Cytotoxicity evaluation of Activ GP and Resilon sealers in vitro

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Objective. This study is to evaluate the cytotoxicity of Activ GP and RealSeal sealers in a cell culture system in vitro, and to compare them with traditional AH 26 and Kerr sealers.
Study design. Samples of 0.5 mg freshly mixed or set RealSeal, Activ GP, AH 26, and Kerr sealers were eluted with 200, 400, 800, and 1,200 μL cell culture medium for 24 and 72 hours. L929 cells were seeded into 96-well plates at 3×10^4 cells/well and cultured with 100 μL eluate from each eluate group. Cells cultured with culture medium only served as a control. After 24 hours' incubation the cytotoxicity was evaluated by MTT assay. Cell viability was calculated as the percentage of the control group, and the results were analyzed with 1-way analysis of variance.
Results. For the freshly mixed sealer, cell viability in the AH 26 group was less than in all of the other 3 sealer groups. The Kerr sealer group had greater cell viability than RealSeal and Activ GP groups. For the set sealer, cell viability in the AH 26 group was greater than in all of the other 3 groups. Cell viability in the RealSeal group was less than in the Kerr and Activ GP groups.
Conclusion. Freshly mixed RealSeal and Activ GP sealers have lower cytotoxicity than AH 26 sealer and more cytotoxicity than Kerr sealer. When sealers are set, RealSeal sealer has more cytotoxicity than AH 26 and Kerr sealer. Activ GP sealer has more cytotoxicity than AH 26 and is similar to Kerr sealer. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:e74-e78)**

Resilon and Activ GP have been developed to improve the seal of root canal fillings. Resilon is a thermoplastic synthetic polymer (polyester)–based core filling material, which also contains bioactive glass, bismuth oxychloride, and barium sulfate.[1] Both its physical and its handling characteristics are similar to those of gutta-percha. Resilon is accompanied by a methacrylate-based dual-curable resin composite sealer (Epiphany) for the filling.[1] It has been suggested that the sealer bonds to both the root canal dentin wall and the Resilon core, forming a "monoblock" system within the root canal.[1,2] Early studies using Resilon and Epiphany showed less bacteria leakage as well as less associated periapical inflammation after coronal microbial inoculation in animal study.[2] Subsequent studies demonstrated that microleakage for root canals filled with Resilon was less than or equal to gutta-percha.[3-7]

Similarly, the Activ GP system is said to achieve the same results as Resilon in its ability to form a "monoblock" obturation; however, its composition is different.[8,9] This system is composed of a core material containing gutta-percha which is impregnated and coated on its external surface with glass ionomer. The accompanying sealer is a traditional glass ionomer sealer which can adhere chemically and micromechanically to the Activ GP cones and bond to the dentin.[8,9] Glass ionomer–based sealers had been developed based on glass ionomers' high affinity for bonding to dentin.[10] Coating Activ GP with glass ionomer particles is done to overcome the drawback of inadequate bonding between gutta-percha and glass ionomer sealer.[8,9]

Endodontic filling materials should not only be able to eliminate or minimize the ingress or egress of bacteria and their byproducts, but also have a favorable tissue response that promotes healing of the periapical tissues. Resilon cone has better biocompatibility, and Active GP cone's is similar to gutta-percha. The purpose of the present study was to evaluate the cytotoxicity of RealSeal, which is 1 of 2 commercially available forms of the Resilon/Epiphany system, and Activ GP sealers and to compare them with traditional root canal sealers AH 26 and Kerr sealer.

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MATERIALS AND METHODS

L929 mouse fibroblasts were obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were grown in Eagle Minimum Essential Medium (EMEM; ATCC), supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT), and 1% antibiotic/antimycotic cocktail (300 units/mL penicillin, 300 μg/mL streptomycin, 5 μg/mL amphotericin B; Gibco BRL, Gaithersburg, MD) under standard cell culture conditions (37°C, 100% humidity, 95% air, and 5% CO_2).

Root canal sealers tested in this study were RealSeal sealer (SybronEndo, Glendora, CA), Activ GP glass ionomer sealer (Brasseler, Savannah, GA), AH 26 sealer (Dentsply De Trey, Konstanz, Germany); and Kerr sealer (Kerr Corporation, Romulus, MI).

The cytotoxicity of the different sealers was tested in 2 ways. In one set of experiments, set sealer was used. Sealers were mixed according to the manufacturers' instruction, placed into 24-well plates at 0.5 g/well, and incubated for 72 hours in a cell culture incubator to allow the sealer to become set. In another set of experiments, freshly (immediately) mixed sealers were placed into the 24-well plates at 0.5 g/well. The freshly mixed and set sealers were incubated with 4 different amounts of cell culture medium, 200 μL, 400 μL, 800 μL, and 1,200 μL, for 24 and 72 hours (1 day and 3 days).

For the cell cytotoxicity assay, L929 cells were seeded into 96-well plates at 3×10^4 cells/well and incubated for 24 hours to allow adhesion. Then 100 μL of the sealer elute from the different elute groups was added to the culture wells. Cells without treatment served as a control group. After an incubation period of 24 hours, cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer's instructions (ATCC).

