Effects of five different resin-based sealers on L929 and Saos-2 cell viability

Funda Kont Cobankara, Hasan Orucoglu[1], Hayriye Esra Ulker[2*], Cihan Yildirim[3], Muhammed Yalcin[4], Abdulkadir Sengun[5]

Departments of Endodontics, Faculty of Dentistry, Selcuk University, Konya, [1]Abant Izzet Baysal University, Bolu, [3]Gaziantep University, Gaziantep, Departments of Restorative Dentistry, Faculty of Dentistry, [2]Selcuk University, Konya, [4]Inonu University, Malatya, [5]Kirikkale University, Kirikkale, Turkey

ABSTRACT

The aim of this study was to evaluate the cytotoxic effects of five different resin-based root canal sealers: EndoREZ, Epiphany SE, EZ-Fill, MMSeal and AHPlus. Set materials were extracted in culture medium and cytotoxicity was determined in two cell lines, human osteosarcoma cell line (Saos-2) and mouse skin fibroblast cells (L929). The cells were incubated in contact with elutes for 24 h. The cell mitochondrial activity was evaluated by the methylthiazole tetrazolium assay. Results with demonstrated that all sealers showed a reduced vital cell number in comparison with the control group ($P < 0.05$). For L929 cells, the ranking of the most to the least toxic material was: EZ-Fill (12.0%) = EndoREZ (12.1%) = AHPlus (12.4%) > MMSeal (44%) = Epiphany SE (46.2 %). For Saos-2 cells revealed that cell survival with extracts of EndoREZ, Epiphany SE, EZ-Fill, MMSeal and AHPlus was 33.9%, 32.9 %, 33.1%, 35.3% and 34.6%, respectively, all tested sealers showed moderate cytotoxicity. Based on the results obtained from the present study, all tested resin-based sealers appear to show toxicity potential to both cells in spite of different toxicity degrees. Therefore, better sealers need to be developed with acceptable biological properties for root canal filling.

Key words: Cytotoxicity, Mouse Skin Fibroblast Cell, Resin-Based Sealers, Sarcoma Osteogenic-2

Access this article online

Website: www.jpediatrdent.org

DOI: 10.4103/WKMP-0028.117443

Quick Response Code:

INTRODUCTION

Successful endodontic treatment depends on thorough debridement of the root canal(s) and total filling of the canal space with an inert material. In canal filling, the use of sealer in conjunction with a core material is considered imperative to produce the highest quality filling. The adequate combination of sealing ability and biocompatibility of a sealer is important for a favorable prognosis in treatment.[1]

During filling, sealers may be extruded into the periradicular area through a large apical foramen, accessory and lateral canals and dentinal tubules. In doing so, sealers come in direct contact with the periodontal ligament, alveolar bone, neurovascular structures and inflammatory cells that may be present when the periradicular tissues are under the influence of microbial contamination.[2] Consequently, the biocompatibility of endodontic, materials is important for the clinical success of endodontic treatment. Periapical alterations or irritations resulting from endodontic treatment also may be caused by adverse effects from substances liberated from materials, in addition to over-instrumentation and infection.[3] In such condition, they could cause not only degeneration of the tissue lying underneath the sealer, but could also delay wound healing.[4]

In general, the biocompatibility of sealer is assessed with a three-step approach. The first step is to screen a candidate material using a series of *in vitro* cytotoxicity assays. Second, if the material demonstrated is not a cytotoxic agent *in vitro*, it can be implanted in subcutaneous tissue and the local tissue reaction evaluated. Finally, the *in vivo* reaction of the target tissue with the material must be evaluated in animals or human beings.[5]

Using cell lines is a common method of *in vitro* testing of sealers, which allows for a reproducible result that can be controlled in a laboratory setting. *In vitro* testing also allows for the comparison between several materials using the same cells under the same conditions.[6] As previous tests with different methodologies have shown

***Address for correspondence**

Dr. Hayriye Esra Ulker, Selcuk University, Dis Hekimligi Fakultesi, Restoratif Dis Tedavisi A.D, Selcuklu, 42075, Konya, Turkey.
E-mail: botsalie@hotmail.com

variable results, it seems prudent to compare new and old sealers by standardized cell culture methods. The frequently used methylthiazole tetrazolium (MTT) (3, [4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay is a well-recognized method for assessing dental materials' non-specific cytotoxicity. This assay is considered a sensitive index to evaluate the cytotoxicity of dental materials.[7] The advantage of this method is simplicity, rapidity and repeatability and it does not require radioisotopes.[8]

