Highlights

- Novel endodontic root canal sealers appeared in recent studies
- A comprehensive literature search to identify the biological and physico-chemical properties, biocompatibility, cytotoxicity, antimicrobial properties related to various recent novel root canal sealers
- Comparative evaluation with conventional endodontic root canal sealers
- Need for well-designed long-term clinical applications
- Need for further in vitro and in vivo work to clarify the mechanism and for the confirmation of the sustainability of their utilization for clinical practice

Cytotoxicity and biocompatibility of root canal sealers: A review on recent studies

Olcay Özdemir¹,² and Turkan Kopac³

Abstract

Many types of endodontic root canal sealers have been employed for the purpose of filling voids and irregularities in root canals, as well as reducing/removing bacterial remnants/remains. Sealers are available in various formulations, and research work to find the most appropriate ones is still ongoing. Recently, many kinds of novel root canal sealers have been introduced under various commercial names. However, most sealers are known to exhibit different levels of cytotoxicity on tissues which would result in prolonged wound healing, inflammation, and bone resorption. Preferably, sealers need to have tolerable biological and physico-chemical properties along with biocompatibility. Additives promoting the biocompatibility and bioactivity of sealers are of major concern in clinical applications. The aim of this review was to compare, evaluate, and analyze comparatively the cytotoxic effects, biocompatibility, and antimicrobial properties of recently used root canal sealers. A comprehensive literature search was made to identify their properties involving biocompatibility and cytotoxicity. In general, the sealers reported in recent literature exhibited favorable biological features in comparison to conventional ones. They promoted better cell viability and biocompatibility. The incorporation of additives influences favorably the potential negative effects. However, it has been highlighted that there is a lack of well-designed long-term clinical applications, and more in vitro and in vivo research work would be helpful to confirm the sustainability of the sealers for further clinical practice.

Keywords

Cytotoxicity, biocompatibility, biomaterials, endodontic root canal sealers, physico-chemical properties

Date received: 30 July 2021; revised: 1 December 2021; accepted: 17 December 2021

Introduction

The objective of endodontic therapy is the successful treatment of root canal infection, cleaning, and shaping of the root canals, filling the root canal space, thus preventing the
coronal and apical microorganism and liquid penetration.1–5 Most of the root canals are filled with a proper sealer which is the main component of root canal obturation to build a liquid-tight seal.6,7 Endodontic root canal sealers have been commonly employed for sealing of dentinal tubules to create a homogenous interface between the dentinal walls and the obturation material,5,8 thus contributing to the obturation of canal space.9,10 The main functions of sealers include filling the void and irregularities involved in the root canal, destroying the remaining bacteria left after cleaning, and shaping of the root canal by its germicidal action.7,11

A diverse range of root canal sealers have been used, along with a solid or a semisolid filler. They are prepared in various forms such as epoxy resin, zinc oxide–eugenol, calcium hydroxide, silicone, bioceramic, glass ionomer-based sealers with different setting formations6,8,11–13 (Figure 1). Although many formulations are available, research work to find the most appropriate ones is still carried out.14 Recently, many kinds of novel root canal sealers were introduced under various commercial names.7,11,15–19 The inclusion of agents into dental products to achieve tissue-remineralization and antibacterial functions are considerable.20 Additives improving the biocompatibility and bioactivity characteristics of sealers are of major concern in clinical applications.12,13 These improving strategies are highly promising for outcomes of root canal treatment and can potentially prevent and control endodontic pathologies.20

Biological interaction is explained by cytotoxicity, cytocompatibility or biocompatibility, cell plasticity, differentiation potential, and bioactive properties.21 Cytotoxicity is defined as the toxic effects of materials on vital tissues.18 The definition of biocompatibility is that having compatible and harmless properties of a material to the vital tissues.22 Biocompatible materials have stable and advantageous host responses during the application and do not influence an immunological or toxic response when contact with the tissue or tissue fluids.22,23 On the other hand, bioactive materials have a structure that can form a bond between the material and tissue.18,24 The research concerning biological interaction is crucial in determining the clinical impact of the materials.18,25,26

Most of the endodontic root canal sealers are known to have some toxic properties or might be exhibiting different levels of cytotoxicity on tissues, which would result in prolonged wound healing, inflammation, and bone resorption.7,27 Preferably, root canal sealers need to have tolerable biological and physico-chemical properties along with biocompatibility6 (Figure 2).

