Article published online: 2021-11-01

ORIGINAL ARTICLE

Evaluation of cytotoxicity of six different flowable composites with the methyl tetrazolium test method

Muhammet Yalcin, Mustafa Ulker[1], Esra Ulker[1], Abdulkadir Sengun[2]

Departments of Restorative Dentistry, İnönü University, Faculty of Dentistry, Malatya, [1]Selçuk University, Faculty of Dentistry, Konya, [2]Kırıkkale University, Faculty of Dentistry, Kırıkkale, Turkey

Address for correspondence:
Dr. Muhammet Yalcin,
Departments of Restorative Dentistry,
İnönü University, Faculty of Dentistry,
Malatya, Turkey.
E-mail: dt.muhammet@gmail.com

ABSTRACT

Background: The aim of this study was to investigate the cytotoxicity of six different flowable composites with the methyl tetrazolium test (MTT). **Materials and Methods:** For MTT, six different flowable composites (Bisco Aelite, Bisco Inc., USA; Esthet X Flow, Dentsply, USA; Filtek™ Supreme XT Flowable Restorative, 3M Espe, USA; Gradia® Direct Flo, GC, USA; Estelite® Flow Quick, Tokuyama Dental Corporation, Japan; and Clearfil Majesty Flow, Kuraray Medical Inc., Japan) were prepared according to the manufacturer's instructions in standard Teflon disks (2 mm × 5 mm) and the samples were extracted in 7 ml of Basal Medium Eagle with 10% new born calf serum and 5% penicillin streptomycin gentamisin for 24 h. In the experiments: The L929 cells were plated (25.000 cells/ml) in wells of 96 well-dishes and maintained in a humidified incubator for 24 h at 37°C, 5% CO_2, and 95% of air. After 24 h incubation of the cells, the incubation medium was replaced by the immersed medium in which the samples were stored. Then L929 cells were incubated in contact with evaluates for 24 h. The cell mitochondrial activity was evaluated by the MTT. 12 well used for each specimen and MTT tests applied 2 times. The data were submitted to the statistically analyzed by one-way ANOVA and Tukey's honestly significant difference (HSD) tests. **Results:** According to the results of MTT test with L-929 fibroblasts demonstrated that all freshly prepared flowable composites did not reduce vital cell numbers (P>0.05) in comparison to control group. **Conclusion:** This study revealed important information for clinical applications of flowable composites in dentistry.

Key words
Flowable composites, methyltetrazolium test, toxicity

INTRODUCTION

The polymers have been used at various areas such as cosmetics, drug systems,[1] and dental applications.[2-5] Polymerization reactions have been constituted by free radicals. In chemistry, free radicals have been known as atom, molecule, or ion with the unpaired electrons. The free radicals have caused disease[6-8] and these have been responsible for toxicity.[9]

In this study, the cytotoxicity of test materials (Bisco Aelite, Bisco Inc., USA; Esthet X Flow, Dentsply, Germany; Filtek™ Supreme XT Flowable Restorative, 3M Espe, USA; Gradia® Direct Flo, GC, USA; Estelite® Flow Quick, Tokuyama Dental Corporation, Japan; and Clearfil Majesty Flow, Kuraray Medical Inc., Japan) were investigated using the methyl tetrazolium test (MTT) method.

Access this article online	
Quick Response Code:	Website: www.ejgd.org
	DOI: 10.4103/2278-9626.116012

Different types of restorative materials are used as dental filling. These materials contact with the oral mucosa, body fluids, dentin and periodontium. The biocompatibility of these materials has great importance and any adverse reactions due to the leaching of components from these dental materials into the oral cavity is a clinical concern.[10] Therefore, a thorough scientific evaluation of the biological behavior of these materials must be performed.

Flow composites were introduced as restorative dental material, which have low viscosity and are easy to handle. Flowable resins were recommended for fillings in hard-to-reach areas, lining, and repair of composite resins and veneers due to their low filler loading and flowable nature.[11] They contain monomers, inorganic filler, and diluent for low viscosity with different percentages.

Cell-culture studies have demonstrated that the components of resin composites are hazardous because

all elicit significant toxicity in direct contact with fibroblasts.[12] Since, flowable composite restorative materials are made flowable by the addition of lower molecular weight resin diluents they may exhibit increased mass release and therefore increased cytotoxicity.[13]

Animal studies have shown that these materials might produce biological effects on dental pulp, although microleakage and bacterial invasion complicate evaluation of these results.[14] The aim of this study was to investigate the cytotoxicity of flowable composites on fibroblast cells with MTT assay *in vitro*.

