Cytotoxicity and Degree of Conversion of Orthodontic Adhesives

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ABSTRACT

Objectives: To test the hypothesis that there is no difference in the cytotoxicity related to the modes of polymerization of five commercially available orthodontic bonding resins, with and without an oxygen-inhibited layer (OIL), and to evaluate the degree of conversion (DC) of these resins and correlate this to cytotoxicity.

Materials and Methods: Five commercially available orthodontic bonding resins were tested for cytotoxicity and DC. Thirty-six disks of standardized dimensions, for each resin, were used for cytotoxicity assessment. Half of them were washed with 99% acetone to remove the OIL (washed resins), and the remaining disks were left intact (intact resins). Glass disks were used as a control. Vero cells were exposed to intact and washed resins on day 1. Cell viability was determined by tetrazolium bromide reduction assay 1, 3, and 6 days after exposure. The DC of the adhesive specimens of each resin, prepared with a procedure identical to the clinical bonding process, was assessed by Fourier transform infrared spectroscopy.

Results: Single-cured systems were comparatively less cytotoxic than dual-cured systems. With removal of the OIL, increased cell viability was noted only with two resins on all three days. Resins tested showed differences in DC. A positive correlation was demonstrated by two resins.

Conclusion: The hypothesis is rejected. Single-cured systems are superior to dual-cured systems in exhibiting comparatively less toxicity and higher DC. A significant positive correlation was not established between cytotoxicity and DC. (Angle Orthod. 2009;79:1133–1138.)

KEY WORDS: Cytotoxicity; Degree of conversion; Oxygen-inhibited layer; Fourier transform infrared spectroscopy; MTT assay

INTRODUCTION

The practice of bonding orthodontic brackets has avoided many of the limitations associated with traditional banding of teeth. Its success has led to the evolution of a plethora of bonding materials for use in orthodontics.

Polymerization processes of dental resin-based materials are usually incomplete under clinical conditions, and almost every component can be detected in extracts of polymerized materials, even when mixed and cured according to the manufacturer’s instructions. Leaching from resin composites may occur at two different times: during the setting period of the resin and later when the resin is degraded.1

Leaching during the first process is related to the degree of conversion (DC).1 It is the extent to which...
carbon double bonds (C=C) of the monomer are converted into carbon single bonds (C–C), to form polymers during the polymerization reaction. The degree to which this conversion of reactive species occurs may affect the compatibility of the resin with the oral tissues. Therefore, a reduction in remaining double bonds to the lowest possible level is considered a desirable feature of polymerization system. Light-cured and chemically cured adhesives have been shown to demonstrate differences in the percentage of DC, with the chemically cured adhesives exhibiting higher DC. Thus, modes of polymerization might indirectly influence the cytotoxic properties of the resins.

The leached components (monomer Bis-GMA and comonomer TEGDMA and other resin components) have been implicated in a variety of cytotoxic responses observed in tissues, as evident from previous studies. In vitro studies have revealed that TEGDMA causes large deletions of DNA sequences, leading to chromosomal aberrations, and it also induces an increase in the number of micronuclei formation in V79 Chinese hamster lung fibroblasts. It has also been shown that Bis-GMA concentrations of 5 μmol/L produce a depression of DNA synthesis. Furthermore, several constituents of composites have been proven to have estrogenic activity. These resins may present an additional hazard in that they may be carcinogenic.

An additional problem with the use of composites is the inhibition of polymerization in surface layers exposed to oxygen. Light-cured or chemically cured dental composite resins leave a soft, sticky superficial layer upon polymerization, commonly referred to as an oxygen-inhibited layer (OIL) because of its origin. It is always present when a composite or bonding resin is exposed to oxygen. The inhibition of polymerization in surface layers exposed to oxygen causes large deletions of DNA sequences, leading to chromosomal aberrations, and it also induces an increase in the number of micronuclei formation in V79 Chinese hamster lung fibroblasts. It has also been shown that Bis-GMA concentrations of 5 μmol/L produce a depression of DNA synthesis. Furthermore, several constituents of composites have been proven to have estrogenic activity. These resins may present an additional hazard in that they may be carcinogenic.

MATERIALS AND METHODS

Five commercially available orthodontic adhesives were tested for cytotoxicity and DC:

1. a chemical-cured adhesive (Unite, 3M/Unitek, Dental Products Division, Monrovia, Calif),
2. a visible light-cured adhesive with primer (TransbondXT, 3M/Unitek),
3. a visible light-cured adhesive without primer (Heliosit, Ivoclar Vivadent AG, Schaan, Liechtenstein, Austria),
4. a tricure resin–modified adhesive (GIC GC Fuji ORTHO LC, GC Corporation, Tokyo, Japan), and
5. a dual-cured adhesive (Phase II, Reliance Orthodontic Products, Itasca, Ill).

