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Comparative Analysis of pH, Calcium Ion Release and Radiopacity of Bioceramic and MTA Fillapex Sealer with Ah Plus – an *In vitro* Study

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ABSTRACT

*The aim of the present study was to evaluate of pH, calcium ion release and radiopacity of Bioceramic sealer and MTA Fillapex compared with AH Plus. METHODS: The release of calcium ion and pH were measured at period of 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, 7 days, 15 days, 30 days with spectrophotometer and pH meter respectively. For the radiopacity analysis, metallic rings with 10-mm diameter and 1-mm thicknes were filled with the sealers. The radiopacity value was determined according to radiographic density (mm Al). RESULTS: Bioceramic sealer showed pH and release of calcium ion greater than that of MTA Fillapex and AH Plus (P <0.001**) during all experimental periods. The Bioceramic sealer showed radiopacity significantly lower than that of AH Plus and MTA Fillapex (P <0.001**). CONCLUSION: AH Plus sealer exhibited superior radiopacity followed by MTA Fillapex and Bioceramic sealer. Bioceramic sealer showed high calcium ion release and alkaline pH in all tested periods followed by MTA Fillapex. AH Plus exhibited lowest calcium release and neutral PH.*

Keywords: *Bioceramic, radiopacity, MTA Fillapex, Calcium*

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INTRODUCTION

The goal of an endodontic treatment is to eliminate microbial challenges from the root canal system and provide a fluid tight seal with a stable biocompatible material to promote the healing of the periapex [1]. Instrumentation accompanied by irrigation and intracanal medication significantly reduce the bacterial population of the infected root canal and prevent re-infection. The root canals are routinely obturated with gutta-percha points in combination with endodontic sealer. The main function of the sealer is to fill the gaps between the gutta-percha points and the wall of the root canal. 2 The use of root canal sealer is considered mandatory to the root canal system because it entombs the remaining microorganism and fills the inaccessible areas of prepared canals [2]. Over the years, different types of root canal sealers, based on zinc oxide eugenol, calcium hydroxide, glass ionomer, epoxy resin and silicone have been introduced to endodontics to improve the sealing ability and to provide excellent antimicrobial action [3]. AH 26 was the first epoxy resin-based sealer which was in use for several decades demonstrated cytotoxic effect because of the release of formaldehyde content as a byproduct during the setting reaction. This lead to the modification of the sealer and they developed AH Plus sealer which is an improved modification of its precursor AH26. AH plus is a 'formaldehyde free' material supplied as two paste system - Epoxide paste and the Amine paste [4]. Mineral trioxide aggregate (MTA) was developed by Mahmoud Torabinejad in 1993 at Loma Lumina university to seal communications between the tooth and its external surfaces [5]. Currently, it is being used in conservative pulpal treatments, as apical barrier in the non-vital immature teeth as root end filling material and as coronal seal in revascularization procedure. It is widely accepted for its biocompatibility and excellent sealing capacity. The MTA powder consist of fine hydrophilic particle such as tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide [6]. Newer endodontic sealers have been developed based on the physicochemical properties of MTA in an attempt to develop a biocompatible sealer with the ideal physical, chemical, and mechanical properties. The grainy texture MTA cement make both manipulation and filling of canals more challenging for the operator. Hence modifications in the original formulation of MTA have been suggested in order to improve its handling properties [7]. MTA FILLAPEX(Angelus) endodontic sealer wasdeveloped in 2010. It has biological properties of MTA and composition after mixture is basically MTA, salicylate resin (1,3 butylene glycol disalicylate resin), bismuth and nanoparticulated silica. Till date, there is a lack of scientific studies evaluating physico-chemical and biological properties of this material [8]. Bioceramic materials have been recently introduced, mainly as repair cement and as root canal sealer [9]. They are based on calcium phosphate and calcium silicate. They are composed of Zirconium oxide, Calcium silicates, Calcium phosphate monobasic, Calcium phosphatemonobasic, Calcium hydroxide, fillers and thickening agents. It is a premixed, radiopaque, injectable and utilizes the moisture present within the dentinal tubules to initiate and complete the setting reaction. It has been reported that the Bioceramic sealer has no shrinkage upon setting, resulting in a gap-free interface between gutta percha and dentin [10]. The pH change of sealers plays a vital role in healing, because pH is associated with antimicrobial effects and deposition of mineralized tissue. It has been found that an alkaline pH of root canal sealer could neutralize the lactic acid from osteoclasts and prevent dissolution of mineralized components of teeth [11]. Calcium hydroxide promote healing by stimulating periapical tissues and also provide antimicrobial action [12]. Radiopacity property is important because an endodontic sealer must be radiopaque enough to be differentiated from neighboring anatomical structures (bone and tooth structures) and other dental materials [13]. According to ANSI/ADA specification No.57, all endodontic sealers should exhibit 2 mm of AL value. It should be more radiopaque than dentin or bone [14].

