In Vitro Assessment of Cytotoxicity of Orthodontic and Dental Composite Resins using Human Gingival Fibroblasts

Raoul Bationo1*, Ablassé Rouamba2, Abdoulaziz Diarra3, Monique Lydie Beugré-Kouassi4, Fabienne Jordana5, Jean Bertin Beugré4

1Bogodogo Teaching Hospital, Burkina Faso; University Felix Houphouet Boigny, Abidjan, Côte d'Ivoire
2Laboratory of Applied Biochemistry and Chemistry, University Joseph Ki-Zerbo, 03 BP 7021, Ouagadougou, Burkina Faso
3Municipal Center of Oral Health of Ouagadougou, Burkina Faso
4Department of Odontology, University Felix Houphouet Boigny, Abidjan, Côte d'Ivoire
5Department of Odontology, University of Nantes, 1 Quai de Tourville, 44035 Nantes, France

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*Corresponding author: Raoul Bationo

Abstract

Objective: The aim of this study was to evaluate and compare the biological effects of seven composite resins (Clearfil Majesty ES-2, Clearfil Majesty ES Flow, Grengloo, Blugloo, Transbond XT, Transbond LR and Filtek Supreme XTE) on human gingival fibroblasts. Methods: Human gingival fibroblasts were cultured up to confluence. Cultures were exposed to composite resins for 24 hours and cell viability measured by MTT assay. Results: All composite resins showed reduced values (85 to 94%) of cell viability compared to the control. Conclusions: Orthodontic and dental composite resins are toxic to human gingival fibroblasts. Dental materials that are used in dentistry should be harmless to oral tissues, so they should not contain any leachable toxic and diffusible substances that can cause some side effects.

Keywords: Cytotoxicity, MTT assay, Gingival fibroblast, Light-cured composite resin.

INTRODUCTION

Dental composites are complex mixed materials which generally consist of an organic polymerizable matrix, reinforcing fillers, which are mainly inorganic and a silane-coupling agent [1]. The polymerizable matrix contains one or more monomers: Bis-GMA and/or UDMA, co-monomers (EGDMA, DEGDMA, TEGDMA) and various additives like initiator, coinitiator, inhibitor of polymerization and photostabilizer [2].

Various components may be released from composite resins into the oral environment. It has been shown that these released components can cause several adverse effects such as mucosal irritation, epithelial proliferation, oral lichenoid reaction, hypersensitivity, and anaphylactoid reactions [3].

Composite resins may cause different reactions in the oral soft tissues such as gingiva. Composites are initially very cytotoxic in in vitro tests of direct contact with fibroblasts. The cytotoxicity seems to be, in the early phase, from the non-polymerized components in the air-inhibited layer that leach out from the materials [4]. Other in vitro studies, which have “aged” the composites in artificial saliva for up to six weeks, have shown that the toxicity diminishes in some materials but remains high for others [5].

The aim of this study was to evaluate and compare the biological effects of composite resins on human gingival fibroblasts.

MATERIALS AND METHODS

Human gingival fibroblasts are amplified from gingiva waste from dental extraction in a patient who gave his consent and had no periodontal pathology. Biopsies are previously cut with surgical blade. RPMI-1640 medium with 10% of fetal bovine serum, 1% of penicillin/streptomycin and 1% of L-glutamine in Petri dishes stored in a humidified incubator at 37 °C under 5% of CO2 in air.

The culture medium is changed every two days up to confluence. Cells are then trypsinized (Trypsin 0.25% in EDTA 0.02%) for 3 minutes at 37°C.
Specimen Preparation

Specimens (10 mm in diameter and 1 mm in thickness) were prepared from seven commercially composite resins (Table-1), cured for 20 seconds using BA Optima 10 LED Curing Light and then tested for cytotoxicity.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Product/Lot</th>
<th>Resin matrix</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil Majesty ES-2/400069</td>
<td>Bis-GMA: 5-15% (CAS No. 1565-94-2); Hydrophobic aromatic dimethacrylate; Hydrophobic aliphatic dimethacrylate</td>
<td>Kuraray</td>
<td></td>
</tr>
<tr>
<td>Clearfil Majesty ES Flow/A60239</td>
<td>TEGDMA: &lt; 10% (CAS No. 109-16-0); Hydrophobic aromatic dimethacrylate</td>
<td>Kuraray</td>
<td></td>
</tr>
<tr>
<td>Grengloo/6623923</td>
<td>TEGDMA: 5-10% (CAS No. 109-16-0); UDMA: 0.1-1% (CAS No. 72869-86-4); HEMA: 1-5% (CAS No. 868-77-9); Bis-EMA6: 1-5% (CAS No. 41637-38-1); GMA: 0.1-1% (CAS No. 106-91-2); EO-TMPTA: 0.1-1% (CAS No. 28961-43-5); 3-trimethoxysilylpropyl methacrylate: 1-5% (CAS No. 2530-85-0)</td>
<td>Ormco</td>
<td></td>
</tr>
<tr>
<td>Blugloo/6556174</td>
<td>UDMA: 1-5% (CAS No. 72869-86-4); Bis-EMA6: 5-10% (CAS No. 41637-38-1); GMA: 1-5% (CAS No. 106-91-2); EO-TMPTA: 1-5% (CAS No. 28961-43-5); 3-trimethoxysilylpropyl methacrylate: 1-5% (CAS No. 2530-85-0)</td>
<td>Ormco</td>
<td></td>
</tr>
<tr>
<td>Transbond XT/N921496</td>
<td>Bis-GMA: 10-20% (CAS No. 1565-94-2); Bis-MEPP: 5-10% (CAS No. 24448-20-2)</td>
<td>3M</td>
<td></td>
</tr>
<tr>
<td>Transbond LR/N919866</td>
<td>Bis-GMA: 5-15% (CAS No. 1565-94-2); TEGDMA: 10% (CAS No. 109-16-0)</td>
<td>3M</td>
<td></td>
</tr>
<tr>
<td>Filtek Supreme XTE/N879475</td>
<td>Bis-GMA: 1-10% (CAS No. 1565-94-2); UDMA: 1-10% (CAS No. 72869-86-4); TEGDMA: &lt; 1% (CAS No. 109-16-0); Bis-EMA6: 1-10% (CAS No. 41637-38-1); PEGDMA: &lt; 5% (CAS No. 25852-47-5)</td>
<td>3M</td>
<td></td>
</tr>
</tbody>
</table>