MATERIALS AND METHODS

The MTT

Six different flowable composites were tested in the experiments: (Bisco Aelite, Bisco Inc., USA; Esthet X Flow, Dentsply, Germany; Filtek™ Supreme XT Flowable Restorative, 3M Espe, USA; Gradia® Direct Flo, GC, USA; Estelite® Flow Quick, Tokuyama Dental Corporation, Japan; and Clearfil Majesty Flow, Kuraray Medical Inc., Japan). Their components and details are listed in Table 1. Test specimens were prepared according to the manufacturer's instructions in standard Teflon molds of 5 mm in diameter and 2 mm in depth. All specimens were prepared and handled under aseptic conditions to limit the influence of biologic contamination on the cell culture tests. Specimen's that required light curing were cured using a standard light curing unit (light emitting diode (LED), Elipar FreeLight 2, 3M Espe Dental Products, St Paul, Minn). Four samples were prepared for each group for cytotoxicity testing. The samples were immersed in 7 ml of culture medium for 24 h at 37°C to extract residual monomer or cytotoxic substances. The culture medium containing material extracts was sterile filtered for use on the cell cultures. L929 cells (ATCC CCl 1, Şap Enstitüsü, Ankara, Turkey) were cultured in Basal Medium Eagle (BME) containing 10% new born calf serum and 100 mg/ml penicillin/streptomycin at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Cell cultures between the 12 and 15 passages were used in this study. Confluent cells were detached with 0.25% trypsin and seeded at a density of 5 × 10 ≥into each of a 96-well plate for 24 h at 37°C and 5% CO_2. After 24 h of incubation, the culture medium was replaced with 200 µL of culture medium containing material extracts of flowable composites. The original culture medium served as control in this study. Cultures were incubated for 24 h at 37°C and 5% CO_2. The viability of cells exposed to material extracts was assessed using the succinic dehydrogenase activity. The succinic dehydrogenase activity has been shown to be reasonably representative of mitochondrial activity in the cells and reflects both cell number and activity.[3] The old medium was removed and cell cultures were rinsed with phosphate buffered saline (PBS), and 0.5-ml aliquots of freshly prepared MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) solution (0.5 mg/ml in BME 9 were added to each well. After a 2 h incubation period (37°C, 5% CO_2), the supernatant was removed and the intracellularly stored MTT formazan was solubilized in 200 µL dimethyl sulfoxide for 30 min at room temperature. The absorbance at 540 nm was spectrophotometrically measured.

The worksheets were incorporated into software, Excel version XP (Microsoft Office XP), and then recalculated as follows: Cell viability percentage $= \frac{(a - b)}{(c - b)} \times 100$ where a is the optical density (OD) value at 540 nm derived from a well added with *a* test chemical, *b* is the mean OD value at 540 nm derived from blank wells, and *c* is the mean OD value at 540 nm derived from control wells (i e., added culture medium as a test chemical).

Twelve replicate cell cultures were exposed to each

Table 1: Test materials, manufacturer and components

Products	Components	Manufacturer
Bisco aelite	Ethoxylated Bis-GMA, glass filler, triethyleneglycol dimethacrylate	Bisco Inc. IL, USA
Esthet X flow	Titanium dioxide, silica amorphous, barium boron fluoroalumino silicate glass, urethane modified Bis-GMA dimethacrylate	Dentsply Caulk, USA
Filtek™ Supreme XT flowable restorative	Bis-GMA, TEGDMA, dimethacrylate polymer UDMA	3M Espe, USA
Gradıa® direct flo	Fluoro-alumino silicate glass, Di-2-methacryloyloxyethyl 2,2,4-trimethylhexamethylene dicarbamate, silica, triethyleneglycol dimethacrylate	GC America Inc., USA
Tokuyama, Estelite® flow guıck	Silica-zirconia filler, Silica-titania filler, Bisphenol A polyethoxy methacrylate, triethylene glycol dimethacrylate, 1,6-bis (methacrylethyloxycarbonylamino) trimethyl hexane, camphorquinone	Tokuyama Dental Corporation, Japan
Clearfil majesty flow	Triethylene glycol dimethacrylate, hydrophobic aromatic dimethacrylate, silanated barium glass filler, Silanated colloidal silica, dl-camphorquinone, accelerators, pigments, others	Kuraray Medical Inc., Japan

Bis-GMA – Bisglycidyl methacrylate; TEGDMA – Triethylene glycol dimethacrylate; UDMA – Urethane dimethacrylate

concentration of a single material in at least two independent experiments. Cell survival in treated groups was compared with that in the untreated controls. Differences between median values were statistically analyzed using the one-way analysis of variance (ANOVA) and Tukey's HSD tests.

