Original papers

A laboratory study evaluating the pH of various modern root canal filling materials

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Abstract

Background. Alkaline pH is responsible for antibacterial activity and the stimulation of periapical tissue healing. It neutralizes the acidic environment of inflammatory tissues in the periapical region of the teeth and favors bone repair by activating tissue enzymes.

Objectives. The aim of this study was to evaluate and compare in vitro the pH of 8 root canal filling materials (sealers and points) -AH Plus Jet (AH), Apexit Plus (AP), Endomethasone N (END), Epiphany (EP), GuttaFlow (GF), gutta-percha (G), Resilon (R), Tubliseal (T).

Material and methods. 0.1 g of each material ($n = 6$) was placed in dialysis tubes and immersed in 20 mL of deionized water. The control contained deionized water (pH 6.6) with an empty tube. The pH values were recorded immediately after immersion (baseline) and after 1, 2, 24, 48, 120, and 192 h with a pH-meter. Data were statistically analyzed using the Student's -t test and 1-way analysis of variance ($p < 0.05$).

Results. Nearly all the materials had pH significantly higher than the control ($p < 0.05$). There were significant differences in the pH between the materials tested at each time point ($p < 0.001$). The highest pH was exhibited by EP, followed by AP and AH. The lowest pH was shown by GF, G and R.

Conclusions. Among the materials studied, only EP, AP and AH Plus were able to elevate the pH level that would allow inactivation of microorganisms in the root canals and promote healing of inflamed periapical tissues. However, the low alkalizing potential of G and R can be modified by the concomitant application of sealers producing alkaline pH.

Key words: pH, endodontics, hydroxyl ion release, root canal obturation

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Increased health awareness among the population and the wish to save their natural teeth, accompanied by up-to-date knowledge and more effective working techniques of dentists, currently make it possible for people to preserve the teeth that in the past would have been extracted. Endodontics is the branch of dentistry concerned with the treatment of diseases of the pulp and periapical tissues. Root canal treatment is a safe and effective means of saving the teeth that otherwise would be lost. Endodontic therapy involves the removal of diseased pulp tissue and the subsequent shaping, cleaning, and hermetic obturation of the root canals to prevent their recontamination. Although this procedure results in removing blood vessels and nerves from the pulp cavity, it can preserve the tooth function successfully for many years provided the treatment is performed properly.[1] In some cases, multiple visits are required to complete endodontic therapy, during which inter-appointment dressings, such as calcium hydroxide are applied into the root canal.[2,3]

Calcium hydroxide releases hydroxyl ion, thus favoring alkaline pH, which is responsible for its antibacterial effect and stimulation of the periapical tissue healing. When used as a temporary dressing, it kills microorganisms actively by damaging the plasma membrane, DNA and proteins of microorganisms.[4] It has been shown that strongly alkaline pH inhibits growth, or even kills *Enterococcus faecalis* – facultative anaerobic Gram-positive cocci responsible for root canal treatment failures.[5] The alkaline pH does not only impede infection development, but also neutralises the acidic environment of inflammatory tissues in the periapical region and favours bone repair by activating tissue enzymes (alkaline phosphatase).[6] The effect of calcium hydroxide seems to be directly proportional to their alkaline potential.[7]

Currently, there is a tendency to limit the number of appointments necessary to complete root canal therapy. It is commonly believed that there is no need to apply temporary dressings into the root canal several times, as a similar effect can be achieved with their single application. Reducing the number of sessions in endodontic treatment eliminates the risk of complications, including the loss of temporary filling or tooth fracture, which can result in treatment failures. Thus, in endodontic therapy a one-visit model is proposed as a standard, with the shaping, cleaning and hermetic obturation of the root canal being performed during one appointment.[8] In light of this fact, it seems important that the functions of inter-appointment dressings could be replaced by the final root canal filling materials.

The primary functions of the root canal filling are obturation and sealing of the root canal space.[9] To fulfil these requirements, the simultaneous use of 2 materials is generally recommended: basic, in the form of central core material (gutta-percha or Resilon), and accessory, in the form of paste sealing spaces between core material and the root canal wall.

Since the studies involving the alkalising abilities of root canal filling materials are relatively scarce, the aim of the current study was to evaluate and compare in vitro the pH of commercially available sealers and points most commonly used in the dental practice.

