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Measuring toxicity levels in root canal sealers

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A recent study[1] performed at the Nordic Institute of Dental Materials (NIOM) in Haslum, Norway looking at the toxicity of root canal sealers *in vitro* has found that Epiphany (Pentron, USA), a multi-methacrylate resin-based sealer, is significantly more toxic to mouse fibroblasts (L-929) than silicone-based RoekoSeal (Coltène Whaledent, Germany) and single methacrylate-based EndoREZ tradent, USA).

In a filter diffusion test, freshly mixed Epiphany and AH Plus (Dentsply, Germany) were rated severely toxic while RoekoSeal and EndoEZ were non-toxic. One of the researchers, Dr Greta Lodiené from the Faculty of Odontology at Kaunas University of Medicine, Lithuania, spoke to *DPREurope* about what this means.

What are the potential consequences of using toxic root canal sealers?

Dr Lodiené: "Biological compatibility of root canal sealers is of importance as these materials frequently come into contact with periapical tissues. The tissue response to these materials may influence the final outcome of the root canal treatment[2]. A sealer should not hinder tissue repair, but aid or stimulate the reorganization of injured structures. Previous studies have shown that the biocompatibility of different material classes and products of root canal sealers vary considerably[3, 4]. "

Why was Epiphany found to be more toxic than the other materials?

"This result can be due to leaching of uncured monomers and setting conditions. Epiphany requires body temperature and total elimination of air contact to set. It sets in 30 minutes in an anaerobic environment, but in the presence of air, setting takes much longer and an uncured layer remains on the surface. This unpolymerized monomer oxygen inhibition layer can form on the surface of resins during polymerization. It significantly influences the biological properties of resins and has been implicated in increased toxicity"[4].

Are dental professionals aware of the toxicity of certain root canal sealers?

"As written in the instructions, all the materials are fully biocompatible. While biocompatibility is a desirable quality, extrapolations to the clinical situation must be made with caution, as the results of such *in vitro* toxicity tests may not correlate with the *in vivo* response. Our knowledge of the relative impact of the toxic influences exerted by the root canal sealer on the treatment outcome in Endodontics is limited, and more extensive studies on the effects on periapical tissues *in vivo* is necessary to place the cytotoxic reactions in perspective."

Are you planning further research in this field?

"Yes. We are further evaluating cytotoxicity of root canal sealers based on various polymers using more specific tests."

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Toxicity evaluation of root canal sealers *in vitro*

G. Lodienė[1,2], E. Morisbak[1], E. Bruzell[1] & D. Ørstavik[3]

[1]NIOM-Nordic Institute of Dental Materials, Haslum, Norway; [2]Department of Dental and Oral Pathology, Faculty of Odontology, Kaunas University of Medicine, Kaunas, Lithuania; and [3]Department of Endodontics, Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo, Institute of Clinical Dentistry, Oslo, Norway

Abstract

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Aim To compare the toxicity of methacrylate resin-based root canal sealers with sealers based on epoxy resin and silicone by two-well established cell culture methods.

Methodology Specimens of AH Plus, EndoREZ, RoekoSeal and Epiphany were prepared for direct contact in the Millipore filter diffusion test and as extracts in the MTT assay. Mouse fibroblasts (L929) were used as toxicity targets. Differences in cytotoxicity between fresh and set specimens and between the extracts of root canal sealers were determined by *t*-test ($P < 0.05$).

Results In the filter diffusion test, freshly mixed Epiphany and AH Plus were rated severely toxic and RoekoSeal and EndoREZ nontoxic. When set, Epiphany was moderately toxic, whereas AH Plus, RoekoSeal and EndoREZ were nontoxic. Epiphany was significantly more toxic than RoekoSeal and EndoREZ ($P < 0.05$). In the MTT assay with set specimens, Epiphany was rated severely toxic; AH Plus and RoekoSeal slightly toxic; and EndoREZ nontoxic. Epiphany was significantly more toxic than the other three materials in this test ($P < 0.001$).

Conclusion The multi-methacrylate resin-based (Epiphany) root canal sealer was significantly more toxic to L-929 cells than the silicone-based Roeko Seal and the single methacrylate-based EndoREZ root canal sealers. AH Plus showed intermediate toxicity.

Keywords: biocompatibility, cell culture, dental materials, dimethylthiazol diphenyltetrazolium bromide assay, endodontics, filter diffusion test.

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Introduction

Biological compatibility of root canal sealers is of importance as these materials frequently come into contact with periapical tissues. The tissue response to these materials may influence the final outcome of the root canal treatment (Waltimo *et al.* 2001). A sealer should not hinder tissue repair, but aid or stimulate the reorganization of injured structures. Previous studies have shown that the biocompatibility of different material classes and products of root canal sealers vary considerably (Huang *et al.* 2002, Zmener 2004).

