IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 23, Issue 4 Ser. 4 (April. 2024), PP 44-51
www.iosrjournals.org

Evaluation Of Cytotoxicity Of Dental Materials Using The Mtt-Tetrazolium Method

Hakan Kamalak[1]
(Department Of Restorative Dentistry, Kahramanmaras Sutcu Imam University, Turkey)

Abstract
Background*: Previous studies have shown that the cytotoxicity of dental materials are examined by direct contact and dentin barrier tests.*
Materials and Methods*: In this experimental study, the cytotoxic effects of thirteen restorative materials on direct contact of three different cell lines were assessed by MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide) assay One hundred fifty six disk-shaped specimens from each material were prepared (according to the manufacturer's information) in standard mold (9 mm diameter and 2 mm thick). The samples were incubated for 24 and 72 h in basal medium supporting the growth of many different mammalian cells and following each incubation, cytotoxicity of the extracts to cultured gingival fibroblast, mesenchymal and neuron cells were measured by MTT assay.*
Results*: Data were statistically analyzed by one way and two-way analysis of variance (ANOVA), at a significance level of p<0.05 and p<0.001 levels. Group ISP, XTB, TNC, TEC, SK, GCE, BEG, EQF, FBP showed low toxic properties, while ISM, F25, GCP and F95 demonstrated low cell survival rates and high toxic properties at incubation periods.*
Conclusion: *The results suggest that MTT analyzes were clearly sufficient to appraise the cytotoxicity of dental materials and to select biocompatible materials.*
Key Words: *MTT-tetrazolium, Fibroblasts, Neuron cells, Mesenchymal , Cytotoxicity*

Date of Submission: 08-04-2024 Date of Acceptance: 18-04-2024

I. Introduction

Biocompatibility is the ability of a material to fulfill its current function in contact with living tissues without causing locally or systemically toxic, mutagenic, allergic, carcinogenic effects and without adversely affecting health. Biocompatible materials designed to interact with biological systems called biomaterials [1-3].

When non-biocompatible materials interact with living systems, tissue reaction and the material is considered toxic. Cytotoxicity, which is the determining factor of biocompatibility, is defined as damage to cell function and structure as a result of disruption of the synthesis chain of macromolecules [2, 4-7].

Cell culture tests are frequently used to evaluate the biocompatibility of materials [8-10]. The procedures under standard conditions in cell culture tests can be repeated, and the measurements can be made by direct observation on cells. Furthermore, the experimental steps can be controlled, easily replicated, and unaffected by individual factors[11-14].The correct evaluation of cytotoxicity requires certain and correct in vitro laboratory tests. So, it is significant to distinguish a suitable appropriate analysis. The MTT assay has been properly accustomed to identify cytotoxicity, as it is appropriately cheap, as well as rapid and basic[15, 16].

Wang et al[17]. expressed the MTT test and live cell calculation as follows in their study, "*the MTT Assay is a susceptible and credible colorimetric testing that evaluates viability, proliferation and activation of cells. The test is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product that is insoluble in water. Viable cells are able to reduce the yellow MTT under tetrazolium ring cleavage to a water-insoluble purple-blue formation which precipitates in the cellular cytosol and can be dissolved after cell lysis, whereas cells being dead following a toxic damage, cannot transform MTT.* This formation production is proportionate to the viable cell number and inversely proportional to the degree of Cytotoxicity" [15, 17-20].

As can be understood from the above information, the cytotoxic effect of longer-term (up to 72h) resin-based composite materials and glass ionomer cements was evaluated in this study.

II. Material And Methods

Thirteen restorative materials were elected for this study: Tetric EvoCeram(TEC), Tetric- N Ceram (TNC), X-tra base (XTB), GC Essentia (GCE), Brilliant EverGlow® (BEG), Synergy (SK), Filtek Bulk Fill Posterior (FBP), GCP Glass Fill (GCP), IonoStar Plus (ISP), IonoStar Molar (ISM), GC Fuji II (F25), GC *Fuji IX* (F9S) and Equia Forte (EQF). As a result of the power analysis, the number of samples was determined as 12 for each material (n=12). The list of samples is listed in Table no 1.

Sample Preparation

Depending on the type of materials, samples were prepared using standard molds with a diameter of 9 mm and a height of 2 mm. Condensation of non-polymerized materials placed in standard molds was achieved with diestema tape and cement glass. Additionally, transparent tape was applied to the surface of the samples to reduce oxygen inhibition. The samples were polymerized with an LED light source (Elipar Freelight II, 3M-ESPE, USA) at 1000 mW/cm2 for 10 seconds. Samples produced as disks (n = 12) were sterilized with ultraviolet light for 24 hours before MTT testing. After each prepared sample was polymerized, it was immersed in the cell culture medium.

