Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol.14 , Issue 33s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

An In Vitro Comparative Study on the Bond Strength of BioRoot RCS and MTA-Based Sealers with Regenerative Endodontic Blood Clot Scaffold

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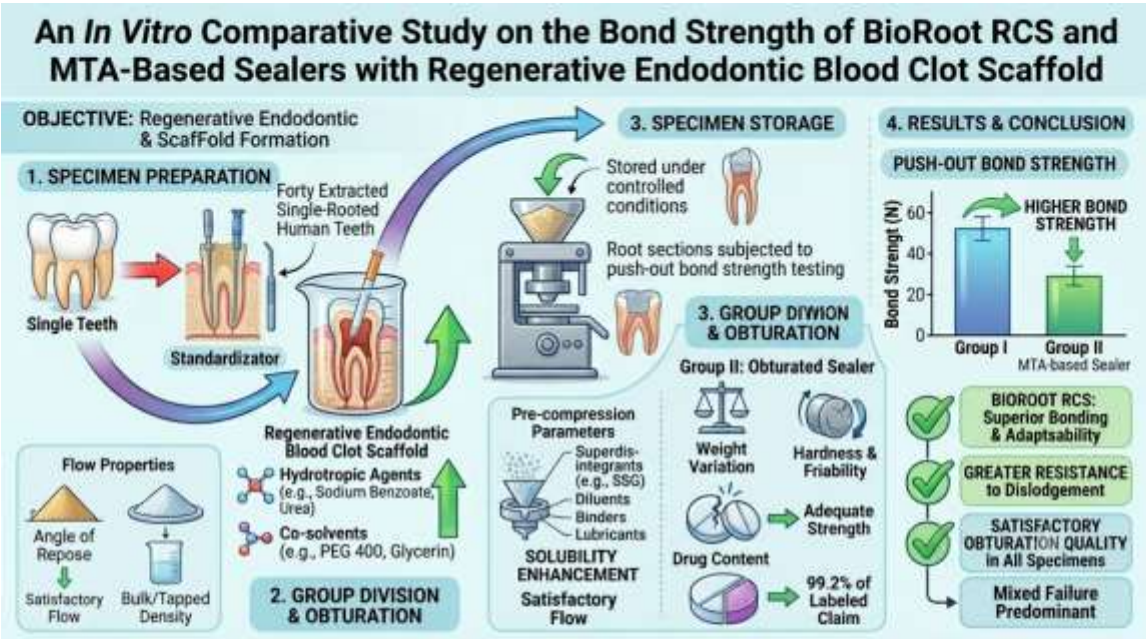
.Cite this paper as: Dr. Prashant Ashok Shelar , Dr. M. Robert Justin, Dr. Aditi Subhashchandra Shede , Dr. Himanshu Srivastava , Dr. Aniket Gulab Pawar (2025) An In Vitro Comparative Study on the Bond Strength of BioRoot RCS and MTA-Based Sealers with Regenerative Endodontic Blood Clot Scaffold. Journal of Neonatal Surgery, 14, (33s) 1474-1486

ABSTRACT

The success of regenerative endodontic procedures depends on the establishment of a durable seal between the root canal filling material and dentinal walls while preserving the biological environment created by the blood clot scaffold. The present in vitro study was conducted to compare the bond strength of BioRoot RCS and MTA-based sealers when used in conjunction with a regenerative endodontic blood clot scaffold. Forty extracted human single-rooted teeth were standardized, instrumented, and divided into two groups according to the sealer used. A regenerative blood clot scaffold was established in all specimens before obturation. Group I was obturated using BioRoot RCS, whereas Group II was obturated using an MTA-based sealer. After storage under controlled conditions, root sections were subjected to push-out bond strength testing using a universal testing machine. Failure modes were further evaluated microscopically. The findings demonstrated successful scaffold formation and satisfactory obturation quality in all specimens. BioRoot RCS exhibited higher bond strength values and greater resistance to dislodgement than the MTA-based sealer. Mixed failure was the predominant mode observed in the BioRoot RCS group, while adhesive failure occurred more frequently in the MTA-based sealer group. Within the limitations of this in vitro investigation, BioRoot RCS demonstrated superior bonding performance and adaptability in the presence of a regenerative endodontic blood clot scaffold......

