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Cytotoxicity of 5 endodontic sealers on L929 cell line and human dental pulp cells

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Abstract

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Aim To investigate the cytotoxicity of five root canal sealers on L929 mouse fibroblasts and primary human dental pulp cells.

Methodology Cylindrical specimens of AH Plus (Dentsply De Trey GmbH, Konstanz, Germany), RoekoSeal (Coltène Whaledent, Langenau, Germany), EndoREZ (Ultradent Products Inc., South Jordan, UT, USA), Epiphany (Pentron Clinical Technologies, LLCC, Wallingford, CT, USA) and Activ GP (Brasseller Inc., USA, Savannah, GA, USA) were kept at 37 °C in a humidified atmosphere of 5% CO_2 for thrice the length of the setting time given by the manufacturer. Extraction of specimens was performed after setting in cell growth medium for 1, 4 and 7 days. Undiluted, 50% and 25% diluted eluates were incubated with cultured cells for 24 and 72 h. Cytotoxicity was assessed using MTS colorimetric bioassay. Kruskal–Wallis test and *post hoc* Dunn's multiple comparison test were used to compare the sealers and diluted/undiluted eluates in terms of cell viability (% of control). Friedman test and *post hoc* Dunn's multiple comparison test were performed to compare extraction periods. Wilcoxon test was utilized in comparing 24- and 72-h readings.

Results Undiluted 1-day eluate of Activ GP was significantly more cytotoxic than all other sealers ($P < 0.0001$). Undiluted 4- and 7-day eluates of Epiphany and Activ GP were significantly more cytotoxic than the other three sealers ($P < 0.0001$). Diluted eluates of Activ GP and Epiphany were generally less toxic than the undiluted ones. The cytotoxicity of Epiphany significantly increased as the extraction period increased ($P < 0.0001$). Epiphany became more toxic with time of exposure to cells. No or minimal cytotoxicity was observed with RoekoSeal, AH Plus and EndoREZ.

Conclusions The sealers exhibited varying degrees of cytotoxicity dependent on their chemical composition.

Keywords: Activ GP, biocompatibility, cytotoxicity, EndoREZ, Epiphany, human dental pulp cell, root canal sealer.

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Introduction

Root canal sealers are in close relation with surrounding soft and hard tissues for long periods of time. In the root canal, sealer may be exposed to tissue fluid and exudates, so that elutable substances, degradation products or corrosion products might gain access to the periradicular tissues (Geurtsen & Leyhausen 1997, Geurtsen 2001, Huang *et al.* 2004). Furthermore, although sealers should be confined within the root canal, their inadvertent extrusion into the periradicular tissues may occur (Gluskin 2005). Thus, toxic sealers and their leaching components can potentially cause tissue injury because of their cellular toxicity and may participate in the development of periapical

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inflammation or the continued existence of a pre-existing periapical lesion, thereby delaying healing and adversely affecting the outcome of treatment. Thus, the biocompatibility of a sealer is of crucial importance (Barbosa *et al.* 1993, Geurtsen 2001, Schwarze *et al.* 2002b).

Biocompatibility of a sealer is determined by various parameters, such as composition and leachable components, setting characteristics, stability of the set sealer and the contact area between the sealer and the adjacent soft and hard tissues (Barbosa *et al.* 1993, Schwarze *et al.* 2002b). For that reason, each sealer must have its biological properties comprehensively evaluated by various laboratory and *in vivo* tests before clinical use to minimize the incidence of local and/or systemic adverse effects (Geurtsen & Leyhausen 1997).

Laboratory assays of cytotoxicity are the initial screening tests in assessing the biocompatibility of a dental material (Dahl 2005). The cytotoxic responses of cells in various culture media vary depending on the chemical composition of the sealer in a given experimental set-up (Geurtsen & Leyhausen 1997, Ersev *et al.* 1999). Compositions of the sealers commonly used in endodontics are based on zinc oxide eugenol, calcium hydroxide, mineral trioxide aggregate, glass–ionomer or polymers, such as epoxy resins, polydimethylsiloxane and methacrylates (Geurtsen 2001). The purpose of this laboratory study was to evaluate the cytotoxic effects of various types of contemporary root canal sealers on an established cell line and primary human dental pulp cells at different intervals after setting.

