Short-Term Cytotoxicity Assessment of Components of the Epiphany Resin-Percha Obturating System by Indirect and Direct Contact Millipore Filter Assays

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Abstract

The Epiphany Resin-Percha Obturating System was assessed for cytotoxicity, compared with gutta-percha and AH-Plus sealer. Specimen disks (Resilon, gutta-percha), filled glass rings (sealers), or imbibed cellulose disks (primer, thinning resin) were placed over Millipore filters in direct or indirect contact with HeLa cell monolayer, incubated for 2 hours, and stained with tetrazolium blue. Cytotoxicity was rated by the surrounding unstained zone: none (0 mm), mild (≤7 mm), moderate (7–12 mm), or marked (>12 mm). Data were analyzed with one-way ANOVA and post hoc pairwise *t* tests. Unstained zones indicating moderate cytotoxicity were significantly larger ($p < 0.05$) for Epiphany primer than for thinning resin and for freshly mixed AH-Plus than for Epiphany sealer. Set sealers (24 and 48 hours), gutta-percha, and Resilon elicited noncytotoxic responses. In conclusion, cytotoxicity of set Epiphany sealer and Resilon was comparable with that of set AH-Plus and gutta-percha. Cytotoxicity of freshly mixed Epiphany sealer, primer, and thinning resin did not exceed that of freshly mixed AH-Plus. *(J Endod 2007;33:24–27)*

Key Words

AH-Plus sealer, cytotoxicity, Epiphany Resin-Percha Obturating System, gutta-percha

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0099-2399/$0 - see front matter

doi:10.1016/j.joen.2006.10.003

Root filling materials should be biocompatible (1), to avoid periradicular inflammation in reaction to leaching toxic products. Such response may delay healing and adversely affect the outcome of treatment (2). In addition, root filling materials should possess adequate sealing properties (1), to seal the root canal system against bacterial ingress (3, 4).

Conventional root filling materials include a core material and a sealer. Gutta-percha, the common core, was reported to be less toxic than other root filling materials tested (5), and is the gold standard for assessing alternative core materials. However, it has poor sealing properties (6). A frequently used type of sealer, the epoxy resin-based AH 26 and AH-Plus (Dentsply DeTrey, Konstanz, Germany), is cytotoxic when freshly mixed (7). AH-Plus, being free of formaldehyde, is less cytotoxic than AH 26 (7). Other sealers are also cytotoxic to a varying degree (2, 8).

The resin-based Epiphany Resin-Percha Obturating System (Pentron Clinical Technologies, Wallingford, CT) includes Resilon, a thermoplastic, synthetic polyester polymer core material, containing fillers of bioactive glass, bismuth oxychloride, and barium sulfate. The Epiphany sealer is a dual curable composite resin, with a matrix of a BisGMA, ethoxylated BisGMA, UDMA, and hydrophilic difunctional methacrylates, and fillers of calcium hydroxide, barium sulfate, barium glass, and silica.

In vitro studies of the Epiphany system have suggested an improved seal against bacterial ingress over gutta-percha and AH 26 sealer (9, 10), and modest reinforcement of teeth against vertical root fracture (11). Both findings have been attributed to bonding of Epiphany sealer to root dentin and the Resilon core, resulting in a monoblock of dentin-sealer-core (10, 11). In an animal study (12), coronally inoculated teeth filled with the Epiphany system developed less periapical inflammation than teeth filled with gutta-percha and AH 26 sealer. The short time allowed for bacterial ingress suggested that the inflammation could be interpreted as a tissue response to the material rather than to bacterial ingress (13–15). Thus, the Epiphany system may have displayed better tissue compatibility than the AH 26 sealer control. All of the above (9, 10–12) suggest potential advantages for the clinical application of the Epiphany system.

The purpose of this study was to evaluate the cytotoxicity of the Epiphany system's components using the indirect contact Millipore filter assay, and in a modified, direct contact assay.

Materials and Methods

The methodology of the indirect contact Millipore filter assay strictly followed the ANSI/ADA (16) and FDI (17) guidelines, defining the sample size ($n = 10$), cell type (HeLa), and test specimens weight (≤3.5 g) and diameter (7 mm).

Cell Cultures

HeLa cells (CCL-2; ATCC, Manassas, VA) were grown in Eagle's α-minimum essential medium with 10% fetal bovine serum and antibiotics (penicillin G 200 μg/ml, streptomycin 200 μg/ml). Cell cultures were incubated at 37°C, 5% CO_2, and 100% humidity, with the medium changed every other day.

Millipore Filters

Millipore filter disks (47 mm diameter, 0.45 μm pore size; Millipore Filter Corporation, Bedford, MA), were placed in 50-mm tissue culture dishes (Falcon, Becton Dickinson, Franklin Lakes, NJ), and covered with 6 ml of cell suspension (1.5×10^5 cells/ml). Culture dishes were incubated for 24 hours at 37°C, 100% humidity, 5% CO_2.

Specimens

Test materials were prepared according to manufacturers' directions, and specimens were formed as follows:

1. Core materials: Resilon and gutta-percha (Obtura Spartan, Fenton, MO) were molded into flat discs.
2. Sealers: Epiphany sealer and AH-Plus were placed in glass rings (5 mm height). They were tested freshly mixed, then incubated at 37°C, 90 ± 10% humidity and tested after 24 and 48 hours. Epiphany sealer specimens were covered on both sides with Mylar sheets to prevent contact with air and light-cured from both sides for 20 seconds before incubation.
3. Primer and thinner: Epiphany primer and thinning resin were imbibed in cellulose disks using a constant volume of 0.1 ml.
4. Epiphany system components combined: The sealer and primer were mixed and placed into glass rings, Resilon was inserted in the center, and the specimens were processed the same as the sealers.

