Short Communication

In Vitro Assessment of Cytotoxicity of Resin-Based Dental Restorative Materials on WEHI 13 Var Fibroblasts

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Purpose: The aim of this study was to assess the cytotoxicity of 4 resin composites' eluates on WEHI 13 Var fibroblasts as they aged in a biologic medium. ***Materials and Methods:*** Cytotoxicity was determined by counting the number of viable cells by trypan blue exclusion. Morphologic changes attributable to cytotoxicity were observed by May-Grunwald-Giemsa cytologic staining and microscopic examination. DNA gel electrophoresis was performed to detect possible genotoxicity and DNA damage. ***Results:*** All resin composite eluates, except that for Targis, caused a pronounced cytotoxicity during the first 72 hours that gradually decreased after 2 weeks of aging. Severe morphologic alterations and pronounced DNA damage were also observed. ***Conclusion:*** These findings indicate that resin-based dental restorative materials release agents cytotoxic and genotoxic to fibroblasts. Cytotoxicity is gradually decreased as the composite resins age in a biologic-relevant medium.
Int J Prosthodont 2006;19:13–16.

Composite resins are extensively used as restorative materials because of esthetic demands and concerns over adverse effects of mercury from amalgam. It is well documented however that several (co)monomers (hydroxyethyl methacrylate, bisphenol glycidyl methacrylate, triethylene glycol dimethacrylate, etc), additives, or polymerization products can be released from set resin composites, with a wide range of toxic potencies on fibroblastic cell lines.[1] This release has often been assumed to be limited to the first 24 hours after polymerization.[2] However, it has been proposed that substantial amounts of resin monomers remain unconverted to polymer for longer periods.[3] The purpose of this study was to evaluate the cytotoxicity and genotoxicity of 4 resin composites on fibroblasts as they aged in a biologic medium.

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Materials and Methods

Materials and Sample Preparation

Two hybrid composites (Tetric Ceram, Ivoclar-Vivadent, Lot G16362; Filtek Z-250, 3M/ESPE, Lot 6020A3), 1 nanohybrid (Simile, Pentron, Lot 124810), and 1 ceromer (Targis, Ivoclar-Vivadent, Lot F44248) were tested. Specimens of each material were prepared aseptically as disks 5 mm in diameter and 1 mm thick, with a surface area of 0.55 cm^2. The first 3 composites were cured for 40 s each, using a light-emitting diode curing unit at 650 mW/cm^2 (bluephase, Ivoclar-Vivadent), while Targis was cured with the laboratory curing unit (Targis power upgrade), according to the manufacturer's instructions. Each specimen was then immersed into 1 mL of culture medium (RPMI 1640, Gibco) for either 24 or 72 hours at 37ºC. Thus, the ratio between the surface area of the specimens and volume of extraction medium was 0.5 cm^2/mL, the lowest in the ISO 10993 Part 5 requirements.[4] In a second series of experiments, eluates were prepared from specimens that had previously remained in RPMI for 1 or 2 weeks.

Table 1 Proportion of Trypan Blue-Positive (Dead) Cells After Exposure of WEHI 13 Var Fibroblasts to 72-Hour Eluates of Various Resin Composites for 24 and 72 Hours

Time Point	Aging Time (days)	Sample	% of Dead Cells (mean)
24	0	Control	1.94
72	0	Control	3.4
24	0	Tetric Ceram	4.9
72	0	Tetric Ceram	19
24	0	Filtek Z250	2
72	0	Filtek Z250	95
24	0	Simile	7.1
72	0	Simile	20
24	0	Targis	5.7
72	0	Targis	3.4
24	7	Control	2.7
72	7	Control	4.1
24	7	Tetric Ceram	9.1
72	7	Tetric Ceram	6.1
24	7	Filtek Z250	3.1
72	7	Filtek Z250	15.6
24	7	Simile	2.3
72	7	Simile	3.4
24	7	Targis	2.4
72	7	Targis	4.3
24	14	Control	4.4
72	14	Control	4.7
24	14	Tetric Ceram	7
72	14	Tetric Ceram	4.6
24	14	Filtek Z250	5.9
72	14	Filtek Z250	2.2
24	14	Simile	4.5
72	14	Simile	4.2
24	14	Targis	4.7
72	14	Targis	1.4

Cytotoxicity

Cytotoxicity was assessed on cultures of WEHI 13 Var fibroblasts (American Type Culture Collection CRL-2148) by counting cell number with a hemocytometer and the proportion of viable cells by trypan blue exclusion (0.4%, Sigma). Cells were seeded (10^4 cells/cm^2) in complete medium (RPMI 1640, with 200 mmol/L L-glutamine, 125 units/mL penicillin, 125 μg/mL streptomycin, 10% fetal bovine serum; Gibco) into 24-well plates and cytotoxicity was evaluated 24 and 72 hours following treatment. The results were expressed as percentage of the control value.

