Turk J Med Sci
35 (2005) 395-399

CLINICAL INVESTIGATION

Analysis of the Cytotoxicity of Four Dentin Bonding Agents on Gingival Fibroblasts

Jülide ÖZEN[1], Arzu ATAY[1], Fulya TOKSOY TOPÇU[2], Ali Uğur URAL[3],
Mehmet DALKIZ[1], Yaşar Meriç TUNCA[2]

[1]Department of Prosthetic Dentistry, Dental Sciences Center, Gülhane Military Medical Academy, Ankara - Turkey
[2]Department Restorative Dentistry and Endodontics, Gülhane Military Medical Academy, Ankara - Turkey
[3]Department of Hematology, Gülhane Military Medical Academy, Ankara - Turkey

Received: April 12, 2005

Abstract: The objective of this study is to evaluate effects of four dentin bonding agents PromptL-Pop, Syntac, Pekabond, Scotchbond-1 on cell proliferation in human gingival fibroblast cultures. Under aseptic conditions, test specimens were placed in the centre of 24-well tissue culture trays. Each well was covered at a concentration of 104 cells/cm2. The cultures were incubated at 37 ºC and cell proliferation was determined by the MTT method 24 h and 72 h after exposure. Statistical analysis was performed applying the Student t test. Statistical analysis of data showed that all materials caused noncytotoxic effects for 24 hours and cytoxic effects for 72 hours. Prompt L-pop displayed the highest number of cells whereas the lowest number of cells was found for Pekabond for 72 hours. These results support the proposal that some bonding agents may cause cytotoxic reactions under in vitro conditions.

Key Words: Dentin bonding agents, gingival fibroblasts, MTT, cell culture

Introduction

Dental adhesive systems were introduced into clinical dentistry after 1960's. Since then the developments in dentin bonding agents have greatly changed the practice of restorative dentistry. Application of dentin bonding agents allows the practitioner to place restorations with high adhesive strength and good marginal seal (1-3).

Research is available regarding the in vitro cytotoxic effect of monomers that are present in dentin bonding systems (3-7). Moreover, the in vitro toxic potential of components of dentin adhesives has also been shown (3-8). Data suggest that pulpal reactions to dentin bonding agents may be influenced by a number of factors, such as composition, clinical application procedure and dentin permeability (8, 9).

Recently, a number of techniques for in vitro cytotoxicity evaluation have been established (3-8). The majority of these assays comprise cell culture systems where, on the occasion of dental material cytotoxicity, fibroblast cell lines are commonly used (6). The outcomes of such experiments are useful predictors for the assessment of dental materials biocompatibility and also provide convenient, controllable and repeatable means for an initial evaluation of biological responses (5, 6).

There is an ongoing debate that the close and prolonged contact of dental materials with gingiva may have the potential to damage this tissue (10). For this reason it is vital to test and determine the cytotoxicity of such materials (11-15). In the present study, the cytotoxicity of four commonly used dentin adhesives was analyzed by using a cell-culture model of primary human gingival fibroblasts.

Materıals and Methods

Test Specimens

The four dentin bonding agents which were tested were as follows: (1) Prompt L-Pop, (2) Syntac, (3) Pekabond, (3) Scotchbond-1. Their components and manufacturers are listed in Table 1. Under germ-poor conditions, the dentin adhesives to be tested were applied into glass tubes (3 mm inner diameter x 1 mm in height), in order to occupy the same volume as the cured bonding agents according to the manufacturers' instructions with the use of an Elipar Freelight II curing light (3M ESPE, Seefeld, Germany).

Cell Culture and MTT Assay

Human gingival fibroblast (GF) cells were used for the cytotoxicity test. Healthy human gingival tissue was obtained from volunteer patients undergoing extraction of the third molar tooth for orthodontic reasons, and each tissue specimen was treated separately. All participants in the study gave informed consent to the experimental procedures and local ethic committee consent for the study protocol was obtained. Immediately after removal, the tissue was placed in Hanks salt solution containing penicillin/ streptomycin and amphotericin B concentrations. Thereafter, biopsies were stored at 4°C for no longer than 6 hours. Specimens were minced into small pieces. Gingival fibroblasts were extracted from the lamina propria using 0.125U/ml of collagenase-P (Boehringer Mannheim, Laval, QC, Canada) (16). Fibroblasts were cultivated at 37°C in Dulbecco's modified Eagles medium (DMEM, Biochrom, Berlin, Germany) and 10% fetal calf serum (FCS), (Gibco, Paisley, U.K.) containing penicillin/streptomycin (Sigma, St. Louis, MO) and amphotericin B (Sigma, St. Louis, MO). Cells were plated in 24-well tissue culture trays (10^4 cells/cm^2). Cells from passage number 2-3 were used.

