Setting Time Affects *In Vitro* Biological Properties of Root Canal Sealers

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Abstract

Introduction: Biocompatibility of root canal sealers is important because of the long-term contact of their eluates and/or degradation products with periapical tissues. The literature still lacks studies about the genotoxic effects of these materials and the influence of setting time on biological properties. The cytotoxicity and genotoxicity of an epoxy resin–based sealer (AH Plus), a single methacrylate-based sealer (EndoRez), and a silicone-based sealer (RoekoSeal) were assessed. **Methods:** Chinese hamster fibroblasts (V79) were cultured and exposed to different dilutions of extracts from the sealers that were left to set for 0, 12, and 24 hours before contact with culture medium. Cell viability was measured by the methyl-thiazol-diphenyltetrazolium assay. Genotoxicity was assessed by the comet assay. Data were statistically analyzed by Kruskal-Wallis and Dunn tests ($P < .05$). **Results:** Root canal sealers were statistically more cytotoxic than the untreated control group, except for the silicon-based sealer. Cell viability ranking was the following (from the most to the least cytotoxic): methacrylate-based > epoxy resin–based > silicone-based. The setting time influenced the epoxy resin–based sealer cytotoxicity (decreased at 12 hours) and the general genotoxicity (increased at 24 hours). DNA damage ranking was the following (from the most to the least genotoxic): methacrylate-based > silicone-based = epoxy resin–based. **Conclusions:** The setting time had influence on the cytotoxicity of the epoxy resin–based sealer and genotoxicity of all tested sealers. The methacrylate-based sealer was the most cytotoxic, and the silicone-based sealer was not cytotoxic. Genotoxicity was observed for all sealers. *(J Endod 2014;40:530–533)*

Key Words

Biocompatibility, cytotoxicity, endodontic sealer, genotoxicity

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The development of endodontic sealers aims to achieve good mechanical properties and biocompatibility. This property is necessary because of the long-term contact of their eluates and/or degradation products with periapical tissues (1, 2). Toxic materials can damage periapical cells and affect the DNA, leading to carcinogenic transformations and/or genome instability (3).

It is known that epoxy resin–based sealers could induce an initial mild inflammatory reaction on surrounding tissues, as well as cytotoxicity (4–6), with slight mutagenic capacity (7). Single methacrylate resin–based sealers were also studied. Whereas some authors reported them as well-tolerated by connective tissues (8), others observed an intense and long-lasting inflammatory reaction (9). A possible cause for this effect could be the presence of urethane dimethacrylate in the structure of the sealer (10). Previous studies also reported that high concentrations of methacrylate monomers might induce DNA damage (11). Recently, silicone-based sealers were introduced, with none or minimal cytotoxicity (12); however, there are no current studies regarding its genotoxic effects. There are a number of studies assessing the cytotoxicity of endodontic sealers, although only a few authors observed the effects of setting time on the genotoxicity of root canal sealers (5, 13–15). With this gap in the knowledge, we hypothesized that different setting times would change both cytotoxicity and genotoxicity of root canal sealers.

The objective of this study was to evaluate the cytotoxic and genotoxic effects of 3 different endodontic sealers by using the methyl-thiazol-diphenyltetrazolium (MTT) assay and the comet assay in a setting time–dependent manner.

Materials and Methods

Cell Culture

Chinese hamster fibroblasts (V79) were cultured in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, penicillin (10,000 IU/mL), and 1% streptomycin (10 mg/mL). Cultures were incubated under an atmosphere of 5% CO_2 at 37°C until confluency. Cells were then detached from the flasks, seeded according to the assay (cytotoxicity or genotoxicity), and incubated again for 24 hours.

Preparation of Extracts

Three sealers were tested, each one with a specific chemical base (Table 1). Sealers were manipulated following the manufacturers' instructions, layered into 24-well plates, and covered by 2.5 mL cell culture medium after waiting 0, 12, and 24 hours for setting. Samples were then reincubated for 24 hours at 37°C. Original extracts (1:1) were serially diluted in the cell culture medium (1:2, 1:4, 1:8, 1:16, and 1:32).

Cytotoxicity Analysis

Cells seeded in 96-well plates (5×10^3 cells/well) were exposed to 200 μL/well of the serial dilutions of the extracts. Untreated cells were used as controls. Cells under the experimental conditions were incubated at 5% CO_2 37°C for 24 hours. The culture medium in each well was replaced by 100 μL MTT solution (Sigma

TABLE 1. Main Components, Setting Time, and Manufacturer of Tested Sealers

Sealer	Composition	Setting time	Manufacturer
AH Plus	Paste A: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments; Paste B: dibenzyldiamine, aminoadamantane, tricyclodecane-diamine, calcium tungstate, zirconium oxide, silica, silicone oil	8 h	Dentsply De Trey GmbH, Konstanz, Germany
RoekoSeal	Polydimethysiloxane, silicone oil, paraffin-base oil, hexachloroplatinic acid (catalytic agent), zirconium dioxide	45–50 min	Coltene-Whaledent, Langenau, Germany
EndoRez	30% urethane dimethacrylate, zinc oxide, barium sulfate, resins, pigments	15–20 min	Ultradent, South Jordan, UT

Aldrich Co, Munich, Germany) and incubated for 1 hour. This solution was removed, and resulting formazan crystals were dissolved by the addition of 100 μL dimethyl sulfoxide (Sigma Aldrich). Plates were shaken for 10 minutes at room temperature and read at 570 nm in a spectrophotometer (ASYS Hitech GmbH, Eugendorf, Austria). Four wells were exposed to each dilution of the extracts in 3 independent experiments. Absorbance readings were normalized to the untreated control cultures and represent the inhibition of succinyl dehydrogenase activity (cellular metabolism). Statistical differences were analyzed by Kruskal-Wallis complemented by Dunn multiple comparisons test, both with significance of $P < .05$.

