through the dentinal tubules and reach the pulp tissue in concentrations considered toxic to the pulp cells, respectively.[3,4]

Recently, the dentists have focused on the adequacy of the biological properties of dentin-bonding systems due to the increased orientation of consumers toward these products and rising demand for improving their quality.[5] The biological behavior of the materials is remarkably affected by the characteristic composition and quantity of the released components. After restorative procedures, the pulp tissue may be damaged as result of the components derived from restoration/lining materials.[6,7] However, the toxic action of components derived from bonding systems can be controlled in the presence of dentin as a biological barrier. The inherent features of dentin like buffer capacity and hydraulic conductance can modify the toxic action of these components, which have intense cytopathic effect raising bonding systems.[8]

The effect of three dentin contacting materials on three-dimensional cultures of pulp-derived cells was evaluated in one study conducted by Galler *et al*. The results of mentioned study showed that the dentin acts as a barrier, decreasing the elicited cytotoxicity with increasing thickness.[8]

To improve contact between the restorative material and the walls of the prepared cavity of the tooth, bonding agents are employed.[9] Dentin-bonding agents should have good biocompatibility as they come into close and prolonged contact with vital dentin.[10] The widely used base substances in the dentin formulations are methacrylate monomers such as 2,2-bis [p-(2-hydroxy-3-methacryl-oxypropoxy) phenyl] propane (bis-GMA) and 2-hydroxyethylmethacrylate

(HEMA), along with other substances such as organic solvents, water, initiators, and inorganic fillers.[11] Toxicity of adhesive materials is closely associated with the dentin permeability as it allows increased diffusion of the released components through the dentin into the pulp. The thickness and age of the remaining dentin are the main factors contributing to permeability.[12]

Evidence of dentinal fluid transudation after the application of two- or one-step total-etch or one-step self-etch adhesives confirm that these systems do not hermetically seal the deep vital dentin.[13,14] After polymerization, these monomers manifest incomplete conversion, and they may be carried out of the polymer matrix, released in the saliva or transferred through the dentinal tubules into the pulp chamber, together with the other components of the system. A wide range of studies has demonstrated that the released monomers cause chemical damage to cultivated cells and pulpal tissue.[15]

From a clinical point of view, the correlation between *in vitro* testing and clinical usage tests suffers from some limitations. However, the *in vitro* cytotoxicity test is paramount to understanding the biologic risk of these materials at the initial setting stage. *In vitro* tests have a number of significant advantages over other types of biocompatibility tests. These tests are relatively quick to perform, generally cost less than animal tests, are standardized, are suitable to large-scale screening, and can be tightly controlled to address specific scientific questions. The overriding disadvantage of *in vitro* tests is their questionable relevance to the final *in vivo* use of the material.

The null hypotheses were: (1) there is no difference between four dentin bonding systems in point of cytotoxicity and (2) the cytotoxicity of dentin-bonding systems is not influenced by remaining dentin thickness (RDT). We sought to evaluate and compare the potential cytotoxic effects of four commonly used dentin adhesives of different generations (4th–7th) with two dentinal thicknesses as a barrier on L929 cells.

MATERIALS AND METHODS

Samples
This experimental study was carried out at Mashhad University of Medical Sciences, Mashhad, Iran, and Kerman University of Medical Sciences, Kerman, Iran. We obtained 80 human, caries-free, premolars extracted for orthodontic reasons from patients aged 18–24 years.

Selection criteria

All the teeth were healthy. The teeth were cleaned using water and pumice slurry to remove any exogenous material and then were examined under a stereomicroscope (Olympus Inc., Melville, NY, USA) at ×40 magnifications to make sure they were free of caries, cracks, and occlusal wear. The teeth were stored in 0.5% chloramine T solution in distilled water (pH 7.8) and used within 3 months following extraction. The crowns of the teeth were removed from CEJ perpendicular to the teeth long axis with a high-speed water-cooled diamond disc.

Dentin-bonding systems

The four dental adhesives included two total-etch bonding systems, Adper Scotchbond Multi-Purpose (SBMP; 3M/ESPE, USA) and Adper Single Bond Plus (SBP; 3M/ESPE, USA), and two self-etch bonding systems, Adper Scotchbond SE (SSE; 3M/ESPE, USA) and Adper Easy One [EO; 3M/ESPE, USA; Table 1].

