Universidad Europea

GRADUATION PROJECT

Dentistry Degree

BIOCOMPATIBILITY OF ENDODONTIC SEALERS

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Abstract

Introduction: The biocompatibility of endodontic sealers is one of the important factors to insure the favorable prognostic of a root canal treatment. The aim of this literature review was to study the biocompatibility of commercialized epoxy resin sealers and the more recent calcium-silicate-based sealers in terms of the analysis of their influence on the inflammatory response and the osteogenic potential they have on the periodontal tissues. **Objectives**: The first objective was to analyze the inflammatory response of the calcium silicate-based sealers compared with epoxy resin sealers. The second objective was to analyze the osteogenic potential of the calcium silicate-based sealers compared with epoxy resin sealers. **Methodology**: Two databases with scientific relevance were used (PubMed via MEDLINE and Web Of Science). After the screening, 9 articles were selected to answer the first objective, and 7 articles were selected for the second objective. The chosen articles corresponded to the eligibility criteria previously determined. **Results**: Regarding the inflammatory response of the tissues, most articles related a higher initial inflammatory response when using epoxy resin and a lower inflammatory response for the calcium-silicate-based sealers. Regarding the osteogenic potential, the epoxy resin sealers have exhibited a negative osteogenic potential while most articles encountered a positive one for the calcium-silicate-based sealers. **Conclusion**: This review showed a difference regarding some features of the biocompatibility of the periodontal ligament depending on the sealers used. When focusing on the inflammatory response and the osteogenic potential, there is a difference between the epoxy resin sealers and the calcium silicate-based sealers. However, the limit of this review was the in-vitro study of the behavior of the different sealers and not the study of their clinical behavior.

Keywords: Dentistry, Endodontics, Biocompatibility, Calcium-Silicate based sealers, Epoxy resin

Resumen
Introducción: La biocompatibilidad de los cementos endodónticos es uno de los factores importantes para asegurar el buen pronóstico de un tratamiento endodóntico. El objetivo de esta revisión fue estudiar la biocompatibilidad de los cementos de resina epóxica comercializados y de los cementos silicato de calcio más recientes mediante el análisis de su influencia en la respuesta inflamatoria y en el potencial osteogénico que tienen sobre los tejidos periodontales. **Objetivos**: El primer objetivo fue analizar la respuesta inflamatoria de los selladores a base de silicato de calcio en comparación con los selladores de resina epóxica. El segundo objetivo fue analizar el potencial osteogénico de los selladores a base de silicato de calcio en comparación con los selladores de resina epoxica. **Material y métodos:** Se utilizaron dos bases de datos con relevancia científica (PubMed vía MEDLINE y Web Of Science). Tras el cribado de los registros, se seleccionaron 9 artículos para responder al primer objetivo y 7 artículos para el segundo objetivo. Los artículos elegidos correspondían a los criterios de elegibilidad previamente determinados. **Resultados**: En cuanto a la respuesta inflamatoria, la mayoría de los artículos relacionaron una mayor respuesta inflamatoria inicial cuando se utilizaba resina epóxica en comparación a los de silicato de calcio. En cuanto al potencial osteogénico, los selladores de resina epóxica han mostrado un potencial osteogénico negativo, mientras que la mayoría de los artículos encontraron uno positivo para los selladores a base de silicato de calcio. **Conclusiones**: Esta revisión mostró una diferencia en cuanto a la biocompatibilidad de los selladores utilizados. Existe una diferencia entre los selladores de resina epoxi y los selladores a base de silicato cálcico de respuesta inflamatoria y del potencial osteogénico. El límite de esta revisión fue el estudio solo in vitro del comportamiento de los diferentes selladores.

Palabras clave: Odontología, Endodoncia, Biocompatibilidad, Cementos silicato de calcio, Resina Epoxica

1 Introduction

1.1 History of Root Canal Treatment

The first root canal procedure was designed in 1838 by Edwin Maynard, and since then, performed millions of times every year and is a part of the daily life of a general or specialized dental professional. Techniques and materials have gone through some changes and improvements. However, some of them remain the same. Even though its forms have changed, gutta-percha was first used in 1867 by the scientist Bowman and is still used nowadays (1,2).

Other materials have disappeared from dental clinics. Arsenic is an example of a material that used to be commonly used to irrigate and devitalize the pulp because of its anti-inflammatory properties but then was proven to be toxic and have unwanted side effects (3). While irrigation has evolved and more options are available, none of them fills all the requirements needed. The success of the root canal treatment relies on the cleaning of the root in order to get a bacteria-free medium and avoid any type of infection after the treatment is done. Nowadays, irrigants are used on a case by case basis: chlorhexidine, digluconate-based, sodium hypochlorite (NaOCl), Ethylene-diamine-tetra-acetic Acid (EDTA), antimicrobial photodynamic therapy (APDT), photon-induced photoacoustic streaming (PIPS), gentle wave irrigation, chlorine dioxide, silver diamine fluoride, tetraclean (4). The world of endodontics, including the materials used, is in constant evolution, also having an impact on endodontic sealers (5).

1.2 Introduction to the Sealers in endodontics

In general, sealers are used with a core such as gutta-percha to create a strong enough barrier and close the canal from the surrounding parts since the use alone of Gutta Percha will not provide a seal effective enough to obstruct the canals. Therefore, the sealer will have to interact appropriately with the dentin present in the root on one side and, on the other side, the cones of gutta-percha (6-7). The American Association of Endodontists defines the endodontic treatment as, “Root canal sealers are used in conjunction with the core filling material to establish an adequate three-dimensional seal and induce hard tissue formation and healing outcomes" (8).

According to Cohen et al., there are certain characteristics which sealers should meet such as *'to exhibit the thickness when mixed to provide good adhesion between it and the canal wall when set, establish a hermetic seal, radiopaque so it can be seen on a radiograph, very fine powder so it can be mixed easily with liquid, no shrinkage on the setting, no staining of the tooth structure, bacteriostatic or at least does not encourage bacterial growth, exhibits a slow set, insoluble in tissues fluids, tissue tolerant that it is not irritating to the peri radicular tissues, soluble in a common solvent if it is necessary to remove the root canal filling'* (9). Characteristics have involved over time but none of the sealers available meet all of those requirements all at once (10–11).

1.3 Types of Sealers

While different categories of sealers are available on the market, their uses and popularity had fluctuated over the years (12).

1.3.1 Medicated sealers

This type of sealer is nowadays completely obsolete. The *medicated sealers* or paraformaldehyde/mercury-based sealers were too toxic and have been prohibited by the US Food Administration (9).

1.3.2 Zinc Eugenol Oxide

It is found on the market and sold by the brand Kerr (Kerr Corporation, Orange, CA, USA) under the name Pulp Canal Sealers (Kerr Corporation, Orange, CA, USA*).* Two working times are available, the basic version with a working time of 1 to 2 hours. The extended version reaches 6 to 8 hours before completing the setting. The pack includes a powder and a liquid acting as a catalyst. The powder is made of Zinc oxide, Precipated Silver, Oleo resin, and Thymol iodide and the liquid contains oil of cloves and Canada Balsam.

Zinc Eugenol Oxide sealers had then seen its composition modified because of staining tissue issues in the product Tubli-Seal (Sybron Endo Corporation, Orange, CA). The base paste contains zinc oxide, oleoresin, bismuth trioxide, thymol iodide, oils and waxes, and the catalyst paste contains eugenol, polymerized resins, and annidalin. (9,13,14)

1.3.3 Eugenol Free Sealers

Sealers eugenol free are also available on the market, known as Nogenol (GC America, Alsip, IL, USA). The standard package includes two tubes, the base, and the catalyst. The properties of this product allow for extended working time and a short setting time once placed into the root canal (15).

1.3.4 Polymeric Calcium Hydroxide Sealers

Polymeric Calcium Hydroxide sealers are also found sold by the brand Kerr under the names SealApex (Kerr, USA). It has a setting time of 45 minutes once placed inside the root canal. SealApex Express (Kerr, USA) has a shorter working time, and Bulk Seal-Apex (Kerr, USA). Those packs contain two bottles, one being the base containing the component Calcium Hydroxide and the second one being the catalyst (16).

1.3.5 Glass Ionomer Sealers

Glass Ionomer Sealers (GIC) at first was released as an acid-base reaction based on the reaction of the glass powder part made of fluoro-alumino-silicate and polycarboxylic acid mixed with water (17). Nowadays, it will be found as an hybrid material made of organic and inorganic content. The pack is made of a powder (fluoro-aluminosilicate glass) content that needs to be mixed with an aqueous solution (acid-containing tartaric acid) (18).

1.3.6 Silicone-based sealers

Silicone-based sealers were created to get better chemical and biological properties. Three models are available on the market: RoekoSeal (Coltene/Whaledent, USA), Guttaflow (Coltene/Whaledent, USA), Guttaflow 2 (Coltene/Whaledent, USA). and Guttaflow Bioseal (Coltene/Whaledent, USA). Guttaflow (Coltene/Whaledent, USA) is an adaptation of Roekoseal (Coltene/Whaledent, USA) under a paste-type RCT filler. Guttaflow 2 (Coltene/Whaledent, USA) is composed of gutta-percha powder (<30 micrometers of size), poly-dimethyl-siloxane, zirconium dioxide, a platinum catalyst, and micro silver particles and has the adjustment to the dentin (19). In its technical manual, the group Coltene describes the GuttaFlow as a "cold flowable gutta-percha". The sealer is used through a premixed syringe. The working time is very short compared to other types of sealers, being less than 15 minutes, and a curing time going up to 30 minutes. (20,21).

