Original Article

Physicochemical properties of a novel experimental alginic acid-enhanced bioceramic-based root canal sealer: A comparative *in vitro* evaluation

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Abstract

Aims: This study aims to evaluate the physicochemical properties of a new experimental alginic acid-incorporated bioceramic-based sealer (Bio-G) compared to commercialized BioRoot RCS.

Materials and Methods: Bio-G sealers with 0%, 3%, and 5% alginic acid concentrations were formulated and tested for flowability, film thickness, radiopacity, working time, setting time, solubility, dimensional stability, and pH. Standardized methodological methods were used and statistical analysis was performed using the one-way ANOVA with *post hoc* Tukey's HSD.

Results: All Bio-G sealer groups met standards for flow (>20 mm) and film thickness (<50 μm). Bio-G (0%-algin) exhibited the highest flow, solubility, and longest setting time, while Bio-G (3%-algin) and Bio-G (5%-algin) had comparable solubility with BioRoot RCS. Radiopacity was lower in all Bio-G sealers than in BioRoot RCS but exceeded the required minimum standard. Moreover, all Bio-G sealer groups maintained an alkaline pH.

Conclusion: The incorporation of alginic acid influenced the physicochemical properties of Bio-G sealers, supporting their potential as alternative bioceramic materials for endodontic applications.

Keywords: Alginic acid; biomaterials; endodontics; material testing; mechanical properties; root canal; silicate

INTRODUCTION

Root canal treatment (RCT) is a widely performed endodontic procedure aimed at eradicating infection, alleviating pain, and restoring the functional integrity of the tooth. The procedure involves the removal of necrotic and inflamed pulpal tissue, followed by thorough chemo-mechanical instrumentation with hand or rotary files and obturation of the root canal system with an inert core material, typically gutta-percha.[1] However, gutta-percha lacks adhesion to the root dentinal walls, necessitating the use of a root canal sealer to establish a hermetic seal, thereby preventing bacterial reinvasion and promoting periapical healing.[2] An ideal root canal sealer should possess excellent physicochemical properties, including dimensional stability without shrinkage, appropriate setting time, insolubility in oral fluids, optimal flowability, acceptable film thickness,

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radiopacity, adequate adhesion to root dentinal walls, and biocompatibility with surrounding tissues.[3]

The current array of root canal sealers is broadly classified into epoxy resin-based, glass ionomer-based, calcium hydroxide-based, zinc oxide eugenol-based, and bioceramic-based formulations. Despite its lack of bioactivity, epoxy resin-based sealers such as AH Plus have historically been considered the gold standard due to their superior sealing ability and mechanical properties.[4] However, the growing emphasis on bioactive endodontic materials has driven a paradigm shift toward bioceramic-based sealers, which offer desirable attributes such as an alkaline pH, chemical stability, minimal shrinkage, biocompatibility, and the ability to induce bioactivity, thereby expediting periapical tissue regeneration.[5,6]

Bioceramic materials, particularly bioactive glass (BG) and glass-ceramics, have gained significant attention in medical and dental applications due to their ability to promote revascularization, enhance osteoblast adhesion, stimulate enzymatic activity, and facilitate the differentiation of mesenchymal stem cells and osteoprogenitor cells.[7] BG has been widely used in dentistry for dentine remineralization, bone grafting, and implant coatings to improve osseointegration.[8,9] Given these advantages, recent research has focused on advancing bioceramic-based root canal sealers by incorporating BG to enhance their performance. For instance, a previous study that incorporated fluoridated BG nanoparticles into AH Plus reported a significant improvement in the push-out bond strength.[10] Similarly, another study found that BG-containing bioceramic-based sealers enhanced the fracture resistance of root canal-treated teeth.[11] These innovations highlight the ongoing efforts to optimize the functionality of BG bioceramic-based sealers for improved endodontic outcomes.

As the part of the ongoing expansion of our project, a novel bioceramic-based root canal sealer (Bio-G) has been developed by incorporating alginic acid into a composite matrix of BG 58S and calcium silicate (Ca_2SiO_4) powders.[12] Alginic acid, a naturally occurring polysaccharide derived from brown algae, is widely recognized for its exceptional biocompatibility and adhesive potential.[13] The rationale behind incorporating alginic acid into Bio-G was to improve its adhesion and structural stability. Previous projects have indicated that alginic acid forms a highly cross-linked network that enhances the sealer's dislodgment resistance and reported favorable adhesive pattern and dentinal tubule penetration.[14] However, the specific impact on the physicochemical properties of alginic acid concentration in Bio-G remains unclear.

