In vitro study on bacterial leakage up to half of the canal length in different post-space preparation methods after root canal obturation

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Abstract

Introduction: A common cause of failure in endodontic treatment is bacterial penetration into the root canal system through microleakage after obturation, particularly when post-space preparation is required. **Objective:** To evaluate bacterial leakage in post-space prepared to half the root canal length using different methods following root canal obturation. **Subjects and Methods:** An *in vitro* study, without a control group, was conducted on 40 mandibular premolars, which were divided into 4 groups according to the obturation method and the timing of post-space preparation. Bacterial leakage was assessed by the turbidity of the BHI solution in each group, and Gram staining was performed if turbidity appeared. **Results:** Group 1 exhibited a delayed bacterial leakage time compared to Group 2. Additionally, Group 3 showed a later leakage time than Group 4. However, Group 1 had an earlier bacterial leakage time compared to Group 3, and Group 2 had an earlier leakage time than Group 4. The differences were statistically significant ($p < 0.05$). Gram-positive bacteria (90%) and Gram-negative bacteria (10%) were identified in the group with lateral compaction obturation. In the group with thermoplasticized Gutta Percha obturation, only Gram-positive bacteria (100%) were found. **Conclusion:** Immediate post-space preparation resulted in a significantly delayed bacterial leakage compared to delayed post-space preparation. Post-space preparation in teeth obturated with thermoplasticized Gutta Percha resulted in a significantly delayed bacterial leakage compared to those obturated using the lateral compaction technique.

Keywords: *Bacterial leakage, post-space preparation, obturation.*

1. BACKGROUND

Root canal treatment often leads to significant loss of dental tissue due to cavity preparation, root canal shaping, and canal preparation. Additionally, the loss of moisture in dentin reduces its restorative capacity and increases the risk of crown fracture. To maintain chewing function and aesthetics, these teeth need to be restored. Clinicians commonly use posts to restore endodontically treated teeth with materials such as prefabricated metal posts, cast posts, carbon fiber posts, and zirconia fiber posts.

Restoring teeth after endodontic treatment typically involves removing part of the root canal obturation (RCO) material to create space for the post. However, this removal may compromise the seal at the root apex, leading to microleakage and potential bacterial invasion. The impact of post-space preparation (PSP) on the seal between RCO material and the canal wall remains controversial.

Several studies have examined bacterial leakage through different RCO materials. Research by Timpawat Siriporn et al. (2001), Medeiros Paulo Leal et al. (2016), and Jafari Farnaz et al. (2016) concluded that bacterial penetration occurred within 24 hours, regardless of the type of RCO cement used.

Currently, there is limited research in Vietnam evaluating bacterial leakage in teeth that have undergone PSP using different RCO methods. Therefore, to further clarify this issue, we conducted the study: ***"In vitro study on bacterial leakage up to half of the canal length in different post-space preparation methods after root canal obturation"***

2. SUBJECTS AND RESEARCH METHODS

2.1. Research subjects

The study was conducted on 40 premolars indicated for extraction due to orthodontic treatment at dental clinics in Hue City.

2.1.1. Inclusion criteria

- Teeth with intact roots and straight canals with an angle ≤ 50° (according to Schneider's classification).
- Teeth that have not undergone root canal treatment and are free from calcification or internal resorption (confirmed by periapical radiographs).
- Teeth with fully developed root apices.
- Selected teeth must be permanent with closed apices and intact roots.

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2.1.2. Exclusion criteria

- Teeth that fractured during post-space preparation or caused root canal obturation material extrusion.

- Teeth with C-shaped canals or abnormal root canal morphology.

2.1.3. Research duration and location

- Research duration: From June 2023 to September 2024.

- Research location: Preclinical Laboratory, Faculty of Odonto-Stomatology, and Department of Microbiology, Hue University of Medicine and Pharmacy.

2.2. Research methods

2.2.1. Study design

This is an *in vitro* experimental study without a control group.

