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# Biocompatibility of Bioceramic Root Canal Sealers: A Review

***Abstract* —** The advantageous technology in endodontics nowadays are bioceramic root canal sealers. The most favourable characteristics of bioceramics root canal sealers are biocompatibility, osteoinductivity and sealing ability. Advantageous characteristics and good bioceramic outcomes make them increasingly presented in the dental market, suitable for use in endodontics. However, the biocompatibility of recently developed bioceramic root canal sealers is not well understood due to the limited scientific evidence and methodological differences in the previous studies. Literature search was conducted from August 2020 until April 2021 through PubMed, Google Scholar and Scopus databases using the combination of terms such as "bioceramic root canal sealers", "biocompatibility", "root canal sealer", "cytotoxicity" and "endodontics" to identify the most relevant articles from year 1991 until 2020. This article aimed to review the bioceramic root canal sealers with regards to the use in endodontics, biocompatibility, and *in vivo* studies. Bioceramic root canal sealers have a variable toxic potential at the cellular and tissue level, based on the current data. The methodological variability of the studies included in this study, as well as the somewhat contradictory findings, make it impossible to draw a conclusion about which type of bioceramic root canal sealer is more biocompatible. Hence, bioceramic root canal sealers were discovered to be biocompatible and comparable to other commercially available root canal sealers.

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## 1 INTRODUCTION

In the early 1990s, the introduction of bioceramic materials as a new category of dental materials can be considered as one of the most notable developments in dental practice. Bioceramics are ceramic materials that have been specially developed for medical and dental use, which included calcium phosphates, alumina, glass ceramics, bioactive glass, hydroxyapatite and zirconia [1]. Ceramics are defined by the American Ceramic Society as mineral, non-substances with a crystalline structure. Ceramics are substances between metals and non-metals, including alumina (aluminum and oxygen combination), calcia (calcium and oxygen combination), and nitride (silicon and nitrogen combination) [2]. Bioactive bioceramics respond with tissue components and can be either bioresorbable, such as calcium phosphate bone replacements or non-bioresorbable, such as calcium silicate or hydraulic cements used in endodontics. The objective of endodontic treatment is to completely fill the root canal space in three dimension and eliminate infection of the root canal from penetration of liquids and microorganisms [3]. The American Dental Association recognized endodontics as a specialty in 1963 and it has been practiced since 200 BC [4]. It is an alternative treatment for severely damaged teeth where the pulp tissue has been infected or died, therefore the tooth is not removed and may continue to function in the mouth. The absence of symptoms such as pain, swelling and sinus tract in teeth without radiographic evidence of periodontal involvement is a sign of the success of treatment [5].

The bioactivity of compounds refers to its ability to generate a coating of hydroxyapatite when in contact with tissue fluid rich in calcium and phosphate. This property makes the material strongly biocompatible, osteoinductive, osteoconductive and contributes to its ability to seal. Dr. M. Torabinejad developed the first generation of bioceramic in endodontics in 1990s,

which was mineral trioxide aggregate (MTA) [6]. One major weakness of MTA is its inability to perform in its pure form as an endodontic sealer. MTA's coarse particle size ranging from 1.5 to 160 micrometers (μm) does not allow it to be dispensed in a mixture that will flow properly. In addition, there is still limited evidence on the potential cytotoxicity of bioceramic root canal sealers and no empirical evidence for its clinical use.

Generally, root filling materials must seal the root canal wall both laterally and apically to prevent the entry of microorganisms or tissue fluids into the root canal system. In an effort to achieve this goal, several root canal sealers have been used in root canal treatment [7]. The properties for a good root canal sealer include being tissue tolerant, possessing antimicrobial activities, ability to provide bacteria-resistant seal and provide good adhesion. It should not be harmful to hard or soft tissues if it overflows into the periapical region [8]. Thus, biocompatibility could be an important factor in choosing the right type of bioceramic root canal sealers for different types of endodontic cases. Biocompatibility is a necessary feature of any root canal sealer since the root filling material has direct contact with the essential tissue at the root apical and lateral foramina or indirectly by surface reconstruction [9]. In other terms, a material is said to be biocompatible if an adverse reaction such as irritation, allergy, toxicity, inflammation, or carcinogenicity is not caused by the material encountering the tissue.

