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ORIGINAL ARTICLE

Determining the setting of root canal sealers using an in vivo animal experimental model

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Abstract

Objectives To present and explore the potential of an animal-based experimental model developed to determine the set of root canal sealers in vivo. The setting of AH Plus, BioC Sealer, TotalFill BC Sealer, and Sealapex was determined using either ISO 6876 or the novel in vivo method proposed in this study.

Material and methods The in vitro setting time of the sealers tested was determined in accordance with ISO 6876:2012. In determining the in vivo set, 24 adult Wistar rats were followed up for two evaluation periods: 1 and 4 weeks. Their upper-right incisor was extracted, and its pulp tissue was removed. The root canal was then filled from retrograde with one of the 4 sealers, and the tooth was re-implanted and fixed with a layer of a flowable composite resin. After 1 or 4 weeks of the surgical procedures, the animals were euthanized, and their incisors were extracted. Two-mm-thick slices of the middle third of the tooth root were obtained and assessed with a Gillmore device, to determine whether or not the sealer had set.

Results The following in vitro results were obtained by using ISO 6876 methodology: AH Plus set after a mean time of 423 ± 20 min and 476 ± 35 min, in metal and plaster molds, respectively. BioC Sealer set after 7 days (in dental plaster molds), whereas TotalFill BC Sealer and Sealapex did not set even after 25 days in both tested conditions (metal or dental plaster molds). Using the novel in vivo methodology, AH Plus, BioC Sealer, and TotalFill BC Sealer set after both 7 and 30 days. In contrast, Sealapex did not set at either time point.

Conclusions AH Plus and BioC Sealer set under both in vitro and in vivo test conditions. TotalFill BC Sealer did not set under in vitro conditions but did after 1 week under in vivo conditions. Sealapex did not set under either in vitro or in vivo conditions.

Clinical relevance The influence of the testing conditions on the setting results is a clear indication that new in vivo experimental models should be useful in future studies on Bioceramics root canal sealers.

Keywords Calcium silicate cements · Mineral trioxide aggregate · Physicochemical properties · Root canal sealer · Setting time

Introduction

Ever since the first ready-to-use bioceramic root canal sealer was introduced in 2007 (iRoot SP, Innovative BioCeramix Inc., Vancouver, BC, Canada), several similar calcium silicate-based root canal sealers have been developed. The goal has been to produce a close-to-ideal sealer, combining the optimal and well-known biological properties of MTA [1–4] with the physicochemical properties of a conventional root canal sealer. This way, adequate consistency and flow would be attained, allowing the calcium silicate root canal sealers to be used with conventional gutta-percha cones and cold filling techniques.

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These hydrophilic calcium silicate root canal sealers instantly attracted the dental community because they were premixed, injectable, hydrophilic, and bioactive root-filling materials. Overall, the body of evidence made available over the last few years has shown that such hydrophilic calcium silicate root canal sealers are biocompatible and bioactive, features mostly attributed to the presence of calcium phosphate in their composition [5–10]. Moreover, it seems that this class of sealers can interact with the surrounding dentinal tissue by both alkalinization and ion release [8, 11]. Their antibacterial activity has already been reported [12, 13], and their high flowability has proven capable of filling the irregular areas of the root canal space [11, 14]. However, other critical physicochemical properties, such as setting ability, have yet to be fully understood and elucidated.

Setting time is an essential physical property for a root canal sealer, from a clinical point of view. As a rule, a fast setting time not rarely renders technical difficulties during the clinical application, whereas either a slow setting time or an incomplete set can result in higher tissue irritation and increased solubility, possibly leading to sealing failures. Therefore, it is important to characterize how calcium silicate-based sealers set since this may provide some indication of whether the sealer is undergoing normal hydration. Commonly, the initial and final setting times of root canal sealers are tested experimentally, based on methods described by the International Organization of Standardization (ISO 6876:2012) [15], by the American National Standards Institute/American Dental Association (ANSI/ADA 57:2000) [16] or by the American Society for Testing and Materials (ASTM C266-07:2007) [17]. These methods consist of basic benchtop tests, using the Gillmore needle apparatus, where a circular needle of a particular size and weight is pressured perpendicularly towards the specimen. The rationale of the test model using the Gillmore needle apparatus is straightforward: if the needle penetrates a sealer specimen to a given depth and leaves a circular indentation, the sealer is considered unset. In contrast, if it fails to penetrate a sealer specimen, leaving no marks, the set has occurred. In other words, the final set status is achieved when the sealer has hardened to a point at which it can sustain a preset load.