Specimen preparation

To avoid touching the predentin, following root removal, the soft tissue of the pulp was cautiously eliminated from the pulp chamber with a sterile excavator. Under air-water coolant, a Class I cavity, approximately 4 mm long and 1.5 mm wide, was obtained using a high-speed cylindrical diamond bur (Brasseler Komet, lemgo, Germany). The teeth were then randomly assigned to two main groups of 40 specimens each based on two remaining dentin thicknesses (0.5 mm and 1.5 mm) between the cavity floor and the pulp side of the dentin. A caliper (Mitutoyo, Japan) was utilized to carefully measure the remaining dentin. Samples in each main group were further subdivided into four subgroups of 10 according to each dentin bonding system. Group 1: Adper SBMP, Group 2: Adper Single Bond Plus (SBP), Group 3: Adper Scotchbond SE (SSE), and Group 4: Adper Easy One (EO). We also merged Groups 1 and 2 as a total-etch bonding group and Groups 3 and 4 as a self-etch bonding group. Before testing, the prepared specimens were sterilized by autoclaving at 121°C for 25 min. For hydration, the sterilized specimens were kept in DMEM for 24 h at 37°C, and the lack of contamination was confirmed.

Adhesive procedures

For each dentin bonding, the adhesives were applied on the cavity based on the manufacturer's instructions and using Demetron LC Curing Light (Kerr, USA 650–700 mW/cm² intensity). Then, Hard Blue Inlay Wax (Kerr, USA) was employed to seal the cavities.

Specimens were immersed in 70% ethanol for 10 min and put in centrifugal tubes containing 4 cc of Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Glasgow, UK) supplemented with 10% Fetal Bovine Serum (FBS, Gibco). The tubes were incubated at 37°C and 100% relative humidity, with 5% CO_2, and 95% air for 24 h. The uncured monomers were released through dentinal tubules into the culture medium. Thereafter, this toxic extract was serially diluted in four dilutions of neat, 1/2 v/v, 1/10 v/v, and 1/100 v/v.

Table 1: Classification and composition of tested dentin adhesives

Dentin adhesive	Classification	Components
Adper Scotchbond Multi-Purpose	Three-step etch and rinse (Fourth generation)	Etchant: Water (55%-65% wt), phosphoric acid (30%-40% Wt), synthetic amorphous silica (5%-10% wt) Primer: HEMA (40%-50% wt), water (35%-45% wt), copolymer of acrylic and itaconic acid (10%-20% wt) Adhesive: BISGMA (60%-70% wt), HEMA (30%-40% wt), DL-Camphorquinone
Adper Single Bond Plus	Two-step etch and rinse (Fifth generation)	Etchant: Water (55%-65% wt), phosphoric acid (30%-40% wt), synthetic amorphous silica (5%-10% wt) Ethyl alcohol (25%-35% wt), silane treated silica (nanofiller) (10%-20% wt), BISGMA (10%-20% wt), HEMA (5%-15% wt), glycerol 1,3-dimethacrylate (5%-10% wt), copolymer of acrylic and itaconic acids (5%-10% wt), water (<5% wt), UDMA (1%-5% wt), DL-Camphorquinone
Adper Scotchbond SE	Two-step self-etch (Sixth generation)	Liquid A: Water (70-80% wt), HEMA (10-20% wt), Surfactant, Pink color Liquid B: Surface treated zirconia, TEGDMA (15%-25% wt), di-hema phosphates (10%-15% wt), phosphoric acids-6-methacryloxy-hexylesters (5%-10% wt), UDMA (1%-10% wt), TMPTMA (5%-15% wt), ethyl 4-dimethyl aminobenzoate (<2% wt), DL-Camphorquinone (<2% wt)
Adper Easy One	One-step self-etch (Seventh generation)	BISGMA (15%-25% wt), HEMA (15%-25% wt), ethanol (10%-15% wt), water (10%-15% wt), phosphoric acid-6-methacryloxy-hexylesters (5%-15% wt), silane treated silica, 1,6-hexanediol dimethacrylate (5%-10% wt), copolymer of acrylic and itaconic acid (1%-5% wt), camphorquinone (1%-3% wt), 2,4,6-trimethylbenzoyldiphenylphosphine oxide (1%-3% wt), ethyl methacrylate (1%-5% wt)

HEMA: 2-hydroxyethylmethacrylate

Cell culture

We used L929 mouse fibroblasts (L929 HUKUK 95030802, Sap Institute, Ankara, Turkey) for the experiment. The cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin solution (Biological Industries). The cultures were supplied with fresh medium every other day and incubated at 37°C and 100% relative humidity, with 5% CO2 and 95% air. Using a mixture of 0.05% trypsin and 0.02% ethylenediaminetetraacetic acid, we detached the confluent. Thereafter, aliquots of the separated cells were subcultured. Cell cultures between the fifth and eighth passages were used in all the experimental procedures.

