Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.792699

Original research

EVALUATION OF CYTOTOXICITY OF DIFFERENT UNIVERSAL BONDS USING THE XCELLIGENCE SYSTEM

*Sevim Atilan Yavuz[1]
Derya Surmelioglu[1]

ABSTRACT

Objectives: The purpose of this study was to explore the cytotoxic effects of five different universal bonding agents on mouse fibroblast cell lines (L929).

Materials and Methods: Five different widely used universal adhesive systems were chosen that have different contents, pH levels, and polymerization methods. A real-time cell analyzer (RT-CES, xCELLigence; Roche Applied Science, Germany, and ACEA Biosciences, USA) was used for cytotoxic evaluation of light-cured polymerized G-Premio Bond (GC Europe, Belgium), Prime&Bond Universal (Dentsply Sirona, USA), Universal Bond Quick (Kuraray, USA), Single Bond Universal (3M ESPE, USA) and self-cured polymerized Tokuyama Universal Bond (Tokuyama, USA) experimental groups. L929 were cultured in Dulbecco's modified Eagle's medium and supplemented with 10% fetal bovine serum and 1 % antibiotics. The assay was performed E-plate-16 and monitored every 15 min for 72 h. Statistical analysis was performed using ANOVA and Tukey's posthoc tests.

Results: All tested universal adhesive systems showed a statistically significant difference in cytotoxicity values in different periods ($p<0.05$). Among the groups compared, G-Premio Bond showed the least cytotoxic effect; and Tokuyama Universal Bond showed the most cytotoxic effect. Different times of all universal adhesive systems significantly increased the count of viable cells compared to the control group ($p<0.05$).

Conclusions: In dentistry, universal adhesive systems can be observed cytotoxic effects to live cells. The evaluation of cytotoxicity with xCELLigence device is a reliable method and should be supported by new studies on this subject.

Keywords: xCELLigence, cytotoxicity, universal adhesive systems, L929.

ORCID IDs of the authors:
S.A.Y. 0000-0002-6192-4931
D.S. 0000-0002-6034-3131

[1] Department of Restorative Dentistry, Faculty of Dentistry, Gaziantep University, Şehitkamil, Gaziantep, Turkey.

Received : 10.09.2020
Accepted : 25.11.2020

How to Cite: Atilan Yavuz S, Surmelioglu D. Evaluation of Cytotoxicity of Different Universal Bonds Using the Xcelligence System. Cumhuriyet Dent J 2020;23:4: -.
***Corresponding Author:**
Department of Restorative Dentistry, Faculty of Dentistry, University, 27310 Sehitkamil/ Gaziantep, Turkey
Phone: +90 342 360 96 00/4304 **Fax:** +90 342 361 03 46 **E-mail:** h.d.gursel@gmail.com

INTRODUCTION

Currently, there is increasing social awareness around the importance of health and wellness. This situation allows improves physicians' working conditions while at the same time providing patients more aesthetic, functional, and longer-lasting restorations. As a result of these developments, new researches are added to the literature with the study of both clinicians and academicians on aesthetic materials.

Dentin bonding agents can improve bonding strength, increase the adhesion of restorations, reduce micro-leakage between the tooth-resin interface, and decrease occlusal stress.[1] Initially, in the clinical resin restorative system, bonding agents were applied in three steps. However, several manufacturers have reduced the bonding procedure to two steps by introducing both the adhesive and the primary solvent in a single bottle.[2] Furthermore, in some systems, the acid, primer, and adhesive are incorporated into the same bottle to make a single-step or self-etching primer bonding system.[3] More recently, single-step universal adhesive systems have been developed which can be used in three different modes such as self-etch, selective etch, and total-etch.[4]

Although these adhesive systems are similar in terms of their monomers' composition, universal adhesive systems differ from other adhesive systems by their monomers that can establish chemical and micromechanical bonds.[5] Another property of universal adhesive is strong adhesion to enamel due to unique COOH or PO_4 monomers that ionically bond to the calcium in the hydroxyapatite crystals of the enamel.[6] Additionally, the MDP monomer, which is only present in universal adhesives, allows universal systems to be used with three different etching techniques.[7]