Materials and methods

Root canal sealers

The sealers and their compositions are given in Table 1.

Cell cultures

Isolation of dental pulp cells

Dental pulp tissue from noncarious permanent teeth that were submitted to endodontic treatment because of prosthodontic reasons at Yeditepe University, Faculty of Dentistry, Istanbul, Turkey, was harvested. The donor patients were between 25 and 37 years old and signed informed consent forms. Following extirpation, dental pulp tissues were delivered to the cell culture laboratory in isolation medium containing DMEM-F12 medium (Sigma, St. Louis, MO, USA) supplemented with 10% foetal bovine serum (Biological Industries, Kibbutz Beit Haemek, Israel), 2 mmol L^{-1} L-glutamine (Biological Industries) and 1% PSA (penicillin, streptomycin and amphotericin solution, Biological Industries). Upon arrival to the laboratory, the tissues were sliced into small pieces using sterile scalpels and seeded into 6-well tissue culture plates (5 or 6 pieces for each well). Two millilitres of cell growth medium containing α-modified

Table 1 Composition and manufacturer of the tested sealers

Sealer	Composition	Setting time	Manufacturer
AH Plus	Paste A: Bisphenol-A epoxy resin, Bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments Paste B: Dibenzyldiamine, aminoadamantane, tricyclodecane-diamine, calcium tungstate, zirconium oxide, silica, silicone oil	8 h	Dentsply De Trey GmbH, Konstanz, Germany
RoekoSeal	Polydimethysiloxane, silicone oil, paraffin-base oil, hexachloroplatinic acid (catalytic agent), zirconium dioxide	45–50 min	Coltène Whaledent, Langenau, Germany
EndoREZ	30% UDMA, zinc oxide, barium sulphate, resins, pigments	15–20 min	Ultradent Products Inc., South Jordan, UT, USA
Epiphany	Resins: Bis-GMA, UDMA, PEGDMA, EBADMA; Fillers: barium sulphate, bismuth oxychloride, calcium hydroxide, silica, silane-treated bariumboraluminosilicate glass (with a small amount of aluminium oxide); Colouring pigment; Dual-cured initiators: cumene hydroperoxide, thiosinamine, champhorquinone: Stabilizer: butylated hydroxytoluene (2,6-di-tert-butyl-4-methylephenol)	45 min	Pentron Clinical Technologies, LLCC, Wallingford, CT, USA
Activ GP	Poly(acrylic acid), tartaric acid, barium aluminosilicate, glass powder dried poly(acrylic acid)	10 min	Brasseller Inc., USA, Savannah, GA, USA

Bis-GMA, bisphenol-A glycidyl methacrylate {2,2 -bis [4-(2-hydroxy-3-methacryloxy-propoxy)-phenyl]-propane}, UDMA, urethane dimethacrylate; EBPADMA, ethoxylated bisphenol-A dimethacrylate; PEGDMA, polyethylene glycol dimethacrylate.

Eagle's medium (α-MEM, Sigma) supplemented with 10% FBS, 2 mmol L^{-1} L-glutamine and 1% PSA was placed in each well, and the plates were incubated at 37 °C in a humid atmosphere of 5% CO_2. The medium was changed every day, and the spreading fibroblast-like cells from the tissue pieces reached 90% confluency within 10 days. For passaging, the cells were trypsinized (trypsin-EDTA solution, 1X, Sigma) for 2 min, centrifuged at 1000 rpm for 5 min and re-suspended in the growth media. Cells from the second passage were used for both the immunocytochemistry and toxicity assays.

