

ORIGINAL ARTICLE

## Effect of Flowable Composite Resin on Cell Viability of Balb/c 3T3 Cells

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### SYNOPSIS

The flowable composite resins have been routinely used in recent clinical dentistry. However, few studies have investigated the biological influence of flowable composite resins commercially available in Japan. In this study, we compared cell viability among 5 products currently available in dental clinical practice. Using mouse-derived Balb/c 3T3 cells, DMEM containing 5% fetal calf serum was adopted as an assay medium. Initially, the flowable resins were dissolved in the assay medium in the presence of dimethyl sulfoxide (DMSO) before photopolymerization, and each solution was diluted multiple times to investigate cell viability.

As a result, all products showed cell death at a dilution ratio of 2. At a dilution ratio of 4 or 8, there was a marked decrease in cell viability. At a dilution ratio of 16, Subsequently, cell viability tests after photopolymerization were performed. Samples were irradiated with an LED-type light irradiator for 5 or 10 seconds, and cell viability was investigated using the three-dimensional culture method with collagen. Cell viability was evaluated using MTT assay. Cell viability in the 10-second irradiation group for all products was higher than in the 5-second irradiation group. Some products showed no significant difference in comparison with the negative control group. There was no relationship between the monomer composition or filler type of any product and cell viability. The results of this study showed that cell viability slightly differed among the 5 clinically available products. There was no product for which cell viability was particularly low.

**Key words:** *flowable composite resin, cell viability, Balb/c 3T3 cells*

### INTRODUCTION

A composite material prepared by dispersing/blending silane coupling agent-treated silica filler in a matrix resin, dental composite resin, was developed in 1962. To date, it has been routinely used as a restorative material for the hard tissue of teeth. In addition to an

improvement in mechanical properties and natural tooth-like esthetic properties, it was necessary to increase the blending ratio of filler to the utmost limit so that the coefficient of thermal expansion might be similar to that for the tooth substance. For this reason, various products have been developed using

nano filler, but there was no fluidity due to an extremely high filler content rate. Initially, composite resins were a chemically polymerized restorative material for anterior tooth molding. However, they have been frequently used in clinical practice with advances in adhesive technology and the development of light-cured resins, and utilized for versatile purposes, such as restoration of the molar region or abutment structure to which a strong occlusal force is added or pit and fissure sealing for caries prevention, with an improvement in physical properties.

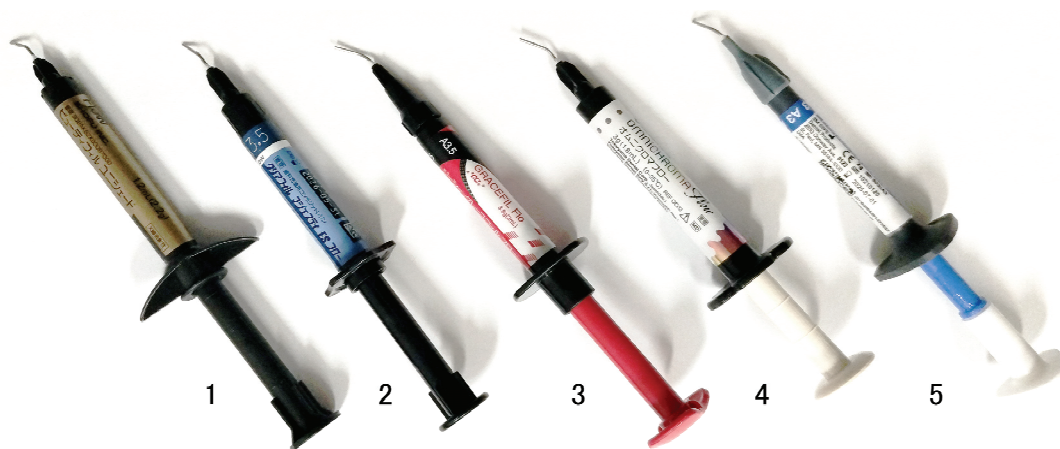
On the other hand, packable composite resins with high viscosity and flowable composite resins with low viscosity were also developed<sup>1-3</sup>. As the viscosity of flowable composite resins is low, they can be directly infused from a syringe using a disposable tip, and their

operability is favorable. Furthermore, products with several levels of fluidity became commercially available, and have been routinely used in recent dental clinical practice. Many studies reported the biological harmfulness of composite resins<sup>4-10</sup>. However, few studies have examined the biological influence of flowable composite resins<sup>11</sup>. In this study, we selected 5 commercially available products among flowable composite resins available in dental clinical practice, and compared cell viability before and after polymerization.