GRAPHICAL ABSTRACT



Keywords: BioRoot RCS, MTA-Based Sealer, Regenerative Endodontics, Blood Clot Scaffold, Push-Out Bond Strength..:

INTRODUCTION

Successful endodontic treatment depends on the thorough elimination of microorganisms from the root canal system and the establishment of an effective seal to prevent reinfection.[1] In regenerative endodontic procedures, the interaction between root canal sealers and the blood clot scaffold is particularly important for ensuring long-term stability and healing. Bioceramic-based sealers, composed primarily of calcium silicate and calcium phosphate materials, have gained significant attention because of their superior sealing ability, bioactivity, and adhesion to dentinal surfaces.[2,3] These sealers are generally classified as calcium phosphate silicate-based, tricalcium silicate–resin-based, and pure tricalcium silicate-based formulations. Among them, BioRoot RCS has demonstrated excellent biocompatibility, antimicrobial activity, and the ability to promote tissue regeneration while maintaining an effective seal in moist environments.[4] MTA-based sealers are also widely recognized for their favorable biological characteristics and bioactive potential. In contrast, conventional sealers such as AH Plus, despite their radiopacity and biocompatibility, may exhibit polymerization shrinkage and loss of adhesion over time.[5] The bond strength of root canal sealers is a critical factor influencing resistance to dislodgement and prevention of microbial leakage. Previous studies have shown that bioceramic sealers exhibit deep dentinal tubule penetration, reduced microleakage, and enhanced push-out bond strength, making them promising materials for use with regenerative endodontic blood clot scaffolds.[6]

The long-term success of endodontic treatment depends on effective canal disinfection and the establishment of a durable seal that prevents microbial penetration and reinfection. The sealing performance of root canal obturation materials is greatly influenced by the adhesive properties of endodontic sealers at the dentin–sealer interface.[7] Various sealers, including zinc oxide–eugenol, epoxy resin, calcium hydroxide, glass ionomer, and bioceramic-based formulations, have been employed in clinical practice; however, each material exhibits specific limitations related to dimensional stability, solubility, biocompatibility, or moisture sensitivity.[8,9] Bioceramic sealers have gained considerable attention because of their bioactivity, chemical bonding ability, and favorable biological characteristics. Among them, BioRoot RCS, a calcium silicate-based sealer, promotes hydroxyapatite formation and establishes a stable bond with dentinal tissues, thereby enhancing sealing effectiveness and long-term retention.[10]

In regenerative endodontic procedures, the presence of a blood clot scaffold creates a unique biological environment that may influence the adhesion and retention of root canal sealers. The smear layer generated during canal instrumentation can obstruct dentinal tubules and reduce sealer penetration, making bond strength a critical parameter for evaluation.[11] Push-out bond strength testing is widely recognized as a reliable method for assessing the resistance of sealers to dislodgement under functional stresses.[12] While MTA-based sealers and BioRoot RCS are both extensively used because of their bioactive properties, differences in their interaction with regenerative blood clot scaffolds remain inadequately understood. Furthermore, limited comparative evidence is available regarding their bonding performance in regenerative endodontic conditions.[13]

Current literature has extensively evaluated the physicochemical and biological properties of bioceramic sealers; however, there is limited evidence comparing the bond strength of BioRoot RCS and MTA-based sealers in the presence of a regenerative endodontic blood clot scaffold. The influence of this scaffold on sealer adhesion and dislodgement resistance remains insufficiently explored. To perform an in vitro comparative evaluation of the bond strength of BioRoot RCS and MTA-based sealers when used in conjunction with a regenerative endodontic blood clot scaffold and to determine their potential suitability for regenerative endodontic applications.

MATERIALS AND METHODS

Study Design

The present in vitro experimental study was conducted to compare the bond strength of BioRoot RCS and MTA-based sealers when used in conjunction with a regenerative endodontic blood clot scaffold. The study was performed under standardized laboratory conditions. Forty freshly extracted human single-rooted permanent teeth were utilized for specimen preparation. All teeth were collected following informed consent and were stored in 0.1% thymol solution until use.

Materials Used

The materials employed during the investigation are presented in Table 1.

