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ORIGINAL ARTICLE

Cytotoxicity evaluation of zinc oxide-eugenol and non-eugenol cements using different fibroblast cell lines

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Abstract

Objectives: Despite being commonly used as temporary cements in dentistry, there is a lack of studies regarding the cytotoxicity of zinc oxide-eugenol (ZOE) and zinc oxide non-eugenol (ZONE) cements. In addition, cytotoxicity evaluation of the materials often involves animal-based cells. Therefore, in this study, a cytotoxicity evaluation of commercially available ZOE and ZONE cements was carried out using both animal and human-based cells. ***Materials and methods.*** The extraction or dilution of the extraction from four commercially available cements (two zinc oxide-eugenol and two zinc oxide non-eugenol) was tested for cytotoxicity, using three different cells and a water-soluble treatzolium salt assay. The results were confirmed using a confocal laser microscope following calcein AM and ethidium homodimer-1 staining. ***Results.*** The results showed that there was a significant difference in cell viability depending on which cell was used, even when the same material was tested. Generally, L929 showed relatively low cell viability with a low EC50 (effective concentration of extracts that caused 50% of cell viability compared to the control) value compared to both HGF-1 and hTERT-hNOF. Such results were also confirmed by a confocal laser microscope. ***Conclusions.*** Careful consideration on interpreting the results for cytotoxicity evaluation of ZOE and ZONE cements is needed when different cells are used.

Key Words: *biocompatibility, cytotoxicity, temporary cements, fibroblasts*

Introduction

Zinc oxide-eugenol (ZOE) cement has been widely used in dentistry since the 1890s, being used for tasks such as temporary restoration and temporary cementation for several reasons, including: easy handling, excellent cavity sealing ability, bactericidal properties and a sedative effect on sensitive teeth [1–3]. Despite many of these advantages, ZOE cements are also known to have an inhibitory effect on resin bonding. Hence, zinc oxide non-eugenol (ZONE) cement has been developed and used in such cases, where it has replaced eugenol with various substitutes as an alternative material [4,5].

Biocompatibility evaluation of dental materials is now an essential step in order for them to be used on patients and *in vitro* cytotoxicity evaluation using the cell culture technique has been a useful method of assessment due to its economical and productive advantages [6–8]. However, previous researches on ZOE and ZONE cements have focused on the mechanical and chemical properties of materials [2,9–11], with a lack of studies related to the cytotoxicity of ZOE cement and especially ZONE cements. Also, many of these studies were carried out using animal-based fibroblast cell lines such as L929 (mouse fibroblast), BHK21/C13 (baby hamster kidney fibroblast) and V79 (Chinese hamster lung fibroblast) [12,13], which showed a relatively high level of cytotoxicity in the ZOE cements.

It is well known from previous studies that the results of cytotoxicity could be dramatically different according to the cell lines used, especially between animal-based cell lines and human-based primary

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Table I. Summary of zinc oxide-eugenol and zinc oxide non-eugenol cements used in this study.

Name	Code	Type	Batch No.	Manufacturer
RelyX Temp E	RTE	ZOE	456562	3M ESPE, Germany
IRM	IRM	ZOE	110202	Dentsply, Caulk, USA
RelyX Temp NE	RTNE	ZONE	465730	3M ESPE, Germany
EsTemp NE	ETNE	ZONE	ET11046	Spident, Korea

ZOE, Zinc oxide-eugenol; ZONE, zinc oxide non-eugenol.

cells [14–16]. Hence, in this study, cytotoxicity evaluations of various ZOE and ZONE products were carried out using both animal-based cell line L929 and the human cell line of human gingival fibroblast. Also, an immortalized form of human gingival fibroblast cell (hTERT-hNOF), introduced in a previous study [17], was used to compare the results of cytotoxicity.

