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The Cell Viability of Dental Materials by The Lactate Dehydrogenase Assay

Hakan Kamalak

Department of Restorative Dentistry, Kahramanmaras Sutcu Imam, Faculty of Dentistry, Onikisubat, Turkey.

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ABSTRACT

Objectives: the objective of this study is to search the cell membrane damage of resin composite materials on cultures of human gingival fibroblasts and neuron cells by the lactate dehydrogenase (LDH) test method.
Methods: A range of thirteen resin composites sterilized under UV light for 2 hours were added to the culture medium of human gingival fibroblasts and neuron for 24 h and 72h. The samples were moved to 48- well-plates in a sterile cabin, one by one, in direct contact with the cells. After 24- and 72-hours incubation period, in the incubator at 37 ºC with 5% CO2; Cell membrane damage was detected by the lactate dehydrogenase (LDH) test method.
Results: In the lactate dehydrogenase (LDH) test method, cytotoxicity is determined by measuring lactate dehydrogenase, which is a cytosolic enzyme released in the event of membrane damage or cytolysis in the cell. A higher LDH value indicates that the toxicity of the material is greater. When the LDH results in Gingival fibroblast cells were examined after 24 hours, F25 was found to be the most toxic, and when the LDH results in neuron cells were examined, ISM and F25 were also found to be the most toxic.
Significance: Dental restorative materials have some toxic effects on cell cultures. The LDH analysis supplies precious instructions about their toxic effects.

Keywords: Lactate dehydrogenase, fibroblasts, neuron cells, cytotoxicity

INTRODUCTION

The polymerized resin-based dental materials contain resin composite, adhesive bonding systems, modified glass ionomer and glass ionomer cements and fissure sealants (atıf). These materials fulfill its current function in contact with living tissues can cause locally or systemically toxic, mutagenic, allergic, carcinogenic effects and with adversely affecting health. [1-3].

The chemical composition of restorative materials deteriorates over time due to intraoral surface interactions with saliva, foods and acids in plaque [4]. Released monomers from dental materials have been found in saliva, dentin, and pulp after placement of resin-based restorative materials [5-7]. Monomers may cause a range of adverse toxic effects on the skin, mucous membranes, eyes or cells[7].

Cell culture experiments are constantly used to evaluate the toxicity of samples, and processes can be repeated under standard conditions or measurements can be made by direct observation on the cells[8-11]. Cell-based toxicity studies were born as an different to animal setups and have become often preferred in toxicology laboratories due to their ease of recourse and relevance with data obtained from in vivo studies [12].

Enzymes leaking into the medium following cell damage or death were also take noticed as indicators of the number of deceased

cells. Among these enzymes, lactate dehydrogenase (LDH), which stands out with its stability, has taken its place among viability tests as a marker of cell death [13].
The objective of our study was to examine the in vitro toxic effects of dental materials on fibroblast and neuron cells by Lactate Dehydrogenase Colorimetric Activity tests.

MATERIALS & METHODS

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) were selected for this assay. As a result of the power analysis, the number of samples was determined as 12 for each material (n=12). The samples of the materials are listed in Table 1.

Sample Preparation

Disc specimens were arranged using cylindrical teflon molds with a diameter of 8 mm and a height of 2 mm. For the condensation of composite materials, pressure was applied with cement glass under pressure. Additionally, transparent tape was placed on the sample surface to limit oxygen inhibition. The overflowing composite materials were cleaned with a cement sipatula. Each of the samples was polymerized with an LED light source for 10 seconds at an average of 1000 mW/cm2. Composite disc samples (n = 12) were then UV-sterilized before LDH testing. The samples were exposed to UV light for 24 hours to make them sterile. Each sample was immersed in cell culture medium immediately after hardening.

Cell Culture

Fibroblast and neuron cell cultures were provided from the ATCC (American Type Culture Collection) global biological resource center. Cells were injected into culture dishes diluted with 10% FBS (Fetal Bovine Serum), 1% antibiotics (containing penicillin-streptomycin-amphotericin B). All cells were incubated at norm circumstances [37°C, 5% (v/v).