## MATERIALS AND METHODS

### 1. Samples

As shown in Fig. 1, commercially available flowable composite resins were used. The name, manufacturer, and lot number of each product are presented in Table 1.



**Fig.1** Five types of flowable composite resins used in this study.

**Table 1** Names, codes, manufacturers, and lot numbers of the five flowable composite resins used in this study\*.

No.	Code	Name (Shade)	Manufactures	Lot Number
1	BE	BEAUTIFUL UniShade Flow (UNI)	Shofu Inc	102205
2	CL	CLEARFILR MajestyR ES Flow Low (A3.5)	Kuraray Noritake Dental Inc.	7B0074
3	GR	GRACEFIL Flow (A3.5)	GC Corp.	2312111
4	OM	OMNICHROMA FLOW (No Shade)	Tokuyama Dental Corp.	0404
5	SU	Supreme Flowable Restorative (A3)	3M Health Care (Solventum)	10210120

\*Concerning the composition of the commercially available flowable composite resins that we used, methacrylate monomer is used according to the respective package inserts, but its type differs among the products. BE: Bis-GMA, Bis-MPEPP and TEGDMA, CL: TEGDMA and other methacrylate monomers, GR: Bis-MEPP, OM: UDMA and nonamethylene diol methacrylate, and SU: Bis-GMA, TEGDMA and other methacrylates are described by the manufacturer's documentation attached to each products. As fillers, BE: glass powder, CL: surface-treated barium glass and silica filler, GR: barium glass, OM: silica zirconia filler, and SU: inorganic filler are described.

## 2. Assay medium

Mouse-derived fibroblast-like cells, Balb/c 3T3 cells (3T3 cells), were used. The 3T3 cells were obtained from the RIKEN BioResource Center (Ibaraki, Japan). Dulbecco's Modified Eagle Medium (DMEM, FUJIFILM Wako Pure Chemical Corp., Osaka) containing 5% fetal calf serum (FCS, HyClone®, UT, USA) was used as an assay medium.

## 3. Cell viability before polymerization

Flowable resins were dissolved in the assay medium without performing photopolymerization, and cells were exposed for a short time to investigate cell viability.

### 1) Preparation of test solution

Five kinds of resin at 100 mg were added to 5 mL of assay medium mixed with 5 mL of dimethyl sulfoxide (DMSO, 99.5%, Wako Pure Chemical Industries Ltd., Osaka, Japan), and repeatedly agitated with a tabletop vibrator/strongly pipetted to prepare an original solution. Each original solution was in a suspended state, and complete transparency was not achieved. However, it was impossible to macroscopically examine the precipitate. In the negative control group, resin-free solution was used. Each original solution was diluted with the assay medium at a dilution ratio of 2 to prepare each test solution.

### 2) Measurement of cell viability

Using a disposable hemocytometer (BT, NanoEnTek Inc., Gyeonggi-do, Korea), 3T3 cells were adjusted to  $1 \times 10^6$  cells/mL with the assay medium. The cell solution was divided by 100  $\mu$ L / well on a 96-well multi-dish (IWAKI, AGC TECHNO GLASS Co., Ltd., Shizuoka, Japan) using a multichannel pipette, and cultured in a CO<sub>2</sub> at 37°C for 24 hours. After confirming normal cell spreading at the bottom of the multi-dish using an inverted phase-contrast microscope (IX-70, Olympus, Tokyo, Japan), the assay medium in

each well was removed, and 100  $\mu$ L/well of test solution was infused. On the other hand, the MTT solution was prepared by dissolving 50 mg of MTT ((3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, DOJINDO Laboratories, Kumamoto, Japan) in 10 mL of PBS(-) and removing solids by passing through a membrane filter. Each test solution was cultured in a CO<sub>2</sub> incubator for 6 hours, discarded, and exchanged for 100  $\mu$ L / well of MTT solution. The MTT solution was placed in a CO<sub>2</sub> incubator for 3 hours. It was discarded, exchanged for 200  $\mu$ L / well of acidic isopropanol solution, and placed at room temperature for 2 hours. After intracellularly synthesized formazan at the well bottom was dissolved, the absorbance of each well was measured at 570 nm using an absorptiometer (Spectra-Max Plus, Molecular Devices, CA, USA).