The obturation procedure is the final stage in endodontic treatment [7]. Even though all efforts focus on confining the root filling materials inside the root canal, some extrusion occurs unintentionally during the obturation process. The extrusion of root filling material has been a subject of debate for several years [10]. It may cause chronic inflammation of the periradicular tissues and can result in delayed wound healing manifested as discomfort, tenderness, and swelling of the affected area if the root filling material comes into contact with soft and hard tissues apically [3]. On the contrary, the extrusion of root filling material ensures patency of the root canal terminus, thus the presence of apical blockage can be verified [11].

In the recent years, the use of this material for obturating the root canal system has been the topic of concern of many researchers. This is due to its property of being able to promote hard tissue formation during healing process, making it a promising material in endodontics. However, the use of some materials in clinical practice has not been supported by robust scientific findings. In the past studies, the evaluation of various bioceramic root canal sealers have not been done simultaneously. The focus of past research was on comparing one or two bioceramic root canal sealers to the conventional root canal sealers with lack of standardization in the cell lines and method of assessing cell viability. In addition to that, biocompatibility of this material through *in vivo* approach has not been thoroughly investigated. Unlike the *in vitro* approach, the *in vivo* is less popular among the researchers which could be due to the complex and more time-consuming procedure but this approach worth investigating to warrant clinical studies in the future.

In the past, conventional root filling material such as gutta-percha was used during obturation procedure. However, in current practice, the use of bioceramic root canal sealers has been the interest of many researchers. Various bioceramic root canal sealers are available in the market such as GuttaFlow Bioseal (Colténe/Whaledent AG, Altstatten, Switzerland), MTA Fillapex (Angelus, Londrina, Brazil), CeraSeal Bioceramic root canal sealer (MetaBiomed Cheongju, Korea), iRoot SP root canal sealer (Innovative BioCeramix Incorporated, Vancouver, Canada), Ortho MTA (BioMTA, Seoul, Republic of Korea) and Dia-Root Bio Canal Sealer (Diadent Group International, Cheongju-si, Republic of Korea). This could be attributed to the excellent physiochemical and biological properties of bioceramic root canal sealers for dental application [12]. In general, both *in vitro* and *in vivo* studies are helpful in providing the preliminary observation on biocompatibility of these recently introduced bioceramic root canal sealers.

## 2 METHODS

We conducted a review from August 2020 until April 2021 through PubMed, Google Scholar and Scopus online databases using the following key words: bioceramic root canal sealers, biocompatibility, root canal sealer, cytotoxicity, and endodontics. The most relevant articles from

year 1991 until 2020 were discovered. The following limits were placed: English language, review, and book chapter. A total of seventy-five articles fit the selection criteria and were reviewed by the authors.

## 3 BIOCERAMIC ROOT CANAL SEALERS

After their initial discovery in the early twentieth century, the chemical and physical properties of bioceramic root canal sealers have gained considerable attention [13]. The quality of root canal sealers is important because certain sealers cause tissue reaction and increase inflammation of the tissue [8]. Root canal sealers are categorized by their major chemical constituents such as zinc oxide eugenol, calcium hydroxide, glass ionomer, silicone, resin, and bioceramics [13]. Table I provides examples of the compositions of the materials.

**Table I**: The Compositions of Bioceramic Root Canal Sealers

| Material | Compositions and manufacturer | | Reference |
|---|---|---|---|
| | Compositions | Manufacturer | |
| GuttaFlow Bioseal | Gutta-percha, zinc oxide, barium sulfate, polydimethylsiloxane, ceramic bioactive glass, zirconia, catalysis of platinum, pigments of color, micro silver | Coltène/Whaledent AG, Altstatten, Switzerland | [8] |
| MTA Fillapex | Salicylate resin, diluting resin, natural resin, bismuth oxide, nano-particulated silica, MTA, pigments | Angelus, Londrina, Brazil | [8] |
| CeraSeal Bioceramic root canal sealer | Calcium silicate's chemical reaction produce crystallization of calcium hydroxide | MetaBiomed Cheongju, Korea | [14] |
| iRoot SP root canal sealer | Zirconium oxide, calcium silicates, calcium phosphate, calcium hydroxide, filler, and thickening agents | Innovative BioCeramix Incorporated, Vancouver, Canada | [15] |
| Ortho MTA | Calcium carbonate, silicon dioxide, aluminum oxide, dibismuth trioxide [MSDS] | BioMTA, Seoul, Republic of Korea | [16] |
| Dia-Root Bio Sealer | Calcium Silicate, Calcium Aluminate, Ytterbium Trifluoride, Zirconium Oxide, Silanamine, 1,1,1-trimethyl-N-(trimethylsilyl)-, hydrolysis products with silica, Hydroxypropyl Methylcellulose, Polyethylene glycol 400, Polyethylene glycol 200, Sorbitan, White Mineral Oil | Diadent Group International, Cheongju-si, Republic of Korea. | [17] |