Cell viability was calculated as the percentage of the control group, and the results were analyzed with 1-way analysis of variance. Post hoc tests were done with Scheffe test. Each experiment was repeated 3 times.

RESULTS

Set sealer

When cells were cultured with 1-day elute of the set sealers, AH 26 had less cytotoxicity than all of the other sealers in the 200 μL elute groups (Fig. 1). Kerr sealer had less cytotoxicity than RealSeal and Activ GP sealers in the 200 μL elute groups (Fig. 1). RealSeal sealer had more cytotoxicity than all of the other sealers in the 400 μL, 800 μL, and 1,200 μL groups (Fig. 1).

When cells were cultured with 3-day elute of set sealers, AH 26 had less cytotoxicity than all of the other sealers in the 200 μL elute groups (Fig. 2). AH 26 and Activ GP sealers had less cytotoxicity than Kerr and RealSeal sealers in the 400 μL elute groups (Fig. 2).

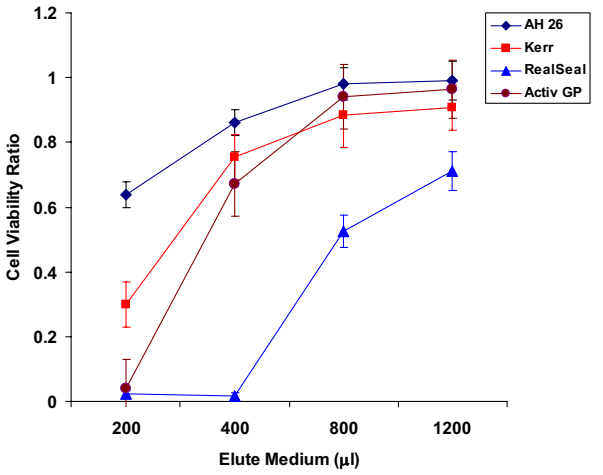

Fig. 1. Cell viability of L929 cells after culture with 1-day elute of set sealers.

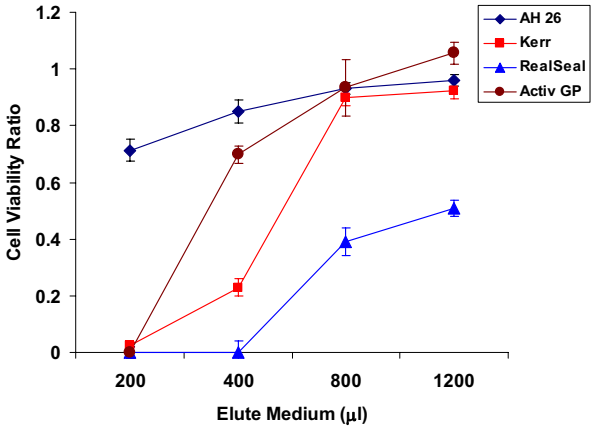

Fig. 2. Cell viability of L929 cells after culture with 3-day elute of set sealers.

RealSeal sealer had more cytotoxicity than all of the other sealers in the 800 μL and 1,200 μL groups (Fig. 2).

Fresh sealer

When cells were cultured with 1-day elute of the freshly sealers, there was no difference between the sealers in the 200 μL groups (Fig. 3). Kerr sealer had less cytotoxicity than all of the other sealers in the 400 μL, 800 μL, and 1,200 μL elute groups (Fig. 3). AH 26 sealer had more cytotoxicity than RealSeal and Activ GP sealers in the 800 μL and 1,200 μL groups (Fig. 3).

When cells were cultured with 3-day elute of the freshly mixed sealers, there was no difference between the sealers in the 200 μL and 400 μL elute groups (Fig. 4). Kerr sealer had less cytotoxicity than all of the other sealers in the 800 μL and 1,200 μL elute groups (Fig.

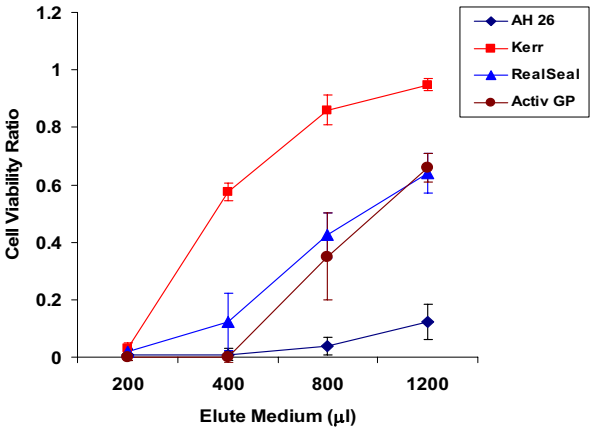

Fig. 3. Cell viability of L929 cells after culture with 1-day elute of freshly mixed sealers.