All adhesive systems such as self-etch, total-etch, and universal adhesive systems have different compositions, pH levels, and polymerization techniques.[8,9] In several studies in the literature, it has been reported that all these parameters are associated with cytotoxicity of adhesive systems in teeth and living tissues.[10-12] Although the purpose of adhesive systems is to provide bonding of restorative materials to enamel/ dentin, it is also important to investigate the potential cytotoxic effects due to the various monomers contain in adhesive systems. Adhesive systems typically include monomers such as bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA), hydroxy ethyl methacrylate (HEMA) and dipenta erythritol penta-acrylate monophosphate (PENTA). Additionally, some contain biphenyl dimethacrylate (BPDM) and polyalkenoic acid.[13,14]

Hydrophobic monomers such as Bis-GMA and UDMA show more cytotoxic effects compared to hydrophilic monomers such as HEMA and TEGDMA.[15] Progress of hydrophilic monomers in dentinal fluid and transporting hydrophobic monomers in dentin tubules can cause cytotoxic effects in pulp. Furthermore, the toxic effects of hydrophilic and hydrophobic groups together are greater than the toxic effects they produce alone.[16] While resin-based dental materials have a local cytotoxic effect on pulp, systemic toxicity has not been observed in previous studies.[17,18]

The acidic characteristic of monomers in universal adhesive systems allows for the simultaneous creation of diffusion channels and the infiltration of these channels. Recently, new universal adhesive systems have been marketed with pH values, but there is not enough search about exposing the dentin complex to these agents. However, it is generally known that due to the cytotoxicity of bonding agents[19-22], there is the possibility of pulpal damage in clinical applications. Therefore, it is recommended for clinicians to use etch-rinse systems in shallow cavities and self-etch systems in deep cavities.[23]

The polymerization of resin monomers is important in terms of biocompatibility and bonding strength in bonding systems. HEMA and UDMA, which are hydrophilic monomers, provide better resin infiltration, increase bond strength and provide sufficient polymerization of monomers.[24,25] The effect of different polymerization types on polymerization is also an important question since it is known that residual monomer release increases due to inadequate polymerization.[26,27] Researches

have been conducted on the effects of universal adhesives with either light-cured or self-cured polymerization types on cytotoxicity because, in the bonding process, unreacted resin monomers can damage the pulp tissue through dentine tubules.[12,28] Although there are significant developments and innovations about the physical and mechanical properties of adhesive systems day to day, the biocompatibility of these systems in tooth and living tissues has not yet been fully characterized. In this study, a real-time cell analyzer (xCELLigence) was used to investigate the time-dependent cytotoxic effects of five different universal dentin bonding agents on a mouse fibroblast cell line (L929). In addition, little is known about the cytotoxicity of universal adhesive systems. This research is the first study in the literature in which the cytotoxicity of different universal adhesive systems is evaluated by the xCELLigence method.

Our study aims to evaluate the cytotoxic effects of five different universal bonding agents on mouse fibroblast cell lines (L929). The null hypothesis was all five different universal adhesive systems with different monomer content, pH level, and polymerization technique will have different cytotoxic effects.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Gaziantep University (process no. 2018/374).

Experimental Groups

Five different universal dentin bonding agents were tested in this experiment: G-Premio Bond (GC Europe, Inc., Leuven, Belgium), Tokuyama Universal Bond (Tokuyama America, Inc., California, USA), Universal Bond Quick (Kuraray America, Inc., Texas, USA), Prime&Bond Universal (Dentsply Sirona, Inc., Pennsylvania, USA) and Single Bond Universal (3M/ESPE, Inc., Minnesota, USA). Materials and ingredients are provided in *Table 1*.