Despite apparently satisfactory performance over many decades, gutta-percha and sealer filling techniques do not represent the universal ideal. Researchers continue to find alternatives that might better seal and mechanically reinforce compromised roots.[9] Until date, newer endodontic materials tend to be resins with very different compositions. With new filling materials being introduced into the market, testing and comparisons to commonly used materials are needed to ascertain, which is most ideal. Currently, many resin-based sealers can be found manufactured under different commercial names such as AHPlus, Epiphany, MMSeal, EZ-Fill and EndoREZ. Experts have stated that the biological basis for endodontic treatment is lagging behind the impressive technological advances in endodontics.[10] However, the majority of the materials lack even basic safety testing, in spite of the requirement that such testing take place before a material can be promoted for clinical use. Because data on comparative cytotoxicity of new resin sealers in different culture systems was scarce,[4,5] the decision was made to evaluate the cytotoxicity of these materials. The aim of the current *in vitro* study was to investigate the cytotoxic effects of five different resin-based sealers on human bone osteosarcoma cell line (Saos-2) and mouse skin fibroblast cells (L929) by MTT assay.

MATERIALS AND METHODS

Five different resin-based sealers were evaluated in this study: AHPlus (Dentsply, DeTrey, Konstanz, Germany), EndoREZ (Ultradent Products, South Jordan, UT, USA), Epiphany SE (Pentron Clinical Technologies, Wallingford, CT, USA), MMSeal (Micro-Mega, Besancon, Cedex, France) and EZ-Fill (Essential Dental Systems, S. Hackensack, NJ, USA). Specifications of the endodontic sealers tested are listed in Table 1. The sealers mixed according to manufacturer's instructions under aseptic conditions to prevent the risk of biological contamination during the cytotoxicity testing. Samples of the materials were prepared in four sterile Teflon rings (2 mm thick × 5 mm diameter). Samples of Epiphany were light cured for 40 s (780 mW/cm^2) from one side. After 6 h of setting tested materials at 100% relative humidity and 37°C, four samples per material were transferred 7 ml of culture medium and incubated in the dark for 24 h at 37°C to extract residual monomer or cytotoxic substances. The culture medium containing material extracts was sterile-filtered for use on the cell cultures.

Cytotoxicity testing

The L929 cells (Mouse C3/An connective tissue, 92123004, Şap Enstitüsü, Ankara, Turkey) were cultured in BME (Basal Medium Eagle) containing 10% newborn calf serum and 100 mg/mL penicillin/streptomycin at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Confluent cells were detached with 0.25% trypsin and seeded at a density of 25 × 10^3 into each well of a 96-well plate for 24 h at 37°C and 5% CO_2.

The Saos-2 cells (Human bone osteosarcoma, 02111901, Şap Enstitüsü, Ankara, Turkey) were cultured in dulbecco's modified eagle's medium containing 10% fetal bovine serum and 100 mg/mL penicillin/streptomycin at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Confluent cells were detached with 0.25% trypsin and seeded at a density of 25 × 10^3 into each well of a 96-well plate for 24 h at 37°C and 5% CO_2.

After 24 h of incubation, the culture medium was replaced with 200 μL of culture medium containing material extracts of endodontic sealers. The original culture medium served as control in this study.

