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# Cytotoxicity of polymerized resin cements on human dental pulp cells in vitro

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## ABSTRACT

**Objectives.** This study was designed to evaluate the cytotoxicity of several resin-based cements (Panavia F, Super-Bond C&B, Chemiace II) after polymerization on cultured human dental pulp cells.

**Methods.** After polymerization, specimens from three resin-based cements were eluted with fresh Dulbecco's modified Eagle's medium (DMEM) without serum for 72 h, at 37 °C, using 0.4 g of each substance per milliliter of fresh medium. Elutes obtained during this step were passed through a 0.22- $\mu$ m filter and diluted with the culture medium by a ratio of 75%, 50%, 25% (v/v). Cytotoxicity of elutes were evaluated by the relative growth rates (RGR) of pulp cells with a modified 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay. The RGR of pulp cells were statistic analyzed by the one-way analysis of variance among the groups.

**Results.** The RGR of cells exposed to 100% concentration of elution of Panavia F, Super-Bond C&B, and Chemiace II were 74.42%, 85.54%, and 82.39%, respectively. The RGR increased along with the elution of cements diluted. There was significant difference between the Panavia F group and Super-Bond C&B group ( $p < 0.01$ ), but there was no significant difference in the cytotoxicity between Chemiace II and Super-Bond C&B.

**Conclusions.** After polymerization, three resin-based cements (Panavia F, Super-Bond C&B, Chemiace II) induced slight cytotoxicity. The sensitivity of cytotoxicity to human pulp cells depended on the resin-based cements and the concentration of the elution. Super-Bond C&B is the least cytotoxic agent among the three resin-based cements.

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## 1. Introduction

The major goals of using resin-based adhesive materials are to enhance the bonding strength between restoration and the tooth structure, reduce the micro-leakage in the dentin–restoration interface and scatter the occlusal stress.

Resin-based cement is necessary to be used for cementing non-metal prosthesis for the advantages of esthetic and strength. As resin-based adhesive materials come into close and prolonged contact with pulpo–dentin complex, their safety influence on pulp tissue is of great interest, especially when the remaining thickness of dentin is thin or pulp

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exposure is noted during tooth preparation [1]. When the remaining thickness of dentin is thin dental adhesive/cement compounds can be eluted from dental materials and can be swallowed by saliva and then they can enter the organism. Dental compounds can be metabolized to very toxic agents in the organism. Furthermore dental compounds can enter the organism by uptake from the blood in the pulp and can then enter the organism by this route. The observed toxicity in cells/organisms is therefore may not be caused by the main molecule but by the toxic intermediates formed in the metabolism of eluted dental compounds.

It has been shown that resin-based adhesive materials exert potential harmful effects to the pulp. The biological safety of dentin-bonding agents has been extensively studied [2,3], but, reports on the biological safety of resin-based cements to cultured human pulp cells are still rare.

"In vitro cytotoxicity test" has the advantage of easy control of experimental factors that are often a problem when performing experiments in vivo. In vitro methods are reproducible, cost-effective, relevant, and suitable for the evaluation of basic biological properties of dental materials.

To evaluate the cytotoxicity of resin-based cements on human dental pulp cells completely, this study evaluate the cytotoxicity of several resin-based cements (Panavia F, Super-Bond C&B, Chemic II) after polymerization on cultured human dental pulp cells.

## 2. Materials and methods

### 2.1. Resin-based cements and elute preparation

Three resin-based cements were evaluated: Panavia F (Kuraray, Japan), Super-Bond C&B (Sun Medical, Japan) and Chemic II (Sun Medical, Japan). The cements were prepared according to the application instructions (Table 1) under aseptic conditions and were applied into polyethylene rings with diameter of 5 mm and height of 2 mm. Samples were polymerized in accordance with the application instructions (Table 1). After polymerization, samples were eluted with fresh Dulbecco's modified Eagle's medium (DMEM) without serum for 72 h, at 37 °C, using 0.4 g of each substance per milliliter of fresh medium. Elutes obtained during this step were passed through a 0.22- $\mu$ m filter and diluted with the culture medium without serum by a ratio of 75%, 50%, 25% (v/v).