## 4. Cell viability after polymerization

### 1) Three-dimensional culture with collagen

Culture medium containing 8 mL of pig-derived Type I collagen (Cellmatrix®, Type I-A, Nitta Gelatin Inc., Osaka, Japan), 1 mL of MEM (10-fold, Nitta Gelatin Inc.), and 1 mL of reconstitution buffer (Nitta Gelatin Inc.) was mixed with 3T3 cells adjusted to  $1 \times 10^5$  cells / mL, and agitated using a tabletop vibrator. The above sol was divided by 200  $\mu$ L / well on the filter bottom of a cell culture insert (Falcon®, 1.0  $\mu$ m, Corning Inc., NY, USA)-set 12-well multi-dish (IWAKI, AGC TECHNO GLASS Co., Ltd.). The culture medium at 300  $\mu$ L was dispensed with a pipette into gaps between each well and the cell culture insert, and cultured in a CO<sub>2</sub> incubator at 37°C for 24 hours culture.

### 2) Preparation of samples

Five kinds of sample were directly filled into silicone tubes measuring 8 mm in inner diameter and 2 mm in height. Subsequently, the samples were irradi-

ated with an LED-type dental light irradiator (PenCure 2000® VL-10, Step exposure mode, Irradiation wavelength: 460nm, Max. 2,000 mW/cm<sup>2</sup>, Medical device notification number: 26B1X00001000513, J. MORITA CORP., Osaka, Japan) for 5 or 10 seconds. Due to aseptic conditions, irradiation was performed, establishing a distance of approximately 2 mm from the tip of the light irradiator and resin surface. The 5-second irradiation condition was a 10-second irradiation condition with 5-second light shielding.

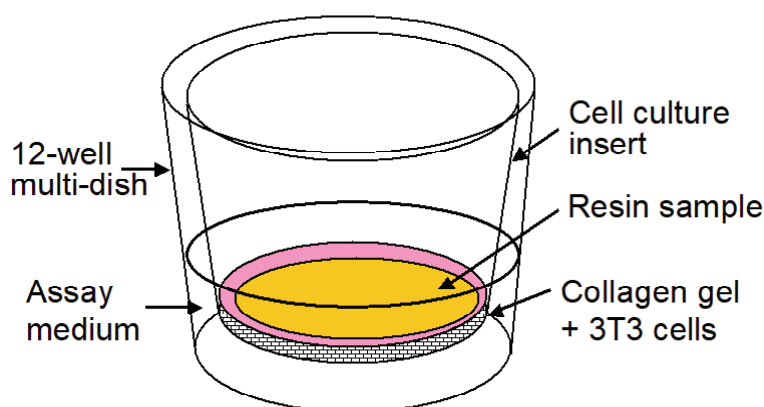
### 3) Evaluation of cell viability

After light irradiation, each resin sample was promptly placed at the top of collagen in the cell culture insert of each well, and brought into contact with cell-encapsulated collagen gel (Fig. 2). Immediately, each sample was placed in a carbon dioxide thermostat at 37°C, and cultured for 24 hours. After the completion of sample exposure, each sample was removed with a tweezers from the cell culture insert, again placed in a CO<sub>2</sub> incubator, and cultured for 2 days. MTT solution at 2 mL / well was

dispensed on a new 12-well multi-dish, and the cell culture insert of each sample cultured for 2 days was placed with tweezers and cultured in a CO<sub>2</sub> incubator for 3 hours. Acidic isopropanol solution at 3 mL / well was dispensed on the 12-well multi-dish, and the cell culture insert of each sample was placed with tweezers. Each sample was dissolved at room temperature for 2 hours, and a tabletop vibrator was used so that formazan might be sufficiently eluted from the cells. Each eluate at 100 mL / well was dispensed on a 96-well multi-dish (IWAKI, AGC TECHNO GLASS Co., Ltd.), and cell viability was measured at an absorbance of 570 nm using an absorptiometer (SpectraMax Plus, Molecular Devices).

### 5. Statistical analysis

The experiment was repeated four times. All data from the absorptiometer are expressed as the mean±standard deviation (SD). Significance was tested using Student's *t*-test. A *p*-value of 0.05 was regarded as significant. For statistical processing, we used Excel 2010 software.



