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Investigation of the cytotoxic effect of current dentine bonding agents on human dental pulp cells

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Abstract

Background An ideal aesthetic restorative material should be attached to the tooth tissues by adhesion, have a smooth surface as possible, should not cause toxic reactions in the pulp and discoloration and microleakage. This study aims at comparatively assess the cytotoxicity of current adhesive systems on human dental pulp cells.

Materials and methods The adequate density of human pulp cells was observed from the ready cell line. The passaging was performed and the 3rd passage cells were selected. Adhesive systems and MTA were used on the cultures. Trypan blue staining was conducted on the cells at the 1st, 2nd, 3rd days and a count of live and dead cells using a light microscope. The dead cells whose membrane integrity was impaired by staining with trypan blue and the viability rate was determined using live and dead cell numbers. Data analysis was performed using IBM SPSS Statistics 22.

Results A significant difference in vialibity rates between adhesive systems was observed on the first day. No significant statistical differences were observed on the 2nd and 3rd days ($p < 0.05$).

Conclusion Futurabond M showed similar biocompatibility with MTA on human pulp cells and it can be applied in cavities with 1–1.5 mm hard tissue between pulp and dentine.

Keywords Stem cell, Dental pulp, Cell viability, Bonding agents

Introduction

During cavity preparation, pulp can be exposed due to caries, trauma or iatrogenic reasons [1–4]. As a result of pulp exposure, tooth pain and inflammation that causes the pulp tissue to turn into a necrotic tissue may occur. In clinical treatment; direct pulp capping or pulpotomy treatment is applied. Direct pulp capping treatment is the process of covering the exposed pulp tissue with a biocompatible material so that the pulp tissue can continue its life in a healthy way in teeth where tissue loss is limited in the crown and has not lost its power to make dentine [2, 5]. Studies have shown that the injured pulp tissue is covered with biocompatible materials to prevent leakage and contamination, so the pulp can produce dentin through cells [6, 7].

Mineral Trioxide Aggregate (MTA) is one first bioceramic based dental material which has been introduced by Torabinejad in 1993 and approved for endodontic usage by FDA in 1998 [8, 9]. In 1999, Torabinejad and Chivian [10] suggested that MTA can be used in

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apexification, as it's a calcium silicate based biocompatible material that can regenerate periodontal tissues, activate cementoblasts and form mineralization [11]. However, MTA has disadvantages such as colour stability of the tooth and diffuculty in manipulation which lead other calcium silicate cements research in dentistry [12].

Nowadays, the increase of aesthetic concerns has led to the development of restorative materials and techniques. An ideal aesthetic restorative material; it should be attached to the tooth tissues by adhesion, have a smooth surface as possible, should not cause toxic reactions in the pulp and shouldn't cause discoloration and microleakage [13].

8th generation dental adhesive systems are the most recently released self-etch adhesive systems. In recent studies, it has been reported that 8th generation adhesive systems have the lowest microleakage values compared to the 6th and 7th generations [14, 15].

Biocompatiblity is one of the most important requirements for materials used in apexification or pulp capping. In recent studies, MTA showed good biocompatibility in terms of cytotoxicity [16–22]. There are many factors that affect the biocompatibility of adhesive systems, many of which are due to the content of the material. In vitro studies frequently focus on the cytotoxicity between the materials and cells. The adhesive systems remain in contact with the pulp cells through dentine for a long time, and as a result of this contact, some changes may occur on the pulp.

Table 1 Components of the test materials

Materials	Contents	Manufacturer	LOT Number
MTA Angelus	*Powder*: Tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide, bismuth oxide *Liquid*: Distilled water	Angelus, Londrina, PR, Brazil	101,321
G Premio Bond	4-META, UDMA MDP Dimethylacrylate Phosphoric acid ester monomer Photoinitiator BHT, MDTP Acetone, water	GC, Tokyo, Japan	1,805,071
Clearfil S^3 Bond Plus	Bis-GMA, HEMA MDP Hydrophilic aliphatic dimethacrylate Hydrophobic aliphatic methacrylate colloidal silica camphoroquinone accelerator ethanol, water	Kuraray, Japan	2T0080
Futurabond M	UDMA, HEMA organic acids camphoroquinone BHT ethanol, water	VOCO, Cuxhaven, Germany	1,818,483

The aim of this study is to investigate the cytotoxic effects of the dentin bonding agents Futurabond M, Clearfil S3 Bond Plus (which are self-etch adhesives), and G Premio Bond (a universal adhesive) on human dental pulp cells in vitro, in comparison with MTA material, and to evaluate the potential use of these bonding agents as a material for direct pulp capping.

