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ORIGINAL ARTICLE

Setting time of calcium silicate-based sealers at different acidic pHs

ABSTRACT

Aim: *To evaluate the effect of root canal sealer type (calcium silicate- or epoxy amine resin-based sealer) and solution/pH (acidified or buffered at pH 7.4, 6.4, 5.4, and 4.4) on their setting time.*
Materials and Method: *Samples of BIO-C Sealer (Angelus), EndoSequence BC Sealer (Brasseler), BioRoot RCS (Septodont), and AH Plus Jet (Dentsply) were manufactured into 10-mm internal diameter rings and exposed to gauzes soaked with different solutions (n=6): PBS at pH 7.4 (control), acidified PBS at 6.4, 5.4, and 4.4, as well as butyric acid buffer at pH 6.4, 5.4, and 4.4. The setting time of the sealers was determined when the indentation of a 100-g-weight Gilmore needle was no longer visible on the top surface of the samples (ISO 6876:2012 guidelines). The data were analyzed by a repeated measures analysis of variance (Mixed ANOVA) (α=0.05).*
Results: *All sealers were set within 24 hours. EndoSequence BC Sealer and BioRoot RCS respectively exhibited the longest and the shortest setting times among all sealers and there was a significant difference between them at all solutions/pH (p<0.05); however, their setting time did not significantly vary regardless of solution/pH (p>0.05). The setting time of the BIO-C Sealer significantly increased when exposed to acidified PBS at pH 4.4 (p<0.01), and butyric acid buffer solutions at pH 6.4, 5.4, and 4.4 (p<0.01). The longest setting times observed for BIO-C Sealer were not significantly different from those observed for EndoSequence BC Sealer (p>0.05). The setting time of AH Plus Jet significantly decreased only when exposed to acidified PBS at pH 4.4 (p<0.05).*
Conclusions: *The setting time of only one calcium silicate-based sealer (BIO-C Sealer) was significantly increased when exposed to some acidic pH conditions. Both the shortest (BioRoot RCS) and the longest (EndoSequence BC Sealer) setting times were observed for calcium silicate-based sealers. BioRoot RCS demonstrated the shortest setting time regardless of solutions/pH and seems the most suitable for root canal treatment in acidic environments.*

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Introduction

Di- and tricalcium silicate-based root canal sealers have shown beneficial effects in endodontics due to their biocompatibility, bioactivity, and stability in moist environments (1, 2); however, dentinal fluid flow can influence the setting reaction and chemically change their surface (1, 3, 4). The setting time of these hydraulic sealers can be negatively affected by the excess moisture and acidic pH of the root canal walls (1, 5). Some manufacturers suggest that the presence of an acidic environment after root canal shaping/instrumentation could delay the hardening of sealers and lead to leakage (6).
The incorporation of additives can alter the physical properties of hydraulic sealers (7, 8). Regarding their presentation, the so-called 'premixed' sealers are supplied in a syringe, in which the product is suspended in a non-aqueous vehicle and requires water from the surroundings to initiate the hydration reaction; in contrast, sealers presented as powder are mixed to a liquid that already contains water to hydrate (1).
Previous studies have investigated the setting time of hydraulic sealers by using different methods (3, 12, 13), moisture concentrations (14, 15), temperatures (16, 17), or residuals of irrigant solutions (18, 19). Although some studies have shown microhardness reduction (20) and solubility increase (21) of some calcium silicate-based sealers exposed to an acidic environment (low pH), there is a lack of studies that investigated their setting time. Therefore, this study aimed to evaluate the effect of root canal sealer type (calcium silicate- or epoxy amine resin-based sealer) and solution/pH (acidified or buffered at pH 7.4, 6.4, 5.4, and 4.4) on their setting time. The null hypotheses tested were that (1) the sealer type and (2) the solution/pH do not significantly affect the setting time of the sealers.

Materials and Method

This study was submitted to the Ethics Committee Research and dispensed under file number 2020-0887.

Preparation of solutions
A commercially available phosphate-buffered saline (PBS) solution at pH 7.4 (Dinâmica Química Contemporânea Ltda) was used as a control. In addition, PBS solution was acidified with butyric acid to obtain solutions at pH 6.4, 5.5, and 4.4, which were verified by using a pH meter (LUCA-210, MS Tecnopon, Piracicaba, SP, Brazil). A buffer solution of butyric acid (0.2 mol/L) and sodium butyrate salt (0.2 mol/L) was also prepared at pH 6.4, 5.4, and 4.4. The concentrations of butyric acid in each solution are shown in Table 1.

