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一、中文摘要

這項研究目的是想了解 5 種不同牙齒顏色複合樹脂基底修復材料的萃取液，對人類牙髓細胞的生物相容性。研究的設計 ：將 5 種不同牙齒顏色複合樹脂基底修復材料(2 種添加樹脂的玻璃離子體修復材料；Fuji II LC 和 Fuji IX, 1 種 compomer ；Dyract, 和 2 種複合樹脂；Tetric 和 Superfil)，聚合後放入細胞培養基液中 2 和 5 天，分別收集材料的萃取液，作用在人類牙髓細胞。評估複合樹脂基底修復材料對人類牙髓細胞的細胞毒性和線粒體活性分析。 結果顯示複合樹脂基底修復材料的萃取液，對人類牙髓細胞俱有細胞毒性。另外, Superfil, Fuji IX, 和 Tetric 顯示了對人類牙髓細胞的線粒體活性的抑制作用。同時被發現 複合樹脂 Superfil 是毒性最強的修復材料。結論是細胞毒性的取決於材料成份種類。 Compomer 或 Fuji II LC 添加樹脂的玻璃離子體修復材料，對人類牙髓細胞初期反應，較其它 3 種修復材料有較好細胞的生物相容性 。

關鍵詞：生物相容性、複合樹脂 、人類牙髓細胞、玻璃離子體、細胞毒性

Abstract

The objective of this study was to determine the cytocompatibility of 5 different extracts of resin-based restorative materials (2 resin-modified glass ionomer cements, 1 compomer, and 2 composite resins) on human pulp cells. Set specimens from 2 resin-modified glass-ionomer cements (Fuji II LC and Fuji IX), 1 compomer (Dyract), and 2 composite resins (Tetric and Superfil) were eluted with culture medium for 2 and 5 days. The effects of resin-based restorative materials on human pulp cells were evaluated with cytotoxicity and mitochondrial activity assays. The results showed that the eluates from resin-modified glass-ionomer, compomer, and composite resins were cytotoxic to primary human pulp cells. In addition, Superfil, Fuji IX, and Tetric demonstrated an inhibitory effect on mitochondrial activity of human pulp cells. It was found that composite resin Superfil was the most toxic restorative material among the chemicals tested. The influence of the cytotoxicity depended on the materials tested. Compomer or light-curing resin-modified glass ionomer may initially react more favorably to pulp cells.

Keywords ： cytocompatibility; composite resins; human pulp cells; glass-ionomer; cytotoxicity

二、緣由與目的

The emphasis on aesthetics has increased in clinical usage, and the number of resin-based restorative materials has

increased over the past decade. However, it has been found that, because of degradation or corrosion, several components are leached out from each resin-based restorative material into the oral environment. This in turn may cause some adverse effects.[1] In addition, direct interactions at the interface between a restoration and the tissues may make biocompatibility an issue. Composite-resin restorative materials cause pulpal inflammation,[2,3] induced by the microleakage of bacteria at the margins of the unetched restoration[4] or by the toxic ingredients of the composite resin.[5-7] Moreover, these compounds are common sensitizing agents and may cause type IV allergic reactions. Allergic reaction reactions among dental personnel are increasingly being reported, and lichenoid reactions have also been attributed to composite materials.[8-10]

Evaluating biologic and toxicologic properties of dental materials is important in relation to the materials' clinical usage.[11] In vitro assays for initial screening of new dental materials are intended for use in humans. Experimentation in vitro has the advantage of easy control of experimental factors that are often a problem when performing experiments in vivo.[12] In vitro methods are simple, reproducible, cost-effective, relevant, and suitable for the evaluation of basic biologic properties of dental materials.

In addition to the evidence that pulpal irritation under composite is produced by microleakage of bacterial byproducts, chemical irritation of the pulp may also occur. However, the cytotoxicity of resin-based restorative materials to cultured human pulp cells has not been adequately studied. The objective of this experiment was to compare 5 resin-based restorative materials (2 resin-modified glass ionomers; 1 compomer; and 2 composite resins) with respect to human pulp cells. Toward this aim, we established an in vitro model system to study the effects of resin-based restorative materials on human pulp cells by cytotoxicity and mitochondrial activity assays.