Given that the physicochemical properties of a root canal sealer might have a direct influence on the quality and longevity of root canal obturation, this study aims to conduct a comparative evaluation of key parameters, including flowability, film thickness, solubility, dimensional stability, pH, radiopacity, working, and setting times of this new experimental algin-enhanced BG-58S Ca_2SiO_4 root canal sealer against a commercialized bioceramic-based sealer, BioRoot RCS (Septodont, Saint Maur-des-Fosses, France). The null hypothesis of this study was that there is no significant difference in the selected physical and chemical properties between the experimental Bio-G sealers with varying alginate concentrations and the commercial BioRoot RCS. By addressing the existing gap in the literature, this study seeks to provide valuable insights into the potential clinical applications of alginic acid-incorporated bioceramic sealers in contemporary endodontic therapy.

MATERIALS AND METHODS

The current study adhered to the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines.[15] A summary of the key steps is presented in the PRILE 2021 flowchart [Figure 1] and a PRILE checklist is provided in Appendix 1.

Ethical approval

The AIMST University Human and Animal Ethics Committee exempted ethical approval for this study as it involved no human participants, animals, or living tissues, and all procedures were purely focused on the physicochemical evaluation of dental materials.

Preparation of experimental sealer materials

Figure 2 summarizes the tested sealers' composition and manufacturer details. The Bio-G sealer was formulated in both powder and liquid components, incorporating 0%, 3%, and 5% alginic acid as per previously published work.[12] The powder consisted of 30 wt.% BG 58S ($SiO_2 \cdot CaO \cdot P_2O_5$), 30 wt.% Ca_2SiO_4, 25 wt.% zirconia dioxide, 10 wt.% calcium carbonate, and alginic acid (0%, 3%, or 5%) as a binder. Meanwhile, the liquid component was prepared by dissolving 2.5 g of calcium chloride in 50 mL of distilled water to obtain a 5% calcium chloride solution. The powder and liquid were mixed at a 1:1 ratio until a homogeneous sealer was formed. On the other hand, BioRoot RCS (control group) was prepared according to the manufacturer's instructions. Each sealer group consisted of ten specimens ($n = 10$).

The evaluation of solubility, flowability, film thickness, solubility, dimensional stability, pH, radiopacity, both working and setting times adhered to the guidelines outlined in the American National Standards Institute/ADA Specification No. 57 and ISO 6876,[16] which define