Due to the significant influence of sealers in endodontic applications, their physical, chemical, and biological features have been the subject matter of many reports in the literature.17,21,28–32 However, no extensive comparative report on the cytotoxicity and biocompatibility of the recently developed sealers has been reported. The aim of this investigation is to assess and analyze the cytotoxic effects and biocompatibility of various root canal sealers in a comparative manner.

**Search strategy and eligibility**

A comprehensive literature search has been done to identify the properties related to various recent root canal sealers.
Review of the recent studies on the cytotoxicity and biocompatibility of endodontic sealers

Based on the historical review of the endodontic sealers about biocompatibility and cytotoxicity characteristics, even though some conflicting evidence was found, low biocompatibility had been presented in zinc oxide eugenol (ZOE) sealers while superior property for silicone-based and tricalcium silicate-based sealers. Moderate biocompatibility was noted in glass ionomer, methacrylate, and salicylate-based. Better biocompatibility was reported in methyl methacrylate-tributyl borane resin compared to other resin-based sealers. Mineral trioxide aggregate (MTA) was the primary bioceramic material introduced in endodontics highlighted by its high bioactivity and biocompatibility.

Cytotoxicity and biocompatibility of various recently utilized endodontic root canal sealers discussed in the present study are shown in Table 1.

Cytotoxicity and biocompatibility potential of endodontic sealers

Various sealers such as GuttaFlow2, MTA Fillapex, GuttaFlow Bioseal were evaluated by Collado-González et al., concerning cytotoxic properties of some sealers on human periodontal ligament stem cells (hPDLSCs) in vitro. As a control for cell attachment and viability, AH Plus was used. Using eluates of sealers, cell viability assay was examined. hPDLSCs were seeded to the surfaces of materials to investigate the cell morphology and binding to the sealers. Analyses were carried out employing scanning electron microscopy (SEM), energy-dispersive X-ray (EDS), inductively coupled plasma mass spectroscopy (ICP-MS) techniques. Results exhibited a high proliferation level, cell spreading, and binding with GuttaFlow Bioseal disks. GuttaFlow2 and GuttaFlow Bioseal indicated less cytotoxic character. The extracts of sealers indicated time and dose-dependent influences on hPDLSCs. GuttaFlow Bioseal had better cytocompatibility, and additional in vitro and in vivo investigations with GuttaFlow Bioseal were proposed to confirm the suitability for clinical applications. Ferreira et al. evaluated the cytotoxic
Table 1. Cytotoxicity and biocompatibility of various endodontic root canal sealers recently utilized.