For the cytotoxicity test, the test specimens were placed in the center of 24-well tissue culture trays. After the 24-hour and 72-hour incubation period, the test specimens were removed from the culture wells and the cytotoxicity of the materials was assessed using the MTT (3-(4,5-dimethyl-thiazoyl)-2,5-diphenyl-SH-tetrazolium bromide) method, after verification of the cell profileration by Trypan blue dye exclusion assay (17, 18). MTT (5 mg/mL in Hanks balanced salt solution) was added to each well, and the microplates were further incubated at 37°C for 4 hours. After the incubation period, 100 µL of acidified isopropanol (0.04 N HCl in isopropanol) was added to the cultures and mixed thoroughly to dissolve the dark blue crystals of formazan. The solubilized reaction products were transferred to a 96-well plate, and the absorbance values of each well were determined with a microplate enzyme-linked immuno-assay (ELISA) reader equipped with a 570-nm filter. Survival rates of the controls were set to represent 100% viability. Untreated cultures were used as controls groups.

Results are presented as percentage cell proliferation determined as 100- (A of experimental well/A of positive control well) X 100. Each experiment was repeated 3 times with representative data presented.

Table 1.Test materials and their composition according to manufacturers.

Dentin Adhesive (Brand Name)	Components	Manufacturer
Prompt L-Pop	Methacrylic phosphates, initatör, stabilizer, fluoride, water.	ESPE Dental AG Seefeld- Germany
Syntac	Maleic acid, HEMA, Metacrylate modified polyacrylic acid, initiators ans stabilizer in an aqueous solution.	Vivadent,USA
Pekabond	Maleic-acid-mono-2-methacryloyl-oxyethylester (0-5%)	Ivoclar Vivadent-USA
Scotchbond-1	BIS-GMA(5-35%) HEMA (5-25%) MMPAS (5-15%) UDMA,GDMA (2-25%) Ethanol (20-60%) Aqua (2-8%)	3M Medica, D-Borken, USA

Statistical analysis was performed applying the Student t test using SPSS 10.0 statistical softwarepackage (SPSSFW, SPSS Inc, Chicago, IL. USA) for Windows. A p value < 0.05 was considered statistically significant.

Results

The results of the cell proliferation for the test materials are given in Table 2. Cell growth of the fibroblast cell cultures was monitored by MTT assay after 24h and 72h exposure period. As shown in Figure 1 and Table 2, there was a statistically significant difference between the respective bonding agents and the control cells after 72h exposure period. Group comparisons for the dental paternals revealed that Prompt L-Pop showed slightly more cells than Syntac, Pekabond and Scotchbond-1. The significantly lowest number of cells was found for Pekabond compared to other test specimens.

Prompt L-Pop cell proliferation rates were about 90% of control cultures. Syntac, Scotchbond-1 reduced cell proliferation rates of about 85% and Pekabond 69% of control cultures ($P < 0.001$). None of the test specimens influenced cell proliferation after the 24h exposure period.

Discussion

Primary gingival fibroblast cultures are more closely related to their original tissue and therefore much easier to identify. Further, these cultures have a nearly unchanged metabolic state relative to their original tissue; the in vitro experiments thus approximate the in vivo situation. For these reasons, this cell culture has been commonly used for cytotoxicity evaluation of biomaterials (6, 12). Therefore, in the present study, an in vitro model of primary gingival fibroblasts placed in direct contact to the testing dentin bonding agents was preferred.

Each method of cytotoxicity measurement had its strengths and weaknesses. The visual method gave accurate results with a minimum amount of equipment, but required some observer training and provided only non-parametric data. Densitometric evaluations were easy to obtain and preserved the monolayer. However, this method was time consuming and less precise. Colorimetric evaluation of solubized formazan dye (MTT Assay) was fast, objective and showed the least variation. Therefore in this study, cytotoxicity was determined by using MTT assay at two observation periods (24 and 72 h). The percentage of viable cells compared with controls represented the level of cytotoxicity of the dentin bonding agents. The cleavage of MTT has several desirable properties for assaying cell survival and proliferation. MTT is cleaved by all living, metabolically active cells that we have tested. The assay can be read a few minutes after the addition of acid-isopropanol, and the color is stable for a few hours at room temperature. The results are also apparent visually, which is very useful if rapid qualitative results are required (18).