Table 1. Materials used in this study

Adhesive Systems	Components	Manufacturer	pH
G-Premio Bond	MDP, 4-MET, Methacrylate monomer, acetone, water, silane	GC Europe (Leuven, Belgium)	1.5
Tokuyama Universal Bond	Bis-GMA, TEGDMA, HEMA, isopropanol, acetone, water	Tokuyama (California, USA)	2.2
Universal Bond Quick	Bis-GMA, HEMA, MDP, hydrophilic amide monomer, ethanol, water, silane	Kuraray (Texas, USA)	2.3
Prime&Bond Universal	PENTA,10-MDP, isopropanol, acetone, water	Dentsply Sirona (Pennsylvania, USA)	~ 2.5
Single Bond Universal	2-HEMA, 10-MDP, dimethacrylate resins, VitrebondTM copolymer, silane, filler, ethanol, water, initiators.	3M ESPE (Minnesota, USA)	2.7

MDP: 10-methacryloyloxydecyl dihydrogen phosphate, 4-META: 4-methacryloyloxyethy trimellitate anhydride, PENTA: Dipentaerythritol pentaacrylate phosphate, TEGDMA: triethylene glycol dimethacrylate, Bis-GMA: bisphenol A-glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate.

Test specimens 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 (ATCC®CRL-6364) without any adhesive material was added.

In order to obtain the cytotoxic values of the adhesive systems used, preparation of test samples, sterilization, preparation of cell culture, and evaluation with the xCELLigence method were performed. All processes were accomplished in accordance with the ISO 10993-5 protocol to ensure standardization.[10] Polymerization of universal dentin bonding systems was achieved by using a LED (Valo Led, Ultradent) light device at times recommended according to the manufacturer's instructions (with the exception of self-cured Tokuyama Universal Bond).

Cell Culture

An established cell lines, mouse fibroblasts L929 (American Type Culture Collection CCL 1), was cultivated in Dulbecco's Modified Eagle's

Medium (DMEM; Invitrogen, Carlsbad, CA, United States) including 10% fetal bovine serum (FBS) and 1% antibiotics (100 IU/ mL penicillin-streptomycin). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

Cells grown in flasks were passaged twice a week until the required cell density for the cytotoxicity test was maintained at 37 °C in a humid atmosphere. A solution containing 0.05% trypsin and 0.53 mM ethylenediamine tetra acetic acid (EDTA) was used to remove the cells from the flasks. All protocols were performed in a laminar flow cabinet with UV light sterilization. DMEM was added to the suspended cells to neutralize the effect of trypsin**.** The medium and manufacturer are shown in *Table 2*.

Table 2. Cell culture medium used in this study

CELL CULTURE MEDIUM	MANUFACTURER
Dulbecco's Modified Eagle Medium	Sigma Aldrich Chemie, Germany
Fetal Bovine Serum	Biochrom KG, Germany
Penicillin /Streptomycin	Biochrom KG, Germany

Cell Count

The hemocytometer was used to calculate the number of cells in milliliters of the suspension, using the following formula:

Total cell count/ ml = hemocytometer count result x 10^4 x medium amount (ml)

Preparation of Extraction Fluid and Extraction Process

In this study, artificial saliva was preferred as extraction fluid. The content of the saliva solution was; 136.8 mM NaCl (sodium chloride), 3.0 mM KCl (potassium chloride), 2.5 mM $CaCl_2.6H_2O$ (calcium chloride), 1.5 mM $MgCl_2.6H_2O$ (magnesium chloride), 0.5 mM $Na_2SO_4.10H_2O$ (sodium sulfate), 4.2 mM $NaHCO_3$ (sodium bicarbonate), and 1.0 mM $K_2HPO_4.3H_2O$ (dipotassium phosphate).

The prepared test samples were placed in an Eppendorf tube, then 1 ml artificial saliva solution was added. Since the extraction liquid to be obtained after all these extraction procedures will be applied to cell cultures, the following procedures were performed in a laminar flow cabinet with UV light sterilization. The extracts were placed in different Eppendorfs tubes due to the various waiting time (24 h, 48 h and 72 h) of the samples.