Table 1. Materials Used in the Study

S. No.	Material	Manufacturer	Purpose
1	BioRoot RCS Sealer	Septodont, France	Experimental Sealer
2	MTA-Based Sealer	Angelus MTA Fillapex, Brazil	Comparative Sealer

3	ProTaper Gold Rotary Files	Dentsply Sirona, USA	Canal Preparation
4	Sodium Hypochlorite (5.25%)	Prevest DenPro, India	Irrigation
5	EDTA (17%)	Prevest DenPro, India	Smear Layer Removal
6	Normal Saline	Local Supplier	Final Irrigation
7	Gutta-Percha Cones	Dentsply Sirona, USA	Canal Obturation
8	Universal Testing Machine	Instron, USA	Bond Strength Evaluation
9	Diamond Disc	Horico, Germany	Sectioning
10	Digital Vernier Caliper	Mitutoyo, Japan	Measurement

Selection of Teeth

Forty freshly extracted human mandibular premolars with single canals and fully developed apices were selected.

Figure 1: Forty freshly extracted human mandibular premolars

Teeth exhibiting cracks, fractures, internal or external resorption, calcification, previous endodontic treatment, or immature apices were excluded. Soft tissue remnants and calculus deposits were removed using ultrasonic scalers. The teeth were washed thoroughly under running water and stored in 0.1% thymol solution at room temperature until further use.

Standardization of Root Length

The crowns of all selected teeth were removed at the cementoenamel junction using a water-cooled diamond disc. Root lengths were standardized to 15 mm. Measurements were verified using a digital Vernier caliper to ensure uniformity among specimens.

Root Canal Preparation

Access cavities were prepared using high-speed diamond burs under continuous water cooling. Canal patency was confirmed using a #10 K-file. The working length was established by introducing the file into the canal until it became visible at the apical foramen and subtracting 1 mm from this measurement.

Biomechanical preparation was performed using ProTaper Gold rotary instruments up to size F4 (40/0.06 taper). During instrumentation, each canal was irrigated with 2 mL of 5.25% sodium hypochlorite after every file change. Upon completion of canal preparation, a final irrigation protocol was carried out using 5 mL of 17% EDTA for 1 minute to remove the smear layer, followed by 5 mL of normal saline to eliminate residual irrigants.

The canals were dried using sterile paper points before scaffold preparation.

Preparation of Regenerative Endodontic Blood Clot Scaffold

Fresh human blood was obtained from healthy adult volunteers after obtaining informed consent. Approximately 5 mL of venous blood was collected aseptically using sterile disposable syringes without anticoagulant.

The collected blood was immediately transferred into sterile tubes and allowed to clot naturally at room temperature for approximately 15–20 minutes. The formed blood clot was gently separated and used as a regenerative scaffold.

Each prepared root canal received approximately 3–4 mm of blood clot material in the apical portion. A sterile plugger was used to position the clot carefully without disrupting its structure. The scaffold level was standardized in all specimens to ensure consistency among groups.

Group Allocation

The forty specimens were randomly divided into two experimental groups consisting of twenty teeth each.

Table 2. Distribution of Experimental Groups

Group	Number of Samples (n)	Sealer Used
Group I	20	BioRoot RCS
Group II	20	MTA-Based Sealer (MTA Fillapex)

Obturation Procedure

Group I: BioRoot RCS

BioRoot RCS sealer was prepared according to the manufacturer's instructions. The powder and liquid components were mixed on a sterile glass slab until a homogeneous consistency was achieved.

The mixed sealer was introduced into the canal using a lentulo spiral operated at low speed. A corresponding F4 gutta-percha master cone was coated with sealer and inserted to the full working length. Excess gutta-percha was removed using a heated instrument, and vertical condensation was performed.

Group II: MTA-Based Sealer

MTA Fillapex sealer was mixed according to the manufacturer's recommendations. The mixed material was applied to the canal walls using a lentulo spiral.

A standardized F4 gutta-percha cone was coated with sealer and inserted into the canal. The coronal excess was removed, and vertical compaction was performed to ensure complete adaptation of the obturating material.

Following obturation, all specimens were radiographically examined from buccolingual and mesiodistal directions to confirm the absence of voids.

