Cytotoxicity evaluation of Active GP and Resilon cones in vitro

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Objective. This study was done to evaluate the cytotoxicity of Activ GP and Resilon cones in an in vitro cell culture system.
Study design. Gutta-percha (GP), Activ GP, and Resilon cones were tested in this study. L929 cells were seeded into 96-well plates at 3×10^4 cells/well. In one set of experiments, 2-mm segments cut from the tip of GP and Resilon cones were placed into the cell culture wells and incubated for 1, 2, and 3 days. In another set of experiments, 2 20-mm segments of GP, Activ GP, and Resilon cones were incubated in 2 mL cell culture medium for 1 week. Then 100 μL elutes were tested for 24 and 48 h. Cell viability was evaluated by MTT assay. Data were analyzed using 1-way analysis of variance.
Results. When GP, Activ GP, and Resilon segments were placed into cell cultures, cell viability in the Resilon group was significantly greater than in the GP and Activ GP groups at any test time. There was no cell viability difference between the Activ GP and GP groups. When the elutes of GP, Activ GP, and Resilon was placed into cell cultures, the results were the same as using segments of the tested material. The cytotoxicity of GP and Activ GP is greater than that of the Resilon cone. There was no cell viability difference between Activ GP and regular GP.
Conclusion. Resilon has better biocompatibility than regular GP and Activ GP cones. The cytotoxicity of Activ GP is similar to that of regular GP. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:e76-e79)**

Resilon and Activ GP have been recently developed to improve the seal of root canal filling. Resilon is a thermoplastic synthetic polymer (polyester)–based core filling material, which also contains bioactive glass, bismuth oxychloride, and barium sulfate.[1] Its physical and handling characteristics are similar to gutta-percha (GP). Resilon has been used with a dual-curable resin composite sealer (Epiphany) for obturation.[1] The sealer bonds to the dentin wall and the Resilon core to form a "monoblock" system within the root canal.[1,2] Early studies showed that root canals filled with Resilon and Epiphany had less bacteria leakage[1] and less periapical inflammation after coronal microbial inoculation in animals.[2] Subsequent studies found that the microleakage from root canals filled with Resilon was less than or equal to those filled with GP.[3-7] Activ GP cones have coated glass ionomer particles on the surface.[8] The Activ GP sealer is a glass ionomer sealer, which can adhere chemically and micromechanically to the Activ GP cones and bond to the dentin.[8]

Even though Resilon and Activ GP have been on the market, there are few studies to investigate their biocompatibility.[9] The aim of the present study was to evaluate the biocompatibility of Activ GP and Resilon cones in a cell culture system.

MATERIAL AND METHODS

L929 mouse fibroblasts were obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were grown in Eagle's minimum essential medium (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/5% CO_2).

Root canal filling materials used in this study were #40, 0.06 taper GP (DiaDent); #40, 0.06 taper Activ GP (Brasseler, Savannah, GA); and #40, 0.06 taper Resilon cones (SybronEndo, Glendora, CA).

The cytotoxicity of GP and Resilon cones was tested in 2 ways. In one set of experiments, GP or Resilon cones were directly added to the cell culture wells.

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L929 cells were seeded into 96-well plates at 3×10^4 cells/well and incubated for 24 h to allow adhesion. Then 2-mm segments cut from the tip of GP or Resion cones were placed into the center of the culture wells. The material covered 3.4% of the culture surface area. Cells were treated in the following groups: 1) no treatment (control); 2) #40, 0.06 taper GP; 3) #40, 0.06 taper Activ GP; 4) #40, .06 taper Resilon cones; and 5) #40, 0.06 taper endosequence rotary files (Brasseler USA, Savannah, GA). The nickel-titanium (NiTi) rotary file was used as a relative inert material to exclude the effect of material contacting on the cells. After 24, 48, and 72 h incubation, cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer's instructions (ATCC).

In another set of experiments, the elute of GP or Resilon cones was used for the cell viability test. Two 20-mm segments cut from the tip of GP, Resilon cones, and NiTi rotary files were incubated in 2 mL cell culture medium for 1 week. The elute of the tested material was as follows: 1) cell culture medium (control); 2) #40, 0.06 taper GP; 3) #40, 0.06 taper Activ GP; 4) #40, 0.06 taper Resilon cones; and 5) #40, 0.06 taper endosequence rotary files. Cells were seeded into 96-well plates at 3×10^4 cells/well and incubated for 24 h to allow adhesion. Then 100 μL elute was added to the cell culture wells. After an incubation period of 24 and 48 h, cell viability was evaluated by the 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

The NiTi rotary file had no effect on the cell viability when file segment or elution of the file was used (Figs. 1 and 2). This result showed that the simple contact of material had no significant effect on cell viability.

When GP or Resilon cones were directly added to the cell culture, cell viability in the Resilon group was significantly greater than in the GP and Activ GP groups at all of the tested times ($P < .05$; Fig. 1). There was no cell viability difference between the GP and Activ GP groups at all of the tested time ($P > .05$; Fig.1).