Immunocytochemistry

Passage two of primary cells were seeded into 6-well plates at a concentration of 10^4 cells/well and incubated overnight. On the following day, the cells were fixed by putting 0.5 mL 2% paraformaldehyde into each well and incubating for 30 min at 4 °C. After fixation, the cells were rinsed with PBS and permeabilized by incubating with 0.1% Triton-X 100/PBS for 5 min. To avoid nonspecific binding of antibodies, the cells were incubated with 2% Goat Serum (Sigma) diluted in PBS for 20 min. Each well was incubated with anti-vimentin antibody overnight at $\mathring{4}C$ and washed twice for 5 min with PBS to remove unbound primary antibodies. After washing, secondary antibodies [goat polyclonal anti-rabbit IgG-Alexa 488, Invitrogen (Carlsbad, CA, USA)] were placed on the cells and incubated for 1 h and washed with PBS twice for 5 min with PBS again. Plates were observed under a fluorescence microscope. The cells presented classical fibroblast-like morphology. Immunocytochemistry analyses revealed that the dental pulp-derived cells were stained positive for vimentin and slightly positive for cytokeratin.

L929 cell line

Mouse fibroblast L929 (American type Culture Collection CCL 1) is an established cell line cloned from strain L. The parent strain was derived from normal subartaneous areolar and adipose tissue of a male C3H/An mouse. The L929 cell cultures were maintained in the same growth medium at 37 °C in a humid atmosphere of 5% CO_2 in the incubator.

Preparation of specimens and eluates

The sealers were prepared according to the manufacturers' instructions under aseptic conditions. Teflon rings (4 mm in diameter and 3 mm in height) covered on both sides with Mylar sheets were used to form cylindrical specimens of each sealer. To ensure proper setting, specimens were kept at 37 °C in a humid atmosphere of 5% CO_2 in an incubator thrice the length of the setting time given by the manufacturer (Eldeniz *et al.* 2007). After setting, extraction was performed by storing each specimen in 2.5 mL of cell growth medium at 37 °C in a humid atmosphere of 5% CO_2 in an incubator for 1, 4 and 7 days. Following filtration, diluted eluates in the growth medium to 50% and 25% were also prepared.

Cytotoxicity assay

L929 and dental pulp cells were seeded into 96-well plates (Corning, Elmira, NY, USA) at a concentration of 3×10^3 cells per well and incubated for 24 h. Then, undiluted, 50% or 25% diluted eluates were added to each experimental well. Cells without eluate treatment served as controls. Following incubation for 24 or 72 h, the cells were checked for the effect of the eluates on their viability (24- or 72-h readings) by using the MTS test (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA). In the MTS test, the cells in each well of the 96-well plate were incubated with 100 mL of growth medium and 20 mL of MTS reagent mixture for 4 h, and MTS absorbencies were measured at 490 nm using a spectrophotometer (Elx800; Bio-tek, Winooski, VT, USA) according to the manufacturer's instructions. Per cent cell viability was calculated by dividing the absorbance values of experimental wells by those of control wells and multiplying by 100. Cytotoxicity was also rated as severely cytotoxic for <30% cell viability, moderately for 30–59%, slightly for 60–90% or not cytotoxic for >90%.

Statistical analysis

Six replicates of each eluate for each reading were performed in two independent experiments. Statistical analysis was performed using the NCSS 2007 Package Program (NCSS, Kaysville, UT, USA). The Kruskal–Wallis test and *post hoc* Dunn's multiple comparison tests were used to compare the sealers and diluted or undiluted eluates. Friedman tests and *post hoc* Dunn's multiple comparison tests were performed to compare different extraction periods. The Wilcoxon test was utilized in comparing the 24- and 72-h readings. The statistical significance level was established at $P < .05$.

Results

Undiluted eluates of the sealers

Viability values of L929 and dental pulp cells treated with the undiluted eluates of the sealers expressed as the percentage of the control group are shown in Figs 1–4.

One-day eluate of Activ GP was significantly more cytotoxic ($P < 0.0001$) to both cells than all other sealers at both the 24- (Figs 1 and 3) and 72-h readings (Figs 2 and 4).

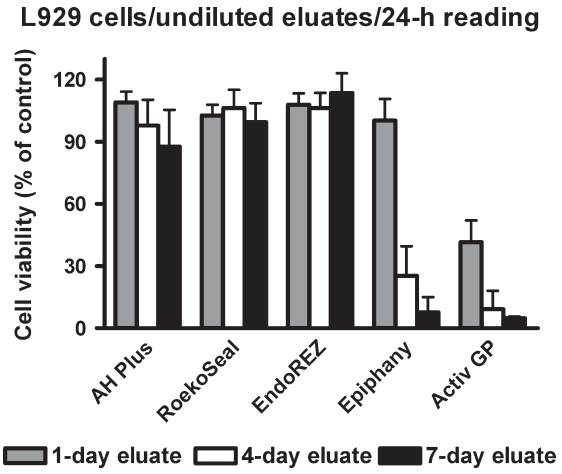

Figure 1 Viability values of L929 cells treated with the undiluted eluates of sealers at the 24-h reading, expressed as the percentage of the control group.