A major problem with these in vitro experimental models is that their results cannot be extrapolated to clinical conditions. The circumstances and environment in which sealers are tested most likely differ from those existing when sealers are clinically used inside the root canal. Sealers are placed into root canals with gutta-percha points. Dentine walls contain natural moisture, which is indispensable for the setting of calcium silicate-based sealers, considering that their setting reaction is triggered by the moisture present in dentinal tubules [18–22]. Any of these factors could affect the setting process of the sealer. Another drawback related to direct indentation tests is the subjectivity involved in interpreting the results. This problem could explain the conflicting results found in the literature regarding the setting ability of calcium silicate-based sealers: whereas some studies demonstrated that these sealers set under controlled in vitro conditions [23–25], others were unable to demonstrate their complete set, even after long experimental periods of time [19, 26, 27]. In short, there is an ongoing debate over the setting ability of hydrophilic premixed calcium silicate root canal sealers, one that has gained even more significance with the growing popularity of bioceramic sealers, and with the introduction of several branded materials on the market. Although setting failures have been reported in vitro, there is no evidence that this also takes place in vivo. Therefore, the present study was designed to address these conflicting results and the clear limitations of the currently available in vitro setups used to assess the setting ability of sealers. Thus, the main purpose of the present study was to present and explore the potential of an animal-based experimental model developed to determine the set of root canal sealers in vivo. The advantages and disadvantages of the methodology are presented and discussed. The setting of a new hydrophilic premixed calcium silicate root canal sealer (BioC Sealer; Angelus, Londrina, PR, Brazil) was evaluated and compared with that of premixed calcium silicate sealer TotalFill BC Sealer (FKG Dentaire, La Chaux-de-Fonts, Switzerland (also marketed as iRoot SP and EndoSequence BC Sealer)), calcium hydroxide-based sealer Sealapex (Kerr, Brea, USA), and epoxy resin-based sealer AH Plus (Dentsply DeTrey, Konstanz, Germany). The null hypotheses tested were (i) that no differences would be found among the setting of the tested sealers and (ii) that no differences would be found between the two tested methodological designs.

Materials and methods

Experiment 1: in vitro tests

The setting time of the 4 tested root canal sealers (AH Plus, BioC Sealer, TotalFill BC Sealer, and Sealapex) was determined in accordance with ISO 6876:2012 [15]. The composition and lot numbers of the evaluated sealers are shown in Table 1. All materials were manipulated according to the manufacturer's instructions. Metal molds measuring 10 mm in diameter and 2 mm in height were used for all the tested sealers (n = 3 per sealer). In addition, type IV dental plaster molds (Durone IV Salmon; Dentsply-Sirona, Charlotte, USA) measuring 10 mm in diameter and 1 mm in height were used for BioC Sealer and TotalFill BC Sealer (materials that specifically require moisture to set) and for AH Plus and Sealapex for comparison (n = 3 per sealer). The plaster molds were stored at 37 °C and 95% relative humidity for 24 h before the setting time experiment. The sealers were manipulated according to their respective manufacturers' specifications,

Table 1 Composition and lot number of the evaluated root canal sealers

Root canal sealer	Composition	Lot number
AH Plus (Dentsply DeTrey, Konstanz, Germany)	Paste A: Bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica and iron oxide pigments Paste B: Dibenzydiamine, aminoadamante, trycyclodecane-diamine, calcium tungstate, zirconium oxide, silica, and silicone oil	1810000182
BioC Sealer (Angelus, Londrina, Brazil)	Calcium silicates, calcium aluminate, calcium oxide, óxido zirconium oxide, iron oxide, silicon dioxide, and dispersing agent	43980
TotalFill BC Sealer (FKG Dentaire, La Chaux-de-Fonts, Switzerland)	Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler, and thickening agents	19001SP
Sealapex (Kerr, Brea, USA)	Catalyst paste: Isobutyl salicylate resin, fumed silica (silicon dioxide), bismuth trioxide, titanium dioxide pigment Base paste: N-ethyltoluenesulfonamidere-sin, fumed sílica (silicone dioxide), zinc oxide, calcium oxide	6653609

placed inside the matrices (metal or dental plaster), and stored at a temperature of 37 °C and 95% air humidity. After 10 min of manipulation, a 100-g Gillmore needle with an active 2-mm tip was placed vertically on the specimen surface. This procedure was repeated every 10 min until the surface of the endodontic sealer was no longer marked by the tip of the Gillmore needle. After 24 h, the tests were performed daily for 25 days.

Experiment 2: in vivo test

Ethical considerations

All procedures were carried out in accordance with conventional guidelines in the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health 85-23, revised 1996). The local Institutional Animal Care and Use Committee (register no. 1041) approved all experimental protocols. This study is reported according to the ARRIVE guidelines (Animal Research: Reporting of in vivo experiments) [28] and PREPARE guidelines (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) [29] with regard to the relevant items. All efforts were made to minimize animal suffering and to reduce the number of animals used with adherence to the 3Rs principles (replacement, reduction, and refinement).

