Biocompatibility Test of Light-cured Composites in Vitro

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The in vitro cytotoxicity to HeLa S3 cells of set specimens of five commercially available light-cured composites was examined by the cell colony-forming method for 6 weeks. Both visible and ultraviolet light-cured composites showed moderate to strong cytotoxicity in the early stage of the experiment, followed by rapid disappearance of cytotoxicity. The light-cured composites showed in vitro biological behaviors similar to those of conventional composites, MFR and composites for core use.

Key words: Visible and ultraviolet light-cured composites, Biocompatibility test

INTRODUCTION

Recently, a number of light-cured composite resins, differing in curing mechanisms from the conventional composite resins, have been developed. They are composite resins available as "one paste" that are filled in the cavity and then light-cured by radiation. This unique light curing system makes this type of resins quite interesting from the biological standpoint. The present experiment was carried out to study the in vitro cytotoxicity of 5 commercially available products for the purpose of examining the biological properties of these light-cured composite resins.

MATERIALS AND METHODS

Cell culture: HeLa S3 cells were cultured in a Eagle's minimum essential medium (MEM) supplemental with 10% calf serum.

Specimen preparation: Five light-cured composite resins, namely 4 of the visible light curing type, Heliosit*, Superlux daylight**, Plurafil super***, and Visio-dispers****, and Nuva-fil***** of the ultraviolet light curing type were examined. Clearfil F****** was used as control of the conventional type. Cylindrical specimens (6 mm in diameter and 5 mm high) were prepared of all composite resins under the curing conditions specified by the manufacturers, that is, the five light-cured composite resins by the use of the


* Shade 20, 20112, Vivadent, Liechtenstein
** Universal, 01099, DMG-Shofu, Japan
*** Universal, 10326, Litema, Baden-Baden
**** Standard, H205, Espe, West Germany
***** Light yellow, 062078, Caulk, USA
****** Universal paste, CU-2268; Catalyst, CC-2168, Kuraray, Japan
respective light source and the conventional composite resin at the specified optimum mixing ratio.

Experimental method: The cylindrical specimen was dipped in 15 ml of fresh culture medium (extract) kept in an Erlenmeyer flask and extracted by the method of dynamic extraction with a freely moving specimen under the conditions of 37°C, 200 rpm in a gyro-rotary shaker†, simulating the dynamic load restorative materials are subjected to in the mouth cavity3-12). Since the specimen is freely swung around by the gyrotory movement of the shaker, it is subjected to a dynamic load caused by the flow of the extract as well as friction with and collision against the flask's wall and thus created is a test environment more closely simulating the influences that the material filled in the mouth cavity is subjected to.

Extraction under these conditions was continued for 2 weeks and the resulting extract was used for the experiment, in which the same specimen was further extracted as above for a total of 6 weeks at 2-week intervals. The extracts taken at the end of each 2-week period were used for cell culture. For the experiment, the extracts obtained as original liquids were diluted to 4 levels, each dilution was used for a 1-week culture and the cell colonies formed were fixed and stained.

The cell colony-forming efficiency was determined by measuring the area colony growth per unit area, using an automated counting system for cell colonies consisting of a combination of photo pattern analyzer†† and personal computer†††. The data for the experimental group at each time of experiment were divided by those for the control group obtained at the same time to determine the relative growth rate, which was finally converted into six-step cytotoxic scores. Relative growth rate, ≥100% ≡ Cytotoxic score, 0 (No effects on the cells); RGR, 75% ~ 99% ≡ Score, 1; RGR, 50% ~ 74% ≡ Score, 2; RGR, 25% ~ 49% ≡ Score, 3; RGR, 1% ~ 24% ≡ Score, 4; RGR, 0% ≡ Score, 5 (No cell growth)5, 9, 12).

RESULTS

1. Extract observation

Samples taken every two weeks until 6 weeks retained their original shade of orange red, indicating that the specimen had no influence on its extract. Further the extract per se retained its transparency, with no indication of cloudiness, nor any precipitates or floating matter. The specimens retained their original shapes throughout the test period, no visible changes in color. Their external appearance also remained unaltered; their surface lustre was well retained, with no indication of discoloration or deposits.

