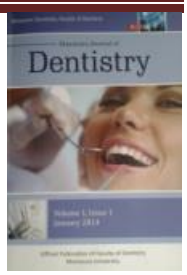
Partially fulfills requirements of obtaining master degree in endodontics



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Abstract:

Aim: The aim of this study was to evaluate the cytotoxicity of Boswellia as a root canal sealer and compare it to four different commercial types of sealers (Endofill, AH plus, MTA fill-apex, Endosequense).

Methods: The endodontic sealers investigated in this study were Endofill, AH plus, MTA fillapex and Endo sequence and Boswellia. The sealers were mixed according to the manufacturer's instructions. For experimental boswellia sealer preparation Tricalcium silicate has been prepared by solid state reaction. the cytotoxicity test were measured for each group and the means were statistically compared.

Results: both MTA Fillapex and endosequence showed an excellent biocompatibility to fibroblast cells over Endofill, AH plus.

Conclusion: The experimental sealer based on boswellia oil -resin showed an intermediate toxicity between the tested sealers.

Introduction

The objective of the canal space obturation is to prevent leakage and protect the periradicular tissue from microorganisms. Therefore, root canal sealer should demonstrate adhesive properties to dentin in order to obliterate irregularities between the root canal wall and the core material. Sealer must provide tight seal between the filling material and dentin to decrease the chance of failure of the root canal treatment. Root canal sealers should have certain characteristics in order to be used. Biocompatibility is one of the most important characteristics of root filling material because, the release of certain chemicals by the sealers may be cause various reactions in the periapical tissue[1,2].

In vitro cytotoxicity assays are extensively used in the preliminary testing of new sealers, since such methods are considered simple, reproducible and reliable for biological evaluations[3,4,5].

Several root canal sealers are currently available on the market and they are classified into five large groups according to their chemical composition: zinc oxide-eugenol-based sealers, sealers containing calcium hydroxide, resin based sealers, glass ionomers-based sealers and those based on silicon[6,7].

Despite the great variety of root canal sealers, there is still no material which fulfills the ideal requirements of the American National Standards Institute/American Dental Association (ANSI/ADA). Thus, the development of new root canal sealers with adequate physico-chemical and biological properties is crucial[8].

Nowadays, many herbal extracts are examined and tested to be used in the dental field. One of the valuable herbs is Boswellia , because of its potential anti-bacterial and ant-inflammatory properties[9].

Recent pharmacological researches showed that boswellia has other medical uses such as: anti-atherosclerosis, analgesic, anti-hyperlipidemic, anti-arthritic and hepatoprotective[10,11].

Badria et al,. 2018 evaluated the biocompatibility, anti-inflammatory and antimicrobial effects of boswellic acid (BA) in comparison to conventional and common root canal irrigants and intracanal medications; e.g. sodium hypochlorite, CHX, calcium hydroxide, TAP, DAP and Ledermix. They concluded that BA showed to be biocompatible with strong anti-inflammatory and antimicrobial activities when compared to other commonly used irrigants and medications. Therefore this study proposed a new efficient and safe irrigant and medicament[12].

In this context, it is important to study the properties of the filling materials in order to establish the appropriate parameters for the development of new products, as well as to evaluate those already available on the markets

Review of Litreature

1. Importance of endodontic sealers :

Endodontic treatment aims to eliminate infection of the root canal and to completely fill the root canal space in three-dimension, in order to prevent apical and coronal penetration of liquids and microorganisms. Most root canals are filled with gutta-percha points in combination with an endodontic sealer which are essential components of root canal obturation to establish a fluid-tight seal.

The main function of a sealer is to fill the spaces between the core material and the walls of root canal and between the gutta-percha cones, in an attempt to form a coherent mass of obturating material without voids. The sealer is expected to fill irregularities and minor discrepancies between the filling and canal walls, accessory canals, and multiple foramina.

By its germicidal action, it is also expected to destroy the remaining bacteria left after cleaning and shaping of the root canal. Although all efforts are concentrated to confine the sealer within the root canal space, some extrusion inadvertently occurs during obturation procedure. When the sealer cement comes in contact with soft and hard tissues

apically, it can cause persistent inflammation of periradicular tissues and may result in delayed wound healing manifesting as pain, tenderness, and swelling of the affected area; hence, biocompatibility of sealers is an important issue in selecting the right type of sealer for different types of endodontic cases[13].

2. Root canal sealers types :

Endodontic sealers have been historically classified in various ways such as: according to eugenol content, usage, absorbance, etc. A comprehensive categorization of the endodontic sealers has been provide according to their composition (Zinc Oxide Eugenol based sealers, Epoxy resin based sealers, Silicon based sealers, MTA based sealers, Calcium-silicate-Phosphate based bioceramic sealers, Methaacrylate resin based sealer, Calcium-phosphate based sealers and Herbal Sealer) [7].