| Materials | Methodology | Major findings | Reference |
|-----------|-------------|----------------|-----------|
| Tubliseal | Evaluation of the effect of addition of antioxidant like pachymic acid | Root canal sealers exhibit cytotoxicity to varying degrees | Arun et al.27 |
| AH Plus   | L929 mouse fibroblast cells in vitro | Pachymic acid significantly reduced sealer-induced cytotoxicity | |
| Sealapex  | Cytotoxicity: MTT-assay | Methacrylate resin-based sealer: severely cytotoxic | |
| EndoREZ   |                          | Calcium hydroxide-based sealers: highly biocompatible | |
| Sealer Plus | L929 fibroblast cells in vitro | Sealapex: the lowest cytotoxicity | Cintra et al.14 |
| AH Plus   | Cell viability: MTT-assay | Pachymic acid: a viable therapeutic agent to overcome potential adverse effects associated with root canal sealers | |
| EndoFill  | Subcutaneous implants in vivo | | |
| SimpliSeal | Histologic analysis | | |
| GuttaFlow Bioseal | hPDLSCs in vitro | | |
| GuttaFlow2 | Cell viability: MTT-assay | Sealer’s extracts cause dose and timedependent effects on hPDLSCs | Collado-González et al.36 |
| MTA Fillapex | Cell Attachment: SEM | GuttaFlow Bioseal: lower cytotoxicity, better cytocompatibility | |
| AH Plus   | L929 mouse fibroblast cells in vitro | Evaluation of physical properties, antimicrobial effect, biocompatibility of D/C methacrylate incorporated experimental sealers | |
| RealSeal (dual polymerization experimental sealer) | Antimicrobial Assay: Enterococcus faecalis | | |
| Dibutyltin (D)/ calcium (C) methacrylate incorporation | Cell viability | | |
| BioRoot RCS | hMSCs in vitro | AH Plus provided a high cytotoxicity | Alsubait et al.46 |
| Endosequence BC | Cytotoxicity: Alamar Blue Assay | 1:2 BR: cell proliferation significantly lower | |
| AH Plus   | Cellular Morphology: SEM | 1:8, 1:32: tricalcium silicate sealers similar cellular proliferation | |
| BioRoot RCS | NIH/3T3 cells in vitro | BioRoot RCS: a positive biological behavior, rather cytocompatible. | Vouzara et al.12 |
| SimpliSeal | Cell viability: modified staining sulforhodamine B assay | SimpliSeal: a strong cytotoxic effect | |
| MTA-Fillapex sealer | | MTA-Fillapex sealer: significant cytotoxicity, acting rather as a resin sealer than as an MTA-based material | |
| AH Plus   | Human osteoblasts in vitro | AH Plus and zinc-oxide eugenol based sealers more cytotoxic | Jung et al.39 |
| Pulp Canal Sealer | Cell viability: MTT-assay | | |
| MTA Fillapex | Living-cell-count | Higher cell viability: BioRoot RCS | |
| BioRoot RCS | Living/dead-staining | | |
| Sealer Plus BC | Cytotoxicity: LDH-assay | | |
| MTA Fillapex | L929 fibroblast cells in vitro | Sealer Plus BC is biocompatible th MTA Fillapex and AH Plus | Benetti et al.35 |
| AH Plus   | Cell viability: Alamar Blue assay | | |
| EndoSeal MTA | Subcutaneous implants in vivo | | |
| Wellroot ST | Histologic analysis | | |
| AH Plus   | hPDLSCs in vitro | AH Plus: lowest cell viability | Lee et al.40 |
| AD Seal   | Cell Viability and Proliferation: MTT-assay | Calcium silicate-based sealers: more biocompatible, less cytotoxic | |
|           | Cellular Morphology: SEM | | |
|           | Inflammatory Response: Enzyme-Linked Immunosorbent Assay | | |
|           | Osteogenic activity: Alkaline Phosphatase staining | | |

(Continued)
response of GuttaFlow Bioseal and compared with epoxy resin sealer. Metabolic activity, cellular viability, the SRB and MTT assays were performed, and GuttaFlow Bioseal presented higher biocompatibility.

Vouzara et al.\textsuperscript{12} investigated the cytotoxic feature of a bioceramic calcium silicate endodontic sealer (BioRoot RCS). They evaluated the influence of the sealer on cell viability and proliferation of cells cultured in comparison to...
calcium oxide and phosphate-containing epoxy (SimpliSeal) and a mineral trioxide aggregate filler containing salicylate (MTA-Fillapex) resin sealers. The tests were accomplished with NIH/3T3 cells grown as monolayer cultures by the application of the extracts of sealers to cells (24h, 7 days). BioRoot RCS exhibited considerably lower cytotoxicity and was rather cytocompatible as compared to the others. SimpliSeal and MTA-Fillapex had significant cytotoxicity.

Cytotoxic properties of a calcium hydroxide-epoxy resin containing sealer (Sealer Plus) were found to be less cytotoxic compared to SimpliSeal, AH Plus, and EndoSeal.14 L929 fibroblasts and MTT assay were utilized for the determination of the cytotoxic features of the sealer extracts with respect to time by Cintra et al.14 Tubes either empty (control) or with materials were employed for testing the rats’ subcutaneous tissues, and histologic analysis was performed after rats died (7, 30 days). Coherent with Cintra et al.,14 Benetti et al.35 reached consistent results that Sealer Plus BC was biocompatible as compared to MTA Fillapex and AH Plus, and less cytotoxic when less-diluted extracts were applied.

Saghiri et al.9 evaluated the cytotoxicity and dimensional changes of a polyurethane expandable sealer (PES) developed recently. They used L929 fibroblasts and a cell viability assay (MTS) for the determination of the cytotoxicity of Sure-Seal Root, AH Plus, and PES dental sealers using extracted single-rooted human teeth. They utilized an advanced choroidal neovascularization model for evaluating the sealers on angiogenesis. SEM analysis was used for the measurement of the sealer penetration through the dentinal tubules. The choroidal neovascularization, MTS, and the penetration depth of PES were found considerably higher. PES exhibited promising results for dentinal tubule adaptation and penetration along with biocompatibility.

