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Evaluation of Cytotoxicity of Dentin Desensitizing Products

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Clinical Relevance

Colgate Sensitive Pro-Relief, Topex and Clinpro White Varnish could be recommended in the treatment of dentin hypersensitivity with regard to their cytocompatibility.

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SUMMARY

Objectives: To evaluate the cytotoxic effects of the dentin desensitizing products (DDPs) used in the treatment of dentin hypersensitivity on cultured human gingival and pulpal fibroblast cells.

Methods and Materials: The cytotoxic effects of DDPs (Smart Protect, Systemp Desensitizer, Seal & Protect, Aqua-Prep F, Isodan, Gluma, BisBlock, D/Sense Crystal, UltraEZ, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish) on cultured human gingival- and pulp-derived fibroblast cells were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test (Serva, Heidelberg, Germany) under two different conditions. In the first test, different dilutions of the DDPs were directly applied onto cultured gingival fibroblast cells, and in the second test, the products were applied onto different-thickness dentin discs (0.5 and 1 mm) placed above cell culture medium, which contained pulp fibroblast cells.

Results: According to the cytotoxicity evaluations of gingival fibroblast cells, the cytotoxicity of all of the DDPs was very high at 50% concentrations ($p<0.05$). Colgate Sensitive Pro-Relief, Clinpro White Varnish, and Topex showed higher cytotoxicity than did the other products ($p<0.05$), decreasing with further dilutions, and these products were found to be less cytotoxic to both types of cells ($p<0.05$) than were the other products with further dilutions. The cytotoxicity to human gingival and pulpal fibroblast cells of Systemp Desensitizer, Aqua-Prep F, Isodan, and Gluma did not show any decrease with further dilutions, and these products were found to be more cytotoxic than the other products ($p<0.05$).

Conclusions: According to the findings of this study, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were less cytotoxic than the other DDPs used in this study.

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INTRODUCTION

Dentin hypersensitivity (DH) is a frequent dental complaint in the adult population, with a prevalence ranging from 4% to 74%, and it occurs in patients between 18 and 70 years of age.[1,2] DH is defined by

short and sharp pain arising from exposed dentin in response to chemical or physical stimuli, typically evaporative, tactile, or osmotic stimuli, that cannot be explained by any other dental problem.[3] Generally, DH occurs as a result of more than one factor. Gingival recession, enamel, and cementum loss are the prevailing causes of oral exposure of dentinal tubules. Dentinal tubule exposure is mainly seen in the cervical regions of the vestibular faces of the teeth.[4,5] Open dentinal tubules provide a direct link between oral environmental factors and the pulpal tissues.[6] Although several theories have been proposed to explain the mechanism of DH, the most widely acknowledged theory is the hydrodynamic theory, proposed by Branström and co-workers. According to this theory, a chemical or physical stimulus allows for dentinal fluid movement within the dentinal tubules in the presence of open dentinal tubules. The theory further proposes that the fluid movement affects the pulpal mechanoreceptors, causing the sensation of pain.[7-9]

Clinical management of DH is based on the prevention or elimination of possible causes of pain. Dentinal tubule occlusion and blockage of nerve activity using desensitizing products are common methods of treatment of DH.[10] Thus, many dentin desensitizing products (DDPs) with different ingredients have been introduced to the market in different forms.

Professional desensitizing products include chemical agents, such as fluoride,[11] oxalates,[12] calcium compounds,[13] potassium nitrate,[14] strontium salts,[15] glutaraldehyde,[16] adhesive materials,[17,18] and arginine-containing desensitizers.[19-22] The desensitizing effects of many of these materials can be reduced over time by refraining from an acidic diet and through daily tooth brushing. Therefore, the success of long-term treatment of DH is thought to depend on occluding and penetrating the dentinal tubules to resist acid attacks as well as on tooth brushing.[23]

In addition to their desensitizing effects, it has been shown that many of these DDPs contain several cytotoxic components, such as glutaraldehyde, 2-hydroxyethyl methacrylate (HEMA), triclosan, resin monomers, and sodium fluoride.[24-27] Some of the desensitizing products on the market that contain resin monomers have similar content to dentin bonding agents. After being applied on the gingiva, bonding agents caused pathological changes in the oral mucosa.[28] When bonding agents come into direct contact with fibroblast cells, negative effects have been found in different *in vitro* studies.[26] For instance, HEMA, which is found in many DDPs, was reported to cause abnormal morphological development and inhibition of cell reproduction in cultured human epithelial cells and pulpal fibroblasts.[24] The cytotoxicity of fluoride, which is frequently used in toothpastes and gargles, in DH treatment has also been reported.[25,29] Fluoride has a repressive effect, particularly on protein synthesis and the mitochondrial activities of human pulpal cells.[30] Glutaraldehyde, which is added to desensitizers to reduce dentin permeability, also has the effect of coagulating protein structures.[16]