**Fig.2** Exposure of resin samples after photopolymerization to 3T3 cells using 3D culture with collagen gel

## Results

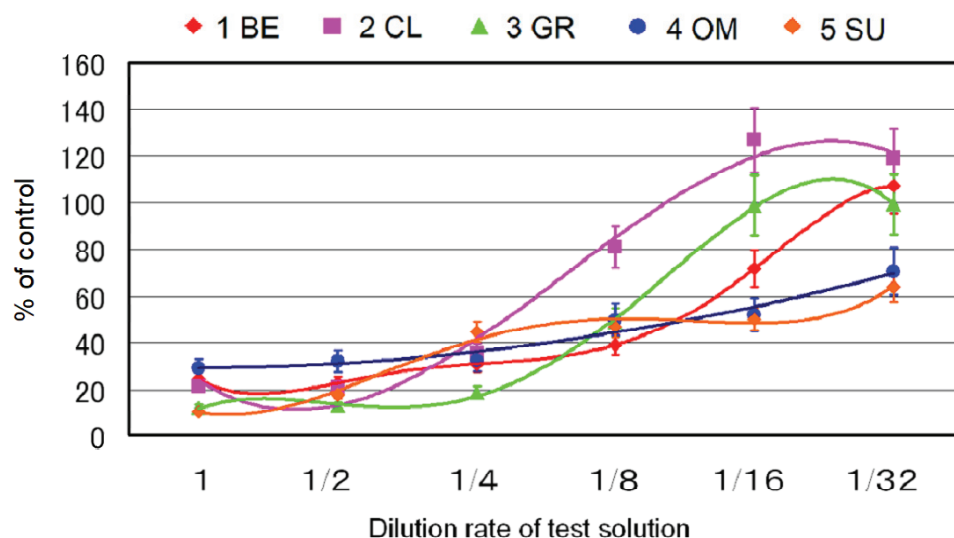
### 1. Cell viability before photopolymerization

The results for the viability of the cells directly exposed to the flowable resins before polymerization are shown in Fig. 3. When original solution was used, cell viability for the 5 resins was <30% even after short-time (6-hour) exposure, showing strong cytotoxicity. Cell viability slightly increased with an increase in the dilution ratio on serial dilution. Cell viability for BE was approximately 39% at a dilution ratio of 8, approximately 72% at a dilution ratio of 16, and  $\geq 100\%$  at a dilution ratio of 32. That for CL was approximately 36% at a dilution ratio of 4, approximately 81% at a dilution ratio of 8, and  $\geq 100\%$  at a dilution ratio of 16. That for GR was low up to a dilution ratio of 4, but it was 49% at a dilution ratio of 8 and  $\geq 100\%$  at a dilution ratio of 16. That for OM was relatively high among the 5 samples up to a dilution ratio of 4, but it was 49% at a dilution ratio of 8, approximately 52% at a dilution ratio of 16, and approximately 70% even at a dilution ratio of 32. That for SU was low up to a dilution ratio of 4, as described

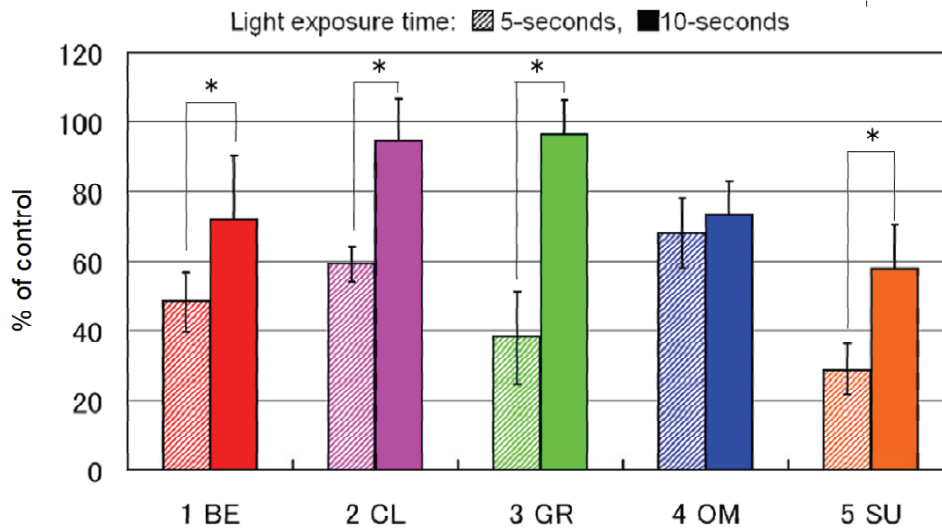
for GR, but it was 47% at a dilution ratio of 8, approximately 50% at a dilution ratio of 16, and approximately 64% at a dilution ratio of 32.