Preparation of cells for morphological evaluation

The cells were plated at 5×10^3 cells/well in 6-well plates with 2 cc of culture medium (DMEM) and incubated for 48 h at 37°C 100% relative humidity with 5% CO_2 and 95% air to obtain a monolayer cell growth. After the cell culture medium was removed, we added to each well 1.8 cc of four different dilutions (neat, 1/2 v/v, 1/10 v/v, 1/100 v/v) of the toxic extract diluted with the culture medium. One well served as control and 1.8 cc of pure cell culture medium was placed in the control well. The plate was incubated for 24 h at 37°C 100% relative humidity with 5% CO_2 and 95% air. Thereafter, the morphologic feature of cells was evaluated using a dissecting microscope (Zeiss, Germany) with ×20 magnification.

(3-(4,5-Dimeth ylthiazol-2-yl)-2,5-dipheny ltetrazolium assay

(3-(4,5-Dimethylth iazol-2-yl)-2,5-diphenylte trazolium (MTT) is a standard colorimetric laboratory assay for quantifying cellular growth, which can be employed for determining the cytotoxicity of potential medicinal agents and other toxic materials. Yellow MTT bromide, a tetrazole) is reduced to purple formazan in the mitochondria of viable cells [Figure 1]. A solubilization solution (usually dimethyl sulfoxide, an acidified ethanol solution or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product in a colored solution. A spectrophotometer can quantify the absorbance of this colored solution at a certain wavelength (usually between 500 and 600 nm). The utilized solvent affects the maximum absorption. Active mitochondrial reductase enzymes can cause this reduction; hence, conversion can be directly associated with the number of viable cells. The efficacy of the agent that causes cell death can be inferred by the comparison of the amount of purple formazan produced by treated cells, as well as the amount of formazan produced by untreated control cells [Figure 2].

Cytotoxicity testing

In general, 5000 cells plus 200 μL of culture medium (DMEM) were added to all the wells of a 96-well cell culture plate and incubated to obtain a monolayer cell growth. After the incubation period, we replaced the culture medium by 200 μL of toxic extract with the aforementioned four serial dilutions, except for the control well, and incubated for 24 h. By discarding the toxic extract, cell exposure halted and cell viability was promptly recorded using MTT assay.
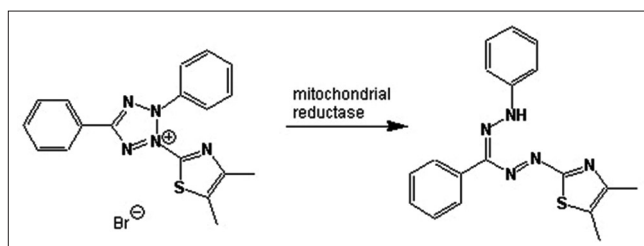

Figure 1: Chemical formulas of (3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide, a tetrazole) reduction to formazan in the mitochondria of living cells.
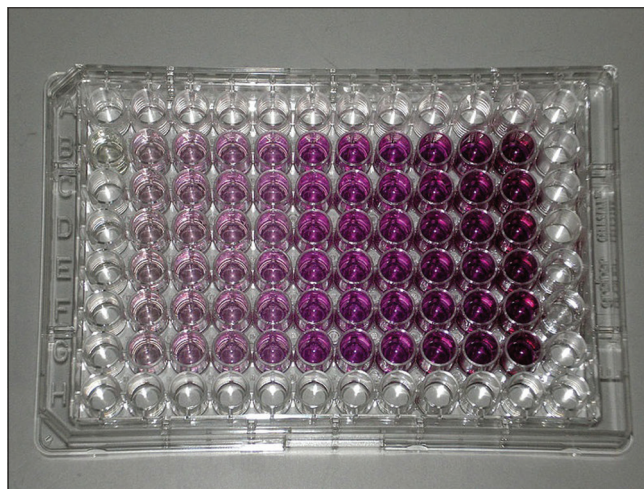

Figure 2: A 96-well microtiter plate used in an (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay. Each column was incubated with different amounts of culture cells, increasing from 500 cells in column 2 (left) to 100.000 cells in column 11 (right), for 72 h. After a short incubation with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium, the purple formazan product was extracted using dimethyl sulfoxide. As can be seen, higher amounts of cells result in higher formazan production and thus a stronger purple color.