Materials and methods

Cells, cell culture and cell proliferation rate

Three different fibroblastic cells were used in this study; mouse fibroblast (L929), human gingival fibroblast (HGF-1) and immortalized human gingival fibroblast (hTERT-hNOF). Both the L929 cell line (NCTC clone 929) and the human gingival fibroblast (HGF-1) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The immortalized human gingival fibroblast was provided by the Department of Oral Pathology, Oral Cancer Research Institute, Yonsei University College of Dentistry, Seoul, where gingival fibroblasts were primarily cultured from healthy human adults followed by transfection with puromycin-resistant retroviral vector plpc-hTERT (Clonetech Laboratories, Mountain View, CA, USA). This resulted in immortalized hTERT transfected normal human oral fibroblast (hTERT-hNOF). It has been confirmed for sub-culturing beyond the 70th passage without signs of replicative senescence and feasibility of biocompatibility evaluation [17].

Each cell was cultured in an appropriate culture medium with 10% foetal bovine serum (FBS, Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (Invitrogen, Grand Island, NY, USA) at 37°C in a fully humidified atmosphere of 5% CO_2. HGF-1 was kept in a low passage of below 10 throughout the experiments.

To assess and compare the proliferation rate of each cell, for the general characteristics and the interpretation of cell viability results following a cytotoxicity test, BrdU incorporation during the DNA synthesis was measured on each cell. Following 24 h of cell culture, 100 μL of 100 mM BrdU solution (Roche Applied Science, Mannheim, Germany) was placed on each well containing the different cells and they were then incubated for 24 h. The cells' DNA were then denatured and incubated with anti-BrdU conjugated with peroxidase for an additional 90 min. Finally, the solution was reacted with tetramethylbenzidine for 30 min and the optical absorbance was read at 370 nm using an ELISA reader (BioTek Instruments, Winooski, VT, USA).

ZOE/ZONE cements and extraction

Various commercially available cements (two ZOE and two ZONE cements) were used in this experiment and are summarized in Table I All samples were checked for expiration dates and stored under manufacturers' recommended conditions throughout the experiment.

Extracts from each type of cement were prepared according to the international standard and adaptation from a previous study [18–20]. Briefly, each type of cement was mixed according to the manufacturers' instructions and then weighed inside a sterilized glass bottle. The fresh serum-free culture medium was added into each bottle according to the extraction ratio set by international standards: 1 mL per 0.2 g of the sample [18]. Each type of cement was then extracted for 24 h at 37°C.

Cytotoxicity test

A cytotoxicity test was carried out according to international standards [19,21]. Briefly, 1×10^4 of each cell was cultured in a standard 96 well (SPL, Gyeongi-do, Korea), in 100 μL of culture medium for 24 h. Following removal of the culture medium, and washing with Dulbecco's phosphate buffer saline (DPBS, Gibco), extractions from each type of cement (as described earlier), or dilutions of extractions using serum-free media (50%, 25%, 12.5% and 6.25%), were then placed on each cell. Extractions and dilutions of extractions were left for 24 h and cell viability was measured using a water-soluble tetrazolium (WST) salt assay (Ez-Cytox, Daeillab, Seoul, Korea). The control cells were exposed to an appropriate fresh culture medium for 24 h, which was then kept at 37°C for 24 h and the cell viability results for each test group were expressed as the percentage of optical density value of each test sample to each control sample following the WST assay.

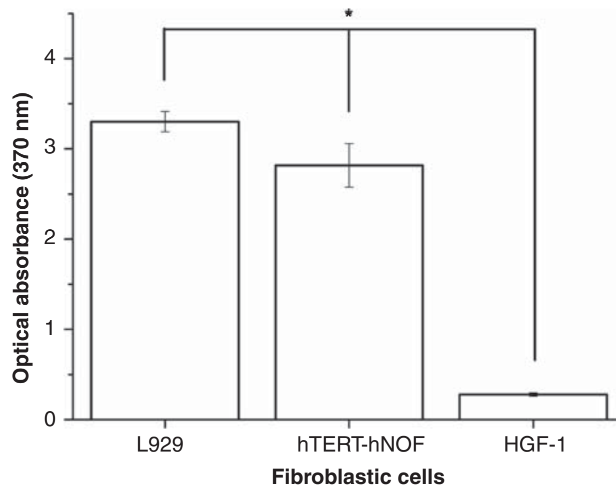

Figure 1. Proliferation rate of each cell measured using the BrdU assay, where value of optical absorbance is proportional to proliferation rate. *Significantly different to each other, $p < 0.05$.