### 3.1 GuttaFlow Bioseal

GuttaFlow is the first flowable, unheated gutta-percha that does not shrink but expands slightly during setting, resulting in an outstanding seal as claimed by the manufacturer. GuttaFlow also exhibits excellent adhesion to both the gutta-percha point (masterpoint) and the dentine wall. Meanwhile, GuttaFlow 2 (Coltène/Whaledent AG, Altstatten, Switzerland) is a revolutionary root canal filling system that combines two materials in one, gutta-percha in powder form with particle size under 30 µm, and sealer [18]. This modern root filling materials operates with cold free-flow gutta-percha.

GuttaFlow Bioseal contains a mixture of gutta-percha, root canal sealer and bioactive substances in which the manufacturer claims that this material can promote regeneration of hard tissue [8]. It is implemented with the development of ceramic technology and was introduced in the

market in the end of 2015, but its efficacy is still unknown due to lack of scientific evidence. GuttaFlow Bioseal is a non-heated flowable type of root filling material and has bioactive materials with the property of bonding with the surrounding tissue in order to facilitate healing. GuttaFlow Bioseal is a smart novel obturation material that can do more than seal and fill the root canal on the market. The bioactive substance supplies natural repair constituents such as calcium and silicates when in contact with fluids. This also stimulates biochemical pathways in the root canal, which provide additional support for regeneration. The advantages of GuttaFlow Bioseal include shorter working time and curing time than GuttaFlow 2 [8]. GuttaFlow Bioseal also provides free-flow gutta-percha with a suitable sealer at room temperature according to the manufacturer's instructions [8]. Nevertheless, GuttaFlow Bioseal showed lower push out bond strength compared to AH Plus (Dentsply, DeTrey, Konstanz, Germany) [19].

The cytotoxicity of GuttaFlow Bioseal have been investigated in the previous studies [8, 18, 20] and the findings showed that the cytotoxicity of GuttaFlow Bioseal and Gutta Flow 2 was lower than MTA Fillapex and AH plus. Due to the excellent properties, GuttaFlow Bioseal is one of the promising materials in endodontics. However, more analysis needs to be carried out in various fields such as *in vivo* studies to support future clinical application.

### 3.2 Mineral Trioxide Aggregate (MTA) Fillapex

Due to the positive biological response to MTA, endodontic sealers based on this chemical composition have been proposed, such as MTA Fillapex (Angelus, Londrina, Paraná, Brazil) [21]. MTA Fillapex was successfully developed in 2010, at trial to combine the biological and sealing properties of MTA cements and the manufacturer states that MTA Fillapex is easy to manage and has excellent operating time and flow, excellent radiopacity and solubility [22]. However, another study was conducted to evaluate the effect of retreatment on the bond strength of MTA Fillapex and AH Plus. The results show that, MTA Fillapex has lower bond strength compared to AH Plus. This finding indicates that retreatment with rotary files and chloroform has no statistically significant effect on the adhesive strength of these sealers [23]. In comparison to standard MTA, MTA Fillapex is composed of salicylate resin, natural resin, diluting resin, bismuth oxide, nanoparticulated silica, MTA, and pigments and is formulated as a paste sealer in a form that can facilitate its proper insertion into the root canal system [18].

MTA Fillapex shows conflicting biocompatibility results; it is cytotoxic in cell lines as reported in numerous studies [18, 20, 21, 24-27] and less cytotoxic in a study by [28] due to the differences in cell types used, experimental conditions, and media. The cytotoxicity of MTA Fillapex could be attributed to the presence of toxic components such as salicylate resin and diluting resin in its formulation [8, 18, 20, 29],. The differences in results reported by these studies could be due to the use of different types of cell lines and various methods of assessing cytotoxicity.