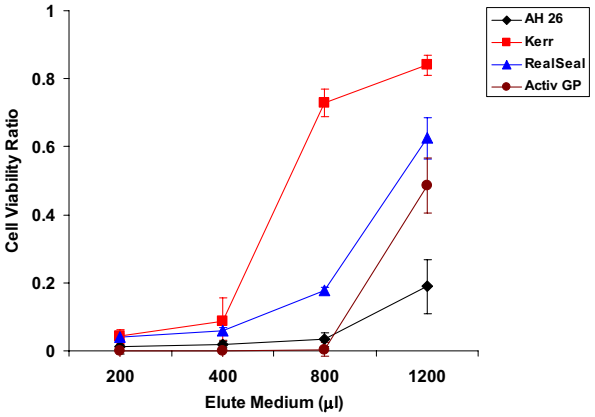

Fig. 4. Cell viability of L929 cells after culture with 3-day elute of freshly mixed sealers.

4). AH 26 sealer had more cytotoxicity than RealSeal and Activ GP sealers in the 1,200 µL groups (Fig. 4).

In summary, for the set sealers, AH 26 had less cytotoxicity and RealSeal sealer had the most cytotoxicity. For the freshly mixed sealers, Kerr had less cytotoxicity and AH 26 had the most cytotoxicity.

DISCUSSION

This study compared the cytotoxicity of RealSeal and Activ GP sealers with the conventional root canal sealer AH 26 and Kerr. In this study model, L929 mouse fibroblasts were placed in the prepared elute of the test sealers. L929 cells are routinely used for cytotoxicity testing.[11-17] This cell line is easy to prepare and culture, and provides more reproducible results without the individual difference and limited life span of primary cells.[18,19] In addition, L929 cells have been shown to be more prone to toxic products than human gingival fibroblasts.[20] The MTT assay is a standard assay to evaluate cytotoxicity of dental and endodontic filling materials.[12-17] This assay measures metabolic activity of cells by the ability of mitochondrial dehydrogenase enzymes to cleave MTT and form purple formazan crystals in the mitochondria of living cells. The level of formazan product created is proportional to the number of surviving cells. Solubilization of the purple formazan produces a colored solution. The absorbance of the colored solution can be quantified by a spectrophotometer. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effect of the agent on the cell viability can be determined.[21,22] The main advantages of this assay are that it is simple, rapid, and precise and does not require the use of radioisotopes.[14,21,22]

AH 26 was found to be the most cytotoxic when freshly mixed and least cytotoxic when set. These findings are consistent with other studies which have demonstrated that AH26 is most cytotoxic initially after mixing and gradually decreases in cytotoxicity with time.[13,23-26] This may be related to the initial release of formaldehyde.[27,28] The Kerr sealer, a eugenol-based sealer, was shown to be the least cytotoxic sealer when freshly mixed. Eugenol has been identified as one of the major ingredients responsible for the material's cytotoxicity.[29,30] This characteristic can also be partially attributed to the several chemical additives used in the various formulations, one of which is resin used for greater dentin adhesion.[31]

To date, a handful of articles have been published characterizing the RealSeal sealer. With the exception of 2, all reflect a rather aggressive cytotoxic effect elicited by the sealer.[13,14,18,24,32,33] This cytotoxic nature can be attributed to the leaching of uncured monomers.[13,14] The cytotoxicity of RealSeal sealer could also be the leaching of filler particles due to degradation of the sealer.[14] RealSeal sealer has a greater solubility than AH 26 sealer.[34] Furthermore, RealSeal sealer setting requires the absence of air.[35] In the presence of air, RealSeal setting is delayed and an unpolymerized monomer oxygen inhibition layer forms on the surface of the resin sealer.[35] It is this unpolymerized layer that affects the biologic properties and may be implicated.[13,24] In an implantation study in which RealSeal was implanted in the mandible of guinea pigs and evaluated for a period of 4 and 12 weeks, the sealer was found to have a favorable biocompatibility profile compared with AH Plus and EndoRez sealers.[36] This may be due to the leached particles which could be quickly cleared by the host, resulting in less local inflammation caused by the sealer.

Glass ionomer sealers have been shown to be well tolerated by tissues.[37,38] However, studies have also

shown glass ionomer cements to be highly toxic to cells in culture.[39] This characteristic is reported to be due to the release of uncured acid from the material. Dentin is believed to play a role in buffering the acid, which might explain its clinical usefulness in successful endodontic treatment outcomes.[40] Moreover, glass ionomer sealers are more soluble in water than other root canal sealers.[41] In the present study, the glass ionomer Activ GP sealer showed moderate toxicity when freshly mixed and less toxicity when set.

The findings of this study demonstrated that in the fresh state the cytotoxicity of the Activ GP and RealSeal sealers is between the Kerr and AH 26 sealers. In the set state, RealSeal sealer has relatively high cytotoxicity compared with the Kerr and AH 26 sealers. This study suggests that caution should be taken not to overfill with root canal sealers. Studies in vitro are the starting point of cytotoxicity evaluation. The results may be used to understand and explain the clinical signs, symptoms, and treatment outcome of root canal filled teeth using the different filling materials. Studies in vivo are needed to investigate the biocompatibility of these sealers and their impact on endodontic treatment outcome.

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