2.2.2. Sample Selection

40 premolars meeting the research criteria were selected and randomly divided into four groups based on the obturation method and timing of PSP:

- **Group 1 (n = 10):** Lateral compaction obturation with AH 26 Plus sealer and immediate PSP.

- **Group 2 (n = 10):** Lateral compaction obturation with AH 26 Plus sealer and delayed PSP.

- **Group 3 (n = 10):** Thermoplasticized GP injection obturation with immediate PSP.

- **Group 4 (n = 10):** Thermoplasticized GP injection obturation with delayed PSP.

In each group, bacterial leakage was assessed based on the turbidity of the Brain Heart Infusion (BHI) solution. Gram staining was performed to classify the bacteria if turbidity appeared in the BHI solution.

2.2.3. Research Procedure

- Step 1: Tooth Preservation

The teeth are cleaned using an ultrasonic scaler to remove any remaining soft tissue on the root surface (if present). The teeth are then immersed in a 0.9% saline solution, which is changed every 24 hours. The solution is stored at room temperature (approximately 25^0C), and the preservation period must not exceed 2 months.

- Step 2: Root Canal Preparation

+ Access opening: The root canal is accessed using an Endo Access bur and smoothed with an Endo Z bur.

+ Working length determination: The working length is determined using a #10 K file.

+ Root canal preparation: The canal is shaped using a ProTaper rotary file system with a crown-down technique, finishing with an F2 file.

- Step 3: Root Canal Obturation (RCO)

Preparation of AH26 Plus sealer: The sealer consists of two tubes (Paste A and B) mixed in a 1:1 ratio, as recommended by the manufacturer.

Lateral compaction obturation method:

+ Cone selection: A ProTaper GP cone (F2) is selected and marked at the working length. The cone is inserted into the canal until it fits snugly at the apical stop.

+ Selection of lateral compaction spreader: The spreader must fit tightly and be 1 - 2 mm shorter than the working length. This study uses a C-type spreader, and the rubber stopper is adjusted accordingly.

+ Drying the canal: The canal is dried using #30 paper points.

+ Applying sealer: A #30 file with a rubber stopper set at the working length is used to apply a thin layer of sealer to the canal walls, particularly in the apical third.

+ Insertion of the master cone: The F2 GP cone is coated with a small amount of sealer and inserted into the canal to the full working length.

+ Lateral compaction: The spreader is inserted with a vertical rotating motion until it reaches the pre-determined length. It is held in place for 10–15 seconds to allow the GP to compact. The spreader is then carefully removed.+ Insertion of accessory cones: Each accessory GP cone is coated with sealer and inserted sequentially into the space created by the spreader until the canal is densely filled.

+ Cutting and sealing: The GP cones are cut using a heated instrument, and temporary sealing is performed using Ceivitron W cement for Group 2.

Thermoplasticized GP obturation method:

+ Selection of injection needle: The appropriate needle size (20, 23, or 25 gauge) is chosen for GP injection. The GP is heated in the gun at 160 - 200°C. The needle is inserted into the canal, reaching 3 - 5 mm from the apex.

+ Applying sealer: A paper point is used to apply AH26 Plus sealer into the canal.

+ Injecting GP: The heated GP in 3 - 4 mm increments for TBOT, with each injection lasting less than 20 seconds.

+ Withdrawing the needle: The needle is removed carefully to prevent GP pullback.

+ Vertical compaction: A heated plugger is used to compact the GP.

+ Radiographic verification: The obturation in the apical third is evaluated using an X-ray. Additional condensation is performed until the apical canal is completely sealed.

+ Filling the remaining canal space: The remaining canal space is filled with more heated GP

and compacted.

+ Temporary sealing: Ceivitron® W cement is used for temporary sealing in Group 4.

All steps are performed using sterilized instruments under aseptic conditions in a biosafety level 2 laboratory in the Department of Microbiology.

