The Effect of Aged Dental Ceramics on Gingival Cell Viability

Yaşlandırılmış Dental Seramiklerin Gingival Hücre Canlılığına Etkisi

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ABSTRACT

Purpose: Limited data is available about the long-term biocompatibility of new dental ceramics. The objective of this study was to evaluate the effects of different dental ceramic materials on cell viability in human gingival fibroblast cultures.

Materials and Methods: Human gingival fibroblast cells were exposed on two feldspathic dental ceramics (Vita Omega and Ivoclar), a lithium disilicate pressable ceramic (Empress-2), and a yttria-stabilized zirconia (Denzir). Cell viability was determined by the mitochondrial suppression method (MTT) before and after aging procedure of the ceramic materials. ANOVA test was used for the statistical analysis.

Results: No significant difference (p>0.05) was observed between ceramic specimens except Empress-2. Significant cytotoxicity was determined in Empress-2 material either before and after aging procedure (p<0.05). Vita Omega, Ivoclar and Denzir were less cytotoxic before and after aging.

Conclusion: Among tested ceramic materials, Empress-2 displayed significant cytotoxicity. Cell suppression levels found for other ceramic materials were within acceptable cytotoxicity ranges determined for alloys and composite resins.

Keywords
Ceramics, Cell viability, Aging

ÖZET


Gereç ve Yöntem: İnsan gingival fibroblastı, 2 farklı feldspathic seramiğe (Vita Omega ve Ivoclar), bir lithium disilicate preslenebilir seramiğe (Empress-2) ve bir yttria-stabilized zirconia seramiğe (Denzir) ekspoz edilmiştir. Hücre canlılığı, yaşlandırma öncesi ve sonrasında mitokondrial suppression yöntemiyle (MTT) belirlenmiştir. İstatiksel analiz olarak ANOVA testi kullanılmıştır.

Sonuçlar: Empress-2 dışında (p<0.05) hiçbir test grubunda yaşlandırma öncesi ve sonrasında istatiksel farklılık görülmemiştir (p>0.05). Empress-2 yaşlandırma öncesi toksisite göstermiştir ve bu toksisite yaşlandırma sonrası biraz daha artmıştır. Vita Omega, Ivoclar ve Denzir yaşlandırma öncesi ve sonrasında Empress-2’ye göre daha az toksisite göstermiştir.

INTRODUCTION

Ceramics are of great importance for teeth restorations. In recent years, several modern, all-ceramic and ceramic materials have been developed in an attempt to achieve the most challenging requirements in restorative dentistry. Since the patients demand tooth-colored restorations and new ceramic types are available ceramics are inevitable solutions for restorative needs.1, 2

The prolonged contact of dental ceramics with the gingiva and the oral mucosa causes that the biocompatibility of these materials is of crucial to their long-term safety. Accordingly, only little information is available on the molecules mediating the mechanism of gingival and periodontal inflammation possibly caused by dental ceramics.3,4 Newer in vitro tests with aging techniques used to estimate long-term biologic responses to materials have been applied to alloys and composites.5 But, only a few published reports have addressed the issue of mass loss and biocompatibility of ceramics.6,7

The purpose of this study was to evaluate how the biological response of different types of dental ceramics varies over time on exposure to gingival fibroblasts. The hypothesis of this study was that different types of dental ceramics have different biological response over time. Therefore in this study we investigated cell viability by fibroblasts in contact with various dental ceramics with aging technique. Changes of the cell viability of these ceramics might indicate early tissue responses different from acute toxicity and, therefore, serve as sensitive indicators of adverse biological effects of dental ceramics.

MATERIALS and METHODS

Sample preparation

The materials tested for cytotoxicity are listed in Table I. Ceramic materials were selected to represent common types of ceramics that have been used clinically. All specimens (n=6, specimen discs; 6mm in diameter and 3 mm thick; exposed to cell-culture medium) were prepared per manufacturer’s directions with heat treatment and were polished using rubber wheels (medium-grit silicone polisher, fine grit silicone polisher; Brasseler USA, Savannah, Ga) and diamond polishing pastes (Tru-luster diamond polishing paste (2 to 5 μm; Brasseler USA). The materials were then cleaned using a laboratory soap on a soft toothbrush (Oral-B no. 35, soft bristles, Belmont, Calif) and disinfected using ultrasonic treatment and isopropyl alcohol in preparation for biologic testing. These procedures have been described in detail previously. After disinfection, specimens were maintained under sterile conditions until being tested for cellular response. Unalloyed Cu was used as the positive control. The medium without ceramic specimen was also kept under the same conditions and for the same period used for the negative control.