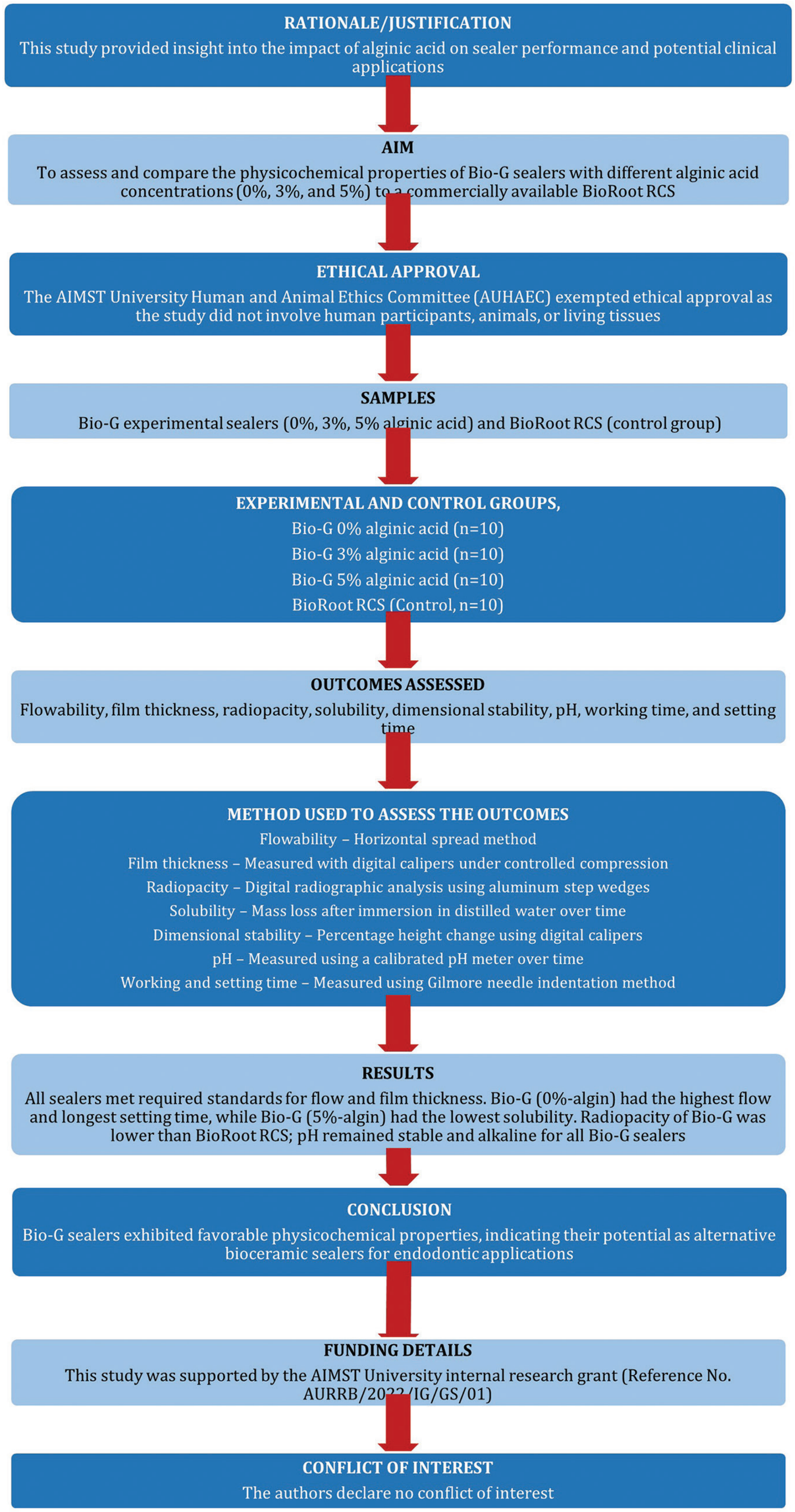

Figure 1: PRILE flowchart of the present study

the requirements and testing methods for root canal filling materials. All analyses were conducted by two calibrated operators working collaboratively to maintain consistency and accuracy.

Cement	Composition	Manufacturer
BioRoot RCS	**Powder**: tricalcium silicate, zirconium oxide, povidone **Liquid**: calcium chloride, polycarboxylate	Septodont, Saint Maur-des-Fosses, France
Bio-G (0% Algin)	**Powder**: calcium silicate, bioactive glass 58S, zirconium dioxide, calcium carbonate, alginic acid powder **Liquid**: deionised water and calcium chloride	I) Universiti Sains Malaysia II) Asian Institute of Medicine, Science and Technology (AIMST) University, Malaysia
Bio-G (3% Algin)		
Bio-G (5% Algin)		

Figure 2: Selected root canal sealers and their respective composition and manufacturer details

Flow

A precise volume of 0.1 mL of the sealers was dispensed onto a glass slab (GLP2 × 2; United Scientific Supplies, Inc., Waukegan, USA). A second glass slab (G1), weighing approximately 20 g, was carefully placed over the sealer, followed by an additional glass slab weighing 100 g, resulting in a total applied mass of 120 g (G1 + sealer + G2). After 10 min, the slabs were removed, and the maximum and minimum diameters of the compressed sealer disc were measured using a digital caliper (19975; Shinwa Rules Co., Ltd., Japan). Each measurement was taken three times per specimen to enhance accuracy and reliability.

