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Cytotoxicity Evaluation of Two Different Composites with/without Fibers and One Nanohybrid Composite

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In this study, cytotoxicity of two different composites with/without fibers (Adoro/Vectris and SculpturePlus/FiberKor) and one nanohybrid composite (Artglass) were investigated and compared. Composites used in the study were prepared as cylindrical discs of 2 mm depth and 8 mm diameter according to ISO 10993 recommendation. Adoro/Vectris and SculpturePlus/FiberKor groups were divided into composite, fiber, and composite+fiber groups. Agar diffusion method was employed, and cytotoxicity rankings were determined using lysis index scores. For statistical analysis, Kruskal-Wallis and Mann-Whitney U tests were used. Amongst the composites, Adoro was found to be less cytotoxic than Sculpture Plus and Artglass materials − which were of the same cytotoxicity ranking. Between the fiber and composite materials, the former were found to be more cytotoxic than the latter; in particular, Vectris was found to be more cytotoxic than FiberKor. It was observed that upon combining with the fibers, the cytotoxic effect of the composites increased. This cytotoxicity enhancement was manifested as an additional effect in Adoro/Vectris group but as a synergistic effect in SculpturePlus/FiberKor group.

Key words: Cytotoxicity, Fiber-reinforced systems, Nanohybrid composite

INTRODUCTION

In prosthetic dentistry, base metal alloys have been used widely in fixed partial denture construction. However, several disadvantages − such as like poor biocompatibility, high corrosion, color change in abutment teeth, and abutment material retention − have goaded researchers to search for alternative materials. Amongst the myriad of presently identified alternative materials, fiber reinforced composites (FRCs) have come into prominence due to their excellent esthetic and mechanical characteristics. Despite these known benefits, a deeper knowledge of the advantages and limitations of these materials would definitely enable clinicians to choose the most suitable fiber reinforced composite − in terms of durability and biocompatibility − in each varied clinical situation[1].

Resin-based prosthetic materials used in dentistry often display cytotoxic properties due to incomplete polymerization[2]. It should be emphasized that in the development of any restorative biomaterial, biocompatibility is another mandatory consideration in addition to durability, esthetics, and ease of clinical manuplation[3].

Currently, two commercially preimpregnated FRC materials are available: FiberKor (Jeneric/Pentron, Wallingford, CT, USA) with its veneering particulate composite, Sculpture Plus, and Vectris (Ivoclar/Vivadent, Schaan, Liechtenstein) with its veneering particulate composite, Adoro. FiberKor is composed of parallel S-glass fibers (made up of silica, magnesia, and alumina) which are preimpregnated with Bis-GMA, PCDMA, and EDMA. Sculpture Plus particulate composite is composed of PCDMA, EDMA, and TEGDMA, with barium glass and fumed silica as filler particles. As for the particulate composite veneering material, it is 75% filled by weight with particles of 0.6-μm average size and is cured by light and heat[4].

As for Vectris, its R-glass fibers are distinguished by three different orientations and which are embedded in Bis -GMA, DDDMA, and UDMA. Vectris Pontic, which has parallel glass fibers similar to the FiberKor orientation, was used in this study. The Adoro particulate composite veneering material is composed of the same basic resin matrix with the addition of silicon dioxide as a filler[4]. Adoro is 72% filled by weight, and is light- and heat-cured[5]. Another prosthetic composite, Artglass, is composed of Bis-GMA and TEGDMA with silicon dioxide, barium alumina, and silica glass as filler particles. It has an average particle size of 0.7 μm, is 68% filled by weight, and is light-cured[6].

With polymerized resin-based dental materials, cytotoxicity arises from the elution of residual monomers and other leachable components, such as initiators and activators. Elutable substances may be created during the clinical service of a resin composite restoration due to chemical and mechanical degradation. Compositional changes occur with time due to intraoral surface interactions with saliva, plaque

acids, and food[9]. The components of resin composites are hazardous in that they all cause significant toxicity in direct contact with fibroblasts. Wataha *et al.*[10] indicated that these components have diverse potencies, and that the risks they pose to the dental pulp depend upon the quantity which permeates the dentin and which accumulates in the pulp.

Cell culture studies are frequently used to assess the cytotoxicity of resin-based materials, their elutes or components[11,12]. Variable levels of cytotoxicity have been shown to be induced by several resin-based materials and their components. However, few studies have evaluated the cytotoxic effects of fiber-reinforced composites.