Table 1
Butyric acid concentrations (mol/L) in each solution at different pH levels

pH	Acidified PBS* (mol/L)	Butyric acid buffer** (mol/L)
6.4	0.039	0.005
5.4	0.049	0.041
4.4	0.197	0.145

**Acidified with butyric acid. **Buffer solution of butyric acid and sodium butyrate.*

Sample preparation
The hydraulic sealers used to prepare the samples are shown in Table 2. The two premixed sealers (BIO-C Sealer and EndoSequence BC Sealer) were directly dispensed from a ready-to-use syringe. The powder/liquid hydraulic sealer (BioRoot RCS) was prepared over a 10-mm-thick smooth glass plate by mixing a full scoop of powder with 5 drops of the liquid for 60 sec with the aid of a number 24 spatula. An epoxy amine resin-based sealer (AH Plus Jet, Dentsply, Charlotte, NC, USA) supplied as a two-component automix syringe was used as a control
Fifty-four samples of each hydraulic sealer were manufactured by applying the sealers into 1-mm-height and 10-mm-internal diameter plaster rings (Durone type IV, Dentsply) previously immersed in 37 °C distilled water for 24 hours. The 54 samples of the epoxy amine resin-based sealer were manufactured into a 2-mm-height and 10-mm-internal diameter stainless steel rings.

Table 2

Brands, manufacturers, types, composition, presentations, and batch numbers of the sealers

Brand	Manufacturer	Type	Composition	Presentation	Batch no.
BIO-C Sealer	Angelus, Londrina, Brazil)	Portland cement (calcium silicate/ aluminate)	Zirconium, iron, and calcium oxides, silicon dioxide, dispersing agents	Premixed	102198
EndoSequence BC Sealer	Brasseler, Savannah, GA, USA	Tricalcium/ dicalcium silicate	Zirconium oxide, monobasic calcium phosphate, calcium hydroxide, fillers, thickeners	Premixed	20003SP
BioRoot RCS	Septodont, Saint-Maur-des-Fossés, France	Tricalcium silicate	Zirconium oxide, calcium chloride (accelerator), polycarboxylate	Powder/liquid	B25218

The 54 samples of each sealer were exposed to 7 different solutions (n=6): PBS at pH 7.4 (control), acidified PBS at 6.4, 5.4, and 4.4, as well as a butyric acid buffer at pH 6.4, 5.4, and 4.4. The bottom surface of each sample was positioned on a layer of nonwoven fabric, which in turn was placed on two gauzes soaked in 2 ml of the respective solution (22). Then, each sample was stored in a 5-cm-diameter plastic container and covered. The specimens were kept in an oven at 37 °C and 95% humidity, as recommended by the International Organization for Standardization (ISO) 6876:2012 (23).

Setting time

The setting time of the sealers was assessed by following the ISO 6876:2012 guidelines (23). A 100-g-weight Gilmore needle (Odeme Dental Research, Luzerna, Brazil) with a 2-mm-diameter flat active tip was manually held 2 cm above from the top surface of the samples and then released. This procedure was performed at regular intervals of 15 min and the samples were considered set when the needle indentation was no longer visible on the surface. The setting time for each sample was recorded in minutes.

Statistical analysis

The Shapiro-Wilk and Levene tests were respectively used to assess the normal distribution of the data and the sphericity of variances. Then, the setting times of the sealers were analyzed by a repeated measures analysis of variance (Mixed ANOVA) followed by post hoc multiple comparisons between groups with Bonferroni correction (α=0.05) (JASP software version 0.15.0, University of Amsterdam, Amsterdam, Netherlands).

