Cytotoxicity of a New Calcium Silicate Endodontic Sealer

Triantafyllia Vouzara, MSc, DDS, Garyfallia Dimosiari, MSc, DDS, Elisabeth A. Koulaouzidou, PhD, MSc, DDS, and Nikolaos Economides, Prof, PhD, MSc, DDS

Abstract

Introduction: Resin sealers with biocompatible and bioactive additives have been used in clinical practice. Recently, a calcium silicate root canal sealer was introduced under the name BioRoot RCS (Septodont, Saint Maur-des-Fossés, France). The aim of this study was to evaluate the effects of BioRoot RCS on cell survival and proliferation of cultured cells in parallel with an epoxy resin sealer with calcium phosphate and calcium oxide and a salicylate resin sealer with mineral trioxide aggregate filler. The tested hypothesis was that BioRoot RCS is significantly less cytotoxic than the other tested sealers. **Methods:** The experiments were performed on NIH/3T3 cells (American Type Culture Collection, Manassas, VA) grown as monolayer cultures at 37°C in atmosphere containing 5% CO_2 in air and 100% relative humidity. The sealers' extracts (24 hours and 1 week) were applied to cells at 1:1 and 1:2 dilutions. The effect was assessed by a modified sulforhodamine B staining assay in reference to controls after 24 and 72 hours of exposure. All experiments were performed at least twice in 6 replicates. Analysis of variance and post hoc comparison tests were used to evaluate the statistical significance of the results at a level of significance of $P = .05$. **Results:** BioRoot RCS was significantly less cytotoxic than the other 2 sealers. MTA-Fillapex (Angelus, Londrina, Brazil) and SimpliSeal (Discuss Dental, LLC, Calver City, CA) exhibited a similar antiproliferative profile with no statistically significant differences in all settings. **Conclusions:** BioRoot RCS showed quite a positive biological behavior. Further investigation is needed in order to clarify the mechanism and the components that contribute to the beneficial results observed. *(J Endod 2018;■:1–4)*

Key Words

BioRoot RCS, cytotoxicity, MTA-Fillapex, root canal sealers, SimpliSeal

Significance

The contact of endodontic sealers with periapical tissues may potentially cause tissue irritation, an adverse effect on the local repair mechanisms, and clinical failure. Cytotoxicity tests are necessary procedures in the preclinical assessment of endodontic sealers.

Endodontic sealers, based on their chemical synthesis, can be defined as zinc oxide eugenol, calcium hydroxide, glass ionomer, silicone, and resin sealers (1). The properties of an ideal root canal sealer have been described by Grossman (2). Biocompatibility is 1 of the basic characteristics that is taken into consideration.

Resin sealers present a variable degree of biocompatibility (3). SimpliSeal (Discuss Dental, LLC, Calver City, CA) is an epoxy resin–based root canal sealer that also contains biocompatible and bioactive fillers such as calcium phosphate and calcium oxide (4). There are contradicting data about its biological profile (4, 5). MTA-Fillapex (Angelus, Londrina, Brazil) is a salicylate resin sealer with additive mineral trioxide aggregate (MTA) and nanoparticulated silica, which has been shown to be rather cytotoxic and genotoxic (6–11).

The favorable results of MTA contributed to the development of a variety of calcium silicate materials, also known as bioceramic products. Their composition includes alumina, zirconia, bioactive glass, glass ceramics, hydroxyapatite, and calcium phosphates. The concept of calcium silicate cements is to exploit the beneficial characteristics of MTA and to also add properties that make them suitable for further applications. They are suggested as pulp capping, root repair, root-end filling, and root canal filling materials. Parirokh et al (12) noted that they may have variable chemical synthesis and named them "bioactive endodontic cements" in order to group them according to their common characteristic, which is bioactivity.

BioRoot RCS (Septodont, Saint Maur-des-Fossés, France) is a root canal sealer based on tricalcium silicate. It seems to be a quite promising endodontic material because it is significantly less cytotoxic and genotoxic compared with other sealers (7, 11, 13, 14) and induces a bioactive result as well (15). However, there are limited data concerning the cytotoxicity potential of BioRoot so far.

