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In vitro evaluation of cytotoxic effects of luting resin cements

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ABSTRACT

Purpose: This *in vitro* study evaluated the cytotoxicity of resin cements with L929 fibroblasts. **Materials and Methods:** The resin cements, Super-Bond C&B, RelyX ARC, Clearfil Esthetic, specimens were prepared and extracted. The L929 cells were plated (25,000 cells/mL) and maintained. The medium was replaced with the resin extracts. Twelve specimens were used for each group. Cell viability was evaluated by 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) tests. The data were analyzed by one-way analysis of variance and Tukey honest significant difference tests. **Results:** Clearfil Esthetic had significantly higher decreasing effect on cell survival and growth (55.64 ± 8.26%), from the other resin cements and the control (P < 001). There are no differences between the survival rates of Super-Bond C&B (72.75 ± 12.33%) and RelyX ARC (62.63 ± 10.98%) (P > 05); however, each of them was cytotoxic when they compared with the control (P < 001). **Conclusion:** Differential toxic effects of resin-based cement on the pulp cells should be considered during the selection.

KEYWORDS: 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, cytotoxicity, fibroblast, resin cements

Introduction

Dental cements have a very long tradition of being used in dentistry for a wide variety of applications.[1] Resin cements are used for the luting of conventional, full and partial ceramic crowns due to their superior properties according to the conventional ones such as high level tensile bond strength, lower oral solubility and higher micromechanical bonding to the prepared tooth surface and restorative materials.[2]

One of the major drawbacks of resin-based materials is the sensitivity reactions.[3,4] The allergic reactions ranging from 0.7% to 2% of the patients, dentists, and dental assistant is reported.[5] On the other hand, *in vivo* and *in vitro* studies have concluded that the released monomers from resinous materials such as triethylene glycol dimethacrylate (TEGDMA) and 2-hydroxyethyl methacrylate (HEMA) may cause the differential damage from gingival margin retraction to the inflammatory reaction reaching the pulp and cell death.[6-8]

Dentin surfaces and the dentinal tubules are exposed to the oral environment after the enamel layer has been removed by the crown preparation. The main purpose of the luting cements is the cementation of the restoration to the prepared tooth surface. Furthermore, these materials have to contribute to protect of the exposed dentin and pulpal tissues from thermal, mechanical and microbial effects during the survival as well as to maintain masticatory function, phonetics, and esthetics.[1,7] It is showed that some elements of the resins have exerted cytotoxic effects on fibroblastic cells *in vitro*.[7]

Dental cements must have superior luting and excellent biocompatibility, because cytotoxicity of dental materials is the critical factor when materials are contacted with adjacent tissues. Although biocompatibility or cytotoxicity is one of the preferring factors for choice of clinicians, cytotoxicity studies of luting dental cements are limited. Consequently,

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the purpose of this investigation was to evaluate the cytotoxic effects of three different resin cements, which have been intensively used in prosthodontics for different clinical applications. The null hypothesis was that there was any different among the tested resin cements.

Materials and Methods

Three resin cements were tested in this study. Material details and ingredients are given in Table 1. Test specimens were prepared according to the manufacturers' recommendations using standard teflon plates of 5 mm in width and 2 mm in depth. All specimens were handled and set under disinfected conditions. Light cured resin cement specimens were polymerized with a standard light emitting diode curing unit (Elipar Free Light 2, 3M ESPE Dental Products, St. Paul, MN, USA). Twelve specimens were prepared for each group.

Material extracting

The specimens were immersed in 7 mL of culture medium for 24 h at 37°C to extract the residual monomer or cytotoxic substances. The culture medium, containing material extracts, was sterilized by filtering and then added to the cell cultures.