xCELLingence System

The xCELLigence system is a method for measuring cell viability in accordance with the manufacturer's instructions. (Roche Diagnostics GmbH, Mannheim, Germany and ACEA Biosciences, Inc., San Diego, CA, USA). The xCELLigence system consists the real time analyser (RTCA), the RTCA single plate (SP) station, the RTCA computer with integrated software and a disposable E-plate 16. While the RTCA SP station fits inside a standard tissue-culture incubator, the measurements are transferred to a software analyser and computer. The E-plate 16 is a disposable device used for performing cell-based assays on the RTCA SP instrument. The single-use E-plate 16 contains gold cell sensor arrays at the bottom which; contributes to the monitoring and testing the cells in each well. The E-plate 16 has a low evaporation lid design: the bottom diameter of each well is 5.0mm ± 0.05mm; with a total volume of 243 ± 5 µL. Approximately 80% of the bottom of each well is covered by circle-on-line-electrodes, which are designed to be used in an environment of +15 to +40 °C, relative humidity 98% maximum without condensation.

Physiological changes of the cells to be examined are determined by the electronic impedance formed in the sensor electrodes. The voltage of the electrodes is about 20 mV (RMS) during the test. In the absence of cells, the main source of electrode impedance is the concentration of ions at both the electrode / solution interface and within the solution. In the presence of cells, which act as insulators, the electrode impedance increases as a result of the change in the local ion environment at the electrode / solution interface. The electrode impedance value changes in proportion to the cell density.

Cytotoxicity Test

In this study, a 16-well E-plate 16 was used; each well had a volume of 250 μL and a base diameter of 5 mm. After 200 μL of the cell suspensions were seeded into the wells (10.000 cells/well) of the E-plate 16 in a laminar flow cabinet with UV light sterilization, and the plates were placed in the incubator. L929 mouse fibroblast cells which were placed on the well of the plates was provided to grow in the appropriate medium and O_2/CO_2 environment, to enter the rapid growth phase (log phase). In addition to the experimental groups, only the cells and medium solution containing no adhesive agents were added to the test plate of the control group. Following this, the E-plate 16 was placed in the incubator. Cytotoxic values of adhesive systems at 24, 48, and 72 h were obtained by applying the extraction liquid at different times (24, 48, and 72 h).

Statistical Analysis

SPSS 22.0 software was used for statistical analysis of the data obtained from this empirical study. The sphericity prerequisite was assessed with Mauchly's test of sphericity. In cases where the sphericity prerequisite was not provided, the difference between repeated measurements was determined by using the Greenhouse-Geise test. ANOVA test was performed for measurements between more than two independent groups. Post-hoc Tukey's test was used for pairwise comparisons in groups with differences. The statistical significance level for all comparisons was $p<0.05$.

RESULTS

Extracts were obtained from wells at 24 h, 48 h, and 72 h for all experimental groups. There were significant differences between all groups following 24 h applications of universal adhesive systems used in the study ($p<0.05$). The highest cytotoxic effect was observed in the Tokuyama Universal Bond group, and the highest cellular viability was found in the G-Premio Bond group. The extract obtained at 48 h showed significant differences between all groups ($p<0.05$). The highest cytotoxic effect was in the Tokuyama Universal Bond group, while the lowest cytotoxic effect was in the G-Premio Bond group. Similarly, in the other hour measurements, there was a significant difference in the 72nd h cytotoxicity values in all groups ($p<0.05$). In the 72 h findings, the most cellular proliferation was observed in the G-Premio Bond group, while the least cellular proliferation was observed in the Tokuyama Bond Universal adhesive system. When all groups were compared time dependent, it was observed that the mean cell proliferation value (%) decreased from 24 h to 72 h. Cell proliferation (%) was seen in all time periods for all groups (24 h, 48 h, and 72 h) shown in *Table 3* and Figure 1.

Table 3. Mean ± Standard error of cell proliferation values of tested universal adhesive systems

Cell Proliferation (%)	24 h	48 h	72 h
Control	0.23 ± 0.04^{Aa}	0.20 ± 0.04^{Ab}	0.15 ± 0.04^{Ac}
G-Premio Bond	0.19 ± 0.04^{Ba}	0.17 ± 0.05^{Bb}	0.12 ± 0.04^{Bc}
Tokuyama Universal Bond	0.10 ± 0.06^{Ca}	0.08 ± 0.04^{Cb}	0.05 ± 0.03^{Cc}
Universal Bond Quick	0.13 ± 0.05^{Da}	0.11 ± 0.05^{Db}	0.07 ± 0.04^{Dc}
Prime&Bond Universal	0.15 ± 0.06^{Ea}	0.12 ± 0.05^{Eb}	0.08 ± 0.05^{Ec}
Single Bond Universal	0.13 ± 0.05^{Fa}	0.10 ± 0.04^{Fb}	0.06 ± 0.04^{Fc}