Cell Culture

All participants in the study gave informed consent to the experimental procedures. Local ethic committee consent was taken for this study. Healthy human gingival tissue was obtained from fifteen volunteer patients undergoing extraction of the third molar region for orthodontic reasons. The subjects were between 17 and 20 years old. They were recruited from nonsmokers of whom 7 were male and 8 were female. Patients suffering from any chronic inflammatory or immunological conditions and systemic infections were not included in the study. Immediately after removal, the tissue was placed in Hanks salt solution containing penicillin/streptomycin and amphotericin B. Thereafter biopsies were stored at 4°C for no longer than 6 hours. Specimens were minced into small pieces (3x3x3 mm). Fibroblasts were cultivated with 5% CO₂ at 37°C in Dulbecco’s modified Eagles medium and 10% fetal calf serum (FCS) containing penicillin/streptomycin and amphotericin B. When the cultures reached 100% confluence, the cells were used for the cell culture. Fibroblasts were plated at 10,000 cells/cm² in
24-well cell-culture plate, followed by immediate placement of the specimens into the cultures. The cells tested were from different humans and were never mixed. The passage number were 4-5. All incubations were carried out at 37 °C in an air atmosphere containing 5% CO₂.

**Cellular Response**

The succinic dehydrogenase (SDH) activity of the cells is representative of the mitochondrial function of the cells. The SDH activity was measured by the 3-(4,5-dimethyl-thiazoyl)-2,5-diphenyl-SH-tetrazolium bromide MTT (Sigma, St.Louis, MO, USA) histochemical method as previously described (10). To measure SDH activity, the test materials were removed from the cell-culture wells after exposure 72h, then cells were detached with 0.25% trypsin containing 1mm ethylene diamine tetra acetic acid (EDTA) for 5 min at 37°C, and subsequently the cells were resuspended in medium at 1x10⁵ cells/μL. After verification of cell viability by trypan blue dye exclusion assay, 200 μL of cell suspension were distributed into each well of 96-well microtiter plates (Costar, Chambridge, MA, USA), and each plate was incubated for 24 hours. Wells containing 200 μL medium alone without cells and reagents were used as negative controls. After treatment for the stated incubation times, 20 μL of MTT solution (5mg/ml) was added to each well, and the microplates were further incubated at 37°C for 4 hours. The unreactive supernatans in the well were discarded, and 100 μL of acidified isopropanol (0.04 N HCl in isopropanol) was added to the cultures and mixed thoroughly to dissolve the dark blue crystals of formazan. The absorbance values (A) of each well were determined with a microplate enzyme-linked immuno-assay (ELISA) reader equipped with a 570-nm filter. The negative control well was used for baseline zero absorbance. Results are presented as percentage cell viability determined as 100- (A of experimental well/A of positive control well) X 100. Each experiment was repeated three times with representative data presented.

**Aging**

The aging procedure was used to accurately simulate long-term use of dental materials,¹¹, ¹² and it presumably encourages an accelerated release of components from the material such that subsequent corrosion is similar to that seen after 8 to 10 months. The specimens were rinsed in sterile water, then submerged into 3% bovine serum albumin (BSA; Sigma) solution for 96 hours. BSA was the most effective “accelerating” solution in previous studies. After BSA treatment, the specimens were rinsed once by being dipped into sterile water and then tested again for cellular response as described previously.

| TABLE I |
|---|---|---|
| **Evaluated dental ceramic materials** |
| **Ceramic MATERIAL (Code)** | **TYPE** | **MANUFACTURER** |
| Vita Omega (Leu-HF) | Metal-ceramic feldspar ceramic | Vita-Zahnfabrik, Bad Sackingen, Germany |
| Ivoclar (Leu-LF) | Metal-ceramic feldspar ceramic | Ivoclar, North America, Amherst, NY |
| IPS-Empress (LDS-2) | Glass ceramic | Ivoclar, Schaan, Liechtenstein |
| Denzir (Y-PSZ) | Yttria-stabilized zirconia ceramic | Decim AB, Skelleftea, Sweden |
Statistical analysis

The analysis of variance (ANOVA) test was performed using a statistical software program (SPSSFW, 9.0; SPSS Inc, Chicago, Ill). P<0.05 and <0.001 were considered statistically significant.

RESULTS

The results of the cytotoxicity test of ceramics are shown in Fig 1, Fig 2, Table II and Table III. The 2 leucite-based feldspathic ceramics (Leu-HF, Leu-LF) and the Y-PSZ caused mild suppression of SDH activity compared with the controls before aging (Fig 1). In contrast, LiDS-2 was severely cytotoxic (p<.05). As the specimens were aged, the LiDS-2 specimen showed a marked improvement in this regard. The LiDS-2 material caused a reduction in SDH activity with aging, whereas the 2 feldspathic materials and Y-PSZ did not change significantly (p>.05) (Fig 2).