1.3.7 Resin sealers

The Resin sealer group introduced by Schroeder et al. is made of Epoxy resin sealers and Methacrylate Resin Sealers. To improve the adhesion to the dentin, research led to the creation of methacrylate resin sealers. *Methacrylate Resin Sealers* were found on the market under four generations. (22,23)

The *first one* was released by the group Hyron in the 1970s and produced up to the 1980s. It was described as being highly hydrophilic, containing mainly poly[2-hydroxyethyl methacrylate] (poly[HEMA]). This first generation was used alone (meaning with no core), and the polymerization happened once the product was placed inside. (9,23)

The unfavorable results led to the *second generation*. It was a dual-cure called EndoREZ (Ultradent, South Jordan, UT, USA). Contrary to the first generation, no adhesive process was necessary. Its main mechanism of action was based on the extensive penetration of the product into the dentinal tubules after eliminating the smear layer (ethylenediaminetetraacetic acid and sodium hypochlorite). However, the bond was described as weak because of the lack of thinness of the sealer, described as bulk. (9,24) A new core material was produced: Resilon (Resilon Research LLC, Madison, CT). Within its composition is found methacrylate resin, bioactive glass, barium sulfate, and bismuth oxychloride. Thanks to its thermoplasticity, it could replace the use of Gutta Percha. They have the characteristic of creating a "monoblock" which means a strong bonding with the core: Resilon and the sealer. Its usage is beneficial when combined with the *third generation* of Methacrylate Resin Sealers (RealSeal/SybronEndo - Epiphany - Fibrefill. Those sealers are dual-curing sealers, meaning that the coronal part will be light-cured for 40 seconds, and the rest will last about 30 minutes. (23,25–27)

The *fourth generatio*n is found as MetaSeal (Parkell, USA), which contains 4-methacryloxyethyl trimellitate anhydride giving the sealer acidic properties and making the sealer self-etching. This methacrylate resin sealer is hydrophilic and dual-cured. Contrary to the third generation, this sealer is self-adhesive, too. Combining self-etching and self-adhesive actions helps the professionals with less chance of errors since one movement is needed to prepare the dentin and the associated tissues. Another type of the fourth generation is RealSeal Se (SybronEndo, Amersfoort, The Netherlands). It has familiar compositions and properties as MetaSeal (Parkell, USA) being hydrophilic, self-etching, and self-adhesiveness. Its use is combined with the core Resilon (Resilon Research LLC, Madison, CT) applying it with lateral or vertical compaction, and has a setting time of 43 minutes. (9,28,29)

Epoxy resin sealers are known to be less expensive than the other options of root canal sealers. Their composition is summarized in table 1. Different types are available on the market: Obturys (OB/ITENA, Villepinte, France) (22), AD SEAL (Meta Biomed, Korea). The group Dentalpsy (Dentsply/ De Trey, Konstanz, Germany) issued AH PLUS (Dentsply/ De Trey, Konstanz, Germany) (9), an epoxy resin sealer used as the gold standard (30) with 240 minutes of WT and 11 minutes of Setting Times and AH 26 (Dentsply/ De Trey, Konstanz, Germany). They are found on the market under three types: double syringe (Base made of Epoxy resin/catalyst), single syringe, or powder (31,32).

AH 26 (Dentsply/ De Trey, Konstanz, Germany) was established by the same laboratory that also introduced epoxy resins in 1938. AH26 (Dentsply/ De Trey, Konstanz, Germany) is made of a powder part (within its composition: hexamethylenetetramine for its polymerization, bismuth trioxide, calcium hydroxide, titanium dioxide) and a resin part (Bisphenol-epoxy resin). This sealer has a working time of 4-6 hours and a setting time of 9-15 hours. AH Plus (Dentsply/ De Trey, Konstanz, Germany) is a paste-to-paste consistency. This sealer is also known as AH PLUS JET when distributed as an automatic mixing syringe. In the European market, it can be sold under the branding TopSeal (14,33,34).

Table 1. Composition of three Epoxy Resin Sealers: AH Plus (Paste A and Paste B) and AD Seal (Base and Catalyst) (35)

Name of the Sealer	Composition	
AH Plus	**Paste A** Epoxy bisphenol-A resin and epoxy bisphenol-F, calcium tungstate, zirconium oxide, silica and iron oxide	**Paste B** Dibenzyl-diamine, aminoadamantane, calcium tungstate, zirconium oxide, silica, and silicone
AD Seal	**Base** Epoxy oligomer resin, ethylene glycol salicylate, calcium phosphate, bismuth subcarbonate, and zirconium oxide	**Catalyst** Poly-aminobenzoate, triethanolamine, calcium phosphate, bismuth subcarbonate, zirconium oxide and calcium oxide

1.3.8 Calcium Silicate Sealers

Calcium Silicate Sealers contain calcium silicate, also a component of mineral tricalcium aggregate (MTA). MTA is a widely used material in dentistry for many indications such as pulpotomy, apexogenesis, root perforations, and root treatments. MTA first was used to seal the end part of an RCT. Because of the good characteristics MTA, these sealers were developed. MTA when used in root canal treatments, allows a good apical seal. This combination allows them to have good adhesiveness to the dentin present in the root canal and reduce fractures. Some of the calcium silicate sealers available on the market are MTA-Fillapex (Angelus, Londrina, Brazil), Endo CPM (Egeo, Buenos Aires, Argentina), iRoot EndoSequence (Innovative BioCreamix Inc., Vancouver, Canada), and EndoSeal MTA (Maruchi, Korea). They are also called bioceramic sealers. Their composition is summarized in table 2. (36–38)

MTA-Fillapex (Angelus, Londrina, Brazil), type is available under a pack of two different acid/base tubes. The first one contains "salicylate resin, bismuth trioxide, and silica, and the second one: silica, titanium dioxide, MTA, and resin." (39)

Endo CPM is a hydrophilic powder/liquid which contains "tricalcium silicate, tricalcium oxide, tricalcium aluminate, other oxides, saline solution, and calcium chloride". The powder is mixed with the liquid allowing the dental professional to choose what consistency is needed. (40)

The iRoot Endosequence sealer is familiar to mineral tricalcium aggregate. It contains calcium silicate, calcium phosphate, calcium hydroxide, niobium oxide, and zirconium oxide. The setting time is 4 hours and up to 10 hours if the roots show dryness. The pack is presented with a syringe and tips to apply the product inside the root canal. (41,42)

EndoSeal MTA (Innovative Bioceramix, Canada) also has familiar properties as MTA. The pack contains the preloaded syringe with one-use tips. EndoSeal MTA (Innovative Bioceramix, Canada) can be combined with gutta-percha and is used after placing the master cone. It is a pozzolan-based material, improving the consistency, the flowability of the product, and the setting time (12 minutes). The radio pacifier bismuth has been replaced by zirconium oxide and shows better characteristics. (43–47)

Table 2. Composition of calcium-silicate-based endodontic sealers (Total Fill BC Sealer, iRoot SP, EndoSeal, MTA FillApex, ProRoot Endo Sealer, Endosequence BC Sealer) (48)

Name of the sealer	Composition
Total Fill BC Sealer	calcium silicates, calcium phosphate monobasic, zirconium oxide, tantalum oxide, and thickening agents
iRoot SP	calcium silicate, calcium phosphate, calcium hydroxide, niobium oxide, and zirconium oxide
EndoSeal	sodium oxide, calcium oxide, potassium oxide, magnesium oxide, iron oxide, aluminum oxide, titanium dioxide, zirconium oxide, silicone dioxide
MTA FillApex	natural resin, salicylate resin, diluting resin, bismuth trioxide, nanoparticulated silica, pigments and MTA
ProRoot Endo Sealer	calcium sulphate, dicalcium silicate, tricalcium silicate, bismuth oxide, and a bit of tricalcium aluminate Liquid
Endosequence BC sealer	phosphate silicate-based sealer

1.4 Biocompatibility in Dentistry

Over time, all of those described sealers had shown different biocompatibility. Since the goal of root canal treatment is to seal the root canal completely from the periradicular tissues, it is important to avoid infection and penetration of bacteria or microorganisms. Even though the preparation of the walls and the instrumentation is fundamental, the materials used also have an impact on the success of the operation (49).
Since the standards have evolved, it is essential to evaluate the endodontic sealers and their biocompatibility to ensure the success of the treatment (50).

The sealer is used as a permanent material for definitive treatment. The extrusion of an incompatible sealer because of overfilling the canal, can have an impact on the tissues and produce an inflammatory response. The extrusion of the sealer will have an impact on the good prognostic of apical periodontitis if the patient suffers from one (51). Most sealers have shown cytotoxic responses and while the level of toxicity will depend on their type and so their formulation, the possible impacts on the good success of the treatment will be a delayed repairing of the wound, an inflammation, an extrusion of bacteria, an inflammation of the tissues leading to bone resorption. The endodontic materials used for the treatment need to be respectful of the surrounding tissues (52).

The Food and Drug Administration defines biocompatibility as *"the ability of a device material to perform with an appropriate host response in a specific situation"* (53). Moreover, the American Dental Association's standards number 41, is the list of characteristics to be considered when evaluating the biocompatibility of materials used. It includes *"cytotoxicity, delayed-type hypersensitivity, irritation, acute and subchronic systemic toxicity, genotoxicity, implantation, and endodontic usage tests"* (54). Essentially, biocompatibility means being compatible with the vital tissues while creating a harmless reaction (without toxic reaction) with a favorable impact on the tissues (55–56).

