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The Effects of Various Dental Restorative Materials on Neuroblastoma Cells

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Abstract

Introduction: Our aim was to investigate the effects of restorative materials such as composite, compomer and glass ionomer, which are frequently used in dentistry, on SH-SY5Y neuroblastoma cells by evaluating cell viability, rate of dead/live cells, oxidative stress parameters, and pro-inflammatory cytokines. Materials and Methods: Equa Forte, Dyract AP, Tokuyama Estelite P Quick, Omnichroma, Filtek Z250, SureFil SDR flow restorative materials were used in our study. SH-SY5Y neuroblastoma cells were cultured with restorative materials. Immunofluorescence labelling was performed on the experimental groups with FDA and PI dyes. Then, ELISA technique was used to detect the levels of TNF-alpha, IL-1-beta, IL-6, SOD, LPO and CAT. One-way ANOVA analysis was used for statistical analysis ($p<0.05$). Results: In the light of the obtained data, it was observed that the dental filling materials were effective in increasing the levels/activities of all parameters including SOD, LPO, CAT, TNF-alpha, IL-1-beta, and IL-6. Immunofluorescence staining micrographs confirmed the viability analysis. Conclusion: Our study shows that biocompatibility cannot be explained by looking at a single cause. Biocompatibility varies with material content, residual monomer amount and solubility. Although all experimental groups have cytotoxic effects, the least effect is seen in the glass ionomer (Equa Forte) group.

Keywords: Capsella bursa-pastoris root, Anticandidal, Antibiofilm, Extraction, *Candida* species.

Introduction

Restorative dentistry involves restoring and maintaining oral health with appropriate restorative treatment to preserve and restore pulp function. Most of these products used in dentistry are triethylene glycol dimethacrylate (TEGDMA), ethylene glycol dimethacrylate (EGDMA), or diethylene glycol dimethacrylate (DEGDMA), B bisphenol A- glycidyl in combination with comonomers methacrylate (Bis-GMA) and urethane various methacrylates such as dimethacrylate (UDMA) (1). For this purpose, restorative materials with different properties are

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produced and there is a strong correlation between the biological compatibility with other oral tissues for the clinical success of these restorative materials (2). Dental filling materials remain in contact with the tissues in their microenvironment for a long time after use. And these materials must go through the certification process before being made available (3). The certification process also includes cytotoxicity tests for clinical risk assessments. For this reason, cytotoxicity test methods are included in international standards (4). Cytotoxicity can be evaluated by methods such as the determination of viable cell proliferation rates and enzyme activities (5).

The cytotoxicity of dental composites is tightly linked to residual monomers released due to degradation processes or incomplete polymerization of materials (6). These residual monomers, dentin dissolves in the tubules with agents such as oral fluids or other external fluids, affecting the soft tissues of the oral cavity and the dentin-pulp complex (7). In other words, as a result of the restoration, the possibility of microleakage causes negative effects on periodontal tissues (8). In light of this information, with both in vitro and in vivo studies, it has been determined that monomers have aimed to reveal their cytotoxic, genotoxic, cellular reactive oxygen production, and general health effects (9). Moreover, studies have shown that amalgam, resin composite and glass ionomers which are used as dental filling materials affect the brain by passing into the blood; however, this study is tissue-based only. However, there is no study on whether the filling materials we used in the study pass the blood-brain barrier.

In current study, our aim was to investigate the effects of restorative materials such as composite, compomer and glass ionomer, which are frequently used in dentistry, on SH-SY5Y neuroblastoma cells by evaluating the oxidative stress parameters, and pro-inflammatory cytokines.

Materials and Methods

Extract Preparation: This study was organized under the International Organization for Standardization (ISO) No: 10993-5:20097 and 10993-12:2021 standards. The experimental groups and the detailed information about dental filling materials and applicated doses are in Table 1.