Material and methods

Table 1 shows the composition of the materials used in the study. All sealers were prepared according to the manufacturer's instructions. Shortly after manipula-

Table 1. Materials used in the study, their compositions and manufacturers

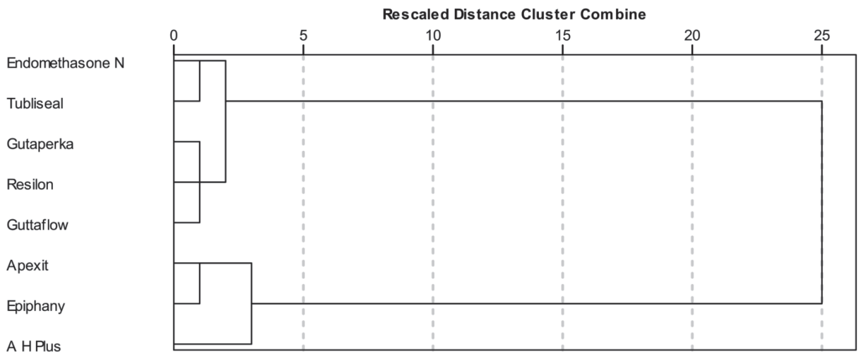
Name	Source	Active ingredients
AH Plus™	Dentsply DeTrey GmbH, Konstanz, Germany	bisphenol-a epoxy resin, bisphenol-f epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments, dibenzyldiamine, aminoadamantane, tricyclodecane-diamine, silicone oil
Apexit® Plus	Ivoclar Vivadent AG, Schaan, Lichtenstein	calcium salts (hydroxide, oxide, phosphate), hydrogenised colophony, disalicylate, bismuth salts (oxide, carbonate), highly dispersed silicon dioxide, alkyl ester of phosphoric acid
Endomethasone N Eugenol	Septodont, Cedex, France Chema – Elektromet, Rzeszów, Poland	zinc oxide, hydrocortisone acetate, thymol iodide, barium sulfate, magnesium stearate, eugenol
Epiphany	Pentron® Clinical Technologies, LLC Wallingford CT, USA	• organic part: bisgma, ethoxylated bisgma, udma, hydrophilic difunctional methacrylates • inorganic part: calcium hydroxide, barium sulphate, barium glass, bismuth oxychloride, silica
Gutta-Flow®	Coltene/Whaledent GmbH+Co. KG, Langenau, Germany	gutta-percha powder, polidimethylosiloxane, silicone oil, platin catalyst, zirconium dioxide, nano-silver, coloring
Gutta-percha points	VDW® GmbH Munchen, Germany	gutta-percha, zinc oxide, barium sulfate, pigment agent
Resilon points	Pentron® Clinical Technologies, LLC Wallingford CT, USA	• organic part: thermoplastic synthetic polymer – polycaprolactone, • inorganic part: biactive glass, bismuth oxychloride, barium sulphate
Tubli-Seal	Kerr Italia S.p.A., Salerno, Italy	zinc oxide, barium sulfate, oleo resin, oils/modifiers, thymol iodide, eugenol