In this study, although direct application was not performed in pulp treatments, it was hypothesized that adhesive systems could affect pulp vitality. It is believed that future studies could benefit from evaluating the potential use of Futurabond M as a capping material in deep cavities.

Materials and methods

Sample preperation

Three different types of materials from 8th generation dental adhesive systems (Clearfil S3 Bond Plus, G Premio Bond and Futurabond M) and MTA were used. Table 1 shows the components of the test materials. 9 samples of each material were prepared in teflon molds (8 mm in diameter, 2 mm high) with the instructions of manufacturer's. The mixture was poured into a sterile Teflon mold, and the light-curing reaction was allowed to complete for 5 min. Materials were prepared in a sterile cabinet under aseptic conditions, according to the manufacturer's instructions. After the samples were kept to complete their polimerisation reactions, they were kept under UV for 8 h before the experiment in order to prevent bacterial, fungal and yeast contamination. In the second experiment, the materials were sterilized by keeping them in autoclave at 121 °C under 1.5 atm pressure for 15 min.

Cells and cell culture conditions

For this study, commercially isolated and registered human dental pulp stem cells (Lonza Group, Switzerland) were kept in a nitrogen tank (-196 oC) until the beginning of the cell transplantation process, and on the day of the experiment, they were removed from the nitrogen tank and dissolved in a water bath and brought to room temperature. The medium for the cultivation and reproduction of cells was prepared as recommended by the manufacturer. The medium from the flasks was removed in the process of passaging. The flasks were washed with 2 ml trypsin EDTA (Sigma- Aldrich T4049, St. Louis, MO, USA) was added to the flasks. After 2 h of incubation, the cells were separated from the flask base. 4 ml TNS (Trypsin neutralization solution) was used to wash the flasks and the cells were seeded into new sterile falcon tubes. The tubes were centrifuged at 1500 rpm for

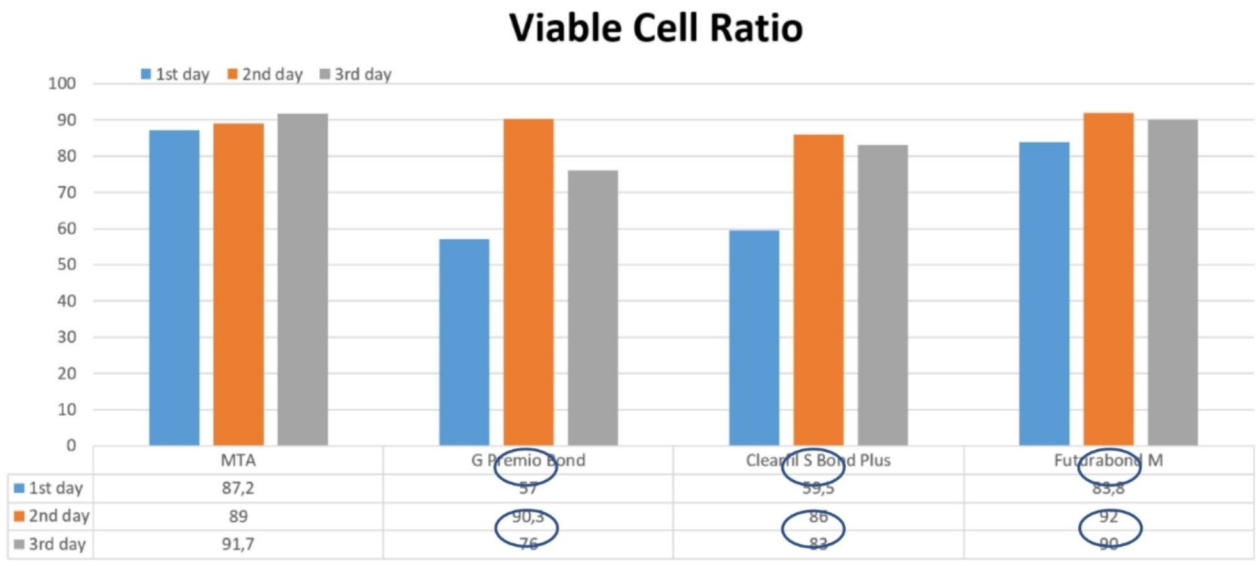

Fig. 1 Viability rates detected in the first experiment on the 1st, 2nd and 3rd days

5 min and cultivated in a new culture flask. Cells at the 3rd passage were used in assays.