Cell culture

L929 cell lines (An1 Mouse C3 [an connective tissue], Şap Enstitüsü, Ankara, Turkey) were cultured in basal medium eagle (BME) containing 10% newborn calf serum and 100 mg/mL penicillin/streptomycin at 37°C in a humidified atmosphere. Cell cultures, between the 12 and 15 passages, were used in the study. Confluent cells were detached with 0.25% trypsin and seeded at a density of 5.3×10^3 into each well of a 96 well-plate. After the 24 h incubation at 37°C and 5% CO_2, the medium was replaced with 200 µL of medium, which containing the extracts of resin cements. After that, the cultures were incubated for 24 h at 37°C and 5% CO_2. The culture medium not including any cement extract was used as a control group.

Cytotoxicity testing

The cell viability was assessed using succinic dehydrogenase activity. The medium was removed and the cell cultures were rinsed with phosphate buffered saline (PBS) and 200 µL aliquots of newly prepared 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) solution (0.5 mg/mL in BME) were added to each well. After 2 h incubation period (37°C, 5% CO_2), the supernatant was removed and the intracellular stored MTT formazan was solubilized in 200 µL dimethyl sulfoxide for 30 min at room temperature. The results were submitted to an enzyme-linked immune-sorbent assay (ELISA) reader (µquant, Bio-Tek Instruments Inc., Winooski, VT, USA) for analysis of optical density at 540 nm. For each material, twelve testing were repeated in two independent experiments.

Statistical analysis

Cell survival in the resin cement groups was compared with that in the untreated controls. Data were expressed as a percentage of the control group. Differences between median values were statistically analyzed using the one-way analysis of variance (ANOVA) and Tukey honest significant difference tests. Significance level was set as $P < 0.05$.

Results

An overview of the results was shown in Table 2. According to results of ANOVA, there were significantly differences among the luting cements in terms of cell survival percentage ($P < 0.001$) [Table 3]. All materials had significantly decreased cell survival when compared to the control group ($P < 0.001$). In general, the rank order with respect to cell viability was found to be as follows: Clearfil Esthetic (55.64 ± 8.26%), RelyX ARC (62.63 ± 10.98%), and Super-Bond C&B (72.75 ± 12.33%) [Figure 1]. Clearfil Esthetic had the most cytotoxic effect on cultured cells ($P < 0.001$). However, the Super-Bond C&B was the less cytotoxic among the tested materials, it was also cytotoxic than the control group ($P < 0.01$).

Discussion

In this study, the mean cell survival rates of L929 cells exposed to the resin cements extracts was 72.75%, 62.63%, and 54.65% in Super-Bond C&B, RelyX ARC, and Clearfil

Table 1: Cements used in this study

Material	Type	Composition	Manufacturer
Super-bond C&B	Self-cure resin cement	4-META, MMA-TBB	Sun Medical Co., Shiga, Japan
RelyX ARC	Dual-cure resin cement	Bis-GMA, TEGDMA, zirconia/silica filler (67.5 wt%)	3M ESPE, St. Paul, USA
Clearfil esthetic	Dual-cure resin cement	BisGMA, TEGDMA	Kuraray Med. Inc., Okayama, Japan

MMA = Methyl methacrylate, 4-META = 4-methacryloxyethyltrimellitic acid anhydride, Bis-GMA = Bisphenol-A-glycidyl methacrylate, TEGDMA = Triethylene glycol dimethacrylate, TBB = Tri-n-butylborane

Table 2: Descriptive statistics of tested materials

Groups	*n*	Mean	Standard deviation	95% confidence interval for mean		Minimum	Maximum
				Lower bound	Upper bound		
Control	12	100.00	11.83	92.48	107.52	83.90	122.72
Super-bond C&B	12	72.75	12.33	64.91	80.58	49.06	89.99
RelyX ARC	12	62.63	10.98	55.66	69.61	44.03	80.27
Clearfil esthetic	12	54.65	8.26	49.41	59.90	36.07	68.91

Table 3: Results of ANOVA analysis

Comparison of groups	Sum of squares	df	Mean square	*F*	Significant
Between	14065.829	3	4688.610	39.003	0.000
Within	5289.313	44	120.212		
Total	19355.142	47			

