Cytotoxicity of provisional crown and bridge restoration materials: an in vitro study

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Abstract: There are numerous materials used to provide temporary coverage for teeth while permanent restorations are being fabricated. The biocompatibility of these materials is important for the protection of teeth and gingiva, but there is little information on this subject. This study aimed to examine possible time-dependent toxic effects of provisional crown and bridge restoration materials, manipulated intraorally or extraorally, on epithelia cells. A total of 20 discs, 4 sample discs from each product having dimensions of 4mm diameter and 2 mm thickness, were prepared. After sterilization, toxicity of these discs was evaluated in the Madin Darby Bovine Kidney (MDBK) cell line. Morphological cell changes were observed microscopically at the 3rd, 6th, 12th, and 24th hours by the filter diffusion test method. After the 3rd hour, Artglass and Structur produced cytotoxic symptoms. Temdent had the least toxic effects at the end of the 12th hour. However, after the 24th hour, the toxic values were similar for all materials. The results of this study show that although the toxicity response of chemically and light curing materials had changed in different time periods, all of them had the same toxic effects at the end of the 24th hour. (J. Oral Sci. 43, 123-128, 2001)

Key words: cytotoxicity; provisional crown and bridge restoration material; cell culture.

Introduction

It is important that prepared teeth be protected and that the patient be kept comfortable while a permanent restoration is being fabricated. By successful management of this phase of the treatment, the dentist can favourably influence the ultimate success of the final restoration and gain the patient’s confidence (1,2). Generally, the serving period of fixed provisional restorations is 20-30 days, but this period may become longer because of technical errors, general health issues and financial problems (3).

The biocompatibility of provisional crown and bridge restoration materials is important, as well as their mechanical and physical properties. Having direct contact with the vital prepared teeth, the intraoral or extraoral setting provisional crown and bridge restoration materials should have no toxic effect both on the pulp and on the gingiva.

Hanks et al. (4) reported that investigators have used cell cultures to determine the biocompatibility of dental materials for the first time. Consequently, the effects of dental materials such as plastics, cements, metals, alloys, and endodontic root canal filling materials on cells in the tissue culture have received much investigation (4,5).

To determine the cytotoxicity of dental materials, two different testing methods (Agar and filter diffusion tests) were suggested for solid materials, in the ISO 7405 standard report. These methods were based on the microscopic evaluations of differences in the cell membrane permeability and metabolic structure (6).

In this study, the toxic properties of five provisional crown and bridge materials used intraorally or extraorally were investigated in vitro by the filter diffusion cell culture testing method.
Materials and Methods

Test Materials

To investigate the cytotoxicity of provisional crown and bridge materials, five different products (Artglass, Structur, Dentalon plus, Temdent, and Tab 2000) were used.

Names, compositions, and manufacturers of these test materials are presented in Table 1.

Preparation of Test Samples

A total of 20 discs, 4 sample discs for each product, having dimensions of 4mm diameter and 2 mm thickness, were prepared according to the manufacturers' instructions. After polymerising these samples in polyethylene rings, they were sterilised in an ultra-violet sterilizator (BS-4012 NbS Di. Co., Turkiye) for 3 min. Then, they were

Table 1 Materials used in this study

<table>
<thead>
<tr>
<th>Material</th>
<th>Type</th>
<th>Composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artglass</td>
<td>light curing resin</td>
<td>Methacrylate acid ester, photoinitiators, stabilizers</td>
<td>Heraeus Kulzer GmbH, Postfach 1242, D-61269, Wehrheim/Ts., Germany</td>
</tr>
<tr>
<td>Structur</td>
<td>chemically curing resin</td>
<td>Methacrylate, benzoyl peroxide, amine</td>
<td>Voco, Postfach 767, D-27457, Cuxhafen, Germany</td>
</tr>
<tr>
<td>Dentalon plus</td>
<td>chemically curing resin</td>
<td>Poly methyl methacrylate N, butyl methacrylate</td>
<td>Heraeus Kulzer GmbH, Postfach 1242, D-61269, Wehrheim/Ts., Germany</td>
</tr>
<tr>
<td>Temdent</td>
<td>chemically curing resin</td>
<td>Poly methyl methacrylate, copolymer methyl methacrylate</td>
<td>Weil-Dental GmbH, Dieselstraße 5-6, D-61191, Rosbach, Germany</td>
</tr>
</tbody>
</table>

