

# Comparative Study of the Cytotoxic Effect of Resilon Against Two Cell Lines

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Resilon is a new material that is a candidate to replace gutta-percha as a root filling material. This study evaluated the antiproliferative effect of Resilon and two commercially available gutta-percha points (Roeko, Dentsply). Two established cell lines (L929 and RPC-C2A) were used for the experiment. Cell survival fraction was estimated by the sulforhodamine-B assay, in reference to controls after 48-h exposure. Non-parametric tests (Kruskal-Wallis followed by Dunn's multiple comparisons) were used to evaluate the statistical significance of the results ( $\alpha=0.05$ ). Cytotoxicity in a descending order was: Resilon > Roeko gutta-percha > Dentsply gutta-percha. At 24-h exposure, no statistically significant differences ( $p>0.05$ ) were observed between tested materials in both cell lines. At 48-h exposure, statistically significant differences ( $p<0.05$ ) were found between Resilon and the other materials in the L929 cell line. In the RPC-C2A cell line Resilon was significantly more cytotoxic than Dentsply gutta-percha ( $p<0.05$ ), but no statistically significant differences ( $p>0.05$ ) were found between Resilon and Roeko gutta-percha. The cytotoxicity of Resilon increased significantly from 24 h to 48 h in both cell lines. Resilon points were more cytotoxic than gutta-percha points. The cytotoxicity was time dependent and increased after 48 h.

Key Words: cytotoxic effect, Resilon, gutta-percha.

## INTRODUCTION

Gutta-percha is the most commonly used solid filling material in endodontic therapy. Commercially available endodontic gutta-percha points contain organic (gutta-percha polymer, resins, wax) and inorganic (zinc oxide, metal sulphates) substances. It is also possible to detect small quantities of colouring and antioxidant agents. These additives improve the strength, plasticity and radiopacity of the material (1,2).

Resilon (Resilon Research LLC, Madison, CT, USA) is a new material that is a candidate to replace gutta-percha as a core root filling material. Its thermo-plastic properties are due to the incorporation of polycaprolactone and it contains methacrylate-based resins. Resilon material behaves similarly to gutta-percha, has the same handling properties and can be

heat-softened or dissolved by solvents, such as chloroform. The overall filler content is approximately 65% by weight. It is used together with Epiphany self-etching primer and sealer (Pentron Clinical Technologies, LLC Wallingford, CT, USA), creating, according to the manufacturers, a "monoblock" root canal filling (3,4).

The biocompatibility of endodontic filling materials is a critical factor for their efficacy. A root model has been suggested for cytotoxicity evaluation of endodontic filling materials in order to simulate clinical conditions (5,6). However, this model does not replicate all clinical situations. Endodontic filling materials should, in theory, remain inside the root canal, but occasionally they are extruded to the periapical tissues through the apical constriction or via an iatrogenic perforation. Therefore, evaluating the biocompatibility of endodontic filling materials based on the biological response of

cell lines is not only useful, but also essential.

Gutta-percha points, generally regarded as an inert material, have shown slight cytotoxicity in some studies (7,8). The cytotoxicity of Resilon has been reported to be similar to that of gutta-percha and lower than that of Epiphany sealer (9). Other authors (10) evaluating the biological response to contemporary endodontic sealers, found similar biological response of fibroblast cell lines to Epiphany sealer/Resilon. The fact that Resilon is a core filling material raises questions about its cytotoxicity compared to gutta-percha points and alpha-phase gutta-percha based systems.

This study evaluated the antiproliferative effect of Resilon, and two commercially available gutta-percha points on two established cell lines (L929, RPC-C2A) by means of the sulforhodamine-B (SRB) assay. The null hypothesis was that there was no difference in cytotoxicity between Resilon and gutta-percha samples.

## MATERIAL AND METHODS

The tested materials were: Resilon points, Roeko gutta-percha points (Coltene-Whaledent, Switzerland), Dentsply gutta-percha points (Dentsply Maillefer, Ballaigues, Switzerland).