### 3.3 CeraSeal Bioceramic Root Canal Sealer

CeraSeal is a bioceramic root canal sealer of the next decade that has excellent sealing capability and biocompatibility. CeraSeal Bioceramic root canal sealer contains calcium hydroxide ($Ca(OH)_2$) with high pH, zirconium oxide and thickening agent which can produce antimicrobial activity that helps in killing microorganisms [14] . CeraSeal Bioceramic root canal sealer is a premixed sealer which can be used easily in daily practice. The new generation of root canal sealers should be biocompatible and well-tolerated by the periradicular tissue, however it remains uncertain if these modern root canal sealers are comparable to traditional sealers in terms of biocompatibility [30].

There are few published studies on the use of this new root canal sealer [14, 30, 31]. This is a topic worth discussing in dental science and clinical practice to warrant its use in endodontics treatment.

### 3.4 iRoot SP Root Canal Sealer

iRoot SP root canal sealer is an aluminium-free bioceramic root canal sealer [13], containing tri- and di-calcium silicates, zirconium oxide and calcium phosphate [12]. The manufacturer claims that this root canal sealer can form hydroxyapatite during the setting process and eventually build a chemical bond between the dentinal wall and the sealer. Bioceramic root canal sealers have many benefits over conventional root canal sealers, including improved biocompatibility, antibacterial properties, nontoxicity, bio-inertness, bioactive substance content, ease of use, and excellent sealing properties [32] but despite that it is a weaker bond strength compared to MTA [33].

The cytotoxicity of iRoot SP root canal sealer has been investigated in previous studies [15, 28]. However, the findings were inconsistent where iRoot SP root canal sealer showed no cytotoxic effects [28] in contrast to a study reported by [15]. This discrepancy could be associated to the different types of cell lines, experimental conditions and methods used, which requires further investigation.

### 3.5 Ortho Mineral Trioxide Aggregate (MTA)

One of the other products manufactured for retrograde filling, perforation repair, orthograde root canal obturation, and direct pulp capping is Ortho MTA (BioMTA, Seoul, Republic of Korea). Based on the manufacturer, Ortho MTA is relatively inexpensive, easy to manipulate and can avoid microleakage by generating a hydroxyapatite interface layer between the Ortho MTA and the canal wall. In addition, when it releases calcium ions through the apical foramen and neutralizes the apical portion of the root, it induces a bioactive function. It thus forms an interfacial layer of hydroxyapatite and releases calcium ions that promote apical periodontium regeneration [16].

Ortho-MTA is a recently developed calcium silicate cement (CSS) with zirconium oxide (ZO) and also considered to have shorter setting times and less heavy metal content [34]. Ortho MTA consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetra-calcium aluminoferrite, gypsum, free calcium oxide and bismuth oxide [16]. However, in the presence of MTA, it causes undesirable physicochemical properties, such as solubilization, difficult handling properties, long setting time and the possibility of discoloration of the tooth structure [35].

The cytotoxicity of Ortho MTA has been studied and it showed that Ortho MTA had lower biocompatibility compared to other materials such as ProRoot MTA (Dentsply, Tulsa, OK, USA) and glass ionomer cement (GIC) [36]. The variations in the initial quantity of different ions emitted from materials can be due to this result. Further research into the study of ions emitted from materials and material constituents is therefore needed. In other findings Ortho MTA had moderate cytotoxicity among the materials tested such as Angelus-MTA (Angelus, Londrina, Brazil) and intermediate restorative material (IRM) (Dentsply, Tulsa, OK, USA) [35]. In other studies, it was shown that the cell viability between Ortho MTA with Biodentine (Septodont, Saint Maur des Fosses, France) and Angelus-MTA was similarly favorable and superior to IRM [34]. Moreover, the study also found that Ortho MTA was slightly more cytotoxic than the other two calcium silicate-based cement (CSCs) which are Endocem MTA (Maruchi, Wonju-si, Korea) and ProRoot MTA. The cytotoxicity of Ortho MTA can be influenced by the toxicity of the raw material itself, which can denature the corresponding cells and proteins released into the medium [16]. Due to the lack of studies and differences in the existing findings on these novel materials, further studies are required to explore the biocompatibility of Ortho MTA for its clinical purposes.