Cytotoxicity Assessment

Vero cells (African green monkey kidney cells) were grown in medium consisting of Eagles’ minimum essential medium containing 5% fetal bovine serum, 100 U of penicillin, 100 μg of streptomycin, and 2 μg of amphotericin B/mL. Five milliliters of these cells in the concentration of 1 × 10⁵ cells/mL were seeded into 25-cm² tissue culture flasks and incubated in 5% CO₂ at 37°C until a confluent monolayer was formed (48 hours). The monolayer was then subcultured with 5 mL trypsin-EDTA to detach the monolayer of cells. Trypsin-EDTA was then added to the detach the surface. The cells were resuspended in 5 mL of growth media and seeded in three 12-well plates for each resin (one plate each for assessment on days 1, 3, and 6), in the concentration of 1 × 10⁵ cells/well. The plates were removed from the incubator after 48 hours at 37°C in 5% CO₂ incubator. After 48 hours, the growth media were pipetted out and 1 mL of maintenance media (media without FCS) was added to all the wells.

Sample Preparation

Thirty-six uniform-size samples (8 mm × 2 mm × 2 mm) prepared in Teflon molds for each resin were cured according to manufacturers’ instructions (40 seconds) using halogen-curing unit 3M ESPE Elipar2500 (3M ESPE, Seefeld, Germany). The distance between the samples and the curing tip was standardized as 2 mm. The entire procedure for sample preparation was done under aseptic conditions in a UV-sterilized laminar air-flow chamber at room temperature.

Experimental Design

The cell culture device described by Tang and associates was used to evaluate cytotoxicity of the resins. Of the five groups of samples (36 disks for each resin) prepared, half the number of samples of each resin were cleaned by wiping their surfaces lightly once with 99% acetone in sterile gauze to remove the
OIL (washed resins). The remaining resin disks were left intact (intact resins). Vero cells were seeded in three 12-well plates for each resin (one plate each for assessment on days 1, 3, and 6). Sterile tissue culture inserts (Falcon 3097) with 0.8-μm pore size Cyclopore PET membranes were placed above the cells in each well. The resin disks were placed on the membrane, allowing the passage of leaching components from the resin to reach the cells, thus exposing them to both intact and washed resins immediately after the removal of OIL on day 1. Sterile glass disks of similar size were used as control.

**MTT Assay for Cell Viability**

The cell viability was assessed on days 1, 3, and 6 by tetrazolium bromide reduction (MTT) assay for mitochondrial activities in all of the test and control groups. The resin disks and membranes were removed from the wells. The cells were cleansed three times with phosphate-buffered saline solution. One hundred microliters of the MTT (Hi Media) solution (0.1 mg/mL of tetrazolium bromide salt dissolved in Basal Medium Eagle, GibcoBRL) was added to each well, and the plates were incubated overnight at 37°C in a 5% carbon dioxide incubator.

During incubation, the yellowish extracellular MTT salt was converted into purplish intracellular formazan by metabolic enzymes in the mitochondria. Propanol in 0.04 mol/L HCl was used to lyse the Vero cells, and the purplish lysate was read using an ELISA reader (Lab Systems, Multiscan EX) with a 560-nm filter. This procedure was done for each of the five resins. In addition, a similar assay was also done for the control group.

**DC Assessment**

Sample preparation and the DC assessment using the FTIR Spectrometer (Perkin-Elmer Corp, Norwalk, Conn) were similar to those described by Gioka et al.³ Thirty maxillary incisor brackets (Gemini 3M) were divided into five groups of six brackets each. With the adhesive applied, the brackets were pressed firmly on a yellowish background surface of 75% reflectance, covered by a cellulose strip to facilitate recovery of the set resin. Light-cured adhesive specimens were cured using a halogen-curing unit (3M ESPE Elipar2500) according to the manufacturer’s instructions. Set adhesive disks, which in clinical conditions correspond to the material in contact with enamel, were pulverized with mortar and pestle over a large surface area avoiding heating effects to produce a fine powder. A standardized quantity of resin powder and KBr was mixed and pressed into a transparent pellet. The pellet was then transferred to the FTIR spectrometer (Perkin-Elmer Spectrum GX). Spectra were recorded under the following conditions: 4000–400 cm⁻¹ wave number range, 4 cm⁻¹ resolution, 30 scans coaddition. The DC was estimated on a relative percentage basis with the two-frequency method and tangent baseline technique. Aliphatic (C=C) bond-stretching vibrations at 1638 cm⁻¹ were chosen as the analytical frequency, and the aromatic (C=C) bond-stretching vibrations at 1605 cm⁻¹ were selected as a reference frequency. For the GCLC, stretching of the methacrylate aliphatic C=C at 1638 cm⁻¹ and stretching of the carbon-oxygen ester bond at 1712 cm⁻¹ were selected as the analytical and the reference frequencies, respectively.