Similarly, in clinical practice, application of these products to the cervical area of a hypersensitive tooth can result in contact with the gingival tissues. In addition, these products pose significant potential toxicity to the pulpal tissues by moving from open dentinal tubules into the pulp.[26] Hence, evaluating the possible cytotoxic effects of these products on gingival and pulpal tissues is important for the health of the teeth and gingival tissues. Although there have been various studies on the effects, mechanisms, and clinical effectiveness of DDPs, the number of studies concerning their biocompatibility has been very limited.[31,32]

The biocompatibility of dental materials has been evaluated using primary human cells, such as periodontal ligament and pulp fibroblast cells, gingival fibroblasts, and odontoblast-like cells, and permanent nonhuman cell lines, such as 3T3 and L-929, as well as primary and immortalized bovine dental papilla-derived cell lines.[23,27,32-36]

In this study, we aimed to evaluate the cytotoxicity of DDPs commonly used in clinical settings for the treatment of DH, and we used an assay system that enabled more clinically relevant assessment of toxicity, in that dentin barriers with different thicknesses were placed between the DDPs and the pulpal fibroblasts, and the cytotoxic effects of the DDPs on the pulpal fibroblasts were assessed by setting a simple mechanism in tissue culture wells.

METHODS AND MATERIALS

Materials

Twelve different DDPs were used in this study, and their brand names, compositions, and batch numbers are shown in Table 1.

Cell Culture

The cytotoxic effects of DDPs on human gingival- and pulp-derived fibroblasts were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-

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Table 1: *Ingredients, Manufacturers' Information, and Lot Numbers for 12 Different Dentin Desensitizing Products (DDPs)*

Groups	Agents	Contents	Manufacturers' Information, Lot Numbers
1	Smart Protect	Antibacterial content in aqueous alcoholic solution (2%-10% glutaraldehyde, 20%-30% isopropyl alcohol, triclosan), and 0.14% fluoride	Detax, Ettlingen, Germany, 090201
2	Systemp Desensitizer	5% glutaraldehyde, <0.1% maleic acid, polyethylene glycol dimethacrylate, and water	Ivoclar Vivadent AG, Schaan, Lichtenstein, M74246
3	Seal & Protect	Di- and trimethacrylate resins, pentaacryloyldipentaerythrytol phosphoric acid, photoinitiators, butylated hydroxytoluene, cetylamin hydrofluoride, triclosan, acetone, and functionalized amorphous silica	Dentsply, Detrey, Konstanz, Germany, 0906004007
4	Aqua-Prep F	10%-30% HEMA and 1%-2% NaF	Bisco Inc, Schaumburg, IL, USA, 1000004372
5	Isodan	0%-40% HEMA, 0%-0.5% NaF, 0%-5% potassium nitrate, sherry flavor, and glycerol	Septodont, St Maur des Fosses, Cedex, France, 46061
6	Gluma	35% HEMA, 5% glutaraldehyde, and water	Heraeus Kulzer GmbH & Co, Hanau, Germany, 010092
7	BisBlock	<5% oxalic acid	Bisco Inc, Schaumburg, IL, USA, 1000003731
8	UltraEZ	3% potassium nitrate, and 0.25% NaF	Ultradent, South Jordan, UT, USA, 53
9	D/Sense Crystal	2.5% potassium binoxalade, 2.5% nitric acid	Centrix, Shelton, CA, USA, 91749
10	Colgate Sensitive Pro-Relief	L-arginine, calcium carbonate, glycerin, water, bicarbonate, hydrated silica, and sodium saccharin	Colgate-Palmolive Co, New York, NY, USA, 9323201110
11	Topex Topical A.P.F. Gel	2.7% NaF, sodium saccharin, kaolin, and glycerin	Sultan Healthcare, Hackensack, NJ, USA, 0513100519
12	Clinpro White Varnish	5% NaF	3M ESPE, St Paul, MN, USA, M13340

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; NaF, sodium fluoride.

lium bromide (MTT) test (Serva, Heidelberg, Germany) under two different conditions. In the first test condition, the DDPs, at various concentrations, were directly applied onto human gingival fibroblasts, while in the second condition, the products were applied onto dentin barriers placed above cell culture medium, which contained human pulp fibroblasts.

Both types of fibroblasts were derived from appropriate tissue samples obtained from healthy subjects who consented to tissue donation for the study, which was approved by the Scientific Research Evaluation Commission of the Medical Faculty of Karadeniz Technical University (file no. 2010/82). In brief, pulpal and gingival tissues were cut into small sizes and were used as explants in tissue culture flasks, with a growth medium containing Dulbecco modified Eagle's Medium (Lonza, Basel, Switzerland) and 10% fetal bovine serum (FBS) plus a penicillin/streptomycin/fungizone mixture (Lonza). The explants were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Growing cells were harvested by 0.25% trypsin and were subcultured until passages three and four to use in the following experiments.