### 3.6 Dia-Root Bio Sealer

The characteristics of the new Dia-Root Bio Sealer pre-filled bioceramic calcium silicate-based MTA sealer in a syringe, highly radiopaque, pre-mixed and non-shrinking root canal sealer. It is commonly used for the prolonged obturation of root canals and effective for all gutta-percha obturation techniques. It has excellent biocompatibility with little inflammation for the tissue healing and stimulates hard tissue regeneration at sites with microbial activity [37]. Dia-Root Bio Sealer also generates Calcium Hydroxide and Calcium Silicate Hydrate and has a high pH for antibacterial activity, such as pH 12 (alkaline condition).

This sealer presented optimal flowability with flow rate of 22 millimeters (mm) to completes the apical sealing. The penetration of this sealer into lateral and accessory canals can be achieved faster because of its low film thickness and high flow rate. It has perfect performance in sealing ability for lateral and multiple root canals, superior adhesion between dentin and gutta-percha points and there is no expansion or shrinkage occur.

Because of its excellent sealing ability, Dia-Root Bio Sealer can be considered a potential alternative to other bioceramic root canal sealers. Nevertheless, minimal evidence about Dia-Root Bio Sealer is accessible for researchers [17].

## 4 *IN VITRO* VERSUS *IN VIVO* APPROACH

*In vitro* study is one of the methods used to identify the characteristics of human and animal cells in a controlled environment that is free of systematic variations. *In vivo* study is performed inside living organisms; experiments are commonly performed in animal models or in humans in the case of clinical trials. Various animals have been used for *in vivo* studies such as mice [12], [24], rats [29], rabbits [42, 43] and

sheep [44]. Conducting research *in vivo* in animals is complex, more costly, more time-consuming and requires skilled manpower [45, 46]. However, in recent practice, researchers have gained interest in zebrafish as animal models [47-49], considering its low cost, availability of complete sequenced genome and ability to produce transparent embryos [50]. Embryos of zebrafish are evaluated on the basis of changes in growth of embryos and larvae, abnormalities during organ formation and heartbeat counts for cardiac assessment [48].

Previous studies as mentioned in Table II have used *in vitro* technique for the evaluation of cell viability due to its reduced experimental cost, less time consumption to get results, potential for automation and the higher relevance to test human cells compared to *in vivo* animal tests [51]. *In vitro* tests can be repeated in multiple wells, over several days to ensure that enough cells have replicated to provide sufficient information for the research [52].

There are many differences between *in vitro* and *in vivo* techniques; in vitro studies can only be used to assess certain cell types and to evaluate the molecular mechanisms, meanwhile *in vivo* studies require approval prior to conducting animal experimentation to allow the evaluation of embryo development, embryonic changes, lifetime effects and trans-generation effects. *In vitro* studies are also important to provide data that will be useful in *in vivo* studies based on screening agents [53]. To date, the most common *in vitro* method is for the study of cell viability. However, *in vivo* approaches have been gaining popularity in recent years due to its more accurate portrayal of the clinical situation. Perhaps, more *in vivo* studies can be applied in future research work to confirm the results of *in vitro* studies.

## 5 TWO-DIMENSIONAL (2D) VERSUS THREE-DIMENSIONAL (3D) CELL CULTURE OBSERVATION

Cell culture is a widely used tool in *in vitro* studies to help our understanding of cell biology. This includes disease mechanisms, tissue morphology, protein production, tissue engineering development and drug action [38]. Cytotoxicity analysis is typically performed using standard 2D culture systems. It can be argued that certain sealers have major toxic activity as observed from *in vitro* studies, but this toxicity does not manifest in real life clinical circumstances primarily due to the disparity between *in vitro* and *in vivo* conditions. This disparity could be attributed to the types of root filling materials, type of cells, duration of cell exposure to extracts, extract dilutions and size of the specimen. Generally, 2D cell culture models have been used in many biological studies since the 1900s and remains a significant form of the cell culture; however, 3D cell culture model has been gaining popularity due it is easy to use protocols, microplate formats with high density, and automation and multimode detection systems compatibility [39, 40].

