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Physical properties of MTA, BioAggregate and Biodentine in simulated conditions: A micro-CT analysis

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The aim of this study was to evaluate the physical properties of calcium-silicate based cements as retrograde filling in different pH and blood conditions using micro-CT. Eighty-four teeth were obturated and after root-end resection, 3 mm-deep root-end cavity was ultrasonically prepared. The samples were divided into four test groups (n=21). Cements were freshly prepared and analyzed with micro-CT before and after exposed. The test groups were exposed for four days to environments containing acid, alkali, or blood. An acidic pH significantly reduced the volume of all materials after four days and differed from the other environments. Biodentine has the highest percentage of loss in volume and density after treatment in an acidic environment. Porosity formation in the acidic medium was observed in Biodentine amongst all materials. The three-dimensional structures of all materials changed after exposed to an acidic pH, while fewer changes were observed in the structures of materials treated with blood and alkali.

Keywords: Endodontics, Microcomputed tomography, Porosity, Retrograde obturation

INTRODUCTION

After endodontic treatment, unsuccessful outcomes such as persistent apical infection may occur. In this case, apical surgery, which will involve the removal of the apical part associated with the periapical infection, is performed[1]. Following the removal of the infected root, it is required to seal the root apex with a filler agent to block the contact between the root canal system and the surrounding tissues and ensure recovery. On the other hand, since the apex is extremely large in teeth with nonvital immature roots, it would not be proper to seal the root canal with conventional root canal filling techniques[2,3].

The ideal apical sealing material must have the adequate physical, chemical, and biological properties. The physical properties include short hardening time, greater radiopacity than the dentin (ISO 2001), and dissolubility less than 3% (ADA 57 2000)[4]. Dissolution of the apical sealings is an undesirable situation since it causes a bacterial leak, and the desired success in treatment may not be achieved. Usually, a modified ISO6876 specification[5] or an ANSI/ADA no. 57/2000 specification[6] are used to evaluate the dissolubility. However, in this study, micro-CT, which is a new method, was used in the evaluation of the dissolubility of the apical sealing materials, and their volumetric values were obtained.

In certain clinical conditions, such as the presence of infection, the periradicular area has an acidic pH that may affect the sealing properties of the recently placed apical filling. It was reported that an area with low pH decreases the durability and hardness of the tested material by affecting the hardening reaction and dissolubility[7,8]. Saghiri *et al.*[9] reported that acidic pH causes MTA to start leaking after a shorter time, compared to neutral pH.

The majority of devital teeth are infected. Thus, the root canal system must be disinfected first. Calcium hydroxide, which is set as a creamy preparation, is used as an intracanal medicament to completely disinfect the root canals[10]. Freshly prepared calcium hydroxide alkalizes the pH of the environment[11]. It is impossible to completely remove calcium hydroxide from the dentin walls. Therefore, this area with alkaline pH can affect the physical properties of freshly prepared and applied apical sealing materials[12]. Lotfi and Stefopoulos[13,14] reported that when root canals are treated with calcium hydroxide, alkaline pH affected apical sealing materials.

There are no studies that investigated the relationship between pH values and increasing porosity which could affect the physical properties of apical sealing materials. Porosity and other microstructural defects in a certain material reduce the distension of the material and decrease the tensile strength, thus, the shortcoming deteriorates its durability against environmental stress. These combinations can have destructive effects, as new microcracks occur and spread within the material. Therefore, the aim of this study is to evaluate the changes in volume, density, and porosity of ProRoot MTA (White; Dentsply Maillefer, Ballaigues, Switzerland, lot14050801), MTA-Angelus (Angelus, Londrina, Brazil, lot37081), BioAggregate (Innovative Bioceramix, Vancouver, Canada, lotM72058) and Biodentine (Septodont, Saint-Maur-des-Fossés, France, lotB13822) under different conditions by using micro-CT. We hypothesized that an environment with low/

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acidic pH is detrimental to those materials and causes changes in volume, density, and porosity than any other types of environmental conditions.

MATERIALS AND METHODS

The parameters investigated were volume, density, and porosity; the materials investigated were ProRoot MTA, MTA-Angelus, resulting in, and Biodentine. Their powder and liquid compositions are outlined Table 1. Four groups were prepared with these materials by manually mixing with conventional placement. The study protocol was reviewed and approved by the ethical committee at the Inonu University Institute of Health Sciences Clinical Research (2015/42).

