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ORIGINAL ARTICLE

Comparative Evaluation of Physicochemical Properties and Apical Sealing Ability of a Resin Sealer Modified with Pachymic Acid

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ABSTRACT

Objective: The addition of pachymic acid (PA) to AH Plus (an epoxy resin sealer) offsets the cytotoxicity of the latter. Prior to the clinical implementation of this formulation, a thorough knowledge of its physicochemical properties and sealing ability becomes mandatory. Hence, this in vitro study aimed to characterize and evaluate the physicochemical properties and apical sealing ability of AH Plus (AHP) with and without the addition of PA.

Methods: Flow, setting time, film thickness, solubility and radiopacity of AHP (group 1) and AHP modified with PA (AHP/PA, group 2) were evaluated in accordance with the guidelines put forth by ISO 6876:2012. The percentage was determined under each parameter. Apical sealing ability was assessed using fluid filtration device. An independent samples t-test was used for inter- and intra-group comparisons of mean fluid flow (MFF).

Results: Incorporating PA to AHP decreased its flow, setting time and film thickness by 24.34%, 2.14% and 31.71% respectively. The solubility of group 2 increased on day 1 by 85.71% and decreased on days 3, 7 and 14 by 46.67%, 34.79% and 13.8% respectively. The radiopacity of AHP was not altered by the addition of PA. MFF rates of group 2 was significantly higher than group 1 on day 1, but not significantly different on day 7.

Conclusion: AHP/PA exhibited physicochemical properties that were within the requirements of ISO and with time, and showed fluid flow similar to AHP.

Keywords: AH Plus, apical sealing ability, pachymic acid, physicochemical properties, resin-based sealers, root canal sealer

HIGHLIGHTS

- This is the first study to evaluate the effects of addition of pachymic acid to AH Plus sealer.
- The addition of pachymic acid to AH Plus did not affect the physiochemical properties of the latter, thereby satisfying the ISO requirements specified for root canal sealers.
- The sealing ability of the modified sealer improves with time.
- Considering the beneficial anti-inflammatory effects of pachymic acid, the results of the study suggest that it could be a valuable addition to AH Plus.

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INTRODUCTION

The success of endodontic treatment lies in meticulous shaping and disinfection of the root canal, followed by its three-dimensional obturation. This procedure should seal all possible portals of communication between the canal and the surrounding periapical tissues. Gutta-percha (GP) and root canal sealers are more commonly used to achieve this. The flow of sealer plugs the gap between the canal wall and core material and fills anatomic intricacies (1). Various sealers have been developed for this purpose and are classified based on their major chemical component. Among the commercially available sealers, epoxy resin-based sealers (ERS) are widely used for obturation and AH Plus (Dentsply, DeTrey GmbH, Konstanz, Germany) is considered a benchmark among them (2).

Despite all efforts to confine the sealer within the root canal space, there is a sound chance for leachable substances or degradation products from the sealer to enter the periapical tissues via the apical foramina, lateral and accessory canals. Inadvertent extrusion of the sealer during obturation brings them in close contact with the periapical tissues and they continue to remain so for extended periods of time, which can cause inflammation, leading to increased postoperative discomfort and even failure of root canal treatment (1).

The cytotoxicity of freshly mixed AH Plus is well documented and is attributed to the release of formaldehyde, presence of bisphenol A glycidyl ether (a mutagenic component) and glutathione (GSH) depletion (3). Its contact with periapical tissues can induce inflammation and oxidative stress in that region (4).

From the time of their inception into clinical practice, the composition of sealers has been constantly experimented with the addition of various substances such as calcium hydroxide (5), amoxicillin (6), chlorhexidine, cetrimide (7), quaternary ammonium polyethylenimine nanoparticles (QPEI-np) (8) and benzalkonium chloride (2) to improve their antimicrobial activity. Hinokitiol was added to AH Plus to provide an anti-inflammatory effect (9). Studies have also shown that ERS incorporated with antioxidants such as peroxisome proliferator–activated receptor gamma (PPAR) agonists and N-acetyl-L-cysteine (NAC), are effective in scavenging reactive oxygen species (ROS), thereby reducing sealer-induced cytotoxicity (3, 10).