Cytotoxicity results were also confirmed by exposing each type of cell to extraction of EsTemp NE (Spident, Incheon, Korea) for 1, 3, 6, 12 and 24 h, followed by staining with calcein AM and ethidium homodimer-1 (Molecular Probes, Eugene, OR, USA) for observation under a confocal laser microscope (LSM700, Carl Zeiss, Thornwood, NY, USA). Intense green fluorescence was observed from live cells and bright red fluorescence was observed from dead cells.

Statistics

Statistical analysis was carried out using a three-way ANOVA and a Tukey method was adapted as a post-hoc test, where significance was declared at $p < 0.05$. The SPSS PASW 18.0 program (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Results

Proliferation rate

The proliferation rate of each type of cell was measured using the BrdU assay (Figure 1) where a high value of optical absorbance indicates the high rate of cellular proliferation. The results showed that the proliferation rate of each cell was statistically different ($p < 0.05$) between all groups. L929 had the highest cellular proliferation rate, followed by hTERT-hNOF cells, whereas HGF-1 cells had the lowest cellular proliferation rate, whereby the optical absorbance value was dramatically smaller compared to the other two cells.

Cytotoxicity test

The cytotoxicity test results using WST assay are shown in Figure 2. In terms of undiluted extract of each type of cement (Figure 2A), generally low level of cell viability (below 40%) was evident in all cell lines exposed to extract each test sample, except the HGF-1 exposed to the extraction of ETNE (cell viability of 66.19%). In terms of 50% diluted extract of ETNE (Figure 2B), both hTERT-hNOF and HGF-1 showed a significantly higher level ($p < 0.05$) of cell viability (78.70% and 101.28%, respectively) compared to L929 (10.62%), which was the general trend for all of the other results. The results of cell viability were significantly different between each of the fibroblast cell types ($p < 0.05$) for most of the samples, but no statistical difference in cell viability was identified between hTERT-hNOF and HGF-1 in some of the test groups (RTE, RTNE, ETNE in Figure 2C, RTE, IRM, ETNE in Figure 2D and IRM, ETNE in Figure 2E). IRM was shown to be the most cytotoxic when 25% (Figure 2C) and 12.5% (Figure 2D) of extraction was exposed to L929 ($p <0.05$), but the effective concentration of extracts for IRM that caused 50% of cell viability compared to the control (EC50, horizontal dotted line in Figure 2) was also different between cell lines. EC50 for IRM using L929 was relatively low, between 12.5% and 6.25% (Figures 2D and E), whereas using HGF or hTERT-hNOF was relatively high, between 50% and 25% (Figures 2B and C).

The result of the cytotoxicity test using WST assay was confirmed by a confocal laser microscope following calcein AM and ethidium homodimer-1 staining. The results are shown in Figure 3, where live cells appear as green and dead cells as red. It was noted that a significant number of dead cells (red) for L929 appeared after 12 h of exposure to the undiluted extraction of ETNE (Figure 3Q), whereas both HGF-1 and hTERT-hNOF cells appeared to be relatively viable (green) in that time period (Figures 3E and K). In terms of cellular morphology, both HGF-1 and hTERT-hNOF were observed to have maintained their elongated shape following 6 h of exposure to an undiluted extraction of ETNE (Figures 3D and J), whereas L929 appeared to be rounded