Table 1: Resin based sealers tested

Sealers	Lot. number	Manufacturer	Composition according to manufacturer
Endo REZ	B36G2	Ultra dent Products Inc., South Jordan, UT, USA	30% UDMA, zinc oxide, barium sulfate, pigments
Epiphany SE	154986	Pentron Clinical Technologies, LLC, Wallingford, CT, USA	Mixture of EBPADMA, HEMA, BISGMA and acidic methacrylate resins, silane-treated barium borosilicate glasses*, silica, hydroxyapatite, Ca-Al-F-silicate, bismuth oxychloride with amines, peroxide, photoinitiator, stabilizers and pigment. *contains a small amount of aluminum oxide
EZ-Fill	071806	Essential Dental Systems, S.Hackensack, NJ, USA	Powder: Bismuth oxide, hexamethylenetetramine, silver powder. Gel: Bisphenol A diglycidyl ether
MMSeal	031406	Micro-Mega, Besancon, Cedex, France	Base: Epoxy oligomer resin (29%), ethylene glycol salicylate (18%), calcium phosphate (17%), bismuth subcarbonate (26%), zirconium oxide (10%). Catalyst: Poly aminobenzoate (31%), triethanolamine (5%), calcium phosphate (29%), bismuth subcarbonate (21%), zirconium oxide (10%), calcium oxide (4%)
AHPlus	0701001662	Dentsply, De Trey GmbH, Konstanz, Germany	Paste A: Epoxy resin, calcium tungstate, zirconium oxide, erosil, iron oxide. Paste B: Adamantane amine, N,N-dibenzoyl-5-oxanonane, TCD-diamine, calcium tungstate, zirconium oxide, erosil, silicone oil

SE: Standard error, UDMA: Urethane dimethacrylate, TCD: Tricyclodecane

The viability of cells exposed to material extracts was assessed by measuring succinic dehydrogenase activity. The succinic dehydrogenase activity has been shown to be reasonably representative of mitochondrial activity in the cells and reflects both cell number and activity.[11] The old medium was removed and cell cultures were rinsed with sterile phosphate-buffered saline and 0.5 ml of a freshly prepared MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) dye solution (Sigma, Taufkirchen, Germany) (0.5 mg/ml in BME) were added to each well. After incubation for 2 h (at 37°C, with 5% CO_2), the supernatant was removed and the intracellularly stored MTT formazan was solubilized in 200 µL dimethyl sulfoxide for 30 min at room temperature. The spectrophotometric absorbance was measured at 540 nm using a spectrophotometer (µQuant, Bio-Tek Instruments, Winooski, VT, USA). Twelve wells were used for each specimen. Testing was repeated twice to ensure reproducibility.

Statistical analysis

The one-way analysis of variance test was used to determine variance for each sealer and control group. The significant differences between groups were analyzed using the Tukey-HSD procedure, with the value of statistical significant being set at $P < 0.05$. All computations were made using Statistical Package for the Social Sciences (SPSS) 10.0 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS

The results of the MTT assays are given in Table 2 for the L929 and Saos-2 cells. Results with both L929 fibroblasts and Saos-2 cells demonstrated that all tested sealers reduced vital cell number in comparison with the control group ($P < 0.05$) [Figures 1 and 2]. For L929 cells revealed that cell survival with extracts of EndoREZ, Epiphany SE, EZ-Fill, MMSeal and AHPlus was 12.1 ± 0.9%, 46.2 ± 4.1%, 12.0 ± 0.8%, 44.0 ± 5.5% and 12.4 ± 1.1%, respectively. The ranking of the most to the least toxic material was: EZ-Fill = EndoREZ = AHPlus >> MMSeal = Epiphany SE. For Saos-2 cells revealed that cell survival with extracts of EndoREZ, Epiphany SE, EZ-Fill, MMSeal and AHPlus were 33.9 ± 3.8%, 32.9 ± 3.2 %, 33.1 ± 5.6 %, 35.3 ± 3.9 % and 34.6 ± 4.2 %, respectively. For Saos-2 cells, all tested sealers showed moderate cytotoxicity [Table 2].

DISCUSSION

In this study, the MTT assay was used to evaluate the cytotoxicity of five different resin-based sealers. MTT is a water-soluble, tetrazolium salt yielding a yellowish solution when prepared in media or salt solution. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenase enzymes in living cells. The test results reflect not only the cell number, but also the vital cell metabolic level. However, different methodologies or cell lines may affect the results, which can create problems in comparing the data from different sources.[8,12] Therefore, a set of standardized-assay procedures was established and used for all test materials evaluated in this study so that the results are comparable.