DC was determined according to the equation % DC = 100(1 – RDB), where RDB is residual double bonds.

\[
RDB = \frac{Ap(C=C)\cdot Am(C=\cdot C)}{Am(C=C)\cdot Ap(C=\cdot C)}
\]

where

- \(Ap(C=C)\): net peak absorbance area of set material at 1638 cm⁻¹
- \(Am(C=\cdot C)\): net peak absorbance area of unset material at 1605 cm⁻¹
- \(Am(C=C)\): net peak absorbance area of unset material at 1638 cm⁻¹
- \(Ap(C=\cdot C)\): net peak absorbance area of set material at 1605 cm⁻¹.

**Statistical Analysis**

The mean and standard deviation values, for both percentage of viability and DC expressed by each of the five groups, were obtained. The viabilities of the Vero cells in all the test groups were expressed as a percentage of the viability recorded in their individual controls. Data obtained were analyzed by two-way analysis of variance (ANOVA) for repeated measurements. Pearson correlation was established between the DC and the viability expressed by the intact resins on day 1. All statistical procedures were carried out using SPSS for Windows (version 11.5).

**RESULTS**

Table 1 depicts the means and standard deviations of the readings in each test group (intact and washed) of the five materials evaluated on days 1, 3, and 6. Figures 1 and 2 show the cell viability, in percentage of their individual control, as estimated by MTT assay on days 1, 3, and 6 after exposure to intact and washed resins, respectively. Statistical results of two-way ANOVA are shown in Table 2. The DC results for the five adhesives tested are shown in Table 3.
Table 1. Viability of Vero Cells, Expressed as the Percentage of Controls, Under the Influence of Intact and Washed Resins In Vitroa

<table>
<thead>
<tr>
<th>Material Tested</th>
<th>Day 1 (24 h)</th>
<th>Day 2 (48 h)</th>
<th>Day 3 (72 h)</th>
<th>Day 4 (96 h)</th>
<th>Day 5 (120 h)</th>
<th>Day 6 (144 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Washed</td>
<td>Intact</td>
<td>Washed</td>
<td>Intact</td>
<td>Washed</td>
</tr>
<tr>
<td>Unite (chemically cured)</td>
<td>21.3 (+4.03)</td>
<td>38.35 (+6.22)</td>
<td>47.77 (+5.89)</td>
<td>72.48 (+6.05)</td>
<td>47.49 (+5.77)</td>
<td>72.14 (+6.09)</td>
</tr>
<tr>
<td>Transbond XT (light cured with primer)</td>
<td>48.02 (+5.93)</td>
<td>72.85 (+6.12)</td>
<td>58.23 (+20.12)</td>
<td>36.27 (+7.90)</td>
<td>48.64 (+5.95)</td>
<td>36.13 (+8.03)</td>
</tr>
<tr>
<td>Heliosit (light cured without primer)</td>
<td>61.2 (+10.95)</td>
<td>80.6 (+8.40)</td>
<td>37.14 (+8.18)</td>
<td>63.8 (+15.16)</td>
<td>38.15 (+3.34)</td>
<td>47.5 (+7.40)</td>
</tr>
<tr>
<td>GC Fuji ORTHO LC (resin-modified GIC)</td>
<td>37.66 (+3.50)</td>
<td>25.79 (+5.22)</td>
<td>34.99 (+4.40)</td>
<td>14.43 (+2.74)</td>
<td>23.5 (+3.98)</td>
<td>5.48 (+2.09)</td>
</tr>
<tr>
<td>Phase II (dual cured, two paste)</td>
<td>34.92 (+4.32)</td>
<td>23.37 (+4.08)</td>
<td>26.35 (+3.99)</td>
<td>14.67 (+2.98)</td>
<td>23.31 (+3.86)</td>
<td>12.66 (+2.69)</td>
</tr>
</tbody>
</table>

a Mean values ± standard deviations, n = 6 for all readings.