Cell Culture

Human gingival fibroblast, neuron and mesenchymal cell lines were obtained from the ATCC (American Type Culture Collection) global biological resource center. Cells were inoculated with the Dulbecco's modified Eagle's medium integrated with 10% FBS (Fetal Bovine Serum), 1% antibiotic (containing penicillin-streptomycin-amphotericin B) into culture dishes (flask) with a surface area of 25 cm^2. All cells were incubated in standard conditions [37°C in a humidified atmosphere of 5% (v/v) CO2].

Preparation of Cell Production Containers

Cells with active logarithmic growth covering 90-95% of the surface were separated from the flask base similar to the passage process and cell suspension was prepared with fresh nutrient medium. The prepared 200 mL cell suspension was evenly distributed in all compartments of the well plates where the materials were to be placed. After the addition of fresh medium, the samples were allowed to incubate again. After seven days of incubation at 5% CO2 and 37°C humid temperature, whether the cells completely filled the eyes of the plates and the spindle characteristic structure of the fibroblasts were examined by microscope. Composite specimens sterilized under ultraviolet (UV) light for 2 hours; One by one, with the help of a sterile press, they were transported to the cell production containers in a sterile cabinet in direct contact with the cells. Analyzes were performed after 72 hours of incubation in a 37°C, 5% CO2 incubator.

Cytotoxicity Test

Cells (1×10^4) were sprinkled in each well of a 96-well plate and incubated for 24 h and 72 h at 37 °C. Cultures were then liabled to 100 µL of the extract medium. Fresh cell medium was used as control. After 24 h and 72 h, cell viability was examined with MTT assay. The MTT solution—(3-{4,5-dimethylthiazol-2-yl}-2,5-diphenyl tetrazolium bromide) was added to each well of the culture and the cells were incubated for 4 h at 37 °C. After four hours of MTT incubation, blue formazan crystals (visible intracellularly in the optical microscope) were dissolved by the addition of dimethyl sulfoxide (sigma, USA). Living cells with active metabolism converted MTT to a purple colored formazan product with an absorbance close to 550 nm. The absorbance value was read with a spectrophotometer device (µQuant, BadFriedrichshall, Biotek) and viable cell count was obtained. Determining viable cell levels

The following formula was used for;

Viability rate (%) = (Sample absorbance value) / (Control group absorbance value)×100.

Statistical Analysis

Power analysis was preferred to determine the number of samples (n=12). IBM SPSS Statistics 22 (IBM SPSS, Türkiye) program was used to evaluate the results obtained from the research. One-way and two-way analysis of variance (ANOVA) methods were used to interpret the data. Statistical significance was evaluated at $p<0.05$ and $p<0.001$ levels.

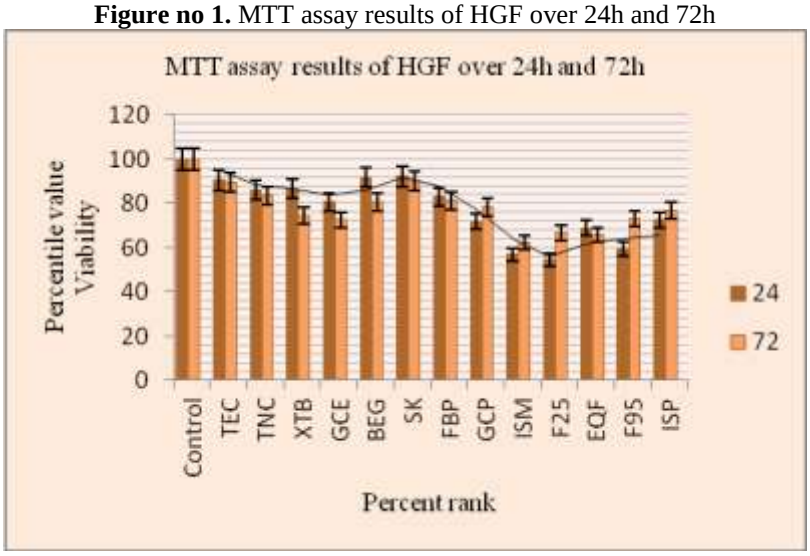
III. Result

For the MTT assay, the viability of the control cultures (cells treated with growth media only) was adjust at 100 %. The viability of HGF, mesenchymal and neuron cells at 24 and 72 hours after treatment are shown in *Figure no 1, in Figure no 2, and Figure no 3*. The data obtained from the MTT assay are shown in *Table no 2, Table no 3 and Table no 4*. When the viability rate of three different cells was evaluated after 24

and 72 hours, data showed that the viability of cells decreased as compared to the control (100% viability) at 24 and 72 hours.