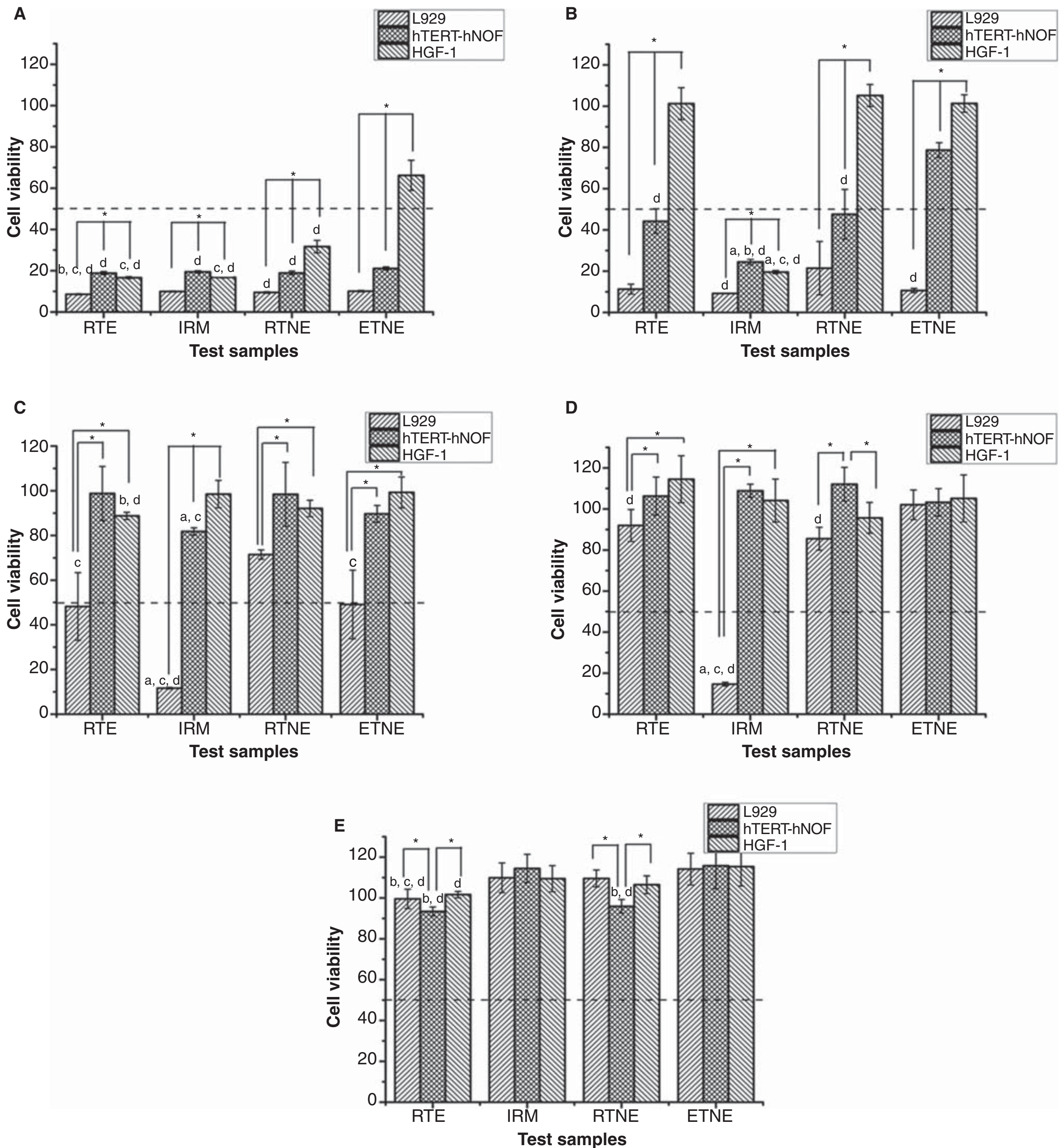

Figure 2. Cell viability of each cell following exposure to extraction or dilution of extraction for different ZOE and ZONE cements. (A) 100% extraction, (B) 50% extraction, (C) 25% extraction, (D) 12.5% extraction, (E) 6.25% extraction. Each lower case letter indicates significantly lower ($p < 0.05$) cell viability within the same cell line compared to (a) RTE, (b) IRM, (c) RTNE, and (d) ETNE. Horizontal dotted line represents the effective concentration of extracts that caused 50% of cell viability compared to control (EC50); * indicates significantly different cell viability between cell lines, within the same tested material, $p < 0.05$.

following the same period of exposure time to an undiluted extraction of ETNE (Figure 3P).