Preparation of Dentin Discs

One hundred thirty sound human third molars were used for this study. They were stored at 4°C in distilled water containing 0.2% sodium azide with thymol to inhibit microbial growth until they were used. While hydrated, the crowns of the teeth were cut mesiodistally parallel to the long axis of the teeth to prepare dentin discs with thicknesses of 0.5 ± 0.05 mm and 1 ± 0.05 mm by means of a low-speed precision cutting machine (Micra Cut 125, Metkon, Bursa, Turkey). Dentin discs were examined with an optical microscope (Olympus Metallurgical Microscope), and only dentin discs that were nearest to the pulp tissue but that did not contain any pulp tissue were used. The pulpal surfaces of the dentin discs were marked to prevent

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the buccal surfaces of the discs from being confused with the pulpal surfaces. Each specimen was then immersed in ethylenediamine tetraacetic acid (14%) for two minutes to remove the smear layer from both surfaces and was then rinsed under tap water for five minutes.[33] The pulpal surfaces of the dentin discs were then marked again and were kept in a saline solution.

Serial dilutions (50%, 20%, 10%, and 1%) of the products (Smart Protect [group 1], Systemp Desensitizer [group 2], Seal & Protect [group 3], Aqua-Prep F [group 4], Isodan [group 5], Gluma [group 6], BisBlock [group 7], and D/Sense Crystal [group 9]) were used directly in culture medium. In the cases of solid or semisolid products, such as UltraEZ (group 8), Colgate Sensitive Pro-Relief (group 10), Topex (group 11), and Clinpro White Varnish (group 12), 0.1 or 1 g of each product was diluted in 0.1 or 1 mL of culture medium, followed by vortexing and incubation at 37°C and 5% CO_2 atmosphere for 24 hours.[37] The resulting elutions were passed through a 0.45-μm filter and were diluted to 50%, 20%, 10%, and 1% in the culture medium.[33] During this procedure, the medium was protected from light to prevent any polymerization.

Cytotoxicity Tests

Direct Contact of DDPs with Human Gingival Fibroblasts—Cells were diluted in growth medium and were seeded into 96-well plates (1×10^4 cells per well) to obtain subconfluent monolayers of cells, following incubation at 37°C and 5% CO_2 atmosphere for 24 hours. The medium was aspirated from all of the wells and was replaced with 100 μL per well of the test solution (50%, 20%, 10%, 1%), prepared as described above. During the test procedures, the products were protected from light to prevent any polymerization. The plates with control wells (ie, no test product) were incubated for another 24 hours. The contents of the plates were decanted, and the wells were washed twice with culture medium without FBS before adding MTT (0.5 mg/mL final concentration) into all of the wells.

The plates were kept in a CO_2 incubator for three hours. Optical density (OD) was determined by dissolving the MTT-formazan product with dimethyl sulfoxide (Merck, Darmstadt, Germany). Only viable cells owning functional mitochondria are able to reduce MTT to insoluble purple formazan crystals.[38] The spectrophotometric absorbance was measured at 570 and 630 nm using an enzyme-linked immunosorbent assay microplate reader (Sunrise, Tecan, Switzerland).

This experiment was conducted twice. In each experiment, each test product and control group was prepared in quadruplicate. The OD values obtained for the same dilution were recorded and averaged as a single measurement. The mortality percentage of the cells (MP) was calculated from the following formula:[39]

$$\mathrm{MP}\% = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100.$$

Cytotoxicity of DDPs to Pulpal Fibroblasts Using Dentin Discs: Preparation of Silicone Molds—A device was designed to place the dentin barriers in 24-well plates in a stable manner so that they would be able to contact the pulpal fibroblast cells and so that the products could be applied on the dentin discs. The diameter of the device was designed in such a manner that it would be equal to the diameter of one well of a 24-well plate. In addition, in the middle of the upper part of this device, a metal stick was designed, the apex of which was 3×3 mm. The marked pulpal surfaces of the dentin discs, with thicknesses of 0.5 mm and 1 mm, were placed immediately below the metal stick in a manner that would allow them to come into contact with the lower part of the device. The surrounding area of the metal stick was thoroughly filled with hydrophilic vinyl silicone (Bisico S1, Bisico Bielefelder Dentalsilicone GmBH, Bielefeld, Germany).

So that the wells could be easily handled and so that they could be placed in 24 wells, a small hole was created on the silicone mold with a hand tool before the silicone hardened. After the silicone hardened, the upper part of the apparatus was discarded, and the dentin discs that were within the silicone molds were obtained (Figures 1 and 2). Dentin discs with the same thicknesses were randomly divided into 13 groups and were placed in silicone molds using the device in a manner that would yield five samples for each group. The contact areas of the dentin discs with the impression material were tightly and hermetically sealed with dental wax. It was determined that there was no leakage between the dentin discs and impression material. One hundred thirty dentin discs in total, which were 0.5 mm and 1 mm in thickness, were inside the silicone molds and were sterilized with ethylene oxide gas. The toxicity of the DDPs to pulpal fibroblasts was evaluated in 24-well plates seeded with the cells. Dentin discs fitting the diameters of the wells of 24-well plates were pushed carefully with pliers until they contacted the medium from the upper part toward the well.

