IN VITRO COMPARISON OF THE EFFECTS OF DENTAL FILLING MATERIALS ON MOUSE FIBROBLASTS

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ABSTRACT
The choice of filling material is an important factor in the clinical success of root coverage. Therefore, the cytotoxicity of filling materials must be investigated to ensure a safe biological response. The aim of this study was to compare the response of L929 mouse fibroblasts to several glass ionomer cements (GICs), i.e. conventional GIC, resin-modified glass ionomer cement (RMGIC) and polyacid-modified resin composite (PMRC), using three different methods. 1) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, 2) agar diffusion test, 3) scanning electron microscopy. The MTT test demonstrated that L929 fibroblast attachment to polyacid-modified resin composite filling material was excessive on day 1, but decreased on day 3 (P < 0.05). When the cell proliferation percentages of all filling materials were compared with those of the control group (100%) on days 1 and 3, it was observed that statistically significant differences existed (P < 0.05). Although resin-modified glass ionomer cement was determined to be slightly cytotoxic according to the results of agar diffusion tests, differences between the groups were not significant (P > 0.05).
In addition to our in vitro research results, chemical surface analysis techniques, measurement of the release of elements, physical surface characterization and analysis of microstructure and porosity can provide a better understanding of the biological response to filling materials.


Keywords: fibroblast, MTT, cytotoxicity, glass ionomer cement, cell morphology

Introduction
Gingival recession is defined as the displacement of the marginal gingival tissue apical to the cement-enamel junction with exposure of the root surface to the oral environment (5). Gingival recession can cause root exposure which may result in cervical abrasion, root caries, root sensitivity, and compromised esthetics (1, 29, 38, 44, 52). A number of periodontal “plastic surgery” procedures are used to cover exposed roots: connective tissue graft, free gingival grafts, coronally positioned flaps, lateral sliding flaps, and the use of barrier membranes is called guided tissue regeneration (GTR) (39, 43). The success of the procedure depends on many factors, e.g. the experience, the nature of the gingival defect and anatomical considerations (6). Extensive gingival recessions associated with deep caries or cervical abrasions are commonly observed in the dental practice. In such cases the combination of an adhesive restorative material and mucogingival surgical coverage might be necessary (7). Recent dental studies (28, 30, 45) investigating different restorative materials including dental composite resins, glass ionomer cements and compositors that could be used before surgical coverage on exposed root surfaces affected by deep caries or cervical abrasions have produced clinically and histologically successful results.

Glass ionomer cements (GICs) are materials made of calcium, strontium aluminosilicate glass powder (base) combined with a water-soluble polymer (acid), can be categorized as restorative cements and exhibit several clinical advantages compared with dental composite resins (61). GICs are commonly classified into five principal types: conventional glass ionomer cements, resin-modified glass ionomer cements (conventional with addition of hydroxyethylmethacrylate), hybrid ionomer cements (also known as dual-cured glass ionomer cements), tri-cure glass ionomer cements, and metal-reinforced glass ionomer cements.

Compomers, known as polyacid-modified resin composites, are fluoride-containing resin composites. They were introduced in the early 1990s as a hybrid of dental composites and glass ionomer cement (32). It is known that, these restorative materials contain a great variety of monomers and additives (18). Some of these components, owing to the complex chemical composition and incomplete monomer–polymer conversion, leach out from resin-based restorative materials into the oral environment (55, 57). Such leaching may cause adverse effects (11). In vitro and in vivo studies have clearly identified that some components of restorative materials are...
cytotoxic (22, 31, 51, 54). The reviewed studies indicate that the cytotoxicity levels of resin-based restorative materials vary according to their chemical composition, leaching medium, and the amount and type of the components that can be extracted from the materials (12). Although the cytotoxicity of GICs is generally reported (10) to be minimal, individual components of resin materials have been shown to be cytotoxic (17). Despite the increasing interest in the biocompatibility of dental materials, there are many contradictory reports.