Table 1. The experimental groups of our study

Group Codes	Doses	Materials	Composition	Manufacturer
Cnt	None	Control Group		
A	100 µl	Equıa Forte	Fluoroaluminosilicate glass, polybasic carboxylic acid, polyacrylic acid, water, iron oxide	GC Corporation, Tokyo, Japan
B	100 µl	Dyract AP	UDMA (Urethane dimethacrylate), Iron oxide pigments. TCB Resin (Tetracarboxylic acid-hydroxyethylmethacrylate-ester), Butyl hydroxy toluene Alkanoyl-poly-methacrylate, Strontium fluoride Strontium-fluoro-silicate glass, Photo initiators.	Dentsply De Trey, Konstanz, Germany
C	100 µl	Estelite P Quick	TEGDMA, 2-Propenoic acid, 2-methyl-, (1-methylethylidene, bis[4,1-phenyleneoxy(2-hydroxy-3,1-propanediyl)] ester, titanium dioxide, 2,6-di-tert-butyl-p-cresol; p-methoxyphenol	Tokuyama Dental Corporation, Tokyo, Japan
D	100 µl	Omnichroma	UDMA/TEGDMA Monomers, spherical SiO2-ZrO2	Tokuyama Dental Corporation, Tokyo, Japan
E	100 µl	Filtek Z250	Bis-GMA (Bisphenol glycidylmethacrylate), Non-agglomerated silica nanoparticles UDMA (urethane dimethacrylate), Bis-EMA (Ethoxylated bisphenol dimethacrylate), TEGDMA (Triethlene glycol dimethacrylate	3M ESPE, St Paul, MN, USA
F	100 µl	SureFil SDR flow	Modified UDMA, Bis-EMA, TEGDMA	Dentsply DeTrey, Konstanz,Germany

Equia Forte (GC Corporation, Tokyo, Japan) was placed in a 0.5 mm thick 6x10 cm Teflon mold after mixing in an amalgamator. Polymerization and coating application were performed according to the manufacturer's data. Compomer and composite groups (Dyract AP, Estelite P Quick, Omnichroma, Filtek Z250, and SureFil SDR flow) were also placed in a 0.5 mm thick, 6x10 mm Teflon mold and polymerized following the manufacturer's recommendations. For cytotoxicity testing, samples were placed in a 50 ml extraction flask containing 60 cm2 of test substance covered with 20 ml of minimally basic medium. After 24 hours at 37 degrees, 100 µl of solution samples were taken and applied.

Cell Culture Protocol and Cell Viability Assay: The SH-SY5Y neuroblastoma cell line was purchased from ATCC. Cells were seeded using Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic and propagated under appropriate conditions (37°C and 5% CO2). After obtaining sufficient confluence, cells were removed from the flask with the help of trypsin (Gibco, Pittsburgh, USA), centrifuged and trypsin removed. Then, the cells were stained with trypan blue (Sigma-Aldrich, USA) and the total cell number was calculated by counting under an inverted microscope.

Cell Viability Detection Kit-8 (CVDK-8) was used for cell viability analysis. For this analysis, 96-well plates were seeded with 7x103 cells per well. Then, the cells reached sufficient confluence, 100 µl of the prepared extracts were given to each well and incubated for 24 hours. After then, cells were incubated with 10 µl of CVDK-8 solution for 4 hours following the manufacturer's instruction. At the end of the period, spectrophotometric measurements were perfomed by ELISA reader (Multiskan GO, Thermo Scientific) at a wavelength of 450 nm.

Immunofluorescent Staining Technique: 24-well plates were seeded with 28x103 cells in each well and were expected to be confluent. After sufficient confluence was achieved, the drug was applied at the determined doses and incubated for 24 hours. Culture media were then removed, washed twice with PBS, fixed in a 4% formaldehyde solution for 4 minutes at room temperature. After then, formaldehyde was moved away and washed twice with PBS. For permeabilization, cells were incubated with 99.9% methanol at room temperature for 20 minutes and washed twice with PBS after incubation. Dyes prepared as 5 ml fluorescent diacetate (FDA, ThermoFisher, Cat No: F1303) and 5 ml propidium iodide (PI, ThermoFisher, Cat No: P1304MP) in 100 ml PBS were added to the wells and photographed with the help of an inverted microscope (Invitrogen Evos FL).

ELISA Technique: Cells seeded in 24-well plates were incubated with drugs for 24 hours, and the medium was collected. Commercially available enzyme-linked immunosorbent assay (ELISA) kits (Table 2) were used following the manufacturer's instruction.