Pachymic acid (PA, ChemFaces Biochemical Co., Ltd, Wuhan, China), a triterpenoid, extracted from the fruiting body of Fomitopsis nigra mushroom, has been shown to possess anti-inflammatory effects (11). Kim et al. (12) demonstrated that the antioxidant effect of PA restored cell viability and alkaline phosphatase activity in mouse osteoblast (MC-3T3 E1) cells exposed to AH Plus. Arun et al. (13) reported that incorporation of PA to AH Plus significantly reduced the cytotoxicity associated with the latter, when tested on L929 mouse fibroblast cells. Though such additions may be beneficial, the original sealer formulation prescribed by the manufacturer is altered. So, a detailed analysis of its properties and sealing ability becomes mandatory. Hence, this study aimed to assess in vitro, the physicochemical properties and apical sealing ability of AH Plus with and without the addition of 0.5% PA.

MATERIALS AND METHODS

The study was duly presented to the Institutional Review Board and approval was obtained.

Preparation of test samples

AH Plus (Group 1, AHP) was prepared by mixing 1g of paste A and 1.18g of paste B according to the manufacturer's instructions. AH Plus modified with 0.5% pachymic acid (Group 2, AHP/PA) was prepared by adding 1.6 mg of pachymic acid to the mixed sealer.

The study consisted of two parameters, namely evaluation of physicochemical properties and apical sealing ability of the modified sealer, in comparison to AH Plus.

Evaluation of physicochemical properties

The following physical properties were evaluated according to ISO 6876:2012 (14). All the experiments except for solubility and radiopacity were performed thrice and their mean was recorded.

Flow

0.05 mL of sealer was dropped on a glass plate. After 3 minutes from the start of mixing, another glass plate was kept centrally upon the sealer and a mass of 100 g was placed on it. After 7 min, the largest and smallest diameters of the compressed sealer discs were measured using a digital vernier caliper (Mitutoyo Corporation, Tokyo, Japan). When the diameters were within 1 mm of each other, their average was recorded as the flow value.

Setting time

Sealers were mixed and filled in glass plate-supported teflon molds having an internal diameter and height of 10x2 mm. The whole assembly was placed in a cabinet (37°C and >95% RH) after 2 min from the end of mixing. When the manufacturer's setting time approached, a Gilmore indenter weighing 100 g, having a flat ended cylindrical needle tip and a diameter of 2 mm was lowered onto the sealer surface. The procedure was halted when indentations were visible on the sealer surface. This was repeated on a new location every one hour upto 20 hours and then once every five minutes until indentations were not visible. The time elapsed from the start of sealer manipulation until the procedure was halted was taken as the setting time.

Film thickness

Two optically flat, square glass plates having dimensions of 15x15x5 mm were placed on top of each other and their combined thickness was measured to an accuracy of 1 µm. 20 mg of freshly mixed sealer was kept centrally between the plates. After 3 minutes from the start of mixing, a load of 150 N was applied on the top plate to make sure that the gap between the two plates is completely filled with sealer. After 7 minutes, the thickness of plates together with sealer was recorded with a digital micrometer (Mitutoyo Corporation, Tokyo, Japan). The film thickness was determined by the difference in the thickness of the plates with and without the sealer.

Solubility

Sealers discs with a diameter and height of 20x1.5mm were made and placed inside an incubator at 37 °C and >95% relative humidity until set. The set sealer samples were weighed with an analytical balance (W1) and immersed in test tubes having 10 mL distilled water. They were then taken out at 1, 3, 7 and 14 days, dried using absorbent paper and kept inside a desiccator for 24 h. After drying (W2), the solubility (S) was determined [S=(W1–W2)/W1x100]. The test was performed twice and mean value obtained.

Radiopacity

10x1 mm sealer discs were prepared and placed in the middle of an intra-oral occlusal, E-speed X-ray film, adjacent to an aluminium (Al) step wedge (50 mm length, 20 mm width, 1-9 mm thick, with equidistant steps of 1 mm). The entire set up was radiographed (65±5 kV and 400 mm target-film distance) and the film was processed and dried. Using an optical density meter (PTW Freiburg GmbH, Germany), the density of the specimen's image was compared with that of the step wedge and the radiopacity was expressed in millimeters of Al.