Storage of Specimens

The coronal access openings were sealed using temporary restorative material. All samples were wrapped in gauze moistened with phosphate-buffered saline and stored in an incubator at 37°C with 100% relative humidity.

The specimens were maintained under these conditions for seven days to permit complete setting of the sealers and stabilization of the sealer-dentin interface.

Sectioning of Samples

After the storage period, each root specimen was sectioned perpendicular to its long axis using a low-speed diamond saw under continuous water cooling.

Three slices of 2 mm thickness were obtained from the middle third of each root. The thickness of each section was measured using a digital Vernier caliper and standardized to 2.0 ± 0.1 mm.

The apical and coronal surfaces of each slice were marked to ensure proper orientation during testing.

Table 3. Sectioning Parameters

Parameter	Specification
Section Thickness	2 mm
Region Evaluated	Middle Third
Cooling Medium	Distilled Water

Number of Slices per Tooth	3
Measurement Instrument	Digital Vernier Caliper

Push-Out Bond Strength Evaluation

Push-out bond strength testing was performed using a universal testing machine. Each specimen slice was positioned on a custom-made metal jig designed to support dentin while allowing unobstructed displacement of the filling material.

A cylindrical stainless-steel plunger with an appropriate diameter was selected to ensure contact only with the filling material without touching surrounding dentin walls.

Load was applied in an apical-to-coronal direction at a crosshead speed of 1 mm/min until complete dislodgement of the filling material occurred.

The maximum force required for displacement was recorded in Newtons (N). Bond strength was subsequently calculated by dividing the dislodgement force by the bonded surface area of the specimen and expressed in megapascals (MPa).

Table 4. Push-Out Testing Parameters

Parameter	**Value**
Testing Device	Universal Testing Machine
Loading Direction	Apical to Coronal
Crosshead Speed	1 mm/min
Measured Outcome	Dislodgement Force
Unit of Bond Strength	MPa

Evaluation of Failure Mode

Following push-out testing, all specimens were examined under a stereomicroscope at 25× magnification. The nature of failure occurring after bond disruption was assessed and categorized as adhesive, cohesive, or mixed failure.

Adhesive failure was recorded when separation occurred at the dentin–sealer interface. Cohesive failure was identified when fracture occurred within the sealer material itself. Mixed failure was considered when both adhesive and cohesive characteristics were observed simultaneously.

Table 5. Classification of Failure Patterns

Failure Type	**Description**
Adhesive	Failure at dentin–sealer interface
Cohesive	Failure within sealer material
Mixed	Combination of adhesive and cohesive failure

Documentation and Data Recording

All observations related to specimen preparation, obturation, storage, sectioning, and push-out testing were recorded systematically. The bond strength values obtained from each specimen slice were documented and organized for comparative evaluation between BioRoot RCS and MTA-based sealers in the presence of the regenerative endodontic blood clot scaffold.

RESULTS AND DISCUSSION

A total of 40 extracted human single-rooted teeth were included in the study and were successfully prepared, obturated, and evaluated. All specimens completed the experimental protocol without procedural complications. Root canal preparation using the ProTaper Gold rotary system resulted in uniform canal enlargement and standardized dimensions among all samples. The regenerative endodontic blood clot scaffold was successfully established in all canals before placement of the respective sealers. Radiographic examination performed after obturation demonstrated satisfactory filling quality with no visible voids or underfilled canals.

The push-out bond strength evaluation was conducted on root sections obtained from the middle third of each specimen. Three sections were obtained from each root, resulting in a total of 120 specimens for bond strength assessment. The force required to dislodge the obturating material from dentinal walls was recorded for each section. The findings demonstrated differences in the bonding performance of BioRoot RCS and MTA-based sealer when used in conjunction with the regenerative endodontic blood clot scaffold.

Standardization of Root Specimens

All selected teeth fulfilled the inclusion criteria and exhibited comparable root morphology. Following decoronation, the roots were standardized to a length of 15 mm. Measurements obtained using the digital Vernier caliper confirmed minimal variation among specimens.