3. Cytotoxicity of zinc oxide based sealers:

Early sealers were modified zinc oxide-eugenol (ZOE) cements based on Grossman or Rickerts's formula that were widely used throughout the world. Unlike the resin-based sealers, setting reaction of ZOE-based sealers is a chelation reaction occurring between eugenol and the zinc ion of the zinc oxide. This reaction might also occur with the zinc oxide phase of Gutta Percha (GP) along with the calcium ions of dentin. This might explain the decreased setting shrinkage associated with the ZOE-based sealers[14].

Michaud et al. evaluated the three-dimensional expansion of Gutta-percha (GP), at various powder/liquid ratios, of a zinc oxide-eugenol (ZOE)-based sealer using spiral computed tomography (SCT). They concluded that, increasing the ratio of eugenol in sealer resulted in volumetric increase of GP. It is cerebrated that the free eugenol component of freshly mixed ZOE sealer can seep out and cause various cytotoxic effects on human gingival fibroblasts, periodontal ligament (PDL) cells, and osteoblast-like cells[15].

However, ***Haseih et al,*** reported that leakage of eugenol into periapical tissues is very low, and it dramatically decreases over time[16].

Sealing properties of ZnOE sealers were inferior in comparison to other sealers due to the relatively high solubility of the ZOE sealer; so, adhesion between GP and ZOE is weak[17].

Eugenol is cytotoxic and the same has been shown frequently for ZOE with different cell culture systems, especially after mixing, but also in a set state. Even higher cytotoxicity was observed with formaldehyde-containing ZOE sealers, which were classified as highly/extremely cytotoxic[18]. An ZOE sealer in the pulp chamber disinfected the dental tubules to a depth of 250 µm[19] and had a good antimicrobial property compared to other sealers[20].

Benjamin and Willershausen 1990, tested the cytotoxicity of six different zinc oxide-eugenol root canal sealers by determining the incorporation of [l-14C] leucine in human gingival fibroblasts that had been in contact with root canal sealers after setting for 24 and 48 h. The biopsies were obtained from healthy patients. In some cases there was a significant difference shown in the fibroblasts' protein synthesis ([l-14C]leucine incorporation) between the group that was coveted with fibroblasts 24 h and the group that was covered 48 h after mixing. Pulp Canal Sealer showed at the middle phase of the trial a toxic increase, but, after the 15th day recovery of the fibroblasts was noticed. In this group constant toxic potential increase was observed with Wach's Formula and Tubli-Seal[21].

Gulati N et al 1991, compared the tissue toxic response of two zinc-oxide-based root canal sealers. The sealers tested were zinc-oxide eugenol and zinc-oxide glycerine. Fifteen albino rats were used for the study and were injected subcutaneously in the preset state. The tissue response was assessed by counting the polymorphonuclear cells at Day 1, Day 7 and Day 15 of the study period. The results showed that the toxicity was greater for the eugenol-containing sealer and increased during the three time intervals. For the non-eugenol sealer the response was milder and reached a peak by 7 days after which it decreased[22].

Abou Hashieh et al 1999, investigated the amount of eugenol released from a zinc oxide-eugenol-based sealer at the apex of teeth filled according to two techniques: the single-cone and the Thermafil. The results of this work show that the level of eugenol released from a zinc oxide-eugenol-based sealer beyond the apex is very low and decreases over time[16].

Huang FM et al 2002, determined the cytotoxicity of three different types of root canal sealer on human periodontal ligament (PDL) cells and a permanent hamster cell line (V79 cells). Set specimens from two resin based sealers (AH26 and AHPlus), three zinc oxide-eugenol-based sealers (Canals, Endomethasone and N2) and one calcium hydroxide-based sealer (Sealapex) were eluted with culture medium for 1, 2, 3 and 7 days. Calcium hydroxide-based sealer was the least toxic sealer amongst the chemicals tested in both cultures. [23].

Martins et al 2013, evaluated, in vitro, the cytotoxicity of six root canal sealers after 12, 24 and 72 h of contact time, using an endothelial ECV-304 cell line. Based on the results, Endofill and GuttaFlow were the most and the least cytotoxic sealers, respectively[24].

Cintra et al 2017, evaluated the cytotoxicity and biocompatibility of Sealer Plus, a new resin epoxy-based endodontic sealer containing calcium hydroxide. AH Plus, Endofill, and SimpliSeal endodontics sealers were used for comparison. L929 fibroblasts were cultured, and an MTT assay was used to determine the cytotoxicity of the sealer extracts at 6, 24, 48, and 72 hours. In general, Sealer Plus promoted greater cell viability and was more biocompatible compared with the other sealers[25].

Jagtap et al 2018, evaluated and compare the cytotoxic effects of different types of root canal. The sealers were eluted with culture medium for 1 hour, 7 days, and 14 days. The MTA Fillapex was found to be the most cytotoxic sealer. Use of resin-based material as a root canal sealer may result in a more favorable response to PDL fibroblasts[26].