### *Cell Lines and Culture Conditions*

Two fibroblast cell lines, L929 (mouse skin fibroblasts) and RPC-C2A (rat pulp cells) were used for cytotoxicity evaluation. L929 cells were obtained from ICRF, London, UK, and RPC-C2A cells were kindly donated by Prof. S. Kasugai (Department of Pharmacology, Faculty of Dentistry, Tokyo Medical and Dental University, Japan). Cells were grown as monolayer cultures in T-75 flasks (Costar/Corning, Cambridge, UK), were subcultured twice a week at 37°C in an atmosphere containing 5% CO<sub>2</sub> in air and 100% relative humidity and maintained at low passage number (5-20). Cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Glasgow, UK), supplemented with 10% fetal bovine serum (FBS, Gibco), 100 µg/mL streptomycin and 100 IU/mL penicillin.

### *Cell Inoculation*

Adhered cells at a logarithmic growth phase, were detached by the addition of 2-3 mL of a 0.05%

trypsin (Gibco, 1:250) and 0.02% EDTA mixture and incubated for 2-5 min at 37°C. Cells were plated in 96-well plates (Costar-Corning) at a density of 4,000 cells *per well* in 100 µL culture medium, and were maintained for 24 h in an incubator to resume exponential growth.

### *Preparation of Test Materials*

The following three materials were tested: Resilon points, Dentsply gutta-percha points and Roeko gutta-percha points. As much as 0.2 g of each point were placed in sterile vials containing 6 mL DMEM and incubated at 37°C for 24 h. One hundred microliter of the extract medium sterile filtrated by a 0.22-µm syringe filter was added to the cells (final volume of 200 µL) and incubated for either 24 h or 48 h. Control wells were treated with 100 µL DMEM. Six replicate wells for each material were prepared. At the established time points, cell numbers were estimated by means of the sulforhodamine-B (SRB) assay.

### *Sulforhodamine-B (SRB) Colorimetric Assay*

The SRB assay was undertaken as described by Skehan et al. (11) and modified by Papazisis et al. (12). Briefly, the culture medium was aspirated prior to fixation and 75 µL of 10% cold (4°C) trichloroacetic acid were gently added to the wells. Microplates were left for 30 min at 4°C, washed five times with deionised water and left drying at room temperature for at least 24 h. Subsequently, 70 µL 0.4% (w/v) sulforhodamine B (Sigma Aldrich Corp., St. Louis, MO, USA) in 1% acetic acid solution were added to each well and left at room temperature for 20 min. The SRB was removed and the plates were washed five times with 1% acetic acid before air-drying. Bound SRB was dissolved in 200 µL 10 mM unbuffered tris-base solution (Merck, Darmstadt, Germany) and the plates were taken to a plate shaker for at least 10 min. Absorbance was read at 492 nm by subtracting the background measurement of 620 nm. The test optical density (OD) was defined as the mean absorbance of each individual well minus the blank value ('blank' is the mean OD of the background control wells). Mean values and coefficient of variation (CV) were calculated. Experiments were performed in sixplicates for each material and incubation period and each experiment was carried out at least twice. The results were expressed as "survival fraction", which

was calculated as the percentage of test OD to control OD (plain medium was added to the control wells).

Non-parametric tests (Kruskal-Wallis followed by Dunn's multiple comparisons) were used to evaluate the statistical significance of the results ( $\alpha=0.05$ ).

## RESULTS

The results are presented in Figure 1. Representative photographs of cells are shown in Figure 2.