Film thickness

Two square glass plates (200 mm^2 area, 5 mm thickness) were used. The initial thickness of the glass plates was recorded using a digital caliper. A 0.015 g amount of sealer was placed between the glass plates, and a 2 kg load (abs-sl-weight-set-small; PCS Instruments, United Kingdom) was applied to ensure uniform compression. After 10 min, the final thickness of the plates with the compressed sealer was recorded using a digital caliper, and the film thickness was calculated by determining the difference between the initial and final measurements.[17]

Solubility

Test specimens were prepared using Teflon rings (20 mm diameter, 1.5 mm thickness). The sealers were placed into the rings at the room temperature (27°C), with nylon threads embedded to facilitate suspension. The specimens were allowed to be set for 72 h, after which they were removed and weighed using a digital balance (WN-FAN, Worner Lab, or OEM, Zhejiang, China) to obtain the initial mass. Each specimen was then suspended using the nylon thread in a capped receptacle containing 50 mL of phosphate-buffered saline (PBS), ensuring that they remained fully immersed without contacting the receptacle walls. Considering the hydrophilic nature of Ca_2SiO_4-based sealers, PBS was selected as the storage medium instead of distilled water, as recommended by the ISO standards, to better simulate the clinical conditions. The samples were stored under these conditions for 1, 7, and 14 days, respectively, after which they were removed, gently dried using absorbent paper, and reweighed to determine their postimmersion mass. Solubility was calculated as the percentage of mass loss before and after immersion.

Dimensional stability

Each specimen was molded into cylindrical silicone molds (6 mm diameter and 10 mm height) and allowed to set for 72 h. Once fully set, specimens were polished using 600-grit sandpaper to ensure surface uniformity. The initial height (H_1) was measured using a digital caliper, after which each specimen was placed in 20 mL of PBS in a small beaker and incubated at 37°C in an incubator (ICS200; Yamato Scientific Co., Ltd., Japan) to simulate the oral conditions. The height (H_2) was remeasured on days 1, 7, and 14.[18] The dimensional change (DC) was calculated using the formula:

$$DC = (H_2\text{-}H_1/H_1) \times 100\%$$

pH

Three polyethene tubes (1 mm in diameter and 10 mm in length) were individually filled with the respective sealer using a disposable syringe fitted with a hypodermic needle. Each tube was then placed in a separate test tube containing 10 mL of distilled water and incubated at 37°C. The pH of each specimen was measured at 1, 7, and 14 days using a calibrated pH meter (Field-Scout SoilStik; Spectrum Technologies, Inc., China). At the end of each time interval, the sealers were removed from the tubes and transferred to fresh receptacles containing 10 mL of distilled water for continued analysis. The pH meter was calibrated before each measurement using standard buffer solutions with known pH values of 4 and 7.[18,19]

Radiopacity

Root canal sealers were dispensed into metal rings (10 mm diameter and 1 mm thickness), which were positioned on flat, smooth glass plates. These specimens were then stored in an incubator at 37°C to allow complete setting. Once set, the plates were removed, and the thickness of each test specimen was verified using a pachymeter (Mitutoyo Corp, Tokyo, Japan) to ensure uniformity.[17] Only specimens meeting the required thickness criteria were selected for radiopacity evaluation. The selected specimens were placed on Kodak Insight occlusal film (Kodak Comp, Rochester, NY) alongside an aluminum step wedge (graded from 2 mm to 16 mm Al) for radiographic analysis. The films were exposed using an X-ray unit (Gnatus XR 6010; Gnatus, Ribeirão Preto, SP, Brazil) operating at 60 kV, 10 mA, with 0.3 s exposure time at a 30 cm focus-to-film distance.[17] Radiopacity measurements were conducted using digital image analysis with ImageJ 1.48 v software (National Institutes of Health, Bethesda, MD, USA) and converted into millimeters of aluminum (mm Al) using the formula:

$A \times 2/B +$ mm/Al (immediately below RDm)

Where:

- A = Radiographic density of the material (RDm) – Radiographic density of the aluminum step immediately below RDm
- B = Radiographic density of the aluminum step immediately above RDm – Radiographic density of the aluminum step immediately below RDm.

Working time

The working time was assessed following the flowability test protocol. Freshly mixed sealers were tested at 30-s intervals, measuring the maximum and minimum diameters of the sealer disc using a digital caliper. Working time was defined as the interval at which the mean diameter of the sealer disc decreased by 10% of the initial flow value.[20]

Setting time

Silicone molds (10 mm diameter and 2 mm height) were prepared and filled with the sealers. The molds were stored in an incubator at 37°C with 95% humidity for 72 h. Setting time was assessed using a 100-g Gilmore needle with a 2-mm flat end, applied vertically onto the surface of the sealer. The setting time was determined as the point when the indenter needle failed to create an indentation on the material's surface. It was performed at 10-min intervals starting 1 h after the mixing. Before each test, the needle tip was cleaned to ensure accuracy. The setting time was recorded as the duration from the start of mixing until the sealer had fully set.[20]

Statistical analysis

IBM SPSS version 29.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Shapiro–Wilk test was employed to check for normality and homogeneity of variance. Since data were normally distributed, one-way ANOVA and *post hoc* Tukey's HSD tests were performed to determine the difference between the groups. The level of significance was set at $P = 0.05$.