Preparation of Cell Production Containers

200 mL cell suspension was prepared with fresh nutrient medium. The prepared suspension was distributed equally to all compartments where the materials would be placed. Samples were allowed to incubate again. After seven days of incubation at 5% CO2 and 37°C humidity, it was examined whether the cells completely filled the wells of the plates. Samples were sterilized under ultraviolet (UV) light for 2 hours; With the help of a sterile press, they were placed one by one into cell production containers in a sterile cabinet, in direct contact with the cells. Analyzes were performed after 24 and 72 h of incubation in a 37°C, 5% CO2 incubator.

Lactate Dehydrogenase Colorimetric Activity Test

In the lactate dehydrogenase (LDH) test method, which is a colorimetric cytotoxicity evaluation method, cytotoxicity is determined by measuring lactate dehydrogenase, a cytosolic enzyme released in the event of membrane damage or cytolysis in the cell. LDH is a stable cytoplasmic enzyme found in cells; It is released into the cell culture medium when cell lysis and membrane damage occur. It is a rapid and simple test that determines changes in the plasma membrane after incubation with a test material.
LDH reduces pyruvate to lactate by oxidation of nicotinamide adenine dinucleotide (NADH) to NAD+ (Formula 2-1). The absorbance of NADH at 340 nm is measured in a spectrophotometer. The decrease in absorbance due to NADH consumption is proportional to cell damage. Triton X-100 is generally utilized as a positive control in the LDH test. The cytotoxicity of the test substance was determined by assuming that the damage

occurred was 100% in the cells incubated with the positive control.

STATISTICAL ANALYSIS

Power analysis was used to determine the sample size (n=12). IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used to interpret the data obtained. One-way and two-way analysis of variance (ANOVA) methods were used to analyze the data. Statistical significance was evaluated at $p<0.05$ and $p<0.001$ grades.

RESULT

In the lactate dehydrogenase (LDH) test method, cytotoxicity is determined by measuring lactate dehydrogenase, which is a cytosolic enzyme released in the event of membrane damage or cytolysis in the cell. A higher LDH value indicates that the toxicity of the material is greater. When we look at the LDH results in Gingival fibroblast cells after 24 hours, it is 14.4 in the TEC group, 15.6 in TNC, 15.7 in XTB, 17.4 in GCE, 13.9 in BEG, 13.3 in SK, 16.4 in FBP, It was found to be 18.1 in GCP, 26.2 in ISM, 31.6 in F25, 20.4 in EQF, 24.9 in F95 and 17.7 in ISP. (Table 2, Figure 1,2)

When we look at the LDH results in Gingival fibroblast cells after 72 hours, it is 12.5 in the TEC group, 17.6 in TNC, 23.9 in XTB, 25.2 in GCE, 18.6 in BEG, 14.1 in SK, 18.1 in FBP, was found to be 20.3 in GCP, 31.2 in ISM, 28.3 in F25, 30.1 in EQF, 24.3 in F95 and 21.8 in ISP. Cell membrane damage was found the most in the ISM group and the least in the SK group. After 72 hours, it was observed that there was an increase in cell membrane damage in the TEC, TNC, XTB, GCE, BEG, SK, FBP, GCP, ISM, EQF, ISP groups and a decrease in F25, F95. (Table 2, Figure 1,2).

When we look at the LDH results in neuronal cells after 72 hours, it is 13.3 in the TEC group, 23.6 in the TNC, 19.4 in the XTB, 16.3 in the GCE, 17.1 in the BEG, 17.6 in the SK, 21.9 in the FBP, It was found to be 26.6 in GCP, 36.7 in ISM, 31.8 in F25, 32.1 in EQF, 27.6 in F95 and 21.3 in ISP. Cell membrane damage was found to be highest in the ISM group and least in the XTB group. (Table 3, Figure 3,4).