Evaluation of apical sealing ability

Group 1 (AHP), group 2 (AHP/PA) and a group 3 as control were evaluated for apical sealing ability. 24 recently extracted, single-rooted human mandibular premolars (n=8 per group) with relatively straight roots, as confirmed by bucco-lingual and mesio-distal radiographs were collected. Teeth with caries, multiple

canals, excessive canal curvature, cracks, resorption, fractures and/or incomplete apex formation were excluded. Standardized 10 mm root sections from the apex were obtained using carborundum discs. Working lengths were established 1mm short of the apical foramen using size 10 K-file (Mani Inc. Tochigi, Japan). Enlargement of the canal was done in crown-down manner using rotary NiTi files (ProTaper, Dentsply Maillefer, Ballaigues, Switzerland) with irrigation using 5.25% NaOCl. Apical third was prepared to size 40 (6%) and the canals were irrigated with 17% EDTA for 1 min in order to dissolve the smear layer. Distilled water was used a final rinse and the canals were dried using paper points. The sealers were introduced (groups 1 and 2 only) with a lentulospiral and the canals were obturated with GP using lateral condensation technique. The roots were stored in 100% humidity for a period of 24 hours.

In groups 1 and 2, the roots were coated with nail varnish, excluding one mm surrounding the apical foramen and were positioned in a fluid filtration device to evaluate their apical sealing ability. Apical side of the root was placed inside a plastic tube, which was further fitted to an 18-gauge stainless steel tube. Cyanoacrylate glue was applied circumferentially between the plastic tube and the root, in order to obtain an airtight seal. All pipettes, plastic tubes and syringes at the apical end of the sample were completely filled with distilled water. A constant pressure of 10 psi was maintained with an air pressure regulator throughout the experiment. A micro syringe was used to suck back water for approximately 2 mm to create an air bubble inside the micropipette. The bubble was then positioned appropriately and its movement was measured (in µl/min) at an interval of 2 minutes for a period of 8 minutes. Thus, four readings were obtained per sample. Their mean was taken as the mean fluid filtration rate (MFF) of that particular sample. Samples in group 3 (control) were obturated using GP alone (without sealer), used as positive controls and their MFF was measured. The same samples were then completely coated with nail varnish including the apex, used as negative controls and their MFF was evaluated. All the samples were stored at 37 °C for one week, following which MFF measurements were made again. The values were tabulated and statistically analyzed.

Statistical analysis

To assess whether the MFF values were normally distributed, Shapiro-Wilk's test was employed. Homogeneity of variances was assessed using Levene's test for equality of variances. An independent samples t-test was used for inter- and intragroup comparisons.

RESULTS

Physicochemical properties

The mean flow, setting time and film thickness of both the groups are given in Table 1. AHP/PA showed a decrease in flow, setting time and film thickness by 24.34%, 2.14% and 31.71% respectively compared to AHP. Both the sealers had a radiopacity equivalent to 9 mm of Al, which corresponds to a reading of 0.38 in optical density meter. The mean solubility of the groups is given in Table 2. AHP/PA showed an increase in solubility on day 1 compared to AHP, whereas on days 3, 7 and 14, it showed a decrease.

Apical sealing ability

The mean and standard deviation (S.D) of fluid flow rates of both the groups on days 1 and 7 are given in Table 3. Positive controls showed a rapid movement of the air bubble immediately when the pressure was applied. Hence, their fluid flow rate was immeasurable. Negative controls showed no fluid movement, which confirmed the functioning and reliability of the experimental set-up. Since the mean values for the control (group 3) was '0', further statistical analysis was carried out using only the mean values of groups 1 and 2. The null hypothesis was that, the incorporation of pachymic acid to AH Plus shall not significantly change its apical sealing ability.

As assessed by Shapiro-Wilk's test, MFF scores of both the groups at two time intervals were normally distributed (P>0.05). As assessed by Levene's test for equality of variances, there was homogeneity of variances (P=0.465 and 0.05). There was a statistically significant difference in the fluid flow rates between the groups [t(14)=-3.366, P=0.005] at day 1, with AHP/PA showing a significantly higher fluid flow compared to AHP. But at day 7, the scores were not statistically significant {t(14)=-1.390, P=0.186}. Intragroup comparison showed no significant difference between days 1 and 7 in both the groups.