Light Microscopy

Cells were stained with May-Grunwald-Giemsa (10%, Merck) cytologic stain to determine morphologic changes and nuclear/cytoplasmic equivalence.

DNA Gel Electrophoresis

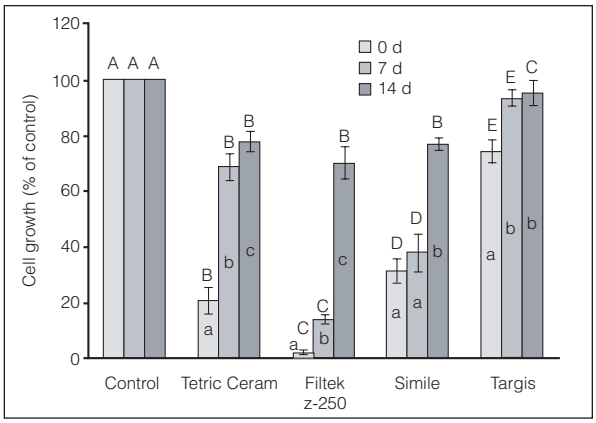
Cells exposed to resin extracts during the first hours after polymerization were collected, lysed, and processed for genomic DNA analysis following the method of Fady et al.[5] Extracted DNA was suspended in 10 mmol/L Tris HCl + 1 mmol/L EDTA (pH = 8) buffer and electrophoresed in 1.8% agarose gel to determine possible genotoxicity. Etoposide, a conventional apoptotic agent, was used at different concentrations as a positive control.

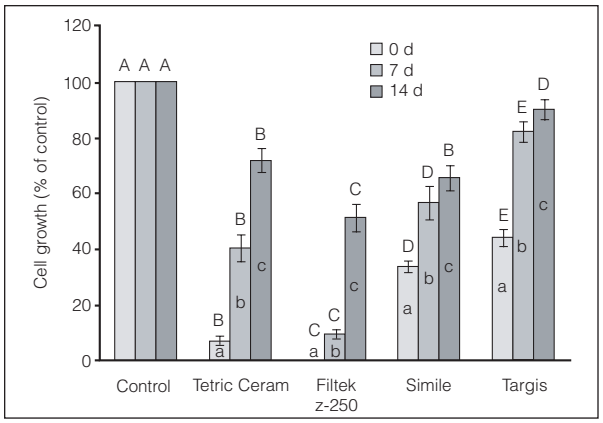
Statistical Analysis

Groups were compared using analysis of variance and Tukey multiple comparison intervals (alpha = .05) and paired-samples *t* test.

Results

The results thus far showed that all resin composite eluates, except that for Targis, caused a pronounced cytotoxicity during the first 72 hours, as shown by the low rate of cell growth and the high proportion of trypan blue-positive (ie, dead) cells (Table 1). Eluates obtained from the same specimens after 1 week of aging showed comparatively less but still marked cytotoxicity. In contrast, resins aged for 2 weeks were much less cytotoxic (Figs 1a and 1b).





Figs 1a and 1b Cytotoxicity of samples 72 hours after treatment, under conditions of no aging (0 days) and aging for 7 or 14 days into RPMI. Fig 1a *(left)* represents the 24-hour eluate, while Fig 1b *(right)* represents the 72-hour eluate, which was found to be more cytotoxic. Error bars indicate ± 1 standard deviation (n = 6). Within each of the materials, identical lowercase letters indicate no statistically significant differences among the different aging times (analysis of variance and Tukey, alpha = .05). Among different materials, identical uppercase letters on the 0-, 7-, and 14-day values separately indicate no statistical differences between the various materials and the control (paired-samples *t* test).