Nair et al 2018, evaluated and compare the cytotoxicity and genotoxicity of two bioceramic root canal sealers: EndoSequence BC and iRoot SP with zinc oxide eugenol sealers on fibroblast cell line. The sealers tested were zinc oxide eugenol, EndoSequence BC, and iRoot SP. Among the three tested materials, zinc oxide eugenol showed maximum cytotoxicity to the cells (30.64% viable cells), followed by EndoSequence BC (71.33% viable cells) and iRoot SP (75.11% viable cells). All the three tested sealers showed varying degrees of cytotoxicity and genotoxicity while using fibro-blast cell line. Zinc oxide eugenol was most toxic in both the assays and iRoot SP showed least toxicity, followed closely by EndoSequence BC[27].

Jung et al 2018, evaluated the effect of an epoxy resin-based (AH-Plus), a zinc oxide eugenol containing (Pulp-Canal-Sealer) and two calcium silicate containing (MTA-Fillapex and BioRoot-RCS) sealers on primary human osteoblasts (hOB) in freshly mixed and set state. Pulp Canal Sealer and MTA-Fillapex showed no biocompatibility in contact with osteoblasts at all. BioRoot-RCS had a positive influence on the cell metabolism (bioactivity) and is biocompatible[28].

Jung et al 2019, evaluated the biocompatibility of two comparatively new calcium silicate containing sealers (MTA-Fillapex and BioRoot-RCS) with that of two established sealers (AH-Plus, epoxy resin-based; Pulp-Canal-Sealer, zinc oxide eugenol containing). In contact with BioRoot-RCS a regeneration of the PDL-cells were observed over time. This sealer showed the lowest toxicity in a freshly mixed and set state. MTA-Fillapex and Pulp-Canal-Sealer were cytotoxic in a fresh as well as in a set state, whereas AH-Plus was cytotoxic in a freshly mixed state, but not when the sealer was set. BioRoot-RCS is biocompatible and bioactive because it seems to have a positive influence on the PDL-cell metabolism. Pulp Canal Sealer and MTA-Fillapex showed no biocompatibility in contact with PDL-cells at all. Freshly mixed AH Plus is less biocompatible on PDL than in a set state[29].

Cytotoxicity of resin-based sealers:

AH Plus consists of a paste-paste system, delivered in two tubes in a new double barrel syringe. The epoxide paste contains radiopaque fillers and aerosil. The amine paste consists of three different types of amines, radiopaque fillers, and aerosol[30

AH Plus showed the greatest stability in solution, as compared to the conventional sealers[31].

AH Plus has a film thickness of 26 mm, which is clearly below the value of less than 50 mm required by the ISO standard for root canal sealing materials[30]. AH Plus has been designed to be slightly thixotropic. A flow of 36 mm also perfectly meets the requirements of the ISO standard (>25 mm).

It is known from the literature that pure epoxy resins develop mutagenic activities under the conditions of the Ames test. Therefore, the epoxide paste (paste A) and amine paste (paste B) were studied in the Ames test, in which the aqueous extracts did not induce any mutagenic effects. In numerous *in vivo* studies, the pure epoxy resins never showed any genotoxic effects[32]. Recently, the antimicrobial effects of endodontic sealers (Endion, AH-26, AH-Plus, Procosol, and Ketac Endo) were investigated after 2, 20, and 40 days. AH Plus produced slight inhibition on *Streptococcus mutants* at 20 days and on *Actinomyces israelii* at every time interval. No effect was found on *Candida albicans* and *Staphylococcus aureus*[33]. The studies showed that AH26 and Endomethasone sealers released formaldehyde after setting. Only a minimum release was observed for AH Plus (3.9 ppm), followed by EZ-Fill (540 ppm) endodontic cement and AH26 (1347 ppm) endodontic cement which yielded the greatest formaldehyde release[34].

AH Plus has greater adhesion to root dentin than Epiphany as it is an epoxy resin-based sealer. AH Plus has better penetration into the micro-irregularities because of its creep capacity and long setting time, which increases the mechanical interlocking between sealer and root dentin and the cohesion of sealer causes Resilon to be more resistant to fracture[35].

Kirsten *et al.* investigated the mutagenicity of resin-based endodontic sealers by evaluating their potential to induce DNA double-strand breaks (DSBs) on extrusion into the periapical tissue and found that there were no indications for increased risk of genotoxicity of resin-based root canal sealers caused by the induction of DNA DSBs[36].