**Different letters within columns and lines indicate statistically significant differences. (Uppercases represent columnar differences intergroup, while lowercases represent linear differences intragroup).*

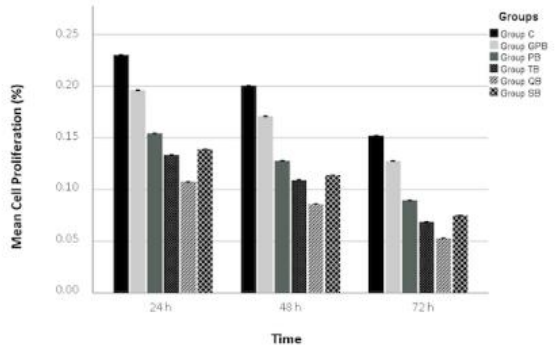

Figure 1. Comparison of mean cell proliferation (%) of all groups at 24, 48 and 72 h

When all groups were compared with the control group, there was a statistically significant difference in all time values in terms of cytotoxicity ($p<0.05$). The lowest cytotoxicity value was observed at 24 h of G-Premio Bond, while the highest cytotoxicity value was found at 72 h of Tokuyama Universal Bond. Cell proliferation (%) seen between control and other groups in all time periods (24 h, 48 h, and 72 h) are shown in Figures 2, 3, 4, 5, 6.

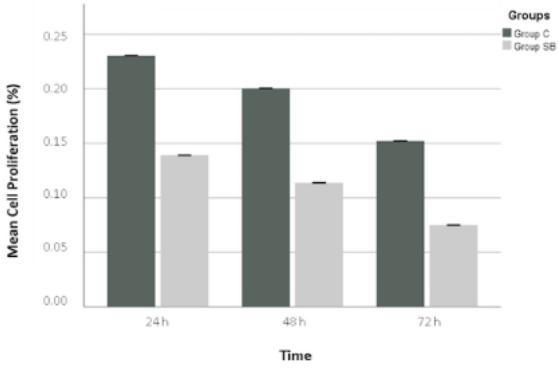

Figure 2. Comparison of mean cell proliferation (%) between control and G-Premio Bond groups at 24, 48 and 72 h

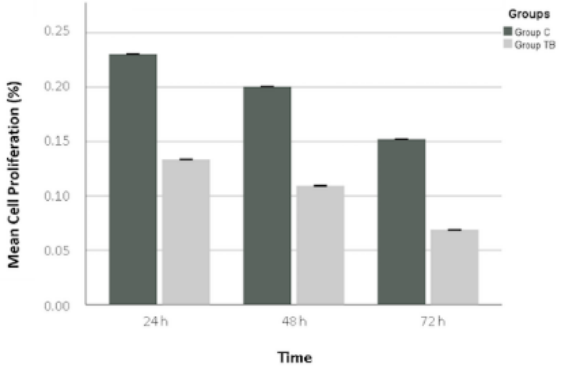

Figure 3. Comparison of mean cell proliferation (%) between control and Prime&Bond Universal groups at 24, 48 and 72 h

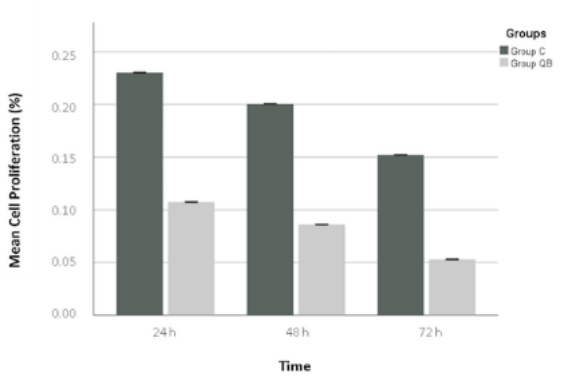

Figure 4. Comparison of mean cell proliferation (%) between control and Single Bond Universal groups at 24, 48 and 72 h