Animal model

Twenty-four adult male and female Wistar rats, aged 10 weeks and weighing 200–300 g, were used in this study. The rats were kept in temperature-controlled rooms, inside individual cages, and received water and food ad libitum throughout the study. They were randomly assigned to 4 groups (n = 6 per sealer) according to the sealer tested (AH Plus, BioC Sealer, TotalFill BC Sealer, and Sealapex) and were followed up for 2 evaluation periods—1 or 4 weeks—resulting in 3 animals per group per evaluation period. A senior veterinarian conducted all the nutritional recommendations and was in charge of the care and pre- and postoperative fasting of the animals, carried out in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and with the current international legislation on animal use in experimental research.

Surgical and endodontic procedures

The animals were anesthetized intraperitoneally with 1 mL/100 g of a solution containing 10% ketamine (1 mL/kg; Virbac; São Paulo, SP, Brazil), 2% xylazine (0.5 mL/kg; FortDodge; Rio de Janeiro, RJ, Brazil), 5% midazolam (0.6 mL/kg; Roche; Rio de Janeiro, RJ, Brazil), tramadol (0.2 mL/kg; Sun; Goiânia, GO, Brazil), and 0.9% saline solution (8.5 mL). During the postoperative period, the rats received analgesia with 5 mg/kg of meloxicam (Eurofarma; São Paulo, SP, Brazil) subcutaneously every 24 h, starting immediately after the surgical procedure and for 2 additional days.

The endodontic procedures were performed under microscopic observation at × 12.5 magnification (DFVasconcelos; São Paulo, SP, Brazil). Each animal was positioned on an operative board specially designed for the experiment, in a dorsal position with its head immobilized. Mouth opening was achieved by using a device designed to fix the upper and lower incisors with orthodontic elastics connected to opposite bars positioned on the extremities of the operative board. In addition, metal rings involved by rubber cannula were used to hold the anterior teeth. Subsequently, syndesmotomy of the periodontal tissue was performed using a syndesmotome (Duflex; Rio de Janeiro, RJ, Brazil) and the upper-right incisor was extracted with a clinical probe adapted to this tooth, taking care to hold it by the crown to preserve the periodontal fibers of its radicular portion (Fig. 1a). The pulp tissue was removed with a size 10 K-file (Dentsply Sirona Endodontics; Ballaigues, Switzerland) (Fig. 1b and c) and a 25/0.04 rotary NiTi instrument (Fig. 1d and e) and the root canal was irrigated with 5 mL of 0.9% saline solution, with a