Table 2 Toxicity % of test materials related to the periods

<table>
<thead>
<tr>
<th>Test materials</th>
<th>3rd hour</th>
<th>6th hour</th>
<th>12th hour</th>
<th>24th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artglass</td>
<td>100**</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Structur</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Dentalon plus</td>
<td>-</td>
<td>66</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Temdent</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Tab 2000</td>
<td>-</td>
<td>-</td>
<td>83</td>
<td>100</td>
</tr>
<tr>
<td>Positive control (ammonium molybdate)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Negative control (cell control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Hours after inoculation.

**Toxicity % = \frac{\text{Number of morphologically changed cells}}{\text{Number of morphologically changed cells and sound cells}} \times 100
placed and held in macro-welled plates prepared for cell culture for a period of 24 hours.

Cell Culture

In this study, the Madin Darby Bovine Kidney (MDBK: HUKUK, 95-121-801) epithelial monolayer cell culture was used. Testing was accomplished according to the filter diffusion testing method reported in the ISO standard 7405. The MDBK epithelial cell suspension was prepared as $2 \times 10^5$ cell/ml in Eagle's Minimum Essential Medium (EMEM; Gibco Paisley Scotland, UK) supplemented with 10% Foetal Calf Serum (FCS; Paesel GmbH and Co., Frankfurt, Germany). A 1ml volume of the cell suspension was placed in each well of a 24-well macro plate, and incubated at 37°C in a humidified 5% CO2 for 24 hours. After obtaining the monolayered cells, sterile cellulose acetate membrane filters with 0.20 nm pore diameters (Sartorius GmbH, Gottingen, Germany) were placed carefully onto the surface of cells reproduced in the 24-well macro plate. Subsequently, using 4 wells for each test sample on a membrane filter, standard test samples were placed in order on membrane filters and plates were incubated at 37°C in a humidified atmosphere of 5% CO2.

Toxicity controls related to these samples were made using a microscope (Inverted Tissue Culture Microscope, Olympus, Tokyo, Japan) taking into account the morphological changes that occurred in cells at the 3rd, 6th, 12th, and 24th hours following the inoculation. During the testing procedure, the probable toxicity controls of the membrane filters were also performed.

As a positive control, 1 M Ammonium molybdate ((NH$_4$)$_6$ MO$_7$ O$_{24}$ 4H$_2$O, Merck, Germany) solution absorbed sterile blotting paper was used. As a negative control, distilled water absorbed sterile blotting paper of the same diameter as the samples were used.

Results

The toxicity percentage of test materials was evaluated relative to the periods of the 3rd, 6th, 12th, and 24th hours in the native cell cultures used (Table 2). Evaluations were based on the morphological changes that occurred in the cells. The main parameters of the toxicity were denoted as spherical cells, colonisation in the periphery, and degenerative changes of the cells that came off.

After the 3rd hour, Artglass and Structur produced cytotoxic symptoms (Figs. 1, 2). Dentalon plus did not produce any cytotoxic symptoms at the 3rd hour, however, did have cytotoxic symptoms in 3 of 4 wells at the 6th hour and in all of the 4 wells at the 12th hour (Fig. 3). Temdent and Tab 2000 did not produce cytotoxic symptoms at the 3rd and 6th hours, but did have cytotoxic effects at the 12th hour (Figs. 4, 5). At the 12th hour, Temdent was evaluated as the least cytotoxic material. However, at the end of the 24th hour, toxicity levels of test materials were comparable. Ammonium molybdate, used as the positive control, had a cytotoxic effect in all of the cells after the 3rd hour (Fig. 6). Meanwhile, membrane filters used during the test did not produce any toxic effect, and the cells existing in the negative control wells stayed vital during the testing procedure (Fig. 7).