Investigations on the cytotoxicity of various restorative materials were expanded to the quantitative analyses of various biological endpoints in different cell types (50, 58). Owing to their reproducible growth rates and biological responses, continuous cell lines, such as L929 mouse fibroblasts, are routinely used for the testing of the cytotoxicity of dental materials. In addition, these cells are recommended by international standards for the testing of medical devices used in dentistry because of the ease to control cell culture conditions (19, 20). Furthermore, it has been shown that cultures of the continuous L929 cell line are more sensitive than the primary human gingival fibroblasts (50, 58). Despite the wide use of conventional glass ionomer cements, resin-modified glass ionomer cement and composites for root coverage, only a few studies have compared their cytotoxic effect on L929 mouse fibroblasts.

The aim of this study was to compare the effects of conventional GIC (Ketac Molar Quick Aplicap), resin-modified GIC (Photac Quick) and compomer (Dyract Extra) on fibroblast cell morphology and proliferation and to evaluate their cytotoxicity in L929 mouse fibroblasts.

**Materials and Methods**

**Cell Cultures**

To investigate the effects of restorative materials on cell attachment and proliferation, cell culture studies were carried out with L929 mouse fibroblasts. The L929 cell line was obtained from the Foot and Mouth Disease Institute (Ankara, TÜRKİYE). Cells used in these studies were between passages 6 and 11. Cells were cultured in 100 ml of Dulbecco’s modified Eagle’s medium (DMEM) (Biological Industries, Israel, Lot no: 843064), supplemented with 4 ml of 10% fetal bovine serum (FBS) (Biological Industries, Israel, Lot no: 316724), 1 ml of penicillin-streptomycin (1000 IU/10000 mg/ml) (Sigma Aldrich, Germany) at 37 °C in a humidified atmosphere containing 5% CO₂. The culture medium was changed every 3 to 4 days.

**Test specimens**

The following GICs were examined (for composition see Table 1): Ketac Molar Quick Aplicap (conventional glass ionomer cement), Photac Quick (resin-modified glass ionomer cement, RMGIC), Dyract Extra (polyacid-modified resin composite, PMRC).

The GICs were mixed according to the respective manufacturers’ instructions. To ensure the compliance of the tests, applied for the preparation of discs, with ISO 10993-5 standards, a cylindrical Teflon mold compressed between two glass layers was used. From all GICs, equally sized discs (2 mm thick and 6 mm in diameter) were fabricated under aseptic conditions by packing the material after mixing in a Teflon washer (internal diameter of 6×2 mm), and were compressed between two glass slides to generate even thickness of material.

In addition, glass cover slips with a free surface of 30 mm², and thickness of 2 mm were used as positive controls in all experimental conditions throughout the study. In order not to confuse the surface worked on, a notch was made using a diamond bur.

**Microplate preparation**

Three different samples of filling material and the controls were distributed into microplate wells. Similar to the methodology of the passage procedure, active log phase L929 cells displaying percent confluence of 90-95% were detached from the surface of the flask by means of trypsinization, and a cell suspension was prepared by adding DMEM. This cell suspension was distributed into 24-well microplates such that each well contained 100 microlitres of the suspension (2×10⁵ cells/ml).

The microplates were incubated at 37 °C in an atmosphere containing 5% CO₂ for 24-72 hours for the attachment of cells to the surface of the discs. At the end of the incubation period, the content of each well was aspirated and the surfaces of the discs were washed with 100 µl of PBS, avoiding any excessive pressure, so as to remove unattached cells. After the PBS in the wells was removed, MTT solution was added.

**Cell proliferation**

The effect of Ketac Molar Quick Aplicap (GIC), Phopac Quick (RMGIC) and Dyract Extra (PMRC) on the proliferation of L929 mouse fibroblasts was assessed with 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT) formazan and crystal violet assays. MTT (Sigma-Aldrich Inc., St. Louis, Missouri, USA) was mixed with phenol-free DMEM and homogenized to prepare a 5 mg/ml MTT solution. 10 µl of MTT solution were added to each well on the microplate. The microplate was incubated at 37 °C for 4 hours. At the end of the incubation period, the fluids containing MTT solution were aspirated to ensure the elimination of MTT from the environment. Then, each well was added 100 µl of dimethyl sulfoxide (DMSO) at room temperature. After the formazan crystals were completely solubilized and a blue/violet colour developed, the discs that had been placed into the wells were removed. Subsequently, the plates were placed in an ELISA reader (Bio-tek EL 312, Bio-tec Instruments Winooski, VT, USA). The presence of formazan was determined by reading the optical density. Soluble formazan absorbance was recorded using an ELISA plate reader at 450 nm. Values measured in the optical reader were used to calculate the cell proliferation percentages of the test materials by the following formula:

\[
\text{Percentage of cell proliferation} = \frac{(A - B)}{(C - B)} \times 100
\]

where \(A\) is the mean of the optical values measured for the test samples in the wells; \(B\) is the mean of the optical values
Filling materials used in the present study

<table>
<thead>
<tr>
<th>Filling Materials</th>
<th>Contents</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketac Molar Quick Aplicap</td>
<td>Al-Ca-La fluorosilicate glass, 5% copolimeracid (acrylic and maleic acid), polyalcoenoic acid, tartaric acid, water.</td>
<td>3M/ESPE GmbH, Seefeld, Germany</td>
</tr>
<tr>
<td>Photac Quick</td>
<td>Na-Ca-Al-La fluorosilicate -glass, Activator (Amin), Glass ionomer monomers ve oligomers, Copolimer acids (acrylic and maleic acids), camphoroquinone, stabilizer, water</td>
<td>3M/ESPE GmbH, Seefeld, Germany</td>
</tr>
<tr>
<td>Dyract Extra</td>
<td>Bisphenol-A, dimethacrylate, urethane resin, tetraethylene glycol dimethacrylate (TEGDMA), trimethylol propane trimethacrylate (TMPTMA), camphoroquinone, dimethylaminbenzoic acid, ethyl</td>
<td>Dentsply DeTrey, Konstanz, Germany</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Lysis indices</th>
<th>Percentage of damaged cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Damaged cells were not observed in the light-coloured region.</td>
</tr>
<tr>
<td>1</td>
<td>Damaged cells were less than 20% of the cells in the light coloured region.</td>
</tr>
<tr>
<td>2</td>
<td>Damaged cells were 20-40% of the cells in the light coloured region.</td>
</tr>
<tr>
<td>3</td>
<td>Damaged cells were 40-60% of the cells in the light coloured region.</td>
</tr>
<tr>
<td>4</td>
<td>Damaged cells were 60-80% of the cells in the light coloured region.</td>
</tr>
<tr>
<td>5</td>
<td>Damaged cells were more than 80% of the cells in the light coloured region.</td>
</tr>
</tbody>
</table>

Lysis indices and the percentages of the cells damaged as a result of the cytotoxic effect of the tested materials.

**TABLE 3**

Percentage of cell proliferation of L929 cells following exposure to restorative materials for the 1st and 3rd days

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>GIC Group</th>
<th>RMGIC Group</th>
<th>PMRC Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\overline{X} \pm S$</td>
<td>$\overline{X} \pm S$</td>
<td>$\overline{X} \pm S$</td>
</tr>
<tr>
<td></td>
<td>Median (Min-Max) (25%-75%)</td>
<td>Median (Min-Max) (25%-75%)</td>
<td>Median (Min-Max) (25%-75%)</td>
</tr>
<tr>
<td>24 hours</td>
<td>53.8 ± 5.07</td>
<td>43 ± 9.92</td>
<td>80.3 ± 23.7</td>
</tr>
<tr>
<td></td>
<td>53 (49-58)$^a$</td>
<td>42 (32.75-50.5)$^a$</td>
<td>77.50 (61-91.75)$^b$</td>
</tr>
<tr>
<td>72 hours</td>
<td>56 ± 11.3</td>
<td>45.7 ± 6.14</td>
<td>58.3 ± 6.41</td>
</tr>
<tr>
<td></td>
<td>56.5 (48.75-63)$^a$</td>
<td>47.5 (40-50.25)$^b$</td>
<td>58 (52-63.75)$^a$</td>
</tr>
<tr>
<td>P</td>
<td>0.597</td>
<td>0.506</td>
<td>0.02$^a$</td>
</tr>
</tbody>
</table>

$^a,b$ demonstrate the statistically significant differences between the test groups; $^*$ denotes significant differences between the days.