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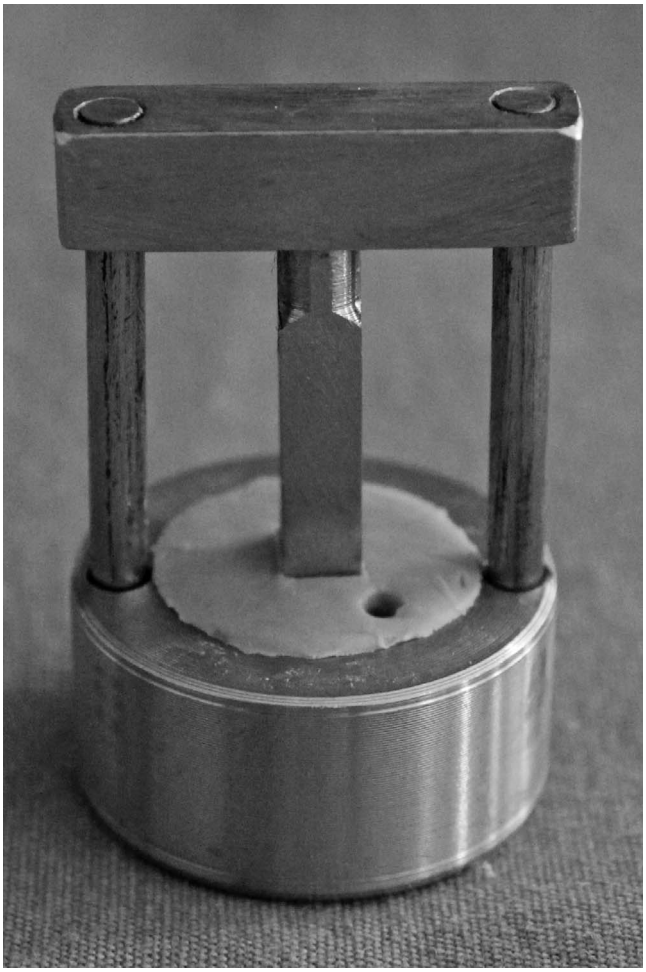


Figure 1. *The device that was designed to hold the dentin discs.*

Each test product was placed in the gap on the dentin barrier without being diluted. The 24-well plates were incubated at 37°C in 5% CO_2 for 24 hours. Dentin discs within the silicone molds were discarded using pliers at the end of the test. The MTT test was performed as described above.

Statistical Analysis of Cytotoxicity

The analysis of the data was performed using statistical software for Windows (SPSS, version 11.5, SPSS Inc, Chicago, IL, USA). The mean and standard deviation (SD) values of MP are shown in Table 2. Multiple comparisons among the groups, in terms of mortality percentage values, were performed by two-way analysis of variance (ANOVA). The significance of the differences between groups within dilutions in gingival fibroblasts was investigated with the Bonferroni correction test. The significance of the differences among groups within

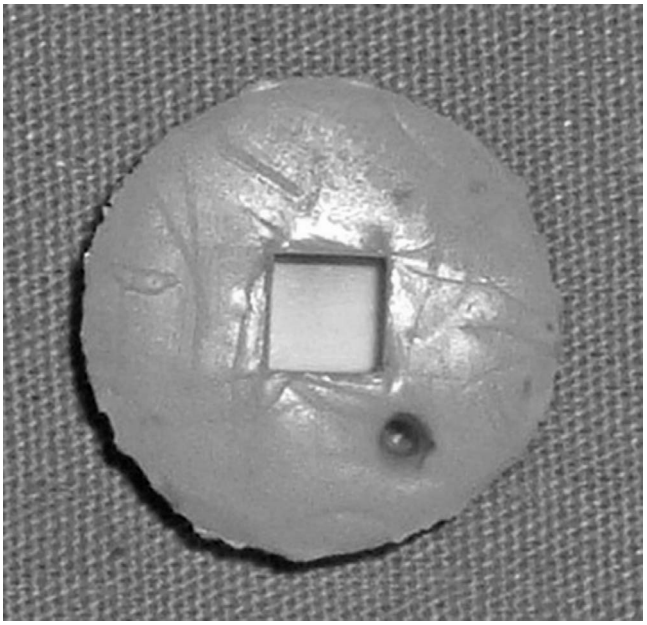


Figure 2. *Dentin discs within silicone molds.*

different-thickness dentin discs in pulpal fibroblasts was investigated with the Fisher least significant difference (LSD) test. Significant differences were found between the material groups and the control groups. The significance level was set at 95% for all of the statistical tests.