1.4.1 Cytotoxicity

Cytotoxicity is defined as *" the effect of being toxic to cells caused by toxic agents is called cytotoxicity. Exposing cells to a cytotoxic compound may result in various outcomes in the cell. At this point, the cells may actively progress into the death phase"* by Erman Salih Istifli and Hasan Basri Ila. Since endodontic sealers aim to seal the root canal, there is direct contact with the tooth and a real proximity with the peri-radicular tissues. Good integration into the body is directly related to their composition and could affect the proliferation of the cells and impact the healing of the apical wound. Cytocompatibility in dentistry and especially in permanent root canal treatment therapy is important to study since the body is going to interact with the materials for good (38,50,57).

1.4.2 Delayed-type hypersensitivity (DTH)

DTH is also known in the medical world as type IV of hypersensitivity reactions. DHT is a sensitivity reaction to a material usually happening after 48 hours and up to 72 hours after the treatment. DHT can happen at subtoxic concentrations. The allergic type IV mechanism is localized on the exposed area (mainly peri radicular tissues for root canal treatment). The cells are usually exposed to the reaction of the immune cells (T cells, macrophages, and monocytes). It is a lymphocyte-mediated reaction more than a mediated antibody damage (type I). Assessing the DHT of the sealer and its material will help with preventing allergic reactions (58–60).

1.4.3 Irritation

Irritation and dermatitis can either happen in the patient or when the dental professional is handling the material (61). The dentist can be irritated and develop contact with urticaria even when using gloves. With poor handling and not using adequate protection, the doctor has a risk of skin reaction (62).

1.4.4 Genotoxicity

A material is defined as genotoxic when it affects the self-repairing process of the cell because it has an impact on the cell genome (63).

1.4.5 Inflammatory response

A dental procedure such as root canal treatment can be the origin of an inflammatory response. For the most part, the treatment can lead to extraversion of the sealer and cause unwanted reactions with the tissues. Investigations outlined that even with the absence of inflammation or infection at the apical area before the treatment, the interaction of the sealer can be at the origin of the inflammation (60). The abnormal presence of the sealer in the tissues will lead to an increase in the polymorphonuclear cells and proinflammatory and anti-inflammatory cytokines. The inflammatory response will evolve polymorphonuclear cells such as lymphocytes and macrophages. Macrophages are a cell population whose role in the immune response benefits homeostasis and so, the repair of inflammation. On another hand, lymphocytes and especially the type T-Lymphocytes have a key role in the immune response helping with the regulation and adaptation of natural immunity. The increase of the T-Lymphocytes in the immunity reaction is followed by the increase of proinflammatory cytokines also called interleukins. Their presence is crucial for the host defenses (82-83).

1.4.6 Osteogenic potential

One of the roles of endodontic treatment is to induce the healing of a possible periapical affectation. Better and faster outcomes of the treatment would also be happening if the sealer had the ability to improve the osteogenesis of the area and its cell proliferation. In order to obtain the osseous and periodontal healing of the patient, the periodontal ligament stem cells need to be able to proliferate and differentiate into osteoblasts. If the sealer is bioactive and demonstrates a positive osteogenic potential, osteoblasts could differentiate faster and ameliorate the result of the endodontic procedure. Since an extrusion of the sealer through the apical foramen is always possible, this close contact with the tissues could benefit the healing process as the sealer would enhance the differentiation of the surrounding cells. (65,66)

Inflammation such as apical periodontitis, usually the reason for a root canal treatment, usually leads to the affection of the tissues and their possible loss. Its self-regeneration is usually insufficient; therefore, treatment is necessary. The proliferation of the human periodontal stem cells opens on their own proliferation and the rise of other types of cells such as osteoblasts, or even progenitor cells. (67)

Thus, the study of the inflammatory response and the osteogenic potential of sealers is essential in order to understand more the benefits of each sealer and enhance their use.

2 Objectives

The goal of this review was to analyze the biocompatibility of the calcium silicate-based sealers and their interaction with the tissues in comparison to the epoxy resin sealers.

- The first objective was to analyze the inflammatory response of the calcium silicate-based sealers in comparison with epoxy resin sealers.

- The second objective was to analyze the cytotoxicity of the calcium silicate-based sealers in comparison with epoxy resin sealers.

3 Materials and methods

In order to conduct the review of the PICOS (Population, Intervention, Comparison, Outcome, Study type) strategy was used. The parameters of the PICO (summarized in table 3) in order to find the question for both research questions were as follow: Population: periodontal tissues, Intervention: calcium-silicate-based sealers; Comparison: epoxy resin sealers. What changed between the two was the Outcome: the inflammatory response for the first research question, and the osteogenic potential for the second research question.

The research questions used for the objectives were as follows:

- *First research question: Are there differences between the new silicate-based sealers and epoxy resin sealers in terms of inflammatory response in the periodontal tissues?*

- *Second research question: Are there differences between the new silicate-based sealers and epoxy resin sealers in terms of osteogenic response in the periodontal tissues?*

Table 3 – Summary of the PICO strategy

Letter	First objective	Second objective
P (Population)	Periodontal tissues	Periodontal tissues
I (Intervention)	Silicate-based sealers	Silicate-based sealers
C (Comparison)	Epoxy resin sealers	Epoxy resin sealers
O (Outcomes)	Inflammatory response	Osteogenic response

The research strategy was carried out using two scientific databases: MedLine (via PubMed) and Web of Science. For the first objective, the research equation was performed on the 16th of March 2023 and the second on the 17th of March 2023. For the second objective, the research question was performed on the 20th of March 2023. A summary of the research equations used, their date, and the databases used, is summarized in Table 4 for the in inflammatory response and Table 5 for the osteogenic response.

Table 4. Summarizing the date of the research, the data base and the search equation used for the inflammatory response

Date of research	Database	Search Equation
16/03/2023	MedLine (via PubMed)	((root canal treatment) OR (epoxy resin sealer)) AND (silicate based sealer)) AND (inflammatory response)
17/03/2023	Web of Science	TS=(root canal filling materials* OR root canal sealer* OR root canalobturation) AND TS=(endodontics) AND TS=(inflammatory response* OR materials testing*)

Table 5. Summarizing the date of the research, the data base and the search equation used for the osteogenic response.

Date of research	Database	Search Equation
20/03/2023	MedLine (via PubMed)	(((root canal treatment) OR (epoxy resin sealer)) AND (silicate based sealer)) AND ((osteogenic response) OR (osteogenic))
20/03/2023	Web of Science	TS=(root canal filling materials* OR root canal sealer* OR root canalobturation) AND TS=(endodontics) AND TS=(osteogenic response* OR materials testing*)

For the first objective, the inclusion criteria were: articles including the inflammatory response of the periodontal ligament cells to calcium-silicate based sealers or to epoxy resins sealers, publication range of 10 years from the 16th of March 2023. The exclusion criteria were articles mentioning experimental sealers unavailable on the market.

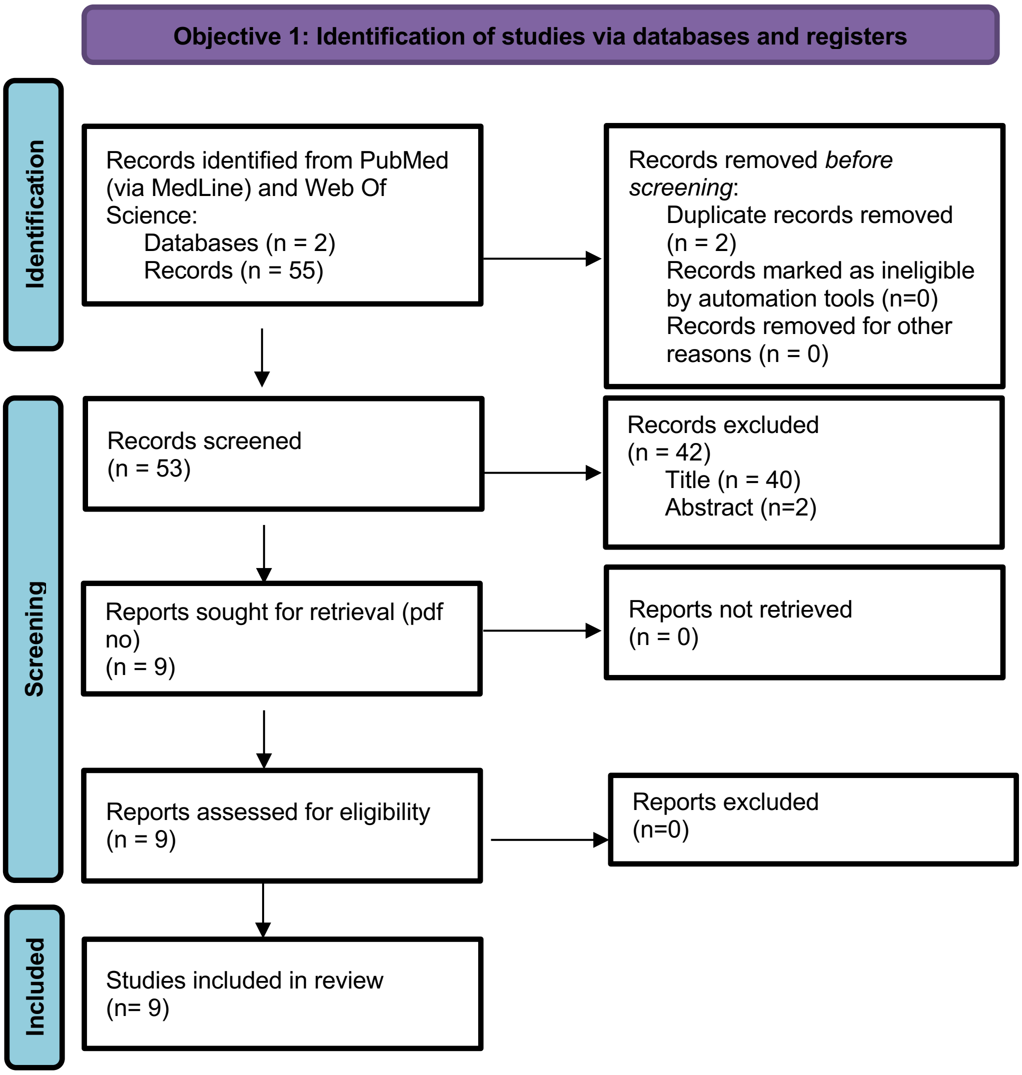
For the second objective, the inclusion criteria established were those articles that included the osteogenic response of the periodontal ligament cells to calcium-silicate-based sealers or to epoxy resins sealers, publication range of 10 years and English language. The exclusion criteria were articles mentioning experimental sealers which are not launched on the market. The research was performed the 20th of March 2023.

