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Cytotoxicity of Epiphany® and Resilon® with a root model

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Abstract

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Aim To record the cytotoxicity of Resilon and Epiphany (Pentron clinical technologies, Wallingford, CT, USA) using a root model.

Methodology Thirty teeth with single roots were sectioned at the enamel–cementum junction, the root canals prepared and each root then sterilized before filling with the lateral condensation technique using one of three filling materials ($n = 10$ per group): Resilon and Epiphany, Sealite (Septodont, Pierre Rolland, Merignac, France) and gutta-percha, Roekoseal Automix (Coltène/Whaledent, Langenau, Germany) and gutta-percha. The roots were stored at 37 °C in an incubator to allow for setting of the root filling materials. The apices of the roots were dipped in 1 mL of MEM culture medium for 1, 2, 7 and 30 days renewing the medium every day. After 24 h contact between the medium and the filled roots, the medium was used to measure the cytotoxicity on mouse fibroblasts L 929 with the MTT assay that recorded the mitochondrial activity of the target cells. An additional test according to ISO 10993-5 standards was undertaken to compare Resilon and Epiphany.

Results The root model showed no statistically significant differences between the sealers at 7 and 30 days (NS). Epiphany and Resilon were the most cytotoxic materials at 1 and 2 days ($P < 0.001$). Unlike Epiphany, Resilon was not cytotoxic when tested according to ISO 10993-5 standards.

Conclusions The cytotoxicity of Resilon + Epiphany, due mainly to Epiphany, decreased after 2 days to reach a level comparable with commonly used root canal sealers.

Keywords: cytotoxicity, filling material, root canal.

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Introduction

After cleaning and shaping the root canal (Shilder 1974), a root filling is required to prevent reinfection (Shilder 1967). The root filling is required to both seal the bacteria remaining within the canal and to prevent the influx of periapical tissue derived fluid from entering the canal. For these reasons, new materials aimed at increasing the effectiveness of canal filling are developed. However, these new materials should meet certain requirements before being marketed.

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Resilon associated with Epiphany (Pentron Clinical Technologies, Wallingford, CT, USA), a thermoplastic synthetic polymer-based root filling material, has recently been introduced. Some of its mechanical and physical properties have been evaluated with controversial results. For example, Resilon seems to increase the fracture resistance of root-filled teeth (Teixeira *et al.* 2004), but also shows a poor Epiphany/dentine adhesion (Gesi *et al.* 2005) and a low bond strength of Resilon to Next-, a methacrylate-based root canal sealant (Hiraishi *et al.* 2005).

In contrast, the biological properties of Resilon and Epiphany are not well documented. The results of biocompatibility studies appear in product disclosure statements and in some conference abstracts but few detailed journal articles deal with the subject. Only one

animal study, performed on dogs, evaluated the periapical response to this material and concluded that Resilon + Epiphany (Pentron Clinical Technologies) induced less periapical periodontitis than gutta-percha and AH 26 (Shipper *et al.* 2005). This favourable result was probably due to the good sealing efficiency of this resinous material (Shipper *et al.* 2004). The purpose of this study was to evaluate the cytotoxicity of Resilon + Epiphany using a root model (Camps & About 2003).

Materials and methods

Three root canal filling materials were tested:

- Epiphany (Pentron clinical technologies) + Resilon cones (Pentron clinical technologies; batch no. 123836).
- Roekoseal Automix (Coltène/Whaledent, Langenau, Germany; batch no. 6503664) + gutta-percha points (Denstply Maillefer, Ballaigues, Swizerland; batch no. 010500).
- Sealite regular (Pierre Rolland, Mengnac, France; batch no. 3895) + gutta-percha points (Denstply Maillefer).

RoekoSeal, a silicone-based root canal sealer, was used as a negative control because it is known to be noncytotoxic (Dartar Öztan *et al.* 2003). Sealite was used as a clinical reference because of the long clinical history of zinc oxide–eugenol-based root canal sealers (Pertot *et al.* 1992).