Table 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 Cement	Powder: 95 % Strontium Fluoroalumino-Silicate Glass, 5 % Polyacrylic Acid Liquid: 40 % Aqueous Polyacrylic Acid	-	F9S
EQUIA Forte	GC Co, Tokyo, Japan	Glass Hybrid	Powder: 95 % Strontium Fluoroalumino-Silicate Glass, 5 % Polyacrylic Acid Liquid: 40 % Aqueous Polyacrylic Acid	-	EQF

Table 2: Gingival fibroblast cells LDH numerical values at 24- and 72-hours n=12, * p 0.05, ** p 0.001

	24		St.d	Sig	72		St.d	Sig
Control	12,8	±	1,3		12,5	±	1,2	
TEC	14,4	±	1,2		15,1	±	1,4	
TNC	15,6	±	1,4		17,6	±	1,5	
XTB	15,7	±	1,5		23,9	±	2,5	**
GCE	17,4	±	1,6		25,2	±	2,4	**
BEG	13,9	±	1,2		18,6	±	1,7	*
SK	13,3	±	1,4		14,1	±	1,4	
FBP	16,4	±	1,6		18,1	±	1,6	
GCP	18,1	±	1,5	*	20,3	±	1,9	**
ISM	26,2	±	2,4	**	31,2	±	2,4	**
F25	31,6	±	2,9	**	28,3	±	2,9	**
EQF	20,4	±	1,8	**	30,1	±	1,8	**
F95	24,9	±	2,2	**	24,3	±	2	**
ISP	17,7	±	1,6		21,8	±	1,9	**

Table 3: Neuron cells LDH numerical values at 24- and 72-hours n=12, * p 0.05, ** p 0.001

	24		St.d	Sig	72		St.d	Sig
Control	12,9	±	1,3		13,3	±	1,2	
TEC	16,30	±	1,2		23,6	±	2,4	
TNC	14,90	±	1,4		19,4	±	1,7	
XTB	15,30	±	1,5		16,3	±	1,6	
GCE	18,20	±	1,6		17,1	±	1,4	
BEG	19,50	±	1,2		17,6	±	1,7	
SK	17,10	±	1,4		21,9	±	2	*
FBP	20,60	±	1,6	*	24,8	±	2,2	**
GCP	28,30	±	1,5	**	26,6	±	2,2	**
ISM	34,70	±	2,4	**	36,7	±	3,4	**
F25	32,10	±	2,9	**	31,8	±	2,9	**
EQF	20,10	±	1,8	*	32,1	±	2,8	**
F95	22,10	±	2,2	**	27,6	±	2,3	**
ISP	15,90	±	1,6		21,3	±	1,9	*

Fig. 1. LDH assay results of HGF over 24h (* p 0.05, ** p 0.001)

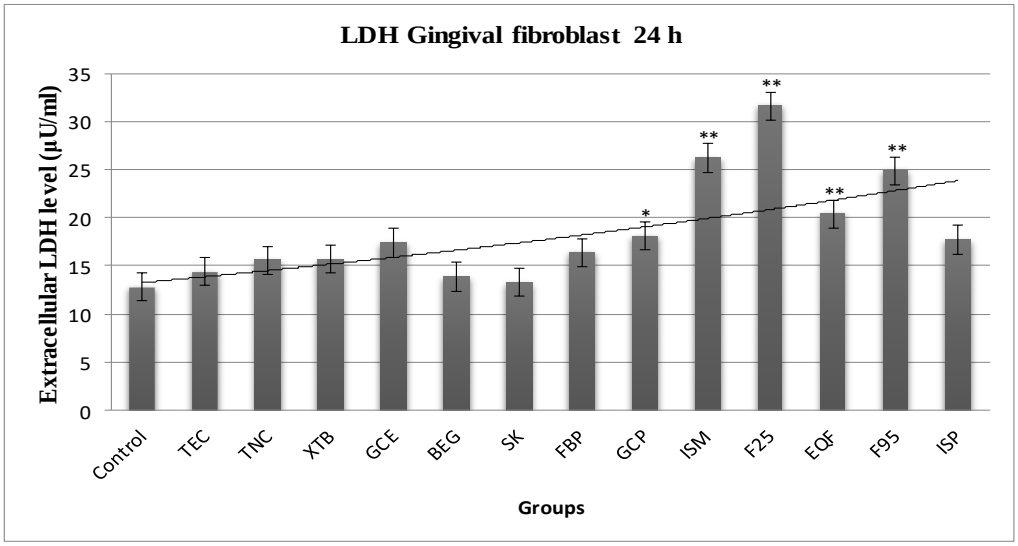


Fig. 2. LDH assay results of HGF over 72h (* p 0.05, ** p 0.001)