Discussion

In the evaluation of the biocompatibility of dental restorative materials, it is obvious that cytotoxic tests are very useful, because they decrease the need of animal and human tests. These tests not only are not affected by factors of individuals, repeatability, and parametric
comparability among materials, but also give information about material-cell interactions. These attributes have increased the interest in cell culture studies. The interaction of cells with material components at the molecular level is possibly responsible for reactions at the tissue level, such as inflammation, necrosis, immunogenesis, and carcinogenesis. Cell culture tests are informative about determining the in vivo response (7).

The direct contact of the material with the cell is the only critical thing in these tests. While evaluating the pulp response, this method may not give accurate information about the toxicity of the material, since there exists a dentin layer, which has a variable thickness between the pulp tissue and the material. However, in this study the filter diffusion test method was appropriate because of the direct contact of the crown margin with the marginal gingiva. In the filter diffusion method, the filter was in contact with growing cells on one side and with material on the other side. Therefore, a filtering material should diffuse into the filter pores in order to show the toxic effect (8). For this study, a filter having pores of 2 nm in diameter was selected. 1 M ammonium molybdate, which was reported as giving positive results by Keech et al. (9), was used as positive control.

Cytotoxicity of resins was postulated to have a relation...
with the reaction by-products or with the unreacted components released from the materials (10). While numerous investigators have reported a variety of tissue reactions to methyl methacrylate, including tissue sensitivity, fibrosarcoma formation, and cytoxicity (11-14), it has also been shown that like methyl methacrylate, formaldehyde and benzoic acid in traditional denture base resins could be potentially toxic substances (10,15). Formaldehyde formation by surface oxidation of unreacted double bonds in resins could cause tissue reaction (15). The most commonly encountered problems in clinics are local inflammatory reactions due to toxic, irritant or allergenic components of dental materials. Not following the clinical and laboratory instructions of the provisional restoration materials could cause desquamative gingivitis, plasma cell gingivitis, and ulcerative gingivostomatitis (16). The barrier function of tissues directly in contact with the margin of the restoration is very important, because the impaired structure of mucosal integrity could act as a co-factor. Besides trauma, thermal damage could also impair this function of the mucosa. It is probable that there becomes irreversible tissue damage after exposing to a temperature of 47°C or higher for a long time. In vitro studies on provisional crown and bridge restorations fabricated intraorally indicate that temperatures of 47-65°C may be present in the bulk material (17). Taking into account the chemical polymerisation of the provisional restoration materials and the exothermic reaction of these, the way the provisional restorations are made becomes an important consideration. Although the direct methods are easy to handle and quick in setting, in order to prevent direct contact of mucosa with the residual monomer and relatively high concentrated monomer, it will be logical to prefer the indirect method.

In this study, it was observed that although the toxicity response of chemically and light curing materials changed at the different time periods, all of them had the same results at the end of the 24th hour. The release rate difference of the components that caused toxicity might be related to the polymerisation level, amount of fillers, and final density of materials. Moreover, results have shown that the release of the cytotoxic component continued after the polymerisation. The methyl methacrylate, which was reported as the primary cause of the tissue reactions, was proved to be 1-4% in autopolymerising resins immediately after polymerisation, and when stored in 50°C water for a certain period it was observed that this percentage was decreased (18). In other studies, Baker et al. (19) and Lefebvre et al. (20) have evaluated the release of methyl methacrylate in human saliva. They found that the greater part of this component had been released in the first hour, and in order to decrease the toxic potential, before the application of these restorations to patients it is preferable to store them in warm water for 24 hours. The same situation is supported by the results of this study. Since the cytotoxicity was observed in all of the materials at the end of the first day, it seems that using the indirect method is more appropriate and it might be preferable to store the materials in warm water for at least 24 hours after curing.

This study demonstrated that the morphological changes in cells included death, and this should be used as one of the parameters of the cytotoxic criteria. Determination of the advanced cell culture studies and toxic or non-toxic components released from the material, and investigation of the interaction in the vital cells at the molecular level should be done in further studies.

**Conclusions**

Within the limits of this study, the following conclusions are drawn:

1. Provisional crown and bridge restoration materials, either chemically or light curing, had cytotoxicity until the end of 24 hours.
2. All of these materials caused cell death at the end of 24 hours.
3. In order to promote tissue health, it is appropriate to use the fabricated provisional restorations 24 hours after polymerisation.

**References**