At 24-h exposure, Resilon exhibited a higher cytotoxic effect to both cell lines than the other tested materials, though without statistical significance ( $p>0.05$ ). At 48-h exposure, statistically significant differences ( $p<0.05$ ) were found between Resilon and the other materials in the L929 cell line. In the RPC-C2A cell line Resilon was significantly more cytotoxic than Dentsply gutta-percha ( $p<0.05$ ), but no statistically significant

differences ( $p>0.05$ ) were found between Resilon and Roeko gutta-percha. The cytotoxicity of Resilon

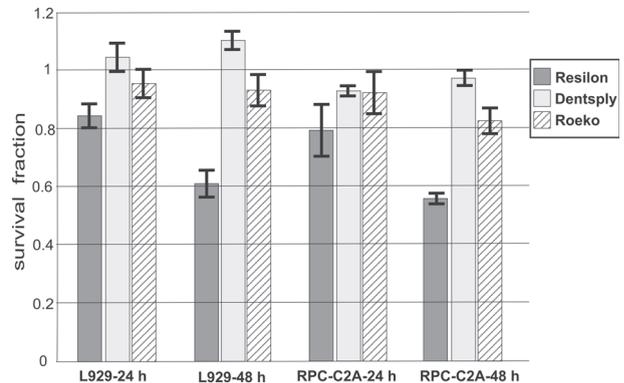


Figure 1. Survival fraction means and SD of Resilon, Dentsply gutta-percha and Roeko gutta-percha in L929 and RPC-C2A cells.

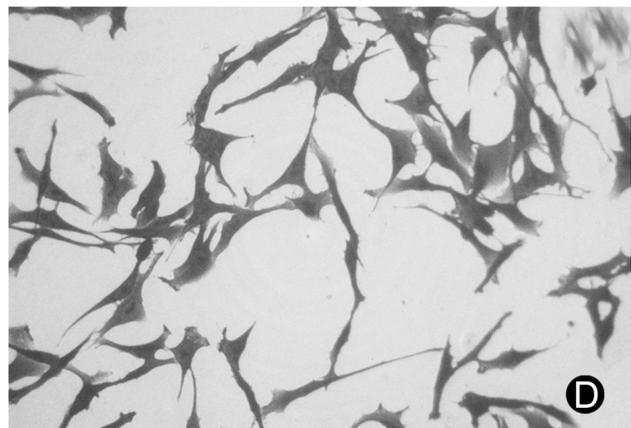
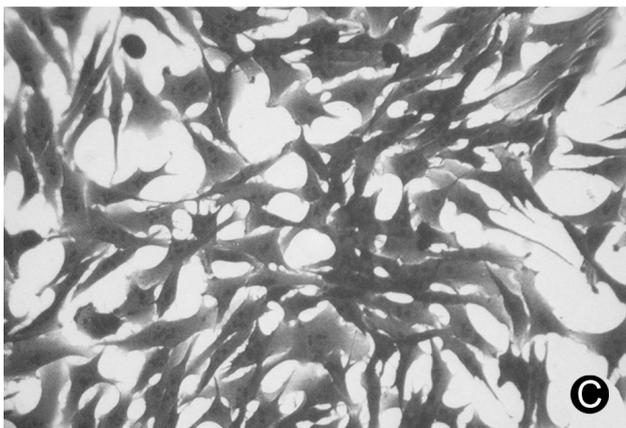
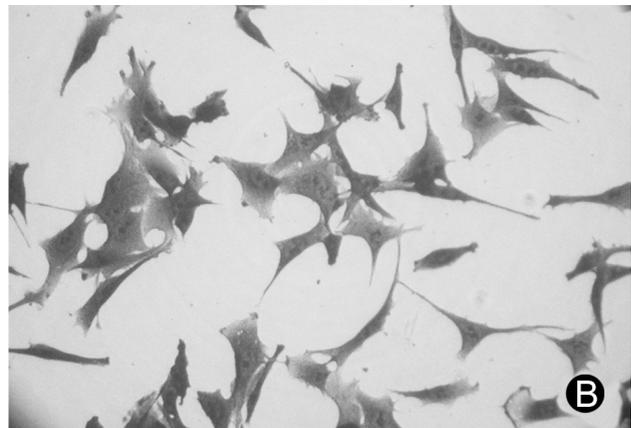
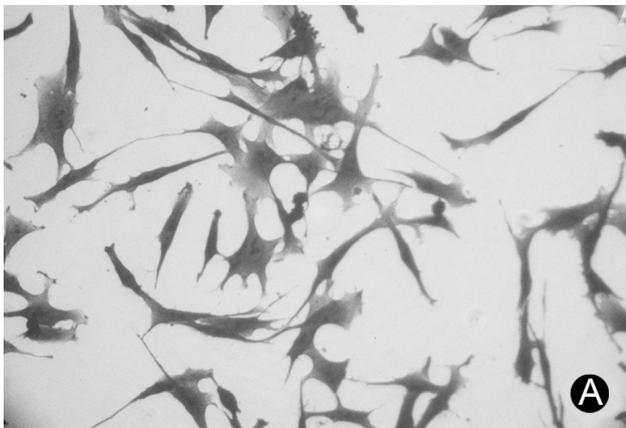