Four groups were established for elution of three resin-based cements separately: 100% dilution (original elution),

$$\text{Relative growth rate (RGR)} = \frac{\text{average of tested group OD} - \text{average of blank control OD}}{\text{average of negative control OD} - \text{average of blank control OD}} \times 100\%$$

75% dilution, 50% dilution, and 25% dilution. Sodium doecyl sulfate (SDS) was used as positive control and DMEM without serum as negative control.

### 2.2. Culture of human dental pulp cells

Fresh healthy extracted teeth, impacted third molars and premolars removed for orthodontic purposes, were obtained clinically from individuals aged 10 to 25 years following

informed consent (Peking University Medical Ethical Committee permission number 0416). For dental pulp cell culture, freshly extracted teeth were immediately delivered to the laboratory and periodontal ligament was gently separated from the surface of the root. The pulp tissues were separated by a periodontal curette from the pulp chamber, which was revealed by cutting around the cemento-enamel junction with sterilized dental fissure burs and carefully split with a hammer. And then, the pulp tissues were cut into 0.5–1 mm<sup>3</sup> pieces, rinsed three times in phosphate buffered saline supplemented with 100 U/mL penicillin and 100  $\mu$ g/mL streptomycin. The pieces were placed into tissue culture dishes and incubated at 37 °C in DMEM supplemented with 10% fetal calf serum (FCS, Life Technologies, Grand Island, NY, USA), 100 U/mL penicillin and 100  $\mu$ g/mL streptomycin, in a humidified atmosphere of 5% carbon dioxide in air. When the growth of pulp cells approached confluence, they were detached with 0.25% trypsin and 0.05% ethylenediamine tetraacetic acid (EDTA) for 2 min and passaged at a ratio of 1:2. Pulp cells between the third and eighth passages were used in this study.

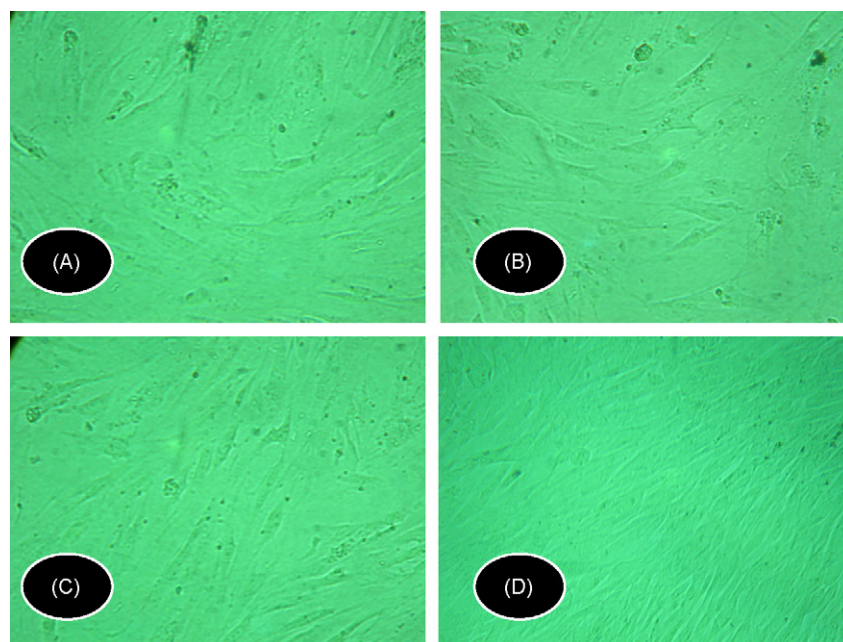
### 2.3. Cytotoxicity test

Confluent cells were detached with 0.25% trypsin and 0.05% EDTA for 2 min, and cells were seeded at an initial density of  $2 \times 10^4$  cells/well in 100  $\mu$ L of fresh DMEM with 20% FCS into 96-well culture plates. After 24 h attachment, various elutes of resin-based cements in 100  $\mu$ L volumes were added. In negative control group, DMEM without serum was added. Only DMEM with 10% FCS was in blank control group. Cells were further incubated until the growth of pulp cells in negative control group approached confluence and then 20  $\mu$ L MTT dye at a concentration of 5 mg/mL was added to each well. Plates were incubated in a CO<sub>2</sub> incubator for 4 h. Mitochondrial dehydrogenase of viable cells can reduce MTT to insoluble formazan. The insoluble formazan so produced was dissolved with 200  $\mu$ L of di-methyl sulfoxide (DMSO), and the optical density (OD) read against a standard reagent blank at OD<sub>570</sub> using a dias micro-plate well reader. The optical density values of the experimental groups were divided by the control and expressed as a percentage of control. The cell cytotoxicity was evaluated according to the relative growth rate of the cells, and the relative growth rates (equals to cell viability relative to controls) were calculated according to the following equation:

Cytotoxicity was rated based on cell viability relative to controls as [4]:

- Non-cytotoxic >90% cell viability;
- Slightly cytotoxic = 60–90% cell viability;
- Moderately cytotoxic = 30–59% cell viability;
- Severely cytotoxic  $\leq$ 30% cell viability.