Cell survival of L929 cells was evaluated in methyl tetrazolium test after exposure to flowable composites. Data are expressed as a percentage of the control cultures. Cell survival rates were calculated from independent experimental cultures.

Cell morphology evaluation

Morphologic alteration of L929 cells was observed directly using an inverted microscope (TS100 Nikon Eclipse, Japan) (×10) and photographed by a camera (Nikon Eclipse, Tokyo, Japan).

RESULTS

According to the results of MTT test with L-929 fibroblasts demonstrated that all freshly prepared flowable composites did not reduce vital cell numbers (P>0.05) in comparison to control group [Figure 1].

DISCUSSION

The test materials was not found toxic compared with cell cultures exposed to the control group (P>0.05). The survival rate of test materials was ranked: Gradia® Direct Flo, GC, USA < Filtek™ Supreme XT Flowable Restorative, 3M Espe, USA < Esthet X Flow, Dentsply, Germany < Clearfil Majesty Flow, Kuraray Medical Inc., Japan < Estelite® Flow Quick, Tokuyama Dental Corporation, Japan < Bisco Aelite, Bisco Inc., USA.

Flowable composites are commonly used in dentistry because its ease of use and low viscosity. We should consider that these materials may release substances which may cause a reaction in adjacent dental pulp, gingiva and alveolar bone. There are several ways that materials may influence the health of soft-tissues by delivering water soluble components into saliva and the oral cavity as well as by interacting directly with adjacent tissues.[15]

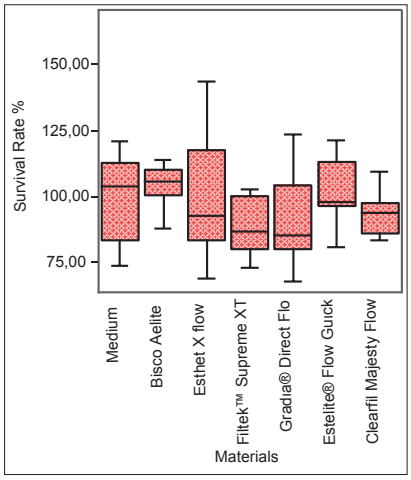

Figure 1: Descriptive values of cell viability by methyltetrazolium test assay

L929 fibrolast cells were used in the current study because they are an ISO-approved cell type and are most common cell type in the pulp, which would be the target of the chemicals released from flowable materials if the odontoblastic layer had been destroyed.[13] Because of its excellent reproducibility, the L929 cell line was preferred to primary gingival fibroblasts[16] (cytotoxicity was tested using the direct method where the material specimens were in direct contact with the cells in a biological solution). L929 fibroblasts were investigated with MTT assay. The MTT assay is a good indicator of cell viability. This assay is based on the reduction of the MTT by those cells that remain viable after exposure and incubation with a test chemical or device.

The aim of this study was to investigate the cytotoxicity of 6 different flowable composites. Present test shows that all flowable composites have no toxic effect on L929 cells. The cytotoxicity of the flowable composites may result from its chemical composition, which has more monomer and less filler. Initial investigations concerning the issue of biocompatibility soon made clear that composites can liberate a wide spectrum of residual compounds due to deficiencies in monomer-conversion during polymerization.[17] Unbound free monomers seem to be directly responsible for the cytotoxicity of resin composites on pulp and gingival cells, and they are probably also implicated in the allergic potential of the material.[18] Apart from the elution of residual monomers immediately after placement, diverse chemical (e.g., solvolysis, hydrolysis, and alcoholysis), and physical (e.g., wear and erosion) reactions at the salivary surface of fillings furthermore promote a constant disintegration and dissolution of resin polymers.[19]

Un bound monomers and/or additives are eluted by solvents or polymer degradation within the 1st h after initial polymerization.[20] Based on the high-performance liquid chromatography results, it can be presumed that the cytotoxicity of the materials could be related to the amount of triethylene glycol dimethacrylate (TEGDMA) that was leached from flowable composites[21] and residual uncured monomer or oligomer.[22] Leaching from composites resins is essentially complete in 24 h.[18] Therefore, most toxic effects from resin composites occur during the 1st 24 h. Resin-based materials continue to release measurable amounts of composite components beyond the initial 24-h period although the rate of release decreases with time.[23]

CONCLUSIONS

The results of this study indicate the flowable composites were no toxic characteristic. This study revealed important information for the clinical applications of flowable composites in dentistry.