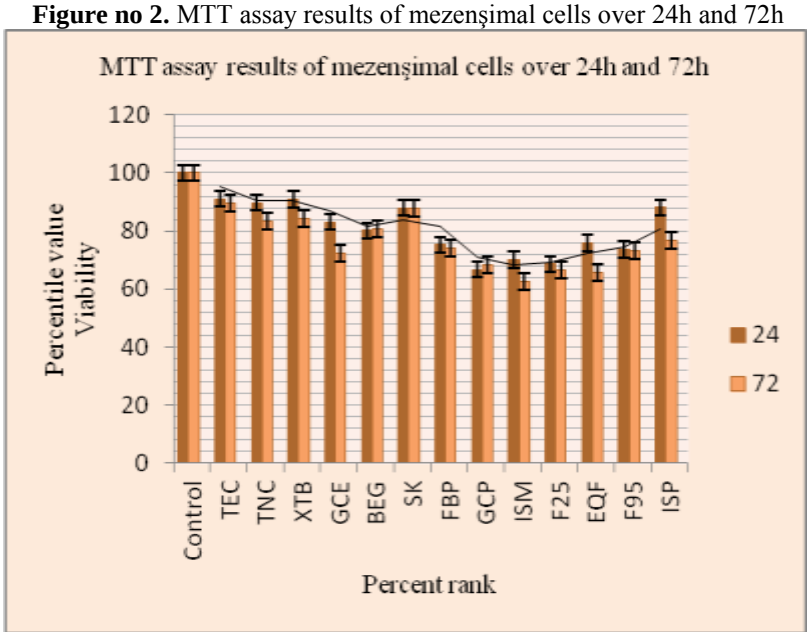
SK, BEG and TEG ejected for 24h and 72h showed almost no cytotoxic effect, while F25, ISM and F95 showed partial cytotoxic effect after 72 h. Also, when the cytotoxicity values obtained after 24 hours and 72 hours were analyzed and compared with the control group (culture medium only), no statistically significant difference was found ($p<0.05$).

Figure no 1. MTT assay results of HGF over 24h and 72h



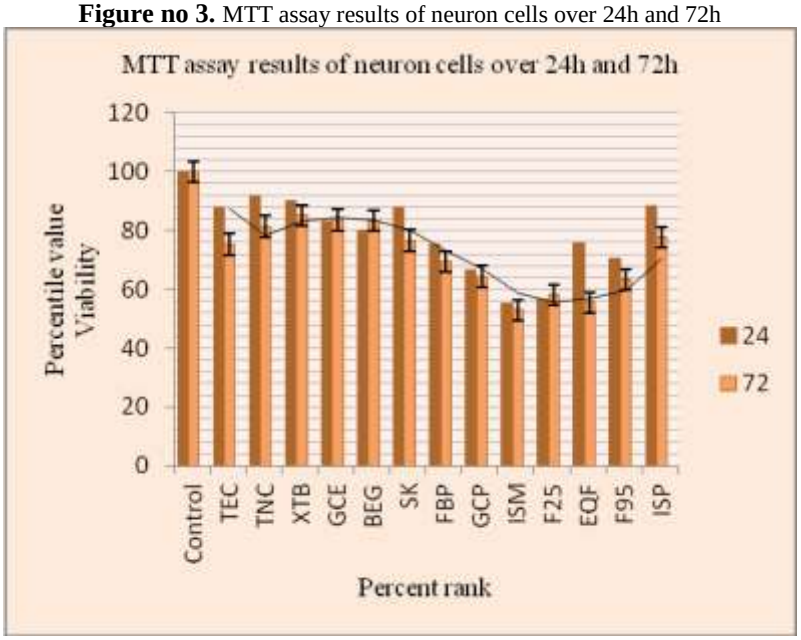
The effects of viability of HGF cells compared to controls at 24 and 72 hours after treatment. The results were calculated as the viability (percent control) compared to the negative control (100% viability) and presented as mean ± SD. n=12, * p <0,05, ** p< 0,001

Figure no 2. MTT assay results of mezenşimal cells over 24h and 72h



The effects of viability of mezenşimal cells compared to controls at 24 and 72 hours after treatment. The results were calculated as the viability (percent control) compared to the negative control (100% viability) and presented as mean ± SD. n=12, * p <0,05, ** p< 0,001

Figure no 3. MTT assay results of neuron cells over 24h and 72h



The effects of viability of neuron cells compared to controls at 24 and 72 hours after treatment. The results were calculated as the viability (percent control) compared to the negative control (100% viability) and presented as mean ± SD. n=12, * p <0,05, ** p< 0,001