RESULTS

Flow, film thickness, radiopacity, working time, and setting time

The data for flow, film thickness, radiopacity, working time, and setting time are summarized in Table 1. All sealer groups showed flow values >20 mm, which satisfied the ISO 6876:2012 standards. Bio-G (0%-algin) demonstrated a significantly higher ($P < 0.05$) mean flow value (22.7 ± 1.8 mm), whereas BioRoot RCS had the lowest mean flow value (20.8 ± 1.2 mm). *Post hoc* comparisons indicated no significant difference between BioRoot RCS and Bio-G (5%-algin). Film thickness varied significantly among the sealer groups ($P < 0.05$), with BioRoot RCS displaying

Table 1: Flow, film thickness, radiopacity, working time, and setting time of tested sealer materials

	Bioroot RCS	Bio-G (0%-algin)	Bio-G (3%-algin)	Bio-G (5%-algin)	*P*	ISO standard
Flow (mm)	20.8±1.2[a]	22.7±1.8	21.3±0.6	20.9±0.9[a]	0.012*	>20
FT (μm)	53.5±4.3	22.1±4.5	31.6±2.2	47.0±3.0	0.001*	<50
Radiopacity (mm Al)	5.87±0.6	4.11±0.2[b]	4.07±0.1[b]	4.07±0.3[b]	0.001*	>3
WT (min)	26.1±3.7[c]	33.5±2.1	29.7±1.7	25.5±0.7[c]	0.001*	-
ST (min)	300.5±11.1[d]	355.4±9.2	300.8±8.8[d]	270.5±12.1	0.001*	-

*Significance level at 0.05. The same superscript lowercase letters within the row indicate no statistical difference ($P>0.05$), FT: Film thickness, WT: Working time, ST: Setting time, RCS: Root canal sealers the highest mean thickness value (53.5 ± 4.3 μm), while the Bio-G groups demonstrated acceptable film thickness values of <50 μm. A significant difference ($P < 0.05$) was noted between the Bio-G groups, indicating that increasing algin concentration had an impact on film thickness. All Bio-G sealers exhibited significantly lower radiopacity than BioRoot RCS ($P < 0.05$), while there was no significant difference among them.

A significant difference in working time was observed among the sealer groups ($P < 0.05$), with Bio-G (0%-algin) having the longest mean working time (33.5 ± 2.1 min), followed by Bio-G (3%-algin) (29.7 ± 1.7 min). Nevertheless, BioRoot RCS (26.1 ± 3.7 min) and Bio-G (5%-algin) (25.5 ± 0.7 min) showed no significant difference ($P > 0.05$). The setting time of the tested sealers ranged from 270.5 min to 355.4 min, with Bio-G (0%-algin) exhibiting the longest mean setting time, while Bio-G (5%-algin) showed the shortest ($P < 0.05$). No significant difference was noted between BioRoot RCS and Bio-G (3%-algin), respectively.

Solubility, dimensional stability, and pH

The solubility, DC, and pH of the sealer materials were assessed on days 1, 7, and 14 [Table 2]. The solubility of Bio-G (0%-algin) exhibited an increasing trend over time, with significantly higher solubility ($P < 0.05$) compared to BioRoot RCS, Bio-G (3%-algin), and Bio-G (5%-algin). In contrast, BioRoot RCS, Bio-G (3%-algin), and Bio-G (5%-algin) all exhibited a decrease in solubility, but no significant differences were observed within each material group ($P > 0.05$), respectively.

BioRoot RCS exhibited the highest DC ($P < 0.05$) on Day 14, followed by Bio-G (5% algin), Bio-G (3% algin), and Bio-G (0% algin), with the latter remaining unchanged from Day 1 to Day 14. Both BioRoot RCS and Bio-G (5% algin) demonstrated significant DC s ($P < 0.05$) over time. Regarding pH, all sealer groups showed a decreasing trend; however, no significant differences ($P > 0.05$) were observed among the Bio-G sealers across day 1, day 7, and day 14. Similarly, on day 14, no significant differences ($P > 0.05$) were noted among all sealer groups.