Table 2: Manufacturer's names and catalog numbers of the test kits used in this study

ELISA Kits	Producer	Catalog Number
Human IL-1-beta ELISA Kit	BT Laboratory	E0143Hu
Human IL-6 ELISA Kit	BT Laboratory	E0090Hu
Human TNF alpha	BT Laboratory	E0082Hu
Human SOD ELISA Kit	BT Laboratory	E4502Hu
Human CAT ELISA Kit	BT Laboratory	E3053Hu
Human LPO ELISA Kit	Elabscience	E-BC-K176-M

Statistical Analysis: The results were expressed as means ± SEM. Statistical significance was evaluated by one-way ANOVA followed by Tukey posttest for more than 2 independent numerical data. All data were analyzed using GraphPad Prism, version 5.0 for Windows (Graph Pad Software, San Diego, California, USA). The level of $p<0.05$ was considered statistically significant.

Results

Cell Viability Analysis Findings: Cell viability assay was performed with CVDK-8 to test whether dental filling materials (A, B, C, D, E, and F) had cytotoxic effects on SH-SY5Y neuroblastoma cell line or not. According to the results, it was detected that cell viability rates decreased in all groups (A, B, C, D, E, and F) compared to control at the end of 24 hours. The most decrease was observed in Group C concerning viability rates (approximately 53%). Following Group C, the survival rate decreased by 50%, 47% 45%, 44%, and 38% Group E, D, F, B, and A, respectively (Figure 1).

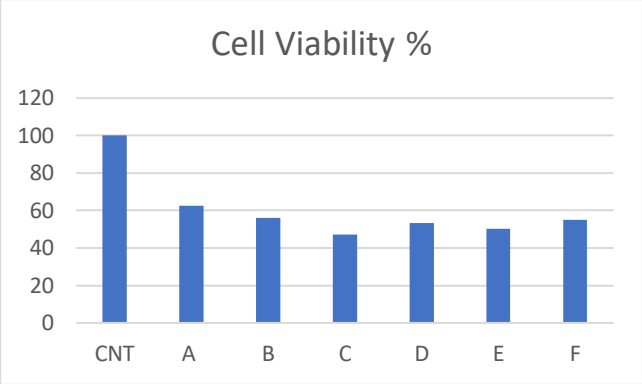

Figure 1: Cell viability test of experimental groups. The viability of the control group was accepted as 100% and the averages of the experimental groups were calculated.

Immunofluorescent Staining Results: To demonstrate the effect of dental filling materials on SH-SY5Y neuroblastoma cells, immunofluorescence labeling was performed with FDA and PI dyes. Findings were similar to cell viability results. All filling materials caused a dramatic increase in cell death. While the most dead cells were observed in Groups C and E, the least cell death was observed in Groups A and B (Figure 2).