Either established permanent cell lines, such as L929 cells or primary cells can be used to test cytotoxicity of dental materials when employing *in vitro* methods of experimentation. The advantage of permanent cell lines is they will continue to grow as long as sustenance is available for them. Primary cells have a predetermined life span and will eventually reach a plateau of growth and then die, even if the conditions for growth are acceptable.[6] The criteria for evaluating the clinical success and failure of endodontic treatment rely heavily on radiographic interpretation of bone density.[13] In addition, because the materials tested would more likely come into contact with human bone cells *in vivo*, evaluation of the response of bone cells to filling materials is important. In this study, in order to more closely represent clinical conditions for cytotoxicity

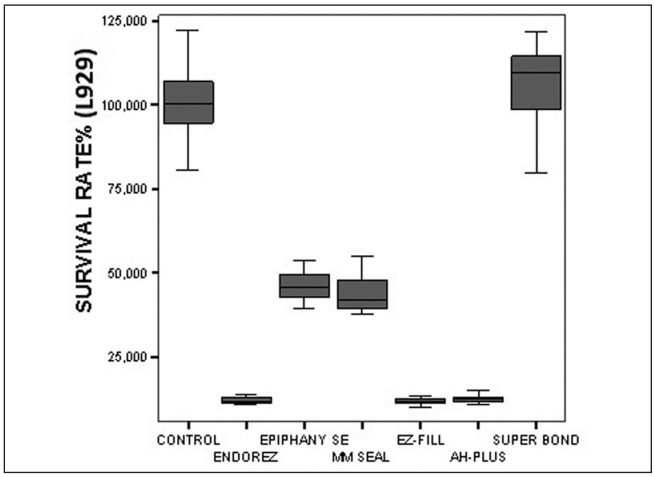

Figure 1: Cytotoxic effects of five different resin-based sealers on mouse skin fibroblast cells by methylthiazole tetrazolium assay. Percentage of absorbance at each elute was calculated and compared with that of control

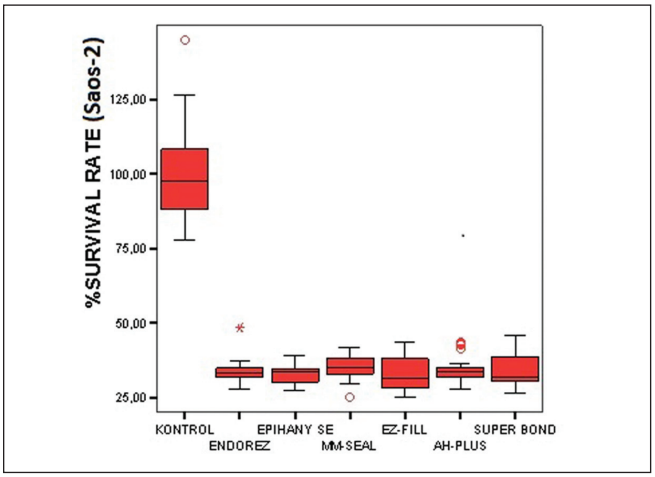

Figure 2: Cytotoxic effects of five different resin-based sealers on sarcoma osteogenic-2 cells by methylthiazole tetrazolium assay. Percentage of absorbance at each elute was calculated and compared with that of control

Table 2: Cytotoxic effect of five different resin based root canal sealers on L929 and Saos-2 cells expressed in percentage of viable cells compared with control in freshly samples. The rating of cytotoxicity for each sealer according to cell type indicated in the last columns

Cell viability % (mean±SD) (n=24)		
Sealer	**L929 cells**	**Saos-2 cells**
EndoREZ	12.1±0.9[b]	33.9±3.8[a]
EpiphanySE	46.2±4.1[a]	32.9±3.2[a]
EZ-fill	12.0±0.8[b]	33.1±5.6[a]
MMSeal	44.0±5.5[a]	35.3±3.9[a]
AHPlus	12.4±1.1[b]	34.6±4.2[a]

Within each set of columns, same superscript letters indicate that do not differ significantly (ANOVA, Tukey-HSD, α=0.05), SD: Standard deviation

evaluation of sealers, human bone osteosarcoma cells (Saos-2) were used, together with the mouse skin fibroblasts (L929) commonly used in cytotoxicity evaluations.