Considering the eligibility criteria and in order to execute a further selection, the title, abstract, summary, and full access to the text were considered for both objectives.
The main information selected from the investigations were: authors, publication date, sealers evaluated, control sealers, sample, sealer presentation (fresh or set), results, and conclusion.

4 Results

For the first objective, 55 articles were found in the two databases included in this work. Two were duplicated. Thus, 53 articles were screened by title. Based on their title and the reading of their abstracts, 44 of them were excluded. For instance, 21 articles had their focus on the push-out bond strength and the resistance/microleakages to fractures after the use of different sealers. Among the rest of them were excluded since their motive was such as the use of omega-3 in endodontics treatments, the use of Zinc-based sealers and so neither epoxy nor silicate-based, testing different humidity, the prevention of a second infection, testing different irrigants over a BulkFill resin, the comparison of rotatory instrumentation, the use of chlorhexidine or phosphates, the use of Resilon. Based on the abstract reading of the 11 pre-selected articles, 2 of them were excluded because of the missing sealers of interest or the non-mention of the anti-inflammation potential. After the full reading of 9 articles, they all corresponded to the inclusion criteria without showing any of the exclusion ones. The summary of the research strategy is disposed of in diagram 1.

Diagram 1. PRISMA-type flow chart summarizing the selection process to include the final reviewed articles for the objective 1 (68)



For the second objective, 43 articles were found in the two same databases mentioned before. There were no duplicates. After the screening, 35 were excluded. 31 were, based on the title, 18 articles happened to mainly focus on the bond provided by the cement or its resistance to fractures. Among the others, 2 articles studied immature teeth and so do not fulfill the purpose of this current study. One article's objective was to investigate the benefice of omega-3 on tissues after going through a root canal treatment. Another article's main objective was to compare retreatments and their outcomes depending on the use of rotatory instruments. Both were not beneficial to the development of this review. Others analyze the use of micro brushes and irrigants, sodium hypochlorite on Bulk resins, the addition of phosphate in chlorhexidine, the composition of different sealers, the use of Resilon, and the accuracy of studies on microleakage. Therefore, they would not have been constructive since they were not meeting the inclusion criteria mentioned before. After reading the abstract, one was excluded because of the focus on pulpal cells which does not correspond to the inclusion criteria. 11 of them were selected for their complete reading. After taking into consideration their full text, 4 articles did not outstand the inclusion criteria because of their language or would not serve the purpose of the current review. For reason that their objective was the antibacterial potential of epoxy sealers (not the osteogenic potential) and the use of MTA on the cementum and the canal itself. The motive of one article focuses on the addition of antimicrobial agents inside the sealers, therefore not available on the market as it is an experimenting composition. The last one did not include a comparison with epoxy resin sealers. Among those 11, 7 of them corresponded to the inclusion criteria and were compatible with the review. This method led to the use of 7 articles for the second objective. The summary of the research strategy is disposed of in Diagram 2.

Diagram 2. Chart summarizing the identification and the screening process to include the final reviewed articles for the objective 2 (68)

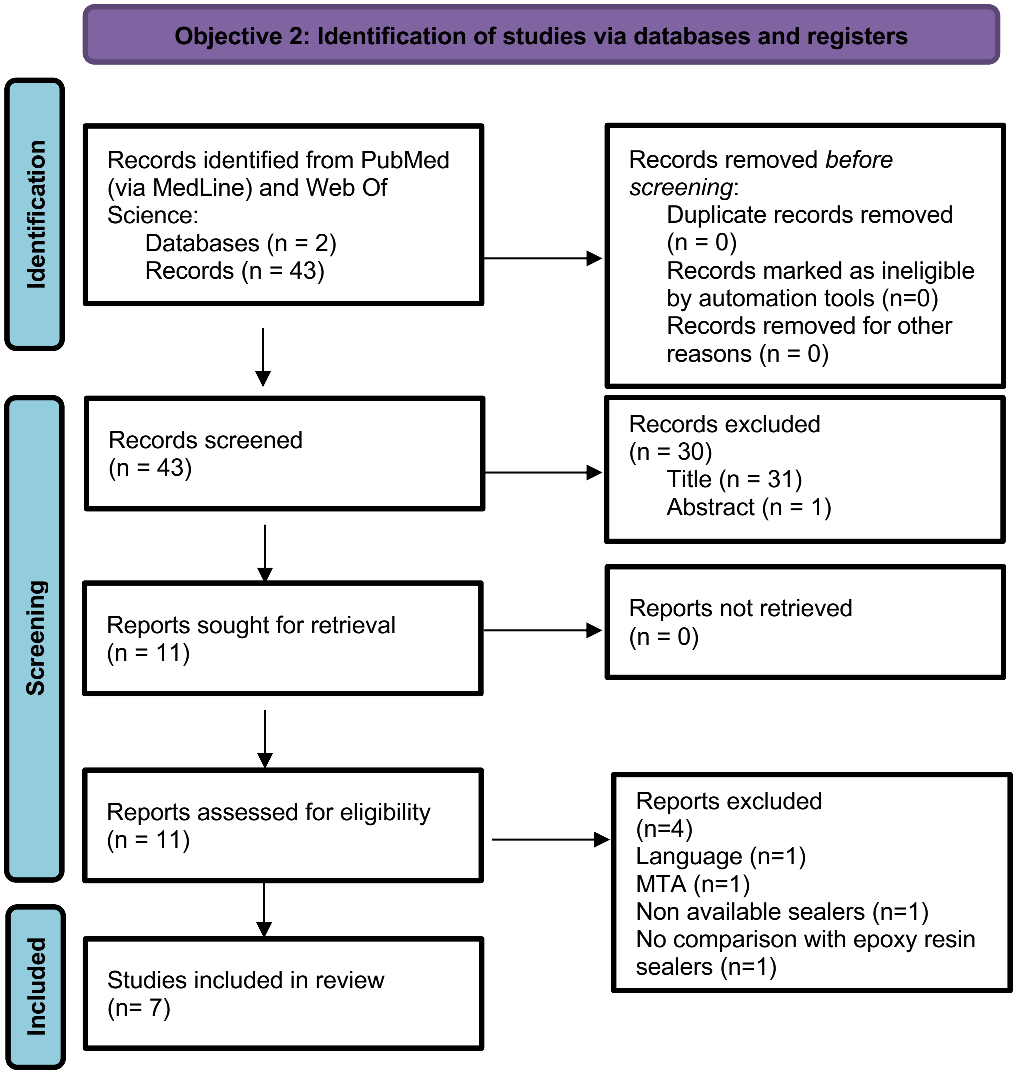


Table 6 summarized the information obtained from the articles included in the review to answer the first objective. The categories included the year of publication, the authors, the sealers mentioned, the cells allowing the test of the inflammatory response, the concentration of the sealers, the test performed to obtain the results, the exposure time, the type of sealer whether it was set or unset (fresh) at the time of the investigation, and the outcomes. The date range of those investigations is 2013 to 2022. The sealers included in the calcium-silicate-based subgroup are BioRootTM RCS, TotalFill, BC Sealer, MTA FillApex, EndoSequence BC, CeraSeal, EndoSeal, ProRoot, EndoSeal MTA, NanoCeramic Sealer, WellRoot ST, SmartPaste Bio. The sealers included in the epoxy resin sealer group are AH Plus, AcroSeal, and AD Seal. The cells used in order to test the inflammatory response of each sealer were: Human periodontal ligament fibroblasts (hPDLFs), Rats' cells for the origin of their mandible, their femur, or the cell line MC3T3-E1 used to evaluate the osteoblast differentiation. The tests performed were an Enzyme-Linked Immunosorbent Assay (ELISA Assay), Real-Time Polymerase Chain Reaction (Real-Time PCR), Histopathological Assay, a study of the thickness of the fibrous plates, and cytokines quantification. All the sealers were evaluated undiluted or with a fourth of their original concentration. The exposure time of the cells to the sealers was from a minimum of 24 hours and up to 90 days. The outcomes highlighted different results regarding the inflammation depending on the sealer's type.

Table 6. Summarizing the year, authors, sealers, cells, concentration, test performed, exposure time, type of cement, and outcomes for the results of the articles assessed for objective 1.

Year	Authors	Silicate-based Sealers	Epoxy resin sealers	Cells	Concentration	Test performed	Exposure time	Type of cement (fresh/set)	Outcomes once assessed
2022	Wuersching et al. (69)	BioRootTM RCS, and TotalFill BC Sealer,	AH Plus	Human periodontal ligament fibroblasts (hPDLFs)	Undiluted	ELISA Assay	24h 7 days	Fresh	AH Plus produces the highest anti-inflammatory response. AH Plus and BioRoot produces increase in PGE2. AH Plus produces an increase of IL-6 especially after a week.