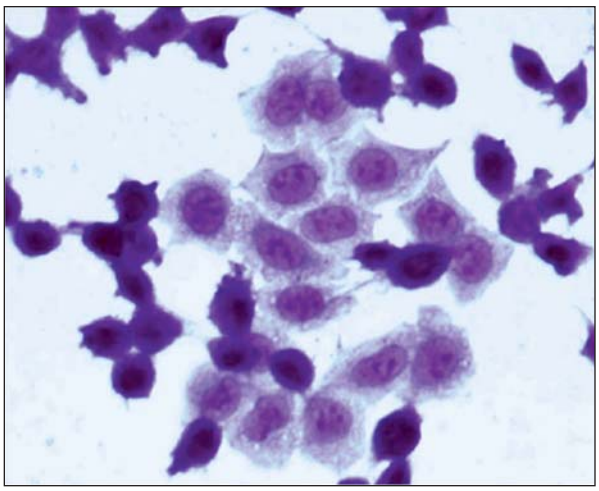


Fig 2a Control WEHI 13 Var fibroblasts stained with May-Grunwald-Giemsa after 72 hours in culture (original maginification × 400).

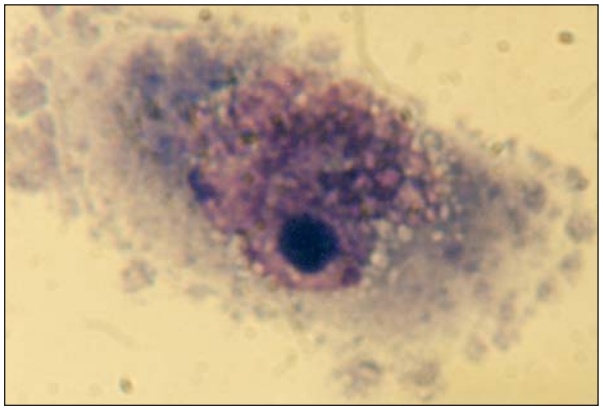


Fig 2b Morphologic changes seen in cells exposed to Simile's eluates. Cells are enlarged and multinucleate, with vacuoles in their cytoplasm, multiple membrane blebs, and protrusions (original magnification ×1,000).

Light microscopic analysis revealed extensive disruption of cell morphology, with giant cells presenting multiple micronuclei, vacuolization of their cytoplasm, and disruption of their plasma membrane appearing in treated cultures. In contrast, cells exposed to Fitek Z-250 were extensively elongated, acquiring characteristic atractoid morphology (Figs 2a to 2d). DNA gel electrophoresis performed at 72 hours following treatment revealed several bands of damaged DNA. These findings indicate severe genotoxicity (Figs 3a and 3b).

Conclusion

From the data presented, it is clear that eluates derived within the first hours following polymerization of composites were extremely toxic. However, after 2 weeks of aging, the resin eluates were moderately cytotoxic. Therefore, it is essential to evaluate cytotoxicity for longer periods that resemble clinical conditions. Eluates of Targis were only slightly cytotoxic, apparently a result of the extensive light/heat laboratory polymerization of this material. The latter yields fewer residual monomers. Finally, resin eluates caused a pronounced disruption of cell morphology and severe genotoxicity. Experiments are in progress to uncover the molecular basis of this phenomenon.

Acknowledgment

Dr A. A. Bakopoulou wishes to thank the Alexandros S. Onassis Public Benefit Foundation in Athens, Greece, for providing her with a graduate fellowship.

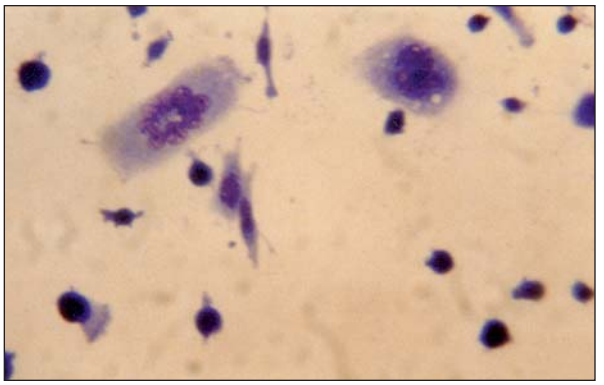


Fig 2c Cells exposed to Tetric Ceram's eluates. Giant multinucleated cells consist of about 20% to 30% of the total population (original magnification × 400).