### 2. Cell viability after photopolymerization

The results for the viability of the three-dimensionally cultured cells exposed to the flowable resins after or during polymerization following light irradiation are shown in Fig. 4. Cell viability in the 10-second irradiation group for all products was higher than in the 5-second irradiation group. Cell viability for BE in the 5-second irradiation group was approximately 48% of the value in the control group, but that in the 10-second irradiation group was approximately 72%. Cell viability for CL in the 5-second irradiation group was approximately 59% of the value in the control group, but that in the 10-second irradiation group was approximately 95%. Cell viability for GR in the 5-second irradiation group was approximately 38% of the value in the control group, but that in the 10-second irradiation group was approximately



**Fig.3** The results of 3T3 cell viability exposed to the flowable resins before polymerization. (bar=SD)



**Fig.4** The results of 3T3 cell viability exposed to the flowable resins after or during polymerization. Significant differences were observed between 5-second and 10-second irradiation for all samples except OM. (bar=SD)

97%. Cell viability for OM in both the 5- and 10-second irradiation groups was approximately 73% of the value in the control group. Cell viability for SU in the 5-second irradiation group was approximately 29% of the value in the control group, but that in the 10-second irradiation group was approximately 58%.

## DISCUSSION

The results for cell viability showed strong cytotoxicity before photopolymerization. Even after 6-hour exposure, cell death was observed in all test product-containing solutions diluted at a ratio of 2. Even the test solutions diluted at a ratio of 4 or 8 showed strong cytotoxicity. Even among the test solutions diluted at a ratio of  $\geq 16$ , some products showed a decrease in cell viability. The results of three-dimensional culture after photopolymerization for 5 or 10 seconds showed that cell viability for all products after 10-second irradiation was higher than after 5-second irradiation. After 10-second irradiation, there were no significant differences in cell viability between CL or GR and a negative control. In the package inserts of all products, it is described that 10-second irra-

diation with an LED-type light irradiator is necessary.

In this study, with respect to the state before photopolymerization, the cells were exposed to the 5 resins dissolved in DMSO and assay medium. Furthermore, the resins became solid after photopolymerization, and could not be dissolved; the three-dimensional culture method with collagen was used. To obtain results in the case of insufficient photopolymerization, 5-second irradiation was also performed. Many *in vitro* and *in vivo* studies reported the biological properties of composite resins 4-12. However, few studies have reported flowable composite resins that are currently commercially available 12. Light-cured composite resins show strong cytotoxicity before light irradiation. As demonstrated by the results of this study, cytotoxicity reduces with the prolongation of the light irradiation time. This tendency was similar to that previously reported 4.

As all products investigated in this experiment may influence human lives and health, they are classified as Class II as administrative medical devices: composite resins for dental filling based on the "Pharmaceutical and Medical

Device Act", and the medical device certification number is granted. Thus, they became commercially available after various tests to be required were performed; therefore, basically, the biological safety of these products is not problematic.

The operability of flowable composite resins is favorable, and they are routinely used. An improvement in fillers or resins contained to improve fluidity has facilitated both an excellent mechanical strength after hardening and favorable esthetics 14-22. Therefore, the data on the biological properties of flowable composite resins may slightly differ from those on the biological properties of conventional composite resins. In this study, we used 5 products that are routinely used in clinical practice, but cell viability before and after photopolymerization slightly differed among the products. In the future, more detailed data must be obtained by changing the experimental conditions. In the products used in this study, there was no relationship between monomer composition or filler type and cell viability. In this study, only 5 products that are routinely used in clinical practice were adopted, but cell viability slightly differed among the products. There was no product for which cell viability was particularly low.

## CONCLUSION

We investigated cell viability by 5 products of the flowable composite resins in dental clinical practice. There was no relationship between monomer composition or filler type and cell viability. In this study, cell viability slightly differed among the products. There was no product for which cell viability was particularly low.

## ACKNOWLEDGMENTS

This study used the Translational Research Institute for Medical Innovation (TRIMI) Research Laboratory Center, Osaka Dental University.

We would like to express our sin-

cere gratitude to Dr. Masaaki Morikawa (Morikawa Dental Clinic, Yao Honmachi, Osaka, Japan) for providing us with all the flowable composite resins used in the experiment.

## CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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(Received, Augusts 12, 2024/  
Accepted, September 25, 2024)

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