Specimen preparations

The minimum sample size for the mean extrusion parameter was statistically analyzed (power=0.90, α=0.05), and it was determined that the minimum number for each group should be 21 and for the subgroup 7. Eighty-four extracted human anterior teeth were selected for this study and stored in 0.1% thymol solution until they were used. The inclusion criteria were as follows: roots with no cracks, caries, immature apices, resorptive defects, or root fillings. An access cavity was performed in each tooth with a #2 size round tungsten bur (Diatech, 9450 Altstatten, Switzerland) under water cooling. Canal patency was achieved by passing a size #10 K-file (VDW, München, Germany) into the root canal until the tip was visible at the apical foramen. Root canals were passively negotiated using stainless steel K-files #10 to the working length; a reproducible glide path was confirmed. The canal was enlarged to F3 size by a single experienced operator with ProTaper Universal Series rotary nickel-titanium files (Dentsply Maillefer), then apical third shaping to a size 30.06 taper. Next, the sequence was completed and manually finished with a size 40 K-file. Root canals were irrigated between each preparation step with 2 mL of 2.5% sodium hypochlorite, using disposable syringes with a 30-G needle. After, root canals were dried with paper points and obturated with lateral compaction, using gutta-percha and AH Plus (Dentsply, DeTrey, Konstanz, Germany) sealer. The excess part of the cone in the coronal section was cut with a GuttaCut (VDW). The crowns were sealed with Cavit (3M ESPE, Neuss, Germany) temporary restorative material and stored at 37°C and 100% humidity for 1 week. The teeth were digitally radiographed at 0.08 s of exposition time in the buccolingual and mesiodistal directions. The digital radiographs were evaluated regarding the quality of obturation.

The crowns of the teeth were separated using diamond discs, and root samples were standardized at a 15mm-length. After, a diamond bur in a high-speed handpiece under copious water spray was used to cut 3 mm of the apical part of the roots perpendicular to the long axis. An ultrasonic device (Variosurg3, NSK-Nakanishi, Tokyo, Japan) with ultrasonic retro tip E32D (NSK-Nakanishi) was used to prepare the apical cavities 3mm-deep with select "E" ENDO mode and 40% power used. A featherlike back-and-forth motion was used with the ultrasonic tips, cooled by a constant stream of water spray in the whole procedure.

Specimens were randomly assigned to 4 groups (*n*=21). Group 1: ProRoot MTA, Group 2: MTA-Angelus, Group 3: BioAggregate, and Group 4: Biodentine. Each group of 21 specimens was divided into 3 subgroups (*n*=7) according to environmental conditions: exposure to 1 mmol/L butyric acid (Sigma; pH 4.4), exposure to phosphate-buffered saline (PBS), which contained buffered potassium hydroxide at a pH value of 10.4, and exposure to blood.

Cements were mixed according to the manufacturers' directions. Root-end cavities were obturated immediately before imaging. After performing the first scan, Eppendorf tubes with two pieces of 2.5×2.5 cm gauze with different liquids were prepared; butyric acid with a buffered pH of 4.4, PBS with a buffered pH of 10.4, and those with human blood were placed at the bottom of each tube (Fig. 1). Wet gauze was placed on top of the tube to prevent tooth movement and continuously keep the tooth exposed to the medium. All the specimens exposed to the environment were freshly replaced every 24 h for 4 days in a 37°C incubator.

Table 1 Composition of calcium silicate-based cements

Material	Composition of powder			Composition of liquid	
	Cement	Radyoopacifier	Additives	Main component	Additives
ProRoot MTA	Tricalcium silicate	Bismuth oxide	Calcium sulfate dihydrate	water	—
MTA-Angelus	Tricalcium silicate	Bismuth oxide	Calcium Carbonate, Iron Oxide	water	—
BioAggregate	Tricalcium silicate	Tantalum oxide	Calcium phosphate monobasic, tantalum pentoxide	water	—
Biodentine	Tricalcium silicate	Zirconium oxide	Calcium Carbonate, Iron Oxide	water	Water reducing agent Calcium chloride

Micro-CT imaging

Each root sample was scanned in micro-CT SkyScan 1172 (Bruker, Kontich, Belgium) at a 9.9-µm resolution. Images were reconstructed using NRecon V1.6.16.6 software (Bruker MicroCT), providing approximately 250 slices per sample, and analyzed using CTan v1.16.4.1 software (Bruker MicroCT). Freshly prepared cements were applied to root-end cavities and immediately scanned. During the scanning process, each root was wrapped in parafilm on the coronal side to prevent changes in direction. Specimens were scanned at 100 kV, using 1 mm-thick aluminum-chopper filter with a frame averaging select 2. If the root samples deviated from the long axis, the images were reoriented using DataViewer v1.5.2.7 (Bruker MicroCT) to show the exact inclination of the root.