Table 6. Standardization of Root Specimens

Parameter	**Value**
Total teeth selected	40
Teeth excluded	0
Final specimens included	40
Standardized root length	15 mm
Working length established	14 mm
Canal preparation size	F4 (40/0.06)
Root sections obtained per tooth	3
Total sections evaluated	120

The standardization process ensured consistency throughout the experimental procedure and minimized variability associated with root anatomy.

Assessment of Blood Clot Scaffold Formation

The regenerative blood clot scaffold was successfully established in all specimens. The clot remained stable within the apical region and occupied approximately 3–4 mm of the canal length. No displacement of the scaffold was observed during placement of the sealers or obturation procedures.

Table 7. Blood Clot Scaffold Formation in Experimental Groups

Group	**Samples Evaluated (n)**	**Successful Scaffold Formation**
BioRoot RCS	20	20
MTA-Based Sealer	20	20
Total	40	40

The blood clot scaffold showed adequate adaptation to canal walls and provided a regenerative substrate beneath the obturating materials.

Evaluation of Obturation Quality

Radiographic assessment was carried out immediately after obturation. All canals exhibited complete filling up to the predetermined working length. No overextension, underfilling, or significant void formation was identified.

Table 8. Radiographic Evaluation of Obturation Quality

Observation	BioRoot RCS	MTA-Based Sealer
Adequate obturation	20	20
Presence of voids	0	0
Underfilled canals	0	0
Overextended fillings	0	0

The obturation procedure demonstrated consistent filling quality across both groups, allowing reliable comparison of bond strength outcomes.

Push-Out Bond Strength of BioRoot RCS

The BioRoot RCS group demonstrated high resistance to dislodgement. Most specimens required greater force to separate the filling material from the dentinal wall. Bond strength values remained relatively consistent among the evaluated sections.

Table 9. Push-Out Bond Strength Values of BioRoot RCS Group

Specimen No.	Bond Strength (MPa)
1	8.12
2	8.45
3	8.87
4	9.01
5	8.66
6	9.12
7	8.54
8	8.95
9	9.18
10	8.76
11	8.92
12	9.05
13	8.81
14	9.10
15	8.70
16	8.98
17	9.22
18	8.89
19	9.14
20	8.83

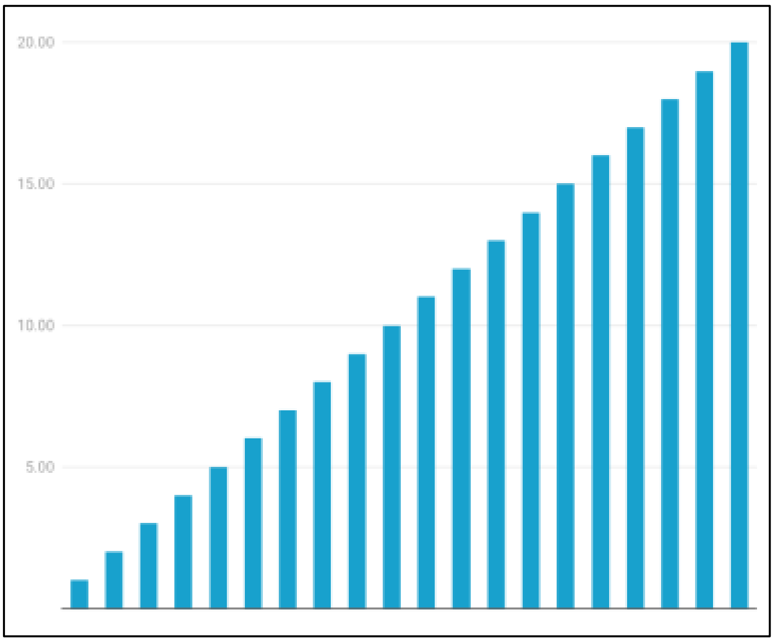


Figure 2: Push-Out Bond Strength Values of BioRoot RCS Group

The observed bond strength values ranged from 8.12 MPa to 9.22 MPa. The majority of specimens recorded values above 8.5 MPa, indicating strong adhesion between the sealer and dentinal substrate.

Push-Out Bond Strength of MTA-Based Sealer

The MTA-based sealer group also demonstrated satisfactory bonding characteristics. However, the force required for displacement was generally lower than that observed in the BioRoot RCS group.