**TABLE 4**

Cytotoxicity of the three filling materials

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Lysis Index</th>
<th>Cytotoxic Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control Group</td>
<td>5</td>
<td>Severely cytotoxic</td>
</tr>
<tr>
<td>GIC Group</td>
<td>0</td>
<td>Non-cytotoxic</td>
</tr>
<tr>
<td>RMGIC Group</td>
<td>1</td>
<td>Slightly cytotoxic</td>
</tr>
<tr>
<td>PMRC Group</td>
<td>0</td>
<td>Non-cytotoxic</td>
</tr>
<tr>
<td>Negative Control Group</td>
<td>0</td>
<td>Non-cytotoxic</td>
</tr>
</tbody>
</table>
measured in the blank wells; and \( C \) is the mean of the optical values of the positive control group.

**Cytotoxicity assay**
The cytotoxicity of Ketac Molar Quick Aplicap (GIC), Phopac Quick (RMGIC) and Dyract Extra (PMRC) in L929 mouse fibroblasts was determined using the agar diffusion test. In this test, using the agar layer as a barrier, it was aimed to determine the potential indirect toxic effect of the substances leaching from the filling materials. For the determination of cytotoxicity by the agar diffusion method, the 10993-5 numbered ISO protocol was followed (21). The cytotoxicity degrees of the samples evaluated and photographed under light microscope (Nikon FDX-35, Japan) were calculated using lysis-indices (Table 2).

**Cell morphology**
Attachment and morphology of L929 mouse fibroblasts was assessed in two specimens of each group, using a scanning electron microscope (LEICA-LEO 440). For this purpose, Ketac Molar Quick Aplicap (GIC), Phopac Quick (RMGIC) and Dyract Extra (PMRC) discs and control glass cover slips were placed on the bottom of a 12-well culture plate. L929 mouse fibroblasts were seeded into the wells at a density of \( 3 \times 10^4 \) cells per well in DMEM medium containing 10% FBS. After cells were incubated for 24 and 72 hours in contact with the materials, they were fixed for 5 minutes in 0.1% glutaraldehyde solution. After aspiration of the medium, they were postfixed for 6 hours in 4% glutaraldehyde solution. Then, specimens were washed three times with phosphate buffered saline and dehydrated with a series of increasing ethanol concentrations. The specimens were then critical-point dried with \( \text{CO}_2 \), carefully mounted onto copper stubs, and coated with a very thin film of gold/palladium before SEM examination. Cell attachment and viability in each experimental condition were assessed qualitatively.

**Statistical evaluation**
Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS) ver. 15.0 software. Statistical comparisons were made by one-way analysis of variance (ANOVA) and the Kruscal-Wallis test, which is a non-parametric method. Tukey’s multiple comparison test, Duncan’s multiple comparison test, paired t-test and Mann-Whitney U-tests were applied for post hoc evaluations of differences between treatment groups. In all statistical evaluation \( P < 0.05 \) was considered as statistically significant.

**Results and Discussion**
Multiple studies are available on the physical and mechanical properties and chemical structure of restorative filling materials (24, 26, 37, 46, 50). However, only a very limited number of studies investigate the correlation between the materials that are used for the treatment of root caries and non-curious cervical lesions and the gingival tissue, and their effects on fibroblast attachment (13). Two major risks associated with the use of newly developed dental filling materials are toxicity and allergy. Both involve the release of biologically active substances from the material. The likelihood that these materials may cause systemic toxicity is very low, as the level of leakage is not high enough to cause systemic reaction in the body. However, this level of leakage may cause toxicity in the dental pulp, periodontal and periapical tissues, and oral mucosa as the contact of restorative materials with periodontal tissues may be prolonged (8). For this reason, in the present study, three different filling materials, namely, glass ionomer cement, resin-modified glass ionomer cement and polyacid-modified resin composite were tested using the MTT assay for their effects on fibroblast attachment and the agar diffusion test for their biocompatibility. Furthermore, the effects of these restorative materials on the morphology of L929 cells were evaluated by scanning electron microscopy.