Table 2: Solubility, dimensional stability, and pH of tested sealer materials after 1, 7, and 14 days

	Day 1	Day 7	Day 14
Solubility (%)* (ISO standard: <3%)			
Bioroot RCS	3.0 ± 0.4^{A}	$2.8\pm0.1^{D,a}$	$2.8\pm0.3^{F,a}$
Bio-G (0% algin)	3.4 ± 0.2	3.8 ± 0.2^{b}	3.9 ± 0.2^{b}
Bio-G (3% algin)	$3.0\pm0.1^{A,c}$	$2.9\pm0.1^{D,c}$	$2.8\pm0.2^{F,c}$
Bio-G (5% algin)	$2.9\pm0.1^{A,d}$	$2.8\pm0.1^{D,d}$	$2.7\pm0.2^{F,d}$
Dimensional change (%)* (ISO standard: <1% shrinkage or <0.1% expansion)			
Bioroot RCS	0.08 ± 0.1	0.13 ± 0.1	0.24 ± 0.2
Bio-G (0% algin)	$0.07\pm0.1^{B,e}$	$0.08\pm0.1^{D,e}$	$0.07\pm0.2^{a,e}$
Bio-G (3% algin)	0.09 ± 0.1^{f}	$0.08\pm0.1^{D,f}$	0.11 ± 0.2
Bio-G (5% algin)	0.07 ± 0.1^{B}	0.10 ± 0.1	0.21 ± 0.2
pH*			
Bioroot RCS	12.1 ± 0.1	11.9 ± 0.9	11.1 ± 0.3^{G}
Bio-G (0% algin)	$11.2\pm0.3^{C,g}$	$11.1\pm0.2^{E,g}$	10.9 ± 0.1^{G}
Bio-G (3% algin)	$11.1\pm0.3^{C,h}$	$11.0\pm0.1^{E,h}$	10.8 ± 0.1^{G}
Bio-G (5% algin)	$11.1\pm0.2^{C,i}$	$11.1\pm0.2^{E,i}$	$11.0\pm0.1^{G,i}$

*Significance level at 0.05. Same superscript lowercase letters within rows indicate no statistical difference ($P>0.05$); Same superscript uppercase letters within columns indicate no statistical difference ($P>0.05$), RCS: Root canal sealers

DISCUSSION

In the present study, the null hypothesis was rejected as there were significant differences between experimental Bio-G sealers and commercialized BioRoot RCS in the selected physical and chemical properties. All tested sealers in the present study exhibited a flow rate exceeding 20 mm, meeting the ISO 6876 standard. The high zirconium oxide content in all sealer groups potentially enhances their hydrophilicity, further improving the flow characteristics.[12,21] Nonetheless, the slightly reduced flowability of Bio-G with increasing alginate concentration can be attributed to the presence of free hydroxyl and carboxyl groups along the alginic acid backbone. These functional groups are highly reactive, promoting strong cross-linking with other particles, which may restrict the sealer's flow.[22]

The flowability of a root canal sealer is influenced by its film thickness, with thinner films allowing better adaptation and penetration into the root canal walls. In contrast, greater film thickness reduces flowability, potentially hindering the sealer's ability to fill anatomical irregularities such as isthmuses and lateral canals. BioRoot RCS exhibited a slightly thicker film than the optimal <50 μm threshold recommended by ISO 6876:2012, consistent with findings from previous studies.[23,24] In the present study, higher film thickness was associated with lower flowability across the tested sealers, aligning with the existing literature.[24] Nonetheless, the difference in film thickness and flowability among BioRoot RCS and Bio-G sealers may be attributed to their chemical composition and particle size differences.[12,21]

Radiopacity is a crucial property of root canal sealers, allowing them to be distinguishable on radiographs. The current findings indicated that all tested sealers met the minimum radiopacity threshold of 3 mm Al, as specified by the ISO standards. Notably, the greater radiopacity of BioRoot RCS is consistent with previous energy-dispersive X-ray spectroscopy analysis, which reported a higher zirconium content in BioRoot RCS than Bio-G sealers, serving as an effective radiopacifier.[12]