- Step 4: Post-space preparation (PSP)

+ Immediate PSP: Performed immediately after RCO.

+ Delayed PSP: Performed after 24 hours.

+ Procedure: Peeso reamers #2, #3, and #4 are sequentially used.

- Step 5: Preparation of research equipment

+ Glass vials (10 mL) with rubber stoppers (24.3 mm diameter × 46.5 mm length) are numbered according to the research groups. Each stopper is perforated centrally using a heated instrument.

+ Tooth placement: Each tooth is inserted under pressure until the cementoenamel junction is at the stopper's surface. The tooth crown remains outside the vial, while the root apex is inside.

+ A 10 ml syringe was attached to a rubber stopper. The rubber stopper was adjusted to fit tightly against the inner wall of the syringe, creating a sealed chamber around the tooth crown.

+ Super glue (502 glue) is applied to the interface between the tooth and the stopper to seal the junction.

+ The prepared samples, including glass vials, teeth, and 10 mL syringe chambers, are sterilized with ethylene gas for 12 hours. The glass vials are then filled with 5 mL of BHI solution, ensuring that 2 mm of the root apex is submerged. All samples are incubated at 37°C for 5 days.

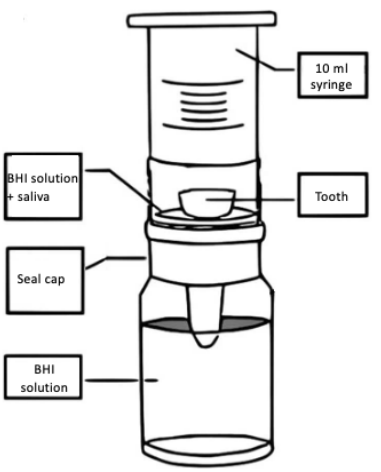

Figure 1. Diagram of the apparatus used to test bacterial leakage.

- Step 6: Bacterial leakage assessment

The upper part of the apparatus (the 10 mL syringe chamber) in each research sample is filled with 5 mL of a mixture of normal volunteer saliva and BHI solution in a 3:1 ratio. The BHI solution (BHI broth - Himedia, India) is prepared by dissolving 0.37 g of powder in 100 mL of distilled water following the manufacturer's instructions. This solution is then sterilized at 121^0 C under pressure for 15 minutes in a wet autoclave, cooled, and used immediately or stored at 4-8^0 C. The entire setup is then incubated at 37^0C. Saliva samples were collected from volunteers who had not taken any antibiotics within the past month. The saliva was completely replaced every 3 days.

The entire system is incubated at 37^0C, and the BHI solution is monitored daily for turbidity over 60 days to assess bacterial leakage.

2.3. Evaluation method

Bacterial leakage is assessed in 2 ways: Samples are monitored every 24 hours. If turbidity appears, the number of days until turbidity appears is recorded.

- Observation of BHI solution turbidity:

+ A cloudy or turbid BHI solution is considered positive (+), indicating bacterial growth.

+ An obvious BHI solution is considered negative (-), indicating no bacterial growth.

- If turbidity appears in the BHI solution, the sample group undergoes Gram staining and is examined under an optical microscope with an oil immersion lens (100x**)** to differentiate between 2 bacterial groups:

+ Gram-positive bacteria (stained purple with gentian violet).

+ Gram-negative bacteria (stained red with safranin).

3. RESULTS

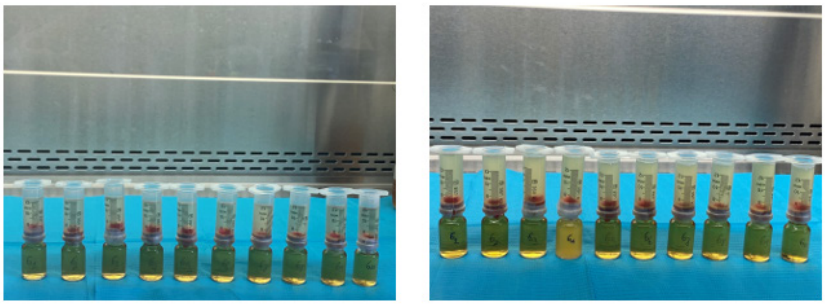

Figure 2. Research samples before and after the appearance of turbidity

Table 1. Bacterial leakage time and bacterial types on root with immediate and delayed PSP using the lateral compaction obturation method.