In short, MTT assay solution (1 mg/ml) was poured in each well. After incubation for 4 h, 200 μL of dimethyl sulfoxide and 25 μL of buffered glycine were also added to each well. To solve the formazan crystals completely, the plates were shaken at 1500 rpm for 5 min. All the cell cultures were performed in eight replicates for each dilution of the toxic extract and the control group. An Elisa Reader device (Stateax, USA) was employed to quantify optic density (OD) of the resultant suspension at 540 nm wavelength. For the control group, the mean OD was set to represent 100% viability. We expressed the outcomes of the experimental groups as the percentage of the control. Cell viability was expressed as a percentage using the following formula: Percentage of viable cells = (A/B) ×100 where A denotes the number of viable cells in the experimental well and B indicates the number of viable cells in the control well.

Outcomes

The primary outcomes of our analyses were the presence of difference between four dentin bonding systems in point of cytotoxicity. Furthermore, the secondary outcomes from the analyses were the influence of cytotoxicity of dentin-bonding systems by RDT.

Statistical methods

To analyze the data, two-way analysis of variance (ANOVA) and *t*-test were performed. Tukey's test was run for *post hoc* comparisons. The significance threshold was set at 0.05 for all the tests. The data were analyzed by SPSS, version 13.0 (StataCorp. Version 13. College Station, TX: StataCorp LP; 2013). Moreover, the normality of the variables' distribution was examined using the K-S test before performing the statistical analyses.

Research ethics

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran (ethical code: IR.MUMS.REC.1393.762). All the ethical considerations were observed in the present study.

RESULTS

Two-way ANOVA revealed that RDT and adhesive type significantly affected cell viability ($P \leq 0.005$). *Post hoc* test showed that the mean percentage of cell viability was significantly higher for EO group in comparison with the other three groups [$P \leq 0.005$].

Total-etch versus self-etch dentin bonding system

Cell viability was significantly higher in the self-etch group relative to the total-etch dentin bonding group. In all the dilutions of the total-etch group, cell viability was significantly higher with 1.5 mm RDT compared to 0.5 mm RDT. In the self-etch group, only in 1/2 dilution, cell viability was significantly higher with 1.5 mm in comparison with 0.5 mm RDT [$P = 0.002$].

Results of morphological evaluation of cytotoxicity

Morphological evaluation of L929 cell samples was performed using a dissecting microscope (Zeiss, Germany) with ×20 magnification. Cells were spindle shape or polygonal with extended cellular processes in the control group. They also had good density, and evidence of cell rounding and detachment was scarce [Figure 3]. Density, round appearance, and loss of extended cellular processes in all the bonding agents and 0.5 mm RDT with neat dilution significantly diminished, and some cells were found floating in the culture medium [Figure 4]. As was noted in the morphological view of L929 cells, cell density in 1.5 mm RDT slightly decreased, and a mild retraction and some round appearances were found [Figure 5].

DISCUSSION

There are various cell culture techniques to assess cell damage caused by dental biomaterials. Commonly, L929 cells cell line is well established and has been used for cytotoxicity evaluation of biomaterials.[16] Our study was performed to evaluate the cytotoxicity of total etch and self-etch dentin bonding systems

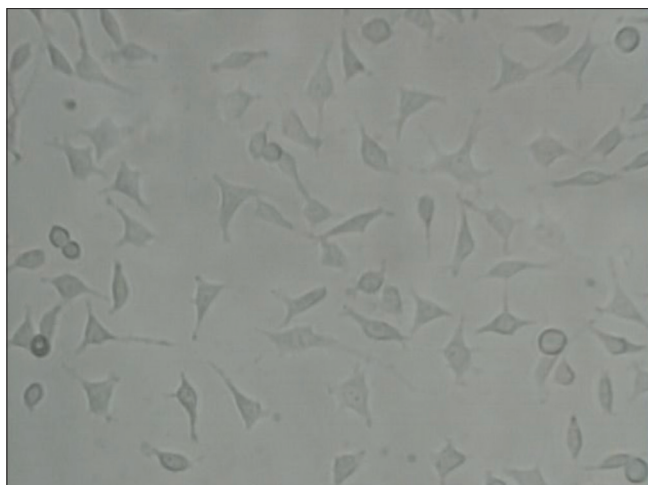**Figure 3:** Morphological view of L929 cells in control group.