Root model

Thirty intact freshly extracted teeth, with single root, were stored at 4 °C in PBS + penicillin 100 IU mL^{-1} + streptomycin 100 μg mL^{-1} and used according to French ethical laws (Journal Officiel de la République Française 2004). The crowns were removed at the cementodentinal junction with a diamond disk under water coolant. A size 10 K-file was introduced into the canal to radiographically measure the working length and to check the patency of the foramen. The root canals were prepared by the same operator to the cementodentinal junction with ProFile instruments (Dentsply Maillefer) using a reduction handpiece coupled to an electric motor (Table 1). A size 10 K-file was used between each ProFile to ensure apical patency. Two millilitre of 2.5% NaOCl were delivered with a 27-gauge needle between each file. The final rinse was performed with saline.

Table 1 Sequence of instruments used for canal preparation

ProFile size 30 taper 06
ProFile size 25 taper 06
ProFile size 20 taper 06
ProFile size 25 taper 04
ProFile size 20 taper 04
ProFile size 20 taper 06
ProFile size 25 taper 06
ProFile size 30 taper 06

The teeth were then sterilized at 135 °C for 35 min (De Wald 1997). After sterilization the 30 teeth were divided randomly into three groups of 10 teeth to be filled using a lateral condensation technique (Resilon + Epiphany $n = 10$, Roekoseal Automix + gutta-percha $n = 10$ or Sealite + gutta-percha $n = 10$). This was completed under sterile conditions in a laminar flow hood. The teeth were then stored for 1 day in an incubator, lying on a gauze sponge saturated with PBS, at 37 °C and 100% humidity to allow setting of the root filling materials.

The apex of the roots was dipped into 1 mL of culture medium for 30 days and the medium was renewed every day to simulate periodontal ligament clearance. The medium was minimum essential medium (MEM, Gibco, Cergy-Pontoise, France) with 10% foetal calf serum (Biowhittaker, Gagny, France) supplemented with penicillin 100 IU mL^{-1} and streptomycin 100 μg mL^{-1}. The medium which remained in contact with the apex for 24 h after 1, 2, 7 and 30 days was called the test medium and was used to measure the cytotoxicity with the MTT assay. No dilution was made because a pilot study showed a low cytotoxicity under these conditions.

L 929 fibroblasts were plated at 30 000 cells cm^{-2} in 96-well plates (Falcon 3072; Becton Dickinson, Oxford, UK) with 200 μL of culture medium. The 96-well dishes were then placed into a humid incubator with an atmosphere of 5% CO_2, 95% air for 24 h prior to use. After this 24 h period, the medium from the 96-well plates was removed and replaced by 200 μL of the test medium. At that time, the 96-well plates were placed in an incubator again for 24 h. A succinyl dehydrogenase assay (MTT) was performed on the dishes after 24 h of incubation (i.e. 48 h after the beginning of the experiment). The medium was removed and immediately replaced with 100 μL per well of a 0.5% solution of 3-(4,5-dimethylthiazol-2-yl)-2,(-diphenyl tetrazolium bromide) in the medium (Sigma, Chemical Company, St Louis, MO, USA). After incubation for 2 h at 37 °C, the supernatant was

discarded, and the formazan crystals were solubilized with dimethylsulfoxide (100 μL per well; Sigma Chemical Co.). The absorbance of each 96-well dish was determined using an automatic microplate spectrophotometer (E 960, Bioblock, Strasbourg, F) at 550 nm. The absorbance of the wells containing the same medium was averaged as a single measurement and calculated against the control medium.

Additional MTT study according to ISO 10993-5 standards

In order to compare Resilon and Epiphany, an additional test was performed according to the ISO standard 10993-5 (1994). Ten samples of Epiphany were prepared according to the manufacturer's recommendations. The samples were covered with glass slide cover slips and stored for 1 day in an incubator prior to sterilization with UV rays. The samples were stored for 30 days in 1-mL culture medium. The culture medium was the same as used in the previous root model: minimum essential medium (Gibco) with 10% foetal calf serum (Biowhittaker) supplemented with penicillin 100 IU mL^{-1}, streptomycin 100 μg mL^{-1}. According to ISO standards, the ratio between the sample surface and the medium volume was 0.5 cm^2 mL^{-1}. The medium was renewed every day to simulate periodontal ligament clearance. The medium that remained in contact with the samples for 24 h after 1, 2, 7 and 30 days was called the test medium and was used to measure the cytotoxicity with the MTT assay as previously described. Resilon cones were also tested under the same conditions.