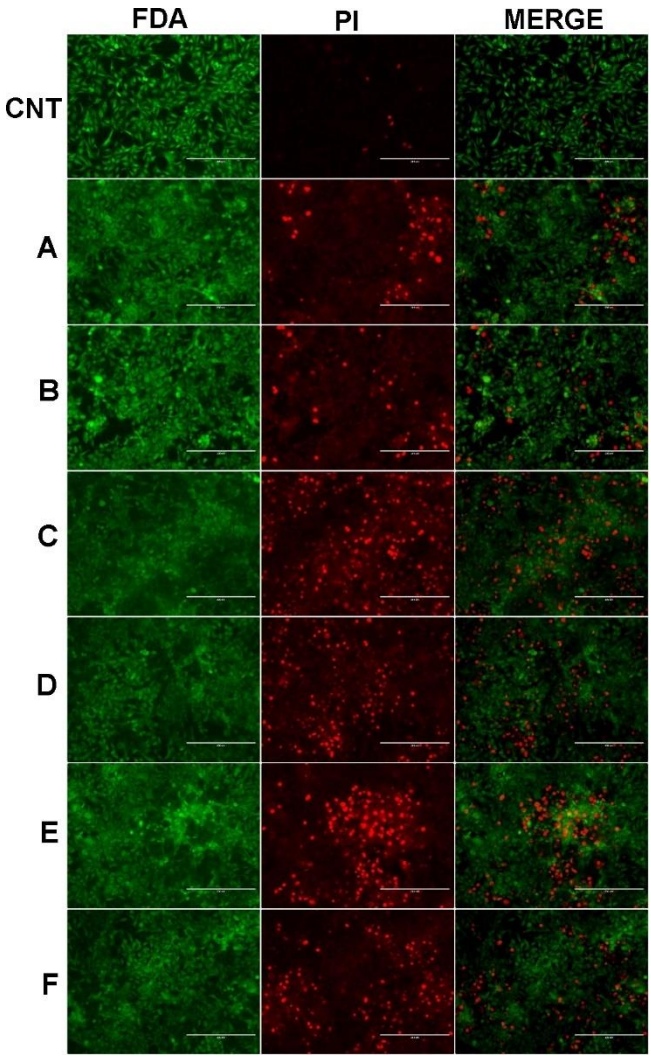

Figure 2: FDA and PI immunofluorescence labeling in experimental groups. Red cells (PI positive) indicate dead cells, and green cells (FDA positive) indicate live cells.

ELISA Results: Cell medium was collected from all experimental groups, and pro-inflammatory (TNF-Alpha, IL-1-beta, and IL-6) and oxidative stress parameters (LPO, SOD, CAT) were examined (Figure 3).

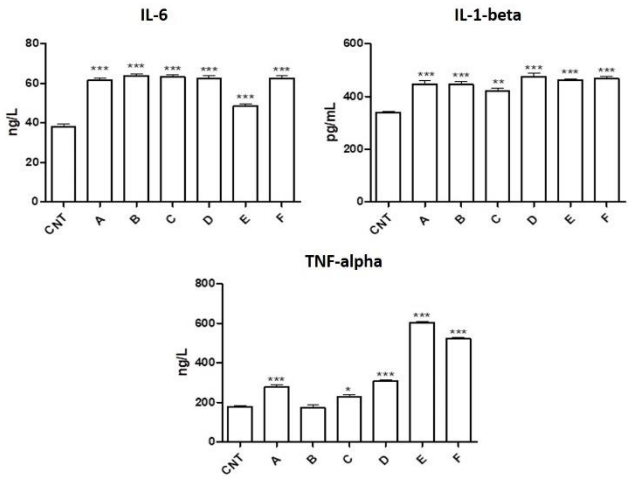

Figure 3: ELISA results of pro-inflammatory cytokines

Compared to the control group, it was determined that IL-6, IL-1-beta, and TNF-alpha levels were increased. It was observed that the highest increase in IL-6 levels was in group C, and the least increase was in group E. When IL-1-beta levels were compared, it was seen that the highest increase was in the D group and the least increase was in the C group. When the TNF-alpha levels were compared, it was observed that the highest increase was observed in the E group, while the value of the B group was very close to the control.

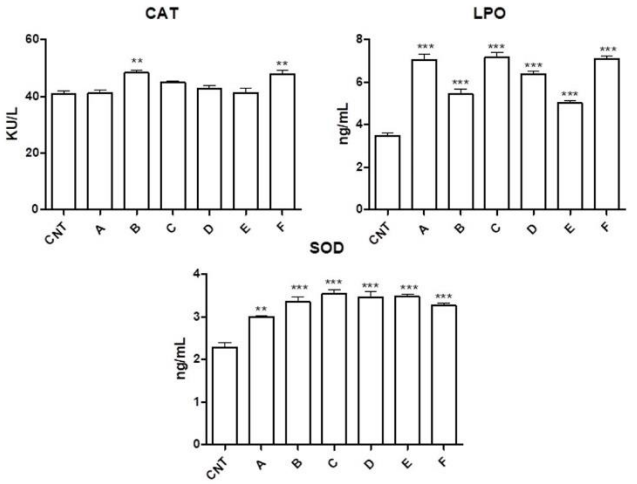

Figure 4: ELISA results of oxidative stress parameters

Compared to the control group, LPO, SOD, and CAT levels were increased. When LPO levels were compared, the greatest increase was seen in groups A, E, and D, while groups B and E were higher than the control but less than groups A, E, and D. When SOD levels were compared, the highest increase was observed in group C, and the least increase was observed in group A. When CAT levels were compared, the highest increase was seen in groups B and F, while the ratio of groups E, A, and D was close to control (Figure 4).