The strong link between sealer solubility and periapical re-infection indicates that water solubility of new sealers should be studied. So, **Azadi *et al***[37]. studied the water solubility of five root canal sealers [AH26, Topseal, 2-Seal, Acroseal, and Roeko Seal Automix (RSA)] and found that the solubilities of the sealers AH26, Acroseal, Topseal, 2-Seal, and RSA were 0.28%, 0.36%, 0.07%, 0.037%, and 0.141%, respectively, after 24 h. After 28 days, their solubilities were 1.75%, 0.746%, 0.082%, 0.04%, and 0.517%, respectively, and the authors came to the conclusion that all the tested materials met the standards (maximum weight loss of 3% within 24 h). However, the results of 2-Seal followed by Topseal were the most favorable ones. According to **Franco *et al.*** [38]. the oxygen inhibits vinyl polymerization in composite resins. **Pecora *et al***[39]. found an adhesion of 4 MPa for AH Plus to dentin. After Er: YAG laser treatment of the root canal, the adhesion increased to about 7 MPa. Recently, Gogos demonstrated that a product identical to AH Plus exhibits a significant self-adhesion to dentin of 6.24 ± 1.43 MPa[40]. Due to its excellent properties, such as low solubility, small expansion, adhesion to dentin, and very good sealing ability, AH Plus is considered as a benchmark "Gold Standard[30].

Al-Hiyasat et al 2010, investigated the cytotoxic effects of four resin-based root canal sealers, namely, AHPlus, an epoxy resin; EndoREZ, a single-methacrylate-based sealer; Epiphany, a multi-methacrylate resin-based sealer; and Metaseal, one of the latest generation methacrylate 4-META-containing resin-based sealers. The result was that all materials were cytotoxic to different degrees. AHPlus was

the least cytotoxic followed by EndoRez, Epiphany and Metaseal, which was the most cytotoxic[41] .

Ehsani et al 2012, evaluated and compared the cytotoxicity induced by two resin-based sealers, 2Seal and AH Plus, in

two osteoblast-like cell lines, MG-63 and Saos-2. This may reflect higher sensitivity of the MG-63 cell line compared to Saos-2 toward cytotoxicity induced by these two sealers, or different kinetics of toxicant release from the sealers[42].

Cobankara FK et al 2013, evaluated the cytotoxic effects of five different resin-based root canal sealers: EndoREZ, Epiphany SE, EZ-Fill, MMSeal and AHPlus. Based on the results obtained from the present study, all tested resin-based sealers appear to show toxicity potential to both cells in spite of different toxicity degrees. Therefore, better sealers need to be developed with acceptable biological properties for root canal filling[43].

Gorduysus et al 2014, the cytotoxicity of fresh samples of AH26 and AH Plus were tested using the ^{51}Cr-release method at 12 and 72 h. Heteroploid L929 mouse fibroblasts were used as target cells. Both AH26 and AH Plus had gamma particle readings that were significantly lower than the positive control (formocresol) and significantly higher than the negative control (labeled, but material free cell suspension), as assessed by Wilcoxon Sum-Rank Tests[44].

Ashraf et al 2018, assessd the cytotoxicity of two experimental endodontic sealers in comparison with AH-26 resin sealer. This in vitro study was conducted on conventional and experimental root canal sealers: AH-26, an epoxy resin experimental sealer A (ES-A) composed of calcium tungstate, zirconium oxide, aerosil, bismuth oxide, titanium oxide, hexamine and an epoxy resin and experimental sealer B (ES-B) with compositions similar to ES-A except for the presence of imidazoline as a catalyst.
The experimental sealers containing nano-particles were mixed with 37.5% of an epoxy resin. The result was found that the set ES-A had the least cytotoxicity from the first hour but the cytotoxicity of ES-B and AH-26 extraction decreased over time. In fresh form, except for 100% concentration, ES-A showed the least cytotoxicity compared to the other two sealers. All three sealers had high cytotoxicity in 100% concentration but had low cytotoxicity in 10% and 1% concentrations[45].

4. Cytotoxicity of MTA based sealers:

MTA based sealer. This sealer produces calcium hydroxide, which is released in solution and induces formation of hydroxyapatite structures in simulated body fluid. Newer developments of MTA include its use as a root canal sealer. Currently, three MTA sealer formulations are available: Endo CPM Sealer, MTA Obtura, and ProRoot Endo Sealer[46-48].

The composition of CPM sealer after mixing is reported to be 50% MTA (SiO2, K2O, Al2O3, SO3, CaO, and Bi2O3), 7% SiO2, 10% CaCO3, 10% Bi2O3, 10% BaSO4, 1% propylene glycol alginate, 1% propylene glycol, 1% sodium citrate, and 10% calcium chloride[49].

MTA Obtura is a mixture of white MTA with a proprietary viscous liquid[50]. ProRoot Endo Sealer is calcium silicate-based endodontic sealer. The major components of the powder of ProRoot Endo Sealer are

tricalcium silicate and dicalcium silicate, with inclusion of calcium sulfate as setting retardant, bismuth oxide as radiopacifier, and a small amount of tricalcium aluminate.

Tricalcium aluminate is necessary for the initial hydration reaction of the cement. The liquid component consists of

viscous aqueous solution of a water-soluble polymer and to improve The liquid component consists of viscous aqueous solution of a water soluble polymer to improve the workability[51].