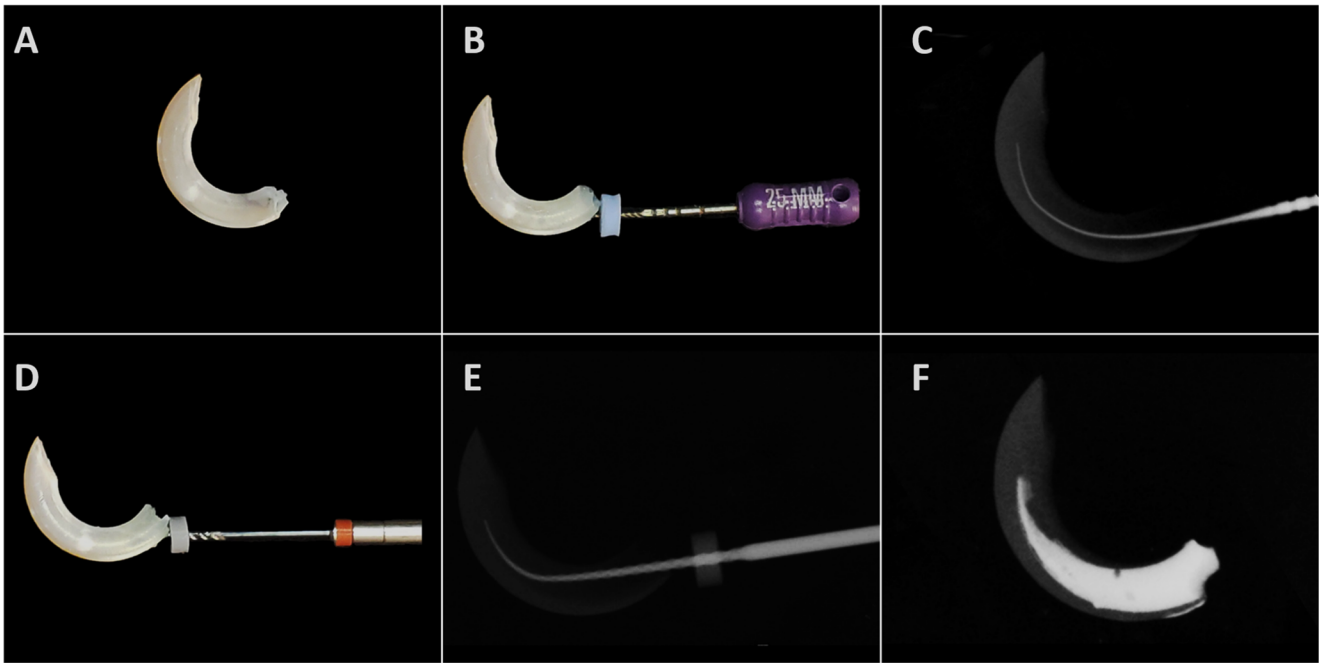

Fig. 1 Sequence depicting the removal of pulp tissue with hand and rotary files and filling the root canal with the root canal sealers tested. **a** Extracted upper-right incisor; **b** removal of pulp tissue with a size 10 K-file; **c** radiographic image with a size 10 K-file in the root canal; **d** removal of remaining pulp tissue with a 25/0.04 rotary NiTi instrument; **e** radiographic image with a size 25/0.04 rotary NiTi instrument in the root canal; **f** root canal filled with root canal sealer

NaviTip 30G needle (Ultradent Products; South Jordan, UT, USA). The root canal was dried with a sterile paper point (Dentsply Sirona Endodontics) and space was filled with one of the four experimental sealers, after manipulating them according to their respective manufacturer's instructions (Fig. 1f). The tooth was re-implanted into the socket, its enamel surface was etched with 35% phosphoric acid (Ultra Etch; Ultradent Products), and it was then fixed with a layer of a flowable composite resin (Filtek Bulk Fill Flow 3M; Maplewood, MN, USA). A single coat of Scotchbond (3 M) was applied, dried, and polymerized for 10 s. The resin was adapted in between the two anterior crowns, so that it would pass through the interproximal space, to keep the crowns united after polymerization of the material.

The animals were euthanized (3 per experimental group) after 1 or 4 weeks of the surgical procedure, and their right incisors were extracted to assess the sealer setting.

Evaluation of sealer setting

After tooth extraction, 2-mm-thick slices of the middle third of the root were obtained using an Exakt 310 CP Series diamond band saw (Exakt Apparatebbau; Norderstedt, Germany). These slices were polished superficially with a Sof-lex Pop-on kit (3 M). The specimens were assessed by a blinded investigator, who did not know which type of sealer was used in each specimens, using a Gillmore device specially designed for this experiment (Odeme Dental Research; Luzerna, SC, Brazil), to determine whether or not the sealer had set. The Gillmore needle size was calculated to be proportionate to the specimen size (rat incisor slice from the middle third, with a mean dimension of 2.82 mm and 1.43 mm in the vestibulo-lingual and mesio-distal diameter, respectively), following the method described by ISO 6876:2012, resulting in its tip weighing 50 g and measuring 1 mm in diameter. The setting of the material was checked by lowering the indenter needle positioned vertically over the sealer surface and observing whether or not it left a complete circular indentation on the sealer. When the needle failed to penetrate the sealer, the material was deemed as set. Conversely, when it left a definitive mark on the sealer surface, the material was deemed as unset. Two experimental times (1 or 4 weeks) were used to determine whether the set of the material was reached (Fig. 2).

Results

Experiment 1: in vitro tests

The AH Plus Sealer was set after a mean time of 423 ± 20 min and 476 ± 35 min, in metal and dental plaster molds, respectively. The BioC Sealer was set after 7 days (only in dental plaster molds), whereas both the TotalFill BC Sealer and the Sealapex sealer did not set even after 25 days in both tested conditions (metal and dental plaster molds) (Table 2).