2019	Bin-Na Lee et al. (70)	MTA Fillapex, EndoSequence BC	AH Plus	MC3T3-E1 Osteoblasts precursors	Undiluted	Real-Time PCR	0,1 and 2 days	Fresh	MTA Fillapex and EndoSequence BC help with the inflammatory response while AH Plus produces it.
2019	L. H. Almeida et al. (71)	EndoSequence BC, MTA Fillapex	AH Plus	Cells of rats' femur	Undiluted	Histopathological Assay	7, 30 and 90 days	Fresh	Inflammation decreases after a month in the three sealers. Acute/chronic inflammatory response lower in EndoSequence BC. Mainly moderate for MTA Fillapex after a week. Mild inflammatory reaction for AH Plus.

2020	Hanseul Oh et al. (72)	CeraSeal EndoSeal TCS	AH Plus	hPDLFs	Undiluted	ELISA Assay	1 day	Fresh and Set	AH Plus when fresh produces the highest anti-inflammatory response. When set, all sealers produced similar anti-inflammatory response (IL-6 concentration).
2020	Alexis Gaudin et al.(73)	BioRoot RCS, ProRoot, MTA Fillapex,	AH Plus	hPDLFs	Undiluted	Cytokines quantification Multiplex bead-based assay	1 day	Fresh	AH Plus produces the highest secretion of IL-6 and IL-8, followed by MTA Fillapex. Proroot and Bioroot had a negative inflammatory reaction. BioRoot RCS produces the highest number of anti-

									inflammatory mediators, followed by ProRoot.
2016	Miriam Z Scelza et al. (74)	MTA Fillapex	AH Plus	Cells of rats' mandible	Undiluted	Histopathol ogical Assay	7, 14, and 28 days	Fresh	At first, MTA Fillapex and AH Plus produces an inflammatory response. Higher inflammatory response with AH Plus after 28 days.
2019	Ju Kyung Lee et al. (75)	EndoSeal MTA Nano-ceramic Sealer, Wellroot ST,	AH Plus and AD Seal	hPDLFs	¼ diluted sealer	ELISA Assay	1 day	Fresh	AH Plus produces the highest anti-inflammatory response. Significantly higher than the other sealers.
2016	Carlos Roberto	Smartpast e Bio	AcroSeal	Rats' cells	Undiluted	Fibrous capusule's	7, 15, 30, 60 days	Fresh	AcroSeal shows medium inflammatory response

	Enmernciano et al. (76)					thickness was study			which tends to decrease after one month. Smartpaste Bio shows medium inflammatory response which tends to decrease after two weeks.
2013	Cauana Olivia Tavares et al. (77)	MTA FillApex	AH Plus	Rats’ cells	Undiluted	Histological studies	7 and 60 days		MTA FillApex and AH Plus have shown an anti-inflammatory response. AH Plus more favorable than MTA FillApex.

Table 7 summarizes the articles included to respond to the second objective. The subcategories chosen are the date of release, the name of the authors, the type cells on which the test was performed, the test performed, the time of exposure, the dilution of the sealer used, whether it was fresh or set, and the main outcomes. The publishing dates go from 2016 and up to 2022. The sealers tested are from the calcium-silicate-based group and epoxy resin group. The calcium-silicate-based group contains BioRoot TM, TotalFill, BC, MTA Fillapex, EndoSequence BC, ProRoot, CeraSeal, EndoSeal, AH Plus Bioceramic, Nanoceramic, and WellRoot ST, MTA Fillapex. The epoxy resin sealer tested are AH Plus, AcroSeal and AD Seal. Those diluted or undiluted sealers were tested on Human Periodontal Ligament Stem Cells (hPDLSC), Osteoblast inducers cells, MC3T3-E1, and osteoblast inducers cells, and progenitor cells called Human Mesenchymal Stem Cells (hMSC). The tests performed were Real-Time PCR: alkaline phosphatase (ALP), osteocalcin (OCN), and RUNX2 genes measurements, Fluorescence microscopy of DMP-1 expression, and Alizarin Red Staining (ARS) over a different period from 45 minutes to 21 days. The outcomes once assessed mention the results obtained on whether the sealers happened to present an osteogenic potential or not and their level of mineralization for some of the studies.

Table 7. Summarizing the year, authors, sealers, cells, concentration, test performed, exposure time, type of cement and outcomes for the results of objective 2.

Year	Authors	Silicate-based Sealers	Epoxy resin Sealers	Cells	Concentration	Test performed	Exposure time	Type of cement (fresh/set)	Outcomes once assessed
2022	Wuersching et al. (69)	BioRoot RCS, and TotalFill BC Sealer,	AH Plus	hMSC	Undiluted	Alizarin Red Staining	7 days	Fresh	AH Plus produces the death of all hMSC and did not show any osteogenic potential. BioRoot RCS produces cell differentiation and calcium deposits with a highly positive osteogenic potential.
2019	Lee et al. (70)	MTA Fillapex, EndoSequence BC	AH Plus,	MC3T3-E1	Undiluted	Real-Time PCR (ALP and OCN measurements)	0,1 and 2 days	Fresh	MTA Fillapex and EndoSequence BC induce osteogenicity.

2019	Giacomino et al. (66)	EndoSequence BC, ProRoot,	AH Plus	IDG-SW3 (Osteoblast inducers cells)	Undiluted	Fluorescence microscopy of DMP-1 expression, Real-Time PCR, ARS.	7 days, 21 days,	Fresh	ProRoot and EndoSequence BC, induces mineralization. Osteogenic potential induced more by EndoSequence BC, than ProRoot while AH Plus did not show any.
2020	Oh et al. (72)	CeraSeal, EndoSeal TCS,	AH Plus	hPDLSC	Undiluted	Real-Time PCR (ALP, OCN and RUNX2 genes) ALP and ARS staining	3 and 7, 14 days.	Fresh	OCN and RUNX2 day 7 are, a lot higher for EndoSeal TCS. Unfavorable potential for AH Plus. EndoSeal TCS +++ and CeraSeal induce ostogenicity.
2022	Sanz et al. (78)	AH Plus Bioceramic Sealer,	AH Plus	hPDLSC	Undiluted	Real-Time PCR	3, 7, 14 and 21 days	Fresh	AH Plus Bioceramic Sealer and EndoSequence BC induces the osteogenic potential.

		EndoSequence BC,							AH Plus has a negative osteogenic potential. EndoSequence BC has a higher mineralization.
2019	Lee et al. (75)	EndoSeal MTA, Nano-ceramic Sealer, Wellroot ST	AH Plus, and AD Seal	hPDLSC	Undiluted	Real-Time PCR ALP staining	3, 6, 9 days	Fresh	EndoSeal MTA, Nano-ceramic Sealer and Wellroot ST all positive and similar results for RUNX2 concentration. ALP staining similar and time dependent for all three. Nano-ceramic Sealer has a higher initial potential. AH Plus has a negative potential.

2016	Suciu et al. (79)	MTA FillApex	AH Plus AcroSeal	Human osteoblasts Dental follicle derived adult mesenchymal stem cells	Undiluted	45 minutes, 1, 5, 8, 9, 14 days	Fresh	After 45 minutes, AcroSeal shows the highest number of osteoblasts and Dental follicle derived adult mesenchymal stem cells. AH Plus has the lower amount. After 24 hours, AH Plus has the highest amount of osteoblast and MTA FillApex the highest amount of Dental follicle derived adult mesenchymal stem cells. AcroSeal has a favorable increase of Dental follicle derived adult mesenchymal stem cells after 9 days.

5 Discussion

Epoxy resin sealers and calcium silicate sealers are commonly used in root canal treatments in endodontics. Both sealers have different chemical compositions, setting reactions, and properties, which can affect their biocompatibility. While the epoxy resin AH Plus is known to be a gold standard used by endodontists, its in-vitro biocompatibility could be questionable. (80)

Thus, the two objectives of this work were to analyze the published literature regarding two biocompatibility characteristics, the inflammatory response, and the osteogenic potential of epoxy resin sealers in comparison with the new silicate-based sealers recently launched in the market.

Regarding the first objective, 9 articles that analyzing properties in cells of the periodontal ligament were selected. The studies were all based on the comparison of epoxy resin-containing sealers and calcium silicates-based sealers.

Four authors (69,72,73,75) tested the sealers on Human Periodontal Ligament Fibroblasts cells. These aforementioned cells are a good indicator when comparing the anti-inflammatory properties of the sealers at the periodontal level as they have similar in-vitro characteristics such as osteoblast. (81)
However, the remaining 5 authors (70,71,74,76,77), used rats to study the anti-inflammatory efficacy of the sealers. The rat cells allow the study of the material biocompatibility and the reaction of the tissues and surroundings.(71)

It should be noted that there are limitation between studies that use different methodologies. The the most performed test is the ELISA Assay (69,72,75), followed by the Histopathological Assay (71,74,77). Among the articles, other tests such as Real-Time PCR (70), Cytokines quantification Multiplex bead-based assay (73), and Fibrous capsule’s thickness were performed (76).

Additionally, toxicity levels are different whether the sealer was fresh or set. All 9 articles studied (69–77) fresh sealers and only Hanseul Oh et al. (72) added results of a previously set sealer.

The most frequent time exposure to a sealer was 24 hours and 7 days. Studies included results up to 60 days. Non standardized time could have an impact on the conclusion of each author.

Regarding the epoxy sealer group, three types have been studied AH Plus, AD Seal and AcroSeal (epoxy resin is present in its composition).

Most of the articled included studies of the epoxy resin sealer AH Plus (69–75,77). One article (75) studied AD Seal and also one article (76) studied AcroSeal. All of those epoxy sealers were compared with silicate-based sealers.