When placed in the canal, it releases calcium activity and causes cell attachment and proliferation, increases the pH, modulates cytokines like interleukin (IL) 4, IL6, IL8, IL10, and hence causes proliferation, migration, and differentiation of hard tissue producing hydroxyapatite which aids in the formation of physical bond between sealer and MTA[52,53].

The polymer did not seem to affect the biocompatibility of the materials and the hydration characteristics were similar to those reported for MTA[54]. Sealers based on MTA have been reported to be biocompatible, stimulate mineralization[49]. and encourage apatite-like crystalline deposits along the apical- and middle-thirds of canal walls[51]. These materials exhibited higher push-out strengths after storage in simulated body fluid[55]. and had similar sealing properties to epoxy resin-based sealer when evaluated using the fluid filtration system[49

Fluoride-doped MTA demonstrated stable sealing up to 6 months, and was significantly better than conventional MTA sealers and comparable to AH Plus. The study supports the suitability of MTA sealers in association with warm GP for root filling[56]. **Loise *et al.*** evaluated the biocompatibility and bioactivity of a new MTA-based endodontic sealer, MTA Fillapex (MTA-F; Angelus, Londrina, Brazil), in human cell culture and came to the conclusion that after setting, the cytotoxicity of MTA-F decreases and the sealer presents suitable bioactivity to stimulate hydroxyapatite crystal nucleation[57]. **Gomes-Filho *et al.*** evaluated the rat subcutaneous tissue reaction to implanted polyethylene tubes filled with MTA Fillapex and compared it with MTA-Angelus, and concluded that MTA Fillapex was biocompatible and stimulated mineralization[58]. **Bortolini *et al*** evaluated *in vitro* the intratubular penetration and permeability of Endo CPM Sealer in teeth contaminated with *Enterococcus faecalis* and concluded that Endo CPM sealer showed greater permeability to *E. faecalis* [59].

Morgental *et al.* found that MTA Fillapex and Endo CPM Sealer has a good antibacterial effect on *E. feacalis* before setting, but not after setting despite having high pH[60].

Bin *et al.* studied the cytotoxicity and genotoxicity of MTA canal sealer (Fillapex) compared with white MTA cement and AH Plus, and found that white MTA group was the less cytotoxic material in this study. Both AH Plus and

Fillapex MTA sealer showed the lowest cell viability rates and caused an increased micronucleus formation[61].

Considering the elastic modulus of dentin which is about 14-18.6 GPa[62]. The reinforcing effect of MTA may be explained by its similar elastic modulus to dentin. This hypothesis also explains the gradual increase in the fracture resistance of MTA-filled teeth found by Hatibovic-

Kofman *et al*[63]. Aalso, fracture resistance of MTA-filled teeth is time dependant.

The alkalinity of MTA can theoretically weaken root dentin similar to the findings on calcium

hydroxide[64]. Another hypothesis is that a combination of little tensile strength of MTA and lack of bonding to dentin can weaken the dentin[63]. Regardless of the excellent biologic properties of MTA, the thin dentinal walls still make these teeth more prone to fracture and a reinforcing technique in these weak roots is necessary.

The novel sealer based on MTA has efficacious sealing ability. In contact with a simulated body fluid, the MTAs release calcium ions in solution and encourage the deposition of calcium phosphate crystals.

Bin et al 2012, evaluated the cytotoxicity and genotoxicity of MTA canal sealer (Fillapex) compared with white MTA cement and AH Plus. Chinese hamster fibroblasts (V79) were placed in contact with different dilutions of culture media previously exposed to such materials. The results showed that both AH Plus and Fillapex MTA sealer showed the lowest cell viability rates and caused an increased micronucleus formation when compared with control untreated group[61].

Gomes-Filho et al 2013, evaluated the healing of periapical lesions in canine teeth after a single session of endodontic treatment with MTA Fillapex compared with Sealapex or Endo-CPM-Sealer. The result was that all 3 materials produced similar patterns of healing; in particular, persistent inflammation and absence of complete periapical tissue healing were consistently noted[65].

Silva et al 2013, evaluated the cytotoxicity, radiopacity, pH, and flow of a calcium silicate-based and an epoxy resin based endodontic sealer, MTA Fillapex and AH Plus, respectively. Cytotoxicity, radiopacity, and flow evaluation were performed following ISO requirements. The result was that in all tested periods, MTA Fillapex was more cytotoxic than AH Plus. Although MTA Fillapex was more cytotoxic than AH Plus, it showed suitable physicochemical properties for an endodontic sealer[66].

Kim and Shin 2014, assessd the cytotoxicity of EndoSeal, a novel mineral trioxide aggregate-based root canal sealer in comparison with two commonly used sealers, AH Plus and Sealapex. The results suggest that EndoSeal has a satisfactory cytocompatibility[67].