Statistical analysis

To compare the sealers within the root model, one ANOVA for each exposure time, followed by a Duncan's multiple range test was performed at the 95% confidence level. To compare Resilon and Epiphany according to the ISO standards, one ANOVA was performed for each exposure time at the 95% confidence level.

Results

Root model

At 7 and 30 days, no statistically significant difference was found amongst the sealers (NS): none of them was cytotoxic (Table 2). At 1 day, Resilon + Epiphany (53%) was more cytotoxic than Roekoseal + gutta-percha (12%) that was in turn more cytotoxic than Sealite + gutta-percha (3%; $P < 0.001$). At 2 days, Roekoseal + gutta-percha (6%) and Sealite + gutta-percha (1%) were not statistically different, however, both of them were less cytotoxic than Resilon + Epiphany (31%; $P = 0.001$).

Table 2 Cytotoxicity of three root canal sealers determined by the root model technique. The sealers in the same column, with the same superscript letter are not statistically different. Cytotoxicity (percentage of cell mortality)

Root canal sealer	1 day $P = 0.001$	2 days $P = 0.001$	7 days NS	30 days NS
Resilon + Epiphany	53 ± 2[a]	31 ± 2[a]	0 ± 1	0 ± 2
Roekoseal + gutta -percha	12 ± 5[b]	6 ± 4[b]	0 ± 4	0 ± 3
Sealite + gutta-percha	3 ± 4[c]	1 ± 4[b]	0 ± 4	0 ± 1

Table 3 Cytotoxicity of Resilon and Epiphany determined with MTT test according to ISO 10993-5 standards (1994). Cytotoxicity (percentage of cell mortality)

Root canal sealer	1 day ($P = 0.001$)	2 days ($P = 0.001$)	7 days ($P = 0.001$)	30 days (NS)
Resilon	9 ± 2	0 ± 2	0 ± 2	0 ± 2
Epiphany	94 ± 7	40 ± 6	5 ± 2	0 ± 3

Additional MTT study according to ISO standards 10993-5

At 30 days, no statistically significant difference was found between Resilon and Epiphany (NS), none of them was cytotoxic (Table 3). Epiphany was statistically more cytotoxic than Resilon at 1, 2 and 7 days ($P = 0.001$). The cytotoxicity of Epiphany at 7 days was lower than 20% and was therefore negligible.

Discussion

Evaluation of the biological effects of dental materials is of great importance because their systematic side-effects may take years before to appear (Geurtsen 2003). The ISO standards 10993-5 (1994) for testing biocompatibility of medical devices are applicable to all such devices with no particular specificity for the dental devices. Therefore, the test designs may be clinically irrelevant for dental practice and protocols closer to clinical conditions should be developed. The results of this work confirm a previous study that demonstrated that the cytotoxicity of root canal sealers evaluated according to ISO standards is much higher than that

recorded by a method simulating the clinical situation (Camps & About 2003). For example, despite an equivalent liquid volume, the 1 day cytotoxicity of Epiphany was 96% with ISO standards and only 57% with the root model, as the cytotoxicity of Resilon is negligible. In line with this concept, the 1 day cytotoxicity of Roekoseal reported here corresponds to the results of Dartar Öztan *et al.* (2003) but the volume of culture medium used in the root model of the present study was four times smaller. As cytotoxicity varies in a dose-dependent manner, variations in the protocol always lead to variations in the outcome of the study (Abou Ashieh *et al.* 1998). Aware of this limitation, the writers of ISO 10993 (1994) standards recommended that specialist services should be used to interpret the results.