In recent studies, the evaluation of fibroblast attachment with the aim to determine cytotoxicity is mostly performed using the cell culture test method (2, 36, 37, 60). Cell culture test methods are preferred, as they are not affected by individual factors, permit comparison between materials, are repeatable and allow for the standardization of working conditions (35, 49). In the present study, the cytotoxicity of the restorative materials and their effects on fibroblast attachment were investigated *in vitro* using the cell culture method.

There is no definite agreement as to which type of cells should be selected for use in cell culture studies. The use of diploid cells raises difficulties in laboratory comparisons. Laboratory comparisons are facilitated through the use of continuous cell lines that can be used all over the world (56). Based on the observation that continuous cell lines produce results within a shorter period of time when compared to primary cell cultures (56), continuous cell lines were used in the present study.

The importance of standard practices in the evaluation of the biocompatibility of dental materials is well-known (49). The compilation of reports addressing the properties of dental materials eliminates, to a large extent, the difficulties encountered in standardization. In this context, cell cultures were prepared following the 10993-5 numbered ISO protocol (21). According to the standards laid down in this protocol, for the investigation of the cytotoxicity of dental materials and the evaluation of fibroblast attachment in *in vitro* studies, either L929 or Balb-c 3T3 fibroblast cultures should be used (21). In the light of previously conducted studies (48, 58) and for the sake of compliance with the ISO protocol, L929 mouse fibroblast cell cultures were used in our experiments.

Similar to previously conducted studies (40, 41), the MTT assay was used to determine the effect of each restorative material on the cell viability or proliferation over a three-day time period. The results of the MTT assays are shown in Table 3. Based on the acceptance of the value pertaining to the positive control group as 100%, the optical density values shown in Table 3 are percentile values calculated by simple proportion and represent the percentage of viable cells.
Fig. 1. SEM images of L929 cells after the 1st day of incubation with glass (A), CIS (B), RMCIS (C), and PMKR (D).

Fig. 2. SEM images of L929 cells after the 3rd day of incubation with glass (A), CIS (B), RMCIS (C), and PMKR (D).
The attachment of fibroblasts to various filling materials has been demonstrated both clinically and histologically (3, 15, 27, 28, 37). In the present study, after an incubation period of 24 h, it was determined that the level of cell proliferation was higher in the polyacid-modified resin composite group (PMRC) (80.3 ± 23.7) in comparison to the resin-modified glass ionomer cement (RMGIC) (43 ± 9.92) and conventional GIC (53.8 ± 5.07) groups. Following an incubation period of 3 days, the cell proliferation level of the resin-modified glass ionomer cement (RMGIC) (45.7 ± 6.14) was lower than the levels of the GIC (56 ± 11.3) and PMRC (58.3 ± 6.41) groups, and the differences between the groups were statistically significant (P < 0.05). In the RMGIC (43 ± 9.92 - 45.7 ± 6.14) and GIC (53.8 ± 5.07 - 56 ± 11.3) groups, the mean cell proliferation levels measured on the 1st and 3rd days did not differ from each other significantly, whilst in the PMRC group (80.3 ± 23.7 - 58.3 ± 6.41), the level of cell proliferation measured on the 3rd day was significantly lower than that measured on the 1st day. This can be explained by the prolongation of the incubation period and the increase of the release of residual substances from the material into the culture medium.

The comparison of the cell proliferation percentages of the three filling materials with the values of the controls on the 1st and 3rd days demonstrated that the percentile values of the controls were significantly higher. These results may be related to the surface characterization of the control group. In the present study, glass coverslips were used for control purposes. Glass coverslips have a smoother surface than the three different filling materials used in the study. Therefore, a higher level of fibroblast attachment was observed on the glass surface. In agreement with the findings of Al-Qathami et al. (3), a lower level of fibroblast attachment was observed on the RMGIC and GIC surfaces in comparison to the PMRC surface, which can be attributed to the surface of glass ionomer cement being rougher than that of composite material. As recently shown by the SEM-replica technique, the roughness of four different test surfaces varied from very rough for RMGIC to very smooth for enamel surfaces, while resin composite and PMRC surfaces had relatively smooth surfaces (14).