Discussion

A cytotoxicity test using the cell culture technique is one of the important steps in the biocompatibility evaluation of dental materials, which is cheaper in comparison to the *in vivo* tests and can be tested on a large number of materials at the same time [22,23].

In this study, cytotoxicity evaluation on commercially available zinc oxide-eugenol (ZOE) and zinc oxide non-eugenol (ZONE) cements was carried out. Both ZOE and ZONE cements showed varying degrees of cytotoxicity, where the results using

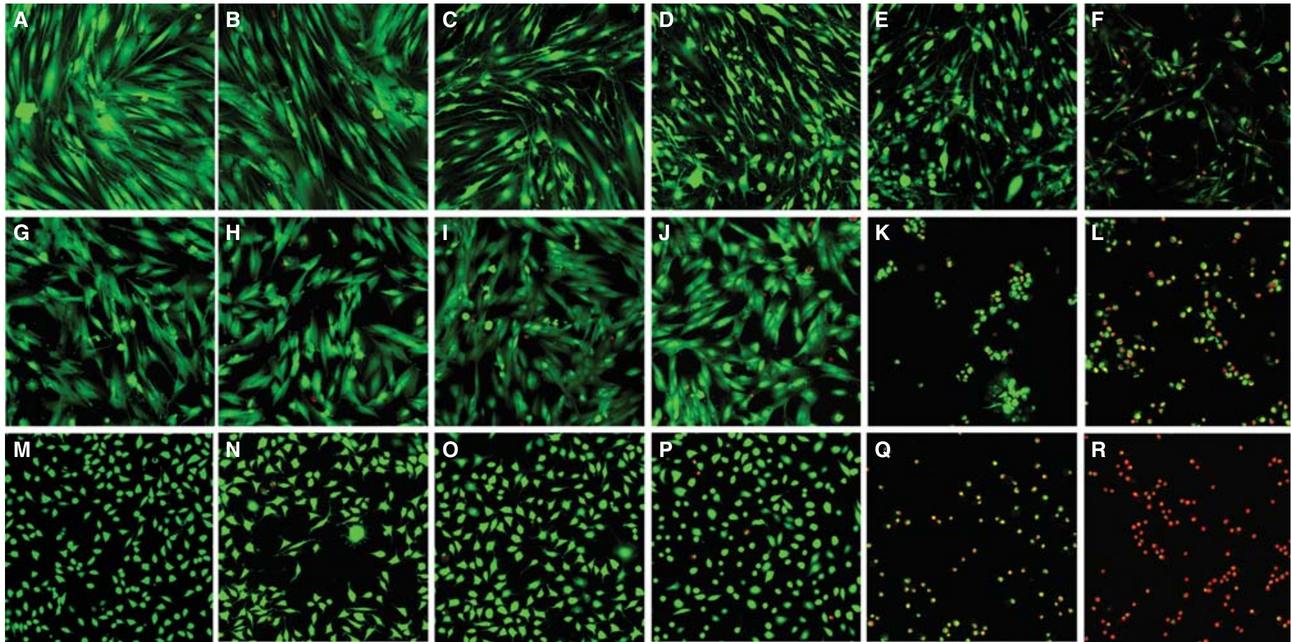

Figure 3. Confocal laser microscopy imaging following calcein AM and ethidium homodimer-1 staining of HGF-1 (A–F), hTERT-hNOF (G–L) and L929 (M–R) after different duration of exposure for extraction of EsTemp NE. Cells were exposed to the extraction for 0 h (A, G, M), 1 h (B, H, N), 3 h (C, I, O), 6 h (D, J, P), 12 h (E, K, Q) and 24 h (F, L, R). Live cells were stained green and dead cells were stained red.