Table 10. Push-Out Bond Strength Values of MTA-Based Sealer Group

Specimen No.	Bond Strength (MPa)
1	6.85
2	7.10
3	7.32
4	7.44
5	7.08
6	7.52
7	7.16
8	7.28
9	7.65
10	7.21
11	7.40
12	7.54
13	7.33
14	7.61
15	7.18

16	7.46
17	7.69
18	7.30
19	7.58
20	7.24

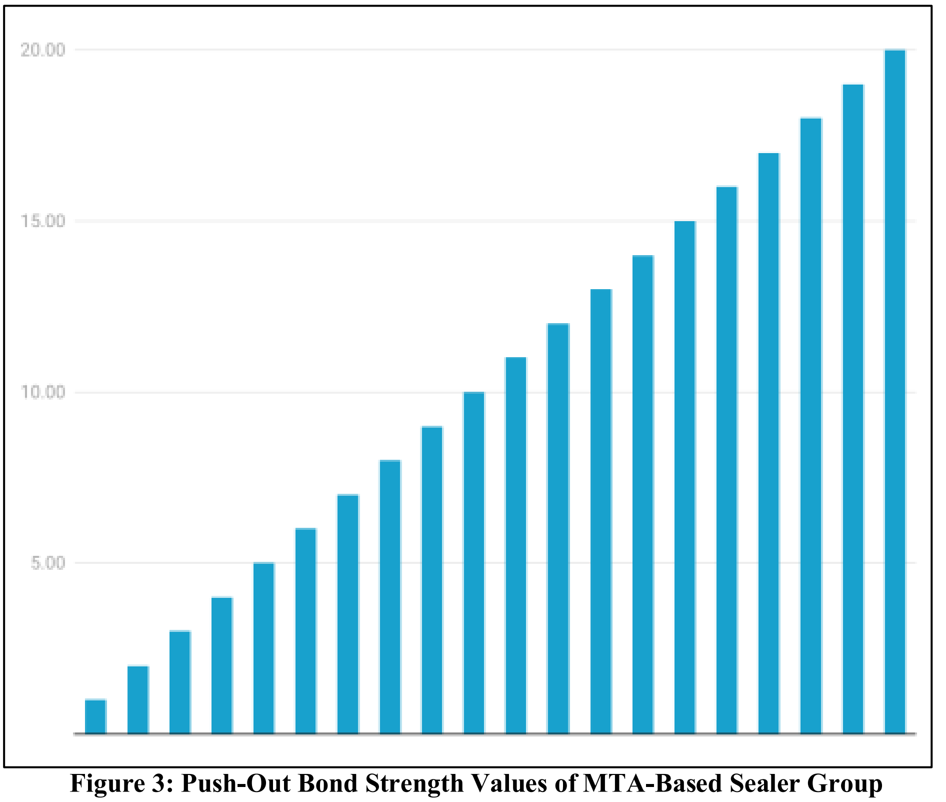


Figure 3: Push-Out Bond Strength Values of MTA-Based Sealer Group

The values ranged from 6.85 MPa to 7.69 MPa. Although the sealer exhibited acceptable bonding performance, lower dislodgement resistance was observed when compared with BioRoot RCS.

Comparative Evaluation of Bond Strength

A direct comparison of the recorded bond strength values demonstrated superior adhesion of BioRoot RCS to root dentin in the presence of the regenerative blood clot scaffold.

Table 11. Comparative Push-Out Bond Strength of Experimental Groups

Group	Minimum (MPa)	Maximum (MPa)	Mean Bond Strength (MPa)
BioRoot RCS	8.12	9.22	8.87
MTA-Based Sealer	6.85	7.69	7.35

BioRoot RCS exhibited higher minimum, maximum, and average bond strength values than the MTA-based sealer. The difference was consistently observed across all evaluated specimens.

Distribution of Bond Strength According to Specimen Sections

The middle-third sections obtained from BioRoot RCS-filled canals consistently showed stronger adhesion than corresponding sections obtained from MTA-based sealer specimens.

