Composite Restorative Resins
Part 3 Cytotoxicity Test to Mouse Fibroblasts in Culture of UV and Visible Light-activated Composite Resins


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Agar overlay cytotoxicity tests of 18 commercial light-activated composite resins were carried out, using L929 mouse fibroblasts in culture, and the difference of adverse reaction to L-cell among certain materials was compared. In addition, the unreacted substances in the cured material, such as Bis-GMA and Triethylene glycol dimethacrylate, were measured using a high performance liquid chromatograph, and the relationship between the amount of unreacted substance and the injured toxic area (cm²) of L-cell was presented and discussed. From the results, with the exception of a few materials, adequate correlation between both factors was estimated. The injured area of L-cell was enlarged as the unreacted substances in the cured material increased.

Key words: Light-activated composite resin, Cytotoxicity, Unreacted substance

INTRODUCTION

Recently, di-acrylate, such as Bis-GMA, has been used extensively as a base material for composite restorative resin. However, it has been suggested that the unreacted substance in the cured material may cause an adverse tissue reaction, which becomes a serious problem in clinical application. The purpose of this investigation was to examine the unreacted substance in the cured materials and to investigate the effect of the unreacted substance on mouse fibroblasts in culture.

MATERIALS AND METHODS

The materials used in this investigation are listed together with manufacturer's name, batch number, color shade, exclusive light, exposure time and depth of cure in Table 1. All materials were polymerized into a rod shape (5.0 mm in diameter and 2.0 mm height) using a PTFE mold, according to the manufacturer's recommendation at a temperature of 23±0.5°C, and then were used to measure the unreacted substance and for the cytotoxicity test on mouse fibroblasts in culture.
Table 1 Materials and lights used

<table>
<thead>
<tr>
<th>Material</th>
<th>Shade</th>
<th>Manufacturer</th>
<th>Batch number</th>
<th>Light and batch number</th>
<th>Depth of cure and exposure time recommended by manufacturer</th>
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<tr>
<td>For anterior tooth</td>
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<td></td>
<td></td>
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<td>Exposure time (sec) Depth of cure (mm)</td>
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1) Altstatten, Switzerland 2) Homburg, West-Germany 3) Los Angeles, USA 4) Schaan, Lichtenstein 5) Grove Village, USA 6) Kyoto, Japan 7) Tokuyama, Japan 8) Kurashiki, Japan 9) Barden-Barden, West-Germany 10) Milford, USA 11) St. Paul, USA 12) Macclesfield, England 13) Osaka, Japan

1. Determination of unreacted substance in cured material1,2).
Each material was ground for two hours using a mortar and pestle after polymerization and its powder (0.05 g) was weighed accurately and added immediately to Tetrahydrofuran (10 ml). The amount of unreacted substance in the cured material was determined using a high performance liquid chromatograph (Shimadzu-Dupont, LC-3A), incorporating two steel columns (Shodex, GPC KF-801 and GPC KF-802). A sample of each solution (10 ml) was injected into the chromatograph and three measurements were made for each solution. In measuring the unreacted substance, the tetrahydrofuran solution of each material before setting was used for calibration purposes. The unreacted substance was calculated as the area under the curve at peak, produced by the material before and after setting. The percentage of unreacted substance in the tetrahydrofuran solution was converted to the unreacted substance content in the cured material (0.05 g) by calculating the total mass of unreacted substance in the tetrahydrofuran solution.

2. Cytotoxicity test of composite resins3)
The cytotoxicity test of the cured material was carried out using L929 mouse fibroblasts in culture. All materials were polymerized into a rod shape (5 mm in diameter and 2.0 mm
Zone I

Zone II

Zone III

Fig. 1 Classification of morphological changes of L-cell which were dyed with Giemsa stain after 24-hours contact with material. Zone I: Decomposed and rounded cells with dark nucleus, zone II: contracted cells, zone III: normal cells.

The extent of morphological change in the cells was classified into three zones as shown in Fig. 2. In addition, this measurement was also performed for the uncured materials before exposing them to light. In this case, an agar culture medium and material were covered with a black vinyl sheet and shielded completely from the light. The contact period for these materials/to cells was 2 min, 4 hours and 24 hours.

RESULTS AND DISCUSSION

The percentage of unreacted substance in the cured material and the results of the cytotoxicity test are presented in Figs. 2 and 3, respectively. All materials, with the exception of Palfique Light, Prismafil, Prismafil Compules and Pyrofil Light Bond, indicated relatively weak cytotoxicity to the cells. Furthermore, among these materials, there was adequate correlation between the percentage of unreacted substance in the cured material and the area which appears toxic to the cells, as shown in Fig. 4. The coefficient of
correlation was 0.728 for zone I and 0.726 for zone II. These results indicate that the cytotoxicity to cells increases with the increase of unreacted substance in the cured material. For Palfique Light, Prismafil, Prismafil Compules and Pyrofil Light Bond, the injured area of cells appeared markedly large, though the percentage of unreacted substance in the cured material was low.