Group	Bacterial leakage time				Bacterial type			
	n	Mean ± SD (Day)	Median (Day)	Min - Max (Day)	Gram-positive		Gram-negative	
					n	%	n	%
1	10	16.60 ± 15.35	11.50	10 - 60	9	90.0	1	10.0
2	10	13.40 ± 16.60	8.00	5 - 60	9	90.0	1	10.0
p		0.040			1.000		1.000	

** Mann-Whitney U Test*

Group 1 exhibited a later bacterial leakage time compared to Group 2. The difference was statistically significant ($p < 0.05$). Both groups was detected Gram-positive bacteria (90%) and Gram-negative bacteria (10%).

Table 2. Bacterial leakage time and bacterial types on root with immediate and delayed PSP using the thermoplasticized GP obturation method

Group	Bacterial leakage time				Bacterial type			
	n	Mean ± SD (Day)	Median (Day)	Min – Max (Day)	Gram-positive		Gram-negative	
					n	%	n	%
3	10	23.70 ± 19.28	14.50	12 - 60	10	100.0	0	0.0
4	10	17.00 ± 15.24	12.00	10 - 60	10	100.0	0	0.0
p		0.030			-		-	

** Mann-Whitney U Test*

Group 3 exhibited a later bacterial leakage time compared to Group 4. The difference was statistically significant ($p < 0.05$). Both groups was detected Gram-positive bacteria in all 10/10 turbid samples (100%).

Table 3: Bacterial leakage time and bacterial types on root after immediate PSP using the lateral compaction and the thermoplasticized GP obturation method

Group	Bacterial leakage time				Bacterial type			
	n	Mean ± SD (Day)	Median (Day)	Min – Max (Day)	Gram-positive		Gram-negative	
					n	%	n	%
1	10	16.60 ± 15.35	11.50	10 - 60	9	90.0	1	10.0
3	10	23.70 ± 19.28	14.50	12 - 60	10	100.0	0	0.0
p		0.033			1.000		1.000	

** Mann-Whitney U Test*

Group 1 exhibited earlier bacterial leakage compared to Group 3. The difference was statistically significant ($p < 0.05$). Group 1 showed the presence of both Gram-positive and Gram-negative bacteria, whereas Group 3 only had Gram-positive bacteria in all 10/10 (100%) turbid samples.

Table 4. Bacterial leakage time and bacterial types on root after delayed PSP using the lateral compaction and the thermoplasticized GP obturation method

Group	Bacterial leakage time				Bacterial type			
	n	Mean ± SD (Day)	Median (Day)	Min – Max (Day)	Gram-positive		Gram-negative	
					n	%	n	%
2	10	13.40 ± 16.60	8.00	5 - 60	9	90.0	1	10.0
4	10	17.00 ± 15.24	12.00	10 - 60	10	100.0	0	0.0
p	0.036				1.000		1.000	

** Mann-Whitney U Test*

Group 2 exhibited earlier bacterial leakage compared to Group 4. The difference was statistically significant ($p < 0.05$). Group 2 showed the presence of both Gram-positive and Gram-negative bacteria, whereas Group 4 only had Gram-positive bacteria in all 10/10 (100%) turbid samples.