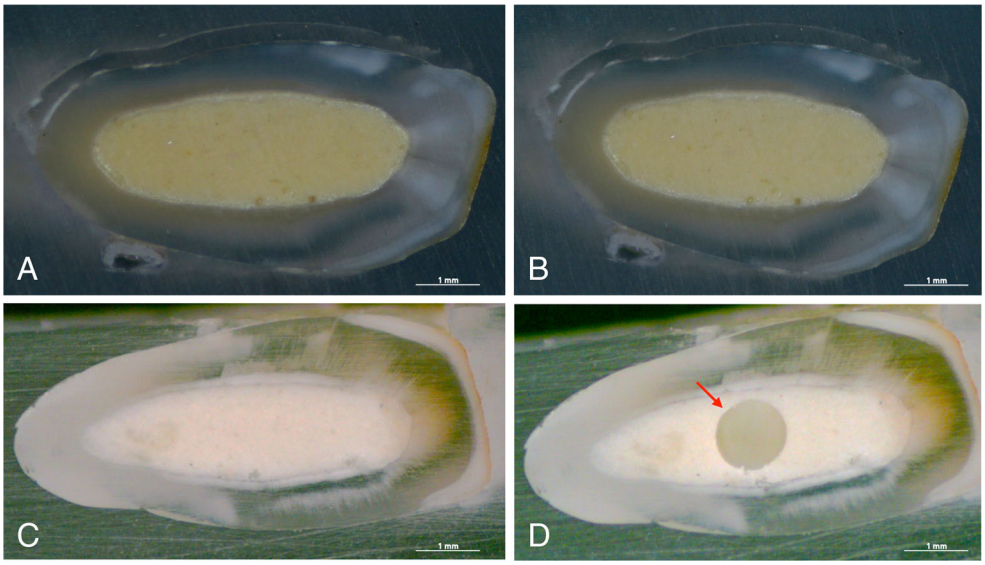

Fig. 2 Example of dentinal slices, obtained at the middle third of maxillary rodent teeth, of **a** a sealer before setting test and **b** the same sample after setting test demonstrating the complete setting, and **c** a sealer before setting test and **d** the same sample after setting test demonstrating the absence of setting with a complete circular indentation on the sealer (arrow). Magnification × 50

Experiment 2: in vivo test

The AH Plus, BioC Sealer, and TotalFill BC Sealer were set at both time points, 7 and 30 days. In contrast, Sealapex remained unset even after 7 and after 30 days (Table 3).

Discussion

The results of the in vitro experiment showed that the setting times of the evaluated sealers were different from those informed by their manufacturers. In fact, only AH Plus Sealer set in the in vitro setup after a period of time close to that reported in previous studies [27, 30–32] and to that informed by its manufacturer. BioC Sealer set after 7 days in dental plaster molds. In contrast, TotalFill BC Sealer and Sealapex did not set, even after 25 days, when the test was conducted following ISO 6876:2012 specifications [15]. Due to the differences in the setting of the tested root canal sealers, the first null hypotheses were rejected. Loushine et al. [18] obtained similar results, namely that both MTA Fillapex and EndoSequence BC Sealer, calcium silicate-based sealers, did not set under in vitro conditions unless they were immersed in Hank's Balanced Salt Solution. Also, previous studies demonstrated that Sealapex did not set in in vitro conditions [24, 33]. In the present study, in vitro setting time was tested under two different conditions: metal and dental plaster molds. ISO 6876:2012 [15] specifications recommend that hydraulic materials, such as BioC Sealer and TotalFill BC Sealer, should be tested using dental plaster molds, as these materials require moisture to completely set. The use of dental plaster molds was also used to evaluate hydraulic materials in other previously published studies [18, 20, 34]. As moisture theoretically influence the setting reaction of a sealer, in the present study, AH Plus and Sealepex were also tested using this model. This failure of some sealers to set under in vitro conditions prompted the testing of these materials under in vivo conditions to determine their set.

The in vivo results of the present study showed that AH Plus, BioC Sealer, and TotalFill BC Sealer set after 1 week. In contrast, Sealapex allowed the needle to penetrate into the surface of the material and leave a complete circular

Table 2 In vitro setting time of the tested sealers using metal molds for all tested sealers and dental plaster molds for BioC Sealer and TotalFill BC Sealer (materials that specifically require moisture to set—according to the ISO guidelines)

Setting time	Metal molds		Dental plaster molds	
Group	*N*	Setting time	*N*	Setting time
AH Plus	3	423 ± 20 (min)	3	476 ± 35 (min)
BioC Sealer	3	Did not set	3	7 ± 0 (days)
TotalFill BC Sealer	3	Did not set	3	Did not set
Sealapex	3	Did not set	3	Did not set

Table 3 In vivo setting test performed after 7 or 28 days after root canal filling

Reached set	After 7 days?					After 28 days?				
		No		Yes			No		Yes	
Group	*N*	*n*	%	*n*	%	*N*	*n*	%	*n*	%
AH Plus	3	0	0	3	100	3	0	0	3	100
BioC Sealer	3	0	0	3	100	3	0	0	3	100
TotalFill BC Sealer	3	0	0	3	100	3	0	0	3	100
Sealapex	3	3	100	0	0	3	3	100	0	0
Total	12	3	25	9	75	12	3	25	9	75

indentation, which indicated that this sealer remained unset even after 4 weeks. Therefore, the second null hypothesis was also rejected. This marked difference in the setting results between in vitro and in vivo setups indicates that experimental conditions are able to affect results markedly. Presumably, controlled exposure to moisture and/or air may be necessary for these sealers to set [18, 35]. When a sealer is on a glass slab, it has a large surface area exposed to moisture/air; conversely, inside the canal, it has little surface area exposure. An in vitro study demonstrated that sealers set more quickly on their exposed surface and much more slowly below the surface [36]. In addition, exposure to moisture is different under in vitro versus in vivo conditions. This difference may be critical to the setting of hydraulic root canal sealers.