Results

The setting time significantly changed in function of both sealer type and solution pH ($p<0.05$) (Table 3). All samples were found set within 24 hours. All tested sealers exposed to PBS at pH 6.4 showed significantly different setting times ($p<0.05$).
The setting time of both EndoSequence BC Sealer and BioRoot RCS did not significantly vary regardless of solution/pH ($p>0.05$). EndoSequence BC Sealer and BioRoot RCS respectively exhibited the longest and the shortest setting times among all sealers and there was a significant difference between them at all solutions/pH ($p<0.05$). The setting time of BioRoot RCS at the neutral solution (PBS 7.4) was significantly lower than the other sealers ($p<0.05$) (Fig. 1).
The setting time of the BIO-C Sealer significantly increased when exposed to acidified PBS at pH 4.4 ($p<0.01$), and butyric acid buffer solutions at pH 6.4, 5.4, and 4.4 ($p<0.01$). The longest setting times observed for BIO-C Sealer were not significantly different from those observed for EndoSequence BC Sealer ($p>0.05$). The setting time of AH Plus Jet significantly decreased only

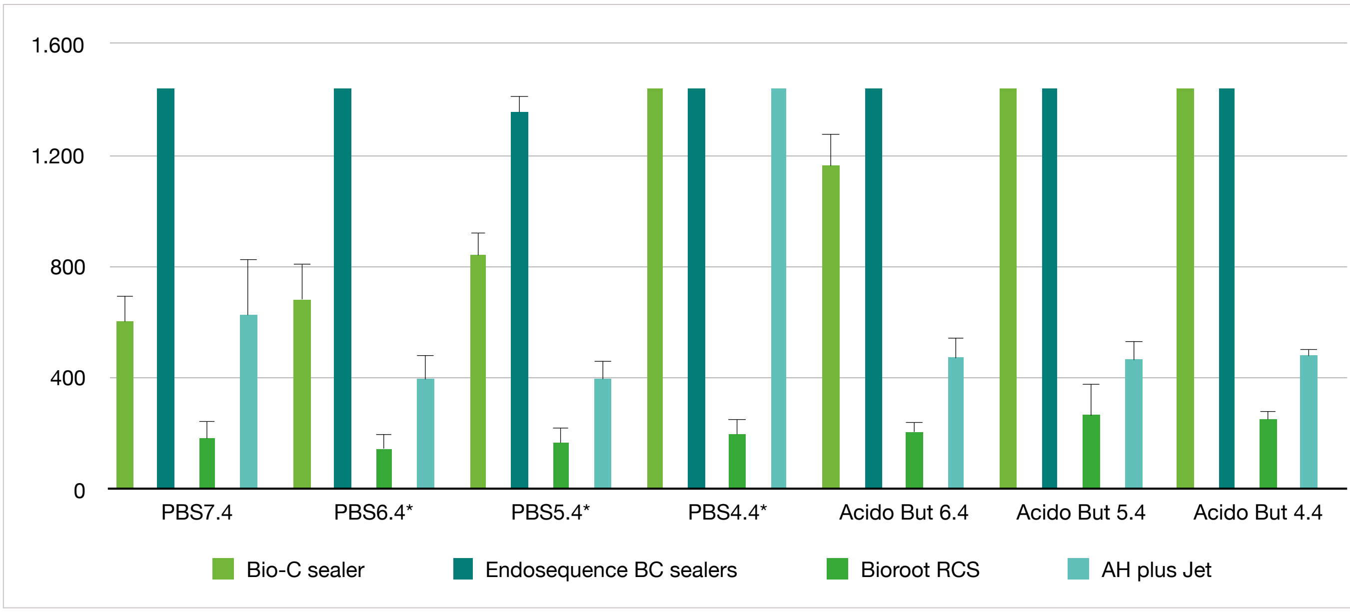

Figure 1
Setting times (min) of sealers exposed to different solutions/pH.
**Solutions without buffer effect.*

when exposed to acidified PBS at pH 4.4 ($p<0.05$).
Except for EndoSequence BC Sealer, the setting times of all sealers tended to increase when exposed to solutions buffered with butyric acid in comparison to acidified PBS solutions at each pH.