4. DISCUSSION

*** Discussion on RCO methods before PSP**

The data on the impact of post-space preparation (PSP) timing after root canal obturation (RCO) with gutta-percha (GP) remains controversial. In our study, results from Table 1 show that with the lateral compaction obturation method using AH 26 Plus, the immediate PSP group up to half the canal length (Group 1) had significantly later bacterial leakage than the delayed PSP group (Group 2). This result aligns with the study by Nikhil (2011), where the leakage time of the immediate PSP group (28.20 ± 0.92 days) was later than that of the delayed PSP group (20.10 ± 0.88 days) [1].

The delayed bacterial leakage in the immediate PSP group can be explained as follows: in the immediate PSP group, the RCO material is still in its working phase, allowing it to continue setting without creating microcracks. In contrast, in the delayed PSP group, the RCO material has fully set, and the rotational force of PSP instruments can disrupt the bonding interface between the RCO material and the canal walls, leading to microleakage [2]. Additionally, immediate PSP in clinical practice reduces the number of treatment sessions, maintains aseptic conditions, and thus lowers the risk of reinfection in the root canal system [3]. Immediate PSP also minimizes the risk of errors, such as loss of apical stop and over-enlargement of the canal, as the clinician is already familiar with the canal morphology and working length throughout the treatment process [4].

Results from Table 2 show that with the thermoplasticized GP obturation method, the immediate PSP group up to half the canal length (Group 3) had significantly later bacterial leakage than the delayed PSP group (Group 4). Inferring from the setting time of the RCO material affecting bacterial leakage, the group obturated with thermoplasticized GP was similarly affected. For thermoplasticized GP, the setting process begins after 25 minutes at a temperature of 37°C and is fully set after 1 hour. A Soo (2014) study found that the average RCO time with thermoplasticized GP was 6.11 minutes [5]. Therefore, when PSP is performed immediately, the thermoplasticized GP is not fully set, allowing it to seal any cracks formed during the PSP process.

Tables 1 and 2 show that both study groups exhibited bacterial leakage (10/10 samples). Gram-positive bacteria were predominantly present in both groups (90.0%). This result differs from Neda's (2022) study, in which the author found that the immediate PSP group had a higher level of leakage despite a later onset [6]. This discrepancy may be because, during the PSP process, the RCO material, which has not yet fully set, undergoes physical and chemical changes under the influence of PSP instruments, thereby increasing bacterial leakage potential [7]. A study by Miletie (2002) identifying specific bacterial strains found that the isolated samples mainly contained Gram-positive streptococci, such as Streptococcus mutans and Streptococcus mitis. Meanwhile, Lactobacillus acidophilus (Gram-positive bacillus) was less frequent and often co-detected with streptococcal strains [8].

*** Discussion on PSP Timing**

The results from Table 3 show that when immediate PSP was performed, the bacterial leakage time in the group with lateral compaction obturation using AH26 was earlier than in the group with thermoplasticized GP obturation. This result is consistent with the study by Chen (2011) [9]. This can be explained by the fact that the lateral

compaction obturation method with AH 26 Plus creates a less homogeneous material mass, and its adaptation to the root canal walls is inferior to that of thermoplasticized GP, even though lateral condensation is better for apical sealing [10]. According to Soo (2014), the root canal is not fully filled with GP in the lateral compaction obturation method because accessory cones cannot fill the gaps created by the spreader, particularly at 1mm and 3mm from the apex. At 3mm from the apex, the void ratio in the lateral condensation method is significantly higher than in the thermoplasticized GP method (1.10 ± 1.43 vs. 0.64 ± 0.91). The voids within the obturation material allow pulp tissue residues containing bacteria to grow, increasing leakage [5]. Hanan (2016) found no significant difference between lateral condensation with AH 26 Plus and thermoplasticized GP methods. This may be because the author only performed obturation at approximately 5mm without PSP, which did not cause a material fracture, resulting in a different outcome than our study.