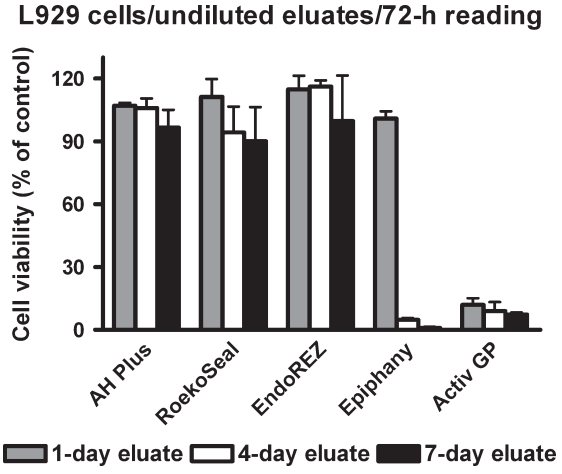

Figure 2 Viability values of L929 cells treated with the undiluted eluates of sealers at the 72-h reading, expressed as the percentage of the control group.

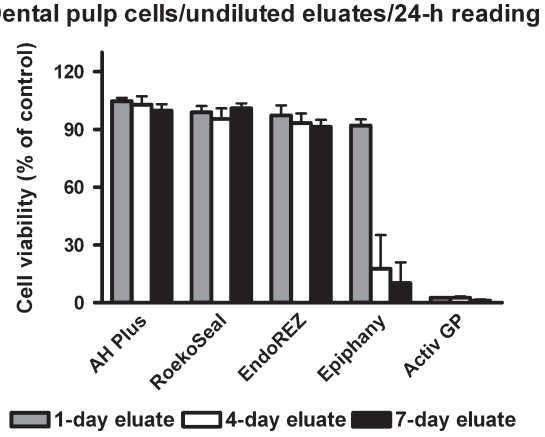

Figure 3 Viability values of dental pulp cells treated with the undiluted eluates of sealers at the 24-h reading, expressed as the percentage of the control group.

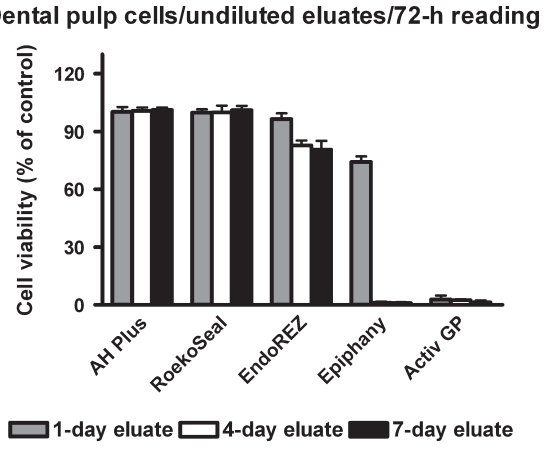

Figure 4 Viability values of dental pulp cells treated with the undiluted eluates of sealers at the 72-h reading, expressed as the percentage of the control group.

Four- and 7-day eluates of Epiphany and Activ GP were significantly more cytotoxic ($P < 0.0001$) to both cells than the other three sealers at 24- (Figs 1 and 3) and 72-h readings (Figs 2 and 4).

At the 24-h reading, Epiphany and Activ GP showed a significant increase ($P < 0.0001$) in cytotoxicity to both cells as the extraction period increased ($P < 0.005$) (Figs 1 and 3).

At the 72-h reading, Epiphany ($P < 0.0001$) showed a significant increase in cytotoxicity ($P < 0.01$) to L929 cells (Fig. 2) whilst EndoREZ and Epiphany showed a significant increase in cytotoxicity to dental pulp cells ($P < 0.01$) (Fig. 4).