Discussion

The acidity of the environment can chemically influence the physical properties and the setting time of calcium silicate-based root canal sealers (8, 20, 24, 25). In this study, the setting time of the sealers significantly varied in function of sealer type and solutions/pH; therefore, null hypotheses had to be rejected.
The evaluation of the setting time of root canal sealers is crucial since prolonged setting times are associated with increased solubility dissolution and disintegration of the sealers, which potentially compromises the sealing integrity of the root canal (26). Moreover, a delayed setting can cause tissue irritation since most root canal sealers ex-

Table 3
Mean (±standard deviations) setting times (min) of the sealers

	PBS	ACIDIFIED PBS*			BUTYRIC ACID BUFFER		
	pH 7.4	pH 6.4*	pH 5.4*	pH 4.4*	pH 6.4	pH 5.4	pH 4.4
BIO-C Sealer	601.33 (±34.70)^{Ba}	683.83 (±47.79)^{Ca}	840.50 (±299.03)^{Ba}	1440.00 (±0)^{Bb}	1166.83 (±423.19)^{Cb}	1440.00 (±0)^{Cb}	1440.00 (±0)^{Cb}
EndoSequence BC Sealer	1440.00 (±0)^{Ca}	1440.00 (±0)^{Da}	1353.00 (±213.11)^{Ca}	1440.00 (±0)^{Ba}	1440.00 (±0)^{Ca}	1440.00 (±0)^{Ca}	1440.00 (±0)^{Ca}
BioRoot RCS	180.50 (±23.05)^{Aa}	143.50 (±19.19)^{Aa}	172.33 (±20.68)^{Aa}	197.50 (±20.45)^{Aa}	206.00 (±13.77)^{Aa}	264.50 (±42.83)^{Aa}	254.17 (±10.98)^{Aa}
AH Plus Jet	631.17 (±74.76)^{Ba}	397.00 (±32.44)^{Ba}	395.67 (±24.48)^{Aa}	1440.00 (±0)^{Bb}	478.33 (±26.94)^{Ba}	466.50 (±25.29)^{Ba}	484.83 (±9.33)^{Ba}

**Solutions without buffer effect. Means with identical superscript uppercase letters in the same columns are not significantly different ($p>0.05$). Means with identical superscript lowercase letters in the same rows are not significantly different ($p>0.05$).*

hibit some level of cytotoxicity before reaching the final setting (14, 27). Ultimately, prolonged setting time may also affect the cementation and lead to the displacement of root canal posts.

Several guidelines have been suggested to replicate clinical conditions on investigations of root canal sealer setting, such as ISO 6876:2012 (11, 28, 29), the American National Standards Institute/American Dental Association (ANSI/ADA) 57:2000, and American Society for Testing and Materials (ASTM) C266-07:2007 (14, 17). Moreover, some studies have assessed the setting time of sealers through rheological tests (18) or experimental models with rats (12). This study used the well-established ISO 6876:2012, which is technically simple and easily reproducible; however, modifications were made to evaluate the sealers under acidic environments.

The bottom surface of the samples was positioned on moistened gauzes to ensure contact between sealers and solutions, as described by Wang et al. (23); however, a layer of nonwoven fabric was positioned in between to prevent the sealer to flow into the gauze fibers and ensure its stability. Each sample was individually stored in plastic containers with covers to ensure the maintenance of particular humid environments provided by different solutions.

Although some studies have used distilled water as a soaking medium (5, 18, 29), other researchers reported different results when compared to PBS (30, 21); thus, PBS at pH 7.4 was used in this study to mimic neutral tissue conditions.

Different from Silva et al. (12) which used pH correctors, PBS was acidified with butyric acid in this study to simulate the acidic environment without potential reactions with other compounds of the sealers (4). Butyric acid is a short-chain fatty acid commonly produced by endodontic pathogens and the most prevalent acid found in endodontic infections (31). Solutions buffered with butyric acid at pH 6.4, 5.4, and 4.4 were also employed for comparative purposes (5, 22).

In addition to pH levels, the concentrations of butyric acid used to prepare the solutions were also taken into account since they can affect the setting time of the sealers. Each sealer sample was exposed to gauzes soaked in 2 ml of solution. This amount was previously determined in a pilot study to avoid excess moisture that could negatively affect the setting of the sealers (14).

According to the manufacturers, the setting times of the tested sealers are 120 to 240 min for BIO-C Sealer, 240 min for EndoSequence BC Sealer, 240 min for BioRoot RCS, and 1440 min for AH Plus Jet.