The purpose of the present study was to evaluate the cytotoxicity of 3 root canal sealers: epoxy resin sealer with calcium phosphate and calcium oxide (ie, SimpliSeal), salicylate resin sealer with MTA filler (ie, MTA-Fillapex), and calcium silicate sealer (ie, BioRoot RCS). The tested hypothesis was that BioRoot RCS is significantly less cytotoxic than the other tested sealers.

From the Department of Dental Tissues Pathology and Therapeutics (Basic Dental Sciences, Endodontology and Operative Dentistry), School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Address requests for reprints to Dr Elisabeth A. Koulaouzidou, Dental Tissues Pathology and Therapeutics, Aristotle University of Thessaloniki, 1 Xenofontos str 55132 Kalamaria, Thessaloniki, Greece. E-mail address: koulaouz@dent.auth.gr

0099-2399/$ - see front matter

https://doi.org/10.1016/j.joen.2018.01.015

Materials and Methods

Materials Tested

SimpliSeal, MTA-Fillapex, and BioRoot RCS were the sealers tested. SimpliSeal contains zirconium dioxide, epoxy resin, calcium oxide, calcium phosphate, bismuth subcarbonate, ethylene glycol monosalicylate, and triethanolamine. MTA-Fillapex composition includes salicylate resin, natural resin, bismuth trioxide, fumed silica, titanium dioxide, and MTA. BioRoot RCS consists of tricalcium silicate, zirconium oxide, and calcium chloride. NIH/3T3 cells were supplied by the American Type Culture Collection (Manassas, VA).

Experimental Design

The sealers' extracts (24 hours and 1 week) were applied to the cells at 1:1 and 1:2 dilutions. Cytotoxicity was measured at 24 hours and 72 hours of exposure in reference to controls. All experiments were performed at least twice in 6 replicates.

Procedures Used

Root Canal Sealers' Extracts. The root canal sealers were prepared according to manufacturers' instructions and placed in Petri dishes. The amount of each sealer was 2 g and covered a surface area of 2 cm^2. They were sterilized under ultraviolet rays for 15 minutes and stored in an incubator at 37°C for 48 hours to set. Ten milliliters of culture medium (Dulbecco modified Eagle medium [DMEM]; Life Technologies, Grand Island, NY) was added in the sealers. The extraction ratio was 0.2 g of sealer per 1 mL of the culture medium. The specimens were incubated for 24 hours and 1 week to extract the eluates (according to ISO 10993). The extracts were filtered sterile at 0.22 μm before their contact with the cells.

Cell Culture. The cells were grown in 75-cm^2 culture flasks at 37°C in a humidified atmosphere with 5% CO_2. DMEM supplemented with 10% fetal bovine serum and antibiotics (100 IU/mL penicillin and 100 mg/mL streptomycin) was used as the culture medium (20 mL culture medium per flask). Cell viability was measured with the trypan blue (Sigma-Aldrich, St Louis, MO) exclusion method in a hemocytometer (Newbauer Improved Bright-line; HBG, Giessen, Hessen, Germany). Adherent cells in a logarithmic growth phase were seeded (100 μL/well) in 96-well flat-bottom microtiter plates (Corning Costar, New York, NY) at a concentration of 3000 cells/well. The sealers' extracts were added to the cells at 1:1 and 1:2 dilutions (100 μL/well) using the growth medium as the dilution material. Cell cultures with 200 μL DMEM served as the negative controls.

Cell Viability. Cell viability and cell proliferation were measured at 24 hours and 72 hours of exposure, with a modified staining sulforhodamine B assay (Sigma-Aldrich) in reference to controls. The results were expressed as fraction unaffected, which is the proportion of cells that were not affected from the agent and derived from the following equation: fraction unaffected = ODx/ODc. ODx represented the test optical density, and ODc represented the control optical density. The mean of the 6 measurements for each concentration tested, the standard deviation, and the coefficient of variation were calculated. Dose-response curves were plotted for each agent. In Figures 1 and 2, a representative test is displayed.