Gaudin et al.38 studied cytotoxicity and influence of cytokine production of some calcium silicate-based endodontic sealers, including MTA Fillapex, ProRoot ES, and BioRoot RCS in different dimensions cell culture models. They assessed the influence of sealers on the production of cytokine and survival of hPDLSCs. AH Plus was the control material. The cells were cultured in either 2- or 3-dimensional medium (24h) with the eluates of sealers. The toxicity of eluates was determined by the application of an in vitro root model. Cell viability and cytokine quantification were evaluated. BioRoot RCS exhibited good cell viability in 2-d culture conditions, whereas ProRoot ES did not indicate any effect. MTA Fillapex exhibited potent cytokotoxicity even at the lowest dilutions. Encapsulation of cells by PuraMatrix lowered the cytotoxicity. In the 3-d model, MTA Fillapex, ProRoot ES, BioRoot RCS exhibited cytocompatibility patterns. Calcium silicate sealers showed unsimilar production of proinflammatory cytokine. In conclusion, ProRoot ES and BioRoot RCS did not induce pro-inflammatory cytokines but enhanced anti-inflammatory cytokine secretion.

The evaluation of the influence of antioxidant (pachymic acid) addition on the cytotoxic properties of different sealers in vitro, including ZOE (Tubliseal), epoxy resin (AH Plus), calcium hydroxide (Sealapex), and methacrylate resin (EndoREZ) based sealers showed that the pachymic acid addition led to a significant decrease of cytotoxicity except for the EndoREZ.27 The experimental groups and subgroups by mixing the sealers were evaluated using mouse fibroblast cells (L929) with cell survival by methyl thiazole tetrazolium assay (24h). All sealers exhibited cytotoxicity to varying degrees, while Sealapex was the lowest cytotoxic, following AH Plus, Tubliseal, and EndoREZ. Therefore, it was proposed as a viable therapeutic material to avoid the potential detrimental consequences of the sealers. Methacrylate resin containing sealer showed a severely cytotoxic effect, while calcium hydroxide-containing types were significantly biocompatible. Other research conducted on human osteoblasts using various sealers including AH Plus (epoxy resin-based), Pulp Canal Sealer (zinc-oxide eugenol containing), and MTA-Fillapex, BioRoot-RCS (calcium silicate-based) in an unset and set condition indicated that zinc-oxide eugenol containing sealer and unset AH Plus presented higher cytotoxicity.39 In addition, it was concluded that BioRoot RCS influenced the cell metabolism and was slightly cytotoxic; however, compared with others, it had a better performance on cell viability.

Lee et al.40 compared the effects of various calcium silicate-based sealers, including Wellroot ST, Nano-ceramic Sealer, EndoSeal MTA, and epoxy resin-based sealers such as AH Plus and AD Seal on different aspects such as inflammatory response, cell viability, and osteogenic potential on hPDLSCs in vitro. Furthermore, Seo et al.41 compared conventional resin-based to various calcium silicate-based sealers like BioRoot RCS, EndoSequence BC, Endoseal MTA about biocompatibility and mineralization activity on human dental pulp stem cells (hDPSCs). As a result of the mentioned studies, calcium silicate-based sealers appeared to be more biocompatible and less cytotoxic than epoxy resin-based sealers on different kinds of stem cells.

Biocompatibility of a calcium hydroxide-epoxy resin containing sealer (Sealer Plus) was found to be enhanced better cell viability and biocompatibility compared to SimpliSeal, AH Plus, and EndoSeal sealers.14 In addition, Sealer Plus BC was presented better biocompatibility as compared to MTA Fillapex and AH Plus.35

Osteogenic potential of endodontic sealers

Giacomino et al.8 studied the osteogenic potential and biocompatibility of ProRoot ES and EndoSequence BC Sealer bioceramic sealers. They hypothesized that the sealers had superior features as compared with Roth and AH Plus types. In the method, murine osteoblast precursor cell line (IDG-SW3) was used at different concentrations (7 days).
Cell survival was identified via ATP quantification. The osteogenic potential was analyzed by fluorescence microscopy (FM) through the use of osteogenesis markers (ALP, DMP-1, Phex). According to the results, both bioceramic sealers (EndoSequence BC Sealer, ProRoot ES) had excellent biocompatibility and promoted significantly osteoblastic differentiation, while EndoSequence BC Sealer indicated higher responses in vitro.