三、結果與討論

Materials and chemicals

As shown in Table I, the resin-based restorative materials tested were 2 resin-modified glass ionomers (Fuju II LC and Fuji IX, GC Corporation, Tokyo, Japan); 1 compomer (Dyract, Dentsply Ltd, Konstanz, Germany); and 2 composite resins (Tetric and Superfil, Dentsply Ltd, Konstanz, Germany).

Table I. Manufacturers and types of resin-based restorative materials tested

Product	Type	Polymerization	Manufacturers
Dyract	Compomer	Light-cured	Dentsply Ltd., Konstanz, Germany
Fuji II LC	Resin-modified glass ionomer	Light-cured	GC Corporation, Tokyo, Japan
Fuji IX	Resin-modified glass ionomer	Self-cured	GC Corporation, Tokyo, Japan
Tetric	Composite resin	Light-cured	Dentsply Ltd, Konstanz, Germany

Superfil	Composite resin	Light-cured	Dentsply Ltd, Konstanz, Germany

All tissue culture biologics were purchased from Gibco Laboratories (Grand Island, NY). Dimethyl sulfoxide and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemical Co (St. Louis, Mo).

Sample fabrication

Triplicate sample disks of the rosin-based restorative materials were fabricated under aseptic conditions in glass molds (2 × 10 × 60 mm). Samples were fabricated and polymerized in accordance with the manufacturer's instructions (Table I). Excess flash was trimmed away with a sterile scalpel.

Eluate preparation

The fresh samples were those tested immediately after disk transfer. All specimens were extracted twice consecutively in 6 mL Dulbecco's modified Eagle's medium (DMEM) without serum. After each elution period, the extracts were removed, and the vials were filled again with fresh medium. Extraction was performed in an atmosphere of 5% CO_2 and 95% air at 37°C. All extracts were filtered for sterilization until used for each assay.

Cell culture

Human dental pulp cells were cultured by using an explant technique as described previously.[13-15] Briefly, human premolars were extracted for the correction of malocclusion. The tooth root was removed by horizontal section below the cementoenamel junction with a no. 330 bur in high speed with water spray. The pulp tissue was removed aseptically, rinsed with DMEM, and placed in a 35-mm Petri dish. Pulp tissue was minced with a number 15 blade into small fragments and grown in DMEM supplemented with 10% fetal calf serum and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL fungizone). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Confluent cells were detached with 0.25% trypsin and 0.05% ethylenediamine tetra-acetic acid for 5 minutes, and aliquots of separated cells were subcultured. Cell cultures between the third and eighth passages were used in this study.

Cytotoxicity assay

An MTT colorimetric assay was developed to monitor mammalian cell survival and proliferation in vitro.[16] The MTT assay was measured by dehydrogenase activity as described by Mosmann,[16] with minor modification.[17] Briefly, 1×10^4 cells per well were seeded to 96-well plates and left overnight to attach. Various eluates in 100-µL volumes were added, and cells were treated for 24 hours. After treatment, 50 µL MTT solution (1 mg/mL in phosphate-buffered saline) was added to each well and incubated for another 4 hours at 37°C. To each well, 150 µL dimethyl sulfoxide was added. Plates were then shaken until crystals were dissolved. Reduced MTT was then measured spectrophotometrically in a dual-beam microtiter plate reader at 570 nm with a

650-nm reference. The optical density values of the experimental groups were divided by the control and expressed as a percentage of control.

Mitochondrial activity

Effects of various eluates on the mitochondrial function were measured by a colorimetric assay as described by Mosmann.[16] This assay measures the conversion of a yellow water-soluble MTT dye into a purple formazan product by active mitochondria via an electron current. As in our recent study,[15,18] cells were seeded 2 × 10^4 cells per well into 96-well culture plates. After overnight attachment, cells were treated with various eluates and 10 µL MTT solution was added to each well for 2 hours. On termination of the experiment, all the medium was discarded by inverting and tapping the plates, and 100 µL dimethyl sulfoxide was added to each well. The functional mitochondrial activity of eluate-treated cells was calculated as a percentage of control.