Resilon + Epiphany was the most cytotoxic filling materials at 1 and 2 days ($P = 0.001$). This difference between the filling materials disappeared with time and was no longer found at 7 and 30 days. Therefore, it can be concluded that Resilon + Epiphany is no longer cytotoxic at 7 days, as is Roekoseal Automix and Sealite that had a good osseous biocompatibility (Pertot *et al.* 1992) and a long history of clinical use. The results of the ISO standards study revealed that the Resilon cones themselves were noncytotoxic but that Epiphany was cytotoxic at 1, 2 and 7 days. Therefore it can be concluded that the cytotoxicity of Resilon + Epiphany observed with the root model was due to Epiphany. The oxygen inhibition layer at the surface of any polymerising resin leaves an uncured monomer layer (Peutzfeld 1997). Anaerobic conditions also shorten the setting time of Epiphany from 1 week to 30 min (Nielsen *et al.* 2006). Therefore, the cytotoxicity demonstrated in this study is likely due to leaching of uncured monomers from the bulk of resin because Epiphany set under anaerobic conditions and no curing system leads to a 100% degree of conversion (Peutzfeld 1997). The dentine adhesive resins leach uncured monomers and other chemicals (Geurtsen *et al.* 1999) that may have cytotoxic interactive effects (Rathanasathien *et al.* 1995). In addition, a well-fitting single master Resilon cone leaves no contact between Epiphany and the culture medium in round canals. This may be different in oval-shaped canals or in case of apical transportation. The cytotoxicity of the endodontic sealers was performed on L929 because the cell type does not influence the outcome of the cytotoxicity studies (Huang *et al.* 2002). The resinous monomers have various effects on eukaryotic cells. They are known to be cytotoxic at concentrations ranging from 0.3 to 88 mmol L^{-1} according to the monomer structure (Yoshii 1997). In addition, they have deleterious effects even at sub-cytotoxic concentrations. They may reduce the mitochondrial activity of pulp macrophages (Rakich *et al.* 1998) and suppress interleukin-1 and tumour necrosis factor secretion (Rakich *et al.* 1999). They may also cause T lymphocyte immunosuppression (Jontell *et al.* 1995) and complement activation (Payne & Horbett 1987), thus playing an important role in inflammatory response. At noncytotoxic concentrations they also interfere with the functions of secretory cells such as odontoblast-like cells (About *et al.* 2005).

The decrease in cytotoxicity of the root canal sealers over time, after complete curing and elution, is a typical feature of cytotoxicity studies (Schwarze *et al.* 2002). The main component of the unreacted monomers leach during the first day (Ferracane 1994), thus, it is not surprising to find that the cytoxicity of Resilon + Epiphany decreases over time and is no longer noticeable after 7 days.

In restorative dentistry, a debate began some years ago, when direct pulp capping with resin was proposed (Cox *et al.* 1998) and opposed (Pameijer & Stanley 1998). In the present study, Resilon + Epiphany had the same cytotoxicity after 7 days as the two other sealers that have been used with success. The only concern may be the cytotoxicity of Epiphany itself in case of overfilling because of the larger surface of contact between the resin and the surrounding tissue.

Conclusions

Resilon + Epiphany was cytotoxic *ex vivo* for 2 days in a root model. After 2 days, Resilon + Epiphany was no more cytotoxic than two other filling materials used clinically with success. This temporary cytotoxicity is due to Epiphany. The material surface/medium ratio used in ISO standards is too high, making this method clinically irrelevant because cytotoxicity is a dose-dependent phenomenon. The root model is more clinically relevant but did not allow to differentiation between the cytotoxicity of Resilon and that of Epiphany. Therefore, both methods are useful to determine the cytotoxicity of root canal filling materials.

References

Abou Ashieh I, Camps J, Dejou J, Franquin JC (1998) Eugenol diffusion through dentin related to dentin hydraulic conductance. *Dental Materials* **14**, 229–36.

About I, Camps J, Burger AS, Mitsiadis TA, Butlet WT, Franquin JC (2005) Polymerized bonding agents and the differentiation *in vitro* of human pulp cells into odontoblast-like cells. *Dental Materials* **21**, 156–63.

Camps J, About I (2003) Cytotoxicity testing of endodontic sealers: a new method. *Journal of Endodontics* **29**, 583–6.

Cox CF, Hafez AA, Akimoto N, Otsuki M, Suzuki S, Tarim B (1998) Biocompatibility of primer, adhesive and resin composite systems on non-exposed and exposed pulps of non-human primate teeth. *American Journal of Dentistry* **11**, S55–63.

Dartar Öztan M, Yilmaz S, Kalayci A, Zaimoglu L (2003) A comparison of the *in vitro* cytotoxicity of two root canal sealers. *Journal of Oral Rehabilitation* **30**, 426–9.

De Wald JP (1997) The use of extracted teeth for in vitro bonding studies. A review of infection control considerations. *Dental Materials* **13**, 74–81.

Ferracane JL (1994) Elution of leachable components from composites. *Journal of Oral Rehabilitation* **21**, 441–52.