López-García et al.42 evaluated compatibility and mineralization potential of two premixed bioceramic root canal sealers, including Bio-C Sealer and TotalFill BC Sealer on hPDLSCs in comparison with epoxy-resin-based one. In the methodology, the researchers conducted cell viability assay, cell migration, cell morphology, cell attachment and surface morphology, and Alizarin Red assay for calcium mineralization. The results presented that TotalFill BC sealer and Bio-C sealer demonstrated better performance on biocompatibility compared to AH Plus.

The effect of biological and physico-chemical properties of endodontic sealers on biocompatibility, cytotoxicity, osteogenecity, and antimicrobial activity

Jo et al.43 evaluated bioactive endodontic sealers (Well Root ST, Endoseal MTA, Nishika Canal Sealer BG) with a resin-based control sealer (AH Plus) concerning chemical, physical, and biological properties, and the results were indicated that Nishika BG showed prominent compatibility, osteogenecity, and angiogenecity as compared to other root canal sealers in vitro. Almeida et al.44 carried out a systematic review on the comparison of biological and physico-chemical features of calcium silicate-containing sealers with the regular ones in vitro. They compared many properties, including radiopacity, bond strength, solubility, setting- working time, flow, ion release, biocompatibility, antimicrobial activity, cytotoxic properties, and pH. The sealers had satisfactory biological and physico-chemical in vitro properties. Generally, the results were better than those of regular sealers. It was highlighted that calcium silicate-containing sealers presented suitable physico-chemical and biological features.

Chen et al.45 investigated the influence of temperature and cytotoxicity on the physico-chemical features and the chemical composition of a calcium silicate, including EndoSequence BC Sealer HiFlow as compared to EndoSequence BC Sealer. The cell viability was evaluated using hPDLSCs. Several measurements such as the radiopacity, microhardness, setting time, film thickness, viscosity were performed. FTIR spectroscopy was utilized for the chemical analysis. Cell viability was shown to decrease significantly on day 3 for both materials (1:4). HiFlow was better performed for film thickness and flow/viscosity, especially at higher temperatures, influenced by the warm vertical compaction technique. On the other hand, Alsubait et al.46 compared dose-dependent property on the cytotoxicity of EndoSequence BC and BioRoot RCS sealers with using AH Plus as a control and indicated that both tricalcium silicate sealers led to similar cellular proliferation that were better than AH Plus.

According to the evaluation of biological and physical features of dual polymerization sealers containing metal methacrylates, calcium methacrylate sealers were proposed as a good option for antimicrobial activity.47 The research work was conducted on the biocompatibility and the antimicrobial features of the dibutyltin or calcium methacrylate incorporated sealers. RealSeal was the control material. Sealers were assessed according to their radiopacity, film thickness, conversion degree, antimicrobial effect to E. faecalis, and cell viability. The film thickness of dibutyltin/calcium methacrylate incorporation in experimental sealers was higher than the other sealers, and the antimicrobial property was improved by the effect of metal methacrylate incorporation. Dibutyltin methacrylate added sealers and RealSeal provided higher cytotoxicity, while calcium methacrylate provided moderate cytoxicity.

The recent studies discussed above are mostly the results of in vitro findings. However, the behavior of materials used in biomedical applications would be different in the biological environment, and a considerable gap exists between the in vitro and in vivo analysis results. The influence of particles in biological medium needs to be further revealed, addressing the critical issues related to cytotoxic aspects of particles intended for use. The effects of various factors should be taken into account during particle-bio molecule interactions.48

Conclusions

In general, the novel endodontic root canal sealers reported in the recent literature exhibited favorable biological features in comparison to conventional ones. They promoted better cell viability and were more biocompatible. For example, calcium silicate-containing sealers indicated optimal physico-chemical and biological characteristics in vitro and exhibited better properties than conventional sealers. They exhibited better performance on flow/viscosity and film thickness, especially at higher temperatures due to the warm vertical compaction techniques applied commonly. Silicone-based sealers enhanced anti-inflammatory cytokine secretion by PDLSCs. Investigation of the cytotoxicity of calcium silicate containing bioceramic sealer showed quite a positive biological behavior and was rather cytocompatible. Bioceramic sealers were found significantly more biocompatible and better tolerated by osteoclast precursor cells in vitro. They promoted osteoblastic differentiation that might be found in some sealers. A recently utilized polyurethane expandable sealer exhibited...
promising results in terms of dentinal tubule adaptation, penetration, and biocompatibility.