Teixeira et al 2017, evaluated the cytotoxicity of root canal sealers under conditions closely resembling a clinical reality. The present study showed that good results were present in AH Plus and Sealapex, but not the Endofill group after 48 h. The method

used enabled evaluation of the cytotoxicity of the studied sealers that diffused through the root apex[68].

Saygili et al 2017, evaluated the cytotoxicity of different sealers

including GuttaFlow Bioseal, GuttaFlow 2, AHPlus and M TA Fillapex on L929 murine fibroblasts. GuttaFlow sealers are less cytotoxic than MTA Fillapex and AH-Plus. At all experimental time points, there was no significant difference

in the cell viability between the GuttaFlow Bioseal group and the control group[69].

Jung et al 2018, evaluated the effect of an epoxy resin-based (AH-Plus), a zinc oxide eugenol containing (Pulp-Canal-Sealer) and two calcium silicate containing (MTA-Fillapex and BioRoot-RCS) sealers on

primary human osteoblasts (hOB) in freshly mixed and set state. MTA-Fillapex and Pulp-Canal-Sealer were cytotoxic in a fresh as well as in a set state. BioRoot-RCS showed the lowest toxicity in both states; where as a regeneration of the cells could be observed over time. Contact of freshly mixed AH-Plus to osteoblasts should be avoided. PulpCanal Sealer and MTA-Fillapex showed no biocompatibility in contact with osteoblasts at all. BioRoot-RCS had a positive influence on the cell metabolism (bioactivity) and is biocompatible[28].

5. Cytotoxicity of bio-ceramic based sealers:

Endosequence BC Sealer, also known as iRoot SP Injectable Root Canal Sealer, is an example of a calcium phosphate silicate-based cement[70]. Its major inorganic components include tricalcium silicate, dicalcium silicate, calcium phosphates, colloidal silica, and calcium hydroxide. It uses zirconium oxide as the radiopacifier and contains water-free thickening vehicles to enable the sealer to be delivered in the form of a premixed paste[71]. Hydroxyapatite is co-precipitated within the calcium silicate hydrate phase to produce a composite-like structure, reinforcing the set cement[72]. The introduction of a premixed calcium phosphate silicate-based sealer eliminates the potential of heterogeneous consistency during on-site mixing. Because the sealer is premixed with non-aqueous but water-miscible carriers, the water-free paste will not set during storage in the syringe and only hardens on exposure to an aqueous environment[73].

EndoSequence BC Sealer uses the moisture within the dentinal tubules after canal irrigation to initiate and complete the setting reaction. Moreover, the presence of smear plugs and/or tubular sclerosis can affect the amount of moisture present[74]. The setting time of EndoSequence BC Sealer is 4 h and it may be extended in overly dry canals[71 The pH of Endosequense BC Sealer during the setting process is higher than 12, which increases its bactericidal properties[75 The amount of Ca $^{2+}$released from Endosequence BC Sealer was far higher (2.585 mg/l) than that released from AH Plus (0.797 mg/l), mainly after 7 days[76].

Loushine et al 2011, investigated the setting time and micohardness of a premixed calcium phosphate silicate-

based sealer in the presence of different moisture contents (0%-9 wt%). The moisture content that produced the most optimal setting properties was used to prepare set EndoSequence BC Sealer for cytotoxicity in comparison with AH Plus, and they concluded that cytotoxicity of AH Plus gradually decreased and became noncytotoxic, whereas BC Sealer remained moderately cytotoxic over the 6-week period. Hence, it shows bioceramic sealer is non-toxic and biocompatible[77].

Zoufan et al 2011, conducted a study which evaluated the cytotoxicity of GuttaFlow and EndoSequence BC sealers and compared them with AH Plus and Tubli-Seal sealers. The GuttaFlow and EndoSequence BC sealers had lower cytotoxicity than the AH Plus and Tubli-Seal sealers[78]. According to **Ghoneim et al. 2011**, bioceramic-based sealer (i.e., iRoot SP) is a promising sealer in terms of increasing *in vitro* resistance to the fracture of endodontically treated roots, particularly when accompanied with ActiV GP cones[79].

Deyan Kossev and Valeri Stefanov 2009, found that when bioceramic-based sealers BioAggregate or iRoot SP are extruded, the pain is relatively small or totally absent. Such lack of pain may be explained based on the characteristics of these new materials. During hardening, they "produce" hydroxylapatite and after the end of hardening process they exhibit the same features as non-resorbable hydroxylapatite-based bioceramics used for bone replacement in oral surgery. Due to the hydroxylapatite formed, they are also osseo-conductive. During setting, hard ceramic-based sealers expand. Expansion of BioAggregate and iRoot SP and iRoot BP is significant (0.20%). These new bioceramic sealers also form chemical bond with the canal's dentin walls. That is why no space is left between the sealer and dentin walls[80].

