Evaluation of the cytotoxic and genotoxic effects of different universal adhesive systems

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Abstract

Objectives: The objective of this study was to evaluate and compare the cytotoxicity and genotoxicity of different universal adhesive systems in the mouse fibroblast cell line L929.

Materials and Methods: L929 (mouse fibroblast) cells were exposed to GPB (G-Premio Bond) (GC Europe, Belgium), Prime&Bond Universal (Dentsply Sirona, USA), Universal Bond Quick (Kuraray, USA), Single Bond (SB) Universal (3M ESPE, USA), and Tokuyama Universal Bond (TB) (Tokuyama, USA). Cell viability was assessed by the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide test, whereas oxidative DNA damage was assessed by determining the 8-hydroxydeoxyguanosine level using an enzyme-linked immunoassay kit. Statistical analysis was performed by one-way analysis of variance, followed by Bonferroni post hoc tests.

Results: Cytotoxic and genotoxic effects of TB and SB Universal groups were significantly higher than the other groups (P < 0.05). Among the adhesives tested, GPB (93.0 ± 1.3) had the least cytotoxicity, while TB (67.3 ± 3.0) had the most cytotoxic effect. In terms of genotoxicity, GPB (2.2 ± 0.3) had the least genotoxic effect, while Tokuyama Bond Universal (4.17 ± 0.4) had the most genotoxic effect.

Conclusions: Universal adhesive systems used in dentistry have cytotoxic and genotoxic effects in live cells. Universal adhesive systems should, therefore, be used with caution due to their cytotoxic and genotoxic effects in clinical applications.

Keywords: 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; 8 OHdG level; adhesive systems; cytotoxicity; genotoxicity

INTRODUCTION

Developments in dental restorative materials aim to obtain the most ideal material that can restore hard tissue loss. Restorative materials have prolonged contact with soft tissues and fluids in the oral cavity. Hence, besides mechanical and physical properties, biocompatibility should be taken into account when selecting a newly developed restorative material.¹,²

Adhesive systems play an important role in the restorative dentistry and are divided into two types: self-etch and etch-rinse. Moreover, depending on the application stages, these systems are subdivided into single-, two-, and three-step systems.³ In recent years, a single-step, self-etch adhesive system, named universal or multimode, has been developed; it can be applied with both self-etch and etch-rinse techniques.⁴ This new system allows clinicians to use the most suitable etching technique. Universal adhesive systems can be applied with three different etching techniques by the help of 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer content.⁵

Although adhesive systems have been used for the advantage of strict adhesion to the enamel and dentin, they have the disadvantage of genotoxic effects resulting from different resin
Monomers present in these systems. Adhesive systems typically include various monomers, such as bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA), hydroxyethyl methacrylate (HEMA), and dipentaerythritol pentaacrylate monophosphate; these monomers form the main organic matrix of most composite resins and dental adhesives.9,10

Monomers, such as HEMA, TEGDMA, Bis-GMA, and UDMA, are able to be transported throughout the dentinal tubules and result in toxic effects in the dental pulp. Moreover, hydrophilic and hydrophobic groups together have greater toxicity than either group alone.8 HEMA and Bis-GMA leach from polymerized adhesive systems and cause genotoxic effects in the human gingival fibroblasts.9,10 HEMA has also been shown to cause cell apoptosis11 and necrosis,12 while Bis-GMA has been shown to have teratogenic effects.13 TEGDMA, another resin monomer, may cause serious DNA damage in the mammalian cells, as indicated by micronucleus induction, gene mutation, and large DNA sequence deletions in hamster fibroblasts.13,14 Although research on the genotoxicity of UDMA is still scarce and controversial, Schweikl et al.15 found that UDMA does not have mutagenic activity in the Ames test, but it did show the ability to induce micronuclei.

Oxidative damage to DNA can be detected by chemical, physical, and enzymatic methods. The levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) have been used to evaluate DNA damage. Cytotoxicity caused by oxidative stress can be confirmed by assessing 8-OHdG levels. Hence, 8-OHdG has often been used as a biomarker of oxidative damage.16

Resin monomers increase reactive oxygen species (ROS), which have potential genotoxic effects.9,16 ROS cause many chronic degenerative diseases, including cancer, as they exhibit genotoxic effects, resulting in damage to purines and pyrimidines. If oxidative stress persists, oxidative damage to lipids, proteins, and nucleic acids accumulates and eventually results in biological effects ranging from the alteration of signal transduction pathways and gene expression levels to cell transformation, cell mutagenesis, and cell death.16 Hence, attention is being paid to the long-term effects, such as genotoxicity, of dental materials.

