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Biocompatibility of Two Different Universal Adhesives

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Abstract

The aim of this study is to evaluate the cytotoxicity with agar diffusion test of two different universal adhesive systems with different content, pH, and polymerization techniques on mouse fibroblast cell lines (L929). G-Premio Bond (GC Europe, Belgium) and Tokuyama Universal Bond (Tokuyama, USA) were supplied from common universal adhesive systems. G-Premio Bond, which is light- polymerized, and Tokuyama Universal Bond adhesive systems, which are chemical-polymerized, are polymerized according to the manufacturer's instructions. All materials were incubated for 72 hours in Dulbecco's modified Eagle medium (DMEM) solution. L929 cells were placed in each wells to be $1.5x10^5$ cells / ml and adhesive systems to be examined for cytotoxicity were applied. Waiting for the added agar to freeze, adhesive systems were installed and cell viability examined in an inverted microscope. Waiting for the added agar to freeze, adhesive systems were placed and cell viability examined in an inverted microscope. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test. A statistically significant difference was found between the two different Universal adhesive systems in terms of cell viability values. Cell viability was significantly lower in the Tokuyama Universal Bond group compared to the G-Premio Bond group ($p < 0.05$). In treatments using universal adhesive systems, cytotoxic effects can be observed against live cells. The compatibility of the results should be supported by different cytotoxicity detection methods.

Research Article

INTRODUCTION

In adhesive dentistry, the strength of the materials developed with new technologies compared to the existing dental materials, their aesthetic feature, the resistance against the forces exposed from the surrounding tissues and the harmful effects on living tissues are still being investigated. In several studies in the literature, it has been reported that monomer is released during and after polymerization of adhesive systems [1-2]. For this reason, materials that will be produced with new technologies have mechanical, physical, functional and aesthetic properties, as well as being biocompatible as well as other properties. Today, research continues to minimize the toxic effect on environmental living tissues, one of the most important effects of adhesive systems [3-4].

With the changes in the structure of adhesive systems from the past to the present, the application technique and attachment efficiency constantly change. Seven generations of adhesive systems have been introduced in the last 20 years. Universal adhesive products, which started to be produced in 2010, are among the latest developments in adhesive dentistry [5-6]. The basic principle of universal adhesives that can be used in three different modes such as self-etch, etch-rinse and selective-etch is to make a simpler application by including etch-rinse and self-etch adhesive systems.

When the components of adhesive systems are examined; bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEG-DMA), hydroxy ethyl methacrylate (HEMA) and dipenta erythtol penta-acrylate monophosphate (PENTA) [7-8]. Although adhesive systems are similar in terms of the monomers they contain, Universal adhesive systems differ from other adhesive systems with their monomers that can establish chemical and micro-mechanical bonds [6]. 10-methacryloloxidesyl dihydrogen phosphate (MDP) is one of the special functional monomers and it is one of the ingredients not found in other generation adhesive systems. MDP monomer, which is only available in Universal adhesives, allows Universal systems to be used with three different pickling techniques [9].

It is known that the adhesive systems in dentistry have different content, pH and polymerization methods [10-11]. Studies have shown that all these parameters have an effect on the

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cytotoxicity of the agent used [12-14]. Along with the studies in the literature, it is known that low pH of adhesives cause a decrease in cytotoxicity value [15].

Although there are important developments and innovations about the physical and mechanical properties of adhesive systems, the biocompatibility of these systems on tooth and surrounding living tissues has not been fully defined. In addition, there are very few studies on the cytotoxicity of universal adhesive systems. Accordingly, the aim of the study was to evaluate the two different universal adhesive systems with different contents, pH and polymerization techniques, by evaluating the cytotoxicity of L929 cells with agar diffusion test.

MATERIALS and METHODS

For this study, the Gaziantep University Clinical Ethics Committee was applied and the ethics committee approval was received with the 2018/373 decision number.

Preparation of bonds

In the study, G-Premio Bond (Gc Europe) and Tokuyama Universal Bond (Tokuyama) universal adhesive systems, which will be evaluated cytotoxicity on L929 (mouse fibroblast) cell lines, are provided. L929 cell line (nothing applied) was used as the control group. According to the manufacturer's instructions, Tokuyama Universal bond was polymerized with chemicals, the G-Premio Bond LED (Valo Led, Ultradent) was polymerized with a lamp device. The contents of the adhesive systems used and the manufacturer companies are shown in Table 1.