One of the key factors determining the sealing ability and retention of the material after PSP is the setting time of the obturation material [9]. Neda's study (2022), which compared microleakage at different time points using two RCO materials, Endoseal MTA and AH Plus, found that Endoseal showed no difference between immediate and delayed PSP. In contrast, AH Plus exhibited a significant difference [6]. According to the author, Endoseal MTA has a faster setting time (4 minutes), so the material is already fully set when immediate PSP is performed. As a result, any fractures and voids created cannot be sealed, similar to delayed PSP [6]. In contrast, AH Plus takes up to 8 hours to fully set at 37°C, leading to differences in sealing properties between immediate and delayed PSP [6]. Reyhani (2015) reported similar findings, showing no difference between immediate and delayed PSP when using MTA material. Lyon (2008) used Resilon as an RCO material, but the differences between PSP time points were insignificant. According to Benjamin (2006), the setting time of Resilon at body temperature is 30 minutes. Thus, to assess bacterial leakage levels in different RCO materials, it is essential to consider their complete setting or curing time to select an appropriate PSP timing.

Based on Tables 3 and 4, the lateral compaction obturation method group exhibited both types of bacteria, with Gram-positive bacteria appearing in most perforated samples. Ozcan's study (2011) analyzed the antibacterial effectiveness of AH Plus and GuttaFlow by isolating Enterococcus faecalis and found that resin-based RCO material (AH Plus) significantly inhibited bacterial growth compared to polydimethylsiloxane-based material (GuttaFlow). This highlights that selecting the RCO material is more crucial than the RCO method in eliminating bacteria [11]. Mohammadi (2007) compared the antibacterial effectiveness of AH 26 Plus, Realseal, and GuttaFlow against Staphylococcus aureus and Streptococcus mutans. The results showed that resin-based AH 26 Plus exhibited superior antibacterial activity against both bacterial species compared to other materials [12]. Melker (2006) demonstrated that RCO using GP containing tetracycline inhibited 4 bacterial species: Actinomyces israelii, Actinomyces naeslundii, Enterococcus faecalis, and Fusobacterium nucleatum. Kowalski (2023) pointed out that GP alone does not provide long-term antibacterial effects. However, combining GP with other RCO materials has shown promising results. Thus, the choice of RCO cement and incorporating antibiotics into GP plays a vital role in preventing bacterial growth after endodontic treatment.

The results from Table 4 show that with delayed PSP, the bacterial leakage time in the lateral compaction obturation group with AH26 was earlier than in the thermoplasticized GP group. Chen (2011) reported similar findings when comparing the two RCO methods at the PSP time point after seven days [9]. Kala (2015) compared the apical sealing ability of different materials under delayed PSP conditions and found significant differences between the materials.

One of the desirable properties of RCO is its ability to adhere to the dentinal walls of the root canal, which is crucial in both static and dynamic situations. Strong adhesion helps eliminate gaps, allowing the RCO material to penetrate effectively and resist dislodgment during subsequent procedures like PSP [13].

Jorge (2007) observed under an electron microscope that after seven days of RCO, the gap between GP, AH 26 Plus, and the root canal wall was relatively large. Polymerization shrinkage created enough stress to cause the RCO material to detach from the dentin of the root canal wall, allowing bacterial leakage [14].

Moinuddin (2019) suggested that uniformity between RCO materials helps reduce bacterial leakage. Although RCO materials can bond to dentin, none can bond to GP, leaving gaps for bacterial growth. The authors conducted a push-out test on

slices taken from different positions in the canal. They found that the thermoplasticized GP group bonded better to the root canal wall in the middle and cervical thirds than the lateral compaction obturation group [15].

Thus, based on electron microscopy and push-out testing, bacterial leakage is influenced by the shrinkage of RCO materials after setting and their adhesion to GP. This explains why the thermoplasticized GP group exhibited slower bacterial leakage than our study's lateral compaction obturation group.

5. CONCLUSION

The immediate post-space preparation (PSP) group exhibited significantly delayed bacterial leakage compared to the delayed preparation group. Additionally, teeth obturated with the thermoplasticized GP method demonstrated significantly delayed bacterial leakage compared to those with the lateral compaction method.

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