DISCUSSION

Primary gingival fibroblast cultures are more closely related to their original tissue and therefore much easier to identify. Further, these cultures have a nearly unchanged metabolic state relative to their original tissue; the in vitro experiments thus approximate the in vivo situation. For these reasons, this cell culture has been commonly used for cell viability evaluation of biomaterials.13, 14 In the present study, an in vitro model of primary gingival fibroblasts placed in direct contact to the testing dental ceramics was preferred.

Each method of cytotoxicity has advantages and disadvantages. The visual method gave accurate results with a minimum amount of equipment, but required some observer training and provided only non-parametric data. Densitometric evaluations were easy to obtain and preserved the monolayer. However, this method was time consuming and less precise. Colorimetric evaluation of solubilized formazan dye (MTT Assay) was fast, objective and the least variable. Therefore, in this study, cytotoxicity was determined by using MTT assay at two observation periods (24 and 72 h). The percentage of viable cells compared with controls represented the level of cytotoxicity of the dental ceramics. The cleavage of MTT has several desirable properties for assaying cell survival and proliferation. MTT is cleaved by all living, metabolically active cells that we have tested. The assay can be read a few minutes after the addition of acid-isopropanol, and the color is stable for a few hours at room temperature. The results are also apparent visually, which is very useful if rapid qualitative results are required.10
The dental ceramics used in the current work were selected because they are commonly used in clinical practice for all ceramic crowns. In this study, all but the LiDS-2 ceramic materials have shown similar reaction to those of the control cultures in the MTT assay before and after aging. Leu-HF, Leu-LF and Y-PSZ have shown similar levels of toxicity, which is considered by most researchers in the biocompatibility field to be acceptable clinically. By these same standards, the initial cellular response to LiDS-2 would not be acceptable, although its improvement with aging has been viewed favorably in the past in interpreting the relevance of in vitro biologic tests. The results were consistent with former reports. The clinical biologic risk from this type of material depends on the ability of normal daily activities, such as tooth-brushing, to disturb the “protective” surface layers. This type of risk should be investigated further. Although trends in SDH activity were also observed with other materials, for example Leu-HF, Leu-LF and Y-PSZ these changes were smaller, and their impact on clinical risk can not be assessed without further study to establish if they continue with time or are reproducible.

Evaluation of mass release from ceramics is not common in the literature, although there are several studies that have demonstrated such mass release. Therefore, future studies should include characterization of the ceramic materials before and after aging to attempt to understand the causes of the changes in cytotoxicity observed and the relevance of these changes to clinical practice.

### TABLE II

**Cell viability values of tested ceramic materials before aging process**

<table>
<thead>
<tr>
<th>Pre-Aging</th>
<th>CONTROL</th>
<th>LIDS-2</th>
<th>LEU-LF</th>
<th>Y-PSZ</th>
<th>LEU HF</th>
<th>COPPER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24h</td>
<td>72h</td>
<td>24h</td>
<td>72h</td>
<td>24h</td>
<td>72h</td>
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<tr>
<td>MTT</td>
<td>100</td>
<td>100</td>
<td>82</td>
<td>71</td>
<td>99</td>
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<tr>
<td>TBARS</td>
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<td>±0.04</td>
<td>±0.04</td>
<td>±0.05</td>
<td>±0.03</td>
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<td>IL-6</td>
<td>0.66</td>
<td>1.83</td>
<td>0.87</td>
<td>1.3</td>
<td>0.8</td>
<td>1.0</td>
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<tr>
<td>IL-8</td>
<td>±0.07</td>
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<td>±0.20</td>
<td>±0.36</td>
<td>±0.9</td>
<td>±1.1</td>
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</table>

### TABLE III

**Cell viability values of tested ceramic materials after aging process**

<table>
<thead>
<tr>
<th>Post-Aging</th>
<th>CONTROL</th>
<th>LIDS-2</th>
<th>LEU-LF</th>
<th>Y-PSZ</th>
<th>LEU HF</th>
<th>COPPER</th>
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</thead>
<tbody>
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<td>72h</td>
<td>24h</td>
<td>72h</td>
<td>24h</td>
<td>72h</td>
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<tr>
<td>MTT</td>
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<td>±0.13</td>
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<tr>
<td>IL-6</td>
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<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>IL-8</td>
<td>±0.4</td>
<td>±0.2</td>
<td>±0.4</td>
<td>±0.4</td>
<td>±0.5</td>
<td>±0.2</td>
</tr>
</tbody>
</table>

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REFERENCES


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