In this study, Prompt L-Pop and Syntac revealed similar results with the MTT assay. The cytotoxicity of Scotchbond-1 was lower than both the Prompt L-Pop and Syntac. The toxicity of Pekabond was found to be the highest. The results also demonstrated that the duration of exposure had a strong effect on the toxicity of dentin

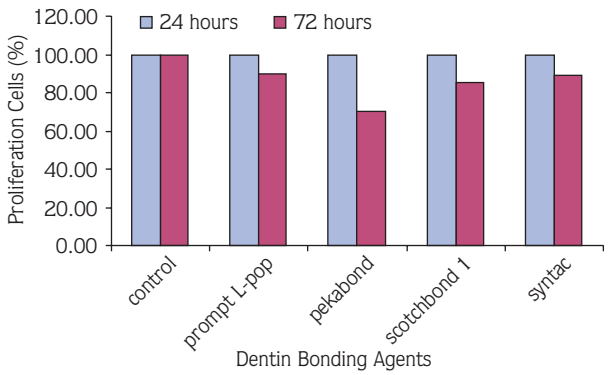

Figure 1. Percentage of cell proliferation after diffusing each dentin bonding system.

Table 2. The results of Mean Values and Standard Deviations (SD) of the test materials and statistical analysis. Cell proliferation was analyzed using the Student t test. *Significant ($P < 0.05$).

Test Specimens	24 hours	72 hours
Control	100±4.29	100±4.52
Prompt L-Pop	100±8.14	90.13±6.34*
Syntac	99.72±3.89	89.01±7.12*
Pekabond	99.93±1.33	69.82±7.29*
Scotchbond-1	99.78±2.79	85.26±7.42*

bonding agents, as the longer period of exposure resulted in a higher incidence of toxicity.

Szep et al (6) found that six dentin bonding agents were cytotoxic (Ariston Liner, Etch & Prime 3.0, Optibond Solo, Prime & Bond NT, Scotchbond 1, and Syntac Sprint) and Koliniotou et al. (3) also showed that six dentin bonding agents (Scotchbond 1, F-2000, Solobond, Heliobond, Bond 1, Syntac) were cytotoxic. Kaga et al. (19) reported that clinical exposure to the primers and adhesives of dentin bonding agents should be minimized. Ruey-Song et al (20) suggested that dentin bonding agents exert potential harmful effects in the pulp. They concluded that differential toxic effects of dentin bonding agents on the pulp cells should be considered during selection of a suitable dentin bonding agent for operative restoration. Ratanasathien et al. (5) reported that the different resins which are present in dentin bonding agents can interact to alter cytotoxicity in vitro. These interactions may cause the resins to be more or less toxic than the sum of the individual toxicities. As the interactions occurred at concentrations which have been shown to be released from resins in vitro, they may be relevant to pulpal irritation in vivo.

The cytotoxicity of dentin bonding agents confirmed in studies may cause inflammation of the oral mucosa. BisGMA was found to dissolve easily and may lead to inflammation in direct contact with pulpal or gingival tissues (21, 22). In another study, direct toxic effects in the form of irritation of the oral mucosa were found four days after the application of the dentin adhesives GLUMA and Scotchbond to class V cavities. The materials had been inadvertently applied even to tissues outside the cavity surfaces (23).

It can be concluded from this in vitro study that all four dentin bonding agents tested are cytotoxic to different extents. Pekabond showed the highest cytotoxicity followed by Scotchbond-1, Syntac, Prompt L-Pop. It is anticipated that all dentin bonding agents tested could cause reactions in deep cavities in vivo in the absence of an adequate lining. Further investigations are necessary to show whether incubation in cell culture medium represents a model to study cytotoxicity on bonding agents in the oral environment and whether persistent toxicity within this biological model can be equivalent to in vivo conditions.