Discussion

Restoration of a deep cavity with a single layer of direct restorative material (more than 2.5 mm thick) has been reported to cause a significant reduction in material properties that may affect its lifespan (10). It was evaluated the effect of filler type, shade, exposure time, and cured radiant exposure on the degree of convergence of composite resins (11). Same researchers tested samples of composite resins cured through different thicknesses of already polymerized composite resins for different exposure times (20, 40, 60, and 80 s) with an irradiation level of 800 mw/cm2. They finalized that the most important factor in the degree of convergence of composite resins is thickness. A degree of convergence thickness of more than 2 mm causes significant degree of convergence lowering. Also, Rueggeberg et al. concluded that to provide an adequately polymerized composite resin it must have a 2mm rise cured for 60 seconds with irradiation levels of at least 400 mw/cm2 (12).

Flury et al. (13) tested the effect of different composite resin thicknesses on the Vickers microhardness of different composite resin types. They reported a reduction in Vickers microhardness values of conventional composite resins at a depth of more than 2 mm. In addition, Price et al. compared the effect of resin thickness on microhardness when cured with PAC or QTH lcus. The researchers reported that the thickness of composite resin has a significant effect on the hardness of composite resin. When using one of the tested lcus, only 2 mm thick specimens showed equivalent hardness values of the upper and lower surfaces at all time intervals. This indicates adequate

degree of convergence of the lower surface of the restoration (14).

Increasing the thickness of the composite resin restoration results in more curing light absorption and scattering and less light penetration within the layers of the cured material. Therefore, the overall curing light energy decreases with the increase of composite resin thickness. Accordingly, the degree of convergence value of the material decreases (15, 16). Therefore, for cavity preparation exceeding 2 mm, the incremental layering technique is considered standard for composite resin placement. It is reported that this technique allows the composite resin layers to be exposed to sufficient light and lower polymerization shrinkage (17, 18). In our study, all materials were polymerized and surface treated in accordance with the manufacturer's instructions.

Composite resin materials can be packaged according to their consistency and classified as flowable composite resins (19). Flowable composite resin has low viscosity due to the low filler level or the addition of modifiers such as surfactants (20). It is used to increase the adaptability of composite resin restoration to cavity walls and floors with very fine-tip syringes. While trying to restore the function and aesthetics of the tooth, the packable composite resin cannot be inserted into the cavity with a syringe due to its high viscosity (15, 19).

Monomer and filler type, filler content, and filler and polymer matrix refractive index all have an impact on the ability to transmit light through the composite resin layers (21). Therefore, it is reported that different composite resin compositions, filler size, weight, volume, and filler-matrix ratio have a significant effect on the degree of convergence and microhardness of composite resins (22, 23) The decrease in viability was most observed in group C (approximately 53%). When the TNF-alpha levels were compared, it was seen that the highest increase was in the E group, and the value of the B group was very close to the control. When CAT levels were compared, the highest increase was seen in groups B and F, while the ratio of groups E, A, and D was close to control.

As a summary, dental filling materials induce inflammatory response and oxidative stress in neuroblastoma cells. Current literature also support that root-end filling materials effect and increase the expression of inflammatory cytokines (24, 25). Two studies also reported that dental filling materials such as dental amalgam, glass ionomer and resin composite had a critical effect on the activities of some oxidative stress process enzymes including LPO, SOD, CAT, and GSH (26, 27).

The results of our study should be supported by performing both in vivo and in vitro tests for future studies. It has been shown that all restorative materials with different contents and chemical structures used in our study have cytotoxic effects on neuroblastoma cells. However, their potential to cause pulpal problems should be investigated by performing long-term tests.

Conclusions

Our study shows that biocompatibility cannot be explained by looking at a single reason. Biocompatibility varies according to the content of the material, the amount of residual monomer, and its solubility. Although all the experimental groups have cytotoxic effects, the least effect is seen in the glass ionomer (Equa Forte) group. More detailed studies researching the reasons of inflammatory response and oxidative stress should be performed for the biocompatibility of filling materials.

Declaration of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

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Conceptualization: SAK, MD; Formal Analysis: PB, MD, ED, SAK; Investigation: PB, MD; Methodology:

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