Cell culture

In this study, L929 cell lines was obtained from the Sivas Cumhuriyet University Advanced Technology Center Hepokur Cancer Research Laboratory. “Dulbecco's Modified Eagle Medium” (DMEM), 10% fetal bovine serum (FBS), 1% antibiotic (100 IU / mL penicillin-streptomycin) and 2 mM glutamine were used for the medium for cells. Passages three times a week until the required cell density for the cytotoxicity test was obtained. Cells were grown at 37 ^{0}C by 5% CO_2 and 95% humidity by reproducing in a CO_2 incubator. Cells were separated from flask with 0.05% trypsin-EDTA solution. All these procedures were carried out in a laminar flow cabinet. DMEM was added to the suspended cells for neutralize the effect of trypsin.

Agar diffusion test

Cells produced in flasks were transferred to sterile plates with 6 wells, 1.5x10^5 cells / ml in each well, and incubated at 37ºC for 24 h in an incubator with 5% CO_2. The medium was discarded after the cells completely covered the swabs. Serum containing medium and dissolved agar (0.5-2% by mass agarose = suitable for mammalian cells) were used as media. Before agar freezes, G-Premio Bond and Tokuyama Universal Bond adhesive systems were placed in the middle of the wells. Incubated for 24 h at 37ºC in an incubator with 5% CO_2. In addition, DMSO impregnated Whatman paper was used as a negative control and live cells were used as a positive control. After 24 hours of incubation, neutral red, a vital dye, was dropped into the wells. Cytotoxicity was determined by invert microscope (Nikon FDX-35, JapanThe viability rates of the cells were determined based on the scale in Table 2.

Statistical analysis

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

RESULTS

Cytotoxic analysis was performed on cells by agar diffusion test. While using live cell lines as positive control, DMSO impregnated paper was used as negative control. Bonds were compared to negative and positive controls. Substances above 40% melting index are considered toxic. Therefore, no toxic area was observed in the bonds we applied. But when we compare the two bonds with each other, we observe that Tokuyama bond has a more toxic effect than G-Premio bond. If we say to Tokuyama bond cell scale 1, we can say 0 to the cell beard of G-Premio bond (Figure 1).

Table 1. Materials used in this study

Adhesive systems	Components	Manufacturer	pH
G-Premio Bond	MDP, 4-MET, MEPS, Methacrylate monomer, acetone, water, silica	GC Europe (Lueven, Belgium)	1.5
Tokuyama Universal Bond	3D-SR monomer, MTU-6, HEMA, Bis-GMA, TEGDMA, acetone, water	Tokuyama (California, USA)	2.2

Table 2. Melt index of cells

Scale	Color Lightening Index	Cell Melting Index
0	No color bleaching under or around the sample	No visible melting
1	There is lightening within the borders.	20% of the area diameter has melting.
2	There is lightening around 5 mm.	20-40% of the area diameter has melting.
3	There is a lightening around 10 mm.	40-60% of the area diameter has melting.
4	There is lightening more than 10 mm in the surrounding area.	60-80% of the area diameter has melting.
5	There is color lightening covering the entire Petri dish.	80% and more of the area diameter has melting.

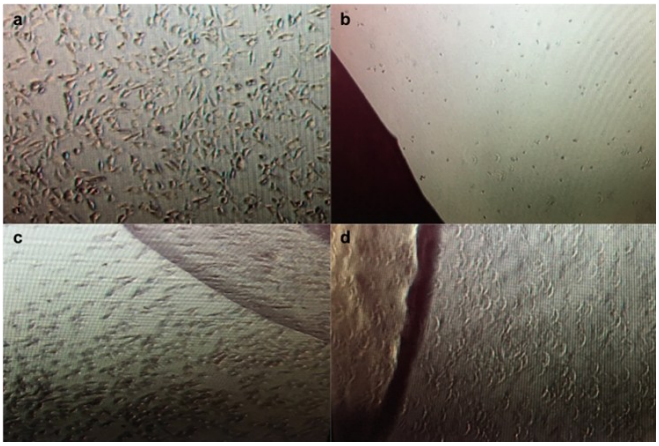

Figure 1: a. Positive Control, b. Negative Control, c. Tokuyoma Universal Bond, d. G Premio Bond; Cytotoxic view of L929 cells with Agar Diffusion Test