Genotoxicity Analysis

The comet assay was performed following a standard protocol (16). Chemicals were obtained from Sigma (St Louis, MO). Dilutions that allowed cell viability of approximately 50% in the MTT assay were chosen for this test. The basic principle of the single-cell gel (comet) assay is the migration of DNA fragments in an agarose matrix under electrophoresis. Under a microscope, cells resemble a comet, with a nucleus and a tail containing DNA fragments or strands migrating toward the anode.

The negative control group was treated with cell culture medium (Dulbecco modified Eagle medium), and positive control group was established by using ethyl methane sulfonate at 5 mmol/L. Three slides were prepared per treatment. After incubation with the extracts, cells were suspended in culture medium (approximately 1×10^4 cells/mL), and 10 μL of this suspension was added to 120 μL 0.5% low melting point agarose at 37°C. This mixture was layered onto a precoated slide with 1.5% regular agarose and covered with a coverslip. The agarose was gelled in a refrigerator, the coverslip was removed, and slides were immersed in lysis solution (2.5 mol/L NaCl, 100 mmol/L EDTA, 10 mmol/L Tris-HCl buffer pH 10, with 1% Triton X-100, and 10% dimethyl sulfoxide) for 2 hours at 4°C. After immersion in alkaline buffer (0.3 mmol/L NaOH, 1 mmol/L EDTA pH >13) for 20 minutes, slides were electrophoresed for another 20 minutes at 25 V (0.86 V/cm, 300 mA), neutralized in 0.4 mol/L Tris-HCl (pH 7.5) for 15 minutes, and fixed in absolute ethanol. Staining was performed with 300 μL DAPI solution (4′,6-diamidino-2-phenylindole dihydrochloride) for 5 minutes. At least 50 randomly captured comets per treatment (25 cells from each slide) were examined at ×400 magnification by using a fluorescence microscope (Leica Leitz, Wetzlar, Germany). Images were analyzed by software (Comet Assay IV v4.3; Perceptive Instruments Ltd, Bury St Edmunds, UK). Tail moment was calculated by the image analysis system as the product of the tail length (DNA migration) and the fraction of DNA in the comet tail (% DNA in the tail). At least 2 slides derived from independent experiments were analyzed, and the statistical differences were analyzed by Kruskal-

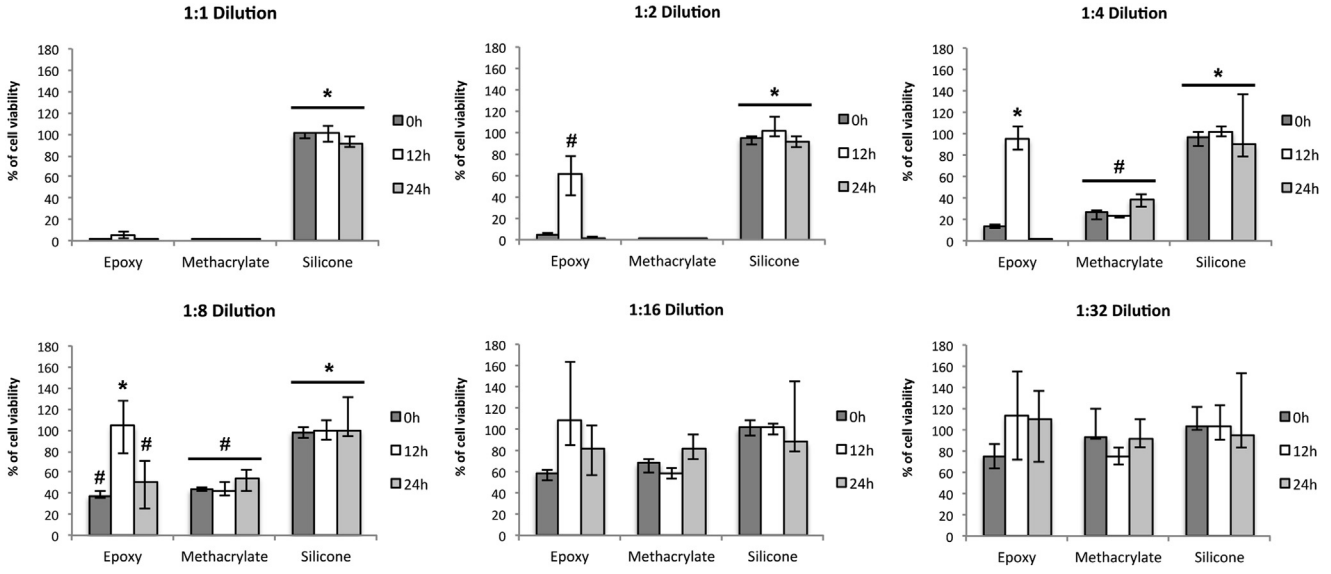

Figure 1. Graphical representation of the sealers' cytotoxicity in V79 cells after exposure to extracts, according to dilution and setting time. *Columns* represent the median cell viability expressed as percentage. *Bars* represent 25% and 75% percentiles. Original extracts (1:1) were serially diluted in fresh medium. Cell cultures were exposed for 24 hours, and cellular survival in treated and untreated cell cultures was determined in quadruplicate in 3 independent experiments (n = 12). Symbols (*, #) indicate the statistical differences among groups.