Although there are many studies on the cytotoxicity of resin monomers in adhesive systems, only a few studies have examined the genotoxicity of dentin-bonding agents.8,7,15 This empirical study aimed to compare the genotoxic and cytotoxic effects of five different universal adhesive systems, which are widely used in adhesive dentistry, in the mouse fibroblast cell line L929 by the enzyme-linked immunosassay (ELISA) and the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) test. Little is known about the genotoxicity of universal adhesive systems. To the best of our knowledge, this is the first study in the literature to evaluate the genotoxicity of different universal adhesive systems by the ELISA.

MATERIALS AND METHODS

The present study was ethically approved by the Research Ethics Committee of Gaziantep University (2018/373).

Samples preparations

The dental-bonding agents tested were G-Premio Bond Universal (GPB) (GC Europe Inc., Leuven, Belgium), Tokuyama Universal Bond (TB) (Tokuyama America Inc., California, USA), Quick Universal Bond (QB) (Kuraray America Inc., Texas, USA), Prime&Bond Universal (PB) (Dentsply Sirona, Inc., Pennsylvania, USA), and Single Bond Universal (SB) (3M/ESPE, Inc. MN, USA). Although Quick Bond Universal and SB Universal are ethanol based, GPB Universal, PM Universal, and TB contained acetone as the solvent. Detailed information about the dentin-bonding systems used in this study is shown in Table 1. Tested adhesives were prepared according to the manufacturers’ instructions. In addition to the five different experimental groups, a control group containing only the L929 fibroblast cell line (American Type Culture Collection [ATCC® CRL-6364]) without any adhesive material was added. To obtain the cytotoxic values of the adhesive systems used, preparation of test samples, sterilization, preparation of cell culture, and evaluation with the XTT and ELISA (Elabscience, Cat

| Table 1: Materials used in this study |
|------------------------------------|
| **Adhesives** | **Main components** | **pH** | **Manufacturer** |
| GPB | 10-MDP, 4-META, MDP, dimethacrylate resins, distilled water, acetone, photo initiators, silica fine powder | 1.5 | GC Europe (Leuven, Belgium) |
| Tokuyama universal bond (TB) | Liquid A: phosphate monomer, Bis-GMA, TEGDMA, HEMA, MTU-6, others Liquid B: acetone, isopropanol, water, acryl borate catalyst, peroxide, others | 2.2 | Tokuyama (California, USA) |
| Clearfil universal bond quick (QB) | Bis-GMA, HEMA, 10-MDP, ethanol hydrophilic amide monomers, Colloidal silica, silane coupling agent, sodium fluoride, di camphorquinone, water | 2.3 | Kuraray (Texas, USA) |
| Prime and bond universal (PB) | PENTA, 10-MDP, mono-di- and trimethacrylate resins, acetone, water | 2.5 | Dentsply Sirona (PA, USA) |
| Single bond universal (SB) | 2-HEMA, 10-MDP, dimethacrylate resins, Vitrebond™ copolymer, silane, filler, ethanol, water, initiators | 2.7 | 3M ESPE, St. Paul, MN, USA |

UDMA: Diurethane dimethacrylate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, 4-META: 4-methacryloyloxyethyl trimellitate anhydride, PENTA: Dipentaerythritol pentaacrylate phosphate, Bis-GMA: Bisphenol A-glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, MDTP: 10-methacryloyloxydecyl dihydrogen thiophosphate, TEGDMA: Triethylene glycol dimethacrylate, GPB: G-premio bond
No.: E-EL-0028) tests were performed. All processes were accomplished in accordance with the ISO 10993-5 protocol to ensure standardization. Polymerization of universal dentin-bonding systems was achieved by using a LED (Valo Led, Ultradent) light device at times recommended by the manufacturer’s guides (with the exception of self-cured TB).

**Cell culture**
In our study, 1929 mouse fibroblasts (ATCC, CCL-1) cells from the ATCC were used. Cells were grown in high-glucose DMEM (Gibco, Grand Island, NY, USA), 10% FBS (Capricorn, USA), 1% penicillin-streptomycin (100 IU/ml) in 5% CO₂, medium, at 37°C, in an incubator. Adhesive systems were prepared under sterile conditions, placed to tubes containing 5 mL DMEM, and vortexed after incubation at 37°C for 24 h. After filtering the boundaries with Whatman paper, the filtrate was used.

**Cytotoxicity test (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide assay)**
Cytotoxicity effects of adhesive systems on L929 cell lines were determined by XTT assay 24 h after treatment. The cell viability that was measured in a microplate reader in the reference range of 475 nm was determined according to the intensity of the orange color observed at the end of the incubation period. All absorbance was compared to control samples (cells without any test compound) which represented 100% viability.