The incorporation of metallic methacrylates into endodontic sealers was proposed. Dibutylin and calcium methacrylate incorporation promoted the antimicrobial effect of sealers. Dibutylin caused a high, while calcium methacrylate resulted in moderate cytotoxicity. Calcium methacrylate sealers were proposed as a good option for antimicrobial activity. Additives used in conventional sealers influence favorably the potential negative effects related with root canal sealers and significantly reduce sealer-induced cytotoxicity. Calcium hydroxide–based sealers appear to be highly biocompatible, among the other zinc oxide eugenol, epoxy, and methacrylate resin sealers. It was highlighted that root canal sealers might exhibit varying levels of cytotoxic consequences. Methacrylate sealer appears to be severely cytotoxic. The extracts from sealers give rise to time and dose-dependent influences on hPDLCs.

More in vitro and in vivo studies would be helpful to confirm the sustainability of the investigated sealers for clinical practice. It was also highlighted that there is a lack of well-designed long-term clinical applications. Further investigations would help to clarify the mechanisms contributing to the observed beneficial results. Additional protocols are needed in the evaluation of the chemical features of sealers for the interpretation of their biological behavior. Basic research protocol findings could further help to contribute to the successful sustainability of in vivo and clinical applications.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iDs
Olcay Özdemir https://orcid.org/0000-0001-8867-1551
Turkan Kopac https://orcid.org/0000-0002-1642-7987

References
1. Rover G, Belladonna FG, Bortoluzzi EA, De-Deus G, Silva EJNL and Teixeira CS. Influence of access cavity design on root canal detection, instrumentation efficacy, and fracture resistance assessed in maxillary molars. J Endod 2017; 43: 1657–1662.
2. Yee RD, Newton CW, Patterson SS and Swartz ML. The effect of canal preparation on the formation and leakage characteristics of the apical dentin plug. J Endod 1984; 10: 308–317.
3. Siqueira Junior JF, Roças IDN, Marceliano-Alves MF, Pérez AR and Ricucci D. Unprepared root canal surface areas: causes, clinical implications, and therapeutic strategies. Braz Oral Res 2018; 32: 665.
4. Azim AA, Aksel H, Zhuang T, Mashtare T, Babu JP and Huang GT. Efficacy of 4 irrigation protocols in killing bacteria colonized in dentinal tubules examined by a novel confocal laser scanning microscope analysis. J Endod 2016; 42(6): 928–934.
5. Marciano MA, Bramante CM, Duarte MA, Delgado RJ, Ordinola-Zapata R and Garcia RB. Evaluation of single root canals filled using the lateral compaction, tagger’s hybrid, Microseal and Guttaflow techniques. Braz Dent J 2010; 21: 411–415.
6. Huang F-M, Tai K-W, Chou M-Y and 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: 153–158.
7. Kaur A, Shah N, Logani A and Mishra N. Biotoxicity of commonly used root canal sealers: a meta-analysis. J Conserv Dent 2015; 18(2): 83–88.
8. Giacomino CM, Wealleans JA, Kuhn N and Diogenes A. Comparative biocompatibility and osteogenic potential of two bioceramic sealers. J Endod 2019; 45(1): 51–56.
9. Saghir MA, Karamifar K, Nath D, Gutmann JL and Sheibani N. A novel polyurethane expandable root canal sealer. J Endod 2021; 47(4): 612–620.
10. Özdemir O, Koçak S, Hazar E, Sağlam BC, Coşkun E and Koçak MM. Dentinal tubule penetration of gutta-percha with syringe-mix resin sealer using different obturation techniques: a confocal laser scanning microscopy study. Aust Endod J. Epub ahead of print 13 July 2021. DOI: 10.1111/aej.12546.
11. Al-Haddad A and Che Ab Aziz ZA. Bioceramic-based root canal sealers: a review. Int J Biomater 2016; 2016: 9753210.
12. Vouzara T, Dimosiari G, Koulaouzidou EA and Economides N. Cytotoxicity of a new calcium silicate endodontic sealer. J Endod 2018; 44(5): 849–852.
13. Komabayashi T, Colmenar D, Cvach N, Bhat A, Primus C and Imai Y. Comprehensive review of current endodontic sealers. Dent Mater J 2020; 39(5): 703–720.
14. Cintra LTA, Benetti F, de Azevedo Queiroz ÍO, et al. Evaluation of the cytotoxicity and biocompatibility of new resin epoxy-based endodontic sealer containing calcium hydroxide. J Endod 2017; 43(12): 2088–2092.
15. Uzunoglu-özüyrek E, Küçükkaya Eren S and Karahan S. Effect of root canal sealers on the fracture resistance of endodontically treated teeth: a systematic review of in vitro studies. Clin Oral Investig 2018, 22(7): 2475–2485.
16. Al-Shwaimi E, Bogari D, Ajaj R, Al-Shahrani S, Almas K and Majeed A. In vitro antimicrobial effectiveness of root canal sealers against Enterococcus faecalis: a systematic review. J Endod 2016; 42: 1588–1597.
17. Donnermeyer D, Bürklein S, Dammashke T and Schäfer E. Endodontic sealers based on calcium silicates: a systematic review. Odontology 2019; 107(4): 421–436.
18. Maru V, Dixit U, Patil RSB and Parekh R. Cytotoxicity and bioactivity of mineral trioxide aggregate and bioactive endodontic type cements: a systematic review. Int J Clin Pediatr Dent 2021; 14(1): 30–39.
19. Marin-Bauza GA, Reichert-Junior FJ, Souza-Gabriel AE, Sousa-Neto MD, Miranda CE and Silva-Sousa YT.
Physicochemical properties of methacrylate resin-based root canal sealers. *J Endod* 2010; 36: 1531–1536.