ANOVA = Analysis of variance

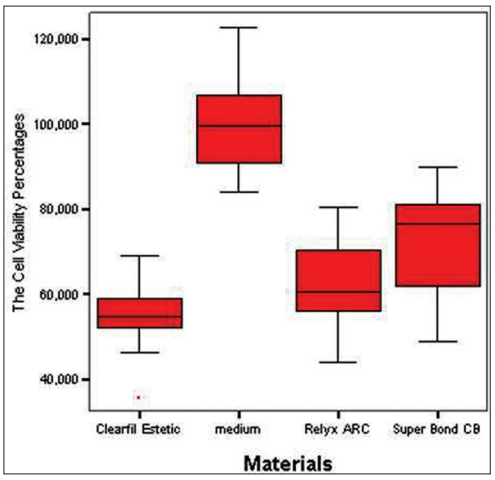

Figure 1: Mean cell survival percent of resin cements with standard deviations bar according to control group

Esthetic, respectively. There were significantly differences among the tested materials by means of cytotoxicity. Therefore, the null hypothesis was rejected.

The cytotoxicity of dental materials is also important for health of the dental stuff. In general, the risk of the adverse effects of dental materials is much greater for the dental assistant than patients, because the dental staffs is frequently exposed to the materials during manipulation of the materials when they are being preparation, application or removed. Furthermore, there is knowledge indicating that the dental materials used by dentist have some risk to the patient and dental staff. It is the clinicians' problem to choose whether this evidence is deserved and to assessment the risk of these materials in dental practice.[9,10]

There are different ways in which these materials can affect the configurations of oral tissues, by carrying water-soluble components into the oral fluids as well as by relations directly with pulpal or gingival tissue.[9,11] Dental cements bond a restoration to freshly prepared dentin. There is critical importance for freshly opened dentinal tubules after tooth preparation[12] thus; it is very critic to assess the toxicity of luting cements, because these materials contact to pulpal chamber and neighboring to periodontal tissue and alveolar bone. Toxic elements released from these cements may result a reaction in adjacent tissues. The degree of the tissue damage varies with contents of the resin cement[7] and the interaction of different ingredient's with the surrounding gingival tissue and dentin resulting in pulpal reactions.[13]

The different cytotoxicity ranges for the tested cements may result from their chemical content and manipulation method.

The main advantages of cell culture tests are no ethical considerations, high reproducibility, simply, not expensive, and their standardization. There is no single specific test method is available to evaluate one type of adverse reaction.[9] The MTT is demonstrated to be suitable to estimate cell densities in small culture. Thus, we prefer the MTT assay procedure.

Franz *et al*.[14] showed that L929 cell line presents comparable results to primary human gingival fibroblasts and therefore may be an alternative model for *in vitro* screening of gingival toxicity. Variations of the experimental setup were more effective on the toxicity results than origins of the fibroblast cells. In this study, the L929 cell line was chosen, because of its advantages such as easy handling, reproducibility of results, and availability. The results of this study demonstrated that the tested resin cements or released substances from these materials are able to elicit the biological responses on surrounding tissues and pulp cells. Although the direct correlation between the occurrence of clinical effects and *in vitro* results is controversial, the resin cements are in close contact to the gingival and pulpal tissues during cementation and function increase the risk of damage. In most cases, a small part of polymerized resin cements remains in the gingival sulcus or between the residual crest and pontic. The degree of tissue damage depends on the amount of cement which in contact with oral tissues and on the amount of released components into the gingival sulcus and the time of the exposure. On the other hand, individual differences in allergic reaction to cytotoxic materials may also exist. Therefore, the immediate elimination of residues after the cementation is important to avoid from the potential toxic reactions.[15]