L929 cells were in agreement with many previous studies [13,24].

The cytotoxicity of ZOE and ZONE cements are known to be caused by the ingredient of each product and the release of components such as eugenol from ZOE cements [25,26] and the zinc ion that is released from both ZOE and ZONE cements [25,27]. However, such previous studies used animal-based cell lines [12,13] and, thus, two human-based cell lines, HGF-1 and immortalized gingival fibroblast (hTERT-hNOF), were used in this study to compare the results with an animal-based mouse fibroblast (L929).

The L929 cell line is a mouse fibroblast that is commonly used for the cytotoxicity evaluation of dental materials [28–30]. It has a heteroploid chromosome pattern and the use of such cells has often been questioned due to the possibility of different reactions against external materials, whereas this may result in more cytotoxic results compared to when human cells are used [16,31]. The HGF-1 cell used in this study was a primary culture that was taken from a healthy human patient and deposited in a commercial cell line bank of ATCC [32]. Such diploid cells will have different mitochondrial function and are known to be more tolerant to toxic products compared to other aneuploidy cells [31,33].

In addition to two cell lines, L929 and HGF-1, this study used immortalized human gingival fibroblast (hTERT-hNOF) which was formed by an hTERT transfection on the primary culture of a healthy adult [17]. Such cells maintained diploid status throughout proliferation and sub-culturing, even beyond the 70th passage [17].

First, the BrdU assay was carried out on each cell before the cytotoxicity evaluation for measurement of individual proliferation rates in order to understand the general characteristics of each cell line and interpret the cytotoxicity test results. The results showed that L929 had the highest proliferation rate, followed by hTERT-hNOF and HGF-1. However, despite having the highest proliferation rate, less of L929 generally survived compared to HGF-1 or hTERT-hNOF, when the cells were exposed to the extraction or dilution of extraction from ZOE and ZONE cements. Hence, the proliferation rate does not seem to affect cell viability following exposure to the extraction or dilution of extraction from the cements. Also, it was noted that hTERT-hNOF had a relatively high proliferation rate, similar to L929, and such characteristics will be ideal in terms of economical and productive views of culturing cells for a cytotoxicity evaluation compared to HGF-1.

In terms of cytotoxicity evaluation, variable levels of cytotoxicity were evident with different ZOE and ZONE cements. However, in general there was no significant difference between the cytotoxicity of ZOE and ZONE cements (for example, between RTE and RTNE which were from the same manufacturer). Although further research may be needed, the majority of cytotoxicity may be due to zinc ion present in both ZOE and ZONE cements, which is more easily extracted from the mixed compound compared to eugenol that is only present in ZOE cements [25–27].

In terms of cell lines, L929 was less tolerant to extraction or dilution of extraction compared to HGF-1, showing a high level of cytotoxicity as expected. These results were in agreement with previous studies that used animal-based fibroblast cell lines such as those from the kidney of a baby hamster (BHK21/C13) and a lung of a Chinese hamster (V79) [12,13].

However, the cytottoxicity test result using hTERT-hNOF was similar to HGF-1. Also, the EC50 of IRM using the L929 cell was very low compared to the EC50 for IRM using both hTERT-hNOF and HGF-1.

The result was also confirmed using confocal laser microscope imaging of the cells following calcein AM and ethidium homodimer-1 staining. Following exposure to an undiluted extraction of ETNE, the majority of cell deaths for L929 cells in 12 h was observed and compared to the relatively higher number of cells that survived during the same period of exposure for HGF-1 and hTERT-hNOF. Additionally, a change in cellular morphology from elongated to rounded shape was noted comparably early for L929 following exposure to undiluted extraction of ETNE, when compared to both HGF-1 and hTERT-hNOF. Such results again confirmed the different tolerance of each cell to the extraction of dental materials and this type of test will be useful for future cytotoxicity evaluations of dental or other biomaterials that are in short contact with the human body (less than 24 h), and where the choice of cells will also need to be considered.