TABLE 1. Mean flow, setting time and film thickness of both the groups

Properties	Groups	
	Group 1 (AHP)	Group 2 (AHP/PA)
Flow (in mm)	26.49	20.16
Setting time (in h)	23.3	22.8
Film thickness (in µm)	21.66	15

TABLE 2. Mean solubility of the sealers

Days	Groups		% difference between the groups
	Group 1 (AHP)	Group 2 (AHP/PA)	
1	0.035	0.065	-85.71
3	-0.075	-0.04	46.67
7	-0.115	-0.075	34.79
14	-0.145	-0.125	13.8

*A decrease in solubility is denoted as a negative numerical value.

TABLE 3. Mean and standard deviation of fluid flow rates of all the groups on day 1 and 7

Days	Groups	n	Mean (in µL/min)	SD
Day 1	Group 1 (AHP)	8	0.190±0.01*	0.016
	Group 2 (AHP/PA)	8	0.213±0.01*	0.011
Day 7	Group 1 (AHP)	8	0.188±0.01	0.018
	Group 2 (AHP/PA)	8	0.206±0.03	0.030

*Denotes significant difference between the groups (P<0.05); SD: Standard deviation.

DISCUSSION

To determine the optimal concentration of PA that can be incorporated to AHP, a pilot study was conducted with varying concentrations of PA, ranging from 0.25%-1%. It was inferred that the addition of 0.5% PA did not jeopardize the flow, setting time and solubility of AHP. Hence, this concentration was chosen for the current study. The physicochemical tests were done according to ISO 6876:2012 as it permits reproducibility and further comparison between studies.

The flow of a sealer indicates its ability to glide into the canal and its ramifications which unless filled creates a communication channel between the main canal and periodontal ligament (15). When determined in accordance with ISO, each disc should have a diameter of not less than 17 mm. The addition of PA decreased the flow of AH Plus, but the values remained well within ISO specifications. Similar results were obtained when amoxicillin (6), hinokitiol (9), benzalkonium chloride (2), quaternary ammonium polyethylenimine nanoparticles (8), cetrimide (7) and calcium hydroxide (5) were added to AH Plus. However, Ruiz-Linares et al (2013) (7) found contradictory results when CHX was incorporated into AH Plus. Presence of epoxy resin facilitates higher flow values for AH Plus sealer. Since the sealer's composition has a direct effect on its flow (16), addition of pachymic acid to AH Plus sealer, could have thickened the final mass thereby increasing the viscosity of AH Plus. Other factors such as particle size of the sealer, manipulation time, shear rate, composition, temperature, internal diameter and insertion rate could also influence flow (17). In case of large root canals with wide apical foramen, where the risk of sealer extrusion is more, this combination of AHP/PA can be considered as a safe alternative to the use of AHP alone.

The setting time of the sealer should be as short as feasible due to the complexity in maintaining the canal dry. An increased setting time will also raise the likelihood of the unset sealer contacting the periapex and causing consequent damage to the tissues (16). The setting time of both AHP (23.3 hours) and AHP/PA (22.8 hours) were within ISO specifications, the values of AHP/PA being minimally lesser than AHP. Similar findings were reported when amoxicillin (6), chlorhexidine (7) and a combination of chlorhexidine and cetrimide (7) were added to AH Plus. However, contradictory results were found when benzalkonium chloride (2), hinokitiol (9), quaternary ammonium polyethylenimine nanoparticles (8) and cetrimide (7) were incorporated into AH Plus. In this study, the setting time observed was higher than the setting time observed by other authors (7, 18). This could be caused by the differences in the weight of the needles used in the tests, which varied from 100 g to 453.6 g (19). Various other aspects like room temperature, particle size, components of the sealer, the part of the sealer tube and relative humidity could also influence the setting time (20). The reactive amine groups present in AHP could have been consumed faster in the setting reaction of AHP/PA. Thus, it can be postulated that the incorporation of pachymic acid hastened the setting reaction of AH Plus. Also, the form, structure, composition and concentration of pachymic acid may also have contributed to the accelerated polymerization process of AH Plus.