20. Baras BH, Melo MAS, Thumbigere-Math V, et al. Novel bioactive and therapeutic root canal sealers with antibacterial and remineralization properties. *Materials* 2020; 13(5): 1096.

21. Sanz JL, Guerrero-Gironés J, Pecci-Lloret MP, Pecci-Lloret MR and Melo M. Biological interactions between calcium silicate-based endodontic biomaterials and periodontal ligament stem cells: a systematic review of in vitro studies. *Int Endod J* 2021; 54: 2025–2043.

22. Washio A, Morotomi T, Yoshii S and Kitamura C. Bioactive glass-based endodontic sealer as a promising root canal filling material without semisolid core materials. *Materials* 2019; 12(23): 3967.

23. Marin E, Boschetto F and Pezzotti G. Biomaterials and biocompatibility: an historical overview. *J Biomed Mater Res* 2020; 108: 1617–1633.

24. Vallittu PK, Boccaccini AR, Hupa L and Watts DC. Bioactive dental materials—do they exist, and what does bioactivity mean? *Dent Mater* 2018; 34(5): 693–694.

25. Sanz JL, Rodriguez-Lozano FJ, Lopez-Gines C, Monleon D, Llena C and Forner L. Dental stem cell signaling pathway activation in response to hydraulic calcium silicate-based endodontic cements: a systematic review of in vitro studies. *Dent Mater* 2021; 37: e256–e268.

26. Liu Y, Liu XM, Bi J, et al. Cell migration and osteo/odontogenesis stimulation of iRoot FS as a potential apical barrier material in apexification. *Int Endod J* 2020; 53(4): 467–477.

27. Arun S, Sampath V, Mahalaxmi S and Rajkumar K. A comparative evaluation of the effect of the addition of pachymic acid on the cytotoxicity of 4 different root canal sealers—an in vitro study. *J Endod* 2017; 43(1): 96–99.

28. Silva EJNL, Cardoso ML, Rodrigues JP, De-Deus G and Fidalgo TKDS. Solubility of bioerodable- and epoxy resin-based root canal sealers: a systematic review and meta-analysis. *Aust Endod J* 2021; 47: 690–702.

29. Sponchiado Junior EC, Vieira WDA, Normando AGC, et al. Calcium silicate-based sealers do not reduce the risk and intensity of postoperative pain after root canal treatment when compared with epoxy resin-based sealers: a systematic review and meta-analysis. *Eur J Dent* 2021; 15(2): 347–359.