Determining both short- and long-term cytotoxicity of sealers might be important. The short-term toxicity of sealers may induce milder *in vivo* inflammatory responses in the periradicular area, which in turn may cause less postobturation symptoms, such as swelling and pain. Furthermore, the healing process likely may occur earlier, compared to conditions in which the cytotoxicity of a sealer lasted for longer periods of time.[14] However, a common finding observed with the traditional sealers is that sealer toxicity is significantly reduced or even eliminated after setting.[15-20] Overextended sealers represent chemical irritation as virtually all endodontic sealers are highly toxic when freshly prepared.[15] The cytotoxicity testing of freshly mixed sealers is clinically relevant as they are introduced into canals in the unset state. Huang *et al.*[21] stated that the difference in toxicity patterns at the various elution times for different sealers may be related to the degree of setting. Therefore, a sealer with a long setting time may show longer periods of cytotoxic effect. Consequently, early cytotoxic effect of the sealers seems more important than late cytotoxic effect. In the presented study, only freshly prepared sealers were used to investigate early cytotoxic effect by simulating the common clinical condition in which the sealer is extruded out of the space during canal filling.

The percentage of viable cells represents the level of cytotoxicity of the test materials. In this study, to determine the cytotoxicity, we compared the number of viable cells with the control cells. The choice of these five resin-based sealers was based on their currently increasing popularity and on the manufacturers' indications of their low toxicity. Our results indicate that although toxicity varied according to cell lines and sealers, immediately after mixing, all sealers used in the study had a cytotoxic effect. For L929 cells, EndoREZ, EZ-Fill, and AHPlus were strongly cytotoxic, whereas Epiphany SE and MMSeal were moderately cytotoxic. For Saos-2 cells, all tested sealers showed moderate cytotoxicity. The different response between L929 and Saos-2 cells to various sealers is difficult to explain and is probably due to differences in the origin of cells. Thus, we may recommend using different permanent cell lines and/or primary cells for screening the cytotoxic effects of sealers.

AHPlus is a two-component paste sealer, based on polymerization reaction of epoxy resin-amines. In the studies by Merdad *et al.*[22] and Lodiene *et al.*,[23] AHPlus showed cytotoxic effect immediately after mixing, but none or an undetectable amount 24 h after mixing. In a study by Azar *et al.*,[14] the freshly mixed AHPlus was cytotoxic, but its initial cytotoxicity was undetectable after 4 h. The short-term cytotoxicity of AHPlus has been attributed to release of formaldehyde[24] and to a lesser extent, to amines added to accelerate the polymerization reaction.[24,25]

EZ-Fill is composed of powder and gel and it has a hydrophobic nature and epoxy resin chemistry. Cohen *et al.*[26] showed that for AHPlus and EZ-Fill the amounts of formaldehyde release are 3.9 and 540 ppm, respectively. Therefore, cytotoxicity of a sealer might be not being based only on formaldehyde release because both sealers showed strong cytotoxic effect for L929 cells while they showed moderate cytotocity for Saos-2 in the present study. Bisphenol a diglycidyl ether was identified as a mutagenic component of resin-based materials, which may also be cytotoxic.[27,28] The gel component of EZ-Fill contains bisphenol a diglycidyl ether, according to the manufacturer. Therefore, bisphenol a diglycidyl ether also could be particularly responsible for EZ-Fill's cytotoxic effect.

EndoREZ is a hydrophilic, two-component, chemical-set material containing zinc oxide, barium sulfate, resins and pigments in a matrix of urethane dimethacrylate (UDMA) resin. In the present study, EndoREZ showed a strong toxic effect on L929 cells while it showed moderate cytotoxic effect on Saos-2 cells. In an *in vitro* study, Bouillaguet *et al.*[29] reported that EndoREZ presented significant cytotoxic risks when freshly mixed to Balb/c 3T3 mouse fibroblasts. In an animal study, subcutaneous implantation of EndoREZ in the connective tissue of rats caused mild to severe tissue reactions that subsided after 30 days. Also, Zmener[30] demonstrated that after subcutaneous implantation of fresh EndoREZ, components such as zinc and barium were present in tissues in direct contact with the sealer. Some of the early studies reported various degrees of toxicity from different concentrations of zinc and barium.[31,32] Also, in an *in vitro* study, Reichl *et al.* stated that the following range of increased toxicity was found: Hydroxyethyl-methacrylate (HEMA) < Triethyleneglycoldimethacrylate (TEGDMA) < UDMA < Bisphenolglycidyldimethacrylate (BisGMA). Therefore, UDMA in the EndoREZ structure, together with components such as zinc and barium, also could be responsible for the cytotoxic effect.[33]