Table 2. pH of 8 endodontic materials tested at different times

Material		Time (h) 0	1	2	24	48	120	192
AH Plus (AH)	**mean**	10.04a	10.06a	10.09a	9.99	9.78	9.53	9.11
	SD	0.18	0.17	0.17	0.28	0.43	0.57	0.78
	minimum	9.87	9.90	9.95	9.72	9.16	8.67	8.01
	median	9.97	9.98	10.02	9.86	9.67	9.40	8.98
	maximum	10.31	10.31	10.34	10.40	10.34	10.19	10.02
Apexit (AP)	**mean**	9.92a	10.11a	10.20a	10.98a	11.09a	11.20a	11.26a
	SD	0.11	0,11	0,13	0,05	0,06	0,06	0,09
	minimum	9.73	9,93	9,98	10,89	11,00	11,12	11,10
	median	9.91	10,11	10,22	10,99	11,10	11,22	11,29
	maximum	10.07	10,23	10,34	11,06	11,17	11,26	11,35
Endomethasone N (END)	**mean**	7.09b	7.39b	7.41b	7.47*b	7.49b	7.32b	7.20§b
	SD	0.14	0.29	0.18	0.18	0.15	0.11	0.13
	minimum	6.89	7.01	7.20	7.21	7.27	7.12	6.98
	median	7.10	7.41	7.42	7.48	7.47	7.33	7.23
	maximum	7.28	7.75	7.70	7.71	7.69	7.45	7.32
Epiphany (EP)	**mean**	9.99a	10.71	11.04	11.28a	11.29a	11.21a	11.23a
	SD	0.22	0,14	0,26	0,09	0,07	0,04	0,05
	minimum	9.70	10,54	10,78	11,16	11,20	11,15	11,16
	median	10.07	10,74	11,05	11,29	11,28	11,21	11,25
	maximum	10.18	10,89	11,30	11,38	11,37	11,28	11,28
GuttaFlow (GF)	**mean**	6.51*d	6.53cd	6.56cd	6.39cd	6.07#§	5.63#	5.02
	SD	0.05	0.04	0.07	0.17	0.18	0 38	0.31
	minimum	6.46	6.49	6.41	6.17	5.86	5.15	4.57
	median	6.49	6.52	6.59	6.45	6.10	5.80	5.11
	maximum	6.58	6.58	6.60	6.54	6.27	5.98	5.36
Gutta-percha (G)	**mean**	6.16*c	6.41ce	6.33*c	6.42ce	6.55#cd	6.15#cd	6.27*#d
	SD	0.13	0.10	0.07	0.14	0.11	0.04	0.05
	minimum	6.05	6.30	6.24	6.29	6.40	6.11	6.20
	median	6.11	6.37	6.32	6.37	6.55	6.13	6.26
	maximum	6.36	6.57	6.42	6.65	6.70	6.21	6.35
Resilon (R)	**mean**	5.89c	6.47cf	6.53ce	7.04#*§	6.83*ce	6.61*ce	6.86#bce
	SD	0.17	0.09	0.09	0.24	0.23	0.09	0.06
	minimum	5.70	6.35	6.40	6.85	6.68	6.52	6.80
	median	5.84	6.47	6.54	6.92	6.70	6.60	6.86
	maximum	6.10	6.58	6.65	7.45	7.20	6.73	6.95
Tubliseal (T)	**mean**	7.04b	7.39b	7.41b	7.45§b	7.31b*	7.23§*b	7.11*bc
	SD	0.16	0.36	0.14	0.20	0.17	0.20	0.21
	minimum	6.85	6.94	7.17	7.12	7.09	7.01	6.89
	median	7.06	7.47	7.46	7.53	7.30	7.24	7.06
	maximum	7.20	7.83	7.57	7.63	7.60	7.54	7.44
control		**6.6 d**	**6.6 def**	**6.6 *de**	**6.6 #de**	**6.6 §de**	**6.6 §de**	**6.6 de**
p-values		*p = 0.006 a-d p > 0.05	a-f p > 0.05	*p = 0.043 a-e p > 0.05	#p = 0.002 *p = 0.006 §p = 0.011 a-e p > 0.05	§p = 0.001 *p = 0.006 #p = 0.007 a-e p > 0.05	§p = 0.002 *p = 0.004 #p = 0.027 a -e p > 0.05	*p = 0.001 §p = 0.023 #p = 0.038 a-e p > 0.05

The values which have not been tagged with identical letters and symbols in the columns indicate statistically significant differences at a level of p < 0.001; The values which have been tagged with identical letters a,b,c,d,e,f, in the columns are not statistically significant (p > 0.05) ; SD - standard deviation.

Fig. 1. Dendrogram illustrating the similarities in pH value of examined materials



The tested materials which are closest to each other in pH level are connected by vertical lines and form a cluster. The position of the lines on the scale (at the top of the figure) indicates the distances between clusters: the closer to the scale center, the greater similarity in pH level (details in the text).

tion, 0.1 g of each material was placed into dialysis tubes (Sigma Aldrich Chemie, Steinheim, Germany) and transferred into separate plastic vials, containing 20 mL of deionized water. A total of 6 samples were used for each material. The vials were hermetically sealed and kept in an incubator at 37°C.