30. Sanz JL, Rodriguez-Lozano FJ, Llena C, Sauro S and Forner L. Bioactivity of bioerodable materials used in the dentin pulp complex therapy: a systematic review. *Materials* 2019; 12(7): 1015.

31. Šimundić Munitić M, Poklepović Perić T, Utrobičić A, Bagić P and Puljak L. Antimicrobial efficacy of commercially available endodontic bioerodable root canal sealers: a systematic review. *PloS One* 2019; 14(10): e0232575.

32. Gandolfi MG, Parrilli AP, Fini M, Prati C and Dummer PM. 3D micro-CT analysis of the interface voids associated with Thermafil root fillings used with AH plus or a flowable MTA sealer. *Int Endod J* 2013; 46(3): 253–263.

33. Fonseca DA, Paula AB, Marto CM, et al. Biocompatibility of root canal sealers: a systematic review of in vitro and in vivo studies. *Materials* 2019; 12(24): 4113.

34. Pelliccioni G, Vellani C, Gatto M, Gandolfi M, Marchetti C and Prati C. Proroot mineral trioxide aggregate cement used as a retrograde filling without addition of water: an in vitro evaluation of its microleakage. *J Endod* 2007; 33(9): 1082–1085.

35. Benetti F, de Azevedo Queiróz ÍO, Oliveira PHC, et al. Cytotoxicity and biocompatibility of a new bioceramic endodontic sealer containing calcium hydroxide. *Braz Oral Res* 2019; 33: e042.

36. Collado-González M, Tomás-Catalá CJ, Oñate-Sánchez RE, Moraleda JM and Rodriguez-Lozano FJ. Cytotoxicity of GuttaFlow bioseal, GuttaFlow2, MTA Fillapex, and AH Plus on human periodontal ligament stem cells. *J Endod* 2017; 43: 816–822.

37. Ferreira I, Larano M, Marto CM, et al. GuttaFlow® bioseal cytotoxicity assessment: in vitro study. *Molecules* 2020; 25: 4297.

38. Gaudin A, Tolar M and Peters OA. Cytokine production and cytotoxicity of calcium silicate-based sealers in 2- and 3-dimensional cell culture models. *J Endod* 2020; 46(6): 818–826.

39. Jung S, Sielker S, Hanisch MR, Libbricht V, Schäfer E and Dammaschke T. Cytotoxic effects of four different root canal sealers on human osteoblasts. *PLoS One* 2018; 13(3): e0194467.

40. Lee JK, Kim S, Lee S, Kim HC and Kim E. In vitro comparison of biocompatibility of calcium silicate-based root canal sealers. *Materials* 2019; 12(15): 2411.

41. Seo DG, Lee D, Kim YM, Song D and Kim SY. Biocompatibility and mineralization activity of three calcium silicate-based root canal sealers compared to conventional resin-based sealer in human dental pulp stem cells. *Materials* 2019; 12(15): 2482.

42. López-García S, Pecci-Lloret MR, Guerrero-Gironés J, et al. Comparative cytocompatibility and mineralization potential of Bio-C Sealer and TotalFill BC Sealer. *Materials* 2019; 12(19): 3087.

43. Jo SB, Kim HK, Lee HN, et al. Physical properties and biofunctionalities of bioactive root canal sealers in vitro. *Biomater* 2020; 10(9): 1750.

44. Almeida SLH, Moraes RR, Morgental RD and Pappen FG. Are premixed calcium silicate-based endodontic sealers comparable to conventional materials? A systematic review of in vitro studies. *J Endod* 2017; 43(4): 527–535.

45. Chen B, Haapasalo M, Mobuchon C, Li X, Ma J and Shen Y. Cytotoxicity and the effect of temperature on physical properties and chemical composition of a new calcium silicate-based root canal sealer. *J Endod* 2020; 46(4): 531–538.

46. Alsabait SA, Al Ajlan R, Mitwalli H, et al. Cytotoxicity of different concentrations of three root canal sealers on human mesenchymal stem cells. *Biomolecules* 2018; 8(3): 68.

47. Rossato TCA, Gallas JA, da Rosa WLO, et al. Experimental sealers containing metal methacrylates: physical and biological properties. *J Endod* 2017; 43: 1725–1729.

48. Kopac T. Protein corona, understanding the nanoparticle-protein interactions and future perspectives: a critical review. *Int J Biol Macromol* 2021; 169: 290–301.