Statistical analysis

Five replicates of each concentration were performed in each test. All assays were repeated 3 times to ensure reproducibility. Statistical analysis was carried out by means of the program SAS for Unix 6.09 (SAS Institute, Cary, NC) by 1-way analysis of variance (ANOVA). Tests of differences of the treatments were analyzed by Duncan's test, and a value of $P < .05$ was considered statistically significant.

The results showed that resin-modified glass ionomer, compomer, and composite resin were cytotoxic to primary human pulp cell cultures by MTT assay. The sensitivity of cytotoxicity to human pulp cells depended on the materials tested.

Eluates from 5 resin-based restorative materials were cytotoxic to primary human pulp cell cultures when collected from disks at all time periods ($P < .05$), and Superfil was the most cytotoxic at day 2 ($P < .05$)(Table II).

Table II. Viable human pulp cells after incubation with eluates of five resin-based restorative materials (percentage of control)

Eluate (time)	Dyract	Fuji II LC	Fuji IX	Tetric	Superfil
Day 2	58 ± 1*†	59 ± 2*†	43 ± 2*†	58 ± 2*	29 ± 3*
Day 5	67 ± 4*	73 ± 2*	63 ± 2*	43 ± 0*†	25 ± 4*

*Statistically significant in comparison with control, $P < .05$.
†Statistically significant between day 2 and day 5, $P < .05$.

Eluates of Dyract, Fuji II LC, and Fuji IX showed decreased cytotoxic response from day 2 to 5 ($P < .05$)(Table II□). This phenomenon showed that the leaching of toxic substances was markedly diminished at the 5-day extraction period. In general, the rank order with respect to cytotoxicity at day 5 was as follows: Superfil > Tetric > Fuji IX > Fuji II LC = Dyract. The result showed that eluates from disks of Tetric produced a significantly greater decrease in viable cell numbers than other eluates from day 2 to day 5 ($P < .05$)(Table II□).

Superfil, Fuji IX, and Tetric demonstrated an inhibitory effect on mitochondrial activity of human pulp cells ($P < .05$)(Table III).

Table III. Mitochondrial activity of human pulp cells after incubation with eluates of five resin-based restorative materials (percentage of control)

Eluate (time)	Dyract	Fuji II LC	Fuji IX	Tetric	Superfil
Day 2	84 ± 1	82 ± 2	65 ± 2*	79 ± 2*	58 ± 3*
Day 5	95 ± 2	100 ± 1	78 ± 1*	80 ± 3*	70 ± 3*

*Statistically significant in comparison with control, $P < .05$.

However, treatment of the cells with eluates of Dyract and Fuji II LC did not significantly inhibited the mitochondria activity of the cells ($P > .05$).

Many cell culture techniques have been applied to assess the cytotoxicity of dental materials. These methods are based on cell cultures with established or diploid cell lines and a few tissue explant techniques. However, an increasingly number of authors have stated that in vitro toxicity tests should be performed with the most appropriate cells (ie, cells homologous to the human tissues of ultimate concern).[19,20] Any material used as a restorative will come into contact with or close proximity to the dental pulp because pulp cells are critical to a healthy dental pulp. In the current study, cultured human pulp cells were used to evaluate the cytotoxicity of resin-based restorative materials. Under normal conditions, few pulp cells proliferate in normal pulp tissue.[21] Cells in the resting phase seem to reflect the in vivo condition more closely than cells in the growing phase. Therefore, the cytotoxic effects of resin-based restorative materials were examined on confluent cells in the study.

According to a previous study by Ferracane and Condon,[22] most unbound substances are liberated from polymerized resins within 1 day. Therefore, additional extracts were made to stimulate the release with time of substances from set resin-based restorative materials in this study. In addition, the freshly prepared resin-based restorative materials were placed immediately into medium. Clearly, resin-based restorative materials should be tested immediately after mixing and also after a period when it is assumed that they have reached their final chemical structure. Resin-based restorative materials are inserted into the cavity in a freshly mixed, incompletely polymerized stage, and thus it is likely that, during a relatively short period after clinical application of the material, local responses are provoked by unreacted or only partially reacted components. After setting, it is possible that potentially toxic constituents may be released from the materials. The difference in toxicity patterns at the various elution times may be related to the degree of setting. This would be reflected in the rate of component leaching. Thus, the different time extracts might be important to determine long-term cytotoxicity of resin-based restorative materials.