The cytotoxicity of fresh epoxy resin-based sealers is well documented (Schweikl *et al.* 1998, Miletić *et al.* 2000, 2005, Huang *et al.* 2002). Such sealers have shown pronounced cytotoxic effects in direct contact test with cultured cells (Geurtsen & Leyhausen 1997). Epoxy resins exhibit severe cytotoxicity immediately after mixing and after time periods up to several hours after mixing. The cytotoxic effects are reduced to control levels after many days to several months (Miletić *et al.* 2005). However, only minor toxicity has been observed in implantation studies (Mittal *et al.* 1995). The latter observation was corroborated in a study placing sealers in direct contact with periapical

Correspondence: Greta Lodienė, Department of Dental and Oral Pathology, Faculty of Odontology, Kaunas University of Medicine, Eiveniu 2, LT-50009 Kaunas, Lithuania (Tel.: +370 37 326681; fax: +370 37 295237; e-mail: lodieneg@gmail.com).

tissues, which resulted in only limited inflammatory reactions (Leonardo *et al.* 1999a).

A sealer containing a single methacrylate (EndoREZ; Ultradent Products, Inc., South Jordan, UT, USA) has been described as well tolerated by connective tissues (Zmener 2004, Zmener & Pameijer 2004). The sealer has shown biocompatibility to periapical tissues in subhuman primates (Louw *et al.* 2001) and to rat cells and bone tissue (Zmener *et al.* 2005).

The silicone-based endodontic sealer, RoekoSeal (Coltène Whaledent, Langenau, Germany), has been reported to be noncytotoxic (Miletić *et al.* 2005). It appears to be less cytotoxic than sealers based on methacrylate, zinc oxide-eugenol and epoxy resin (Bouillaguet *et al.* 2004). However, another study rated the silicone-based sealer equal to an epoxy resin-based sealer in terms of cytotoxicity (Dartar Öztan *et al.* 2003).

A multi-methacrylate resin-based root filling material (Epiphany/RealSeal; Pentron, Clinical Technologies, LLC, Wallingford, CT, USA), used in combination with a plastic core (Resilon, Resilon Research, North Brandford, CT, USA), has been introduced as having a potential to challenge the use of gutta-percha for root filling (Shipper *et al.* 2004). Several studies have tested the cytotoxicity of this material, with highly variable results (Key *et al.* 2006, Susini *et al.* 2006). Sousa *et al.* (2006) observed bone formation and only minor inflammatory reactions in their investigation of the same multi-methacrylate sealer in guinea pigs.

As previous tests with different methodologies have shown variable results, it seems prudent to compare new and old sealers by standardized cell culture methods. The standardized filter diffusion test and the frequently used MTT [3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, both employing mouse fibroblasts as target cells, are well recognized methods for assessing dental materials' nonspecific cytotoxicity. The aim of this study was to apply these tests in a comparison of the cytotoxicity of the newer, methacrylate resin-based root canal sealers with two other materials based on epoxy and silicone, respectively.

Materials and methods

Root canal sealers

The tested root canal sealers were obtained from manufacturers and listed in Table 1.

Cell culture

Mouse fibroblast cells (L-929, American type Culture Collection CCL 1) were propagated in minimum essential medium (PAA Laboratories GmbH, Pasching, Austria), supplemented with 5% foetal bovine serum (Sigma, St Louis, MO, USA), 2 mmol L^{-1} L-glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (all Cambrex Bio Science, Verviers, Belgium) at 37 °C in air atmosphere containing 5% CO_2 and 95% relative humidity. Cells were passaged by treatment with 0.5 g L^{-1} trypsin, 0.2 g L^{-1} ethylene diamine tetracetic acid (Cambrex Bio Science) in phosphate-buffered saline solution. Cell viability and cell number were measured using the trypan blue exclusion method and Bürke counting chamber.

Specimens

The sealers were prepared according to the manufacturers' instructions under aseptic conditions, and specimens were formed as follows: the materials were placed in nonreactive plastic rings (5 mm diameter). One group of samples was placed in contact with cell monolayers immediately after mixing or setting (fresh condition). Dual-cure root canal sealer specimens were prepared without light- and with light-induced polymerization for 40 s using a dental polymerization lamp (VCL Complete, sds Kerr, Sybron Dental Specialities, Danbury, CT, USA). A second set of test samples was allowed to set for 24 h at 37 °C and 100% humidity (set condition). EndoREZ did not completely set even after 24 h incubation without additional light-induced polymerization, and was therefore tested only as light cured.

Filter diffusion test

The filter diffusion test was performed as described in international standards (International Standard ISO 7405 1997, International Standard ISO 10993-5 1999). Cells were grown for 48 h on cellulose-acetate filter (HAWP 04750; Millipore Corp., Bedford, MA, USA) in 60 × 15 mm culture dishes (Falcon; Beckton Dickinson Lab., Franklin Lakes, NJ, USA). The specimens containing the test material were placed directly on the filters. PTFE (polytetrafluozoethylene) (Guarniflon, Bergamo, Italy) discs served as negative controls and cellulose filters loaded with 35 μL of 4% phenol were used as positive controls. After 2 h incubation at 37 °C in air atmosphere containing 5% CO_2 and 95%