Cell viability assay

The culture medium was transferred into a tube to collect floating cells, while the adherent cells were removed using a 0.25% trypsin treatment for 5 min and afterwards moved to the same tube. After centrifugation, the supernatant was discarded, and cells were suspended in PBS. To ensure the absence of residual cells in the well or supernatant, microscopic examination was conducted. Subsequently, cells were stained with trypan blue (Sigma-Aldrich T8154, St. Louis, MO, USA), and the count of unstained cells was performed using a hemocytometer chamber and were examined with light microscope (Leica MZ 75, Leica, Wetzlar, Germany). The cell viability was calculated as a percentage using the following formula: Percentage of viable cells=(A/B)x100.

Where A is the number of viable cells in the experimental well and B is that in the control well.

The method used was a direct contact test, with the sample sizes of the solid-form MTA Angelus and bonding materials chosen according to the ISO 10993-5 standards, ensuring that the surface/medium ratio was 0.5–6 cm^2/ml. This was to allow the culture medium to completely surround the samples during the release phase and to ensure complete polymerization.

Statistical analysis

The sample size calculation resulted in an 80% power at the 5% statistical significance level and a 10% the difference between the groups and required 9 samples for each group. In the evaluation of data collected statistical analysis for IBM SPSS Statistics 22 (IBM SPSS, Turkey) programs were used. While evaluating the study data, firstly the Pearson Chi-Square test, Fisher's Exact Probability Test were used in cases where the expected value was less than 5 and the number of cells exceeded 20% of the total number of cells. Significance was evaluated at the $p<0.05$ level.

Table 2 Finding obtained as a result of the Chi-Square Test in the first experiment at the end of the 1st, 2nd and 3rd days

Time		Value	df	Asymptotic Significance (2-sided)
24 h	Pearson Chi-Square	154,979[b]	3	,000*
	Likelihood Ratio	145,721	3	,000
	Linear-by-Linear Association	,605	1	,437
	N of Valid Cases	1700		
48 h	Pearson Chi-Square	2,270[c]	3	,518*
	Likelihood Ratio	2,218	3	,528
	Linear-by-Linear Association	,120	1	,730
	N of Valid Cases	800		
72 h	Pearson Chi-Square	33,950[d]	3	,000*
	Likelihood Ratio	33,438	3	,000
	Linear-by-Linear Association	,034	1	,854
	N of Valid Cases	900		

Pearson Chi-Square Test, Fisher's Exact Probability Test *$p<0,05$

[b, c, d] Different letters indicate significant differences between groups

Results

Figure 1; Table 2 show the rates of viable cells at 24, 48 and 72 h at the end of the first experiment. When the viability rates of the applied adhesive materials in the first