DISCUSSION

In the literature, it has been reported that different monomers, which are biological compatibility determinants, are released according to the contents of resin-based dental materials [7, 16]. These monomers can have harmful effects on the oral mucosa, dentine tubules and pulp [17-18]. The cytotoxic effects of adhesives vary with factors such as different monomers contained in adhesive systems that are widely used in restorative dentistry or application of a separate acid step [19]. Silva et al. [20] examined the biocompatibility of the adhesive systems they chose from four different generations, and reported that Universal bond systems had the lowest cytotoxic effect on pulpal cells. In this study, it was aimed to compare the cytotoxic effects of two different universal adhesive systems on the L929 (mouse fibroblast) cells with different contents and pH, which were developed as an adhesive system, using different polymerization methods. To be used in our study, G-Premio Bond (pH <2), which is polymerized with acetone based light, and Tokuyama Universal Bond (pH> 2), which is chemically polymerized, are used.

The hybrid layer formed in single-stage adhesives, such as universal adhesives, acts as a semipermeable membrane due to the high hydrophilic property of the adhesives. With this situation, the resulting microleakage and nanoleakage causes toxic effects on pulpal tissues [21-22]. In our study, both Universal bond systems did not show toxic effects.

It is known that in universal adhesives there are monomers such as HEMA, Bis-GMA, UDMA and PENTA, as well as samples containing biphenyl dimethacrylate (BPDM) and polyalkenioc acid. Hydrophobic monomers (Bis-GMA, UDMA) found in adhesive systems show more cytotoxic effects compared to hydrophilic monomers (HEMA, TEGDMA) [23]. Hydrophilic monomers can progress in dentin fluids and carry hydrophobic monomers in dentin tubules, causing cytotoxic effects in the pulp. The toxic effect of hydrophilic and hydrophobic groups together is higher than the toxic effect they produce alone [24]. HEMA and UDMA, which are hydrophilic monomers, increase bond strength by providing better resin infiltration and provides sufficient polymerization of monomers. It is known that residual monomer released from adhesive agents that have not been adequately polymerized causes toxic effects [25]. Some components of resin-based dental materials are considered to be cytotoxic to cells, while Bis-GMA and UDMA have a high toxic effect, while HEMA and TEGDMA have a moderate toxic effect [24, 26, 27]. In our study, Tokuyama Universal Bond system containing Bis-GMA, TEGDMA and HEMA showed higher toxicity than G-Premio Bond without HEMA and Bis-GMA.

In theory, although the monomers are expected to polymerize with the polymerization process of resin based dental materials, the failure of the methacrylate monomers to react by 15% to 50% causes waste monomer release [28]. The high polymerization of adhesive systems ensures that possible biological risks are reduced. In the study conducted by Schedle et al. [14], the number of cells remaining in the chemical polymerization, which was cytotoxically evaluated after both chemical polymerization and light polymerization of an adhesive system, was found to be lower, that is, its toxic effects

were higher. The toxicity value of Tokuyama Universal Bond, which is chemically polymerized in our study, is compatible with the study of Schedle et al. [14].

Schmalz et al. evaluated the cytotoxicity of the low pH adhesive systems, and reported that the low pH adhesive does not show cytotoxic effect for pulp [1]. Although our study shows that the G-Premio Bond, which has a pH <2, has a cytotoxic effect, contradicts the study in the literature, it has a low cytotoxic effect compared to the Tokuyama Universal Bond and adhesive system with a pH> 2, Schmalz et al. [15] were considered compatible with the study.

Tokuyama Universal Bond, which is chemically polymerized, showed more cytotoxic effects than G-Premio Bond, which is light-cured. If the adhesive systems are not fully polymerized, necessary measures should be taken to ensure adequate polymerization as there will be waste monomer release.

Conflicts of interest

The authors declare that they have no conflict of interests.

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