Fifty per cent diluted eluates of the sealers

Viability values of L929 and dental pulp cells treated with the 50% diluted eluates of the sealers expressed as the percentage of the control group are shown in Figs 5–8.

The 7-day eluate of Epiphany was significantly more toxic to the L929 cells than the 7-day eluates of all other sealers at both readings ($P < 0.0001$) (Figs 5 and 6).

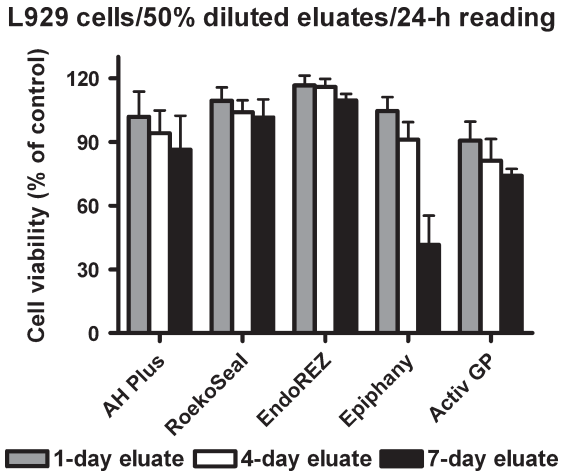

Figure 5 Viability values of L929 cells treated with 50% diluted eluates of sealers at the 24-h reading, expressed as the percentage of the control group.

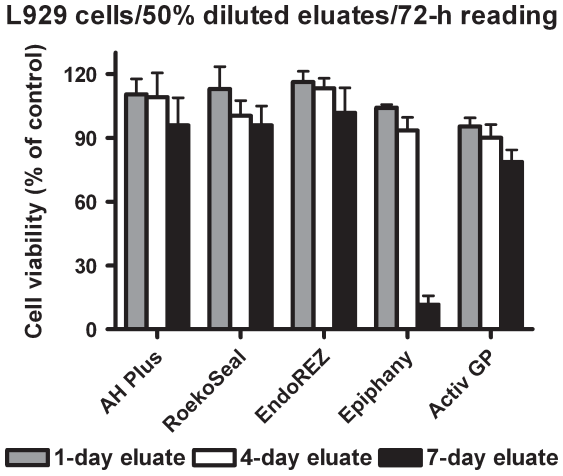

Figure 6 Viability values of L929 cells treated with 50% diluted eluates of sealers at the 72-h reading, expressed as the percentage of the control group.

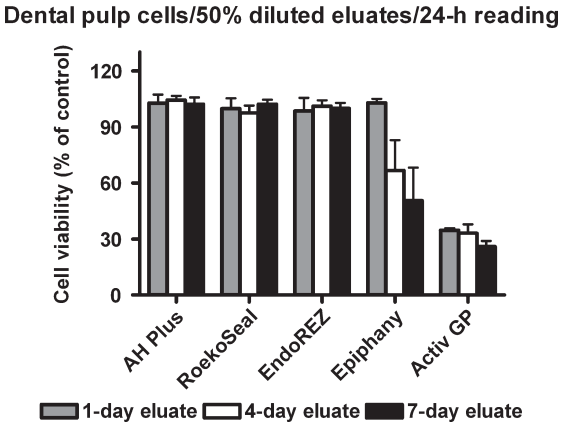

Figure 7 Viability values of dental pulp cells treated with 50% diluted eluates of sealers at the 24-h reading, expressed as the percentage of the control group.

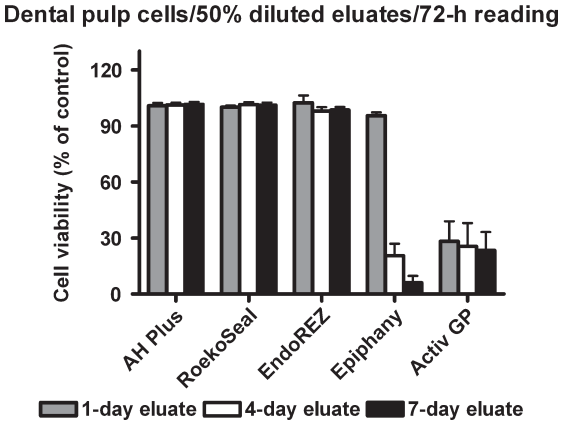

Figure 8 Viability values of dental pulp cells treated with 50% diluted eluates of sealers at the 72-h reading, expressed as the percentage of the control group.