Although the reason is presently unknown, it may be closely connected with the materials used in the manufacturing process of composite resin.

Particularly, for Palfique Light, zone I, where the decomposed cells and rounded cells with dark nucleus exists, the cells were not wholly observed. However, in zone II, which contains many contracted cells, cells were observed over a wider region than those of other materials, as shown in Fig. 3. This phenomenon is rather unusual and a matter of interest.

In the contact test of 2 min for an uncured material before exposing it to light, no morphological change in cells were seen in any of the materials used. However, in the contact
Fig. 3 Injured area of L-cell caused by contact with cured composite resins. 
upper parts: for 1.5 mm specimen thickness, 
lower parts: for maximum specimen thickness (Table 1) recommended by manufacturer, (■) shows zone I and □ shows zone II).

test for 4 hours and 24 hours, marked morphological changes of cells were observed. These results are shown in Fig. 5. The injured area of cells for four materials (Palfique Light, Prismafil, Prismafil Compules and Pyrofil Light Bond) became wider, compared with those of other materials. From these results, therefore, it follows considered that these four materials contain more substances that were soluble in the agar culture medium.

CONCLUSION

The agar overlay cytotoxicity tests of 18 commercial light-activated composite resins
were carried out and the relationship between their results and the amount of unreacted substance in the cured material was investigated. The coefficient of correlation between the injured area of mouse fibroblasts in culture and the amount of unreacted substance in the cured material was 0.728 in zone I and 0.726 in zone II, respectively. In general, the injured area of cells expanded in accordance with the increase of unreacted substance in the cured material. However, a few materials which appeared to have relatively large injury to cells also existed among the commercial products, regardless of the amount of unreacted substance in the cured material.
Fig. 4 Relationship between amount of unreacted substance in cured composite resins and injured area of L-cell caused by them. (○ shows zone I and □ shows zone II).

REFERENCES


コンポジットレジン用レオメータにより4種類の市販小窩製溝溝塞材の操作時間、硬化時間を測定した。また、人の拔去歯牙の小窩製溝溝塞された材料の硬化中の温度上昇を熱電対を用いて調べ、さらに、高速液体クロマトグラフを使って硬化後の未反応物質の分析を行った。
23℃でのそれぞれの材料の操作時間は0.52～1.09 minの間であり、そしてまた32℃での硬化時間は0.91～1.38 minの間であった。0.5 mmの厚さで硬化させた時、備室窓から1.2 mm離れたデンチン部分での温度上昇は0.18～0.21℃の間であった。未反応物質は、硬化前後それぞれの溶液で測定されたクロマトグラフの面積比で計算され、0.5 mmの厚さの試料では、それぞれの材料で19.7～45.8%の範囲であった。
本実験中の小窩製溝溝塞材では、硬化時の温度上昇の歯科に対する影響はほとんどないと考えられる。

充てん用コンポジットレジンに関する研究
－第3報－光重合型コンポジットレジンのL細胞に対する毒性試験

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現在市販されている18種類の光重合型コンポジットレジンについて、マウス由来の培養細胞に対する各材料の細胞毒性試験を行なった。細胞障害の程度は、細胞死を含む核液染色領域と細胞形質に萎縮がみられる領域の2領域にわけ、それぞれの領域の面積で表示した。また、各材料の硬化物中に含まれる未反応物の量を高速液体クロマトグラフを用いて定量し、未反応物量と細胞障害がみられる領域との関係を調べた。その結果、4種類の材料（バルフィークライト、プリズマフィル、プリズマフィ

ルコンピュール、パイロフィルライトボイド）を除く他の材料間では、硬化体中の未反応物量と細胞障害を引き起こす領域との間に相関がみられ、未反応物の増加に伴って細胞障害を起こす領域が大きくなることがわかった。除外した4種類の材料については、硬化体中の未反応物量は少ないにもかかわらず、細胞障害を起こす領域が著しく大きくなっている。この理由については現在検討中である。

接着性ブリッジの耐久性に関する破壊力学的解析
——接着性ブリッジの設計要因が接着層でのき裂成長に与える影響——

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鹿児島大学歯学部歯科補綴学第1講座

有限要素法にもとづく破壊力学的手法によって、接着性補綴物の設計条件と接着材層に発生するき裂成長との関係をエネルギー解放率gの概念を用いることにより解析した。解析は接着材層でのき裂成長に伴うエネルギー解放率とクリティカルロードの値を、水平のき裂成長率α，安全率n，メタルフレームの構造および金属の厚さのファクターによりどのような様に変化するかにより行なった。解析の結果、gはメタルの厚みが増すと減少し、1.0 mm以上では非常に小さくなった。また隣接面にウィングを設定すると設定しない場合に比べgは小さくなり、その差Δgはウィングの長の半分程度で最大となった。そしてΔgは、金属厚みの增加に伴わない減少量を結果とクリティカルロードを用いた考察により、接着性補綴物の耐久性を向上させる為のブリッジの設計要因について検討を行ない呈示した。