RESULTS

Cytotoxicity of Desensitizing Products to Gingival Fibroblasts

Two-way ANOVA revealed significant interactions ($p<0.001$) among the products and concentrations, as shown in Table 2, indicating 1) that cytotoxicity decreased as medium dilution increased and 2) that the toxicities of the products were different.

Table 2: *Two-way Analysis of Variance (ANOVA) Table for Overall Models (Mortality Percentages of Gingival Fibroblasts)*[a]

	Sum of Squares	*df*	Mean Square	*p*
Agents	251,118.06	11	22,828.91	<0.001
Dilutions	58,636.15	3	19,545.38	<0.001
Agents × Dilutions	76,485.76	33	2317.76	<0.001
Error	54,169.01	240	225.705	<0.001
Total	1,798,153.98	288		

[a] *Two-way ANOVA revealed significant interactions (p<.001) among the products and dilutions.*

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Table 3: *Cytotoxicity of Products to Gingival Fibroblasts Within Same Dilutions*[a]

Dentin Desensitizing Products	50% Dilutions	20% Dilutions	10% Dilutions	1% Dilutions
1. Smart Protect	92 (2) [A]	85 (1) [AFG]	80 (2) [AC]	49 (2) [AC]
2. Systemp Desensitizer	95 (1) [A]	94 (0) [AFG]	94 (1) [AE]	92 (1) [B]
3. Seal & Protect	93 (5) [A]	77 (3) [G]	73 (3) [BC]	64 (12) [CD]
4. Aqua-Prep F	97 (0) [A]	97 (0) [AF]	97 (1) [DE]	94 (1) [B]
5. Isodan	98 (2) [A]	98 (1) [AF]	97 (1) [DE]	95 (0) [B]
6. Gluma	96 (0) [A]	94 (1) [AFG]	94 (1) [AE]	92 (0) [B]
7. BisBlock	97 (0) [A]	97 (1) [AF]	91 (13) [AE]	55 (29) [AD]
8. UltraEZ	98 (0) [A]	55 (24) [B]	14 (22) [FM]	3 (36) [E]
9. D/Sense Crystal	97 (1) [A]	97 (1) [F]	97 (1) [GE]	63 (21) [AD]
10. Colgate Sensitive Pro-Relief	72 (29) [B]	5 (21) [C]	0 (36) [H]	0 (32) [E]
11. Topex	95 (4) [A]	13 (23) [C]	0 (27) [H]	0 (21) [E]
12. Clinpro White Varnish	72 (22) [B]	18 (8) [C]	7 (18) [HM]	5 (26) [E]

[a] *According to Bonferroni's test, in each column (each tested dilution), values with different superscript letters indicate significant differences ($p<0.05$) among different groups in the same dilutions. The results are expressed as the means of mortality percentages (S.D.). The mean difference is significant at the 0.05 level.*

The MP values (means and SDs) of different groups with different dilutions in gingival fibroblast cells are shown in Table 3.

Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish showed smaller MP values than did the other groups in all dilutions except the 50% dilution ($p<0.05$), and there were no statistically significant differences between each of these products, except in the case of the dilution of 50% ($p>0.05$). The MP values of Systemp Desensitizer, Aqua-Prep-F, Isodan, and Gluma were all greater in each dilution.

Table 4 shows the decreases in the MP values of the products with the dilutions. The MP values of Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish showed statistically significant differences at 20%, 10%, and 1% dilutions, compared to at 50% dilution. The cytotoxicity of Systemp Desensitizer, Aqua-Prep F, Isodan, and Gluma did not show any statistically significant differences among dilutions. Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish was found to be less cytotoxic to gingival fibroblasts in all dilutions, whereas Systemp Desensitizer, Aqua-Prep-F, Isodan, and Gluma were found to be more cytotoxic in all tested dilutions ($p<0.05$).

Cytotoxicity of DDPs to Pulpal Fibroblasts

The MP values (means and SDs) of different groups with different dentin disc thicknesses on pulpal fibroblasts are summarized in Table 5.

Table 4: *Cytotoxicity of Products to Gingival Fibroblasts Within Different Dilutions*[a]

Dentin Desensitizing Products	50% Dilutions	20% Dilutions	10% Dilutions	1% Dilutions
1. Smart Protect	92 (2) [A]	85 (1) [A]	80 (2) [A]	49 (2) [B]
2. Systemp Desensitizer	95 (1)	94 (0)	9 (1)	92 (1)
3. Seal & Protect	93 (5) [A]	77 (3) [AC]	73 (3) [BC]	64 (12) [BC]
4. Aqua-Prep F	97 (0)	97 (0)	97 (1)	94 (1)
5. Isodan	98 (2)	98 (1)	97 (1)	95 (0)
6. Gluma	96 (0)	94 (1)	94 (1)	92 (0)
7. BisBlock	97 (0) [A]	97 (1) [A]	91 (13) [A]	55 (29) [B]
8. UltraEZ	98 (0) [A]	55 (24) [B]	14 (22) [C]	3 (36) [C]
9. D/Sense Crystal	97 (1) [A]	97 (1) [A]	97 (1) [A]	63 (21) [B]
10. Colgate Sensitive Pro-Relief	72 (29) [A]	5 (21) [B]	0 (36) [B]	0 (32) [B]
11. Topex	95 (4) [A]	13 (23) [B]	0 (27) [B]	0 (21) [B]
12. Clinpro White Varnish	72 (22) [A]	18 (8) [B]	7 (18) [B]	5 (26) [B]