The 1-day eluate of Activ GP ($P < 0.0001$), and 4- and 7-day eluates of Activ GP and Epiphany were significantly more toxic ($P < 0.0001$) to dental pulp cells than all other sealers at both readings (Figs 7 and 8).

Epiphany and Activ GP became significantly more toxic ($P < 0.01$) to both cells with an increasing extraction time at both readings (Figs 5–8).

Twenty-five per cent diluted eluates of the sealers

The 1-, 4- and 7-day eluates of all sealers were not cytotoxic to both the L929 and dental pulp cells at the 24- and 72-h readings.

Undiluted versus diluted eluates of the sealers

The undiluted eluate of Activ GP was significantly more cytotoxic to both cells and at all extraction periods than the diluted eluates at both readings ($P < 0.0001$).

The undiluted 4- and 7-day eluates of Epiphany were significantly more toxic to L929 cells than the diluted eluates at the 24-h reading ($P < 0.0001$). At the 72-h reading, a similar result was observed with the 4-day Epiphany eluates ($P < 0.0001$). Regarding the 7-day eluates of Epiphany, the 25% diluted eluate was significantly less toxic than the undiluted and 50% diluted eluate ($P < 0.0001$).

The undiluted 1-, 4- and 7-day eluates of Epiphany were significantly more toxic to dental pulp cells than the diluted eluates at both readings ($P < 0.005$). Similar significant differences between the undiluted and diluted eluates of EndoREZ were observed at both readings ($P < 0.05$), except between the 1-day eluates at the 24-h reading.

Twenty-four-hour versus 72-hour reading

The viability values of L929 cells at the 72-h reading were significantly lower than the ones at the 24-h reading in the groups of undiluted 1-day eluate of Activ GP ($P < 0.005$) and 4-day eluate of Epiphany ($P < 0.005$), and 50% diluted 7-day eluate of Epiphany ($P < 0.005$). Undiluted 4- and 7-day eluates of Activ GP and 7-day eluate of Epiphany were severely cytotoxic at both readings.

The cell viability values obtained at the 72-h reading for dental pulp cells in undiluted and 50% diluted Epiphany groups were significantly lower than the results at the 24-h reading ($P < 0.05$), except with the undiluted 7-day eluate. Undiluted 7-day eluate of Epiphany and 1-, 4- and 7-day eluates of Activ GP were severely cytotoxic at both readings. Undiluted 4- and 7-day eluates of EndoREZ yielded significantly lower cell viability values at the 72-h reading than at the 24-h reading ($P < 0.05$).

Discussion

AH Plus is an epoxy resin-based sealer and the manufacturer claims that this material no longer releases formaldehyde compared to AH 26. However, the moderate to severe cytotoxic effect of AH Plus immediately after mixing is well documented (Eldeniz *et al.* 2007, Merdad *et al.* 2007, Lodiene *et al.* 2008). This initial cytotoxicity has been attributed to a minimum formaldehyde release, from amines added to accelerate the epoxy polymerization (Cohen *et al.* 2000, Eldeniz *et al.* 2007, Merdad *et al.* 2007) which decreases after setting (Eldeniz *et al.* 2007, Merdad *et al.* 2007), or to the epoxy resin component (Cohen *et al.* 2000, Eldeniz *et al.* 2007). The eluates of set AH Plus were found to be slightly or not cytotoxic under the conditions of the present study.

RoekoSeal is a sealer based on silicone, which is a biocompatible material. Accordingly, many previous studies have shown that RoekoSeal was only slightly or not cytotoxic (Schwarze *et al.* 2002b, Oztan *et al.* 2003, Bouillaguet *et al.* 2006, Susini *et al.* 2006, Eldeniz *et al.* 2007, Lodiene *et al.* 2008). The findings of the present study are in agreement with these previous reports.