Corresponding author:
Jülide ÖZEN
Mebus Evleri İller Sk. 9/9
06580 Tandoğan, Ankara - Turkey
E-mail: julideozen@yahoo.com

References

1. Reeves GW, Fitchie JG, Hembree JH et al. Microleakage of new dentin bonding systems using human and bovine teeth. Oper Dent 20: 230-35, 1995.
2. Retief DH, Mandras RS, Russell CM. Shear bond strength required to prevent microleakage at the dentin restoration interface. Am J Dent 7: 44-46, 1994.
3. Koliniotou-Koubia E, Dioysopoulos P, Koulaouzidou EA et al. In vitro cytotoxicity of six dentin bonding agents. J Oral Rehabil 28: 971-75, 2001.
4. Vajrabhaya L, Pasasuk A, Harnirattisai C. Cytotoxicity evaluation of single component dentin bonding agents. Oper Dent 28: 440-44, 2003.
5. Ratanasathien S, Wataha JC, Hanks CT et al. Cytotoxic interactive effects of dentin bonding components on mouse fibroblasts. J Dent Res 74:1602-6, 1995.
6. Szep S, Kunkel A, Ronge K et al. Cytotoxicty of modern dentin adhesives –in vitro testing on gingival fibroblasts J Biomed Mater Res 63:53-60, 2002.
7. Schmalz G, Schuster U, Koch A et al. Cytotoxicity of low pH dentin-bonding agents in a dentin barrier test in vitro. J Endod 28:188-92, 2002.
8. Pashley DH. Consideration of dentin permeability in cytotoxicity testing. Int Endod J 21; 143-54, 1988.
9. Söderholm KJ. Correlation of in vivo and in vitro performance of adhesive restorative materials: a report of the ASC MD 156 Task Group on test methods for the adhesion of restorative materials. Dent Mater 7; 74-83, 1991.
10. Caughman WF, Caughman GB, Shifflett RA et al. Correlation of cytotoxicity, filler loading and curing time of dental composites. Biomaterials 12:737-40, 1991.
11. Hidemann D, Lambert F. Cell culture of human gingival epithelium. Dtsch Zahnärztl Z 33: 29-32, 1978.
12. Hidemann D, Lambert F. The testing of dental filling materials in cell culture. Dtsch Zahnärztl Z 35:349-53, 1980.
13. Pelka M, Danzl C, Distler A, et al. A new screening test for toxicity testing of dental materials. J Dent 28: 341-45, 2000.

14. Paul C, Ronnda LB, Lloydstone J et al. Culturing keratinocytes and fibroblasts in a three-dimensional mesh results in epidermal differentiation and formation of a basal lamina-anchoring zone. J Invest Dermatol 100: 35-9, 1993.

15. Bumgardner JD, Lucas LC, Tilden AB. Toxicity of copper-based dental alloys in cell culture. J Biomed Mater Res 23: 1103-14, 1989.

16. Tardif F, Ross G, Rouabhia M. Gingival and dermal fibroblasts produce interleukin-1? converting enzyme and interleukin-1? but not interleukin-18 even after stimulation with lipopolysaccharide. J Cell Physiol 198: 125-32, 2004.

17. Sjögren G, Sletten G, Dahl J. Cytotoxicity of dental alloys, metals, and ceramics assessed by Milipore filter, agar overlay, and MTT tests. J Prosthet Dent 84: 229-36, 2000.

18. Edmondson JM, Armstrong LS, Martinez AO. A rapid and single MTT based spectrophotometric assay for determination drug sensitivity in monolayer cultures. J Tissue Cult Methods 11: 15-7, 1988.

19. Kaga M, Noda M, Ferracane JL et al. The in vitro cytotoxicity of eluates from dentin bonding resins and their effect on tyrosine phosphorylation of L929 cells. Dent Mater 17:333-39, 2001.

20. Chen R, Liu C, Tseng W et al. Cytotoxicity of three dentin bonding agents on human dental pulp cells. J Dent 31:223-29, 2003.

21. Hanks CT, Wataha JK, Parsell RR et al. Delineation of cytotoxic concentrations of two dentin bonding agents in vitro. J Endod 18:589-596, 1992.

22. Rathburn MA, Craig RG, Hanks CT et al. Cytotoxicity of a Bis-GMA dental composite before and after leaching in organic solvents. J Biomed Mater Res 25:443-457, 1991.

23. Hensten-Pettersen A. Skin and mucosal reactions associated with dental materials. Eur J Oral Sci 106: 707-712, 1998.