Table 2. Statistical Difference Between the Viabilities Expressed by Intact and Washed Resins on All Three Days, as Analyzed by Two-Way Analysis of Variance

<table>
<thead>
<tr>
<th>Materials Tested</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact resins</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Washed resins</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Cytotoxicity of Resins

All the resins tested demonstrated toxicity. Two-way ANOVA showed statistically significant differences between the viabilities expressed among the intact resins on each of the three days. A similar result was demonstrated by the washed resins. A significantly higher percentage of viability was expressed by the washed resins of Unite (P < .001) and Heliosit (P < .01) than their respective intact groups at all time intervals. But the viability expressed by the washed Heliosit resins decreased over time. Washed resins of Transbond XT demonstrated a significantly (P < .001) higher percentage of viability than the intact resins only on day 1. In both GC Fuji ORTHO LC and Phase II groups, the intact and washed resin groups showed a decrease in viability over time, and the washed resin groups revealed a significantly lower viability (P < .001) at all time intervals compared with intact resins.

Table 3. Means and Standard Deviations of the Percentages of Degree of Conversion (n = 6 for All Readings)

<table>
<thead>
<tr>
<th>Materials Tested</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unite</td>
<td>51.65</td>
<td>0.74</td>
</tr>
<tr>
<td>Transbond XT</td>
<td>48.74</td>
<td>0.29</td>
</tr>
<tr>
<td>Heliosit</td>
<td>48.63</td>
<td>0.34</td>
</tr>
<tr>
<td>GC Fuji ORTHO LC</td>
<td>38.88</td>
<td>3.46</td>
</tr>
</tbody>
</table>

Table 4 shows the Pearson correlations between the cell viability expressed by intact resins on day 1 and the DC. The correlations were negative for all the materials tested except Heliosit and Phase II. But these positive correlations were not statistically significant (P > .05).

DC of Resins

Statistically significant differences in the DC were demonstrated between the materials tested, with Unite demonstrating the highest DC. This was followed by Transbond XT and Heliosit, between which there was no statistically significant difference.

Correlation of Cytotoxicity and DC

Table 4 shows the Pearson correlations between the cell viability expressed by intact resins on day 1 and the DC. The correlations were negative for all the materials tested except Heliosit and Phase II. But these positive correlations were not statistically significant (P > .05).
Intact Resins (With OIL). The intact resins of Unite (chemically cured adhesive) showed the maximum toxicity on day 1, which might be due to the liquid activator component.\textsuperscript{14} The viability expressed by both the intact and washed Unite resins, however, demonstrated an increase with time. This suggests that leaching of residual monomers and the associated toxic effect were almost complete by day 1 (after 24 hours). Heliosit (light-cured adhesive without primer) demonstrated a relatively higher percentage of cell viability on day 1. When compared with Transbond XT (also a light-cured material), the faintly lower toxicity of Heliosit on day 1 can be attributed to the absence of activator, primer, and the solubility of the components—might also have a role on the cytotoxicity of the materials.

The study detected potential toxic effects in orthodontic adhesives, which warrants further in vivo testing. To reduce the potential cytotoxic effects, several precautionary measures can be followed. The clinician should use only as much material as necessary, and care should be taken to remove excess polymerized adhesives, particularly in areas where the adhesives may come in intimate contact with the subgingival and interproximal tissues. Excess activator material has to be removed thoroughly by washing the tooth with a water spray once the adhesive has set. When sealants are applied, they should be painted conservatively and localized to the tooth surface where the bracket is to be placed, avoiding gingival contact wherever possible. As it has been reported that sealants have low abrasion resistance and are removed from tooth surfaces easily by tooth brushing, their potential for demineralization prevention assumes less significance when one considers the toxicity.\textsuperscript{6,15} Although in clinical situations, the volume of liquids passing through the oral cavity, including saliva, water, beverages, and so forth, might dilute the leached components and thus reduce their concentration, the prolonged exposure of tissues and organs to such noxious materials must not be overlooked. Therefore, further studies on the long-term effects of low concentrations of these materials need to be investigated to verify their safety. It further behooves manufacturers to test their products adequately, as suggested by the ADA Council on Materials. Furthermore, manufacturers should request feedback from users regarding reactions in which the product might be implicated since the absence of reactions to a product in the laboratory setting does not preclude reactions by human beings in clinical practice.

CONCLUSIONS

- Cytotoxicity was exhibited by all of the resins tested.
- Single-cure systems exhibited comparatively less...
cytotoxicity and higher DC, thus proving superior to dual-cure systems.

- The role of OIL in producing cytotoxic effects was questionable.
- A positive correlation between cytotoxicity and DC was exhibited by only two resins, thus indicating that factors other than polymerization (such as the presence of activator, primer, and the solubility of the components) might have a role in the cytotoxic properties of the resins.

REFERENCES