Table no 1: Materials used in this study

Materials	Manufacturer	Type	Organic Matrix	Filler % (Wt)	Code
Tetric Evoceram®	Ivoclar Vivadent AG, Schaan, Liechtenstein	Bulk Fill	Dimethacrylate Co-Monomers Bis-GMA, Bis-EMA And UDMA	80	TEC
Tetric® N-Ceram	Ivoclar Vivadent AG, Schaan, Liechtenstein	Bulk Fill	Bis-GMA, Bis-EMA And Urethane Dimethacrylate Monomer (UDMA),	75–77	TNC
X-Tra Base	Voco (Cuxhaven, Germany)	Bulk-Fill Flowable Composite	Bis-GMA, UDMA, TEGDMA	86	XTB
G-Aenial	GC Corporation, Tokyo, Japan	Microfilled Hybrid Composite	Urethane Dimethacrylate (UDMA), Dimethacrylate Co-Monomers.	76	GCE
Brilliant	Coltene, Altstaeten SG, Switzerland	(Nanohybrid Composite)	Bis-GMA, Bis-EMA, TEGDMA.	74	BEG
Synergy	Coltene, Altstaeten SG, Switzerland	(Nanohybrid Composite)	Bis-GMA, Bis-EMA And Urethane Dimethacrylate Monomer (UDMA),	77	SK
Filtek Bulk Fill Posterior	3M ESPE/ USA	Bulk Fill	UDMA, DDDMA, AUDMA	76,5	FBP
GCP Glass Fill	GCP Dental, Vianen, The Netherlands	Glass Carbomer	Fill:Fluoro-Aluminosilicate Glass, Apatite, Polyacids Gloss: Modified Polysiloxanes	-	GCP
Ionostar Plus	VOCO Gmbh, Cuxhaven, Germany Highly Viscous	Glass-İonomer Cements	Fluoro-Aluminosilicate Glass 50–100% Polyacrylic Acid 10–25%, Tartaric Acid < 2.5%	-	ISP
Ionostar Molar	Voco Gmbh, Cuxhaven, Germany	Glass-İonomer Cements	Powder: Fluoro-Alumino-Silicate Glass, Polyacrylic Acid Poder, Pigment Liquid:Polyacrylic Acid, Tartaric Acid, Distilled Water.	-	ISM
Fuji II LC	GC; Tokyo, Japan	Glass-İonomer Cements	Liquid: Polyacrylic Acid Powder: Al2O3-Sio2-Caf2 Glass And HEMA Urethane Dimethacrylate	-	F2S
GC *Fuji IX*	GC Co, Tokyo, Japan	Conventional Glass-İonomer	Powder: 95 % Strontium Fluoroalumino-Silicate Glass, 5 % Polyacrylic Acid	-	F9S

		Cement	Liquid: 40 % Aqueous Polyacrylic Acid		
EQUIA Forte	GC Co, Tokyo, Japan	Glass Hybrid	Powder: 95 % Strontium Fluoroalumino-Silicate Glass, 5 % Polyacrylic Acid Liquid: 40 % Aqueous Polyacrylic Acid	-	EQF

Table no 2. The parameters of MTT assay results of gingival fibroblast cells

	24		**St. d**	**Sig**	**72**		**St.d**	**Sig**
Control	100	±	4		100	±	4	
TEC	90,47	±	3,6		89,54	±	3,6	
TNC	86,25	±	2,9		83,51	±	2,9	
XTB	86,73	±	2	*	74,47	±	2	*
GCE	80,7	±	3,8		72,42	±	3,8	*
BEG	91,8	±	4,5		80,78	±	4,5	*
SK	92,28	±	3,8		89,97	±	3,8	
FBP	82,99	±	3,5		81,12	±	3,5	
GCP	71,69	±	2,6	*	78,31	±	2,6	*
ISM	56,78	±	1,9	**	62,43	±	1,9	**
F25	54,54	±	2	**	66,67	±	2	**
EQF	68,89	±	2,8	**	65,61	±	2,8	**
F95	59,39	±	1,5	**	73,02	±	1,5	*
ISP	72,45	±	2,8	*	76,72	±	2,8	*

Mean – Standard Deviation, Statistical significance level * p <0,05, ** p< 0,001

Table no 3. The parameters of MTT assay results of mesenchymal cells

	24 h		**St.d**	**Sig**	**72 h**		**St.d**	**Sig**
Control	100	±	4,02		100	±	4,02	
TEC	91,05	±	3,59		89,54	±	3,59	
TNC	89,75	±	3,89		83,51	±	2,89	
XTB	90,92	±	4		84,47	±	4	
GCE	83,15	±	3,78		72,42	±	3,78	*
BEG	80,14	±	3,5		80,78	±	4	*
SK	87,87	±	3,8		87,97	±	3,8	
FBP	75,23	±	2,54	*	74,12	±	3,54	*
GCP	66,65	±	2,59	**	68,31	±	2,59	**
ISM	70,13	±	2,89	*	62,43	±	1,89	**
F25	68,65	±	2	**	66,67	±	2	**
EQF	75,83	±	2,78	*	65,61	±	2,78	**
F95	73,64	±	2,5	*	73,02	±	3	*
ISP	88,14	±	2,8		76,72	±	3,8	*