[A] *According to Bonferroni's test, in each row (each tested product), values with different superscript letters indicate significant differences ($p<0.05$) among different dilutions in the same groups. The groups that do not contain any letters indicate no significant differences ($p>0.05$) between different dilutions. The results are expressed as the means of mortality percentages (S.D.). The mean difference is significant at the 0.05 level.*

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Table 5: *Cytotoxicity of Products to Pulpal Fibroblasts*[a]

Products	0.5 mm	1 mm
1. Smart Protect	98 (2) ^{A,1}	97 (1) ^{A,1}
2. Systemp Desensitizer	98 (2) ^{A,1}	97 (0) ^{A,1}
3. Seal & Protect	58 (11) ^{BD,1}	50 (11) ^{B,1}
4. Aqua-Prep F	94 (1) ^{A,1}	92 (6) ^{A,1}
5. Isodan	98 (0) ^{A,1}	98 (0) ^{A,1}
6. Gluma	97 (13) ^{A,1}	82 (0) ^{A,1}
7. BisBlock	49 (1) ^{BC,1}	30 (1) ^{D,1}
8. UltraEZ	39 (5) ^{C,1}	30 (13) ^{D,1}
9. D/Sense Crystal	70 (21) ^{D,1}	68 (7) ^{C,1}
10. Colgate Sensitive Pro-Relief	26 (21) ^{E,1}	15 (12) ^{E,1}
11. Topex	29 (13) ^{E,1}	12 (13) ^{E,2}
12. Clinpro White Varnish	30 (10) ^{E,1}	11 (8) ^{E,2}

[a] *According to Fisher's least significant difference (LSD) test, in each column (each tested dentin thickness), values with different superscript letters indicate significant differences ($p<0.05$) among different groups with the same thickness. The results are expressed as the means of mortality percentages (S.D.). In each row (each tested agent), values with different numbers indicate significant differences ($p<0.05$) among different thicknesses in the same tested products. The results are expressed as the means of mortality percentages (S.D.). The mean difference is significant at the 0.05 level.*

According to two-way ANOVA and the LSD tests, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were found to be less cytotoxic than the other groups at both thicknesses ($p<0.05$), and no statistically significant differences were detected among these three groups ($p>0.05$). In contrast, Smart Protect, Systemp Desensitizer, Aqua-Prep-F, Isodan, and Gluma resulted in the highest mortality values ($\geq$82%) for pulpal fibroblasts exposed to these products through 0.5-mm and 1-mm dentin discs.

DISCUSSION

Many products with different components and mechanisms of action are available on the dental market for the treatment of DH. Considering the way in which they are applied to overcome sensitization, not only the gingival tissues but also the dental pulp, via the dentinal tubules, are almost exposed to DDPs. It is known that DDPs can contain toxic components, such as glutaraldehyde, HEMA, triclosan, methacrylate resins, and sodium fluoride.[24,26,40] Therefore, the cytotoxicity of DDPs to delicate tissues is a significant issue, and their biocompatibility has been the subject of *in vitro* cytotoxicity studies.[33,36,41-43]

For this purpose, primary human periodontal ligament and pulp fibroblast cells, gingival fibroblasts, and odontoblast-like cells, as well as permanent cell lines, such as 3T3 and L-929, and primary and immortalized bovine dental papilla–derived cell lines have been used.[23,27,32-36]

It has been reported that primary cell lines are more suitable for *in vivo* conditions as a result of their specific metabolic activities.[44] The International Organization for Standardization, which provides guidance for the evaluation of the *in vitro* cytotoxicity of dental materials, reported that primary cell lines obtained from live cells can be used on occasions in which specific sensitivity should be determined.[45]

The nature of the testing methods has varied in that cytotoxicity is assessed either by exposing cells directly or indirectly to the test materials.[46,47] In the present study, the cytotoxic effects of DDPs were evaluated on primary gingival fibroblasts by direct contact testing because the gingiva was exposed to these products during improper application of the products.