The setting time of an endodontic sealer is closely linked to its working time and is influenced by its composition. A shorter setting time typically allows for quicker procedural completion but may reduce the available working time for sealer manipulation and placement. In the current study, both the working and setting times of the Bio-G sealer groups decreased as the concentration of algin increased. This can be attributed to the inherent properties of alginic acid, a naturally occurring hydrophilic polysaccharide.[22] When exposed to moisture, alginic acid readily absorbs water and forms a viscous gel-like structure, which might accelerate the initial gelation and setting process. The increased alginate content likely enhances this effect by promoting faster hydration and crosslinking interactions, leading to a more rapid transition from a workable state to a hardened material.[25] This is particularly important when an immediate permanent restoration is required or when a post needs to be placed after root canal filling.[26] In such cases, selecting a sealer with a short setting time is advantageous

The solubility of root canal sealers is a crucial factor influencing the success of RCT, as high solubility can lead to the formation of gaps at the dentine–gutta–percha interface, resulting in microleakage and increasing the risk of endodontic failure.[27] According to ISO 6876 standards, a set sealer should exhibit solubility below 3%. Our findings revealed that only Bio-G sealers containing 3% and 5% algin met these ISO requirements. This reduced solubility may be attributed to the highly cross-linked polymer network formed by alginic acid,[22] which enhances the sealer's structural stability and resistance to dissolution.

The dimensional stability of a root canal sealer is pertinent, as any contraction or shrinkage can compromise marginal adaptation, leading to bacterial leakage.[26] Ideally, sealers should maintain a stable volume or exhibit slight expansion upon setting to ensure optimal sealing. According to ISO standards for dimensional stability, sealers should not shrink by more than 1% or expand beyond 0.1% of their initial mass after setting. However, on day 14, BioRoot RCS, Bio-G 3%-algin, and Bio-G 5%-algin exhibited expansion beyond the ISO-recommended threshold. This could be attributed to water sorption and the inherent expansion properties of Ca_2SiO_4 and alginate, which progressively absorb moisture over time, leading to dimensional growth.[26]

The alkaline pH of root canal sealers is considered one of their key advantages, as it facilitates the formation of apatite-like deposits on the sealer surface upon contact with body fluids. This process enhances bioactivity and promotes a strong chemical bond with the dentinal walls. In addition, the high alkalinity of these sealers supports apical healing, encourages tissue mineralization, and provides bacteriostatic effects, contributing to an antimicrobial environment.[20] Distilled water was chosen as the storage medium for the present pH testing due to its neutral pH, allowing for a more precise assessment of pH fluctuations.[28] Although all sealer groups exhibited a gradual decline in pH over time, their final pH levels remained statistically unchanged by day 14, indicating stability in their alkalinity over the observation period.

This study has several limitations. First, it did not include *in vivo* experiments, which restricts the clinical applicability of the findings. Second, the observation period was relatively short, potentially overlooking long-term performance. Third, the experimental conditions differed from actual clinical settings, where factors such as moisture, bacterial load, and pressure can significantly influence sealer properties. In addition, the test results may be affected by the operator's skill and experience. Meanwhile, the investigation of the physical and biological properties of the materials may be limited due to insufficient information on their components and proportions, which are typically not disclosed by manufacturers. These variations likely contribute to the differing characteristics of root canal sealers. The type and ratio of components in each sealer can also influence its physical properties. Future research should implement longer observation periods, include more extensive physical, mechanical and biological testing, as well as evaluate the antimicrobial properties of the new Bio-G sealer to better simulate real-world conditions. Finally, the sealer's behavior in the specialized environment of the root canal must be thoroughly investigated before progressing to clinical trials.

CONCLUSION

In short, the new experimental Bio-G sealers demonstrated favorable physicochemical properties, meeting the required standards for flow, film thickness, and radiopacity. Higher alginic acid concentrations improved solubility and dimensional stability. Moreover, all Bio-G sealers maintained a consistently alkaline pH and exhibited acceptable working and setting times, making them promising alternatives for endodontic applications.

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Conflicts of interest
There are no conflicts of interest.