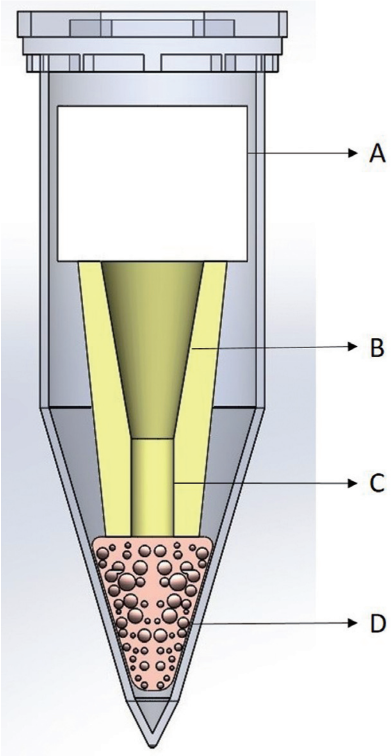

Fig. 1 Schematic diagram of the experimental set up, showing a sealed 1.5 mL Eppendorf tube containing a wet gauze roll (A) to maintain fully saturated humidity and the material contact with the liquid. The root canal filling material (B). Retrograde cavity obturated with test cements (C) was exposed to the appropriate liquid medium with gauze (D) on its lower surface.

The parameters of retrograde filling materials were evaluated, and two images of each material were recorded:

1. Volume loss
2. Density changes
3. Porosity changes

Statistical analysis

A statistical analysis was performed using IBM SPSS Statistics for Windows (Version 22.0, IBM, Armonk, NY, USA). Normality for continuous variables in groups was determined by the Shapiro-Wilk test. Conover-Iman and Kruskal-Wallis tests were used to compare the variables between the groups. A *p*-value ($p<0.05$) indicated strong evidence against the null hypothesis, so the difference is statistically significant.

RESULTS

The median values (min–max) of the volume, density, and porosity in each group are summarized in Table 2. Regarding volume changes, statistically significant differences were found in acidic pH, alkali pH, and blood ($p<0.001$, $p=0.001$, and $p=0.007$). In acidic pH, Biodentine showed a higher percentage of volume loss this parameter (−75.18%), followed by ProRoot MTA (−17.99%), BioAggregate (−16.43%), and MTA-Angelus (−6.53%). In alkali pH and blood, Biodentine showed

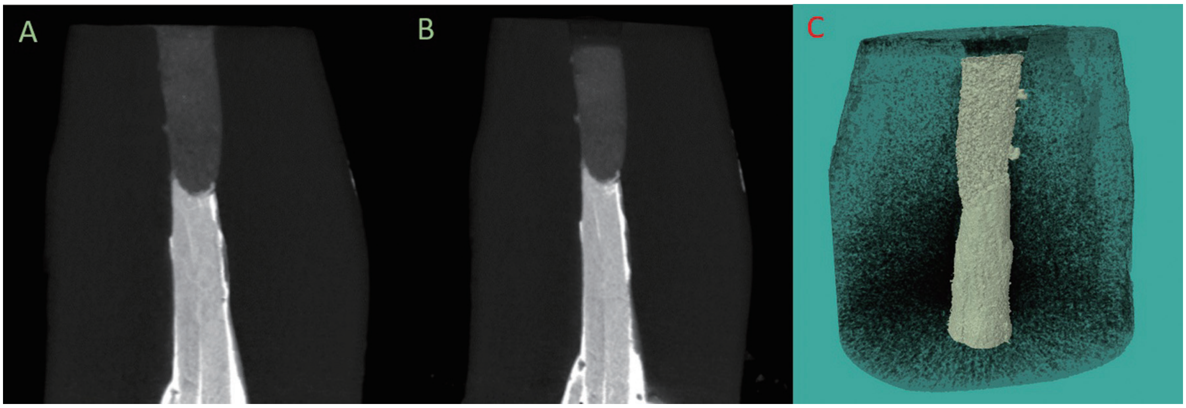

Fig. 2 The volume change was shown on Biodentine in acidic pH.
Before scanning with micro-CT Biodentine fully set on the retrograde cavity (A). The volume loss was observed after exposure to acidic pH (B, C).