When exposed to PBS at pH 7.4, the setting time of BioRoot RCS (180.50±3.05) and AH Plus Jet (631.17±74.76) found in this study were within the range indicated by the manufacturers and corroborated previous findings (4, 9, 17, 28); however, the setting times of BIO-C Sealer (601.33±34.70) and EndoSequence BC Sealer (1.440±0) were much longer than those values indicated by the manufacturers and those findings reported by other studies (11, 17, 28, 29). A high moisture level of the medium could delay the initial setting of these premixed sealers suspended in a non-aqueous vehicle (14). Conversely, the powder of BioRoot RCS is mixed with a liquid that already contains the necessary water to initiate the hydration reaction and, might be less affected by excess moisture or acidity (1).

Tricalcium silicate-based sealers such as BioRoot RCS have a faster setting time than dicalcium silicate-based sealers (32). The powder component of BioRoot RCS contains the calcium chloride additive that significantly reduces the setting time and increases the sealer pH at the initial stages due to the release of calcium ions (4, 10, 33). This pH increase during the setting process seems to neutralize acidic environments. Furthermore, the liquid component of the sealer contains polycarboxylate additives, which are known as “superplasticizers” in engineering and allow the reduction in the water: sealer ratio without affecting the setting time; thus, handling of the sealer is facilitated and physical properties are enhanced (34).

The other two hydraulic sealers evaluated in this study have distinct compositions without setting accelerators. EndoSequence BC Sealer contains monobasic calcium phosphate and calcium hydroxide additives, which reduce the release of free calcium

and do not neutralize the surrounding environment (8). Conversely, BIO-C Sealer is a Portland cement-based sealer that contains calcium silicates and aluminates. The start of the setting reaction is accelerated since the aluminate phase reacts with water to form ettringites and enhances the sealer strength; however, previous studies have demonstrated that these crystals can be negatively affected by acidic environments (5, 22). Furthermore, BIO-C Sealer contains a silicon dioxide additive that reacts with the calcium hydroxide formed during the setting reaction and leads to the formation of a more hydrated calcium silicate gel. This additive also reduces the release of free calcium and the alkalization of the environment, which may delay the setting reaction, particularly in acidic environments (8). In general, with the exception of EndoSequence BC Sealer which exhibited the longest setting time regardless of the solution and pH, the other sealers exhibited longer setting times when exposed to buffered solutions (PBS at pH 7.4 and butyric acid buffer at pH 6.4, 5.4, and 4.4) in comparison to acidified PBS solutions (at pH 6.4, 5.4, and 4.4) that loss the buffering effect when butyric acid is added. Buffer solutions tend to maintain pH when exposed to acids or strong bases (35).
After setting, the calcium silicate-based sealers exhibited a more brittle appearance than AH Plus Jet. Although considered an important property, the setting time itself does not determine the quality of a root canal sealer (36, 37, 38) since solubility and microhardness are also associated with their stability within the root canals. The excessively slow setting may negatively affect some sealer properties; however, a fast setting does not necessarily indicate superiority in all cases. In this study, the setting time of the BIO-C Sealer was the most influenced by acidic solutions.
Some authors suggest the use of calcium hydroxide or antibiotic pastes to reduce infection/purulent secretion and neutralize the acidity that could negatively affect the setting reaction of root canal sealers (24, 36). The use of sodium hypochlorite solution may resolve this issue since no adverse effect on the setting of AH Plus Jet has been observed (18). However, further studies are needed to better understand the residual chemical effects of these root canal cleaning methods on the setting of calcium silicate-based sealers.
Further research that closely resemble clinical conditions are encouraged to detail the effect of acidic pH conditions and additives contained in hydraulic sealers on their physical properties. Moreover, scanning electron microscopy and energy dispersive spectroscopy could provide insights into potential surface changes and chemical characterization of these materials.

Conclusions

The setting time of only one calcium silicate-based sealer (BIO-C Sealer) was significantly increased when exposed to some acidic pH conditions. Both the shortest (BioRoot RCS) and the longest (EndoSequence BC Sealer) setting times were observed for calcium silicate-based sealers. BioRoot RCS demonstrated the shortest setting time regardless of solutions/pH and seems the most suitable for root canal treatment in acidic environments.

Clinical Relevance

Understanding the setting time of root canal sealers in various pH conditions is crucial for dental professionals during endodontic procedures. These insights can aid clinicians in selecting the most appropriate sealer for specific clinical scenarios, ultimately contributing to the success and efficiency of endodontic procedures.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

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