Cytotoxic Effects of Veneer Composite Materials

Martina Schmid-Schwap, MD, DMD^a/Alexander Franz^b/Martin Krainhöfner, MD, DMD^c/Franz König^d/ Gerald Krennmair, MD, DMD^e/Andreas Schedle, MD, DMD^f

> The aim of this study was to evaluate 4 veneer composites—Signum+EM, Gradia Enamel E3, Sinfony E3, and SR Adoro S1—in a standardized test at 2 different conditions. For each composite, 2 groups of specimens (added to cultures immediately after preparation [ie, fresh] or after 7 days of incubation in cell culture medium) were added to L-929 fibroblast cultures for 72 hours. All composites showed reduced cell numbers compared to glass controls. Fresh specimens of Signum+EM exhibited the least cytotoxicity, followed by Gradia Enamel E3 and Sinfony E3 and then SR Adoro S1. For specimens with 7-day incubation, 3 of the composites (Signum+EM, Sinfony E3, and Gradia Enamel E3) showed similarly low cytotoxicity, while cytotoxic results with SR Adoro S1 were significantly higher. With low in vitro toxicity values in conjunction with good mechanical properties, veneer composites appear to offer an interesting alternative for prosthetic rehabilitation. *Int J Prosthodont 2007;20:596–598.*

Biocompatibility of dental materials is of major importance not only to dental practitioners, but also to patients, who have shown growing interest in the materials being used in their mouths. Cytotoxic effects of resin-based materials, which are predominantly triggered by monomer release, were demonstrated in cell culture studies.¹ Toxic effects will increase with the amount of unreacted substance contained in the cured material. In addition to composites, ceramic materials also show biologic effects.²

Very few studies have reported on the toxicity of veneer composites. In obvious contrast to ceramic

veneers, veneer composites show higher elasticity, thus causing less tension at the core material/veneer material-interface. In addition, veneer composites are highly regarded as a cost-efficient alternative to ceramic veneers. The aim of the present study was to evaluate the cytotoxicity of 4 different veneer composites using the same standardized test system. The null hypothesis was that there was no difference in cytotoxicity between the 4 composites tested.

Materials and Methods

Four composites–Gradia Enamel E3, Signum+EM, Sinfony E3, and SR Adoro S1 (Table 1)–were prepared in polyamide blocks (diameter: 5 mm, height: 2 mm), removed immediately after curing, cleaned, polished, and sterilized (Table 2).

Glass specimens were used as negative controls and polyvinyl chloride strips (certified reference material) were used as positive controls. Half of the specimens were added to the cultures immediately after preparation and sterilization (fresh specimens), while the other half were incubated at 37°C, pH 7.2, for 7 days in cell culture medium. Specimens were prepared in triplicate. Experiments were repeated 6 times.

L-929 fibroblasts (5-mL aliquots containing 3×10^4 cells/mL) cultivated in Dulbecco's Modified Eagle's Medium (DMEM) (supplemented with 10% fetal calf serum, 1% glutamine, and 1% penicillin/streptomycin) were exposed to specimens in 6-well plates for 72

^aSenior Resident, Bernhard Gottlieb University Clinic of Dentistry, Department of Prosthodontics, Medical University of Vienna, Austria. ^bResearch Associate, Bernhard Gottlieb University Clinic of Dentistry, Central Research Unit, Medical University of Vienna, Austria.

^cUniversity Assistant, Bernhard Gottlieb University Clinic of Dentistry, Department of Prosthodontics, Medical University of Vienna, Austria. ^dUniversity Assistant, Core Unit for Medical Statistics and Informatics, Section of Medical Statistics, Medical University of Vienna, Austria.

^eAssociate Professor, Bernhard Gottlieb University Clinic of Dentistry, Department of Prosthodontics, Medical University of Vienna, Austria. ^fAssociate Professor, Bernhard Gottlieb University Clinic of Dentistry, Central Research Unit, Medical University of Vienna, Austria.

Correspondence to: Dr Andreas Schedle, Bernhard Gottlieb University Clinic of Dentistry, Währingerstrasse 25a, 1090 Wien, Austria. Fax: 42 77 67159. E-mail: andreas.schedle@ meduniwien.ac.at

Table 1 Materials Studied

Material	Lot no.	Characteristics	Light-curing unit	Manufacturer
Gradia Enamel E3	0406111	Light-cured composite for fixed partial dentures, inlays, and veneers	Curing: Labolight LV-III (halogen light 468 nm)	GC
Signum+EM	010032	Light-curing polyglass for fixed partial dentures	Curing: Heraflash (xenon flash lamp 360-600 nm)	Heraeus Kulzer
SR Adoro S1	G11893	Newly developed, microfilled, light/ heat-curing composite for full- coverage and partial veneers. Suitable for the fabrication of metal-supported and metal-free restorations	Precuring: Demetron Optilux 401 curing unit (Kerr; light intensity = 500 mW/cm ²). Curing: Lumamat 100/Targis Power Upgrade (mercury vapor lamp 400–550 nm)	Ivoclar Vivadent
Sinfony E3	187951	Light-curing composite for the fabrication of fixed partial dentures, inlays, and onlays	Precuring and curing: Visio Beta vario (fluorescent tubes 400–500 nm)	3M ESPE

Table 2Precuring, Curing, and Maximum Temperature of Light-Curing Units According to the Manufacturers'Recommendations

Material	Precuring	Curing*	Maximum temperature
Gradia Enamel E3	-	3 min light curing (specimens covered with insulating gel [Gradia Air Barrier, GC])	42°C
Signum+EM	-	180 s light curing (specimens covered with insulating gel [Insulating Gel, Heraeus Kulzer])	$\approx 60^{\circ}$ C
SR Adoro S1	20 s light curing (specimens covered with polyester foil [Hostaphan, Mitsubishi])	25 min light curing (specimens covered with insulating gel [SR-Gel, Ivoclar Vivadent])	102°C
Sinfony E3	7 min light curing followed by 10 s light curing under vacuum	1 min light curing followed by 14 min light curing under vacuum	$\approx 60^{\circ}$ C

*After curing, all composites were cleaned with steam and water, polished (tool kit, Heraeus Kulzer), and sterilized with ultraviolet radiation.

hours (37°C, 5% carbon dioxide). Cells were then harvested with trypsin, centrifuged, resuspended in 500 μ L DMEM, and counted over a fixed time of 30 seconds with a flow cytometer.