Figure 4: All bonding agents and 0.5 mm remaining dentin thickness with neat dilution.
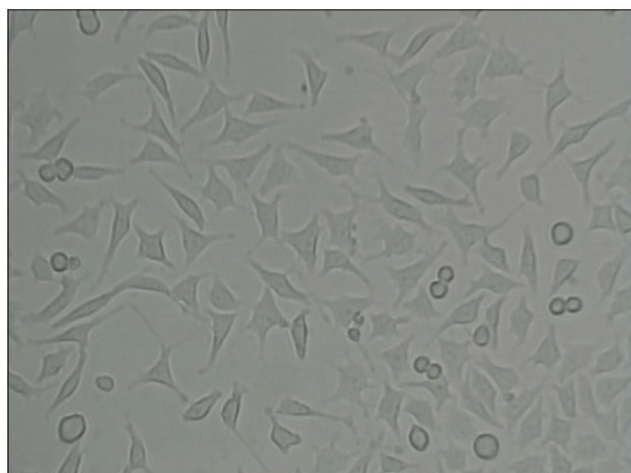

Figure 5: Morphological view of L929 cells in 1.5 mm remaining dentin thickness with other dilutions.

with two different dentin thickness on L929 cell line. Our findings showed that cell viability was higher in the self-etch group than the total-etch dentin bonding group. Moreover, cell viability was higher with 1.5 mm RDT compared to 0.5 mm RDT in all the total-etch dilutions, and it was higher with 1.5 mm in comparison with 0.5 mm RDT only in the 1/2 dilution of self-etch. Based on morphological evaluation, density, round appearance, and extended cellular processes in all the bonding agents decreased.

Similarly, L929 cells cell line were used for cytotoxicity evaluation of biomaterials in one study by Gurpinar *et al*. Based on the obtained results, all self-etching systems were found to be cytotoxic to varying degrees, and more pronounced toxic effects were observed at lower dilution.[17]

Dentin permeability and residual dentin thickness influence on adhesives induced cytotoxicity of unreacted monomers pulp cells.[18] However, all adhesive restorations are not affected by cytotoxic components of the adhesives and/or composites. The number of unconverted monomers is affected by some factors, such as curing time, thickness of resin increment, and light intensity provided by light units.[19]

It has been suggested that 0.5 mm RDT can diminish material toxicity to 75% and 1 mm dentin can lower toxicity to 90% of the control value in the absence of dentin.[20] Hamid and Hume used dentin slices 0.4-3.6 mm in thickness as a barrier to determining the impact of dentin thickness on diffusion of resin monomers from adhesives after 24 h of incubation. They found that dentin thickness was inversely associated with component diffusion and directly related to cross-section fraction of dentinal tubules.[21]

We found that the cytotoxicity of adhesives was lower when they were applied to dentins with 1.5 mm thickness rather than those with 0.5 mm thickness. Our findings were in agreement with the results of Galler *et al*.'s study investigating the effects of 0.1–0.5 mm thick dentin discs as a barrier against the cytotoxicity of different bonding systems.[8] Based on the mentioned study, dentin can protect against acidic self-etching dentin bonding agents, which can be explained to some extent by the buffer capacity of dentin. Superficial dissolution of dentin apatite arising from phosphoric acid on dentin can lead to neutralization of phosphoric acid via binding of the protons to the $OH-$ and PO_4^3- ions of the apatite. Due to the increased permeability of thin dentin layers (100 μm), the toxic reaction will grow stronger either through the remaining protons or the included monomers.[8]

The cell damage caused by resinous monomers and other constituents of adhesive systems can also be affected by diversity in the chemical composition of the materials and the interaction of their various components with the dentinal structure,[22] hence disparate pulp tissue responses.[23] The discrepant cytotoxicity outcomes for the tested adhesives can be explicated by the differences in their rheological properties and application technique.[24] It should be underscored that comparative data on cytotoxicity of current self-etching and total-etch adhesives are limited.[25]