Several different animals have been used in experimental studies, including rats, dogs, cats, and monkeys [37–40]. One of the benefits of using rats is that a large number of animals can be assessed over a short period of time [41, 42]. The biological response progresses faster in rodents due to their faster metabolism compared with that of humans, thus allowing fast results [43]. However, despite these advantages, there are some difficulties related to the intrinsic features of rats, such as their small teeth and mouth, which require proper adaptations, materials, and training. That is the apparent reason why larger animals have been preferred for endodontic studies instead of rats [40, 44].

Some studies using rat models have used molars in their experiments [44–46]. However, pulp space in a rat molar root is very limited, thus making it unfeasible to assess sealer properties using the methods available in the literature. Therefore, the use of maxillary incisors was preferred. Since these rodents have continuously growing and erupting incisors, the papilla had to be drained out to interrupt this process and allow the sealer to remain in direct contact with periradicular tissues [47]. The specimens were obtained from the middle third of the teeth because it corresponds to the portion with the highest volume to contain the sealer, as demonstrated by microtomographic (micro-CT) images. When developing the method, 2D/3D micro-CT images were also used to calculate the mean area/volume of the canal lumen. This data was used to determine the dimensions of the Gillmore device, specially designed for this experiment, with weight and surface area made proportionate to the incisors of the rats using a ratio of the measurements provided by ISO 6876:2012 specifications [15].

The results of the current study confirmed the usefulness of this novel in vivo method, which enables testing under conditions that are closer to those of a clinical setting. The in vivo experimental animal model presented herein may contribute to the future development of new root-filling materials, especially premixed sealers, which are dependent on the environmental moisture conditions of the canal space to reach the final set. Although the testing environment of in vitro studies is standardized and allows controlled conditions of humidity and temperature, it fails to replicate all the uncontrolled clinical variables that can potentially influence the setting reaction of a material placed inside a tooth. The in vivo model proposed here is an attempt at reproducing clinical conditions as closely as possible, especially regarding moisture and other fluids, such as dentinal fluid and blood, which are preconditions for the sealers to set [48]. The proposed in vivo experimental design has its own limitations and it does not perfectly mimic the human teeth environment. On the other hand, it is not possible to test the setting of the root canal sealers in the clinical setting with currently available technology. One clear limitation is that root canal sealers are recommended for orthograde intracanal use. However, in the present study, the root canal sealers were inserted on recently extracted incisor teeth in a retrograde manner, and afterwards, teeth were reimplanted. The decision to perform experiments in this way was based on the anatomical impossibility of performing proper cleaning and shaping procedures in the rat's incisors due to the incisal calcification and extreme canal curvature. Another shortcoming is related to the fact that the method does not allow determining the precise setting time of the sealers insofar as it is a destructive assay. However, even with this less-than-ideal experimental situation, it is beyond doubt that the in vivo scenario of the current method is able to provide important insights regarding the setting of the new root canal sealers—e.g., calcium silicate-based sealers—which are completely subject to the intracanal environment conditions. This is clearly demonstrated in present results where some of the tested sealers did not set in vitro but completely set in the in vivo model, which stands for quite innovative data. One may argue that statistical analysis was not performed; however, statistics do not make sense for in vitro results as using metallic molds, only AH Plus set while the BioC Sealer, Total Fill BC Sealer, and Sealapex did not set and when dental plaster molds, only BioC Sealer set while Total Fill BC Sealer did not set. Moreover, for the in vivo setup for all root canal sealers, 100% of the samples fallen into "set" or "did not set" category so no frequency variation by categories can be evaluated.

Conclusions

AH Plus and BioC Sealer set under both in vitro and in vivo test conditions. TotalFill BC Sealer did not set under in vitro conditions but did after 1 week under in vivo conditions. Sealapex did not set under either in vitro or in vivo testing conditions. The influence of the testing conditions on the setting results is a clear indication that new in vivo experimental setups should be tested in future studies on bioceramics root canal sealers.

Contributions of each author Emmanuel João Nogueira Leal Silva contributed to the idea, hypothesis, and final approval of the version to be published. Iracema C. Ehrhardt contributed to the experimental design. Gerhilde Callou Sampaio contributed to the experimental design. Milla Lessa Cardoso contributed to the experimental design. Diogo da Silva Oliveira contributed to the experimental design. Marcelo J. Uzeda contributed to the experimental design, analysis, and interpretation of data. Monica Diuana Calasans-Maia contributed to the experimental design, analysis, and interpretation of data. Daniele Moreira Cavalcante contributed to the experimental design, analysis, and interpretation of data. Gustavo De-Deus contributed to the idea, hypothesis, and final approval of the version to be published. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and based on the welfare of animals.

Informed consent For this type of study, formal consent is not required.