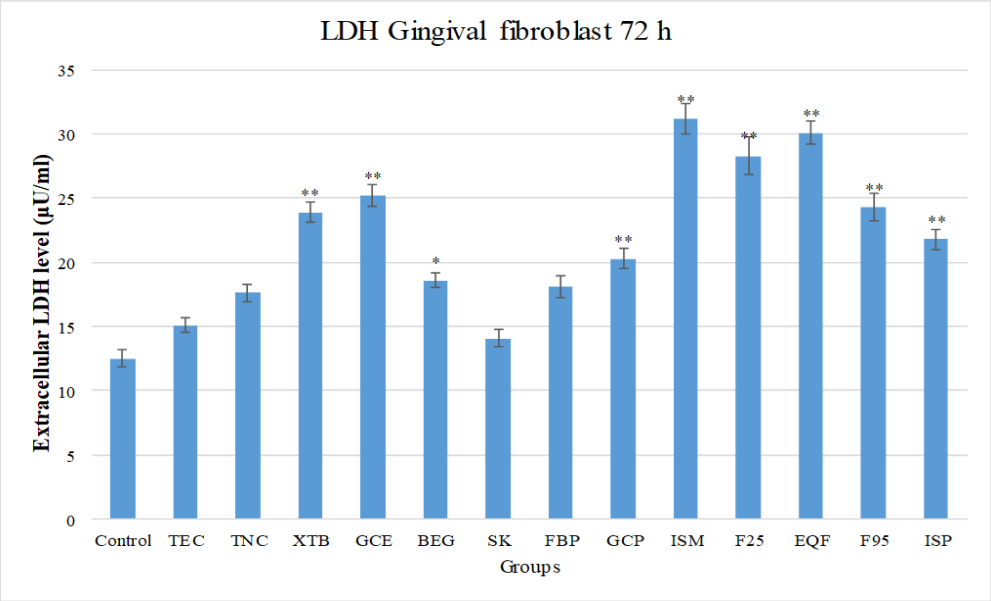


Fig. 3. LDH assay results of neuron cells over 24h (* p 0.05, ** p 0.001)

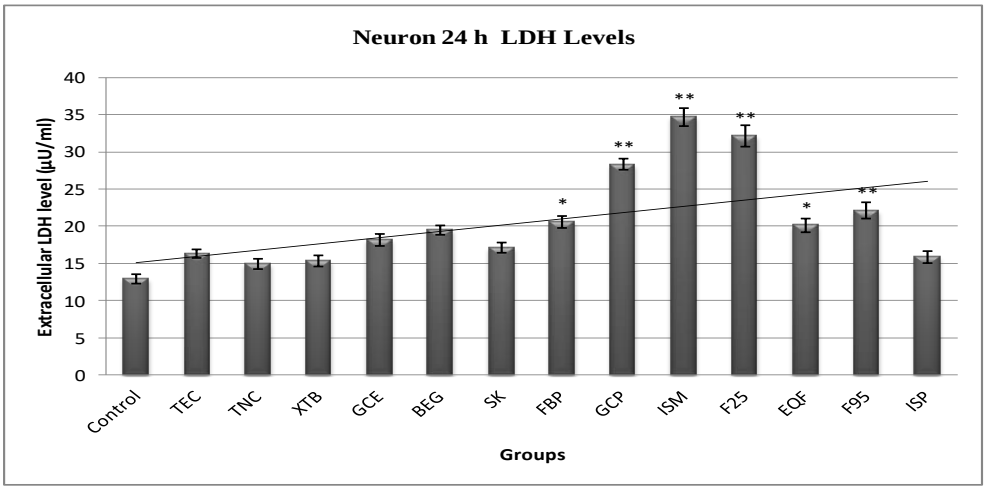
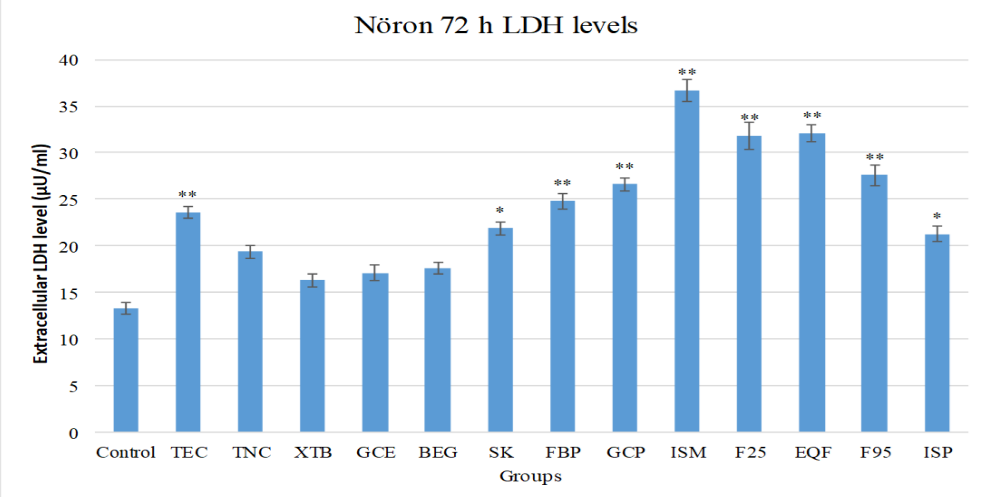