The matrix of resin cements contains of different monomers, such as bisphenol-A-glycidyl methacrylate (Bis-GMA) (bisphenol-A diglycidyl ether dimethacrylate) and/or urethane dimethacrylate (UDMA). Other components of the composite matrix are co-monomers, for example ethylene glycol dimethacrylate, diethylene glycol dimethacrylate (DEGDMA), TEGDMA and additional additives such as co-initiators, photo initiators, inhibitors, and color pigments. TEGDMA decreases the viscosity of the resin matrix, thus allowing increased filler content. These ingredients may altered cell metabolism at concentrations well under the toxic threshold and the observed changes may be assumed a potential mechanism for inducing adverse clinical effects.[16]

Bisphenol-A-glycidyl methacrylate demonstrated greater difficulty in penetrating and has less mobility, but it is the most toxic among the monomers. Furthermore, Bis-GMA goes under hydrolysis, produced methacrylic acid (MAA) as

a metabolite. MAA can provoke cytotoxicity by stimulating the release of tumor necrosis factor[17] or by modification the lipid level of the cell membrane, which affects the membrane permeability.[16] Water-soluble ingredients are used in different resin-cements (TEGDMA) and adhesives (HEMA/TEGDMA), and thus are released from the materials. Swallowed TEGDMA were almost completely absorbed by the organism.

Resin cements were studied with different cytotoxicity tests. It was found that resin cements were differently toxic in various cell types (mouse fibroblasts or pulp fibroblasts).[18] But in most cases, pronounced cell toxicity was observed in accordance with the present study.[19-21] Furthermore, when dentin tissue was located between the test materials and tested cells, the active concentration of some toxic chemical compounds leaching from adhesives decreased the cells.[22] Relatively hydrophilic components, such as TEGDMA in RelyX ARC and Clearfil Esthetic, may further penetrate and diffuse thin dentin to connective tissue of pulp, hours or days after placement.[7,8] The concentration of these materials into the cultured cells was found to be so high that destruction of pulp cells might be possible clinically.[23]

Super-Bond C&B contains 4-methacryloxyethyl trimellitate anhydride (4-META), a high performance bonding monomer, and tri-nbutylborane (TBB) as a catalyst. It has been shown that 4-META may not affect the cytotoxicity induction.[24] MMA is the main monomer of Super-Bond C&B. It is showed that MMA has a low potential cytotoxicity when compared with TEDGMA and Bis-GMA.[24,25] Also, the residual MMA, potential factor of cytotoxicity, was lower after the polymerization and decreases with time due to the TBB. This may explain the reason why the Super-Bond C&B had the lowest toxicity among the tested resin cements. The polymerization initiator of Super-Bond C&B is TBB. Tronstad and Spångberg[26] studied pulp responses to the resins and MMA/TBB used in deep cavities in monkeys. They demonstrated that pulpal response to MMA/TBB-based resin better than the composite resin.

The polymerization mode of resin cements could affect the cytotoxic response of these materials. Amount of residual monomers in the cured materials is related to the polymerization mode of the resin materials. Schmid-Schwap *et al*.[15] showed that the chemically cured resin-based cements had higher toxicity than the dual-cured specimens and the type of curing light could influence the cytotoxicity of resin cements. The results of this study showed that the self-cure resin cement, Super-Bond C&B, had lower toxicity than the dual cured resin cements. These results suggested that the toxic effects of 4-META-TBB monomer on the cells were concentration dependent and lower concentration of the monomer may not be cytotoxic. Moreover, polymerized Super-Bond C&B resin cement contains little monomer and should have less negative effects.[27] Our data are consistent with those of similar studies that found less cytotoxic effects with Super-Bond C&B compare to Clearfil Esthetic and RelyX ARC.[24,28] This study concluded that the tested resin cements enable to cause the tissue damage differing brands and polymerization type.

Conclusion

Super-Bond C&B is the least cytotoxic agent among the tested resin-based cements. It ıs indicated that in clinical application of these cements, different toxic effects on the pulp cells and gingival tissues should be considered. However, further studies using different test methods are needed to be investigated for resin cements. Research efforts should focus on assessing long-term biologic effects of resin cements.

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