When assessing the inflammatory response of the epoxy resin sealers, AH Plus led to a majority consensus about its initial acute inflammatory response being higher than the calcium-silicate sealers when being compared with. (69–76)

Wuersching et al. (69), Scelza et al. (74) and Tavares et al. (77) agreed that the acute temporary response of AH Plus tended to be higher after a week.

On another hand, AD Seal showed a higher cytotoxicity than calcium silicates sealers but significantly lower than AH Plus. AcroSeal shows a medium inflammatory response which tends to decrease after one month but stays more cytotoxic than the calcium silicate sealer Smartpaste bio (75,76).

Among epoxy resin sealers, it has been observed that the epoxy resin sealer AD seal has a lower induce inflammatory response regarding its level of cytokines obtained in the study. AH Plus has a higher inflammatory response than AD Seal. AH Plus is made of 25% and up to 50% of bisphenol A epoxy resin, and AD Seal, is made of less than 20 % epoxy resin. Since it has been mentioned the potential toxicity of this compound earlier in this review, this difference can be the origin of the difference of inflammation among the epoxy resin sealers. (75)

Hanseul et. al. (72) suggested that the inflammatory response obtained might depend on whether the sealer was fresh or set. The unset epoxy sealer AH Plus had shown to lead to a higher anti-inflammatory response. The epoxy resin sealer setting reaction is based on an addition reaction leading to the creation of polymers. This setting reaction is one of the reasons of the acute cytotoxicity of AH Plus.

AH Plus is the sealer generating the most anti-inflammatory response among both groups: calcium-silicates sealers and epoxy resin sealer.

***Regarding the silicate-based sealers*,** the studies included MTA FillApex, BioRoot, ProRoot, TotalFill, Endosequence, CeraSeal, EndoSeal and Nano-ceramic Sealer.

The majority of them, 5 articles evaluated the inflammatory response of MTA FillApex (70,71,73,74,77), 2 articles studied BioRoot (69,73), 1 article studied ProRoot (73), 1 article studied TotalFill (69) 2 studied EndoSequence (70,71) , 1 article studied CeraSeal (72), 1 article studied EndoSeal (72), 1 article studied EndoSeal MTA (75), 1 article studied WellRoot (75), 1 article studied Nano-ceramic Sealer (75) and 1 studied Smartpaste Bio (76).

For MTA FillApex, there is an agreement on the positive inflammatory response of the tissues when in contact with. However, Cauana Olivia Tavares et al. (77) is the only one study of this review disapproving of the initial unfavorable results of AH Plus. The study mentions that AH Plus has more favorable than MTA FillApex. The author calls out their experimental techniques and claims that the possible direct contact of the sealer with the connective tissues can be the origin of an emphasized inflammatory response.
In their article, Almeida et al. (71) introduce the experiment of adding calcium silicate particles inside the MTA FillApex sealer. Without adding those ceramic particles, the

inflammatory response was recorded to be higher. Once adding the particles, the inflammatory response was recorded to be mild. This led to the conclusion that ceramic particles present in the bioceramic sealers are inducing a better response from the tissues surrounding the sealers.

In comparison, It is said that after a day BioRoot produces an increase in PGE2 (Prostaglandin E2) while Alexis Gaudin et al. (73) disagrees mentioning the presence of the highest number of anti-inflammatory mediators produced with BioRoot. Therefore, It has been observed that BioRoot led to an increase of anti-inflammatory mediators and a significantly lower secretion of pro-inflammatory mediators than AH Plus (69,73).

As a comparison with BioRoot, the Calcium-Silicate sealer called ProRoot also had positive outcomes on the inflammatory response, better than AH Plus (73).

In their study, Hanseul Oh et al. (72) commented a better biocompatibility of EndoSeal and CeraSeal since they showed anti-inflammatory response but significantly lower than AH Plus

Regarding EndoSequence, it has been observed the total absence of an acute inflammatory response after 7 days while it was moderate for MTA Fillapex or AH Plus (71,74). In addition, EndoSequence decreased inflammation even with the presence of inflammatory cells. This means that Endosequence might help with inflammation and acts as an inflammatory potential reducer (70,71).

Regarding EndoSeal MTA, Nano-ceramic Sealer, WellRoot, and Seal Apex had shown better results concerting the anti-inflammatory reaction to the tissues than both epoxy resin sealers AD Plus and AH Plus (75,76).
In order to improve the loss of the periodontal tissues due to inflammation just as apical periodontitis, the sealer and its direct contact with the tissues could be helpful if it presents bioactive capacity, and so, the osteogenic potential. Epoxy resin sealers have

been used for many years and are known to be the gold standard. The calcium-silicate sealers are more recent and are known to be biocompatible. (67)
Thus, the second objective of this review was to analyze the published literature regarding the osteogenic potential, of epoxy resin sealers in comparison with the new silicate-based sealers.

Regarding the second objective, 7 articles were reviewed. The studies were all based on the comparison of epoxy resin-containing sealers and calcium silicates-based sealers.
Three articles based their study on Human Periodontal Ligament Stem Cells (72,75,78), 1 article studied progenitor cells (hMSC) (69), 1 study based their results on rat'cells (70), 1 article studied osteoblast inducers cells (66), 1 article studied human osteoblasts (79).

Among the different tests performed, the majority of them being 5 carried out Real-Time PCR (66,70,72,75,78), Alkaline Phosphatase activity measurement and/or Alizarin Red S Staining were performed (66,69,70,72,75,78), 2 articles added osteocalcin measurement (70,72). Finally, 1 article performed fluorescence microscopy (66).

The results obtained were from testing the different sealers using different time frames from 45 minutes to 3 weeks post-exposure. All authors included fresh sealers and studied the osteogenic potential from the exposure to the cells.

Regarding the epoxy resin sealers, the totality of the articles used AH Plus (66,69,70,72,75,78,79). Two articles added another type of epoxy resin sealer AD Seal (75) and AcroSeal (79).
Regarding the calcium-silicate sealers, 2 articles studied MTA FillApex (70,79), 1 article compared BioRoot (69), 1 article studied TotalFill (69), 2 articles studied EndoSequence (70,78), 1 article studied ProRoot (66), Oh et al. studied CeraSeal and EndoSeal (72). 1 article compared EndoSeal MTA, Nano-Ceramic sealer and WellRoot (75). Finally, 1 article studied AH Plus Bioceramic Sealer (78).

Regarding the epoxy resin sealer AH Plus, there is a consensus of the articles mentioning no osteogenic effect or the death of the tested cells reinforcing the idea of the negative osteogenic effect of it (66,69,72,75,78). However, AcroSeal shows a positive osteogenic potential after 9 days on the dental follicle (mesenchymal stem cells) (79).

The epoxy resin sealers happened to have significantly lower to no osteogenic potential when compared to the calcium silicate sealers.

Regarding the calcium-silicates sealers there is an agreement about MTA FillApex and its highly positive osteogenic potential (70,79). As well for EndoSequence where the potential was significantly positive. It was also found the positive mineralization of the area. This happened to be higher than other calcium-silicate based sealer such as ProRoot. (66,70,78).The sealer BioRoot also showed promising results concerning the osteogenic potential with an increase of cells differentiation and positive mineralization of the surface. (69)

When testing the two calcium-silicate sealers EndoSeal and CeraSeal, Hanseul Oh. et al, affirmed the positive potential of both sealers (72). When comparing EndoSeal MTA, Nano-Ceramic sealer and WellRoot, the three of them showed a positive osteogenic potential being initially higher for the Nano-Ceramic one. (75). AH Plus Bioceramic sealer induced comparable positive potential than the previously mentioned sealer EndoSequence (78).

Overall and with an agreement with all the nine articles, the epoxy resin did not show any positive osteogenic potential while all the cited calcium-silicate sealers did.

One of the possible limitations of the present work is that the results show the behavior of the cements in vitro, therefore a review in in vivo studies would be necessary to evaluate the clinical performances.

6 Conclusions

Considering the limitations of the present study, the conclusions of the present work were the following:

- The calcium-silicate-based sealers showed a decreased inflammatory response, especially thanks to their composition. EndoSequence is the sealer inducing the best outcomes. The epoxy resin sealers showed a higher inflammation mainly caused by their compounds and their setting reaction.

- The Epoxy resin sealers demonstrated a negative osteogenic response in the periodontal tissues while the calcium-silicate sealers have all showed a positive osteogenic potential with a potential mineralization of the surface inducing better outcomes of the treatment.