Mean – Standard Deviation, Statistical significance level * p <0,05, ** p< 0,001

Table no 4. The parameters of MTT assay results of neuron cells

	24 h		**St.d**	**Sig**	**72 h**		**St.d**	**Sig**
Control	100	±	4,02		100	±	4,02	
TEC	88,05	±	3,59		75,3	±	2,59	*
TNC	91,75	±	3,89		81,3	±	3,89	
XTB	89,92	±	4		85,2	±	4	
GCE	83,15	±	3,78		83,5	±	3,78	
BEG	80,14	±	3,5	*	83,2	±	3,5	
SK	87,87	±	3,8		76,6	±	3,8	*
FBP	75,23	±	2,54	*	69,5	±	2,54	*
GCP	66,65	±	2,59	**	64,4	±	2,59	**
ISM	55,13	±	1,89	**	52,9	±	2,89	**
F25	56,65	±	2	**	58,1	±	2	**
EQF	75,83	±	2,78	*	55,5	±	2,78	**
F95	70,64	±	2,5	*	63,3	±	2,5	**

ISP	88,14	±	2,8		77,8	±	2,8	*

Mean – Standard Deviation, Statistical significance level * p <0,05, ** p< 0,001

IV. Discussion

In the treatment of teeth with fillings, different restorative materials (Composites, glass ionomer cement and flowable composites, etc.) with improved physical, chemical and biological properties are used. Most of these materials consist of a polymerizable organic resin matrix and particulate ceramic reinforcement fillers bonded with a silane coupling agent [21-23].

The biocompatibility of the materials used is important for the success of the process. While evaluating the biocompatibility of restorative materials, cell culture studies are easy to apply, controllable, reproducible and less costly [24].

In this study, the effects of different brands of restorative materials on stem cells were examined by MTT assay.

The oral cavity and its surroundings are covered with keratinocytes. In the lower layer, it is filled with connective tissue, lamina propria and gingival fibroblasts [25]. Dental filling materials are usually in contact with oral epithelial cells. If the biochemical components in its content penetrate the epithelium, it can interact with stem cells such as fibroblasts. Thus they can cause toxic effects. [26].

Fibroblastic stem cells are the predominant cell type in the pulp and can be affected by substances released from filling materials if the odontoblastic layer is deformed [27].

Human dental pulp stem cells are frequently used in cytotoxic studies because they can be easily obtained from extracted teeth, have no ethical problems, and are long-lasting [28, 29]. In our study, mesenchymal cells and neuron cells were used as well as dental pulp cells and the toxic differences between them were evaluated.

As mentioned above, mouse fibroblast cells, namely L929 cells, are frequently used in the biological evaluation of dental materials in in vitro studies due to their ease of use [30, 31]. In this study, unlike fibroblast cells, mesenchymal cells and neuron cells were also used. Thus, the effect of three different stem cells on cytotoxicity was evaluated in the same study.

In this study, we performed standard tests MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays for cytotoxic evaluations of thirteen dental restorative materials [32]. MTT test is often used to assess cell proliferation and neural toxicity [33]. This test has often been described in the literature and is reliable, reproducible, results in a short time, and is more reliable than other tests[34]. This use has been frequently described in the literature and is reliable, reproducible, and more sensitive than other colorimetric analyzes [35].

Here, we show that TEG, BEG and SK is significantly less cytotoxic to mesenchymal, neuron and human gingival fibroblasts cells, than GCP and F25. In literature studies, the toxic effects of resin-containing composite materials depend on many factors such as the degree of conversion after polymerization, the number of unbound free monomers, the release of ions over time, and microleakage [36].

In this study, when the findings were analyzed, the direct ratio between the cytotoxicity of the materials and the filler rate was determined. For example, TEC's filler rate (80%) is higher than other materials. Cell viability rate of TEC was found higher than the other groups after 24 and 72 hours. It was observed that the cell viability rate of GCP (glass ionomers are known to have a low filling ratio) was lower after 24 and 72 hours. In the light of this information, it was concluded that as the filler ratio increases, the toxic effect on the cell decreases.

In this study, samples were light cured for 20 s. This time allows for a high degree of conversion of materials and a small amount of elutable material. Although it is not possible to know exactly what percent of the conversion rate is, there are unbound monomers when considering the literature studies [37].

Table 1 shows the monomers showing the cytotoxic properties of the materials. Bis GMA has a cytotoxic effect on stem cells [38].

TEGDMA induces apoptotic proteins in pulp fibroblasts [39, 40]. UDMA exerts cytotoxic and growth inhibitory effects by inducing reactive oxygen species, which is an important cause of thiol reduction and cell damage in cells [41].

Analyzing the monomer elution from bulk-fill and conventional resin-containing composites using liquid chromatography, it was observed that it separated BisGMA, BisEMA and TEGDMA from conventional composites [37, 42]. It can be thought that the amounts of the above-mentioned unbound monomers are responsible for the more toxic effects of some of the materials used in our study.

Interestingly, when the results of HGF, mesenchymal and neuron cells were evaluated, it was seen that HGF and neuron cells had similar results, and had more toxic effects than mesenchymal cells.