A 2-stage plan was followed for keeping the width of the confidence interval small even if larger standard deviations were observed. After the first stage with a sample size of 18 observations per composite, all standard deviations were smaller than 15 and therefore the trial was terminated. To pool the 4 test composites and the positive control for each time point of incubation (fresh or 7 days), the Ryan-Einot-Gabriel-Welsch multiplerange test was used to control the multiple-level alpha.³

Results

Mean cell numbers and 2-sided 95% confidence intervals for all composites are shown in Table 3. These results demonstrate that all composites (fresh and after 7day incubation) showed reduced cell numbers compared with glass controls (the 95% confidence intervals for the standardized mean do not include the value 100%).

Analysis of variance showed that the cytotoxic effects of the composites varied significantly (P < .0001). Incubation for 7 days had a significant influence on cy-

totoxicity (P<.0001), and the composites showed varying degrees of reduction of cytotoxicity after incubation (interaction substance versus incubation time, P=.0003).

A rank order of significantly different effects was established for fresh and 7-day-incubated specimens. Fresh specimens of Signum+EM exhibited the least cytotoxicity, followed by a group of 2 composites (Gradia Enamel E3 and Sinfony E3), and then SR Adoro S1. The positive control showed the highest cytotoxicity (Fig 1a). After 7 days of incubation, nearly the same rank order was found: a group of 3 composites (Signum+ EM, Sinfony E3, and Gradia Enamel E3) showed the lowest cytotoxicity, while SR Adoro S1 showed significantly higher results. Again, the positive control exhibited the highest cytotoxicity (Fig 1b).

Discussion

In previous studies, some of the fresh composites tested showed cytotoxic effects similar to those of the positive control and exceeding those of amalgam.¹ These effects decreased after incubation in a cell culture medium until no toxicity was detectable after 6 weeks, at which point elutable species had leached from the composites.

Preincubation time	Composite	No. of observations	Mean	SD	Lower 95% Cl	Upper 95% Cl
Fresh	Signum+ EM	18	92.90	8.33	88.76	97.04
	Gradia Enamel E3	3 18	76.72	8.32	72.59	80.86
	Sinfony E3	18	75.44	9.48	70.72	80.16
	SR Adoro S1	18	68.06	9.71	63.23	72.88
	Positive control	18	10.28	7.37	6.61	13.94
7 days	Signum+ EM	18	92.78	10.15	87.73	97.82
	Gradia Enamel E3	3 18	87.20	7.74	83.35	91.05
	Sinfony E3	18	88.98	4.32	86.84	91.13
	SR Adoro S1	18	78.75	7.29	75.13	82.38
	Positive control	18	10.90	6.38	7.73	14.07

Table 3 No. of L-929 Fibroblasts (Means and 2-Sided 95% Confidence Intervals) for All Composites

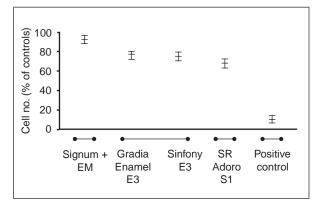


Fig 1a Means and corresponding 95% confidence intervals of freshly prepared specimens grouped by composite with no difference in effects. Cell numbers are expressed as percentage of controls (cultures with glass specimens). Vertical bars represent the 95% confidence intervals for the means calculated by 18 observations. The composites are ranked by their mean toxicity level; composites connected with the same horizontal bar are not significantly different from each other. To check the sensitivity of the test system, positive controls were applied without incubation.

Compared with currently available composites,¹ the veneer composites tested showed lower average toxicity results in fresh conditions. These superior results may be explained by the optimal hardening in the photocuring unit, which allows for optimized luminescence from all directions, in contrast to the lamps for direct composites.

The low in vitro toxicity of composites demonstrated by this study is consistent with clinical data reported for the good gingival health associated with veneered polymer crowns.⁴ Even the 3-year survival rates of metal-free polymer crowns are within an acceptable range, with high acceptance by patients.⁵

Conclusion

With low in vitro toxicity values in conjunction with good mechanical properties, veneer composites may offer an interesting alternative for prosthetic rehabilitation, especially when high elasticity modules are required.

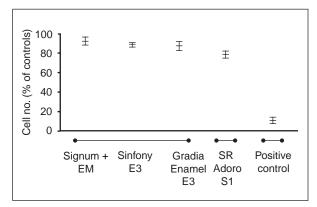


Fig 1b Means and corresponding 95% confidence intervals of 7-day incubated specimens grouped by composite with no difference in effects. Cell numbers are expressed as percentage of controls (cultures with glass specimens). Vertical bars represent the 95% confidence intervals for the means calculated by 18 observations. The composites are ranked by their mean toxicity level; composites connected with the same horizontal bar are not significantly different from each other. To check the sensitivity of the test system, positive controls were applied without incubation.

Acknowledgments

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