7 References

1. *Bradley H. Gettleman, Moein Sadrkhani, Laurence D. Johns,. Endodontic History [Internet]. AAE American Association of Endodontists; 2021. Available from: https://www.aae.org/specialty/paperpoint/endodontic-history/*

2. *American Association of Endodontists. What is a Root Canal? [Internet]. American Association of Endodontists; 2021. Available from: https://www.aae.org/patients/root-canal-treatment/what-is-a-root-canal/*

3. *Hyson JM. A history of arsenic in dentistry. J Calif Dent Assoc. 2007;35(2):135-9.*

4. *Jaskiran Cheema, Goldy Rathee, Gourav Thapak, et al. Root canal irrigants: A comprehensive review. Int J Community Health Med Res. 2020; 6(2):24- 27. doi: 10.21276/ijchmr*

5. *Abrishami A, Manjarrés V, Gutmann JL. Historical Perspectives on the Evolution of Root Canal Sealer/Cements. J Hist Dent. 2022;70(2):107-18.*

6. *Schäfer E, Schrenker C, Zupanc J,et al. Percentage of Gutta-percha Filled Areas in Canals Obturated with Cross-linked Gutta-percha Core-carrier Systems, Single-Cone and Lateral Compaction Technique. J Endod. 2016;42(2):294-8. doi: 10.1016/j.joen.2015.10.018*

7. *Suciu I, Dimitriu B, Ciocardel M, Chirila M, Amza O, Scarlatescu S, et al. Evaluation of the sealer/gutta-percha ratio on sets of root section surfaces of some extracted teeth sealed using the cold lateral condensation technique. J Med Life. 2021;14(3):337-46. doi: 10.25122/jml-2021-0102*

8. *American Association of Endodontists. Treatment Standards. [internet] USA. 2020; available from: https://www.aae.org/specialty/wp-content/uploads/sites/2/2018/04/TreatmentStandards_Whitepaper.pdf*

9. *Rotstein I, Berman LH, Hargreaves KM. Cohen's Pathways of the Pulp. 11th ed. Elsevier; 2016.*

10. *Chen G, Chang YC. Effect of Immediate and Delayed Post Space Preparation on Apical Leakage Using Three Root Canal Obturation Techniques After Rotary Instrumentation. J Formos Med Assoc. 2011;110(7):454-9. doi: 10.1016/S0929-*

6646(11)60067-3

11. Ortiz-Blanco B, Sanz JL, Llena C, Lozano A, et al. Dentin Sealing of Calcium Silicate-Based Sealers in Root Canal Retreatment: A Confocal Laser Microscopy Study. J Funct Biomater. 2022;13(3):114. doi: 10.3390/jfb13030114

12. Mohammed Sulaiman Alaenazi, Saeed Saad Al-Qahtani, Dr. Hmoud Ali Algarn, et al. Contemporary Endodontic Sealers. Journal of Health, Medicine and Nursing. 2018; (46)42-52.

13. Kerr Dental. Instruction for Use, Pulp Canal Sealer & Pulp Canal Sealer EWT IFU [Internet]. Kerr Dental. 2021. Available from: https://www.kerrdental.com/resource-center/pulp-canal-sealer-pulp-canal-sealer-ewt-ifu

14. Komabayashi T, Colmenar D, Cvach N, et al. Comprehensive review of current endodontic sealers. Dent Mater J. 2020; 39(5):703-20. doi: 10.4012/dmj.2019-288

15. GC America Inc. Product Catalog [Internet]. USA. 2016. Available from: https://www.gcamerica.com/GCA_Catalog-2016_10-02-iPad.pdf

16. Mark Dzendzel. Annex VII EC Declaration of Conformity, Endodontic Root Canal Sealers Endo-010 [Internet]. USA Kerr Dental; [2021; cited 2023 January]. Available from: https://www.kerrdental.com/en-eu/endodontics-products/sealapex-endodontic-obturation-fill#docs

17. Watson TF, Atmeh AR, Sajini S, et al. Present and future of glass-ionomers and calcium-silicate cements as bioactive materials in dentistry: Biophotonics-based interfacial analyses in health and disease. Dent Mater J. 2014;30(1):50-61. doi: 10.1016/j.dental.2013.08.202

18. Mohammadi Z, Shalavi S. Clinical applications of glass ionomers in endodontics: a review. Int Dent J. 2012 Oct;62(5):244-50. doi: 10.1111/j.1875-595x.2012.00125.x.

19. Saygili G, Saygili S, Tuglu I, Capar ID. In Vitro Cytotoxicity of GuttaFlow Bioseal, GuttaFlow 2, AH-Plus and MTA Fillapex. Iran Endod J. 2017; 12(3): 354-359. doi: 10.22037/iej.v12i3.15415

20. Attash I, AL-Ashou W. The Effect of Smear Layer on the Push-out Bond Strength of Silicone-based Root Canal Sealers. Al-Rafidain Dent J. 2022;22(2):313-22. doi: 10.33899/rdenj.2021.130285.1106

21. Coltene Duo. GuttaFlow 2, Cold Flowable Gutta-Percha Manuel of instruction of

Guttaflow 2 [Internet]. WhaleDent, USA. [unknown date, cited 2023 Apr]. Available from: https://nam.coltene.com/fileadmin/Data/NAM/Endodontics/30588A_GuttaFlow_2_bro.pdf

22. Kharouf N, Sauro S, Hardan L, Fawzi A, Suhanda IE, Zghal J, et al. Impacts of Resveratrol and Pyrogallol on Physicochemical, Mechanical and Biological Properties of Epoxy-Resin Sealers. Bioengineering. 2022;9(3):85. doi: 10.3390/bioengineering9030085

23. Kim YK, Grandini S, Ames JM, et al. Critical Review on Methacrylate Resin–based Root Canal Sealers. J Endod. 2010;36(3):383‑99. doi: 0.1016/j.joen.2009.10.023

24. Deniz Sungur D, Purali N, Coşgun E, et al. Push-out bond strength and dentinal tubule penetration of different root canal sealers used with coated core materials. Restor Dent Endod. 2016;41(2):114. doi: 10.5395/rde.2016.41.2.114

25. Pandey P, Aggarwal H, Tikku AP, et al. Comparative evaluation of sealing ability of gutta percha and resilon as root canal filling materials- a systematic review. J Oral Biol Craniofacial Res. avr 2020;10(2):220‑6. doi: 10.1016/j.jobcr.2019.12.004

26. Shokouhinejad N, Sabeti M, Gorjestani H, et al. Penetration of Epiphany, Epiphany Self-Etch, and AH Plus into Dentinal Tubules: A Scanning Electron Microscopy Study. J Endod. sept 2011;37(9):1316‑9. doi: 10.1016/j.joen.2011.05.002

27. Shrestha D, Wei X, Wu WC, et al. Resilon: a methacrylate resin-based obturation system. J Dent Sci. 2010;5(2):47‑52. doi: 10.1016/S1991-7902(10)60008-6

28. Sarangi P, Mallick R, Satapathy S, et al. An In vitro Comparison of pushout bond strength of resilon with MetaSEAL and AH plus sealers. Contemp Clin Dent. [internet] 2017;8(4):613. doi: 10.4103/ccd.ccd_666_17

29. Schäfer E, Bering N, Bürklein S. Selected physicochemical properties of AH Plus, EndoREZ and RealSeal SE root canal sealers. Odontology J. 2015;103(1):61‑5. doi: 10.1007/s10266-013-0137-y

30. Silva EJ, Hecksher F, Vieira VT, Vivan RR, Duarte MA, Brasil SC, Antunes HS. Cytotoxicity, antibacterial and physicochemical properties of a new epoxy resin-based endodontic sealer containing calcium hydroxide. J Clin Exp Dent. 2020 Jun 1;12(6):533-539. doi: 10.4317/jced.56534.

31. Lee JK, Kwak SW, Ha JH, et al. Physicochemical Properties of Epoxy Resin-Based and Bioceramic-Based Root Canal Sealers. Bioinorg Chem Appl. 2017. doi:

10.1155/2017/2582849

32. Zhou H min, Shen Y, Zheng W, et al. Physical Properties of 5 Root Canal Sealers. J Endod. 2013;39(10):1281-6. doi: 10.1016/j.joen.2013.06.012

33. Ashraf H, Shafagh P, Mashhadi Abbas F, et al. Biocompatibility of an experimental endodontic sealer (Resil) in comparison with AH26 and AH-Plus in rats: An animal study. J Dent Res Dent Clin Dent Prospects. 2022;16(2):112-7. doi: 10.34172/joddd.2022.019

34. Dentsply International, Inc Dentsply Maillefer. Product catalog. [Internet]. USA. 2016. Available from: https://www.maillefer.com/wp-content/uploads/2016/10/2016-Maillefer-Catalog.pdf

35. Donnermeyer D, Bürklein S, Dammaschke T, et al. Endodontic sealers based on calcium silicates: a systematic review. Odontology. 2019;107(4):421-36. doi: 10.1007/s10266-018-0400-3. Epub 2018 Dec 15

36. Camilleri J, éditeur. Mineral Trioxide Aggregate in Dentistry: From Preparation to Application [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014. doi: 10.1007/s10266-018-0400-3

37. Ismail S, Fayyad DM, Eldaharawy M, et al. Effect of two calcium-silicate sealers and a resin sealer on collagen matrix integrity of root dentin after different treatments. An in vitro and in vivo study. Saudi Endod J. 2022;12(1):67. doi: 10.4103/sej.sej_57_21

38. Zhou H min, Du T feng, Shen Y,et al. In vitro cytotoxicity of calcium silicate-containing endodontic sealers. J Endod. janv 2015;41(1):56-61. doi: 10.1016/j.joen.2014.09.012

39. Lim M, Jung C, Shin DH, Cho Y bum, Song M. Calcium silicate-based root canal sealers: a literature review. Restor Dent Endod. 2020;45(3). doi: 10.5395/rde.2020.45.e35

40. Rawtiya M, Verma K, Singh S, et al. MTA-Based Root Canal Sealers. Yadav NS, editor. J Orofac Res. 2013;(3):16-21. doi: 10.5005/jp-journals-10026-1057

41. Jafari F, Jafari S. Composition and physicochemical properties of calcium silicate based sealers: A review article. J Clin Exp Dent. 2017;9(10). doi: 10.4317/jced.54103

42. EndoSequence, BC SEALER, Instruction for use. [internet]. Brasseler U.S.A.. Available from: https://brasselerusadental.com/wp-

content/uploads/sites/9/2018/01/IFU-0018_Brasseler_USA_ES_BC_Sealer_IFU_REV_C.pdf

43. Silva EJNL, Carvalho NK, Prado MC, et al. Push-out Bond Strength of Injectable Pozzolan-based Root Canal Sealer. J Endod. 2016;42(11):1656-9. doi: 10.1016/j.joen.2016.08.009

44. Cutajar A, Mallia B, Abela S, et al. Replacement of radiopacifier in mineral trioxide aggregate; characterization and determination of physical properties. Dent Mater J. 2011;27(9):879-91. doi: 10.1016/j.dental.2011.04.012

45. Moazami F, Naseri M, Malekzadeh P. Different Application Methods for Endoseal MTA Sealer: A Comparative Study. Iran Endod J. 2020;15(1):44-9. doi: 10.22037/iej.v15i1.27568

46. Adl A, Sadat Shojaee N, Pourhatami N. Evaluation of the Dislodgement Resistance of a New Pozzolan-Based Cement (EndoSeal MTA) Compared to ProRoot MTA and Biodentine in the Presence and Absence of Blood. 2019:1-6. doi: 10.1155/2019/3863069

47. EndoSeal MTA, product catalog [Internet]. Maruchi USA. [unknown date, cited Apr 2023]. Available from: https://maruchiusa.com/pages/endoseal-mta

48. Jafari F, Jafari S. Composition and physicochemical properties of calcium silicate based sealers: A review article. J Clin Exp Dent. 2017;e1249-55. doi: 10.4317/jced.54103.