Before each measurement, the vials were shaken for 5 s to ensure uniform hydroxyl ion distribution. The pH values were recorded immediately after immersion (baseline) and after 1, 2, 24, 48, 120, and 192 h with a pH-meter (ISE 710A, Orion Research Inc., Boston, USA), previously calibrated with solutions of known pH (4, 7, 10). Each sample was measured twice, and the mean value was recorded. The experiment was performed in static conditions (without changing the deionized water).[10] The pH of the deionized water in which an empty tube was immersed was measured in all study periods (control).[11]

Statistical analysis was performed using the software package STATISTICA 8.0 (StatSoft). One-way analysis of variance, ANOVA, for independent samples was applied to compare pH of the materials at each time point. If the difference was significant, individual comparisons were performed by Tukey's multiple comparisons test. The level of significance was set at $p < 0.05$. Hierarchical cluster analysis with a dendrogram, using average linkage between groups, was used as the classification method. Pearson's correlation coefficient was applied to measure the strength and direction of the linear relationship between the pH of the materials and the time of the experiment.

Results

The obtained results are listed in Tables 2 and 3, and presented in Fig. 1. The dendrogram (Fig. 1) presents 3 separate clusters of materials which are most similar to each other in terms of pH. The greatest similarity in pH was found in the following groups: the first cluster consisted of alkaline materials such as AP, EP and AH, the second one was composed of neutral materials -G, R, GF. The third cluster contained acidic materials -END, T.

The mean pH values and SD measured for the study materials at different time points are presented in Table 2. The controls showed no noticeable change over the experimental period.

The majority of the materials demonstrated significantly higher pH as compared to the control, except for GF at baseline and after 1, 2, 24 h, G after 1, 48, 120, 192 h and R after 1, 2, 48, 120, 192 h.

Generally, the pH of the materials differed between individual clusters and these differences were statistically significant ($p < 0.001$).

The highest pH was exhibited by EP, followed by AP and AH. All 3 materials had a very similar pH at baseline (no statistically significant differences, $p > 0.05$). EP had significantly higher pH than AH at all other time points ($p < 0.001$), and compared to AP after 24, 48, 120, 192 h. AH showed statistically lower but still alkaline pH than AP after 24, 48, 120 and 192 h ($p < 0.001$) (Table 2).

The pH of GF, G, and R did not differ significantly after 1 and 2 h ($p > 0.05$). The baseline R showed a lower pH than those of GF and G, but in the last period of the experiment the pH of R increased ($p < 0.001$) (Table 2). In the first 2 h, G had a lower pH than GF, but over time the pH of G rose and was statistically significant after 48, 120, and 192 h (Table 2).

Table 3. Correlations between mean values of materials pH and the duration of the experiment

Material	Pearson's correlation coefficient	Level of significance	Correlation
	r	p	
AH	-0.649	0.000	strong negative
AP	0.769	0.000	strong positive
END	-0.177	0.262	poor negative
EP	0.468	0.002	moderate positive
GF	-0.940	0.000	strong negative
G	-0.256	0.102	poor negative
R	0.395	0.010	moderate positive
T	-0.272	0.082	poor negative

r – Pearson's correlation coefficient; strong correlation $r > 0.6$; moderate correlation $0.3 < r < 0.6$; poor correlation $r < 0.3$; p – level of significance.

END and T were characterised by very similar and statistically insignificant pH values during all experimental periods ($p > 0.05$) (Table 2).

The analysis of the pH values of the materials as a function of time showed that only 2 sealers (EP and AP) were characterised by a gradual increase in pH until the final hours of the experiment. The pH value of other materials, after a slight increase, was either continuously decreasing (AH, GF) or stabilised (G, R, END, T).

A correlation was demonstrated between pH of the materials and time of the experiment. AP, EP and R showed positive and statistically significant correlations ($p < 0.05$) (Table 3). The other materials exhibited negative correlations, which were statistically significant for AH, GF, G and R ($p < 0.05$).