Morphological alteration of the pulp cells was observed directly by phase contrast microscope and photographed by a Nikon camera.



**Fig. 1 – Morphological changes of pulp cells following exposure to resin-based cements elution. (A) Negative control; (B) 100% concentration of Panavia F; (C) 100% concentration of Super-bond C&B; (D) 100% concentration of Chemicace II.**

**Table 1 – Resin-based cements tested and their instructions of application.**

Product	Mixing ratio	Polymerization
Panavia F	Paste A/paste B (1/1)	Light-cured for 20 s
Super-Bond C&B	4 drops of Monomer/1 drop of Catalyst S/1 small cup of standard measuring spoon	Self-cured
Chemicace II	1 scoop (small cup)/1 drop	Lighted-cured for 20 s

#### 2.4. Statistical analysis

Five replicates of each concentration were performed in each test. All assays were repeated four times to ensure reproducibility. The relationships of RGRs among groups were statistical analyzed by one-way analysis of variance.  $p < 0.05$  was considered to be statistically significant.

### 3. Results

Cultured human dental pulp cells were elongated and spindle-shaped in appearance (Fig. 1A). Fig. 1B–D shows following exposure to elutes of three resin-based cements, the pulp cell density decreased and cells appeared more retracted than negative controls, exhibited a loss of normal organization, leading to enlargement of intercellular space.

The RGRs of cultured pulp cells are shown in Table 2. The RGR of cells exposed to 100% concentration of elution of polymerized Panavia F, Super-Bond C&B, and Chemicace II were 74.42%, 85.54%, and 82.39%, respectively, while 100% in negative control group and 0.00% in positive control group.

There was significant difference between the Panavia F group and Super-Bond C&B group ( $p < 0.01$ ), but there was no significant difference in the cytotoxicity between Chemicace II and Super-Bond C&B. There was significant difference

between the experiment group and negative control groups. At each concentration level, Panavia F was significantly more cytotoxic than Super-Bond C&B ( $p < 0.01$ ), and there was no significant difference in the cytotoxicity between Chemicace II and Super-Bond C&B. Panavia F was significantly more cytotoxic than Chemicace II at high concentration (100%, 75%), but at low concentration (50%, 25%), they were not significantly different from each other.

The RGR increased along with the elution of cements diluted (Fig. 2). The higher concentration of elute, the more toxic effects on the pulp cells. There were no statistical significance between adjoining concentrations of elute; but among other concentrations of elute, they were significantly different from each other.

### 4. Discussion

In vitro cytotoxicity tests should be performed with cells homologous to the human tissue of ultimate concern [5–7]. Primary pulp cells are more closely related to their original tissue and have a nearly unchanged metabolic state relative to their original tissue. So, cultured human pulp cells were used to evaluate the cytotoxicity of resin-based cements in this study. Under normal conditions, few pulp cells proliferate in pulp tissue [8]. Cells in the resting stage seem to reflect the in vivo condition more closely than do cells in the growing