49. Ashour A. Factors affecting the success of endodontic treatment (surgical or nonsurgical): a brief review. Int J Med Dev Ctries. 2019;3(9):730–733. doi: 10.24911/IJMDC.51-1548175808

50. Alves Silva EC, Tanomaru-Filho M, da Silva GF, et al. Biocompatibility and Bioactive Potential of New Calcium Silicate-based Endodontic Sealers: Bio-C Sealer and Sealer Plus BC. J Endod. oct 2020;46(10):1470-7. doi: 10.1016/j.joen.2020.07.011

51. Kohsar AH, Hasani M, Karami M, et al. Subcutaneous Tissue Response to Adseal and Sure-Seal Root Sealers in Rats: a Histopathological Study. Maedica. 2022;17(3):654-61. doi: 10.26574/maedica.2022.17.3.654

52. Kapralos V, Böcker J, Vach K, et al. On the biocompatibility of endodontic sealers. Swiss Dent J. 2022;132(9):586-97.

53. U.S. Department of Health and Human Services Food and Drug Administration Center for Devices and Radiological Health Center for Biologics Evaluation and Research.

Use of International Standard ISO 10993-1, « Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process », Guidance for Industry and Food and Drug Administration Staff [Internet]. USA. 2020. Available from: https://www.fda.gov/media/85865/download

54. American Dental Association. Evaluation of biocompatibility of medical devices used in dentistry [Internet]. USA. 2008. Available from: http://161.200.36.106/upload/images2/dent-bio-material/cytotoxicitytest/ISO7405.pdf

55. Özdemir O, Kopac T. Cytotoxicity and biocompatibility of root canal sealers: A review on recent studies. J Appl Biomater Funct Mater. 2022. doi: 10.1177/22808000221076325.

56. Washio A, Morotomi T, Yoshii S, et al. Bioactive Glass-Based Endodontic Sealer as a Promising Root Canal Filling Material without Semisolid Core Materials. Mater Basel Switz. 2019;12(23):3967. doi: 10.3390/ma12233967

57. Salih Istifli E, Tahir Hüsunet M, Basri Ila H. Cell Division, Cytotoxicity, and the Assays Used in the Detection of Cytotoxicity. IntechOpen. 2019. doi: 10.5772/intechopen.88368

58. Melvin Berger Stuart L Abramson, Michael A Kaliner. Delayed Hypersensitivity Reactions Clinical Presentation. Medscape Drug Reference [Internet]. USA. 2018. Available from: https://emedicine.medscape.com/article/136118-overview

59. Pemberton MN, Gibson J. Chlorhexidine and hypersensitivity reactions in dentistry. Br Dent J. 2012;213(11):547-50. doi: 10.1038/sj.bdj.2012.1086

60. Bratel J, Jontell M, Dahlgren U, et al. Effects of root canal sealers on immunocompetent cells in vitro and in vivo. Int Endod J. 1998;31(3):178-88. doi: 10.1046/j.1365-2591.1998.00148.x

61. Christian Gomes Moura C, Cristina Cunha T,et al. A study on biocompatibility of three endodontic sealers: intensity and duration of tissue irritation. Iran Endod J. 2014;9(2):137-43.

62. Afzal Ali, Meltem Sümbüllü, Aasima İshaq, et al. Localized contact urticaria due to epoxy resin-based endodontic sealer. Saudi Endod J. 2022; 12(2): 222-226. doi: 10.4103/sej.sej_251_21

63. Brzovic V, Miletic I, Zeljezic D, et al. In vitro genotoxicity of root canal sealers. Int Endod J. 2009;42(3):253-63. doi: 10.1111/j.1365-2591.2008.01510.x

64. Zhang JM, An J. Cytokines, Inflammation, and Pain. Int Anesthesiol Clin. 2007;45(2):27-37. doi: 10.1097/AIA.0b013e318034194e

65. Winning L, Robinson L, Boyd AR, et al. Osteoblastic differentiation of periodontal ligament stem cells on non-stoichiometric calcium phosphate and titanium surfaces: Osteoblastic Differentiation of PDLSCs on Modified Ti Surfaces. J Biomed Mater Res A. 2017;105(6):1692-702. doi: 10.1002/jbm.a.36044

66. Giacomino CM, Wealleans JA, Kuhn N, et al. Comparative Biocompatibility and Osteogenic Potential of Two Bioceramic Sealers. J Endod. 2019;45(1):51-6. doi: doi: 10.1016/j.joen.2018.08.007

67. Pejcic A, Kojovic D, Mirkovic D, Minic I. Stem cells for periodontal regeneration. Balk J Med Genet BJMG. 2013;16(1):7-12. doi: 10.2478/bjmg-2013-0012

68. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ.[internet] 2021;n71.

69. Wuersching SN, Diegritz C, Hickel R, et al. Comprehensive in vitro comparison of the biological and physicochemical properties of bioactive root canal sealers. Clin Oral Investig. 2022;26(10):6209-22. doi: 10.1007/s00784-022-04570-2

70. Lee BN, Hong JU, Kim SM, et al. Anti-inflammatory and Osteogenic Effects of Calcium Silicate–based Root Canal Sealers. J Endod. 2019;45(1):73-8. doi: 10.1016/j.joen.2018.09.006

71. Almeida LH, Gomes APN, Gastmann AH, Pola NM, et al. Bone tissue response to an MTA -based endodontic sealer, and the effect of the addition of calcium aluminate and silver particles. Int Endod J. oct 2019;52(10):1446-56. doi: 10.1111/iej.13135

72. Oh H, Kim E, Lee S, et al. Comparison of Biocompatibility of Calcium Silicate-Based Sealers and Epoxy Resin-Based Sealer on Human Periodontal Ligament Stem Cells. Materials. 2020;13(22):5242. doi: 10.3390/ma13225242

73. Gaudin A, Tolar M, Peters OA. Cytokine Production and Cytotoxicity of Calcium Silicate–based Sealers in 2- and 3-dimensional Cell Culture Models. J Endod. 2020;46(6):818-26. doi: 10.1016/j.joen.2020.03.011

74. Scelza MZ, Campos CA, Scelza P, et al. Evaluation of Inflammatory Response to Endodontic Sealers in a Bone Defect Animal Model. J Contemp Dent Pract. 2016;17(7):536-41.

75. Lee JK, Kim S, Lee S, et al. In Vitro Comparison of Biocompatibility of Calcium Silicate-Based Root Canal Sealers. 2019;12(15):2411. doi: 10.3390/ma12152411

76. Bueno CRE, Valentim D, Marques VAS, et al. Biocompatibility and biomineralization assessment of bioceramic-, epoxy-, and calcium hydroxide-based sealers. Braz Oral Res. 2016;30(1). doi: 10.1590/1807-3107BOR-2016.vol30.0081

77. Tavares CO, Böttcher DE, Assmann E, et al. Tissue Reactions to a New Mineral Trioxide Aggregate–containing Endodontic Sealer. J Endod. 2013;39(5):653-7. doi: 10.1016/j.joen.2012.10.009

78. Sanz JL, López-García S, Rodríguez-Lozano FJ, et al. Cytocompatibility and bioactive potential of AH Plus Bioceramic Sealer: An in vitro study. Int Endod J. oct 2022;55(10):1066-80. doi: 10.1111/iej.13805

79. Sucio I, Soritau O, Gheorghe I, et al. Biocompatibility Testing on Cell Culture of some Root Canal Sealers used in Endodontics. Romanian Biotechnological Letters, 2016;21(3): 11543-11549.

80. Huang TH, Yang JJ, Li H, Kao CT. The biocompatibility evaluation of epoxy resin-based root canal sealers in vitro. Biomaterials. 2002;23(1):77-83. doi: 10.1016/s0142-9612(01)00081-3

81. Basdra EK, Komposch G. Osteoblast-like properties of human periodontal ligament cells: an in vitro analysis. Eur J Orthod. 1997;19(6):615-21. doi: 10.1093/ejo/19.6.615

82. Ma Z, Wang Y, Zhu X, Zhang C, Li S, Jin L, et al. Role of Polymorphonuclear Neutrophils in the Clearance of Enterococcus faecalis Derived from Saliva and Infected Root Canals. Journal of Endodontics. 2011; 37(3):346–52.

83. KOHSAR AH, HASANI M, KARAMI M, MOOSAZADEH M, DASHTI A, SHIVA A. Subcutaneous Tissue Response to Adseal and Sure-Seal Root Sealers in Rats: a Histopathological Study. Maedica - a Journal of Clinical Medicine. 2022;17(3):654–61.