2D cell culture model is advantageous as it is inexpensive, simple and reproducible; however, the limitations are changes cell's polarity, disturbance of interactions between cellular and extracellular environments and also lack in method for detection of division resulting in lesser cell viability compared with the 3D cell culture [38]. 2D cell culture models can also form a monolayer which can inhibit cell contacts and modify the original characteristics of cell morphology and functionality [25]. Therefore, to overcome these limitations of the 2D cell culture model, 3D cell culture model has been introduced to better mimic *in vivo* conditions.

The advantages of 3D cell culture model include that the cells can be extracted from the culture and can be used for further experiments. Additionally, 3D cell culture models, can better simulate cellular conditions for *in vivo* studies, since the 3D scaffold supports cell growth and cell functions involving morphogenesis, cell metabolism and cell-to-cell interactions [25]. However, 3D cell culture models are complex, with some cell lines needing costly plates covered with different materials, such as polystyrene or covalently bound hydrogel, due to the powerful adhesion capabilities of the cell. The set-up is time-consuming, and results have limited reproducibility [38]. Generally, the 2D cell culture model is still commonly used, but adopting the 3D cell culture model in future research works will allow observation of the behaviors that are characteristic *in vivo* [41].

## 6 CELL VIABILITY ASSAYS

Various assays are used to measure and estimate the number of viable cells in culture. In previous studies for the cytotoxicity analysis of bioceramic root canal sealers, cell viability has been assessed via MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [12,

**Table II**: Summary of Parameters and Results Obtained from Most Recent *In Vitro* Studies for Bioceramic Root Canal Sealers.

| Bioceramic root canal sealers | Parameters and results | | | | References |
|---|---|---|---|---|---|
| | Cell lines | Incubation period | Evaluation method | Cytotoxicity | |
| GuttaFlow Bioseal | • Mouse fibroblast cell lines (L929)<br>• Human periodontal ligament stem cells (hPDLSCs)<br>• MDPC-23 odontoblast cells | • 3h, 1d, 3d and 7d<br>• 24h, 48h, 72h, and 168h<br>• 24h, 72h, and 120h | • MTT assay<br>• Sulforhodamine B (SRB) assay | • Less cytotoxic than AH-Plus, and MTA Fillapex and showed the highest cell viability on L929 cell lines at 7 days<br>• Cell viability was evident after 24 hours in the presence of GuttaFlow Bioseal, at 168 hours, GuttaFlow Bioseal exhibited high cell viability on hPDLSCs<br>• GuttaFlow bioseal shows a lower cytotoxic on MDPC-23 odontoblast cells compared to the AH26 sealer and its dependent on concentration of the materials and time exposure | [8, 18, 54] |
| MTA Fillapex | • Mouse fibroblast cell lines (L929)<br>• Human periodontal ligament stem cells (hPDLSCs)<br>• Immortalized human gingival fibroblast-1 HGF-1 (ATCC CRL-2014)<br>• Balb/c 3T3 cells fibroblasts (American Tissue Type Collection; ATCC, Manassas, | • 3h, 1d, 3d and 7d<br>• 1h, 6h, 20h and 24h<br>• 24h<br>• 3d, 7d, and 14d<br>• 24h, 48h, 72h, and 168h<br>• 24h, 48h and 72h | • MTT assay<br>• Trypan Blue assay<br>• Alamar Blue assay<br>• Sulforhodamine B (SRB) assay | • MTA Fillapex was more cytotoxic on L929 cell lines than GuttaFlow Bioseal, GuttaFlow 2 and AH-Plus<br>• MTA Fillapex more cytotoxic on L929 fibroblasts cell and less biocompatible than AH Plus and Sealer Plus BC<br>• MTA Fillapex showed non cytotoxic on hPDLSCs | [8, 18, 24, 25, 27-29] |

**Table II (Continued)**: Summary of Parameters and Results Obtained from Most Recent *In Vitro* Studies for Bioceramic Root Canal Sealers.