References

1. Damas BA, Wheater MA, Bringas JS, Hoen MM (2011) Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence bioceramic root repair materials. J Endod 37:372–375
2. Leal F, De-Deus G, Brandão C, Luna AS, Fidel SR, Souza EM (2011) Comparison of the root-end seal provided by bioceramic repair cements and White MTA. Int Endod J 44:662–668
3. Parirokh M, Torabinejad M, Dummer PMH (2018) Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part I: vital pulp therapy. Int Endod J 2:177–205
4. Torabinejad M, Parirokh M, Dummer PMH (2018) Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part II: other clinical applications and complications. Int Endod J 3:284–317
5. Chang SW, Lee SY, Kang SK, Kum KY, Kim EC (2014) *In vitro* biocompatibility, inflammatory response, and osteogenic potential of 4 root canal sealers: Sealapex, Sankin Apatite Root Sealer, MTA Fillapex, and iRoot SP root canal sealer. J Endod 40:1642–1648
6. Shokouhinejad N, Nekoofar MH, Razmi H, Sajadi S, Davies TE, Saghiri MA, Gorjestani H, Dummer PM (2012) Bioactivity of EndoSequence root repair material and bioaggregate. Int Endod J 45:1127–1134
7. Chen I, Salhab I, Setzer FC, Kim S, Nah HD (2016) A new calcium silicate-based bioceramic material promotes human osteo- and odontogenic stem cell proliferation and survival via the extracellular signal-regulated kinase signaling pathway. J Endod 3:480–486
8. Moinzadeh AT, Aznar Portoles C, Schembri Wismayer P, Camilleri J (2016) Bioactivity potential of EndoSequence BC RRM Putty. J Endod 4:615–621
9. Lv F, Zhu L, Zhang J, Yu J, Cheng X, Peng B (2017) Evaluation of the in vitro biocompatibility of a new fast-setting ready-to-use root filling and repair material. Int Endod J 6:540–548
10. Giacomino CM, Wealleans JA, Kuhn N, Diogenes A (2019) Comparative biocompatibility and osteogenic potential of two bioceramic sealers. J Endod 45:51–56
11. Candeiro GT, Correia FC, Duarte MA, Ribeiro-Siqueira DC, Gavini G (2012) Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. J Endod 3:842–845
12. Zhang H, Shen Y, Ruse ND, Haapasalo M (2009) Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod 35:1051–1055
13. Lovato KF, Sedgley CM (2011) Antibacterial activity of Endosequence root repair material and proroot MTA against clinical isolates of Enterococcus faecalis. J Endod 37:1542–1546
14. Fernández R, Restrepo JS, Aristizábal DC, Álvarez LG (2016) Evaluation of the filling ability of artificial lateral canals using calcium silicate-based and epoxy resin-based endodontic sealers and two gutta-percha filling techniques. Int Endod J 4:365–373
15. International Organization for Standardization (2012) International Standard ISO 6876:2012: dental root canal sealing materials. Geneva: International Organization for Standardization
16. American Dental Association (2000) ANSI/ADA Specification n° 57 - Endodontic Sealing Material. Chicago: ADA
17. ASTM International C266–07 (2007) Standard test method for time of setting of hydraulic cement paste by Gillmore needles
18. Loushine BA, Bryan TE, Looney SW, Gillen BM, Loushine RJ, Weller RN, Pashley DH, Tay FR (2011) Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. J Endod 5:673–677
19. Han L, Okiji T (2013) Bioactivity evaluation of three calcium silicate-based endodontic materials. Int Endod J 9:808–814
20. Zhou HM, Shen Y, Zheng W, Li L, Zheng YF, Haapasalo M (2013) Physical properties of 5 root canal sealers. J Endod 10:1281–1286
21. Xuereb M, Vella P, Damidot D, Sammut CV, Camilleri J (2015) In situ assessment of the setting of tricalcium silicate-based sealers using a dentin pressure model. J Endod 1:111–124
22. Duarte MAH, Marciano MA, Vivan RR, Tanomaru Filho M, Tanomaru JMG, Camilleri J (2018) Tricalcium silicate-based cements: properties and modifications. Braz Oral Res 1:e70
23. Massi S, Tanomaru-Filho M, Silva GF, Duarte MA, Grizzo LT, Buzalaf MA, Guerreiro-Tanomaru JM (2011) pH, calcium ion release, and setting time of an experimental mineral trioxide aggregate-based root canal sealer. J Endod 6:844–846
24. Viapiana R, Flumignan DL, Guerreiro-Tanomaru JM, Camilleri J, Tanomaru-Filho M (2014) Physicochemical and mechanical properties of zirconium oxide and niobium oxide modified Portland cement-based experimental endodontic sealers. Int Endod J 5:437–448
25. Mendes AT, Silva PBD, Só BB, Hashizume LN, Vivan RR, Rosa RAD, Duarte MAH, Só MVR (2018) Evaluation of physicochemical properties of new calcium silicate-based sealer. Braz Dent J 6:536–540
26. Guo YJ, Du TF, Li HB, Shen Y, Mobuchon C, Hieawy A, Wang ZJ, Yang Y, Ma J, Haapasalo M (2016) Physical properties and hydration behavior of a fast-setting bioceramic endodontic material. BMC Oral Health 20:16–23
27. Lee JK, Kwak SW, Ha JH, Lee W, Kim HC (2017) Physicochemical properties of epoxy resin-based and bioceramic-based root canal sealers. Bioinorg Chem Appl 2582849

28. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010) Animal research: reporting in vivo experiments: the ARRIVE guidelines. J Gene Med 12:561–563
29. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T (2018) PREPARE: guidelines for planning animal research and testing. Lab Anim 52:135–141
30. Versiani MA, Carvalho-Junior JR, Padilha MIAF, Lacey S, Pascon EA, Sousa-Neto MD (2006) A comparative study of physicochemical properties of AH Plus and Epiphany root canal sealants. Int Endod J 6:464–471
31. Flores DS, Rached FJ Jr, Versiani MA, Guedes DF, Sousa-Neto MD, Pécora JD (2011) Evaluation of physicochemical properties of four root canal sealers. Int Endod J 2:126–135
32. Marin-Bauza GA, Rached-Junior FJ, Souza-Gabriel AE, Sousa-Neto MD, Miranda CE, Silva-Sousa YT (2010) Physicochemical properties of methacrylate resin-based root canal sealers. J Endod 9: 1531–1536
33. Andolfatto C, Bonetti-Filho I, Carlos IZ, Guerreiro-Tanomaru JM, Kuga MC, Tormin FBC, Faria G (2017) Cytocompatibility, physical properties, and antibiofilm activity of endodontic sealers with amoxiciline. Microsc Res Tech 80:1036–1048
34. Kaup M, Schäfer E, Dammaschke T (2015) An in vitro study of different material properties of Biodentine compared to ProRoot MTA. Head Face Med 11:16
35. Sousa-Neto MD, Guimarães LF, Saquy PC, Pécora JD (1999) Effect of different grades of gum resins and hydrogenated resins on the solubility, disintegration, and dimensional alterations of Grossman cement. J Endod 7:477–480
36. Higginbotham (1967) A comparative study of the physical properties of five commonly used root canal sealers. Oral Surg Oral Med Oral Pathol 24:89–101
37. Torabinejad M, Kiger RD (1980) Experimentally induced alterations in periapical tissues of the cat. J Dent Res 1:87–96
38. Walton RE, Ardjmand K (1992) Histological evaluation of the presence of bacteria in induced periapical lesions in monkeys. J Endod 5:216–227
39. Cohenca N, Romualdo PC, da Silva LA, da Silva RA, de Queiroz AM, De Rossi A, Nelson-Filho P (2015) Tissue response to root canal irrigation systems in dogs' teeth with apical periodontitis. Clin Oral Investig 5:1147–1156
40. Alsulaimani RS (2018) Immediate and delayed repair of 2 sizes of furcal perforations in dogs' teeth using mineral trioxide aggregate cement. J Endod 6:1000–1006
41. Zmener O (2004) Tissue response to a new methacrylate-based root canal sealer: preliminary observations in the subcutaneous connective tissue of rats. J Endod 5:348–351
42. Garcia L d F, Marques AA, Roselino LM, Pires-de-Souza FC, Consani S (2010) Biocompatibility evaluation of Epiphany/ Resilon root canal filling system in subcutaneous tissue of rats. J Endod 1:110–114
43. Belladonna FG, Calasans-Maia MD, Novellino Alves AT, de Brito Resende RF, Souza EM, Silva EJ, Fidel SR, De-Deus G (2014) Biocompatibility of a self-adhesive gutta-percha-based material in subcutaneous tissue of mice. J Endod 1:1869–1873
44. Brandão PM, de Figueiredo JAP, Morgental RD, Scarparo RK, Hartmann RC, Souza RA (2019) Correction to: Influence of foraminal enlargement on the healing of periapical lesions in rat molars. Clin Oral Investig 4:2001–2003
45. Scarparo RK, Dondoni L, Böttcher DE, Grecca FS, Figueiredo JA, Kantarci A, Van Dyke TE, Batista EL Jr (2014) Intracanal delivery of Resolvin E1 controls inflammation in necrotic immature rat teeth. J Endod 5:678–682
46. Yoneda N, Noiri Y, Matsui S, Kuremoto K, Maezono H, Ishimoto T, Nakano T, Ebisu S, Hayashi M (2017) Development of a root canal treatment model in the rat. Sci Rep 1:3315
47. Calasans-Maia MD, Melo BR, Alves AT, Resende RF, Louro RS, Sartoretto SC, Granjeiro JM, Alves GG (2016) Cytocompatibility and biocompatibility of nanostructured carbonated hydroxyapatite spheres for bone repair. J Appl Oral Sci 6:599–608
48. Hess D, Solomon E, Spears R, He J (2011) Retreatability of a bioceramic root canal sealing material. J Endod 11:1547–1549