EndoREZ is a hydrophilic, dual-cured sealer containing zinc oxide, barium sulphate, resins and pigments in a matrix of urethane dimethacrylate (UDMA) resin and was found to be non cytotoxic in the present study.

Slight toxicity was observed in EndoREZ only with the 4- and 7-day undiluted eluates at the 72-h reading for dental pulp cells (Lodiene *et al.* 2008, Brackett *et al.* 2009). The discrepancy between the results of studies suggesting that this material is cytotoxic (Bouillaguet *et al.* 2006, Eldeniz *et al.* 2007, Ames *et al.* 2009, Al-Hiyasat *et al.* 2010) might be related to variations in experimental conditions, such as biological end-points, target cell type, cell material contact method, preparation of extracts or solid specimens and exposure time (Oztan *et al.* 2003).

Epiphany, RealSeal (Sybron Dental Specialties, Orange, CA, USA) and InnoEndo (Heraeus-Kulzer, Armonk, NY, USA) are brand names of dual-cured hydrophilic multi-methacrylate dental resin-based composite sealers with similar chemical compositions. High water sorption and solubility are common problems associated with the incorporation of hydrophilic resin monomers and have been demonstrated in the set Epiphany sealer (Versiani *et al.* 2006, Donnelly *et al.* 2007). Water sorption and diffusion lead to the plasticizing of the resinous matrix of polymerized composite resins, which in turn precedes and expedites the leaching of unreacted monomers, such as Bis-GMA, UDMA, ethoxylated bisphenol-A dimethacrylate (EBPADMA) and triethyleneglycol-di-methacrylate) (Sideridou & Achilias 2005, Versiani *et al.* 2006, Donnelly *et al.* 2007, Kim *et al.* 2010). The inherently high resin content of Epiphany is a mixture of Bis-GMA, UDMA, EBPADMA and hydrophilic difunctional methacrylates (Teixeira *et al.* 2004, Bouillaguet *et al.* 2006,

Xu *et al.* 2010). Bis-GMA, UDMA and dimethacrylates were reported to be highly cytotoxic (Hanks *et al.* 1991, Ratanasathien *et al.* 1995, Geurtsen *et al.* 1998), whereas EBPADMA is the most toxic of the group (Hanks *et al.* 1991). The chemical structure of the monomers was found to have a direct effect on the amount of eluted monomers, as well as the time needed for the elution (Sideridou & Achilias 2005). In the present study, undiluted 4- and 7-day eluates of Epiphany were found to be severely cytotoxic (Eldeniz *et al.* 2007, Lodiene *et al.* 2008, Camargo *et al.* 2009, Al-Hiyasat *et al.* 2010, Xu *et al.* 2010). The cytotoxicity of Epiphany also significantly increased as the extraction period and exposure time increased (Brackett *et al.* 2008, 2009, Heitman *et al.* 2008, Ames *et al.* 2009), and these results can be attributed to an increased exposure time of the cells to unreacted resin monomers and/or to the interactions between resin components (Ratanasathien *et al.* 1995, Bouillaguet *et al.* 2006). The observed cytotoxicity may also be caused by the leaching of uncured monomers as a result of incomplete polymerization of Epiphany under the conditions of the present study. Complete setting of this sealer is reported to vary from 30 min in an anaerobic environment to 7 days in the presence of air (Nielsen *et al.* 2006). Additionally, an unpolymerized monomer oxygen inhibition layer was observed on the surface (Rueggeberg & Margeson 1990, Nielsen *et al.* 2006, Versiani *et al.* 2006, Merdad *et al.* 2007), which has been implicated in increased resin toxicity (Caughman *et al.* 1991, Tang *et al.* 1999). Monomer to polymer conversion is an important aspect of the cellular toxicity of resins (Caughman *et al.* 1991). Incomplete polymerization of Epiphany has been detected, despite a post-curing time of as long as 2 weeks *in vitro* (Beriat *et al.* 2009). Sub-optimally polymerized resins have been shown to exhibit higher elution of monomers over time (Munksgaard *et al.* 2000). *In vitro* cytotoxicity of Epiphany, however, does not necessarily reflect the long-term risk because many factors, such as immune response and blood circulation, cannot be taken into account in laboratory conditions (Xu *et al.* 2010). Indeed, there are *in vivo* studies that have shown that Epiphany was biocompatible after implantation in rat connective tissue and has intraosseous biocompatibility (Onay *et al.* 2007, Garcia Lda *et al.* 2010). Moreover, the Epiphany/Resilon system was reported to present more favourable periapical tissue reactions than root fillings with gutta-percha and Sealapex or AH 26 in studies performed on dogs (Shipper *et al.* 2005, Leonardo *et al.* 2007). In addition, clinical outcome assessments yielded acceptable results of root canal treatments with the Resilon and Epiphany sealer (Cotton *et al.* 2008).