| Bioceramic root canal sealers | Parameters and results | | | | References |
|---|---|---|---|---|---|
| | Cell lines | Incubation period | Evaluation method | Cytotoxicity | |
| | | | | • MTA Fillapex exhibited significantly less viable cells in comparison to Endosequence BC sealer after the first hour and after 20 hours of incubation, while for the other incubation periods there were no significant differences<br>• MTA Fillapex showed severe cytotoxic activity on immortalized human gingival fibroblast-1 HGF-1 (ATCC CRL-2014)<br>• MTA Fillapex the lowest cytocompatibility on balb/c 3T3 fibroblasts | |
| CeraSeal | • Human periodontal ligament stem cells (hPDLSCs) | • 24h, 48h and 72h<br>• 1d, 3d, and 7d | • MTT assay<br>• Cell Counting Kit-8 (CCK-8) | • Ceraseal displayed higher cell viability on hPDLSCs<br>• CeraSeal showed significantly higher cell viability than EndoSeal TCS and AH-Plus | [14, 30] |
| iRoot SP | • Human periodontal ligament stem cells (hPDLSCs<br>• Human fibroblast cells (MRC-5) | • 3d, 7d and 14d<br>• 24h, 72h and 7d<br>• 48h | • MTT assay | • iRoot SP showed non cytotoxic effect on hPDLSCs after 2 weeks | [15, 28, 32] |

**Table II (Continued)**: Summary of Parameters and Results Obtained from Most Recent *In Vitro* Studies for Bioceramic Root Canal Sealers.

| Bioceramic root canal sealers | Parameters and results | | | | References |
|---|---|---|---|---|---|
| | Cell lines | Incubation period | Evaluation method | Cytotoxicity | |
| | • L929 murine fibroblast cell line | | | • The cytotoxic effect of iRoot SP on human fibroblast cells was concentration dependant. iRoot SP displayed an acceptable biocompatibility<br>• iRoot SP showed least toxicity on L929 murine fibroblast cell line | |
| Ortho MTA | • Mouse 3T3 fibroblast cells<br>• Human Dental Pulp Cells<br>• Preosteoblastlike cell line MC3T3-E1<br>• Human osteosarcoma MG-63 cells | • 24h and 7d<br>• 3h<br>• 3d and 7d<br>• 3h | • MTT assay<br>• Cell Counting Kit-8 aasay<br>• XTT assay | • Minor cytotoxic effects for Ortho MTA on mouse 3T3 fibroblast cells<br>• Ortho MTA showed favorable cell proliferation on human dental pulp cells<br>• Ortho MTA was significantly more cytotoxic than ProRoot and Endocem MTA on osteoblastlike cells<br>• Cell viability of Ortho MTA was lower than ProRoot MTA on human osteosarcoma MG-63 cells | [16, 34-36] |

**Table III**: Biocompatibility of Bioceramic Root Canal Sealers in Endodontics from Most Recent *In Vivo* Studies.

| Animal model | Summary of *in vivo* studies for bioceramic root canal sealers | | | References |
|---|---|---|---|---|
| | Bioceramic root canal sealers | Species | Biocompatibility | |
| Subcutaneous implantation | GuttaFlow Bioseal | Wistar rats | • Lowest inflammation on day 8, similar inflammation on day 30 and decreased over time<br>• A thin, well-defined capsule was seen at the implant-tissue interface<br>• Had the most macrophage filtrate | [55, 56] |
| | MTA Fillapex | Wistar rats | • Showed higher variable macrophages<br>• Showed higher results on the presence of multinucleated giant cells after 30- and 90-days experimental periods<br>• Exhibited samples with severe inflammatory response after 90 days. | [56-58] |
| | iRoot SP | Wistar rats | • Induce the infiltration of inflammatory cells, especially macrophages and multinucleated giant cells. | [57, 59] |

14, 18], alamar blue assay [29], and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay [60].

The MTT assay is commonly used compared to other assay because it is a homogenous cell viability assay, more suitable for high-throughput screening, suitable for most cells, most sensitive in terms of detector of toxicity and inexpensive [61]. MTT assay is a lab test used to measure and determine cell proliferation and the cytotoxicity of potential agents and other toxic materials [62]. The disadvantage of using different assays is that few assays' absorbance results are influenced by the cell number, cell type and the incubation time [61]. Thus, MTT assay is used to assess cell proliferation efficiency, test compounds for cytotoxic effects, and in multiplexing as an internal control to evaluate viable cell number during cell-based assays.