International standards state in their ISO that cell lines other than those that are established or commercially available may be used for a cytotoxicity evaluation if they can lead to more relevant results [19]. The results of this study indicate that there were differences in cytotoxicity test results when animal-based cells were used, compared to when human-based cells were used for the test of cytotoxicity in ZOE and ZONE cements. Also, many previous studies indicated that there were differences in cytotoxicity test results between animal and human-based cells for other types of dental materials, such as root canal sealers [14].

Although animal-based cell lines may be useful in terms of easy availability, and therefore screening purposes, cytotoxicity evaluation using human cells derived directly from clinically relevant tissue will no doubt be more clinically relevant [14,15]. However, such primary cells from humans have the disadvantage of a limited lifespan with replicative senescence and are, therefore, not ideal in terms of economical and reproducible results. Hence, in this study, an additional cell line of hTERT-hNOF was used. Such cell lines have shown relatively similar cytotoxicity evaluation results to HGF-1 over L929, and they have also exhibited a high rate of proliferation with the capability of sub-culturing for a high number of passages.

Therefore, in this study, it was shown that there was a difference in cytotoxicity evaluation results when an animal-based cell line was used compared to when a human-based cell line was used. Future studies comparing the cytotoxicity results of different cell lines in correlation with *in vivo* or clinical biocompatibility evaluations will be useful in terms of considering cell lines for more clinically relevant results. Despite these limitations, the results of the cytotoxicity evaluation for zinc oxide-eugenol and zinc oxide non-eugenol cements were clearly different between animal-based cell line and human-based primary cells. Hence, careful consideration must be given to the selection of cell lines when testing these or other dental materials.

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References

[1] Markowitz K, Moynihan M, Liu MS, Kim S. Biologic properties of eugenol and zinc oxide-eugenol - a Clinically oriented review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1992;73:729–37.

[2] White KC, Cox CF, Kanka J 3rd, Dixon DL, Farmer JB, Snuggs HM. Pulpal response to adhesive resin systems applied to acid-etched vital dentin: damp versus dry primer application. Quintessence Int 1994;25:259–68.

[3] He LH, Purton DG, Swain MV. A suitable base material for composite resin restorations: zinc oxide eugenol. J Dent 2010; 38:290–5.

[4] Hill EE. Dental cements for definitive luting: a review and practical clinical considerations. Dent Clin North Am 2007; 51:643–58, vi.

[5] Barkin ME, Boyd JP, Cohen S. Acute allergic reaction to eugenol. Oral Surg Oral Med Oral Pathol 1984;57:441–2.

[6] Schmalz G. Concepts in biocompatibility testing of dental restorative materials. Clin Oral Investig 1997;1:154–62.

[7] Hanks CT, Wataha JC, Sun ZL. *In vitro* models of biocompatibility: a review. Dent Mater 1996;12:186–93.

[8] Wataha JC. Principles of biocompatibility for dental practitioners. J Prosthet Dent 2001;86:203–9.

[9] Fonseca RB, Martins LR, Quagliatto PS, Soares CJ. Influence of provisional cements on ultimate bond strength of indirect composite restorations to dentin. J Adhes Dent 2005;7:225–30.

[10] Watanabe EK, Yamashita A, Imai M, Yatani H, Suzuki K. Temporary cement remnants as an adhesion inhibiting factor in the interface between resin cements and bovine dentin. Int J Prosthodont 1997;10:440–52.

[11] Woody TL, Davis RD. The effect of eugenol-containing and eugenol-free temporary cements on microleakage in resin bonded restorations. Oper Dent 1992;17:175–80.

[12] 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.

[13] Koulaouzidou EA, Papazisis KT, Economides NA, Beltes P, Kortsaris AH. Antiproliferative effect of mineral trioxide aggregate, zinc oxide-eugenol cement, and glass-ionomer cement against three fibroblastic cell lines. J Endod 2005;31:44–6.