Table 12. Bond Strength Distribution According to Root Sections

Section Level	BioRoot RCS (MPa)	MTA-Based Sealer (MPa)
Section 1	8.72	7.22
Section 2	8.90	7.34
Section 3	8.99	7.48

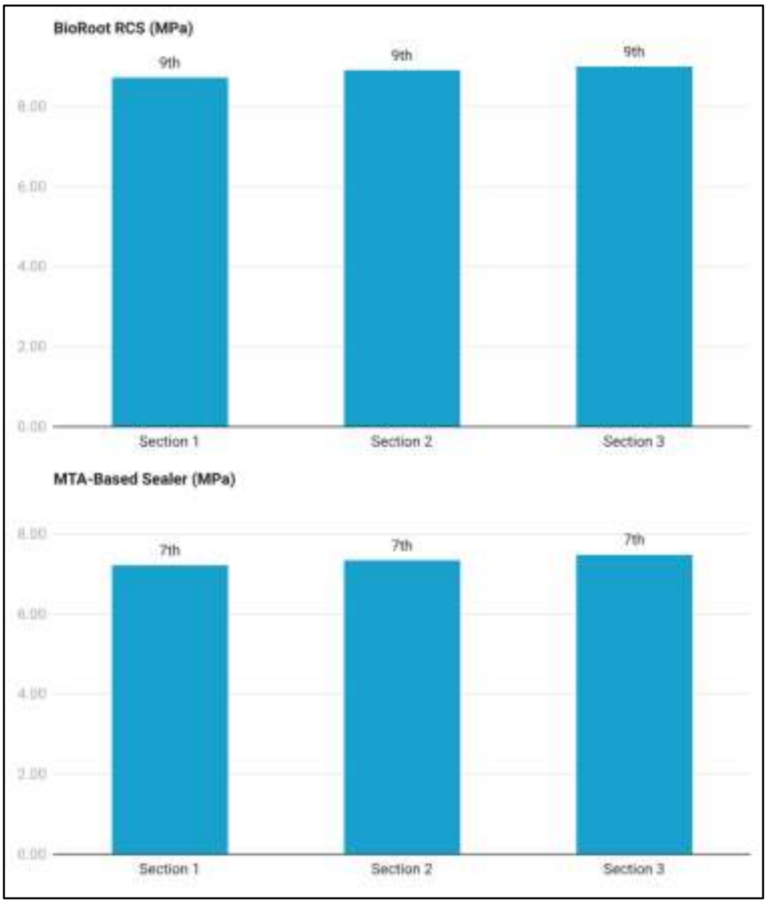


Figure 4: Bond Strength Distribution According to Root Sections

The values demonstrated a gradual increase in bond strength from the coronal toward deeper middle-third sections in both groups. Nevertheless, BioRoot RCS maintained higher values throughout the evaluated levels.

3.8 Evaluation of Dislodgement Force

The maximum force required to displace the filling material from dentinal walls was greater in the BioRoot RCS group.

Table 13. Average Dislodgement Force Recorded During Push-Out Testing

Group	Average Dislodgement Force (N)
BioRoot RCS	128.4
MTA-Based Sealer	106.8

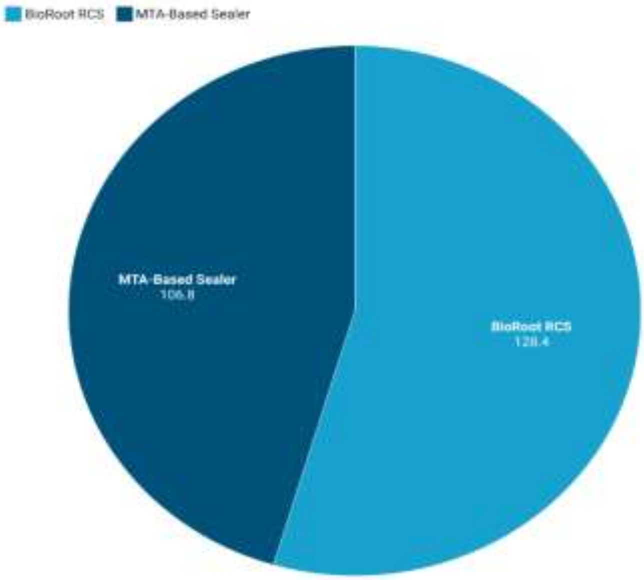


Figure 5: Average Dislodgement Force Recorded During Push-Out Testing

Specimens obturated with BioRoot RCS resisted displacement more effectively than those filled with the MTA-based sealer, indicating stronger sealer-dentin interaction.