Remarkably, cell viability rates of HGF in specimens with F25, ISM, F95, GCP, ISP were as part of higher after 72 h than after 24 h. This finding suggests the possibility that stem cells in contact with composite materials proliferate and the potential for cytotoxicity decreases in some resin-containing composite materials.

A similar situation was detected when the viability of mesenchymal and neuron cells was examined after 72 hours.

Studies have shown the detrimental effects of resin-containing composites on osteoblastic cells [43]. ISM had values close to 56.78%, which was at the limit of severe cytotoxicity, while F95 had values of around 59.35% and could be considered moderately cytotoxic. While ISM had values close to 56.78%, which was at the cytotoxicity limit, F95 had values around 59.35% and could be considered as moderately cytotoxic. XTB, SNC had values considered mildly cytotoxic. None of the materials were classified as "non-cytotoxic" in this test. To our knowledge, there is no similar study examining the viability of three different stem cells in the same study, and this effect should be investigated for longer than 72 hours.

The cytotoxicities of the materials are categorized according to ISO standard 10993-5:2009; non-cytotoxic or slightly, moderately or highly cytotoxic [43]. In this study, the toxic effect of resin-containing composite materials was evaluated as more or less toxic. In this study, the most harmful effects of composites were determined as neuron cells, HGF cells and Mesenchymal cells, respectively.

When the toxicity of three different cells after 24 and 72 hours was evaluated, it was seen that the bulk fill composites were less toxic. Similar results were also found in studies by different researchers [43]. When cytotoxicity studies were examined in the literature, none of the materials were defined as non-toxic, they were defined as less toxic or more toxic.

In this study, we showed that composite materials TNC, XTB, SK and BEG were significantly less cytotoxic to HGF, mesenchymal and neuron stem cells using the MTT assay. More research is needed in the future to confirm these in vitro results. SK may represent a crucial technological advance in overcoming the adverse biocompatibility of resin-based dental restorative materials.

Contrary to the adverse effect of F25 ve ISM on HGF cells and neuron cells, a similar effect was not identified in the mesenchymal stem cells. Low cytotoxic effect was seen for mesenchymal cell lines. BisGMA, UDMA and TEGDMA, which are found in almost all materials, are thought to cause DNA strand breaks in HGF cells [55], thus these materials cause toxic effects [44].

In a recent study, the cytotoxic values of Tetric N-Ceram Bulk-Fil, Xtrafil, and Xtrabase composite resins were examined. The cytotoxicity was evaluated by MTT test on HGF. Tetric N-Ceram Bulk-fill composite resin was found to have higher toxicity [45]. In this study, Tetric N-Ceram Bulk-Fil and Xtrabase composite resin were analyzed to have similar toxic effects.

Putzeys et al [46, 47]. In his study, BisGMA, UDMA, TEGDMA were separated from the Filtek™ Supreme XTE structure. Filtek™ Supreme XTE in contact with human gingival keratinocytes caused a decrease in interleukin 6 secretion. This indicates a defense against infections. that is, this material was found to have a toxic effect. In our study, however, it was observed that Filtek™ Supreme had little toxic effect and increased cell viability in stem cells after 72 hours.

In a study by Cosgun et al., [48] they found that the materials evaluated in terms of cytotoxicity after 24 hours did not show any toxic effect, and after 72 hours, Zirconomer, EQUIA Forte, Fuji IX and Fuji II showed statistically significantly lower cell viability values compared to the control group. Similarly, in our study, EQUIA Forte, Fuji IX and Fuji II were found to have toxic properties. As a result of the study, it was seen that glass ionomer cements had more toxic effects.

V. Conclusion

This study demonstrated that there was a decrease in cell viability over time. In addition, it was observed that dental composite materials had less toxic effects on mesenchymal cells and more toxic effects on neuron and human gingival fibroblast cells.

VI. Declaration by Authors

Acknowledgement: This research was supported by Fırat University Scientific Research Projects (FUBAP) with project number DHF-16.03. Also, I could not have undertaken this article without Ali Taghizadehghalehjoughi and his friends. I would like to express my sincere gratitude to Ali Taghizadehghalehjoughi and his friends who contributed to the preparation and finalization of the experimental setup part of the article. Conflict of Interest: The authors declare no conflict of interest.

References

[1]. Schmalz, G., Concepts In Biocompatibility Testing Of Dental Restorative Materials. Clin Oral Investig, 1997. 1(4): P. 154-162.
[2]. Polyzois, G.L., In Vitro Evaluation Of Dental Materials. Clin Mater, 1994. 16(1): P. 21-60.
[3]. Bekeschus, S., Et Al., Biological Risk Assessment Of Three Dental Composite Materials Following Gas Plasma Exposure. Molecules, 2022. 27(14).
[4]. Pintor, A.V.B., Et Al., Mtt Versus Other Cell Viability Assays To Evaluate The Biocompatibility Of Root Canal Filling Materials: A Systematic Review. Int Endod J, 2020. 53(10): P. 1348-1373.
[5]. Scotti, R., Et Al., Biocompatibility Of Various Root Canal Filling Materials Ex Vivo. Int Endod J, 2008. 41(8): P. 651-657.