The aim of this study was to investigate pH, calcium ion release, and radiopacity of Bioceramic sealer, MTA Fillapex and AH Plus sealer.

MATERIAL AND METHODS

GROUP-1- MTA FILLAPEX SEALER (ANGELUS)

GROUP-2 - AH PLUS (DENSPLY)

GROUP-3 - BIOCERAMIC SEALER (BRASSELER)

pH ANALYSIS & CALCIUM ION RELEASE

To determine the pH and calcium ion release, five freshly prepared samples as per manufacturer instruction of each material was inserted into polyethylene tubes measuring 1.5mm internal diameter and 1 cm length with only one end open. Each sample was placed in the flask containing 10 ml of distilled water and stored at 37 degrees. The tubes were replaced after 3 hours into another flask with an equal amount of new distilled water. This procedure was repeated after 6, 12, 24 and 48 hours, 7, 15 and 30 days. Later the pH was measured by using a calibrated digital pH meter to the above predetermined periods. The same solution used for pH analysis were used to measure the calcium ions release with an atomic absorption spectrophotometer. The concentration of calcium ions was determined with a calcium hollow cathode lamp (422.7nm wavelength and O.7-nm window) operated at 20 Ma.

RADIOPACITY TEST

Tencylindrical samples of the MTA Fillapex and Bioceramic sealer were prepared by pouring into the metallic rings (1mm diameter, 1mm inch thickness). AH plus sealer were mixed according to the manufacturer specification, poured into the metallic ring. All the samples were made to set at 37 degrees and then removed from the ring. The dentin cylinders was prepared from non carious roots of premolar that were used for the purpose of radiographic comparision along with the set sealer and aluminium step wedge. The thickness of dentin cylinders was tested with a dental caliper. Radiographic comparison was performed on all the set sealer and dentin specimens (1mm thickness) by placing them on occlusal films along with an aluminium step wedge that varied in height from 1- 10 mm. The radiographs were taken using radiographic unit operated at 60 KV,10mA and the exposure was set to 0.3 seconds with a focus film distance of 30 cm. Radiopacity was determined by digitizing the radiographs and calculating the radiographic density in millimetres of aluminium step wedge.

RESULTS

pH ANALYSIS

Bioceramic sealer cement presented high alkaline pH in all experimental times, with maximum pH value at the period of one week followed by MTA Fillapex (Table 1). AH Plus cement demonstrated slightly neutral pH. The pH values were not significant among the groups ($p<.995$)

CALCIUM ION RELEASE

Bioceramic sealer presented significantly greater release of Calcium ion than MTA Fillapex in all the experimental periods. The high release of calcium was observed during the first week. AH Plus sealer present the lowest calcium release in all the experimental period. Table 2 describes the amount of Calcium release released at the different experimental times. The calcium ion release were highly significant among the groups (P <0.001**).

RADIOPACITY TEST

The radiopacity value of different sealers are described in Table 3. AH Plus (8.72 ± 0.23 mm Al) had the highest radiopacity followed by MTA Fillapex(6.71±.33 mm Al) and Bioceramic sealer (4.19 ±0 .19mm Al) .This value was above the recommendation by ISO 6786/2001. The dentin presented radiopacity corresponding to 1.070 ± 0.150 mm Al. The radiopacity values were highly significant among the groups(P <0.001**).

TABLE.1. pH(mean, standard deviation)

		Bioceramic Sealer		MTA Fillapex		AH Plus	
		Mean	SD	Mean	SD	Mean	SD
Hours	3 hr	10.28	.48	9.38	.11	6.94	.11
	6 hr	10.74	.54	8.16	.11	6.50	.12
	12 hr	10.04	.21	8.42	.31	6.30	.16
	24 hr	11.04	.28	8.62	.16	6.14	.11
	48 hr	11.08	.41	8.14	.11	5.86	.09
	7 days	11.42	.35	8.18	.18	5.62	.11
	15 days	11.24	.29	7.98	.15	5.68	.08
	30 days	11.28	.40	7.88	.08	6.32	.04
Group Total		10.89	.58	8.34	.48	6.17	.43