In contrast, cytotoxicity to primary pulp fibroblasts was tested by the indirect contact method using dentin discs because dentin, as a result of its tubules, can act as a channel for the components released from a variety of restorative dental materials to reach the pulp tissue.[48] For this reason, dentin discs with different thicknesses were interpositioned between pulpal fibroblast cells and products to simulate clinical conditions. It was reported that 0.5 mm in thickness was optimal for evaluating a range of cytotoxic concentrations,[43] and a dentin barrier that was greater than 0.5 mm in thickness could dramatically reduce dentin permeability.[49] Therefore, in this study dentin discs with thicknesses of 0.5 mm and 1 mm were used.

In this study, the cytotoxicity of DDPs on gingival and pulpal fibroblasts was evaluated by applying the MTT assay. The MTT assay is a commonly used cell viability assay in evaluating the cytotoxicity of dental materials because of its ease of use and high sensitivity.[23,31,45,50,51] The MP value was determined using OD values as a result of the MTT assay.[39]

According to the results of this study, when different dilutions of components released from DDPs were tested on gingival fibroblast cells, Colgate Sensitive Pro-Relief, Clinpro White Varnish, and Topex were less cytotoxic than the other DDPs. In contrast, Systemp Desensitizer, Aqua-Prep F, Isodan, and Gluma were found to be more cytotoxic to both gingival and pulpal fibroblast cells.

It has been reported[24-26] that the cytotoxicity of a material is related to its composition. An example of

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this relationship is HEMA, which is a low–molecular weight hydrophilic monomer that can be released from resin-based materials and penetrate the dentin tissue easily, affecting odontoblast vitality and physiological activity.[52-54] The low dose and long-term use of HEMA inhibits its inflammatory response ability. However, the cytotoxicity of HEMA was reported[55] to depend on time and concentration. Aqua-Prep F, Isodan, and Gluma are known to contain HEMA at rates of 10%-30%, 0%-40%, and 35%, respectively. Thus, the toxicity of Aqua-Prep F, Isodan, and Gluma detected in the present study might be related to their HEMA content.

Furthermore, Aqua-Prep F and Isodan contain sodium fluoride as well as HEMA. Sodium fluoride has been shown to be cytotoxic in low -pH environments.[56,57] Isodan has a low pH (2.2). In high concentrations, sodium fluoride prevented the proliferation of human epithelial tissue cells (HaCaT cells), and the cell reaction to sodium fluoride depends on the cell type.[58,59] Just as the cytotoxicity of Aqua-Prep F and Isodan might be related to HEMA and sodium fluoride, the combined effect of these two materials can also affect cytotoxicity. Topex and Clinpro White Varnish contain higher proportions of sodium fluoride than do Aqua-Prep F and Isodan. However, Topex and Clinpro White Varnish were cytotoxic only in high concentrations (50%), and these groups were not found to be cytotoxic in further dilutions. Therefore, the higher cytotoxicity of Aqua-Prep F and Isodan at all concentrations might be more closely related to HEMA than to sodium fluoride.

In contrast, Gluma, Smart Protect, and Systemp Desensitizer contain glutaraldehyde, which is used as a disinfectant and sterilizing agent against bacteria and viruses (2% solution). Glutaraldehyde causes coagulation of the plasma proteins in tubule fluid, resulting in a reduction in dentinal permeability[60]; thus, glutaraldehyde is added to DDPs and to dentin bonding agents for its desensitizing effects.[16] Therefore, the cytotoxicity of Gluma, Smart Protect, and Systemp Desensitizer found in this study might be related to their glutaraldehyde content, which is toxic to cells.

Similarly, the cytotoxicity of Systemp Desensitizer might be due to its ingredients, which include methacrylated monomers and glutaraldehyde, because methacrylated monomers may cause membrane lipid dissolution on cell membranes.[31,50]

Smart Protect contains triclosan, in addition to glutaraldehyde. Triclosan has been added to the active ingredients of some mouth rinses and dentifrices to prevent and treat gingivitis and plaque.[61] It was reported that triclosan induced cell death by apoptosis and by slowing the growth kinetics of the Smulow-Glickman (S-G) human gingival epithelial cell line,[62] and it was also reported that triclosan inhibited the adipocyte differentiation of human mesenchymal stem cells at high concentrations.[63] The cytotoxicity of Smart Protect could be attributed to glutaraldehyde and triclosan or to the combined effect of these and other ingredients.

Seal & Protect contains triclosan, like Smart Protect, as well as toxic components, such as di- and trimethacrylate resins and pentaacryloyldipentaerythrytol, phosphoric acid, functionalized amorphous silica, photoinitiators, and butylated hydroxytoluene. The ingredients of Seal & Protect have different rates of toxicity.[27,36,64,65] The cytotoxicity of Seal & Protect might be related to its ingredients, or it could be due to the interactions of all its contents.

Furthermore, Seal & Protect is clinically polymerized with blue light. Polymerization of methacrylate resins can reduce the cytotoxicity of resin monomers.[66] However, the application of blue light causes the formation of free radicals in cells and negatively affects cell division, causing oxidative stress in different cell series, as well as DNA modifications.[67-69] In this study, with the aim of eliminating the negative effects of blue light on polymerization, Seal & Protect was not polymerized with light. Therefore, the possible effects of blue light on the cytotoxicity of Seal & Protect could not be determined under these testing conditions.