2. Effects of the extract on cell colony formation

Both light-cured composite resins and conventional composite resins, showed lower relative growth rates after 2 weeks of extraction as the extract concentration increased (Tables 1 and 2, Figure 1). From the 4th week, the relative growth rates values were similar to that of the control group. Thus, all 6 products tested showed moderate to strong

† G24, New Brunswick, USA
†† Biotran III, New Brunswick, USA
††† MB-6890, Hitachi, Japan
BIOMATERIAL TEST OF LIGHT-CURED COMPOSITES

Table 1  Relative growth rate (RGR) as assessed by cell colony area

<table>
<thead>
<tr>
<th>Materials</th>
<th>Max, Mean &amp; Min</th>
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<th>4</th>
<th>6</th>
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<tr>
<td></td>
<td>Max</td>
<td>Mean</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>1 CLEARFIL F</td>
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<td>106</td>
<td>81</td>
<td>56</td>
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<tr>
<td>2 HELIOSIT</td>
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<td>109</td>
<td>88</td>
<td>67</td>
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<tr>
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<td>107</td>
<td>102</td>
<td>97</td>
</tr>
<tr>
<td>4 PLURAFIL SUPER</td>
<td>25%</td>
<td>107</td>
<td>104</td>
<td>101</td>
</tr>
<tr>
<td>5 VISIO-DISPERS</td>
<td>25%</td>
<td>114</td>
<td>113</td>
<td>112</td>
</tr>
<tr>
<td>6 NUVA-FIL</td>
<td>25%</td>
<td>116</td>
<td>110</td>
<td>104</td>
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Confidence limit of population mean (5% level of significance).

\*RGR (\%) = \frac{\text{Maximum, mean and minimum values in the experimental conditions}}{\text{Mean values in control}}

Cytotoxicity after 2 weeks of extraction as the concentration of the extract increased, but negligible cytotoxicity thereafter.

DISCUSSION

There are many reports on in vitro tests and in vivo tests on the pulpal irritation of the composite resins^{13-22}. Since composite resins contain many fillers, low contents of resin monomers were considered to reduce tissue irritation in the early stage of their development. Today, however, it is widely recognized that their pulp irritation is equal to or even higher than that of MMA resins which are typical of the conventional unfilled resins. The results of biological in vitro tests show strong cytotoxicity in the process of
Table 2  Mean scores of cytotoxicity deduced from RGR values*

<table>
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* Based on relative growth rate values as expressed by cell colony area.

![Figure 1](image)

Figure 1  Biocompatibility deduced from the cytotoxic scores. The four bars in each experimental week represent cytotoxic scores for four classes of the original extract concentrations, i.e. 25%, 50%, 75%, and 91%, from left to right. Also, six dots at the left side of the column of each experimental week correspond to cytotoxic scores; namely, the top dot represent Score 0, the second dot from the top; Score 1, and so on.

setting of such resins after mixing\textsuperscript{23-27}. Although cytotoxicity is noted for some time even after setting, the greater part of the cytotoxic component is gradually lost after 20 weeks\textsuperscript{10}. Similar test results were obtained with rather short test periods for the latest composite resins of the MFR type or composites for core use\textsuperscript{28}.

The light-cured composite resins examined in the present experiment showed in vitro
biological behaviors closely similar to those of Clearfil F, Concise, Isopast, Micro jar, Superlux, Core paste 6000, Clearfil core, Corelite, Core Max, Adaptic, new composite resins TMM-Si₃N₄ Resin, TMM-SiO₂ Resin etc.⁷⁻¹⁰,²⁸). The light-cured composite resins in the present experiment had primary monomers similar to those of composite resins such as Heliosit with Bis-GMA and urethane dimethacrylate, Superlux daylight and Plurafil super with urethane dimethacrylate, and Visio-dispers and Nuva-fil with Bis-GMA and Triethylene glycol dimethacrylate²⁹). The fillers used in resins other than Nuva-fil were micro-fillers identical to those contained in composite resins of the MFR type, the average filler size being 0.03 μm and the filler contents being as low as 35% to 39% except for Visio-dispers (66%), which were less than half of that of Clearfil F (approx. 80%). This means that they have a high monomer content which was shown to be the main cause of their cytotoxicity³⁰), the monomer-filler ratio being identical to that of the MFR type²⁸). This suggests that a high resin content increases cytotoxicity. The present results, however, showed that the effects of the new type of composite resins on cell growth were identical to those of Clearfil F, a conventional composite resin. This supports the results of earlier tests on the MFR type of the chemical initiating system. The increase of the content of the resin matrix and the difference in the kind of resin monomer were not directly reflected in change of the cytotoxicity.

The light-cured composite resins contain a component not found in conventional composite resins, i.e. a photosensitizing agent²⁹); visible light-cured composite resins contain α-diketone and ultraviolet light-cured composite resin Nuva-fil, benzoin methylether. Our results demonstrate that these additional components do not contribute to the cytotoxicity of this type of composite resins. Although cytotoxicity was noted for some time after setting, its degree was low, being similar to that of various kinds of composite resins⁵,¹⁰,²⁸), and is in sharp contrast with the marked cytotoxicity resin monomer alone. This may be due to the condition of the set specimen. The specimens were already sufficiently set when extraction was started. Our results appeared to confirm the earlier data showing that the marked cytotoxicity immediately after mixing is attenuated gradually with time in the polymerization process⁵,¹⁰,²⁸). The reason for the moderate to strong cytotoxicity noted in the extract in the early post-setting stage due to dissolving out of the residual monomer into the extract. This may well explain the observed phenomenon. It is probably that after such cytotoxic components were "washed out" in the first extraction, the specimens no longer contained such components in quantities sufficient to dissolve out in the 2nd and 3rd extraction in the 4th and 6th weeks of the experiment, respectively, to exhibit positive cytotoxicity.