Figure 2. Representative photographs of cells (original magnification  $\times 100$ ). A= Control L929 cells at 24 h; B= L929 exposed to Dentsply gutta-percha for 24 h; C= Control RPC-C2A cells at 48 h; D= RPC-C2A exposed to Resilon for 48 h.

increased significantly from 24 h to 48 h in both cell lines.

The cytotoxicity of Roeko gutta-percha increased after 48 h, with a statistically significant difference compared to the 24-h exposure period, only in the RPC-C2A cell line. There was no statistically significant difference between the 24- and 48-h values for Dentsply gutta-percha. The null hypothesis was rejected.

## DISCUSSION

A number of *in vitro* and *in vivo* methodologies have been used to evaluate the biocompatibility of dental materials. *In vitro* methods are simple, rapid, reproducible and inexpensive. The established cell lines most widely used are L929, BHK21/C13, HeLa, Raji, KB, and NCTC-2544. The experimental settings include those that expose the cells to the test materials and measure alterations in cell counts, changes in membrane permeability, metabolic alterations and cytopathogenic changes. The cell density used in this study (4,000 cells/well) was sufficient to ensure that cells would be on the exponential phase of the growth curve throughout the experiment. The recorded data were cell numbers, estimated originally with the SRB assay, which is both accurate and linear in the estimation of cell populations (12).

Gutta-percha filling points, the most frequently used solid core root filling material, only contain about 20% organic material (gutta-percha and wax), with 60 to 75% of the remainder being zinc oxide filler (1,2). Although regarded as an inert material, gutta-percha produces some degree of tissue irritation, probably due to its zinc oxide content. Pascon and Spangberg (7) evaluated the toxicity of commercially available gutta-percha using the radiochromium release assay. All gutta-percha points were toxic at longer observation periods and the researchers attributed this to leakage of zinc ions into the culture medium. Szep et al. (8) compared the cytotoxicity of medicated (calcium hydroxide or chlorhexidine) and non-medicated gutta-percha points using primary human gingival fibroblasts. Comparison of the non-medicated points (DeTrey and Roeko) to each other and to the control cultures showed an increase in the number of dead cells which, however, was not statistically significant. The DeTrey cultures showed a lower, but once again not statistically significant, mitotic rate than the Roeko cultures.

In the present study, the cytotoxicity of Dentsply and Roeko gutta-percha to L929 cells was minimal.

After 48-h exposure, Roeko gutta-percha showed higher but not statistically significant cytotoxic effect to RPC-C2A cells than that of Dentsply gutta-percha. The difference between Roeko gutta-percha and the control in RPC-C2A cells (48-h exposure) was statistically significant. The differences between commercially available gutta-percha points could be attributed to differences in zinc oxide content. Unfortunately, manufacturers usually do not inform the relevant quantities of gutta-percha components, nor is it known whether the composition of the points is consistent over the years, making it impossible to confirm this hypothesis.