Table 2 Comparison of the percentage of changes in analysis results obtained after 4 days, compared to the first analysis

	Environments	ProRoot MTA Median (Min, Max)	MTA-Angelus Median (Min, Max)	BioAggregate Median (Min, Max)	Biodentine Median (Min, Max)	*p*
Volume	Acid	−17.99 ^{a}(−43.15, −1.98)^{x}	−6.53 ^{b}(−15.5, −1.16)^{x}	−16.43 ^{a,b}(−36.94, 4.26)^{x}	−75.18 ^{c}(−87.19, −68.33)^{x}	<0.001*
	Alkali	0.0 ^{a}(0.0, −1.71)^{y}	0.20 ^{a}(−1.41, 19.23)^{y}	1.34 ^{a}(−0.93, 4.89)^{y}	−5.86 ^{b}(−7.3, −1.82)^{y}	0.001*
	Blood	0.84 ^{a}(−0.51, 1.86)^{y}	0.0 ^{a}(−0.51, 2.13)^{y}	−1.15 ^{a}(−18.33, 5.81)^{y}	−8.82 ^{b}(−13.92, −0.61)^{z}	0.007*
	p	0.001*	0.001*	0.025*	0.001*	
Density	Acid	−34.37 ^{a}(−49.19, −7.32)^{x}	−15.11 ^{b}(−23.91, −10.36)^{x}	−16.66 ^{b}(−20.7, −10.32)^{x}	−42.59 ^{a}(−74.85, 32.01)^{x}	<0.001*
	Alkali	0.47 ^{a}(−0.65, 1.56)^{y}	−1.58 ^{b}(−5.41, 5.83)^{y}	0.43 ^{a}(−0.20, 1.55)^{y}	−0.38 ^{a,b}(−1.13, 1.16)^{y}	0.019*
	Blood	1.55 ^{a}(−0.64, 2.33)^{y}	0.44 ^{a}(−0.47, 14.79)^{z}	2.11 ^{a}(−20.7, 3.95)^{y}	−2.42 ^{b}(−7.76, 0.0)^{z}	0.034*
	p	0.001*	<0.001*	0.024*	<0.001*	
Porosity	Acid	0.006 ^{a}(−0.01, 0.17)^{x}	0.09 ^{a}(0.0, 0.93)^{x}	−0.30 ^{b}(−0.78, −0.04)^{x}	−0.009 ^{c}(−0.22, 0.13)^{x}	0.001*
	Alkali	−0.05 ^{a}(−0.01, 0.07)^{x}	−0.01 ^{a}(−0.11, 0.0)^{y}	0.1 ^{a}(−0.67, 0.32)^{x}	0.01 ^{a}(−0.23, 0.06)^{x}	0.185
	Blood	−0.0004 ^{a}(−0.05, 0.01)^{x}	−0.0001 ^{a}(0.0, 0.0)^{z}	−0.13 ^{a}(−1.2, 0.05)^{x}	0.01 ^{a}(−0.18, 0.73)^{x}	0.203
	p	0.201	<0.001	0.074	0.338	

* Median values for each property represented with different superscript lowercase letter; (row; a,b,c) or equation (column; x,y,z) are significantly different.

** Different letters and equation in each column and row indicate statistical differences ($p<0.05$).

*** Row p values represent the comparison of materials in the same environment, and column *p* values represent the results of comparison of the same material in different media.

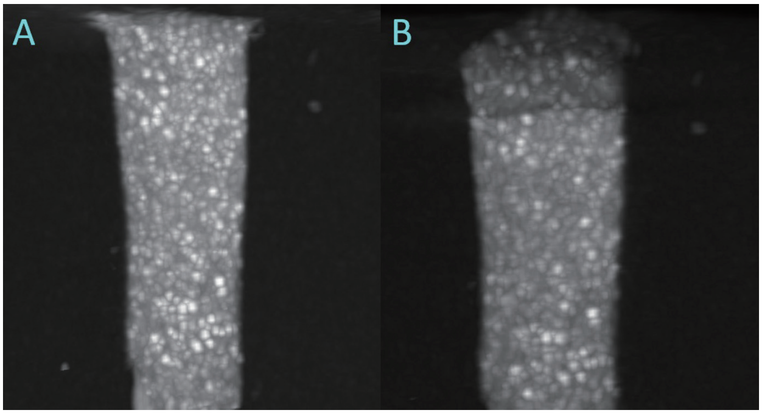

Fig. 3 After exposure to acidic pH, density change on ProRoot MTA was observed by CTVox programme.
Before (A) and after (B) expose the acidic medium. A loss of density was observed when compared (A) to (B).