The cytotoxicity of extracts of 5 resin-based

restorative materials was evaluated with MTT assay in human pulp cells. Our results were in agreement with previous studies that these materials are cytotoxic to many cell lines[7,23,24] as well as to primary cultures in vitro,[23-28] although the cell systems, materials, and methods are different. The cytotoxic nature of these materials was clearly shown.

Conventional composite resins contain a polymerizable organic matrix, inorganic reinforcing fillers, and a silane-coupling agent that bridges the organic and inorganic components. Considerable amounts of triethylene glycol dimetharylate may be released by polymerized resin into water. Bisphenol-A-glycidyl methacrylate, urethane dimethacrylate, ethyleneglycol dimethacrylate, diethyleneglycol dimethacrylate, 1,6-hexanediol dimethacrylate, methyl methacrylate, camphorquinone, and 4-N, N-dimethylamino-benzoic acidethyler have been identified in minor concentrations in aqueous extracts.[6,29] In addition, liberated formaldehyde may have a synergistic effect on cytotoxicity.[30] These may be the reason that composite resin was shown as highly cytotoxic among the materials tested.

Resin-modified glass-ionomer cements contain ion-releasing glass particles, water-soluble polyacrylic acids, light-curable monomers, and additives. Previous studies have shown that resin-modified glass ionomer is cytotoxic to cultured cells.[23-28] Information about the organic components of resin-modified glass-ionomer cement is not readily available from the manufacturers. However, the organic components were shown to segregate high amounts of hydroxypropylmethacrylate, glycidyl-methacrylate monomers, and ethylene glycol.[7] In addition, fluoride release might also contribute to the cytotoxic effects. A recent study demonstrated that fluoride release has significant potential for pulpal toxicity by inhibiting cell growth, proliferation, mitochondrial activity, and protein synthesis.[18]

Compomers have been developed to combine the fluoride release of glass-ionomer cements with the mechanical properties of composite resins. Thus, these materials are composed of an ion-releasing glass. The fillers are partially silanized to couple the glass with the polymer network. These materials are much closer to composite resins than glass-ionomer cements. Aqueous eluate of compomers has been reported to induce moderate injury in cultured cells.[25,27] From examination by gas chromatography/mass spectrometry, it was shown that the extract contained a very high concentration of triethylene glycol dimetharylate and small amounts of comonomers, such as hydroxyethylmethacrylate, and various ethylene glycol compounds.[7] Furthermore, because of the ion-leaching glass fillers, compomer may also release fluoride, especially during the first few days after polymerization.[31] Substances leached from compomer might be the reason that it exhibited cytotoxic effect.

Pulp cells are important for the homeostatic function of pulpal connective tissue and are

responsible for the healing process when pulp tissues are insulted by mechanical, chemical injury, or microbial irritants. After injury, pulp tissue exerts the inherent potential to repair and regenerate. Healing of the traumatized pulp depends on the capacity of adjacent pulp cells to proliferate and migrate into wound site and secrete various matrix proteins.[32] In this study, resin-based materials were found to inhibit growth of human pulp cells. These results indicate that these materials could impair pulp cell function. In addition, impairment of mitochondrial function was also an important mechanism for resin-based materials-induced cytotoxicity in pulp cells.

It is our opinion that in vitro screening tests are very helpful in assaying the biologic effects of dental materials, but they may be limited in their ability to simulate the clinical condition. It may be unrealistic to transfer in vitro findings to in vitro situations. However, it is necessary to continue investigating resin-based materials until some are found that fulfill all the properties of ideal dental restorative materials.

In summary, resin-based restorative materials could constantly release substances after being exposed to an aqueous environment for extended periods, possibly causing moderate cytotoxic reactions and possibly contributing to pulpal irritation. However, cytotoxicity of resin-based restorative materials varies depending on the product tested, especially on the quality of leachable components. An optimum polymerization therefore is necessary for those materials. Furthermore, extractable amounts of components should be reduced. Severely cytotoxic substances should be replaced by less toxic alternatives, if available.

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