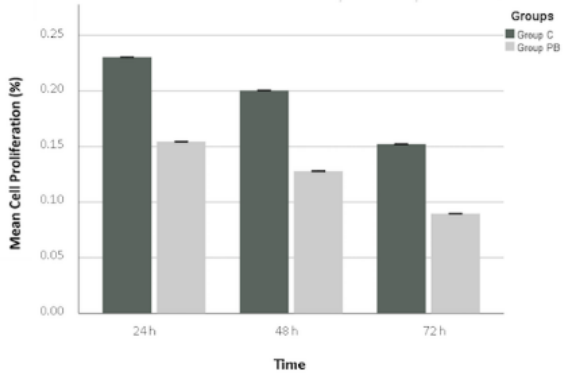

Figure 5. Comparison of mean cell proliferation (%) between control and Tokuyama Universal Bond groups at 24, 48 and 72 h

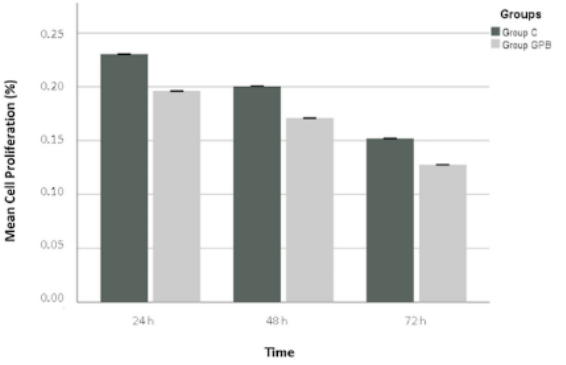

Figure 6. Comparison of mean cell proliferation (%) between control and Universal Bond Quick groups at 24, 48 and 72 h

DISCUSSION

Dental materials which have different contents are offered on the market by the manufacturers in adhesive dentistry with the advancement of technology each passing day. During the evaluation of a newly developed material, its physical, mechanical and biological properties should be taken into consideration.[1,29] The number and variety of tests evaluating the biocompatibility of materials have increased with developed products.[9] The adhesive systems used in restorative dentistry have different cytotoxic properties. Da Silva *et al.*[30] examined the biocompatibility of the

four different generations of adhesive systems and reported that the universal bond systems had the lowest cytotoxic effect on pulpal cells.

Previous research has determined that different universal adhesives have different compositions, such as HEMA, Bis-GMA, UDMA and PENTA, as well as biphenyl dimethacrylate (BPDM), CQ, MDP, polyalkenoic acid, acetone and ethanol, different pH and polymerization methods.[8,9] Several studies have shown that these parameters impact the cytotoxicity of the agent used.[10-12] Therefore, in this experimental study, five different universal adhesive systems with different contents, pH and, different polymerization methods were investigated. Acetone-based G-Premio Bond (pH<2), Tokuyama Universal Bond (pH>2), ethanol-based Prime & Bond Universal (pH>2.5), Universal Bond Quick (pH>2) and Single Bond Universal (pH>2.5), which contain one or more of the resin monomers, were preferred for use in this study.

The cell type in which the dental materials are in contact with the cytotoxicity is important with the in-vitro studies. Especially in dentistry, using Balb 3T3 or L929 mouse fibroblasts cell cultures to evaluate cytotoxicity is recommended.[31-33] L929 is a continuous cell culture line with a wide range of use as a standard in cytotoxicity testing of dental materials.[34] In a previous study examining cytotoxicity, a dental material showed similar effects on L929 mouse fibroblasts and gingival fibroblasts.[12]