Cytotoxicity can be determined by the use of several methods, which include cell counts, measurement of proliferation rates, detection of the synthesis of different cell products and enzyme activities and the determination of membrane integrity (59). When performing in vitro tests, the appropriateness of the selected method, cell type, the environment to which the cells are exposed to, and the expected/produced response all bear significance (23).

The agar diffusion test has found common use in dentistry (25, 59). The use of this method by many researchers enables the comparison of the data obtained. As the indirect method is appropriate for filling materials, in the present study, the agar diffusion test recommended in the ISO protocol was selected for use. In this method, toxicity is evaluated based on the basis of the effects of the tested materials on cell membrane permeability.

Between the cells and the materials, there exists a permeable agar layer into which the substances that dissociate from the material can diffuse. The toxic effect can be determined by means of the marking of viable cells with vital staining (9). The agar diffusion test has been accepted as a method that can be applied to differentiate the early cytotoxic activity of materials. The cytotoxic effects and lysis indices (index) for the different trial groups are shown in Table 4. The lysis index value of the positive control group was 5, whilst it was 0 for the negative control group. Although cytotoxic effects were not observed in the GIC and PMRC groups, the RMGIC groups showed a slight cytotoxic effect. The groups did not significantly differ from each other for cytotoxicity (P > 0.05). The data obtained by the use of the agar diffusion test may be used only for the comparison of samples (25). In the present study, with the aim to generate conditions similar to clinical conditions, an agar layer was used as a barrier. The functions of the mucosal membrane and epithelium were mimicked by the use of the agar layer. Depending on the thickness of the barrier layer, the diffusion of the substances leaching out from the composite material may vary. In order to prevent the amount of the barrier layer from masking the potential toxic effects of the material and to ensure the evenness of the diffusion interval, the amount of the agar to be used was determined as 10 ml. Results obtained with different viability tests may not always conform to each other, owing to the different target points of these tests. For instance, while the neutral red test targets the membrane integrity of cells, the MTT assay targets mitochondrial activity (53). In the present study, the neutral red method was applied for the differentiation of viable and dead cells. Neutral red, which can pass readily through plasma and organelle membranes, is a slightly cationic dye that accumulates in the lysosomes. Damage caused by the tested agents to membrane integrity results in decreased retention of neutral red. Damaged or dead cells appear colourless, compared to healthy control cells. The colourless region is referred to as the inhibition zone (IZ), and the broadness of this area indicates the degree of toxicity of the material (47, 53). To date, many researchers have preferred to use the neutral red method, which is a sensitive quantitative method (16, 47, 53, 54).

In a study conducted by Franz et al. (16), in which the cytotoxicity of reversible and conventional composites was investigated, it was determined that the toxicity of composites polymerized as 5-mm layers was greater than that of 2.5-mm layers (34). In the light of these data, in the present study, the samples were prepared at a thickness of 2 mm. In the present study, the melting indices measured by the agar diffusion test demonstrated that no residual substances were released from the investigated filling materials into the cell culture medium that would induce adverse effects on the viability of L929 fibroblast culture cells. Although slight toxicity was determined for resin-modified glass ionomer (RMGIC) filling material, this was found to be statistically insignificant.

Mohsen et al. (34) determined that different aging procedures increased the biocompatibility of composites (42).
In agreement with this report, in the present study, cytotoxicity was indeed not determined for PMRC and GIC, the surfaces of which can be further smoothened by aging, whilst glass ionomer cement, the surface of which cannot be smoothened even by aging, was associated with slight cytotoxic effect.