[14] Eldeniz AU, Mustafa K, Orstavik D, Dahl JE. Cytotoxicity of new resin-, calcium hydroxide- and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines. Int Endod J 2007;40:329–37.

[15] Groth T, Falck P, Miethke RR. Cytotoxicity of biomaterials - Basic mechanisms and *in vitro* test methods: a review. Altern Lab Anim 1995;23:790–9.

[16] Pissiotis E, Spangberg LSW. Toxicity of pulpispad using 4 different cell-types. Int Endod J 1991;24:249–57.

[17] Illeperuma RP, Park YJ, Kim JM, Bae JY, Che ZM, Son HK, et al. Immortalized gingival fibroblasts as a cytotoxicity test model for dental materials. J Mater Sci Mater Med 2012;23:753–62.

[18] ISO documents. ISO 10993 – 12. Biological evaluation of medical devices — Part 12: sample preparation and reference materials. Geneva: International Standard; 2002.

[19] ISO documents. ISO 10993 - 5. Biological evaluation of medical devices — Part 5: tests for *in vitro* cytotoxicity. Geneva: International Standard; 2009.

[20] Kwon JS, Lee SB, Kim CK, Kim KN. Modified cytotoxicity evaluation of elastomeric impression materials while polymerizing with reduced exposure time. Acta Odontol Scand 2012; 70:597–602.

[21] ISO documents. ISO 10993 - 1. Biological evaluation of medical devices — Part 1: evaluation and testing within a risk management process. Geneva: International Standard; 2009.

[22] Vajrabhaya L, Sithisarn P. Multilayer and monolayer cell cultures in a cytotoxicity assay of root canal sealers. Int Endod J 1997;30:141–4.

[23] Williams DF. On the mechanisms of biocompatibility. Biomaterials 2008;29:2941–53.

[24] Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Cytotoxicity of four root end filling materials. J Endod 1995;21:489–92.

[25] Hume WR. An analysis of the release and the diffusion through dentin of eugenol from zinc oxide-eugenol mixtures. J Dent Res 1984;63:881–4.

[26] Koch MJ. Formaldehyde release from root-canal sealers: influence of method. Int Endod J 1999;32:10–16.

[27] Kaga M, Seale NS, Hanawa T, Ferracane JL, Waite DE, Okabe T. Cytotoxicity of amalgams, alloys, and their elements and phases. Dent Mater 1991;7:68–72.

[28] Chaves CDL, Machado AL, Carlos IZ, Giampaolo ET, Pavarina AC, Vergani CE. Cytotoxicity of monomers, plasticizer and degradation by-products released from dental hard chairside reline resins. Dent Mater 2010;26: 1017–23.

[29] Schedle A, Samorapoompichit P, Rauschfan XH, Franz A, Fureder W, Sperr WR, et al. Response of L-929 fibroblasts, human gingival fibroblasts, and human tissue mast-cells to various metal-cations. J Dent Res 1995;74:1513–20.

[30] Schmid-Schwap M, Franz A, Konig F, Bristela M, Lucas T, Piehslinger E, et al. Cytotoxicity of four categories of dental cements. Dent Mater 2009;25:360–8.

[31] Kasten FH, Felder SM, Gettleman L, Alchediak T. A model culture system with human gingival fibroblasts for evaluating the cytotoxicity of dental materials. In Vitro 1982;18: 650–60.

[32] Mcallister BS, Leeblundberg LMF, Javors MA, Olson MS. Bradykinin receptors and signal-transduction pathways in human fibroblasts - Integral role for extracellular calcium. Arch Biochem Biophys 1993;304:294–301.

[33] Alnazhan S, Spangberg L. Morphological cell changes due to chemical toxicity of a dental material - an Electron-- Microscopic Study on human periodontal-ligament fibroblasts and L929 cells. J Endodont 1990;16:129–34.