Alamar blue assay is also recognized as resazurin reduction assay. The important features of the alamar blue assay are that it is comparatively cheap, it uses a homogeneous format, and is more sensitive than tetrazolium assays [63]. Furthermore, obtaining more data about the cytotoxicity mechanism, it can be multiplexed with other approaches such as measuring caspase activity [61]. The disadvantages of alamar blue assay include, the potential for fluorescent interruption from compounds being assessed and the often-overlooked direct toxic effects on the cells [61].

MTS assay provides a colorimetric method for the sensitive quantification of viable cells. The MTS assay is similar to the generally used MTT assay, with the difference that MTS assay's formazan product is soluble in the cell culture medium [60]. This assay offers ideal properties for evaluating specific *in vitro* cytotoxicity because it is easy to use, rapid, reliable, and affordable [61]. However, the weaknesses for MTS assay are that the absorbance level measured at 490-500 nm is determined by the incubation time, type of cell and number of cells. Moreover, influencing the measured absorbance level is the percentage of MTS detection reagents to cells in culture [61].

Throughout previous research, other uncommon assays have also been used, such as trypan blue assay [24], XTT assay [64], real-time viability assay [65], WST-1 assay [66], and WST-8 assay [67].

## 7 CELL LINES

To date, the use of cell lines for the analysis of cell viability lacks standardization. This may be due to the origin of the cell line itself that is highly susceptible to various forms of contamination. In addition, selection of cell lines must consider the availability of growth factors or media for its maintenance [68]. Due to the lack of standardization, a consensus cannot be reached regarding which cell lines provide the most accurate findings.

In research targeting cell culture, scientists often use cell lines as models because they offer a stable platform, are inexpensive and easy to handle. The oral mucosa protects the oral cavity from harmful environmental influences such as pathogens, chemicals and constant abrasion [69]. Human gingival fibroblast cell lines have been selected for *in vitro* studies because it can provide valuable toxicity information compared to using animal cell lines [70].

In the past years, cytotoxicity assessment of bioceramic root canal sealers have been performed using various human cell lines such as fibroblasts, MRC-5 [15], gingival fibroblasts [27, 71], periodontal ligaments (hPDLs) [18, 20, 21, 28] and dental pulp stem cells (HDPSCs) [26]. Gingival fibroblasts have the potential for scar-less wound healing compared to skin fibroblasts and it is also among the most abundant cells [70], making it suitable for the analysis of biocompatibility for bioceramic root canal sealers. Cytotoxicity has also been investigated in animal cell lines such as mouse fibroblast (L929 murine fibroblasts) [8, 24, 29], balb/c 3T3 cells fibroblasts [25], MC3T3-E1 mouse osteoblast [12] and rat clonal dental pulp cells [72]. However, the interactions on human fibroblasts from gingival and oral mucosa have not been fully understood. Perhaps, the use of these cell lines is deemed more appropriate because of its close resemblance to the oral condition [73-75].

## 8 CONCLUSION

Bioceramic root canal sealers demonstrate positive outcomes. Nevertheless, contradictions in the findings of previous studies indicate that these bioceramic root canal sealers do not meet all the specifications needed as the ideal root canal sealer. The methodological variability of the studies included in this study, as well as the somewhat contradictory findings, make it impossible to draw a conclusion about which type

of bioceramic root canal sealer is more biocompatible. Therefore, a deeper understanding for biocompatibility of bioceramic root canal sealers requires more studies with correctly planned experiments, precise and detailed reporting. In this case, in order to improve the results, the following methodological considerations should be considered during the design phase of the biocompatibility study; (a) the use of human-derived cell lines (namely gingival fibroblasts) should be used first for *in vitro* testing over animal-derived cells or others. Gingival fibroblasts have the potential for scar-less wound healing, and it is also among the most abundant cells, making it suitable for the analysis of biocompatibility for bioceramic root canal sealers; (b) for several factors, the reports and results of different animal models, such as zebrafish, mice, rat, sheep and dog, are contradictory. First, the (pathological) physiological and anatomical differences between different animals may lead to differences in the results of different studies. Hence, it is difficult to compare the results of different studies, and thus cannot be directly applied to humans. To overcome this weakness, it is important to establish a well-defined gold standard of animal models and the associated experiment procedures as well as the parameter assessment.

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## CONFLICTS OF INTEREST

There are no conflicts of interest.

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