**Table 2 – Relative growth rate (RGR, %) and cytotoxic rates (n = 3).**

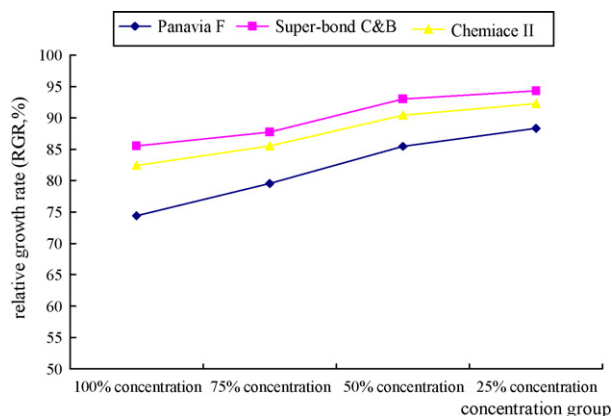
Experimental groups	100% concentration		75% concentration		50% concentration		25% concentration	
	RGR	SD	RGR	SD	RGR	SD	RGR	SD
Panavia F	74.42 <sup>*</sup> Slight	1.64	79.54 <sup>*</sup> Slight	3.59	85.46 <sup>*</sup> Slight	3.89	88.36 <sup>*</sup> Slight	3.59
Super-Bond C&B	85.54 Slight	2.71	87.75 Slight	2.49	93.00 Non	3.38	94.30 Non	3.97
Chemiace II	82.39 Slight	1.45	85.52 Slight	1.04	90.42 Non	1.34	92.3 Non	2.83
Positive control	4.06 (SD 0.02) severely cytotoxic							
Negative control	100 <sup>**</sup> non-cytotoxic							

\* Denote statistically significant differences between Panavia F group and Superbond C&B group with  $p < 0.05$ .  
\*\* Denote significant differences between the experiment groups and negative control with  $p < 0.01$ .

phase [9]. Therefore, the cytotoxicity of resin-based cements was examined on confluent cells in this study.

In the present study, the mean relative growth rate of human pulp cells exposed to 100% dilution concentration of elute was 74.42%, 82.39%, and 85.54% in Panavia F, Chemiace II, and Super-Bond C&B groups, while 100% in negative control group and 0.00% in positive control group. That means that the three resin-based cements have slight cytotoxicity after polymerization. At the same dilution concentration, the rank orders with respect to cytotoxicity were found to be as follow: Panavia F > Chemiace II > Super-Bond C&B. Panavia F was cytotoxic than Super-Bond C&B statistically ( $p < 0.01$ ), and there was no significant difference in the cytotoxicity between Chemiace II and Super-Bond C&B.

Several studies have indicated that cytotoxic effects in cell culture are mainly caused by released monomers. Curing of resin-based cement is usually not complete, unconverted monomers can be released from resin into an adjacent aqueous phase and can diffuse through dentin to the pulp space [10,11]. The sensitivity of cytotoxicity to human pulp cells depended on the resin-based cements tested. This may be due to differences in the content and component of monomers or additives of three resin-based cements.



**Fig. 2 – Relation between RGRs and dilution concentration of resin-based cements.**

Panavia F is dual-cure resin cement based on Bis-GMA. It contains 10-methacryloyloxydecyl dihydrogen phosphate (MDP), a special adhesive monomer. It has been shown that Bisphenyl-A-glycidyl methacrylate (Bis-GMA) is strong cytotoxic to fibroblast [12,13]. Super-bond C&B is self-cure dental adhesive resin cement based on MMA (methyl methacrylate). It contains 4-methacryloxyethyl trimellitate anhydride (4-META), a high performance bonding monomer, and tri-n-butylborane (TBB), a catalyst. Chemiace II is dual-cure resin cement, also contains 4-META. Compared with other poly-functional methacrylate monomers, MMA has a low potential for pulp irritation [14]. It has been shown that 4-META may not affect the cytotoxicity induction [14]. This may be the reason why there was no significant difference in the cytotoxicity between Chemiace II and Super-Bond C&B at the same concentration and both showed slight cytotoxicity.

The sensitivity of cytotoxicity to human pulp cells depended on the concentration of elutes tested, and the cytotoxicities of the resin-based cements decreased as dilution concentration of elutes increase. For example, from 100% concentration to 25% concentration, the mean relative growth rate of Panavia F was 74.42%, 79.54%, 85.46% and 88.36%, respectively. This may be due to difference in the content of monomers or additives of different dilution concentration. As dilution concentration of elutes increase, the content of monomers or additives of dilution decrease. There were no statistical significance between adjoining concentrations of elutes; but among other concentrations of elutes, they were significantly different from each other.

Under the condition of this study, after polymerization, three resin-based cements (Panavia F, Super-Bond C&B, Chemiace II) induced slight cytotoxicity. The sensitivity of cytotoxicity to human pulp cells depended on the resin-based cements and the concentration of the elution. Super-Bond C&B is the least cytotoxic agent among the three resin-based cements. That indicated that during clinical application of resin-based cements, differential toxic effects of resin-based cement on the pulp cells should be considered

during selection of suitable resin-based cements for restoration.

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