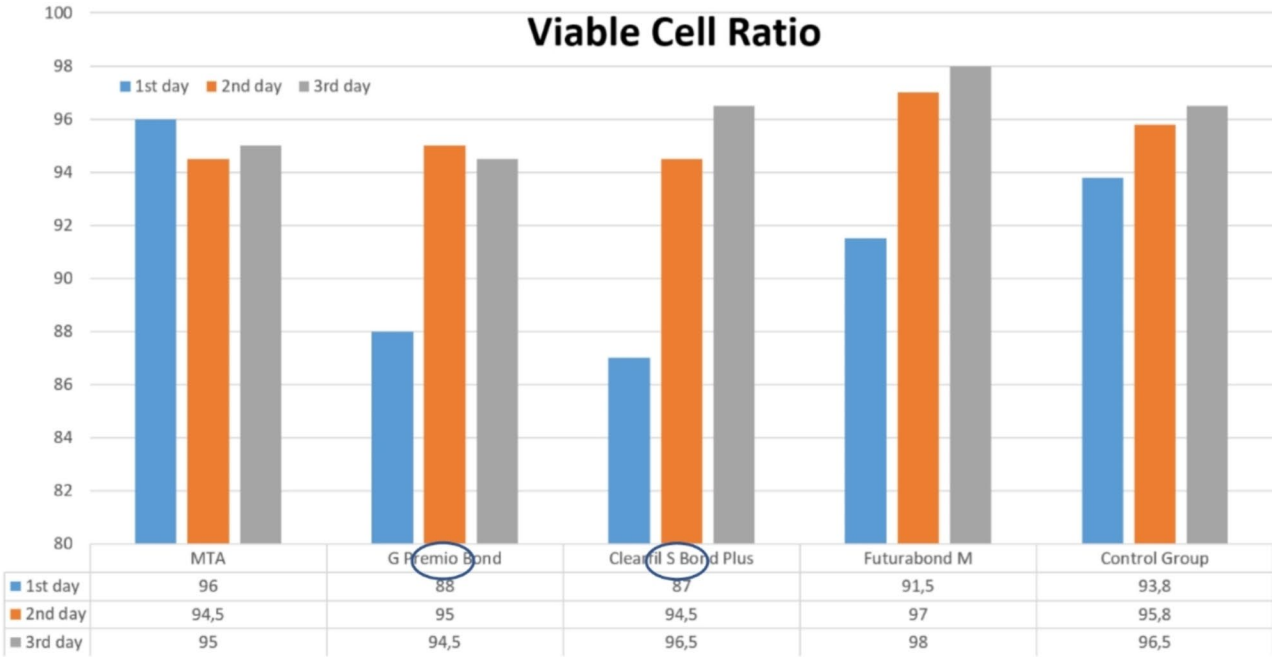

Fig. 2 Viability rates detected in the second experiment on the 1st, 2nd and 3rd days

Table 3 Finding obtained as a result of the Chi-Square Test in the second experiment at the end of the 1st, 2nd and 3rd days

Time		Value	df	Asymptotic Significance (2-sided)
24 h	Pearson Chi-Square	16,418[b]	4	,003*
	Likelihood Ratio	16,545	4	,002
	Linear-by-Linear Association	,197	1	,657
	N of Valid Cases	1200		
48 Saat	Pearson Chi-Square	2,069[c]	4	,723*
	Likelihood Ratio	2,163	4	,706
	Linear-by-Linear Association	,881	1	,348
	N of Valid Cases	1100		
72 h	Pearson Chi-Square	4,250[d]	4	,373*
	Likelihood Ratio	4,394	4	,355
	Linear-by-Linear Association	2,088	1	,148
	N of Valid Cases	1200		

Pearson Chi-Square Test, Fisher's Exact Probability Test *$p<0,05$

[b, c, d] Different letters indicate significant differences between groups

24 h were compared with the control group MTA, it was observed that all bonding agents showed more cytotoxic effects. When the adhesives used were compared, no statistically significant difference was observed between G Premio Bond and Clearfil S3 Bond Plus, while these two systems were found to be more cytotoxic than the other adhesive agent, Futurabond M (p:0.000; $p<0.05$). According to the viability rates obtained, the highest viable cell number was observed with 87.2% in the control group in which MTA was applied, while the lowest viable cell rate was seen in G Premio Bond with 57%. Among the adhesive materials, the highest vitality rate was observed in Futurabond M with 83%, while the vitality the vitality rate of Clearfil S3 Bond Plus was calculated at 59.5%. There was no statistically significant difference between the applied adhesive agents and the control group in 48 h results ($p>0.05$). When the viability rates of the applied adhesive materials in the first 72 h were compared with the control group MTA, it was observed that there was a statistically significant difference between them and all bonding agents showed more toxic effects than MTA (p: 0.000; $p<0.05$).

In the second experiment, a group to which no material was applied was added as the control group instead of MTA Angelus. MTA was used as a comparison group for the evaluation of adhesives. Viability rates obtained from the 5-out general comparison of the statistical results of the cytotoxicity evaluation are as in Fig. 2; Table 3. When the viability rates of dental pulp cells applied with adhesive materials in the first 24 h were compared with the control group, a statistically significant difference was found between them (p:0.003; $p<0.05$). When the materials were evaluated among themselves, it was seen that there was no significant difference between MTA and Futurabond M and the control group ($p>0.05$). Similarly, no significant difference was found between Clearfil S3 Bond Plus and G Premio Bond in terms of cell viability ($p>0.05$). Clearfil S3 Bond Plus and G Premio Bond materials were found to be more toxic compared to the MTA and control groups. There was no significant difference between Futurabond M, which has less toxic effects compared to other adhesive groups used in the study, and Clearfil S3 Bond Plus and G Premio Bond adhesive agents

($p>0.05$). When the viability rates of the cells to which the adhesives were applied at 48th and 72nd hours were compared between the control group and each other, no statistically significant difference was found ($p>0.05$). Although the vitality rate of Futurabond M at the end of 48th and 72nd hours was higher than that of the control group, no significant difference was found between them as a result of the statistical analysis ($p>0.05$).