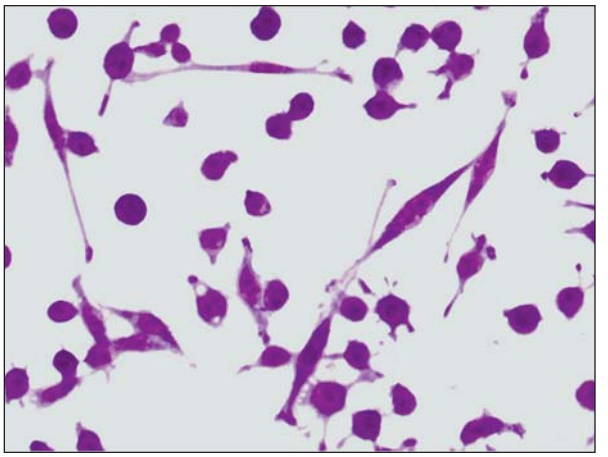
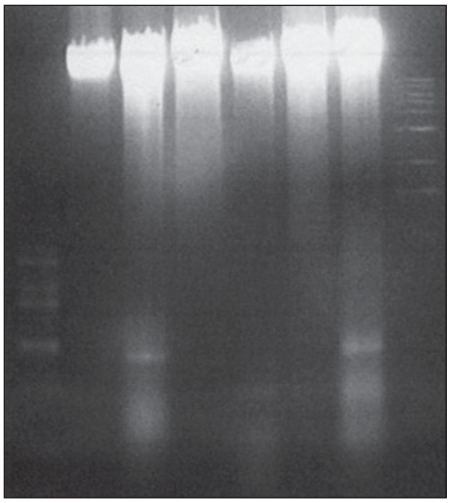


Fig 2d Cells exposed to Filtek Z250's eluates. A great proportion of cells presents an elongated spindle-like phenotype (original magnification × 400).



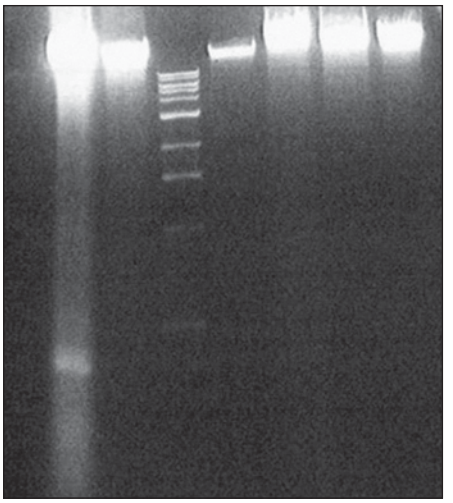


Fig 3a *(left)* Agarose DNA gel electrophoresis. Lane 1: DNA internal size standards > 100 base pairs; lane 2: control at 48 hours; lane 3: FiltekZ250 at 72 hours; lane 4: Simile at 48 hours; lane 5: Simile at 72 hours; lane 6: Tetric Ceram at 48 hours; lane 7: Tetric Ceram at 72 hours; lane 8: DNA internal size standards > 1 kb.

Fig 3b *(right)* Lane 1: Etoposide 10^{-7} mol/L at 48 hours; Lane 2: Etoposide 3.10^{-7} mol/L at 24 hours; lane 3: DNA internal size standards > 1 kb; lane 4: Targis at 48 hours; lane 5: Targis at 72 hours; lane 6: Simile at 48 hours; lane 7: Tetric Ceram at 48 hours.

References

1. Geurtsen W, Lehmann F, Spahl W, Leyhausen G. Cytotoxicity of 35 dental resin composite monomers/additives in permanent 3T3 and three human primary fibroblast cultures. J Biomed Mater Res 1998;41:474-480.
2. Ferracane JL, Condon JR. Rate of elution of leachable components from composite. Dent Mater 1990;6:282-287.
3. Wataha JC, Rueggeberg FA, Lapp CA, et al. In vitro cytotoxicity of resin-containing restorative materials after aging in artificial saliva. Clin Oral Investig 1999;3:144-149.
4. ISO 10993/5: Biological evaluation of medical devices-Part 5: Tests for cytotoxicity: In vitro methods. Geneva: ISO, 1992.
5. Fady C, Gardner A, Jacoby F, et al. Atypical apoptotic cell death induced in L929 targets by exposure to tumour necrosis factor. J Interferon Cytokine Res 1995;15:71-80.