TABLE 2. CALCIUM ION RELEASE(mean, standard deviation)

		Bioceramic Sealer		MTA Fillapex		AH Plus	
		Mean	SD	Mean	SD	Mean	SD
Hours	3 hr	17.99	1.08	13.42	.68	9.04	.59
	6 hr	19.94	1.42	16.04	1.20	10.60	.72
	12 hr	14.95	.92	10.99	1.20	6.57	.64
	24 hr	12.33	.71	14.10	.76	9.55	.89
	48 hr	14.56	.89	16.84	1.11	6.90	.59
	7 days	21.45	1.45	16.42	.98	13.46	.79
	15 days	14.94	1.20	13.15	.77	11.23	.84
	30 days	18.34	1.08	16.33	.62	11.53	.69
Group Total		16.81	3.10	14.66	2.14	9.86	2.32

TABLE.3 RADIOPACITY(mean, standard deviation)

Materials	Mean	SD
Bioceramic Sealer	4.19	.19
MTA Fillapex	6.71	.33
AH Plus	8.72	.23

STATISTICAL ANALYSIS:

The results of the present study were subjected to statistical analysis to interpret the significant differences in pH, calcium ion release and radiopacity values within each group and also between the groups using one way ANOVA and Post hoc tukey tests.

One way analysis of variance (ANOVA) is used to study the overall variance within groups. The mean percentage pH, calcium ion release and radiopacity with standard deviation were calculated. The Post hoc Tukey test was used for intra group and inter group comparison in order to determine which groups differ from each other. The Post hoc Tukey Test is designed to perform a pair wise comparison of the means to identify the specific sub groups in which significant difference expression occurs.

TABLE-4: ONE-WAY ANOVA FOR INTER GROUP COMPARISION OF RADIOPACITY, pH, CALCIUM ION RELEASE SCORES

GROUP	RADIOPACITY	p H	CALCIUM ION RELEASE
	M+SD	M+SD	M+SD
BIOCERAMIC	4.19±0.19	10.89±3.10	16.81±3.10
MTA FILLAPEX	6.70±0.33	8.34±0.47	14.66±2.14
AH PLUS	8.71±0.22	6.17±0.43	9.85±2.31
P-VALUE	0.000**	0.000**	0.000**

***** Denotes significant at 1% confidence level***

TABLE-5: POST HOC TUKEY ANALYSIS FOR THE COMPARISON OF INTERGROUP SCORES

GROUPS	P- VALUE	P- VALUE	P- VALUE
	RADIOPACITY	p H	CALCIUM RELEASE
BIOCERAMIC X MTA FILLAPEX	0.000**	0.000**	0.001
BIOCERAMIC X AH PLUS	0.000**	0.000**	0.001
AH PLUS X MTA FILLAPEX	0.000**	0.000**	0.000**

**** Denotes significant at 1% confidence level**

DISCUSSION:

The main function of a root canal sealer is to fill the irregular spaces between the gutta-percha and the canal walls along with accessory canals, lateral canals and multiple foramina [15]. Clinician select the sealers for their ease and convenience of clinical application [16]. With novel endodontic sealers being successively developed, it is important to understand their physicochemical properties. Laboratory studies based on the physicochemical properties can give a better knowledge on the clinical behavior and handling performance of endodontic sealers. The properties include good sealing, radiopacity, easy handling, dimensional stability, high flow and low solubility. This present study evaluated some of the important properties that are essential for an endodontic sealer. This study evaluated some of the physicochemical andbiological properties such as pH, calcium ion release, radiopacity, flow and cytotoxicity of Bioceramic sealer, AH plus and MTA based sealer. Studies have suggested that the repair and stimulation of mineralized tissue depends on pH and calcium ion release [17]. Calcium reacts with carbonic gas to initiate the calcification process. Therefore, by analyzing the alkalinization ability and calcium releasing, the sealer can be indirectly evaluated for the induction potential of mineralization [18].

The assessment of pH and calcium ion release through the method of immersion of standardized polyethylene tubes in distilled water, is well established and superior to invivo studies. The placement of the materials inside root canals results in inaccurate results due to the difficulty in standardization of the foramen opening and also because of the interference of root dentine itself [18].

Hence in the present invitro study, calcium ion release and pH were analysed by inserting the sealer into the polyethylene tube. Radiopacity is an essential property of a endodontic sealer along with the physical, chemical and biological property to have a clear visibility of penetration of the filling materials into the accessory canals on radiographs [19].The degree of radiopacity in a sealer is mandatory for controlling root canal filling to differentiate it from tooth and periradicular anatomical structures [13]. In this present study, the radiopacity of three sealers were compared.