Fig. 4. LDH assay results of neuron cells over 72h (* p 0.05, ** p 0.001)



DISCUSSION

In cell culture experiments, an experimental setup is prepared by keeping the cells of organisms in in vitro environments, in specifically manufactured containers, by controlling environmental conditions such as temperature and humidity, and by protecting them from contamination. Another advantage of cell culture studies is that cells taken from different kinds of living things, including humans, can be kept alive in a laboratory environment, cells taken from any tissue or organ can be used, and it eliminates many ethical concerns [14-19]. Different in vitro studies are carried out to determine whether dental materials have cytotoxic potential. Cell-derived cytotoxicity studies, animal experiments, rat studies and laboratory studies have become frequently used in laboratories due to their ease of application and suitability for data [20, 21].

The method used to evaluate cell viability and choosed in this study is the determination of lactate dehydrogenase (LDH) activity released from damaged/dead cells into the medium [22]. Lactate dehydrogenase is a cytoplasmic enzyme found in all cells. When cells are discovered to toxic effects, their plasma membrane integrity is disrupted and the LDH enzyme leaks from the cells and into the medium. So, cell damage can be appreciated by measuring LDH enzyme activity after exposure [23].

There are two different methods that can be applied to determine LDH activity. The test reagent used in the first method is toxic to cells, causing LDH release and distorting test values. For this reason, after exposure of the cells to the toxic substance, some medium taken from the wells is transferred to another microplate and the test reagent is added and incubated for a few hours [24]. LDH present in the medium during incubation catalyzes the conversion of pyruvate to lactate. During this enzymatic activity, NADH undergoes oxidation and turns into NAD+. The test is completed by determining the amount of NADH in the examined medium with a microplate reader at 340 nm absorbance [23]. In the second method, resazurin dye and diaphorase enzyme are used. The test reagent is added directly onto the cells. The first test method was preferred in this study.

The stable cytoplasmic enzyme lactate dehydrogenase (LDH) is an important parameter for the detection of oxidative stress, apoptosis, necrosis and cellular damage. It is rapidly released in all cells under suitable conditions and when the plasma membrane is damaged. Kumar et al. In their study, they showed that LDH level increased with cellular damage, which is consistent with our study [25, 26].

Karaoğlanoğlu et al [27]. In his study, cell damage testing was evaluated after 24 and 72 hours. Similarly, in our study, it was evaluated after 24 and 72 hours. Karaoğlanoğlu et al [27]. No toxic effects were observed after 24 hours in their studies. However, in our study, toxic effects were observed in groups and cells after 24 hours.

CONCLUSION

Cytotoxicity tests provide basic information about the behavior of a substance with unknown toxic effects and serve as a source for subsequent animal experiments or clinical trials. Therefore, the accuracy and reliability of the data obtained from relevant studies are important. The reliability of the data obtained from the tests performed depends not only on the method but also on many variables such as the knowledge and experience of the researcher, the cell type used, ambient temperature, test reagent content and medium composition. Success in test results requires all these variables to be taken into consideration together.

Declaration by Authors

Ethical Approval: Not Applicable

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