[6]. Hauman, C.H. And R.M. Love, Biocompatibility Of Dental Materials Used In Contemporary Endodontic Therapy: A Review. Part 2. Root-Canal-Filling Materials. Int Endod J, 2003. 36(3): P. 147-160.
[7]. Zhu, Y.Q. And X.Y. Wang, [Study On The Biocompatibility Of Commonly Used Root Canal Filling Materials]. Shanghai Kou Qiang Yi Xue, 1994. 3(2): P. 78-81.
[8]. Schmalz, G., Use Of Cell Cultures For Toxicity Testing Of Dental Materials--Advantages And Limitations. J Dent, 1994. 22 Suppl 2: P. S6-11.
[9]. Rappaport, H.M., G.E. Lilly, And P. Kapsimalis, Toxicity Of Endodontic Filling Materials. Oral Surg Oral Med Oral Pathol, 1964. 18: P. 785-802.
[10]. Steinlin-Schopfer, J., Et Al., Evaluation Of The Roche Antigen Rapid Test And A Cell Culture-Based Assay Compared To Rrt- Pcr For The Detection Of Sars-Cov-2: A Contribution To The Discussion About Sars-Cov-2 Diagnostic Tests And Contagiousness. J Clin Virol Plus, 2021. 1(1): P. 100020.
[11]. Berridge, M.V., Et Al., Mitochondrial Transfer Between Cells: Methodological Constraints In Cell Culture And Animal Models. Anal Biochem, 2018. 552: P. 75-80.
[12]. Brooks, A.E., Et Al., Culture And Expansion Of Rodent And Porcine Schwann Cells For Preclinical Animal Studies. Methods Mol Biol, 2018. 1739: P. 111-126.
[13]. Hara, Y., Et Al., Dehydropropylpantothenamide Isolated By A Co-Culture Of Nocardia Tenerifensis Ifm 10554(T) In The Presence Of Animal Cells. J Nat Med, 2018. 72(1): P. 280-289.
[14]. Ilie, N., Cytotoxic, Elastic-Plastic And Viscoelastic Behavior Of Aged, Modern Resin-Based Dental Composites. Bioengineering (Basel), 2023. 10(2).
[15]. Weyermann, J., D. Lochmann, And A. Zimmer, A Practical Note On The Use Of Cytotoxicity Assays. Int J Pharm, 2005. 288(2): P. 369-376.
[16]. Ye, X., Et Al., Evaluation Of Genes Involved In Oxidative Phosphorylation In Yeast By Developing A Simple And Rapid Method To Measure Mitochondrial Atp Synthetic Activity. Microb Cell Fact, 2015. 14: P. 56.
[17]. Wang, X., Et Al., Comparison Of Mtt Assay, Flow Cytometry, And Rt-Pcr In The Evaluation Of Cytotoxicity Of Five Prosthodontic Materials. J Biomed Mater Res B Appl Biomater, 2010. 95(2): P. 357-364.
[18]. Parra, D., Et Al., Methodological Characterization Of The 2-Keto [1-13c]Isocaproate Breath Test To Measure In Vivo Human Mitochondrial Function: Application In Alcoholic Liver Disease Assessment. Alcohol Clin Exp Res, 2003. 27(8): P. 1293-1298.
[19]. Mosmann, T., Rapid Colorimetric Assay For Cellular Growth And Survival: Application To Proliferation And Cytotoxicity Assays. J Immunol Methods, 1983. 65(1-2): P. 55-63.
[20]. Husoy, T., T. Syversen, And J. Jenssen, Comparisons Of Four In Vitro Cytotoxicity Tests: The Mtt Assay, Nr Assay, Uridine Incorporation And Protein Measurements. Toxicol In Vitro, 1993. 7(2): P. 149-154.
[21]. Kim, Y., Et Al., Biocompatibility And Bioactivity Of Set Direct Pulp Capping Materials On Human Dental Pulp Stem Cells. Materials (Basel), 2020. 13(18).
[22]. Pituru, S.M., Et Al., A Review On The Biocompatibility Of Pmma-Based Dental Materials For Interim Prosthetic Restorations With A Glimpse Into Their Modern Manufacturing Techniques. Materials (Basel), 2020. 13(13).
[23]. Dreanca, A., Et Al., Systemic And Local Biocompatibility Assessment Of Graphene Composite Dental Materials In Experimental Mandibular Bone Defect. Materials (Basel), 2020. 13(11).
[24]. De Souza Costa, C.A., Et Al., Biocompatibility Of Resin-Based Dental Materials Applied As Liners In Deep Cavities Prepared In Human Teeth. J Biomed Mater Res B Appl Biomater, 2007. 81(1): P. 175-184.
