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

Katsuichiro INOUE*, Hiroyuki ARIKAWA*, Koichi FUJII*, Akira NIIHARA*,
Ryuichiro FUJITA*, Gakuji TSUKADA*, Kayoko KUROKI**, Tomoko OKA*** and
Choji UCHIYAMA***

*Department of Dental Materials Science, Kagoshima University Dental School,
1208-1 Usuki, Kagoshima 890, Japan

**Department of Dental Pharmacology

***Department of Oral Bacteriology, Kyushu Dental College, 2-6-1,
Manazuru, Kokurakita-ku, Kitakyushu 803, Japan

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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 Triethyleneglycol 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 high) using a PTFE mold, according to the manufacturer's recommendation at a temperature of $23 \pm 0.5^\circ\text{C}$, 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

Material	Shade	Manufacturer	Batch number	Light and batch number	Depth of cure and exposure time recommended by manufacturer	
					Exposure time (sec)	Depth of cure (mm)
For anterior tooth						
Brilliant Lux	U	Coltene ¹⁾	090783	Pluraflex HL150, 01737	40	2.0
Durafill	U	Kulzer ²⁾	Jan84136	Translux CL, 15/11462	20	3.0
Estilux	GO	Kulzer ²⁾	Aug83142	Duralux UV-300, 505/1919	40	2.5
First Lite	U	Phase alloy ³⁾	40404	Wite Lite™, 60610	20	2.5
Heliosit	20	Vivadent ⁴⁾	011283	Heliomat, 18518	20	4.0
Light Activated Restorative	L	Teledyne Getz ⁵⁾	603081	Focus™ Activator Light, 35408	20	3.5
Lite-Fil A	U	Shofu ⁶⁾	018401	Daylight Lamp, 0184704	30	3.0
Palfique Light	U	Tokuso ⁷⁾	LU204	Wite Lite™, 60610	30	3.0
Photo Clearfil A	US	Kuraray ⁸⁾	1046	Quick Light, 1205	20	2.5
Plurafil Super	U	Litema ⁹⁾	40545	Pluraflex HL150, 01737	30	3.5
Prismafil	L	Caulk ¹⁰⁾	111683	Prisma Lite, 24520-1	10	3.5
Prismafil Compules	LG	Caulk ¹⁰⁾	0720834	Prisma Lite, 24520-1	10	2.5
Silux	U	3M ¹¹⁾	072484	Optilux, 00287	20	2.5
Super Lux Daylight	U	Shofu ⁶⁾	30344	Daylight Lamp, 0184704	30	3.0
For posterior tooth						
Lite-Fil P	—	Shofu ⁶⁾	018413	Daylight Lamp, 0184704	30	3.0
Occlusin	—	ICI ¹²⁾	450181/1/B	Luxor, 06462	40	3.0
P-30	—	3M ¹¹⁾	4U6P	Optilux, 00287	20	2.5
Pyrofil Light Bond	—	Sankin ¹³⁾	163-502	Suncure Light, 1013	20	3.0

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⁾ Macclcesfield, England ¹³⁾ Osaka, Japan

1. Determination of unreacted substance in cured material^{1,2)}.

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

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.

hight) and placed on the cells which were cultivated in advance on a glass cover slip. The contact time of the materials/to cells was 24 hours. The cells were dyed with Giemsa stain after removal of the material and the morphological changes that took place were investigated using a light microscope.

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

Fig. 2 Percentages of unreacted substance in cured material at 3 days after polymerization.

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

Fig. 5 Injured area of L-cell caused by contact with uncured composite resins before exposing to light.
upper parts: contact with material for 4 hours,
lower parts: contact with material for 24 hours. (▨ shows zone I and □ shows zone II).

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).

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コンポジットレジン用レオメータにより 4 種類の市販小窩裂溝充填材の操作時間、硬化時間を測定した。また、人の抜去歯牙の小窩裂溝に充填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°C 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°C 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、髓室蓋から 1.2 mm 離れたデンチン部分での温度上昇は 0.18~0.21°C の間であった。未反応物質は、硬化前後の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。

本実験中の小窩裂溝充填材では、硬化時の温度上昇の歯髄に対する影響はほとんどないと思われる。

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

井上勝一郎*, 有川裕之*, 藤井孝一*, 新原 明*, 藤田龍一郎*

塚田岳司*, 黒木賀代子**, 岡 智子***, 内山長司***

*鹿児島大学歯学部歯科理工学講座 **九州歯科大学歯科薬物学講座

***九州歯科大学口腔細菌学講座

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、細胞死滅を含む核濃染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(パルフィークライト、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——

篠原直幸

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が、水平的なき裂長さ a^* 、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 はウィング長の半分程度で最大となった。そして Δg は、金属厚みの増加に伴ない減少した。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。