Epiphany SE is a new version of the resin-based sealer Epiphany, in which the priming step is eliminated. Epiphany SE, containing a variety of compounds [Table 1], exhibited moderately cytotoxic potential to both cell lines. Chang *et al.*[34] have shown that HEMA alone can suppress cellular growth and cell cycle progression. Therefore, this result can be due to UDMA, HEMA, and BisGMA components in the structure of Epiphany SE. The toxicity of Epiphany has been also revealed in previous studies.[6,23,35]

The new resin sealer MMSeal has been developed recently. Our study showed that MMSeal has a moderately toxic potential to both cell lines. No published data is available about *in vitro* cytotoxicity of this sealer. Therefore, validation and extension of our results await further investigation.

Based on the results obtained from the present study, all tested resin-based sealers appear to have toxic potential to both cells in spite of different toxicity degree. Previous studies have reported a variety of cytotoxicity for these materials. Our results are in agreement with results of Ashraf *et al.*[36] and Silva *et al.*[37] who have reported that ah plus, epiphany and endorez exhibit similar toxicity in relation to cell survival. Therefore, manufacturers need to develop better sealers with acceptable biological properties for filling. In addition, because the results of *in vitro* assays may not be directly comparable with the *in vivo* conditions, where all healing parameters are functioning, long-term controlled and randomized success-and-failure clinical studies on patients are also necessary to assess *in vivo* responses when different sealers are extruded into the periradicular tissue. However, these types of studies can give dentists an opportunity to compare the relative toxicity of these filling materials.