In the test using the elutes of GP or Resilon cones, there was no significant cell viability difference between the GP and Activ GP groups ($P > .05$; Fig. 2). Cell viability in the GP and the Activ GP groups was significantly less than in the Resilon group ($P < .05$; Fig. 2).

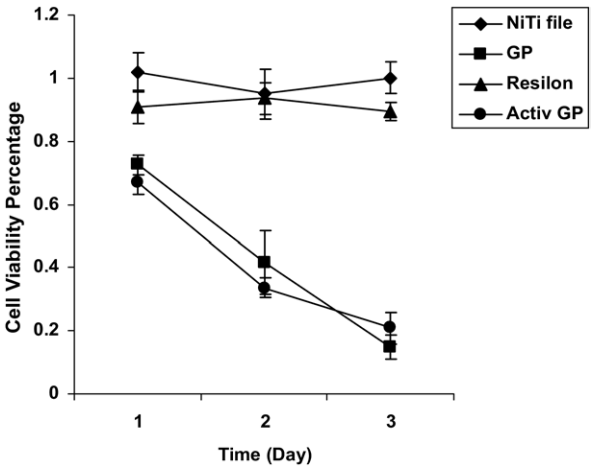

Fig. 1. Cell viability after cells were cultured with segments of gutta-percha (GP), Resilon cone, and nickel-titanium (NiTi) rotary file.

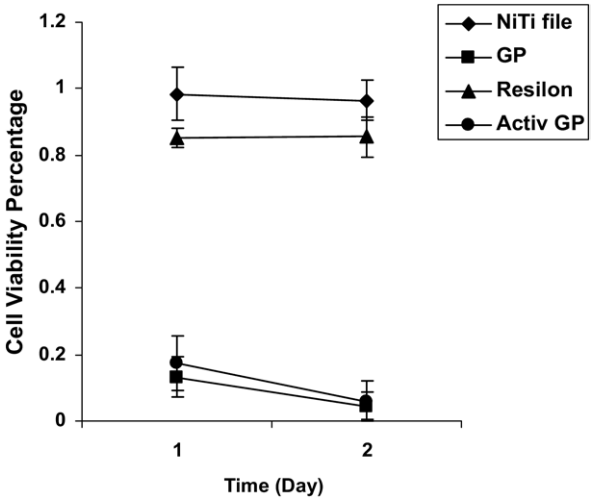

Fig. 2. Cell viability after cells were cultured with elute of gutta-percha (GP), Resilon cone, and nickel-titanium (NiTi) rotary file.

DISCUSSION

L929 mouse fibroblasts were chosen in this study. This cell line is easy to prepare and culture without the individual difference of primary cells. L929 cells are routinely used for cytotoxicity testing.[10-16] The MTT assay is a standard assay to evaluate cytotoxicity of dental materials.[11-15] MTT is reduced to purple formazan in the mitochondria of living 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.[17,18]

Gutta-percha has been the most widely used root canal filling material. Pure GP is biocompatible;[10] however, GP cones have been shown to possess cytotoxicity.[10,19,20] The composition of most GP cones includes zinc oxide.[10] The cytotoxicity of GP cones results from the leakage of zinc ions.[10] The antibacterial activity of GP cones is also attributed to the zinc oxide content.[21,22] In the present study, when GP was directly placed into the cell culture, cell viability was reduced. The cell viability decrease is not due simply to the material contacting with the cells, because the contact of NiTi file had no effect on cell viability. The cytotoxicity is due to leaching substances from GP cones. The results from the elute of GP confirmed that leaching substances reduces cell viability. These results agree with earlier findings that GP cones cause cytotoxic reactions.[10,19,20,23-26] Whether the leaching substance is zinc oxide[10] or other GP components needs to be further determined. Furthermore, the preparation of the evaluated material plays an important role in the outcome of in vitro cytotoxicity assay.[10,27,28] This could explain why GP cones were tested to be biocompatible in several other studies.[9,27,29,30]

Chlorhexidine, calcium hydroxide, and iodoform have been added to GP cones as antibacterial additives.[25,31-37] Although the cytotoxicity of calcium hydroxide–based GP is similar to that of conventional GP cones,[24,25,34] chlorhexidine-containing GP cones showed more cytotoxicity than conventional GP cones.[25,34] Glass ionomer particles were incorporated on the surface of the Activ GP to achieve a bond with the glass ionomer sealer.[8] The present study found that the cytotoxicity of glass ionomer particle–coated Activ GP is similar to that of conventional GP cones. The addition of glass ionomer particles has no effect on the cytotoxicity of GP cones. Glass ionomer cement sealer has been demonstrated clinically to have a successful endodontic treatment outcome,[38] although it is more soluble in water than other root canal sealers.[39]

Resilon is composed of 57% polyester polymer polycaprolactone and 42% bioactive glass and radiopaque fillers.[40] Polycaprolactone is biocompatible and has been developed for tissue engineering and biomedical applications.[41-44] The present study found that the polycaprolactone-based Resilon is biocompatible, in line with another recent study that used a root model.[11]

In summary, Resilon has better biocompatibility than GP cones. The biocompatibility of Activ GP is similar to that of regular GP.

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