There are two primary causes of pulpal injury after restoration: tissue irritation induced by the material and the microleakage in the tooth-restoration interface. Many investigators are of the opinion that of these two factors, microleakage is of the greater importance³¹,³²). We, however, are of the opinion that quite a few of the cases of pulpal injury are attributable to the restorative material used. Since in the early stage of restorative procedure an unset material or compound is filled in the cavity, its strong cytotoxicity remains for some time after setting, thus the possibility of tissue irritation by and cytotoxicity of the material can not be excluded.
CONCLUSION

The in vitro effects of light-cured composite resins on HeLa S3 cells were studied.
1. Throughout the 6-week period, no change was noted with regard to the extract samples taken periodically or the specimens.
2. Both visible light-cured composite resins and ultraviolet light-cured composite resin showed in the early stage of the experiment moderate to strong cytotoxicity similar to their conventional counterpart, but it disappeared rather rapidly thereafter until it was no longer noticeable 6 weeks later.

The above results were similar to the reported data on the conventional materials.

ACKNOWLEDGMENT

The authors are grateful to Dr. Y. Ohgitani and Ms. Y. Ohta for their assistance throughout the present project.

REFERENCES


29) Technical notes of the respective manufacturers' products


による上向き切削・研削および下向き切削・研削の仕上
面に及ぼす影響を調べた。本研究から次の結論が得られ
た。
1) 切削工具（カーバイドバー7664）によるマイクロ
フィル型コンポジットレジンの切削仕上面は従来フィラ
ー型コンポジットレジンに対して著しく改善された。こ
れは分散相フィラーの粒径とそれに基づくコンポジットレ
ジンの不均質性によって切削抵抗とその変動が影響され
ることを示唆する。
2) 研削工具（ダイヤモンド工具 F 201 R）による研
削仕上面はカーバイドバーによるそれよりも改変される
と共に、コンポジットレジン間の差異が減少した。これ
は負のすくい角の増加によって切れ込みが減少したことに
起因すると考えられる。
3) 両工具において、上向き切削・研削の仕上面の方
が下向き切削・研削のそれよりも良くなった。これは接
線切削・研削抵抗が上向き切削・研削では切削・研削予
定面の上方に働く、下向き切削・研削では逆に下方に働く
ことから説明できる。

光線重合レジンの細胞毒性 (in vitro)

中村正明, 今井弘一, 大島 浩, 工藤貴也, 吉岡宣史朗, 川原充幸
大阪歯科大学歯科理工学教室

光線重合レジン5製品、すなわち、可視光線重合タイ
プ4製品、紫外線重合タイプ1製品の硬化試料がHeLa
S3細胞に対しておよそ6時間影響を調べるために、in vitro
環境下で細胞コロニー形成を観察して実験を行った。
2週間の単位浸漬時間を合計6週間におわたって、従来か
ら行っている口腔内の動的環境のシミュレーションを目
ざした試験試験を続け、各時期の浸漬液を実験に用
いた。テストした各材料で初期に浸漬液の濃度があがる
と中等度から強い細胞毒性を示した。しかし、その後は
細胞毒性は急速に消失していった。この結果は同時にテス
トした従来型のクリアフィルFと似ていたが、以前に
テストしたMFRタイプなどを含む従来型製品とも軽
を一にするものであった。以上の実験結果はテストした
2種類の光線重合レジンが細胞に対して従来型製品とき
わめてよく似た影響を及ぼすことを見ている。

本研究の一部は文部省科学研究費補助の試験研究
(56870103) による。

貴金属修復物の高温における変形機構
（第2報）高温たわみに対する鉄, インジウム, スズ添加の影響

安藤信夫, 中山正彦
日本歯科大学理工学教室

鉄、インジウムおよび/あるいは、スズを2種の母合
金、90 Au-10 Pd, 80 Au-20 Pt, に添加し、母合金に
つき10種の合金を試作した。添加元素の合計は、0.75％
であった。高温たわみ（試験片を1050℃まで加熱した後
の最終たわみ量）および、変形開始温度（20 μm のたわ
みを生じる温度）を測定し、これらの性質に対する添加
元素の影響を調べた。