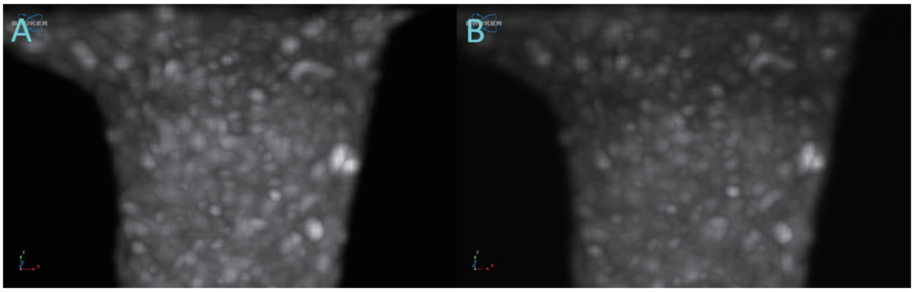

Fig. 4 After exposure to acidic pH, internal changes in MTA-Angelus were observed by CTVox programme.
In acidic pH, larger particles (A) were converted into smaller particles (B), which formed an increasing amount of gaps.

significantly greater differences in volume loss than the other materials (−5.86 and −8.82%) (Fig. 2).

Regarding the percentage of density, statistically significant differences were found in acidic pH, alkali pH, and blood (p<0.001, p=0.019, and p=0.034). In acidic pH, Biodentine (−42.59%) and ProRoot MTA (−34.37%) had significantly more density loss compared with MTA-Angelus (−15.11%) and BioAggregate (−16.66%) (Fig. 3). In alkali pH, MTA-Angelus showed more significant density loss than the other materials (−1.58%). In blood, Biodentine showed more significant density loss than the other materials (−2.42%).

Regarding the percentage of porosity, statistically significant differences were found in acidic pH (p=0.001, Fig. 4). In acidic pH, Biodentine showed significantly less porosity than the other materials (−0.009%). There were no significant differences in alkali pH and blood.

DISCUSSION

The root repair materials may come into contact with inflammatory tissue when used for perforation repair or retrograde filling. In the present study, an attempt was made to mimic a clinical situation by exposing four different apical sealing materials (ProRoot MTA, MTA-Angelus, BioAggregate, and Biodentine) to butyric acid (pH 4.4), PBS (pH 10.4) and blood. In addition, there are very few studies in the literature that investigated the effect of these conditions. In certain clinical situation, calcium-silicate cements may be applied in the presence of infection or inflammation. In this condition the surface of the material would be exposed to an acidic environment15). The application of calcium-silicate cements in lower pH might influence the material's physical and chemical properties. In our study, to simulate the clinical conditions in which inflammatory responses are activated in an acidic environment, butyric acid was used, as it has been reported to be one of the metabolic byproducts of anaerobic bacteria16).

The results in the present study showed that butyric acid significantly decreased the volume and density of each material tested, even at a PBS with 10.4 pH and blood (p<0.05). The possible mechanism for this reduction is that butyric acid disturbed the hydroxyapatite crystal formation and interfered with the hydration reaction during the setting of the silicate materials. Namazikhah *et al.*8) found that the surface microhardness of MTA was impaired in an acidic environment. It could be explained that the material could not harden as well in a low pH environment8,17). In the present study, Biodentine was significantly higher in volume loss compared with the other cements in an acidic pH (p<0.001). In contrast, Biodentine had good properties compared with MTA in microhardness and push-out studies18,19). Nevertheless, Biodentine demonstrated significantly low porosity compared with the other cements in acidic conditions (p=0.001). This can be explained by the fact that materials with low porosity become more durable by itself. The setting reactions of calcium-silicate cements take much longer than the initial hardening. The hydration process continues to take place, the porosity decreases, and the mechanical resistance increases20). Grech *et al.*21) reported that Biodentine had low fluid uptake and sorption values, low setting times, and superior mechanical properties. The fluid uptake and setting time was highest in MTA compared to Biodentine. This was supported by Camilleri *et al.*22) who stated that Biodentine is denser and less porous when compared to MTA, which explains its lower fluid uptake. In contrast, Agrafioti *et al.*23) found no differences between Biodentine and MTA in acidic conditions. The difference in these results may be due to the types of acid used in experiments.