Inconsistent findings are reported on laboratory toxicity of the glass–ionomer-based root canal sealers, Ketac-Endo and Endion (Beltes *et al.* 1997, Telli *et al.* 1999, Willershausen *et al.* 2000, Schwarze *et al.* 2002a,b, Gorduysus *et al.* 2007). Activ GP is a glass–ionomer-based sealer recently marketed to be used in conjunction with gutta-percha cones coated with glass–ionomer. Donadio *et al.* (2009) reported that Activ GP was moderately toxic when freshly mixed and less toxic when set. In the present study, undiluted eluates of Activ GP were found to be severely cytotoxic, whereas 25% diluted eluates were noncytotoxic, suggesting that cytotoxicity is a dose-dependent phenomenon (Susini *et al.* 2006). Undiluted and 50% diluted eluates became more toxic as the extraction period increased; these results can be attributed to the possible release of cytotoxic substances from the disintegrating sealer (Beltes *et al.* 1997, Schwarze *et al.* 2002b), which is known to be highly water soluble (Schafer & Zandbiglari 2003).

However, it is important to keep in mind that the sealer is not completely exposed to fluids in clinical conditions, limiting the dissolution of toxic components (Schafer & Zandbiglari 2003). The cytotoxicity of glass–ionomer cements has also been attributed to the release of uncured acid from the material; again, which can be less effective *in vivo* as dentine is believed to play a role in buffering the acid (Donadio *et al.* 2009). Moreover, *in vivo* studies on glass–ionomer sealers indicated total healing following subcutaneous implantation in connective tissue of rats (Kolokuris *et al.* 1996), satisfactory histocompatibility in the apical area of root filled teeth in dogs (Leonardo *et al.* 1998) and acceptable endodontic outcomes (Friedman *et al.* 1995).

Large amounts of sealer used in laboratory studies result in extensive contact areas with the extraction medium or cells; however, direct contact between a root filling and the surrounding tissues is much less intense, and indirect interactions as a result of diffusion or perfusion are limited to apical foramina, accessory/lateral canals or dentine tubules (Schmalz *et al.* 2001, Schwarze *et al.* 2002b, Camps & About 2003, Susini *et al.* 2006). On the other hand, it must be kept in mind that extrusion of sealers is possible, especially when a periapical lesion alters the anatomy of the apex. In such a clinical scenario, the contact area between the sealer and the target cells, and in turn, the concentration of the leachable cytotoxic components on cells, may

increase greatly (Siqueira 2005). Cytotoxicity increases with concentration in laboratory studies (Camps & About 2003). *In vivo*, however, these leached substances can be cleared by the host, possibly resulting in less local inflammation (Donadio *et al.* 2009). Therefore, caution must be exercised when interpreting the results of laboratory studies. Cumulative laboratory and *in vivo* data are required to give a definite statement about the biocompatibility of root canal sealers.

Conclusion

No or minimal cytotoxicity was observed with Roeko-Seal, AH Plus and EndoREZ. Epiphany became more toxic with time of extraction and exposure to cells. Activ GP exerted a substantial cytotoxic effect. Concentration played a significant role in the cytotoxicity profiles of Epiphany and Activ GP. Nevertheless, caution must be exercised in extrapolating the results of this laboratory study into clinical conditions. More detailed *in vivo* research and long-term clinical assessments are needed to be able to judge the biocompatibility of these root canal sealers.

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