**Assessment of oxidative DNA damage (8-hydroxydeoxyguanosine)**
To assess DNA damage, 8-hydroxy-2′-deoxyguanosine (8-OHdG) test was performed with the ELISA kit. After the cells were grown in plates, they were planted in 10 × 10⁴ wells. As positive control, 75 µM concentrated H₂O₂ was added to the cells. Furthermore, 0.1% phosphate-buffered saline was used as the negative control. Adhesive systems representing IC₅₀ values were added to other wells and incubated for 24 h. The color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The experiment was performed with three different samples, each with duplicate.

**Statistical analysis**
All data were analyzed (SPSS 19.0, IBM, Armonk, NY, USA) using one-way analysis of variance and the least significant difference (Bonferroni) multiple comparison tests. Statistical significance was set at 0.05.

**RESULT**

2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide assay
Cell viability (%) is shown in Table 2 and Figure 1 for all groups. When all groups were compared with the control group, there was a statistically significant difference in terms of cytotoxicity (P < 0.05). The significant differences were observed both between TB-SG groups and the other groups – TB or SG for the cell viability (P < 0.05). Toxicity levels were in the following order: GPB < PB < QB < SB < TB. The lowest cytotoxicity value was observed in GPB (93.0 ± 1.3), while the highest cytotoxicity value was found at TB (67.3 ± 3.0).

**Enzyme-linked immunoassay assay**
8 OHdG levels are shown in Table 3 and Figure 2 for all groups. When all groups were compared with the control group, there was a statistically significant difference in terms of genotoxicity (P < 0.05). There was a significant differences between GPB-PB and TB-SB (P < 0.05), and the significant differences were observed among QB with the other groups in the 8-OHdG levels (P < 0.05). 8-OHdG levels are shown as follows: GPB < PB < QB < SB < TB. The highest genotoxic effect was found at Tokuyama Bond Universal (4.17 ± 0.4), while the lowest genotoxic effect was found at GPB (2.2 ± 0.3).

**Table 2: Cell viability (%), mean±standard deviation**

| Groups            | Cell viability (%) | mean±standard deviation |
|-------------------|--------------------|-------------------------|
| Control           | 98.67± 0.88        |                         |
| GPB               | 93.00±1.30         |                         |
| Tokuyama Bond U   | 67.35±3.07         |                         |
| Quick Bond U      | 89.41±2.66         |                         |
| Prime&Bond U      | 91.64±2.40         |                         |
| Single Bond U     | 70.34±2.88         |                         |

The difference between the data shown with different lower case letters are statistically significant (P<0.05). GPB: G-Premio Bond

**Table 3: 8-hydroxydeoxyguanosine levels (ng/mL), mean±standard deviation**

| Groups            | 8-OHdG levels (ng/mL) | mean±standard deviation |
|-------------------|-----------------------|-------------------------|
| Control           | 0.1±0.06              |                         |
| GPB               | 2.22±0.31             |                         |
| Tokuyama Bond U   | 4.17±0.44             |                         |
| Quick Bond U      | 3.15±0.25             |                         |
| Prime&Bond U      | 2.54±0.28             |                         |
| Single Bond U     | 3.86±0.23             |                         |

The difference between the data shown with different lower case letters are statistically significant (P<0.05). GPB: G-Premio Bond, 8-OHdG: 8-hydroxydeoxyguanosine
DISCUSSION

All adhesive systems have different compositions, pH levels, and polymerization techniques. In the literature, it was stated that different monomers are released from resin-based dental materials before or after polymerization. Monomers released from the materials of different compositions determine biocompatibility. Residual monomers are transported to the portion of saliva, which is in contact with the oral mucosa. They cause harmful effects on the pulp and dentin tubules. Previous studies reported that all these parameters are associated with cytotoxicity of adhesive systems. Animal experiments and cell culture tests are commonly used in cytotoxicity assessments of dental materials. Animal experiments, however, involve prolonged testing and are expensive. Cell culture tests have become an alternative to animal experiments, given their advantages, such as low cost, controllability, and easy processes. The XTT assay test is preferred for measuring cell viability and is useful as an in vitro screening tool to compare the cytotoxicity of dental materials. In addition, during this evaluation, cells are collected from the wells, and information about their genotoxicity is obtained by analyzing the level of oxidative stress (e.g., 8-OHdG). Among the various types of oxidative DNA damage, 8-OHdG is a ubiquitous marker of oxidative stress. An oxidative DNA damage byproduct – 8-OHdG – is physiologically formed and enhanced by chemical carcinogens. Our study compared the cytotoxic and genotoxic effects of adhesive systems that are widely used in adhesive dentistry on L929 mouse fibroblast cell lines.

HEMA, which is a component of many adhesive systems, reaches the pulp due to its high water solubility and low molecular weight. HEMA causes apoptosis of pulp cells based on the amount of residual monomer released. HEMA-containing adhesive systems, such as SB Universal, TB, and Quick Bond Universal, have high cytotoxic values. That's why, the present study included these adhesives.