Some studies explain the significantly higher cytotoxicity of the total-etch adhesive system (SBMP and SBP) than self-etch adhesives system (SSE and EO) in both 0.5 and 1.5 mm RDT.[26] It is broadly accepted that the removal of smear layer with acid etching strengthens the bond between resin and the tooth,[27] widens the entry to the dentinal tubules, and consequently, promotes dentinal permeability and the risk of toxicity. In the teeth previously etched with 37% phosphoric acid, a higher and faster rate of diffusion was noted.[28] Self-etching adhesives do not thoroughly eliminate the smear layer, rather, they demineralize a depth of 5 μm on the surface of adjacent dentin through the smear layer.[29] Since the resinous monomers cannot penetrate deep into the dentinal tubules using self-etching adhesives, they are considered safer than total-etch adhesive systems.[23]

Koulauzidou noted that in rat pulp cells and human long fibroblasts cell viability reduced significantly after exposure to total-etch adhesive in comparison with self-etch adhesives.[30] Another study exhibited that although the self-etch bonding agents did not affect the cell cycle patterns, total-etch bonding agents induced cell cycle arrest.[31] These results were in agreement with our findings showing that cytotoxicity of total-etch adhesive systems was significantly higher than self-etch adhesive systems in both 0.5 and 1.5 mm RDT.

Totally, our findings showed a difference among the four dentin-bonding systems with respect to cytotoxicity and that dentin thickness clearly affected the cytotoxicity of dentin bonding systems.

Furthermore, synergistic reaction occurred in 25 μmol/L of BisGMA disregarding HEMA concentration. In addition, low-molecular-weight resins such as HEMA, 4-META, and TEGDMA may act as solvents for more viscous resins such as BisGMA and UDMA that enhances their diffusibility to cells and tissues.[32] Interactions among multiple components of the current adhesive systems may cause different levels of cytotoxicity than the individual components themselves; therefore, net evaluation of the cytotoxicity of products should be considered.[13]

Different degrees of cytotoxicity were found in all the evaluated materials, which could be due to diversity in composition of the specimens. As regards the list of constituents of the tested dentin adhesives, the materials vary in terms of resin matrix and inorganic filler particles. Considering the scarcity of data on inorganic fillers and the percentage by weight of the matrix, this aspect could not be discussed here.[33]

Since simplified (two-step) etch-and-rinse adhesives exhibit greater permeability after polymerization because they include higher percentages of hydrophilic monomers compared to three-step adhesive, hence facilitating the presence of water-filled areas within hybrid layer and causing the cytotoxic monomer to leach further. This finding explains the significantly higher cytotoxicity of SBP than SBMP.[34]

Moreover, we found that the cytotoxicity of SSE was more pronounced than EO. SSE belongs to the strong self-etch adhesives (pH <1) and has higher acidity compared to mild and intermediary strong systems. The interaction patterns observed in enamel and dentin resemble phosphoric acid treatment after etch and rinse approach. EO is a mild self-etch adhesive (pH ~2.3) and low pH directly influences the self-etch adhesive capacity to dissolve the smear layer and demineralize the subjacent dentin at a higher rate causing more permeability, which explains our findings regarding the higher cytotoxic effect of SSE relative to EO.[11]

Our findings revealed that cytotoxic effects of different dilutions of bonding agents on L929 cells increased at higher concentrations after 24 h of incubation, which was in congruence with the results of other studies.[33] It goes without saying that reduced cytotoxic effects of the materials by dilutions were the results of low concentrations of harmful components.

Diffusion and the toxicity of resinous materials are affected by the remaining dentin thickness, permeability, and dentin location; however, lack of a subjective idea as to these factors is a matter of concern. Therefore, biologic risks associated with the application of dentinal adhesives in deep cavities, close to the pulp, should be minimized. It appears that components of the adhesive systems tested may be capable of causing cellular damage, even when an interposing layer of dentin separates the material from the pulp. Clinicians should therefore consider the application of a lining agent to the depths of their cavity preparations before applying a dental adhesive.

CONCLUSION

The results suggest that the tested adhesive system had some degree of cytotoxic effects on cultured L929 in

compare to control except for EO group. We showed that lower cytotoxicity was found with an increase in the dilution of toxic extract and also cytotoxicity decreased with an increased dentin thickness. Future studies are suggested to examine the relationship between toxicity and the degree of the conversion of adhesive systems. It would also be beneficial to modify the testing device to simulate pulpal pressure and evaluate its influence on the outcomes.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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