Borges et al 2012, compared the changes in the surface structure and elemental distribution, as well as the percentage of ion release, of four calcium silicate-containing endodontic materials with a well-established epoxy resin-based sealer, submitted to a solubility test, and found that AH Plus and MTA-A were in accordance with ANSI/ADA's requirements regarding solubility, while iRoot SP, MTA Fillapex, and Sealapex did not fulfil ANSI/ADA's protocols. High levels of Ca^{2+} ion release were observed in all materials except AH Plus. Scanning electron microscopy (SEM)/Energy-dispersive X-ray spectroscopy (EDX) analysis revealed that all samples had morphological changes in both outer and inner surfaces after the solubility test. High levels of calcium and carbon were also observed at the surface of all materials except AH Plus and MTA-A[81]. Further studies should be conducted to evaluate the by-product components produced during setting to accurately assess the cytotoxicity of EndoSequence BC Sealer.

Candeiro et al 2016, compared the characteristics of bioceramic endodontic sealer Endosequence BC sealer with those of AH Plus sealer. Methodology Cytotoxicity and genotoxicity were analysed on human gingival fibroblasts submitted to cell culture medium conditioned by sealers using the MTT reduction assay and micronucleus formation test (MNT), respectively. Cells grown on fresh medium served as controls. They concluded that the Bioceramic-based sealer had less cytotoxicity and genotoxicity and similar antibacterial effect against E. faecalis in comparison with AH Plus sealer[82].

Baraba et al 2016, investigated the cytotoxicity of two different bioactive root canal sealers: one based on mineral trioxide aggregate, MTA Fillapex and the other based on bioceramics, Endosequence BC Sealer, in culture of mouse L929 fibroblasts. They found that MTA Fillapex and Endosequence BC sealer were both cytotoxic in cultures of mouse L929 fibroblasts[83] .

Chakar et al 2017, evaluated the cytotoxicity of a new bioceramic-based root canal sealer (BioMM) by direct and indirect contact with human fibroblasts and to compare it with a zinc oxide-eugenol sealer, the Pulp Canal Sealer-extended working time (PCS-EWT). Cell viability was assessed through direct and indirect contact between human fibroblasts and sealer. They found that direct contact showed a significantly higher cell viability with BioMM as compared to PCS-EWT. BioMM may be considered minimally cytotoxic if accidentally extruded into the periapical tissues[84].

These results may suggest a good alternative to develop new endodontic sealers, in order to achieve better biological response and healing, when compared to commercially available sealers

7- Roles of Herbal in medicine :

Herbal products have been used since ancient times in folk medicine, involving both eastern and western medicinal traditions. Many plants with biological and anti-microbiological properties have been studied since there has been a relevant increase in the incidence of antibiotic overuse and misuse. In dentistry, Phytomedicines has been used as anti-inflammatory, antibiotic, analgesic and sedative agents. In endodontics because of the cytotoxic reactions of the most of the commercial intracanal medicaments used and their inability to eliminate bacteria from dentinal tubules, trend of recent medicine attends to use biologic medication extracted from natural plants[85].

Boswellia is the genus of trees and the order of Sapindales, comprises of nearly 25 species on total[86,87]. The main species of Boswellia, are namely B. sacra, B. frereana,B. papyrifera, and B. serrata. Boswellia Serrata, also known as the Indian Frankincense, and are known for many pharmacological medicinal applications[88]. Boswellia are moderate-sized flowering plants (trees and shrubs) and are native from tropical regions, predominantly India, Africa and Middle East. Boswellia has been studied for their potential as a natural product for ayurvedic medicinal use[89] .

Originally, its medicinal use in elephants was observed by the ayurvedic healers and eventually got translated to the human health benefits. In ancient times, the boswellia extracts procured from the sap of boswellia tree, were used to treat chronic ailments that respond to anti-inflammatory agents including arthritis, diarrhea, and

pulmonary diseases[90]. Recently, boswellia has gained much attention in the medicinal community because of its active ingredient and its unique chemical signature, "boswellic acid" possess anti-inflammatory properties equivalent to prescription medications without any signs of side effects[91].

Moreover, boswellia have been studied for their potential as a natural product for medicinal use because of their high boswellic acid content particularly AKBA (acetyl-11-keto-beta-boswellic acid) as the predominant active component in boswellia, and most supplements in themarketplace have specialized to maximize that component[7] . However, recent research has indicated that few of the water soluble polysaccharides in boswellia are critical components in initiating and supporting the anti-inflammatory activity, while the lipid-soluble boswellic acid

facilitates a slow and sustained action. Indeed, a specific boswellin polysaccharide extract (Polysal), primarily consists of galactose, arabinose, D-glucuronic acid, and 4-o-methyl-glucuronoarabino-galactan has demonstrated a dose dependent anti-inflammatory potential, similar to the boswellic acids[92] .