BisBlock contains less than 5% oxalic acid, and D/Sense Crystal contains 2.5% nitric acid and 2.5% potassium dioxalate. Oxalic acid forms soluble salts with sodium, potassium, and ammonium ions and insoluble salts with calcium, magnesium, and iron ions.[70,71] In neutral and alkaline environments, calcium and oxalate can bind together, forming different-shaped crystals of calcium oxalate.[70] Topical usage of potassium oxalate results in the deposition of calcium oxalate crystals on the dentin surface. Oxalate reacts with the calcium compounds in dentin and promotes deposition of potassium oxalate. The precipitation of oxalic acid, as calcium oxalate monohydrate, was reported to be an intracellular toxin to normal human proximal tubule cells by inhibition of mitochondrial respiratory function in proximal tubular cells,[72] and it was also reported that the cytotoxicity of oxalate might be due to plasma membrane damage and organelle injury.[73]

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The cytotoxicity of D/Sense Crystal could depend on potassium dioxalate, and the cytotoxicity of BisBlock might result from its oxalic acid content. The higher MP value of D/Sense Crystal, compared to that of BisBlock, on pulpal fibroblasts could be related to its ingredients and to the interactions of these ingredients with each other.

In addition, the manufacturers recommend etching the exposed tooth surface for 15 seconds with 32% phosphoric acid before applying BisBlock in clinical practice. In this study, phosphoric acid was not applied because of its probable toxic effects on fibroblast cells in addition to BisBlock.[65] So the application of 32% phosphoric acid could cause the cytotoxic effects of BisBlock in cases of its misapplication to gingival tissues.

UltraEZ contains 3% potassium nitrate and 0.25% sodium fluoride. An experimental mouth rinse, which contains 7% potassium nitrate and 0.025% sodium fluoride, similar to UltraEZ, was reported to be noncytotoxic to human esophageal squamous cell carcinoma (SCC) cells .[74] In the current study, UltraEZ was cytotoxic at 50% and 20% dilutions, and its cytotoxicity decreased to a considerable extent as the concentration decreased.

Colgate Sensitive Pro-Relief was the most recently introduced of the desensitizing products tested in this study. It contains L-arginine, calcium carbonate, glycerin, water, bicarbonate, hydrated silicate, and sodium saccharine. The essential component of the product is arginine, an amino acid, which is positively charged at a physical pH of 5-7.5 and contains a bicarbonate buffer and calcium carbonate. Colgate Sensitive Pro-Relief only showed cytotoxic effects in 50% dilutions, such as Topex and Clinpro White Varnish. It was reported that Colgate Sensitive Pro-Relief occluded exposed dentinal tubules and tubule plugs containing calcium and phosphate, and these products reached a depth of 2 μm into the dentinal tubules.[20,75]

When cytotoxicity was evaluated using different-thickness dentin discs, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were not cytotoxic. In addition, BisBlock, UltraEZ, Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were found to be less cytotoxic to pulp fibroblasts, compared to the other DDPs. This finding can be explained by the dentinal tubule-occlusive effects of these products, in addition to their compositions.

The cytotoxicity of the DDPs in the current study might lack any clinical significance because of the buffering capacity of dentin and saliva.[76,77] Saliva can dilute the DDPs to some extent, and it can wash the tooth surfaces and gingiva. As the gingival surface is protected by highly keratinized epithelia cells, it is possible that cytotoxicity of the DDPs on the gingival surface might decrease clinically.

In addition, in this *in vitro* study, pulpal clearance and pulpal pressure were not simulated. In addition, the possible consequences of adding a large percentage product solution to the culture media (eg, change of pH and/or osmolality) were not determined in this study. Whether or not this practice can cause significant adverse effects on fibroblast cells could be investigated in future studies. Another important point is that the varied application methods might affect the cytotoxicity of different desensitizers as well (eg, blue light application after applying desensitizers or etching the exposed tooth surface with phosphoric acid before applying desensitizers in clinical practice). Furthermore, the DDPs included more than one cytotoxic component. The cytotoxic effects of the interactions between the components of these products should be investigated under *in vitro* and *in vivo* conditions.

CONCLUSION

Colgate Sensitive Pro-Relief, Topex, and Clinpro White Varnish were found to be less cytotoxic than the other DDPs used in this study. Further studies are necessary to simulate salivary components and their interactions with these products, as well as the effects of pulpal pressure and clearance on the cytotoxicity of DDPs.

Human Subjects Statement

This study was conducted in accordance with all the provisions of the local human subject oversight committee guidelines and policies. The approval code for this study is 2010/82. This study was conducted at Karadeniz Technical University.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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