REFERENCES

1. Wheeler JC, Woods JA, Cox MJ, Cantrell RW, Watkins FH, Edlich RF. Evolution of hydrogel polymers as contact lenses, surface coatings, dressings, and drug delivery systems. J Long Term Eff Med Implants 1996;6:207-17.
2. Truffier-Boutry D, Demoustier-Champagne S, Devaux J, Biebuyck JJ, Mestdagh M, Larbanois P, *et al.* A physico-chemical explanation of the post-polymerization shrinkage in dental resins. Dent Mater 2006;22:405-12.
3. Reichl FX, Simon S, Esters M, Seiss M, Kehe K, Kleinsasser N, *et al.* Cytotoxicity of dental composite (co) monomers and the amalgam component Hg (2+) in human gingival fibroblasts. Arch Toxicol 2006;80:465-72.
4. Sustercic D, Cevc P, Funduk N, Pintar MM. Determination of curing time in visible-light-cured composite resins of different thickness by electron paramagnetic resonance. J Mater Sci Mater Med 1997;8:507-10.
5. Michelsen VB, Moe G, Strøm MB, Jensen E, Lygre H. Quantitative analysis of TEGDMA and HEMA eluted into saliva from two dental composites by use of GC/MS and tailor-made internal standards. Dent Mater 2008;24:724-31.
6. Zyrianov VV, Sumovskaya AY, Shostak AA. Application of electron spin resonance for evaluation of the level of free radicals in the myometrium in full-term pregnancy with normal labour and uterine inertia. J Biosci 2003;28:19-21.
7. Tuusa SM, Puska MA, Lassila LV, Vallittu PK. Residual monomers released from glass-fibre-reinforced composite photopolymerised in contact with bone and blood. J Mater Sci Mater Med 2005;16:15-20.
8. Durner J, Walther UI, Zaspel J, Hickel R, Reichl FX. Metabolism of TEGDMA and HEMA in human cells. Biomaterials 2010;31:818-23.
9. Mutal L, Gani O. Presence of pores and vacuoles in set endodontic sealers. Int Endod J 2005;38:690-6.
10. Milhem MM, Al-Hiyasat AS, Darmani H. Toxicity testing of restorative dental materials using brine shrimp larvae (Artemia salina). J Appl Oral Sci 2008;16:297-301.
11. Park SB, Son WS, Ko CC, García-Godoy F, Park MG, Kim HI, *et al.* Influence of flowable resins on the shear bond strength of orthodontic brackets. Dent Mater J 2009;28:730-4.
12. Schedle A, Franz A, Rausch-Fan X, Spittler A, Lucas T, Samorapoompichit P, *et al.* Cytotoxic effects of dental composites, adhesive substances, compomers and cements. Dent Mater 1998;14:429-40.
13. Wataha JC, Lockwood PE, Bouillaguet S, Noda M. *In vitro* biological response to core and flowable dental restorative materials. Dent Mater 2003;19:25-31.
14. Wennberg A, Mjör IA, Hensten-Pettersen A. Biological evaluation of dental restorative materials – A comparison of different test methods. J Biomed Mater Res 1983;17:23-36.
15. Huang TH, Tsai CY, Chen SL, Kao CT. An evaluation of the cytotoxic effects of orthodontic bonding adhesives upon a primary human oral gingival fibroblast culture and a permanent, human oral cancer-cell line. J Biomed Mater Res 2002;63:814-21.
16. Jonke E, Franz A, Freudenthaler J, König F, Bantleon HP, Schedle A. Cytotoxicity and shear bond strength of four orthodontic adhesive systems. Eur J Orthod 2008;30:495-502.
17. Lovell LG, Newman SM, Donaldson MM, Bowman CN. The effect of light intensity on double bond conversion and flexural strength of a model, unfilled dental resin. Dent Mater 2003;19:458-65.
18. Ferracane JL. Elution of leachable components from composites. J Oral Rehabil 1994;21:441-52.
19. Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. Crit Rev Oral Biol Med 2001;12:136-51.
20. Goldberg M. *In vitro* and *in vivo* studies on the toxicity of dental resin components: A review. Clin Oral Investig 2008;12:1-8.
21. Al-Hiyasat AS, Darmani H, Milhem MM. Cytotoxicity evaluation of dental resin composites and their flowable derivatives. Clin Oral Investig 2005;9:21-5.
22. Caughman WF, Caughman GB, Shiflett RA, Rueggeberg F, Schuster GS. Correlation of cytotoxicity, filler loading and curing time of dental composites. Biomaterials 1991;12:737-40.
23. Wataha JC, Hanks CT, Strawn SE, Fat JC. Cytotoxicity of components of resins and other dental restorative materials. J Oral Rehabil 1994;21:453-62.

How to cite this article: Yalcin M, Ulker M, Ulker E, Sengun A. Evaluation of cytotoxicity of six different flowable composites with the methyl tetrazolium test method. Eur J Gen Dent 2013;2:292-5.

Source of Support: Nil, **Conflict of Interest:** None declared.