[25]. Kydd, W.L. And C.H. Daly, The Biologic And Mechanical Effects Of Stress On Oral Mucosa. J Prosthet Dent, 1982. 47(3): P. 317-329.
[26]. Hensten-Pettersen, A., Skin And Mucosal Reactions Associated With Dental Materials. Eur J Oral Sci, 1998. 106(2 Pt 2): P. 707-712.
[27]. Wataha, J.C., Et Al., In Vitro Biological Response To Core And Flowable Dental Restorative Materials. Dent Mater, 2003. 19(1): P. 25-31.
[28]. Huang, F.M. And Y.C. Chang, Cytotoxicity Of Resin-Based Restorative Materials On Human Pulp Cell Cultures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2002. 94(3): P. 361-365.
[29]. Cao, T., Et Al., Comparison Of Different Test Models For The Assessment Of Cytotoxicity Of Composite Resins. J Appl Toxicol, 2005. 25(2): P. 101-108.
[30]. Henstenpettersen, A. And K. Helgeland, Sensitivity Of Different Human Cell-Lines In The Biologic Evaluation Of Dental Resin-Based Restorative Materials. Scandinavian Journal Of Dental Research, 1981. 89(1): P. 102-107.
[31]. Theilig, C., Et Al., Effects Of Bisgma And Tegdma On Proliferation, Migration, And Tenascin Expression Of Human Fibroblasts And Keratinocytes. Journal Of Biomedical Materials Research, 2000. 53(6): P. 632-639.
[32]. Onet, D., Et Al., The Cytotoxicity Of Dental Restorative Materials On Gingival Stromal Mesenchymal Cells-An In Vitro Study. Curr Health Sci J, 2022. 48(3): P. 331-339.
[33]. Kunert, M., Et Al., The Cytotoxicity And Genotoxicity Of Bioactive Dental Materials. Cells, 2022. 11(20).
[34]. Kuden, C., S.N. Karakas, And S.G. Batmaz, Comparative Chemical Properties, Bioactivity, And Cytotoxicity Of Resin-Modified Calcium Silicate-Based Pulp Capping Materials On Human Dental Pulp Stem Cells. Clin Oral Investig, 2022. 26(11): P. 6839-6853.
[35]. Carrillo-Cotto, R., Et Al., Cytotoxicity Of Contemporary Resin-Based Dental Materials In Contact With Dentin. Eur J Oral Sci, 2020. 128(5): P. 436-443.
[36]. Goldberg, M., In Vitro And In Vivo Studies On The Toxicity Of Dental Resin Components: A Review. Clin Oral Investig, 2008. 12(1): P. 1-8.
[37]. Schubert, A., Et Al., Cytotoxic Effects To Mouse And Human Gingival Fibroblasts Of A Nanohybrid Ormocer Versus Dimethacrylate-Based Composites. Clin Oral Investig, 2019. 23(1): P. 133-139.
[38]. Hanks, C.T., Et Al., Cytotoxic Effects Of Resin Components On Cultured Mammalian Fibroblasts. J Dent Res, 1991. 70(11): P. 1450-1455.
[39]. Batarseh, G., Et Al., Triethylene Glycol Dimethacrylate Induction Of Apoptotic Proteins In Pulp Fibroblasts. Oper Dent, 2014. 39(1): P. E1-8.
[40]. Kanjevac, T.V., Et Al., Cytotoxicity Of Glass Ionomer Cement On Human Exfoliated Deciduous Teeth Stem Cells Correlates With Released Fluoride, Strontium And Aluminum Ion Concentrations. Archives Of Biological Sciences, 2015. 67(2): P. 619-630.
[41]. Chang, H.H., Et Al., The Mechanisms Of Cytotoxicity Of Urethane Dimethacrylate To Chinese Hamster Ovary Cells. Biomaterials, 2010. 31(27): P. 6917-6925.

[42]. Alshali, R.Z., Et Al., Analysis Of Long-Term Monomer Elution From Bulk-Fill And Conventional Resin-Composites Using High Performance Liquid Chromatography. Dent Mater, 2015. 31(12): P. 1587-1598.
[43]. Haugen, H.J., Et Al., Bulk Fill Composites Have Similar Performance To Conventional Dental Composites. International Journal Of Molecular Sciences, 2020. 21(14).
[46]. Putzeys, E., Et Al., Long-Term Elution Of Monomers From Resin-Based Dental Composites. Dent Mater, 2019. 35(3): P. 477-485.
[48]. Cosgun, A., B. Bolgul, And N. Duran, In Vitro Investigation Of Antimicrobial Effects, Nanohardness, And Cytotoxicity Of Different Glass Ionomer Restorative Materials In Dentistry. Niger J Clin Pract, 2019. 22(3): P. 422-431.