It should also be highlighted that a fiber substructure could be set free because of careless treatment/handling by technician or dental practitioner. Against this potential health hazard that could be created by set free fibers, the aim of this study was to investigate and compare the cytotoxic effects of two different composites with/without fibers and one nanohybrid composite.

MATERIALS AND METHODS

Specimen preparation

Table 1 shows the materials used in this study, which were obtained directly from their respective manufacturers. "Dentin" composites corresponding to Vita shade A3 were selected for this study. To facilitate investigation and comparison, Adoro/Vectris and SculpturePlus/FiberKor groups were divided into (a) composite, (b) fiber, and (c) composite+fiber groups. The test was then conducted with five composite specimens, five fiber specimens, and five composite+fiber specimens (Table 2). Each composite+fiber group was formed by placing five fiber bundles at the bottom of the mold and then placing the composite on them.

For each test group, five cylindrical specimens were prepared by placing the material into a stainless steel mold (2 mm deep and 8 mm in diameter). A thin Mylar strip was placed on top of the specimen, followed by a 1-mm glass slide on top of the mold to extrude excess composite material and to eliminate air bubbles. Each specimen was polymerized according to the manufacturer's recommendations. Specimens were then stored for 24 hours at 37℃ and 100% relative humidity.

Cell culture

L929 cells (Alum Institute Culture Collection, Ankara, Turkey) were cultured in 100 ml DMEM (Dulbecco's modified Eagle medium) (Sigma Aldrich Cheme, Germany) supplemented with 2 ml L-Glutamine (Biochrom KG, Berlin, Germany), 2.2 g/L sodium bicarbonate (Sigma, MO), 0.1 mM non-essential amino acids (Sigma, MO), 1 mM sodium

Table 1 Materials used in this study

Material	Manufacturer	Matrix	Filler	Filler size and amount
Artglass	Heraeus/Kulzer, Dormagen, Germany	Bis-GMA, TEGDMA	Boron silicate, barium aluminum	0.7 μm, 68 wt%
Sculpture Plus	Jeneric/Pentron Inc., Wallingford, CT, USA	TEGDMA, EDMA, PCDMA	BaO, SiO_2, zirconium silicate	0.6 μm, 75 wt%
FiberKor	Jeneric/Pentron Inc., Wallingford, CT, USA	Bis-GMA, EDMA, PCDMA	–	–
Adoro	Ivoclar Vivadent, Schaan, Liechtenstein	UDMA	Silicon dioxide	72 wt%
Vectris	Ivoclar Vivadent, Schaan, Liechtenstein	UDMA, Bis-GMA, DDDMA	–	–

Bis-GMA (Bisglycidyl methacrylate), TEGDMA (Triethyleneglycol dimethacrylate), EDMA (Ethylene dimethacrylate), PCDMA (Polycarbonate dimethacrylate), UDMA (Urethane dimethacrylate), DDDMA (Decandiol dimethacrylate)

Table 2 Polymerization methods of the materials

Material	Manufacturer	Polymerization method and time
Adoro/Vectris	Ivoclar Vivadent, Schaan, Liechtenstein	10 seconds in Targis Quick + 25 minutes in Lumamat 100 at 95℃ heat
SculpturePlus/FiberKor	Jeneric/Pentron, Wallingford, CT, USA	3 minutes in Cure-Lite Plus + 20 minutes in Conquest Curing Unit
Artglass	Heraeus Kulzer, Wehrheim, Germany	180 seconds of Xenon strobe light in Artglass UniXS Curing Unit

pyruvate (Sigma, MO), 4 ml fetal calf serum (Biochrom KG, Berlin, Germany), and 1 ml penicilin/streptomycin (10 g/ml) (Biochrom KG, Berlin, Germany). L929 cells were then seeded on tissue culture dishes (15 mm deep and 35 mm in diameter) and incubated in an incubator set (37℃, 5% CO_2).