Discussion

When treating perforations of the dental pulp cavity for many different reasons, the first goal is to maintain pulp vitality. The success of the capping treatment to protect the vitality of the pulp depends on many factors [4, 23, 24]. One of these affects the biocompatibility of the materials used in the treatment and their capacity to create reparative dentine [25].

Biocompatibility can be assessed through in vitro, in vivo (animal experiments), and clinical (usage) test groups. Cytotoxicity tests are the most commonly used in in vitro assessments for this purpose among these groups [26]. The vitality test with trypan blue is also a long-established in vitro test method. Trypan blue, which can bind strongly to proteins, passes through the membranes of cells with impaired membrane integrity, allowing dead cells to be stained blue and detected. The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method measuring succinic dehydrogenase (SDH) activity is also used as a simple method to determine cytotoxicity. In previous studies, it was reported that residual monomers can affect color in experiments with SDH, so we used trypan blue in our study [27–29]. In this study, conducted in vitro, continuous cell lines that have the ability to reproduce indefinitely, are accepted as transformed primary cells and have a more stable phenotype were used. In this study, human dental pulp stem cells were preferred due to their more stable phenotype and faster proliferation. Since the first cells affected by adhesive agents are pulp cells, it is anticipated that using fibroblasts from the pulp will provide more accurate results. Cells used in this study were from the third passage. Cells that have undergone fewer passages are considered to have undergone the least amount of change in terms of their characteristics.

Studies have been conducted for years that indicate that adhesive systems can be used as capping material [30–32]. In many studies with self-etch adhesives and total-etch adhesives, no statistically significant difference was found in terms of cell viability [33–35]. However, they reported that the differences in the application stages and the substances they contain may affect cell viability rates [33, 34]. Caroprese et al. [35] concluded in their study that two-step bonding systems exhibit cytotoxic effects on certain dental pulp cells, whereas self-etch adhesives do not have a significant cytotoxic effect. In this study; we found no significant difference in cell viability between the self-etch adhesive Clearfil S3 Bond Plus and the universal adhesive G Premio Bond at the 24th, 48th and 72nd hours.

In recent studies using adhesive systems, it has been reported that the components of the products affect cytotoxicity, and adhesive systems containing such as UDMA, HEMA, Bis-GMA, 4-META, TED-GMA and silver nanoparticles cause toxicity at different rates [36–40]. In both experiments conducted in this study, the number of live cells observed in the wells treated with Futurabond M was similar to MTA in the first experiment, MTA and control groups in the second experiment, and statistically there was no significant difference between them except for the first 24 h. It is thought that this difference observed between them in the first 24 h may be due to high molecular weight monomers such as UDMA and HEMA in the structure of Futurabond M.

The pH of the adhesive systems can be different. In a recent study, it was stated that as the pH of the adhesive systems decreases, they have negative effects on the apoptosis mechanism in the cell [41]. The pH values of G Premio Bond, Clearfil S3 Bond Plus and Futurabond M used in this study are in the range of 1.5, 2.7 and 3–4 respectively. As a result of the study, we are of the opinion that one of the reasons why Futurabond M is less toxic than other adhesive agents may be related to the pH value of Futurabond M.

In minimally invasive dentistry, preserving tooth tissues as well as maintaining pulp vitality is of significant importance, and careful attention must be paid to the selection of materials used in treatment. As a result of this research, we concluded that adhesive systems, especially in treatments close to the pulp, should be used in the clinic with careful and correct indications, following the manufacturer's recommendations.

When compared to other studies using similar materials, the limitations of this study may be attributed to several factors, such as differences in donors, variations in cell types, preparation of the material and the manner in which it contacts the cells, differences in evaluation methods, and variations in the duration of cell-material contact.

Conclusions

All adhesive systems were found to cause a decrease in pulp vitality compared to the control group. Futurabond M exhibits similar cytotoxic properties and biocompatibility to MTA, however, extensive studies are needed to further investigate the cytotoxic effects of Futurabond M.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-024-04985-1.

Supplementary Material 1

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Author contributions
K.G and C.A. concep-designed the study. C.A performed the main part of the study including collection of samples, delivery of interventions, collection of data and writing of manuscript under the guidance of M.K. S.S interpreted the data and performed qualitative data analysis. The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Ethical approval
The ethical approval has not been obtained for any application made.

Consent for publication
No/Not applicable.

Competing interests
The authors declare no competing interests.

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