Indirect Assay

Millipore filters with HeLa cell monolayer were rinsed once in phosphate-buffered saline (PBS) and placed cell-side down on a culture dish containing nutrient agar (Difco, Becton Dickinson, Sparks, MD). Three or four specimens were placed on the filters, and the dishes were incubated for 2 hours at 37°C, humidified atmosphere of 5% CO_2. After incubation, specimens were discarded; filters were removed, rinsed in PBS, and incubated overnight in a nitro blue tetrazolium dye solution (Aldrich Chemical, Milwaukee, WI). They were then rinsed in distilled water, air-dried, and assessed for staining pattern.

Direct Assay

The reproducibility of the protocol was verified in preliminary tests. Similar materials and procedures were used as in the indirect assay; however, specimens were placed at the bottom of a culture dish and nutrient agar was added flush with the top of the specimen. Millipore filters with HeLa cell monolayer were placed cell-side down over the agar and embedded specimens, and covered with a second agar layer (Fig. 1).

Controls

Negative controls were comprised of filters with cells placed on agar without material specimens, and filters without cells placed on agar with specimens, to assess interactions between test materials

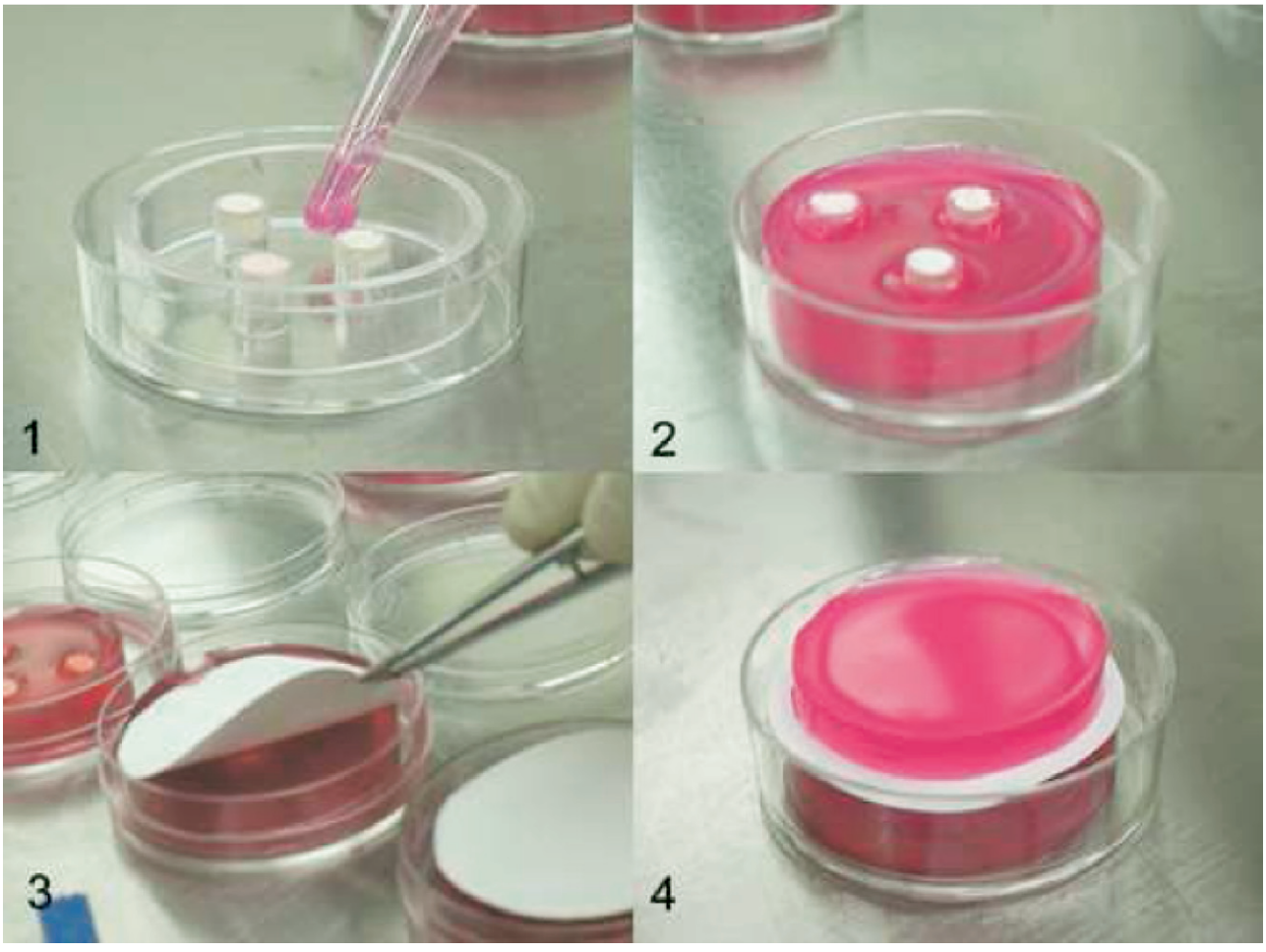

Figure 1. Direct cytotoxicity test with the Millipore filter test. (1, 2) Agar placed around the glass rings filled with the test materials. (3) The Millipore filter with HeLa cells placed cell-side down in the culture dish, over the agar medium and test materials. (4) A second layer of agar placed on top of the filter to provide nutrition to the cells.