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Appendix 1: Preferred Reporting Items for Laboratory Studies in Endodontology 2021: Checklist of items to be included when reporting laboratory studies in Endodontology*

Section/Topic	Item number	Checklist items	Reported on page number
Title	1a	The Title must identify the study as being laboratory-based, e.g. "laboratory investigation" or "*in vitro*," or "*ex vivo*" or another appropriate term	1
	1b	The area/field of interest must be provided (briefly) in the Title	1
Keywords	2a	At least two keywords related to the subject and content of the investigation must be provided	1
Abstract	3a	The rationale/justification of what the investigation contributes to the literature and/or addresses a gap in knowledge must be provided	1
	3b	The aim/objectives of the investigation must be provided	1
	3c	The body of the abstract must describe the materials and methods used in the investigation and include information on data management and statistical analysis	1
	3d	The body of the abstract must describe the most significant scientific results for all experimental and control groups	1
	3e	The main conclusion(s) of the study must be provided	1
Introduction	4a	A background summary of the scientific investigation with relevant information must be provided	1
	4b	The aim(s), purpose(s) or hypothesis(es) of an investigation must be provided ensuring they align with the methods and results	2
Materials and methods	5a	A clear ethics statement and the ethical approval granted by an ethics board, such as an Institutional Review Board or Institutional Animal Care and Use Committee, must be described	2
	5b	When harvesting cells and tissues for research, all the legal, ethical, and welfare rights of human subjects and animal donors must be respected and applicable procedures described	N/A
	5c	The use of reference samples must be included, as well as negative and positive control samples, and the adequacy of the sample size justified	3
	5d	Sufficient information about the methods/materials/supplies/samples/specimens/instruments used in the study must be provided to enable it to be replicated	3–5
	5e	The use of categories must be defined, reliable and be described in detail	3–5
	5f	The numbers of replicated identical samples must be described within each test group. The number of times each test was repeated must be described	3–5
	5g	The details of all the sterilization, disinfection, and handling conditions must be provided, if relevant	N/A
	5h	The process of randomization and allocation concealment, including who generated the random allocation sequence, who decided on which specimens to be included and who assigned specimens to the intervention must be provided (if applicable)	N/A
	5i	The process of blinding the operator who is conducting the experiment (if applicable) and the examiners when assessing the results must be provided	N/A
	5j	Information on the data management and analysis including the statistical tests and software used must be provided	5
Results	6a	The estimated effect size and its precision for all the objective (primary and secondary) for each group including controls must be provided	5–6
	6b	Information on the loss of samples during experimentation and the reasons must be provided, if relevant	N/A
	6c	All the statistical results, including all comparisons between groups must be provided	5–6
Discussion	7a	The relevant literature and status of the hypothesis must be described	6–8
	7b	The true significance of the investigation must be described	6–8
	7c	The strength(s) of the study must be described	6–8
	7d	The limitations of the study must be described	6–8
	7e	The implications for future research must be described	6–8
Conclusion(s)	8a	The rationale for the conclusion(s) must be provided	8
	8b	Explicit conclusion(s) must be provided, i.e. the main "take-away" lessons	8
Funding and support	9a	Sources of funding and other support (such as supply of drugs, equipment) as well as the role of funders must be acknowledged and described	Title page
Conflicts of interest	10a	An explicit statement on conflicts of interest must be provided	Title page
Quality of images	11a	Details of the relevant equipment, software and settings used to acquire the image(s) must be described in the text or legend	N/A
	11b	If an image(s) is included in the manuscript, the reason why the image(s) was acquired and why it is included must be provided in the text	N/A
	11c	The circumstances (conditions) under which the image(s) were viewed and evaluated must be provided in the text	N/A
	11d	The resolution and any magnification of the image(s) or any modifications/enhancements (e.g. brightness, image smoothing, staining, etc.) that were carried out must be described in the text or legend	N/A
	11e	An interpretation of the findings (meaning and implications) from the image(s) must be provided in the text	N/A
	11f	The legend associated with each image must describe clearly what the subject is and what specific feature(s) it illustrates	N/A
	11g	Markers/labels must be used to identify the key information in the image(s) and defined in the legend	N/A
	11h	If relevant, the legend of each image must include an explanation whether it is preexperiment, intra-experiment or postexperiment and, if relevant, how images over time were standardized	N/A

*Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Rôças IN, Siqueira JF Jr., *et al.* PRILE 2021 guidelines for reporting laboratory studies in Endodontology: A consensus-based development. Int Endod J 2021;54:1482-90. N/A: Not available