Biological events (cell proliferation, vitality, toxicity, the demonstration of the physical situation of the cells) can be analysed in real time by the xCELLigence system.[35] The greatest advantage of this system is that the respective number of cells in each well of the E-plate 16, the proliferation, attachment and spreading of the cells can be monitored every 15 min.[36] Data from cells in the wells are monitored simultaneously on the connected computer.[37] This property allows for instant changes, such as stopping the experiment and adding a new substance.[36] With the xCELLigence system, it is also possible to observe and evaluate the real-time analysis without the need for staining or marking the cells. In methods such as MTT, XTT and staining methods, it is not possible to use existing cells for different purposes after end-point analysis. Obtaining limited data with three different measurements in a 72-hour experiment using methods sensitive to human error is another disadvantage. In contrast, it is possible to receive cellular data every 15 seconds with real-time analysis using the xCELLigence system. The basic working principle in obtaining data in new systems is based on impedance measurements; therefore the cells used in the xCELLigence system can be evaluated with another test.[38] For example, when a material is evaluated in terms of cytotoxicity, cells are collected from the wells when needed and information about the genotoxicity is obtained by evaluating the oxidative stress level. While only one parameter is evaluated with classical methods, general data (cell proliferation, cell apoptosis, morphological change in cell membrane) can be examined with xCELLigence. The xCELLigence device offers economical, comprehensive and more accurate study to researchers.[37]

Previous studies have reported that different monomers are released from resin-based dental materials during or after polymerization.[39-41] The monomers released from the materials in the different compositions determine the biocompatibility.[13] Although it is possible to fully polymerize the monomer in theoretically, one study that this transformation is at most 70% with conventional light sources.[42] With the increasing power of light sources, the polymerization percentage of monomers is expected to increase. In addition, as heat is generated during the polymerization of dental materials, the dentinal fluid in the tubules moves towards the pulp and the released monomers reach the pulp. These issues increase the damage to the pulp caused by heat. In a study, an 800 mW light device (20 s) and a 650 mW light device (30 s) were used for polymerizing the resin composite, and the number of live lymphocyte cells was examined at the end of the experiment.[43] High cell viability was detected in the group that was applied for a longer time at low power density compared to the group that was applied for a shorter time at high power density.

In the literature, there have been many studies about the release of monomers time-dependent due to insufficient polymerization. Some studies indicate that 1-7 days are required for full-swing, while in some studies it has been stated that 30 days should be waited.[12,44] In addition, there is a positive connection between the contact time of the adhesive systems to the dentine and the cytotoxic effect of the monomers in the contents of the adhesive. Ratanasathien *et al.*[16] found, that the duration of action of monomers released from adhesive agents has a strong effect on the toxicity of adhesive systems. In this study, measurements at 24 h, 48 h and 72 hours were obtained in order to evaluate the short-medium-long-term cytotoxic effects of adhesive systems, and it was observed that the cytotoxicity varied depending on the time of exposure.

Some components of resin-based dental materials are considered to be cytotoxic; furthermore, cytotoxic effects of Bis-GMA, TEGDMA, UDMA and HEMA have been investigated. In previous studies, the cytotoxicity of monomers has been shown to be the following: Bis-GMA> UDMA> TEGDMA> HEMA.[16,36] The cytotoxicity values obtained as a result of this study are achieved by a similar mechanism. G-Premio Bond and Prime & Bond Universal adhesive systems that did not contain any of these resin monomers showed lower cytotoxicity. Also, Tokuyama Universal Bond which contains resin monomers such as Bis-GMA, UDMA and HEMA, had the highest cytotoxic value.

Koulaouzidou *et al.*[45] examined the cytotoxic effects of three different adhesive systems on fibroblast cells and reported that the XP Bond group showed the highest cytotoxic value. This study identified that UDMA and TEGDMA monomers in the content of the XP Bond system are responsible for this result. The same study reported that the molecular weight and components of resin monomers may also affect this result: HEMA, which has a low molecular weight, has a less toxic effect than Bis-GMA, UDMA and TEGDMA. While Bis-GMA and UDMA showed highly toxic effects, HEMA and TEGDMA had moderately toxic effects.[16,42,46] In present study, the Tokuyama Universal Bond group, which includes Bis-GMA, TEGDMA and HEMA monomers had the highest cytotoxicity value, while G-Premio Bond and Prime&Bond Universal adhesives which do not include any of these monomers, had the lowest cytotoxicity value.