Discussion

The experimental method consisting in placing root canal filling materials in plastic tubes and immersing them in vials with an aqueous medium for a varying period of time in order to evaluate the pH of sealers is well established in literature. The dialysis tubes simulate the single-rooted teeth and, therefore, eliminate the anatomic variables found within the root canals of the teeth. According to Beltes and al., this method offers simplicity, time economy, and guarantees the reproducibility of measurements and easy comparisons of results.[12]

Among the materials tested, Epiphany (9.99–11.29) and Apexit Plus (9.92–11.26) had the highest pH. This may be due to the presence of calcium hydroxide in their composition. When the materials were placed in an aqueous solution, calcium hydroxide dissociated into hydroxyl and calcium ions increasing the pH in the surrounding medium.[13] AH Plus presented a slightly lower but still alkaline pH (10.09–9.11). The pH values observed in the present study were higher than those obtained by other authors. Tanomaru-Filho et al. demonstrated that Epiphany produced the pH of 7.11–9.04 throughout a 28-day observation period.[14] Faria-Junior et al. evaluated a new version of the Epiphany sealer – Epiphany SE (with acidic resin monomers added) and obtained pH values in a range of 5.25–5-72.[15-20] Apexit Plus caused alkalisation at the level of 7.5–10.79 and AH Plus in a range of 6.04–7.81. These discrepancies may be explained by various experimental conditions (different sample mass, evaluation of the release of hydroxyl ions after material setting, replacement of the surrounding medium after each measurement).

Zinc oxide-eugenol sealers, gutta-percha, Resilon and GuttaFlow exhibited neutral or slightly acidic pH. These observations are in agreement with earlier reports.[14,20,21]

Maintaining the alkaline environment during the root canal treatment and after its completion seems to be desirable from the clinical point of view.[7] It has been proven that the growth and development of osteoblasts, i.e. cells crucial for the healing of periapical tissues, depends on the pH in the extracellular fluid. In the acidic environment, osteoblast activity decreases, and even a slight drop in pH can inhibit their function. Precipitation of calcium and phosphate salts in tissues and mineralisation processes, on the other hand, are supported by the alkaline pH.[22]

One of the most frequently used biochemical markers of osteoblast activity and mineralisation processes in bones is alkaline phosphatase (ALP). It liberates free phosphate ions, which in turn react with calcium ions to form calcium phosphate precipitates in the organic bone matrix. Optimal pH for this enzyme activity can be varied in different biological systems, ranging from 8.0 to 10.8.[23]

The alkaline pH of root canal filling materials, dependant on hydroxide ion release, appears to be responsible for their antibacterial effect. Estrela et al. have proved that at a pH greater than 9, bacterial enzymes can be irreversibly inactivated, resulting in loss of their biological activity.[15] As the experiment shows, only materials in the alkaline group could produce the pH level favouring an alkaline phosphatase activity and promoting an antimicrobial action.[2,24]

Therefore, attempts are made to incorporate alkalising substances, such as calcium hydroxide into root canal filling materials. Tanomaru-Filho et al. observed a beneficial effect of adding 20% of calcium hydroxide to Epiphany sealer.[25] This resulted in a significant increase in the release of hydroxyl ions and thereby an elevation of pH values during the 28-day experimental period. Moreover, the addition of calcium hydroxide to Epiphany promoted better consistency for its use as a retrograde filling material, following root-end resection.[14] Duarte et al. also have shown that the $Ca(OH)_2$ addition to AH Plus favored a more alkaline pH. The authors emphasise, however, that when the material is used as a sealer, 10% addition of calcium hydroxide thickens the material too much, and they recommend a 5% incorporation.[20] Da Silva and Leonardo point out that merely the presence of calcium hydroxide in the composition of a sealer does not assure the release of an adequate amount of hydroxyl ions in the final product. The ions may not be released due to the interaction with other material components or after material setting.[26]

Still, it should be remembered that in clinical conditions the alkalising abilities of endodontic materials can be modified by dentine tissues. However, dentine seems to be a stronger buffer for acids than for alkalis. Main buffer properties depend on dentine hydroxyapatites together with water and a layer of adsorbed ions. The released layer adhering to apatite crystals reacts with various chemical compounds used in endodontic therapy and can modify their pH. The whole dentine tissue has been shown to be a more effective buffer than hydroxyapatite alone, indicating a contribution of dentine organic components to its buffer capacity.[27]

Conclusions

Among the materials studied, only Epiphany, Apexit Plus and AH Plus were able to elevate the pH level that would allow the inactivation of microorganisms in the root canals and stimulate healing of inflamed periapical tissues. Gutta-percha, Resilon and GuttaFlow did not increase the pH sufficiently to stimulate biologically beneficial processes. The low alkalising potential of gutta-percha and Resilon can, however, be modified by the concomitant application of sealers producing alkaline pH.

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