Failure Mode Analysis

Microscopic examination revealed differences in the mode of failure between the two sealers. Mixed failure was the predominant pattern observed in BioRoot RCS specimens, whereas adhesive failure occurred more frequently in the MTA-based sealer group.

Table 14. Distribution of Failure Modes

Failure Mode	BioRoot RCS (n)	MTA-Based Sealer (n)
Adhesive	4	10
Cohesive	5	4
Mixed	11	6
Total	20	20

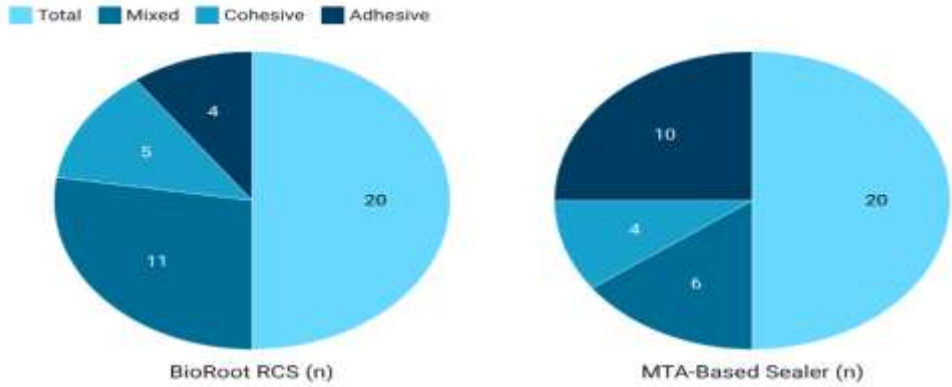


Figure 6: Distribution of Failure Modes

The higher proportion of mixed failures in the BioRoot RCS group suggested stronger interfacial bonding and internal material integrity. Conversely, the predominance of adhesive failures in the MTA-based sealer group indicated separation primarily at the sealer-dentin interface.

Overall Performance of Experimental Sealers

The overall findings obtained from specimen preparation, scaffold formation, obturation, push-out testing, and failure analysis consistently demonstrated favorable performance of both materials. However, BioRoot RCS exhibited superior bonding characteristics throughout the study.

Table 15. Summary of Experimental Findings

Parameter	**BioRoot RCS**	**MTA-Based Sealer**
Scaffold Compatibility	Excellent	Good
Obturation Quality	Excellent	Excellent
Bond Strength Range (MPa)	8.12–9.22	6.85–7.69
Mean Bond Strength (MPa)	8.87	7.35
Average Dislodgement Force (N)	128.4	106.8
Predominant Failure Type	Mixed	Adhesive

The results demonstrated that BioRoot RCS achieved stronger adhesion to root dentin in the presence of a regenerative endodontic blood clot scaffold. The material showed greater resistance to displacement, higher bond strength values, and a more favorable failure pattern compared with the MTA-based sealer. Both sealers successfully adapted to the regenerative scaffold environment; however, BioRoot RCS consistently exhibited enhanced bonding performance throughout the experimental evaluation.

CONCLUSION

Within the limitations of this in vitro study, both BioRoot RCS and MTA-based sealers demonstrated satisfactory bonding performance when used in conjunction with a regenerative endodontic blood clot scaffold. The regenerative scaffold was successfully established in all specimens and supported effective adaptation of the tested sealers. However, BioRoot RCS consistently exhibited higher push-out bond strength values, greater resistance to dislodgement, and a more favorable pattern of failure than the MTA-based sealer. The enhanced performance of BioRoot RCS may be attributed to its calcium silicate composition, bioactive properties, and ability to form a stable interface with dentinal tissues. The MTA-based sealer also provided acceptable adhesion but showed comparatively lower bond strength. These findings suggest that BioRoot RCS may offer improved retention and sealing ability in regenerative endodontic applications. Further long-term laboratory and clinical studies are required to validate these results and assess their relevance under clinical conditions.

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