Agar diffusion method

Cytotoxicity tests which employed agar diffusion method were performed according to ISO 10993-5 recommendation[13]. 0.5 ml of 1% neutral red was added to each tissue dish and incubated for 30 minutes. Excess dye was removed, and test specimens were placed on the agar surface so that the bottom surface of each specimen was in contact with agar. A phenol-impregnated blotting paper was used as positive control and a DMEM-impregnated blotting paper as negative control. The dishes were incubated for 24 hours. Thereafter, the cultures were examined under a microscope by one examiner experienced in the use of this evaluation technique. It should be noted that the identity of the specimens was not made known to the examiner.

Decolorized zones and cell lysis around and/or under the specimens were evaluated according to ISO 10993-13 standard[14]. Five specimens of each group were studied, and each test was repeated twice using the same test specimens. Cell lysis is defined as loss of cell membrane integrity, which is visible in light microscopy. In this study, cell lysis was scored as follows: 0=no cell lysis detectable; 1=less than 20% cell lysis; 2=20% to 40% cell lysis; 3=>40% to <60% cell lysis; 4=60% to 80% cell lysis; 5=more than 80% cell lysis. For each specimen, one score was given and the median score value for all parallels from each specimen was calculated for the lysis zone. Cytotoxicity was then classified as follows: 0−0.5= non cytotoxic; 0.6−1.9=mildly cytotoxic; 2.0−3.9= moderately cytotoxic; 4.0−5.0=markedly cytotoxic. The median (instead of the mean) was calculated to describe the central tendency of the scores because the results were expressed as an index in a ranking scale.

Statistical analysis

Statistical tests were performed using SPSS (Version 9.0, SPSS Inc., Chicago, IL, USA). Data were analyzed statistically using Kruskal-Wallis one-way analysis of variance and Mann-Whitney U-test. Level of significance was set at $p=0.05$.

RESULTS

Fig. 1 shows the results of the cytotoxic effects of the tested materials as lysis index scores. All the materials studied were ranked between mildly cytotoxic and markedly cytotoxic. In the composite group, the cytotoxic ranking of Adoro was significantly lower than those of SculpturePlus and Artglass − which had the same cytotoxic ranking (moderately cytotoxic). Comparison of the cytotoxic rankings between composite and fiber groups showed statistically significant differences ($p<0.05$), where the fibers were found to be more cytotoxic than composites. Among the fibers, Vectris was found to be more cytotoxic than FiberKor. Further, it was observed that the cytotoxic effect of the composites increased after combining with fibers. This cytotoxicity enhancement was manifested as an additional effect in Adoro/Vectris group but as a synergistic effect in SculpturePlus/FiberKor group.

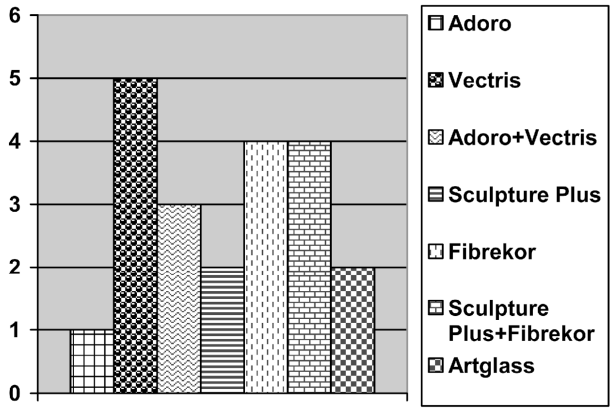

Fig. 1 Lysis index scores of materials tested.

On the overall, lysis index score was 5 (markedly cytotoxic) in positive control group and 0 (non cytotoxic) in negative control group.

DISCUSSION

In laboratory situations, leaching is essentially complete in 24 hours[7] − which means that most toxic effects from resin composites occur during the first 24 hours. However, while initial leaching may happen quickly, continued release of materials may occur. Resin-based materials continue to release measurable amounts of composite components beyond the initial 24-hour period although the rate of release decreases with time[10].

Due to the hydrophilic nature of TEGDMA, significant amounts of this substance leach into an aqueous environment, such as the oral cavity. Thus, it was hypothesized that TEGDMA frequently interferes with oral and/or systemic tissues[15]. Geurtsen *et al.*[11] reported that TEGDMA might cause microsomal peroxidation and might act on liposomes as a surfactant-like agent solubilizing the lipid bilayer of membranes. Further, TEGDMA was reported as an irritant to most tissues in National Institute of Occupational Safety and Health 1995 report[16].