Several studies have reported that Boswellia is relatively safe and more potent. While most anti-inflammatory drugs function as Cox enzyme inhibitors,

boswellia serrata exerts a novel inhibitory effect of pro-inflammatory enzymes (5-lipxygenase) , and effectively

blocks cytokine synthesis, which are the strong contributors to inflammation-associated diseases[93]

Cytokines (leukotrienes) play a major role in promoting a battery of age-associated diseases including joint pain, intestinal disorders, cancer and pulmonary diseases. The anti-inflammatory effects of boswellia have been investigated for their benefits in osteoarthritis (OA), suppress pain & inflammation that is common in many conditions, asthma, swelling, and gut/bowel health. Further, boswellic acid suppress the proliferation of tissues at the site of inflammation and inhibits the breakdown of connective tissues caused by TNF-α, which is a potent inflammatory agent. In addition, boswellia has been proven to improve blood circulation to the joints restoring the integrity of damaged blood vessels, and again it is a natural way of preserving the vascular function unlike the traditional drugs of choice with side effects[94].

Khosravi Samani et al 2011, evaluated anti-inflammatory effects of Frankincense in the treatment of gingivitis, which is a periodontal tissue inflammatory disease. At the end of the study, the indices in all groups showed significant decreases in comparison to the first day, except for the bleeding index in the group without SRP and drug therapy. In addition, no significant difference was observed between powder or extract therapy and between patients received either SRP or treatment alone. Frankincense, a safe and low-cost herbal medicine, may be feasibly applied to improve inflammation based disease of gingival as an adjunct to the conventional mechanical therapy[95].

Garrido et al 2015, evaluated the cytotoxic effect of a new Copaiba oil-based root canal sealer (Biosealer [BS]) on

osteoblast-like Osteo-1 cells. They found that S26, EF and AHP presented decreased cellular viability. BS maintained cellular viability similar to CG. The Copaiba oil-based root canal sealer presented promising results in terms of cytotoxicity which indicated its usefulness as a root canal sealer[8].

Silva et al 2016, evaluated the cytotoxic and genotoxic effects of two experimental root canal sealers, based on extracts from Copaifera multijuga and Ricinus communis (castor oil polymer), comparing them to synthetic resin-based sealers: a single methacrylate-based, a multi-methacrylate-based, and an epoxy resin-based sealers. They found that the single methacrylate-based sealer had the most cytotoxic effects, with significant reduction in cell viability in all dilutions of the extract. The castor oil polymer-based

sealer was, on the other hand, the most biocompatible sealer, with no cytotoxic effects at any concentration. All tested sealers were not genotoxic, excepting the single methacrylate-based sealer[96].

Maraghehpour B et al 2016, evaluated the antibacterial effect of Boswellia serrata (BS and Nigella sativa (NS) on Aggregatibacter actinomycetemcomitans (A.a) known as main pathogen of aggressive periodontitis. They found both BS and NS are effective against A.a which should be taken

into account as appropriate ingredient for oral hygiene products[97].

Governa P et al 2018, inflammatory bowel diseases, which consist of chronic inflammatory conditions of the colon and

the small intestine, are considered a global disease of our modern society. Recently, the interest toward the use of herbal therapies for the management of inflammatory bowel diseases has increased because of their effectiveness and favourable safety profile, compared to conventional drugs. Boswellia serrata Roxb. and Curcuma longa L. are amongst the most promising herbal drugs, however, their clinical use in inflammatory bowel diseases is limited and little is known on their mechanism of action. The aim of this work was to investigate the effects of two phytochemically characterized extracts of *B. serrata* and *C. longa* in an in vitro model of intestinal inflammation. Their impact on cytokine release and reactive oxygen species production, as well as the maintenance of the intestinal barrier function and on intestinal mucosa immune cells infiltration, has been evaluated. The extracts showed a good protective effect on the intestinal epithelium at 1 µg/mL, with TEER values increasing by approximately 1.5 fold, compared to LPS-stimulated cells. *C. longa* showed an anti-inflammatory mechanism of action, reducing IL-8, TNF-α and IL-6 production by approximately 30%, 25% and 40%, respectively, compared to the inflammatory stimuli. *B. serrata* action was linked to its antioxidant effect,

with ROS production being reduced by 25%, compared to H_2O_2 -stimulated Caco-2 cells. *C. longa* and *B. serrata*resulted to be promising agents for the management of inflammatory bowel diseases by modulating in vitro parameters which have been identified in the clinical conditions[98].

Saha S et al 2019, compared antimicrobial activity of endodontic sealers added to herbal extracts. Three sealers mixed with three herbal extracts were evaluated against seven strains of bacteria at various time intervals using Agar Diffusion Test. Three herbal extracts used in this study were methanolic extracts of Licorice, Bakul, and Guduchi The mean zones of inhibition were measured. They found that the Zinc Oxide Eugenol based sealer with herbal extracts produced largest inhibitory zones followed in descending order by Resin based sealer and Calcium hydroxide along with three herbal extracts respectively [99].

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