Resilon bondable material is made from polyester polymers and contains fillers and radiopacifiers in a soft resin matrix. The thermoplasticity of Resilon stems from polycaprolactone, a biodegradable polyester with a moderately low melting point, while its bondability results from the inclusion of resins containing methacryloxy groups. Polycaprolactone is a suitable and biocompatible material that can be used in devices, such as absorbable sutures (13), scaffold for vascular graft development (14) and experimental epidermal substrates for skin regeneration (15). It seems reasonable to attribute the higher cytotoxicity of Resilon compared to gutta-percha primarily to its resin content. With Resilon, the cytotoxic effect to both cell lines was more pronounced after 48 h than after 24 h of exposure, indicating a prolonged and time-dependent effect for the periods evaluated in this study. A possible explanation would be the biodegradability of Resilon. Tay et al. (16,17) found that Resilon was susceptible to alkaline and enzymatic hydrolysis and exhibited extensive surface thinning and weight loss after incubation in hydrolytic enzymes. The biodegradation of polycaprolactone probably exposes the polymer matrix, a phenomenon that increases over time. In gutta-percha specimens, only superficial pores were created by enzymes, with no further degradation changes (16).

Using a root model, Susini et al. (6) compared the cytotoxicity of fillings composed of Epiphany and Resilon with both fillings of Roekoseal and gutta-percha, and fillings of Sealite and gutta-percha. These authors found that Resilon/Epiphany combination was more cytotoxic than the other combinations at 1 and 2 days. After 7 and 30 days, there were no differences in cytotoxicity. In order to determine which material (Resilon or Epiphany) was responsible for the cytotoxic effect, the authors made a comparison according to ISO

10993-5 standards. The results showed that Epiphany was significantly more cytotoxic than Resilon, which the authors considered to have no cytotoxicity. In contrast, Bouillaguet et al (10) reported that Resilon was cytotoxic to fibroblasts to a degree comparable to that of Epiphany sealer, and that both materials were more cytotoxic than AH-plus and Gutta-flow sealers.

In another study (9), the cytotoxicity of Resilon was found to be the same as that of gutta-percha and lower than that of Epiphany. These outcomes are in contrast with the findings of Bouillaguet et al. (10) and those of the present study, in which Resilon exhibited greater cytotoxicity than gutta-percha. This may be due to the different cell types (primary fibroblasts vs. established cell lines) and methodologies (trypan blue staining and hemocytometer counting vs. SRB assay vs. SDH activity) used in each study.

In conclusion, Resilon points were more cytotoxic than gutta-percha cones. The cytotoxicity of Resilon to both cell lines increased significantly from 24 h to 48 h of exposure, indicating that further research on the *in vivo* biocompatibility of this material is necessary.

## RESUMO

Resilon é um material novo com potencial para substituir a gutta-percha como material obturador radicular. Este estudo avaliou o efeito anti-proliferativo do Resilon e de duas marcas comerciais de pontas de gutta-percha (Roeko e Dentsply). Para os fins deste estudo foram utilizadas duas linhagens celulares conhecidas (L929 e RPC-C2A). A fração de sobrevivência celular foi estimada pelo método colorimétrico de sulforodamina B comparado aos controles após exposição por 48 h. A significância estatística dos resultados ( $\alpha=0,05$ ) foi avaliada pelos testes não-paramétricos de Kruskal-Wallis e Dunn para comparações múltiplas. A citotoxicidade dos materiais em ordem decrescente foi: Resilon > gutta-percha Roeko > gutta-percha Dentsply. Após 24 h de exposição, não foram encontradas diferenças estatisticamente significantes ( $p>0,05$ ) entre os materiais testados em ambas as linhagens celulares. Após 48 h, o Resilon apresentou um efeito citotóxico significativamente maior ( $p<0,05$ ) em comparação aos outros dois materiais na linhagem celular L929. Na linhagem RPC-C2A, a citotoxicidade do Resilon foi significativamente maior ( $p<0,05$ ) que a da gutta-percha Dentsply, mas não houve diferenças significantes ( $p<0,05$ ) entre Resilon e gutta-percha Roeko. A citotoxicidade do Resilon aumentou significativamente de 24 para 48 h para ambas as linhagens celulares. As pontas de Resilon foram mais citotóxicas do que as pontas de gutta-percha. A citotoxicidade foi tempo-dependente e aumentou após 48 h de exposição.

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