Some components of resin-based dental materials are considered to be cytotoxic to cells, and this effect is thought to be mainly caused by HEMA, TEGDMA, and UDMA. According to a previous study, a combination of Bis-GMA, TEGDMA, and HEMA showed higher cytotoxic effects on the fibroblast cells. Urcan et al. studied the toxic effects of Bis-GMA, TEGDMA, UDMA, and HEMA, the most common monomers in composite resins. They reported the order of cytotoxicity as Bis-GMA > TEGDMA > UDMA > HEMA. In this study, the TB group, which includes Bis-GMA, TEGDMA, and HEMA, showed the highest cytotoxic value.

Schmalz et al. conducted a 24-h study to analyze the toxic effects of Bis-GMA, TEGDMA, UDMA, and HEMA, the most common monomers in composite resins. The order of cytotoxicity was found to be Bis-GMA > TEGDMA > UDMA > HEMA. They also evaluated the cytotoxicity of adhesive systems with low pH values using the dentin barrier test and reported that the low-pH adhesives did not show cytotoxic effects for the pulp. Contrary to Schmalz et al., our study showed that GPB Universal, which has a pH of <2, exhibits cytotoxic effects. However, cytotoxicity of GPB Universal was significantly less than TB (pH > 2) and SB (pH > 2.5).

The cytotoxicity of SB (pH = 4.3), Clearfil SE Bond (primer pH = 1.9, bond pH = 2.8), Xeno III Bond (pH = 1.0), Clearfil Protect Bond (primer pH = 1.9, bond pH = 2.8), and Adper Prompt Bond (pH = 0.8) was investigated using the MTT method. The lowest cytotoxicity was found to be presented by the Adper Prompt Bond adhesive system, which possesses the lowest pH value. Our results showed that the lowest cytotoxicity was detected in GPB Universal (pH <2) adhesive system, which is consistent with their results. However, it was not possible to evaluate the sole effect of acidity on cytotoxicity, and it is beyond the scope of the current study.

10-MDP promotes inflammatory response and suppresses odontoblastic differentiation of the human pulp cells. In our study, Prime&Bond Universal, Quick Bond Universal, GPB Universal, and SB Universal, which include MDP, showed different cytotoxic values. 4-META is a monomer that is commonly added to universal adhesives for providing adhesion to alloys. There are limited studies investigating the cytotoxicity of 4-META. Nakagawa et al. found that luting material including 4-META possessed high-level biocompatibility on pulp cells. In our study, GPB Universal including 4-META showed a lower cytotoxic value than the other groups.

According to our results, all the universal adhesive systems have significant cytotoxic effects on the L929 mouse fibroblast cell line compared to the control group. Besides, the cytotoxic effects of the adhesive systems on the L929 mouse fibroblast cells were related to their composition, i.e., acidic monomers and other compounds. Furthermore, it is possible that the variations in concentrations affect the
toxicity of each material. The synergistic effects between the components of the dental adhesives may result in higher cytotoxicity.[36]

A limited number of studies in the literature have focused on the genotoxicities of universal adhesive systems. Due to their aerobic metabolism, a small amount of ROS are constantly produced in cells and tissues. Cellular antioxidants, such as glutathione, act to detoxify these reactive molecules; however, when the balance between the oxidants and antioxidants is disturbed, oxidative stress arises. If oxidative stress persists, oxidative damage accumulates in the lipids, proteins, and nucleic acids, resulting in biological effects ranging from changing signal transduction pathways and gene expression levels to cell transformation, mutagenesis, and cell death.[36] Leaks from resin-based materials, such as HEMA, Bis-GMA, and TEGDMA, are possible causes of cellular stress through the formation of ROS. Such leaks have recently been shown to be a possible link between ROS production and cytotoxic activity.[37] HEMA, Bis-GMA, and TEGDMA were reported to have genotoxic effects on human fibroblasts.[38,39] Kleinsasser et al.[40] stated that TEGDMA, UDMA, and HEMA induced a significant elevation in the DNA migration in the Comet assay and that this was a possible sign of genotoxic effects in the human salivary glands and lymphocytes. In this study, TB including HEMA, Bis-GMA, and TEGDMA was observed to have highly genotoxic effects.

This research has some limitations. The only L929 mouse fibroblast cell was used for the assessment of cytotoxicity. Further in-vitro investigations should focus on the cytotoxic effects of these materials on the human-derived cells or the other methods such as dentin barrier test.

CONCLUSIONS

All adhesive systems tested in this study showed cytotoxic and genotoxic effects. To increase the biocompatibility of universal adhesive systems, more materials need to be developed and more studies need to be conducted to better understand the associated biological risks and take necessary measures.

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Conflicts of interest

There are no conflicts of interest.

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