In our study, two materials − Sculpture Plus and FiberKor − which differed only in terms of Bis-GMA and TEGDMA as a matrix monomer, indicated lysis index scores of 2 and 4 respectively. As a result of this finding, it could be concluded that the cytotoxic potential of Bis-GMA was two times greater than that of TEGDMA. Moreover, the lysis index score of Artglass pointed out the antagonistic effect of Bis-GMA and TEGDMA. However, this possible antagonistic effect was not explicitly exhibited in SculpturePlus/FiberKor group which included both Bis-GMA and TEGDMA.

According to Yoshii[17], acrylates were evaluated to be more toxic than their corresponding methacrylates. Based on their findings, the cytotoxicity ranking of monomers was BisGMA> UDMA>HEMA>MMA. They reported that dimethacrylates with 14 or fewer oxyethylene chains showed similar cytotoxicity, while dimethacrylates with 23 oxyethylene chains showed lower cytotoxicity. In our study, Adoro was found to be less cytotoxic in comparison with Artglass according to their compositions − and this finding agreed with that of Yoshii[17]. From the results of our study, it would appear that the Bis-GMA content was responsible for the "moderately cytotoxic" level of Artglass.

Theilig *et al.*[18] reported that "lipophilic" matrix monomer Bis-GMA inhibited cell growth at significantly lower concentrations than the "hydrophilic" co-monomer TEGDMA. This observation was confirmed by previous studies[11,19], whereby Bis-GMA indicated a higher cytotoxic potency than TEGDMA. As a case in point, Ratanasathien *et al.*[19] ranked the cytotoxic effects of the evaluated monomers as Bis-GMA>UDMA>TEGDMA>HEMA. In another study, Hanks *et al.*[20] tested the cytotoxicity of 11 components toward Balb/c 3T3 mouse fibroblasts. They reported that the magnitude of potency variations among the evaluated resin components was more than 100 times. The high activity of Bis-GMA may be due to its migration into the lipid bilayer of phospholipid containing cholesterol in cell membranes[9]. However, other factors such as electrical charge[17] and cationic charge density[21] may also affect interaction with the cell membrane and therefore the resultant cell damage.

Our entire findings were similar with those of Geurtsen *et al.*[11], Yoshii[17], and Theilig *et al.*[18]. However, our results did not agree with those of Ratanasathien *et al.*[19] concerning the cytotoxicity ranking of UDMA. In our study, Adoro − which had UDMA polymer matrix − was found to be the least cytotoxic material. As for Sculpture Plus and FiberKor which had a similar matrix, they differed only in TEGDMA and Bis-GMA content. This chemical structural difference hence caused Sculpture Plus to be moderately cytotoxic, but FiberKor to be markedly cytotoxic. With Vectris and FiberKor, their lysis index scores were 5 and 4 respectively. This could be explained by the higher cytotoxic potential of DDDMA. Furthermore, Adoro/Vectris was found to be less cytotoxic than Vectris. This could be explained by the less Vectris surface area in Adoro/Vectris as compared to the available surface area in Vectris group alone.

By means of agar diffusion method too, Vallittu and Ekstrand[12] evaluated the cytotoxic effects of PMMA and E-glass fibers used for reinforcement. It was found that both of them were non-cytotoxic, which was contradictory to our findings. This could be explained by the possible antagonistic cytotoxic effect between PMMA and E-glass fibers. In this study, both fibers tested were found to be cytotoxic − which could be explained by the Bis-GMA content in their compositions.

Other parameters like residual monomer amount and curing degree were not considered in order to standardize the study. Moreover, standard deviation values were recorded as 0 because in each test group, all samples gave the same index score.

CONCLUSIONS

Within the limitations of this study, the following *in vitro* results were obtained:

1. Adoro was found to be less cytotoxic than Sculpture Plus and Artglass materials, which had the same cytotoxic ranking.

2. Fiber materials were found to be more cytotoxic than composite materials.

3. Vectris was found to be more cytotoxic than FiberKor.

4. The cytotoxic effect of composites increased after combining with fibers. This cytotoxicity enhancement was manifested as an additional effect in Adoro/Vectris group but as a synergistic effect in SculpturePlus/FiberKor group.

With respect to these findings, it was determined that due to the high cytotoxicity of fiber-reinforced composite systems, direct contact between fiber material and intraoral tissues should be avoided during clinical usage and treatment.

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