One of the most recommended methods to measure the radiopacity is the use of an aluminum step wedge as a reference standard. Both International Standards Organization (ISO) and American National Standards Institute/American Dental Association (ANSI/ADA) have published standardized procedures for quantifying the radiopacity of several types of dental materials, referring at least 98% pure aluminum wedge as a reference (ISO 6876/2001)20. The ISO 6876/2001 (2001) standard establishes that root canal sealers should be at least as radiopaque as 3 mm Al. According to the American National Standards Institute and American Dental Association (ANSI/ADA) specification No. 57, endodontic filling materials should present a difference in radiopacity equivalent to at least 2 mm Al in comparison to bone or dentin [21]. Katz et al first used aluminum step wedge to compare the radiopacity of gutta-percha cones and observed the radiopacity of 7.4 mm Al [22]. Specifications for root canal filling materials demand only minimum radiopacity limit, the extreme increase in radiopacity leads to false impression of a dense homogenous filling. Bone, periodontal ligament and dentin radiopacity presents difference in the levels. The radiopacity of 3mm Al stepwedge is ideal for differentiating of lateral canal oburation and overfilling [23]. In the present study, all sealers showed different pH values and calcium ion release at different time interval (TABLE 1). The result shown in increasing order. AH plus<MTA Fillapex <Bioceramicsealer. Bioceramic sealer showed high pH (10.89±.58) with increase after 1 week. The pH of MTA fillapex was 8.4 in the first week and there is reduction after that. AH plussealer had a neutral pH.

It has been reported that high pH in the sealer activates alkaline phosphatase that is present in the tissues, is involved in mineralization process and also neutralize the acids secreted by osteoclasts and in MTA-based materials, the chemical reaction that takes place during setting results is the formation of calcium hydroxide, which subsequently dissociates into calcium and hydroxyl ions which increase the pH [19]. Holland *et al*.(1999)evaluated MTA root canal sealer in dogs teeth and their results showed that MTA sealer consistently induces closure of the main canal foramen by new cementum deposition [24].

Hui Zhang et al (2009) compared the pH values and antibacterial activity against E. faecalis, indicate that there are factors other than pH that are more important for their antibacterial activity [25].

On basis of calcium ion release (TABLE 2), High levels of calcium ion release were observed in Bioceramic sealer (16.81±3.10) >MTA Fillapex(14.66±2.14) but not in AH Plus(9.86±2.32). The calcium oxide and calcium hydroxide-containing sealers such as Bioceramic sealer and MTA fillapex favors an alkaline pH and high calciumrelease.The low calcium release of AH Plus is in agreement with Duarte et al(2004) who observed low values in calcium release of pure AH Plus, similar to zinc oxide eugenol sealer [26].

Milton Carlos et al. (2013) showed same results to the present study with MTA fillapex providing more pH and calcium ion release than AH plus in all time periods but same antibacterial efficacy26.Similarly George CaccioCanderiro et al (2012) analysed the pH and calcium ion release of Bioceramic sealer and AH plus and found similar result to the present study with high pH and calcium ion release in Bioceramic sealer at all time interval [27]. The low calcium release in AH plus is because of the presence only calcium tungstate, calcium releasing compound in its composition [19]. In first week, Bioceramic sealer presented the high calcium release (21.45± 1.45) and this is because of the final setting time of this material that requires 160 - 240 hours in moist medium. It was observed that moisture facilitates the hydration reactions of calcium silicates to produce calcium silicate hydrogel and calcium hydroxide, which partially react with the phosphate to form hydroxyapatite and water. The release of calcium and hydroxyl ions from Bioceramic sealer may result in the formation of an interfacial layer that forms a chemical bond between the sealer and dentinal walls [9].

In the recent study comparing the pH and calcium ion release of Bioceramic sealer and AH plus with pH meter and atomic absorption spectrophotometer, George *et al* concluded the similar result of the present study with high alkaline pH and calcium ion release of Bioceramic sealer at a time period of one week over AH plus sealer [9].

CONCLUSION

Within the limitations of this Invitro study of this present study, it can be concluded that:

1. AH Plus sealer exhibited superior radiopacity followed by MTA Fillapex and Bioceramic sealer
2. Bioceramic sealer exhibited high calcium ion release and alkaline p H in all tested periods followed by MTA Fillapex. AH Plus exhibited lowest calcium release and neutral pH.

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