Statistical Analysis

Statistical differences between the control and proliferation in the presence of the sealers' extracts were analyzed using the SPSS 17 package (SPSS Inc, Chicago, IL). One-way analysis of variance and the post hoc Tukey test were performed. *P* values < .05 were considered statistically significant.

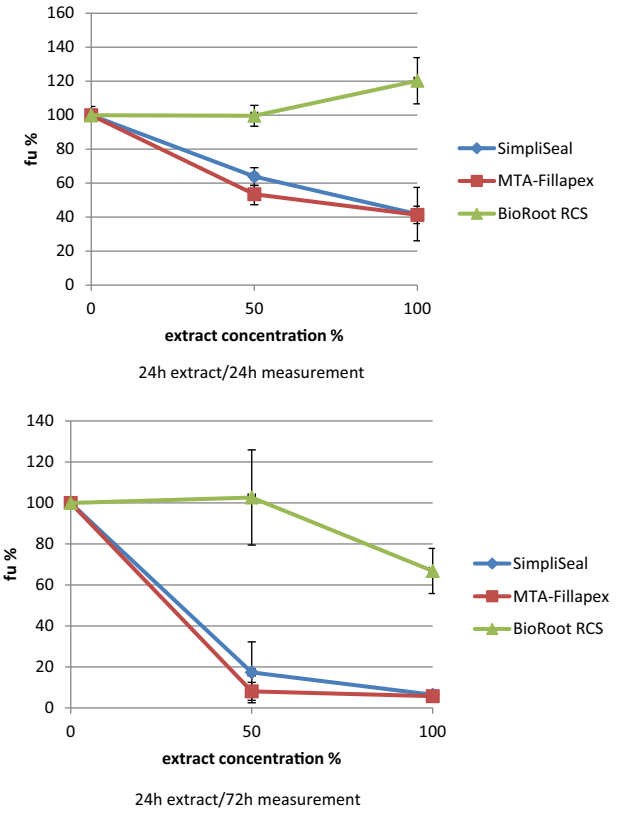

Figure 1. The viability of cells exposed to 24-hour extracts of SimpliSeal, MTA-Fillapex, and BioRoot RCS measured at 24 hours and 72 hours.

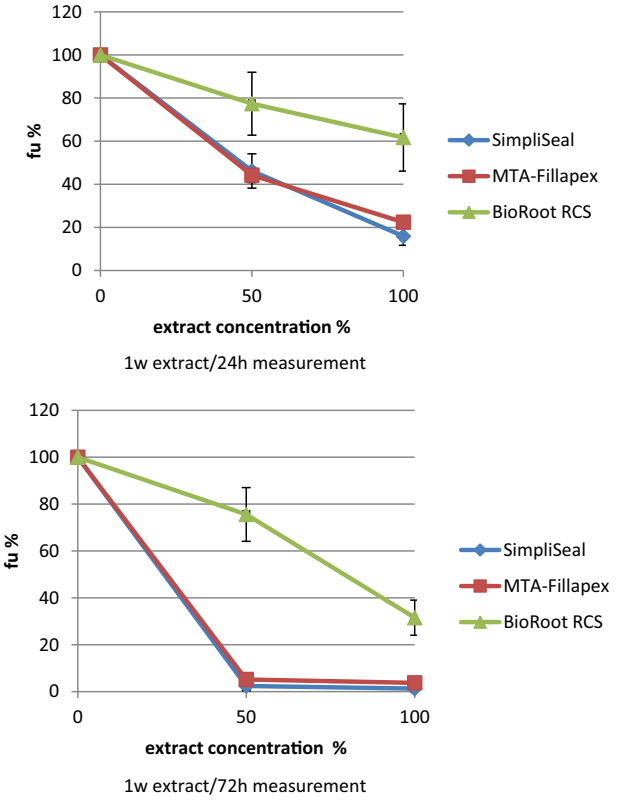

Figure 2. The viability of cells exposed to 1-week extracts of SimpliSeal, MTA-Fillapex, and BioRoot RCS measured at 24 hours and 72 hours.