Several studies reported that camphorquinone, which acts as a photo initiator in adhesive systems, is both cytotoxic and mutagenic.[40,47] In this present study, it is possible that camphorquinone, which is a component of the Prime & Bond Universal adhesive system, may be responsible for the cytotoxic effect of this adhesive agent.

In a study in the literature, the cytotoxicity of composite resin and adhesive systems was examined immediately and following a seven days incubation period, it was reported that all samples were cytotoxicity, but it decreased after seven days.[48] Extraction fluid containing residual monomers released from dental materials was removed to evaluate the samples, and only samples were used. However, in this study, the samples were kept in the extraction liquid for 72 h and cytotoxic effects were determined using the extraction fluid. In the study of Franz *et al.*[48], although the toxic effect of all groups is consistent with this research, it assumed that the reason for the decrease cytotoxic effect at the end of 72 h was performed by removing the extraction fluid of the material.

In the study of Yasuda *et al.*[22], the cytotoxic effect of five different adhesive systems (AQ Bond Plus, Clearfil Tri-S Bond, G-Bond, Adper Prompt and Absolute) in human pulp cells was examined when compared with the polymerized samples and those applied without polymerization, lower cytotoxicity was observed in the polymerized group. Additionally, the cytotoxic effect was significantly higher at 72 h compared to 24 h. This finding is consistent with the present study, in which comparison of 24 h and 72 h groups, revealed significantly more toxic effects present at 72 h.

Schedle *et al.*[12] examined, the cytotoxic effects of composite, adhesive system and / or primer combinations, including experimental

groups for both chemical polymerization and light polymerization of the Optibond adhesive system. On the 2nd and 7th day of the experiment, the number of cells (%) was lower in the chemical polymerization of the Optibond adhesive system. Moreover, in this study, Tokuyama Universal Bond, which is chemically polymerized, was shown to be associated with lower cellular proliferation compared to four other universal adhesive systems polymerized with the LED light device (G-Premio Bond, Prime&Bond Universal, Single Bond Universal and Quick Bond Universal).

In another study[49], the cytotoxicity of Single Bond (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) which have different pH values were investigated by MTT method, and the lowest cytotoxicity was found in the Adper Prompt Bond adhesive system with the lowest pH value. In this study, the G-Premio Bond adhesive system (pH=1.5) had the lowest pH value and demonstrated the lowest cytotoxicity. Similarly, when the Single Bond Universal (pH=2.7) and Universal Bond Quick (pH=2.3) were compared, the Universal Bond Quick adhesive system, which has a lower pH, showed lower cytotoxicity.

According to our results, the null hypothesis that all five different universal adhesive systems with different monomer content, pH level, and polymerization technique will have different cytotoxic effects was accepted.

This experimental study has several limitations, including the inability to fully mimic the in vivo environment of the in vitro study. In addition, cytotoxicity was not followed for time periods longer than 72 h, and mouse fibroblast cells were used. Very few studies to date have evaluated the cytotoxicity of adhesive systems using the xCELLigence system. Ours is the first study to compare the cytotoxic effects of five different universal adhesive systems with the xCELLigence device; however, additional studies are required to clearly elucidate these cytotoxic effects.

CONCLUSIONS

According to the results of this research, all the universal adhesive systems used in were found to have a significant cytotoxic effect on the L929 mouse fibroblast cell line compared to the control group, and it was concluded that this effect increased time-depending (24 h, 48 h and 72 h). It was assumed that the cytotoxic effect of adhesive systems on L929 mouse fibroblast cells is related to the increase in residual monomer concentration released from adhesives.

ACKNOWLEDGMENTS

All procedures performed in this study were in accordance with the ethical standards of the Ethics Committee, University Gaziantep (process no. 2018/374), and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The authors would like to thank Prof. Dr. Esra CAN EROĞLU, Prof. Dr. İhsan HUBBEZOĞLU, Assist. Prof. Dr. Ceylan HEPOKUR for their contributions

CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article**.**

FUNDING

This study was supported by Gaziantep University, Scientifics Research Section with DHF.UT.19.03 project number.

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