REFERENCES

1. Briseño BM, Willershausen B. Root canal sealer cytotoxicity on human gingival fibroblasts: 2. Silicone- and resin-based sealers. J Endod 1991;17:537-40.
2. Al-Awadhi S, Spears R, Gutmann JL, Opperman LA. Cultured primary osteoblast viability and apoptosis in the presence of root canal sealers. J Endod 2004;30:527-33.
3. Murphy WM. The testing of endodontic materials *in vitro*. Int Endod J 1988;21:170-7.
4. Geurtsen W, Leinenbach F, Krage T, Leyhausen G. Cytotoxicity of four root canal sealers in permanent 3T3 cells and primary human periodontal ligament fibroblast cultures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:592-7.
5. Tai KW, Huang FM, Chang YC. Cytotoxic evaluation of root canal filling materials on primary human oral fibroblast cultures and a permanent hamster cell line. J Endod 2001;27:571-3.
6. Key JE, Rahemtulla FG, Eleazer PD. Cytotoxicity of a new root canal filling material on human gingival fibroblasts. J Endod 2006;32:756-8.
7. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
8. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. J Endod 2003;29:654-7.
9. Whitworth JM, Boursin EM. Dissolution of root canal sealer cements in volatile solvents. Int Endod J 2000;33:19-24.
10. Bergenholtz G, Spångberg L. Controversies in endodontics. Crit Rev Oral Biol Med 2004;15:99-114.
11. Wataha JC, Craig RG, Hanks CT. Precision of and new methods for testing *in vitro* alloy cytotoxicity. Dent Mater 1992;8:65-70.
12. Dahl JE, Frangou-Polyzois MJ, Polyzois GL. *In vitro* biocompatibility of denture relining materials. Gerodontology 2006;23:17-22.
13. Rodan GA. Introduction to bone biology. Bone 1992;13 Suppl 1:S3-6.
14. Azar NG, Heidari M, Bahrami ZS, Shokri F. *In vitro* cytotoxicity of a new epoxy resin root canal sealer. J Endod 2000;26:462-5.
15. Spangberg L, Pascon EA. The importance of material preparation for the expression of cytotoxicity during *in vitro* evaluation of biomaterials. J Endod 1988;14:247-50.
16. Barbosa SV, Araki K, Spångberg LS. Cytotoxicity of some modified root canal sealers and their leachable components. Oral Surg Oral Med Oral Pathol 1993;75:357-61.
17. Schmalz G, Hiller KA, Aslan-Dorter F. New developments in the filter test system for cytotoxicity testing. J Mater Sci Mater Med 1994;5:43-51.
18. Siqueira JF Jr. Aetiology of root canal treatment failure: Why well-treated teeth can fail. Int Endod J 2001;34:1-10.
19. Dahl JE. Toxicity of endodontic filling materials. Endod Top 2005;12:39-43.
20. Miletić I, Devcić N, Anić I, Borcić J, Karlović Z, Osmak M. The cytotoxicity of RoekoSeal and AH plus compared during different setting periods. J Endod 2005;31:307-9.
21. Huang FM, Tai KW, Chou MY, Chang YC. Cytotoxicity of resin-, zinc oxide-eugenol-, and calcium hydroxide-based root canal sealers on human periodontal ligament cells and permanent V79 cells. Int Endod J 2002;35:153-8.
22. Merdad K, Pascon AE, Kulkarni G, Santerre P, Friedman S. Short-term cytotoxicity assessment of components of the epiphany resin-percha obturating system by indirect and direct contact millipore filter assays. J Endod 2007;33:24-7.
23. Lodiene G, Morisbak E, Bruzell E, Ørstavik D. Toxicity evaluation of root canal sealers *in vitro*. Int Endod J 2008;41:72-7.
24. Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS. An *in vitro* study of the cytotoxicity of two root canal sealers. J Endod 2000;26:228-9.
25. Leonardo MR, Bezerra da Silva LA, Filho MT, Santana da Silva R. Release of formaldehyde by 4 endodontic sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:221-5.
26. Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS. Formaldehyde evaluation from endodontic materials. Oral Health 1998;88:37-9.
27. Canter DA, Zeiger E, Haworth S, Lawlor T, Mortelmans K, Speck W. Comparative mutagenicity of aliphatic epoxides in *Salmonella*. Mutat Res 1986;172:105-38.
28. Heil J, Reifferscheid G, Waldmann P, Leyhausen G, Geurtsen W. Genotoxicity of dental materials. Mutat Res 1996;368:181-94.
29. Bouillaguet S, Wataha JC, Lockwood PE, Galgano C, Golay A, Krejci I. Cytotoxicity and sealing properties of four classes of endodontic sealers evaluated by succinic dehydrogenase activity and confocal laser scanning microscopy. Eur J Oral Sci 2004;112:182-7.
30. Zmener O. Tissue response to a new methacrylate-based root canal sealer: Preliminary observations in the subcutaneous connective tissue of rats. J Endod 2004;30:348-51.
31. Smith JW, Leeb IJ, Torney DL. A comparison of calcium hydroxide and barium hydroxide as agents for inducing apical closure. J Endod 1984;10:64-70.
32. Meryon SD, Jakeman KJ. The effects *in vitro* of zinc released from dental restorative materials. Int Endod J 1985;18:191-8.
33. Reichl FX, Simon S, Esters M, Seiss M, Kehe K, Kleinsasser N, *et al*. Cytotoxicity of dental composite (co)monomers and the amalgam component Hg(2+) in human gingival fibroblasts. Arch Toxicol 2006;80:465-72.
34. Chang HH, Guo MK, Kasten FH, Chang MC, Huang GF, Wang YL, *et al*. Stimulation of glutathione depletion, ROS production and cell cycle arrest of dental pulp cells and gingival epithelial cells by HEMA. Biomaterials 2005;26:745-53.
35. Susini G, About I, Tran-Hung L, Camps J. Cytotoxicity of epiphany and resilon with a root model. Int Endod J 2006;39:940-4.
36. Ashraf H, Moradimajd N, Mozayeni MA, Dianat O, Mahjour F, Yadegari Z. Cytotoxicity evaluation of three resin-based sealers on an L929 cell line. Dent Res J (Isfahan) 2012;9:549-53.
37. Silva EJ, Santos CC, Zaia AA. Long-term cytotoxic effects of contemporary root canal sealers. J Appl Oral Sci 2013;21:43-7.

How to cite this article: Cobankara FK, Orucoglu H, Ulker HE, Yildirim C, Yalcin M, *et al*. Effects of five different resin-based sealers on L929 and Saos-2 cell viability. J Pediatr Dent 2013;1:37-41.
Source of Support: Nil. **Conflict of Interest:** None declared.