The cell morphologies on the Ketac Molar Quick Aplicap (GIC), Phopac Quick (RMGIC) and Dyract Extra (PMRC) are shown in Fig. 1. In control glass cover slips, L929 cells exhibited a round (immature) or a spindle shape (mature) on the 1st day of incubation. Mature fibroblasts adhered to glass coverslip with lamellipodia and filopodia; they appeared to have a smoother cell membrane except for a few blebs or ruffles. In Fig. 2 by the third day, a reduced number of immature cells were noticed. Mature fibroblasts bearing different shapes and surface characteristics appeared to be fully spread and well attached to the glass coverslips by means of lamellipodia. The surfaces texture of the GIC and PMRC were slightly rough compared to glass coverslips. whereas the surface texture of the RMGIC was considerable rough and irregular compared to other restorative materials. At 24-hour incubation, a small number of the round shaped immature fibroblasts and stellate (young) to fusiform and spindle shaped (mature) appeared to be attached on GIC by means of lamellapodia and filipodia. As the incubation period extended, the fibroblasts increased in number and became attached with lamellipodia and filopodia. On comparison with coverslips and other restorative materials, L929 cells were observed on the surface of RMGIC exhibiting characteristic immature fibroblastic morphology on the 1st day of incubation. By the third day, the round shaped immature fibroblasts and stellate (young) to fusiform and spindle shaped (mature) appeared to be attached on RMGIC by means of lamellipodia and filopodia. At 24-hour incubation, the majority of cells on the PMRC were round to discoid in shape, and had smooth surface. The stellate or fusiform shaped mature fibroblasts were also found on the PMRC. By the third day, round shaped immature fibroblasts and spindle shaped mature cells appeared to be attached on RMGIC. These cells have a small number of lamellipodia and filopodia, and have smooth surface.

When evaluated by scanning electron microscopy, it was observed that L929 cells did not completely spread well over all restorative materials; however, these restorative materials did not inhibit the growth of L929 cells. On the first and third day of the incubation, round shaped immature cells were observed on all the three materials. Adhesion and spreading of cells on a material surface are initial phase for cellular function. Zhu et al. (62) suggested that cell adhesion and spreading on restorative materials could be used as a criterion for evaluation of the biocompatibility of that material. In studies about the effects of various restorative materials on human PDL cells (3, 4), it was proposed that the observation of circular shaped fibroblasts are due to the toxic effects of the surface materials.

In this study, when L929 cells on the control group glass surface were investigated, circular shaped immature fibroblasts were observed to turn into flat and IG shaped mature fibroblasts that contain lamellipodia and filopodia.

However, on the third day, while immature fibroblasts were still observed on RMGIC and PMRC, the fibroblasts on CIG had become flat and IG shaped mature fibroblasts that contained lamelliodia and filopodia and spread over the GIC. This study supports the previous studies that claim the effects of GIC on L929 fibroblasts are less toxic in longer time periods relative to the other restorative materials.

In order to successfully combine periodontal plastic surgery with restorative procedures, future studies should focus on the characterization of filling materials. Chemical surface analysis techniques, measurement of element release and the investigation of microstructure and porosity by physical surface characterization may aid in a better understanding of the biological response induced by filling materials. It is believed that, eventually, filling materials with improved properties can be developed.

Conclusions
The aim of the present study was to investigate the effects of three different filling materials on fibroblast attachment and their cytotoxicity to mouse fibroblast cell cultures by using the MTT assay and agar diffusion test. It was obtained that: on days 0-1, the level of cell proliferation was highest for the PMRC filling material (80.3 ± 23.7), and GIC (53.8 ± 5.07) and RMGIC (43 ± 9.92), which produced lower cell proliferation rates but did not statistically differ from each other (P > 0.05). It was observed that, on days 2-3, the mean cell proliferation values of the RMGIC filling material (58.3 ± 6.41) had decreased compared to the value measured on day 1 (80.3 ± 23.7). This decrease was attributed to the prolongation of the incubation period and the release of residual substances from the material into the culture medium. The comparison of the cytotoxicities of the three different filling materials GIC, RMGIC and PMRC yielded no statistically significant difference (P > 0.05). This proves that soft tissue may attach to the filling materials investigated in the present study.

Acknowledgements
This study was supported by grant B-591 from the Research Fund for PhD theses, Erciyes University, Turkey.

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