Tricalcium silicate is a main constituent of Biodentine, BioAggregate, and MTA and responsible for early strength development. Biodentine is presented as a powder consisting of tricalcium silicate, dicalcium silicate and calcium oxide; calcium carbonate (filler material); and zirconium oxide as a radiopacifier. The liquid for mixing with the cement powder consists of calcium chloride ($CaCl_2$) and a hydrosoluble polymer in order to keep a good flowability with a low water/powder ratio. The addition of $CaCl_2$ to tricalcium silicate could cause acceleration of the hardening of cement. It was reported that the addition of $CaCl_2$ to tricalcium silicate influences not only the degree of hydration, but also the morphology of the main hydration product, which is a calcium silicate hydrate[24]. Bortoluzzi *et al.*[25] showed that $CaCl_2$ also increased the sealing of cement. The presence of water is essential both for the hardening of the product and physical strength of the material. We should note that redundant water in calcium silicate system could give the material porous property, significantly degrading the macroscopic mechanical resistance, while insufficient water content could possibly lead to the lack of homogeneity in mixture. Our results concurred on this point because the low water/powder ratio provides good mechanical strength and negligible porosity, and the material dissolves and becomes unstable when coming in contact with a liquid. In some clinical situations, repair materials might be exposed to a high pH environment. Tronstad *et al.*[26] showed the pulp, dentin, cementum, and periodontal ligaments of vital or necrotic pulp teeth with a closed or open apex in the pH range of 6.4–7. However, after calcium hydroxide treatment, the pH values of the most inner part of circumpulpal dentin changed to a pH range of 11.1–12.2. In this study, we aimed to examine the effect of alkalinity on microleakage properties of these cements by exposing them to the alkaline-soaked pieces of gauze. There are conflicting results regarding the effect of pretreatment with calcium hydroxide on the sealing ability of MTA. Hachmeister *et al.*[27] reported that medication with calcium hydroxide does not affect the sealing ability of gray MTA. Stefopoulos *et al.*[14] suggested two reasons for the negative effect of calcium hydroxide on the sealing ability of MTA. According to their findings the residual calcium hydroxide might merely be a mechanical obstacle to the adaptation of MTA to root canal walls, or it might chemically react with MTA. In this study, we eliminated the effect of a mechanical obstacle by not placing the alkaline source directly into the root canals, and only the apical surfaces of cements were exposed to an alkaline pH. Saghiri *et al.*[28] showed that, at pH values of 8.4 and 9.4, the amount of unhydrated MTA decreases, and the surface hardness, as an indicator of the setting process, increases. If MTA is exposed to pH values higher than 9.4, adverse effects might occur. In this study, both MTAs and BioAggregate volume and porosity changes were not significantly different. However, Biodentine showed statistically significant differences of volume loss ($p<0.001$). In density changes, ProRoot MTA showed better results against MTA-Angelus ($p<0.001$).

In most endodontic treatments, MTA slurry comes into contact with blood, and in the extreme, might become mixed with blood during placement. Torabinejad *et al.*[29] found that root-end-dye microleakage of MTA contaminated with blood did not change significantly. In our study, there is no significant difference in porosity changes. However, Biodentine significantly lost its volume and density after exposure to fresh blood. This result regarding the *in vivo* condition could be explained by the duration of exposure of root-end filling material to blood but could not be exactly simulated *in vitro*. By preventing the blood from coagulating *in vitro*, the clinical situation can be better simulated. Nekoofar *et al.*[30] show the undesirable effect of citrate as an anticoagulant on MTA settings. We used fresh blood for all materials to prevent the adverse effect of coagulation.

CONCLUSIONS

In conclusion, the results showed that acidic environment (pH<7.0) had a negative effect in all materials in this study. While all the materials had high volume loss in an acidic environment, Biodentine had a relatively higher volume loss among them. These results can help clinicians to achieve more reliable results in procedures such as retrograde fillings. Most interactions between environmental conditions and dentin seem to have a negative impact on the toughness of retrograde filling materials. Further research on positive interactions between the dentin and environment is needed to expand our knowledge in regard to development of new products and approaches for more effective and reliable materials.

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