A thin film sealer can provide better wetting of the surface, ease of GP placement and thereby a better seal than a sealer with a greater film thickness (21). ISO advocates a sealer film thickness of no greater than 50 µm. The film thickness of both AHP (21.66 µm) and AHP/PA (15 µm) were well within ISO limits. However, AHP/PA's film thickness was 31.71% lower when compared to AHP. The decrease in film thickness with the addition of pachymic acid could be attributed to the thixotropic behavior of the material (22). The methodology for testing the film thickness, which involves using a weight of approximately 15 Kg could also have resulted in shear thinning of the material under such a high load.

According to ISO, the radiopacity of the sealers should be equal to or greater than 3 mm of Al. The addition of PA did not alter the radiopacity of AH Plus and the values were equivalent to 9 mm of Al. The values of radiopacity recorded in this study were lower than those reported by other authors (18, 23), but were higher than that observed by Vertuan et al., (19) and Ruiz-Linares et al. (7). These differences may probably be due to the variations in methodology applied or the manipulation of the sealer. Being a base-catalytic sealer, its components could get deposited at the bottom of the tube while the initial part possesses reduced amounts of radiopacifiers, resulting in different values (24).

The methodology used in this study is a modification of that proposed by ISO and previously reported by various authors (23, 25). It is based on the evaluation of the specimen's loss of mass prior to and after 7 days of immersion in distilled water, whereas the methodology prescribed by ISO is based on mass of residues produced by the specimens, after evaporation of the liquid in which they are immersed. This study demonstrated that both AHP and AHP/PA presented solubility values that were well within the ISO recommendations (not more than 3%). AH Plus presented lower solubility and similar results were obtained in several other studies (5, 26). During AH Plus's manipulation, polyamines and diepoxide compounds get mixed and covalent bonds are formed between the amine and epoxide groups resulting in a strong, rigid and highly cross-linked polymer, thus reducing its solubility (27).

The addition of PA increased the solubility of AH Plus on day 1. Similar findings were observed when calcium hydroxide (5), hinokitiol (9) and 2% quaternary ammonium polyethylenimine (QPEI) nanoparticles (8) were added to AH Plus. But on days 3, 7 and 14, the solubility of AHP/PA decreased. Similar behavior was noted when benzalkonium chloride was added to AH Plus (2). The differences in solubility with the addition of PA could be due to its chemical interactions with AH Plus. It has been reported that AH Plus exhibited severe cytotoxicity for 4 hours immediately after mixing (28). Whether this initial rise in solubility of AHP/PA could facilitate a burst of PA release, which might be able to counteract the early cytotoxicity of AH Plus sealer needs to be further studied.

Fluid filtration method is advantageous since it is non-destructive and allows repeated measurements in the same sample over a period of time so that the leakage values can be quantified. It works on the principle that, if the canal is sealed completely, no movement of fluid can be detected. MFF of AH Plus was similar to those described by Boulliguet et al. (29). Results of this study showed that the mean fluid flow rates of AHP/PA were higher

than AHP on both the days. But this difference was statistically significant only on day 1, probably due to the initial and final setting phases of the materials. Hence, the null hypothesis was rejected and the alternate hypothesis was accepted.

There was a decrease in fluid flow rate in both the groups over time (day 7) and this could be attributed to the setting of the sealers. The presence of voids called 'dead ends' which progressively gets filled with water and do not allow the fluid to perfuse through, expansion of AH Plus and/or GP could have also led to the reduced leakage rates. A sealer, which is completely set, should not allow perfusion of fluids across it (29). But in this study, fluid flow was still elucidated. This could be attributed to the lack of bonding between the sealer and GP.

CONCLUSION

Within the limitations of this in vitro study, it can be concluded that incorporating PA to AH Plus:

1. Reduced the flow, film thickness and setting time of AH Plus.
2. Increased its solubility on day 1 and decreased on days 3, 7 and 14.
3. Did not alter its radiopacity.
4. Significantly decreased its apical sealing ability on the first day, but on the 7th day, there was no significant difference.

Though the physicochemical properties of AHP/PA presented values that were within the standards set by the ISO, further experiments on dimensional stability and long-term sealing ability should be carried out before considering this combination for clinical applications. Future studies should investigate the biological effects of this modified sealer on periapical inflammation and healing.

Disclosures

Conflict of interest: The authors deny any potential conflict of interest related to the study.

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