Cytotoxicity assay

The cytotoxic effects of the eluated extracts were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded onto 96-well plates and incubated for 24 hours at 37°C in a humidified incubator at 37 °C under 5% of CO₂ in air. The culture medium was then replaced with 100 µL of resin extracts for 24 h incubation.

Cells were also seeded onto 12-well plates in direct contact with resin samples and incubated for 24 hours. Then 100 µL of immersion medium was instilled onto 96-well plates. The culture medium was used as a control.

10 µL (5 mg/mL) of MTT solution was added to each well (96-well plate) and the plates were incubated for 3 hours. The MTT was then removed and 100 µL per well dimethyl sulphoxide (DMSO) was added to each well to dissolve the formazan crystals. Optical densities (OD) were measured at 570 nm in an EPOCH reader and cell viability was calculated using the following formula:

\[
\text{Cell viability} = \frac{\text{OD test group}}{\text{OD control}} \times 100
\]

Experiences are triplicated and repeated for a minimum of three times. The data were presented as mean and SD. The cell viability in types of contact was compared by t-test.

RESULTS

Table-2: Values of optical densities

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Clearfil ES-2</th>
<th>Clearfil ES Flow</th>
<th>Grengloo</th>
<th>Blugloo</th>
<th>Transbond XT</th>
<th>Transbond LR</th>
<th>Filtek Supreme XTE</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>I</td>
</tr>
<tr>
<td>OD</td>
<td>0.56 ± 0.03</td>
<td>0.5 ± 0.02</td>
<td>0.56 ± 0.05</td>
<td>0.58 ± 0.03</td>
<td>0.59 ± 0.04</td>
<td>0.54 ± 0.03</td>
<td>0.55 ± 0.03</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td>P value</td>
<td>0.39</td>
<td>0.31</td>
<td>0.07</td>
<td>0.67</td>
<td>0.77</td>
<td>0.63</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>

D=Direct; I=Indirect
DISCUSSION

Human gingival fibroblasts (HGFs) were used for cytotoxicity testing because they are in close proximity with restorative dental materials in the oral cavity and more clinically relevant. Also HGFs are sensitive cells that can be easily isolated and cultured in normal culture medium [6].

In this experiment the exposure time was 24 h, because it has been shown that monomer release from composite resins is complete in 24h [7]. Therefore, most toxic effects from composite resins occur during the first 24 h.

All our composite resins showed reduced values (85 to 94%) of cell viability compared to the control. According to Sjogren et al., [8], all tested resins have a slight toxicity (cell viability between 60 and 90%) except Clearfil Majesty ES Flow, Grengloo and Filtek Supreme XTE which are classified non-toxic (cell viability >90%) in indirect contact.

It is evidenced in literature that the polymerization rate can significantly affect the cytotoxicity of a composite material, through the diffusion of a large number of unreacted resin monomers [9-11]. In the current study, several monomers contained in the composite material used (such as Bis-GMA, Bis-EMA, UDMA, TEGDMA declared by the manufacturer) are known to diffuse from partially polymerized composite resins and to be cytotoxic in vitro [12, 13].

Malkoc et al., [14] evaluated the cytotoxic effects of five different light-cured orthodontic composites. There were significant similarities in resin matrices when evaluating the ingredient of tested materials. However, Transbond XT also contains Bis-EMA. In addition, a Bis-EMA monomer showed a cytotoxic effect analogous to that of TEGDMA [15]. The mechanism of cytotoxicity induced by TEGDMA in human fibroblasts was studied [16]. Hence, cytotoxicity of Transbond XT could be explained by the presence of Bis-EMA in its matrix.

TEGDMA, Bis-GMA and UDMA biological effects on three HGF cell lines and immortalised human keratinocytes were evaluated and compared by Moharamzadeh et al., [17]. The three resin monomers showed toxic effects on the HGFs and HaCaT cells. Bis-GMA was the most toxic and UDMA was the least toxic of the monomers tested.

CONCLUSION

Resin monomers contained in the composite material are known to diffuse from partially polymerized composite resins and to be cytotoxic in vitro. Orthodontic and dental composite resins are toxic to human gingival fibroblasts. Dental materials that are used in dentistry should be harmless to oral tissues, so they should not contain any leachable toxic and diffusible substances that can cause some side effects.

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REFERENCES