Gesi A, Raffaelli O, Goracci C, Pashley DH, Tay FR, Ferrari M (2005) Interfacial strength of Resilon and gutta-percha to intraradicular dentin. *Journal of Endodontics* **31**, 809–13.

Geurtsen W (2003) Toxicology of dental materials and 'clinical experience'. *Journal of Dental Research 2003* **82**, 500.

Geurtsen W, Spahl W, Müller K, Leyhausen G (1999) Aqueous extracts from dentin adhesives contain cytotoxic chemicals. *Journal Biomedical Material Research* **48**, 772–7.

Hiraishi N, Papacchini F, Loushine RJ et al. (2005) Shear bond strength of Resilon to a methacrylate-based root canal sealer. *International Endodontic Journal* **38**, 753–63.

Huang FM, Tai KW, Chou MY, Chang YC (2002) Cytotoxicity of resin, zinc oxide–eugenol, and calcium hydroxide-based root canal sealers on human periodontal ligament cells and permanent V79 cells. *International Endododontic Journal* **35**, 153–8.

ISO standard 10993-5 (1994) *Biological evaluation of medical devices. Tests for cytotoxicity: in vitro methods*. International Standard Organisation, Geneva, Switzerland.

Jontell M, Hanks CT, Bratel J, Bergenholtz G (1995) Effects of unpolymerized resin components on the function of accessory cells derived from the rat incisor pulp. *Journal of Dental Research* **74**, 1162–7.

Journal Officiel de la République Française (2004). *Loi n°* 2004–800 du 6 août 2004 relative à la bioéthique, Paris, France.

Nielsen BA, Beeler WJ, Vy C, Baumgartner JC (2006) Setting times of Resilon and other sealers in aerobic and anaerobic environments. *Journal of Endodontics* **32**, 130–2.

Pameijer CH, Stanley HR (1998) The disastrous effect of the 'total etch' technique in vital pulp capping in primates. *American Journal of Dentistry* **11**, S45–54.

Payne MS, Horbett TA (1987) Complement activation by hydroxyethtylmethacryalte–ethymethacrylate copolymers. *Journal Biomedical Material Research* **26**, 473–7.

Pertot W, Camps J, Remusat M, Proust JP (1992) In vivo comparison of two root canal sealers implanted into the mandibular bone of rabbit. *Oral Surgery, Oral Medicine, Oral Pathology* **73**, 613–20.

Peutzfeld A (1997) Resin composites in dentistry: the monomer system. *European Journal Oral Science* **105**, 97–116.

Rakich DR, Wataha JC, Lefevre CA, Weller RN (1998) Effects of dentin bonding agents on macrophage mitochondrial activity. *Journal of Endodontics* **24**, 528–31.

Rakich DR, Wataha JC, Lefevre CA, Weller RN (1999) Effects of dentin bonding agents on the secretion of inflammatory mediators from macrophages. *Journal of Endodontics* **25**, 114–7.

Rathanasathien S, Wataha J, Hanks CT, Dennison JB (1995) Cytotoxic interactive effects of dentin bonding components on mouse fibroblasts. *Journal of Dental Research* **74**, 1602–6.

Schwarze T, Leyhausen G, Geurtsen W (2002) Long-term cytocompatibility of various endodontic sealers using a new root canal model. *Journal of Endodontics* **28**, 749–53.

Shilder H (1967) Filling root canal in three dimensions. *Dental Clinics North America* **11**, 723–44.

Shilder H (1974) Cleaning and shaping the root canal. *Dental Clinics North America* **18**, 269–96.

Shipper G, Orstavik D, Teixeira FB, Trope M (2004) An evaluation of microbial leakage in roots filled with a thermoplastic synthetic polymer-based root canal filling material (Resilon). *Journal of Endodontics* **30**, 342–7.

Shipper G, Teixeira FB, Arnold RR, Trope M (2005) Periapical inflammation after coronal microbial inoculation of dog roots filled with gutta-percha or resilon. *Journal of Endodontics* **31**, 91–6.

Teixeira FB, Teixeira EC, Thompson JY, Trope M (2004) Fracture resistance of roots endodontically treated with a new resin filling material. *Journal American Dental Association* **135**, 646–52.

Yoshii E (1997) Cytotoxic effects of acrylates and methacrylates: relationships of monomer structure and cytotoxicity. *Journal Biomedical Material Research* **37**, 517–24.