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Penetration of Biodentine, NeoMTA 2, and NeoPUTTY into dentinal tubules in primary tooth pulpotomy: a confocal laser scanning microscopy analysis

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Abstract

Background Pulpotomy is an accepted treatment option to preserve primary teeth with exposed pulp due to caries or trauma and to ensure proper function until physiologic exfoliation. This study aimed to assess the dentin penetration of NeoMTA 2, NeoPUTTY, and Biodentine using confocal laser scanning microscopy (CLSM).

Methods The present study was performed using 42 freshly extracted lower primary molars. All samples were divided into 3 separate groups and the Biodentine, NeoMTA 2, and NeoPUTTY were placed on the cavity floor, respectively. High-speed diamond discs were used to create 2-mm-thick sections from the furcation region, including a 1- mm crown and 1-mm root. Primary molar tooth sections were placed on a glass slide and examined using a CLSM (LSM 980, Zeiss, Germany). All images were analyzed using ImageJ/Fiji (National Institute of Health, USA) software for the measurement of penetration depth. The penetration depth of the materials was evaluated using one-way ANOVA, and the Tukey test was used for comparisons between groups at $p < 0.05$ significance level.

Results There was no significant difference in penetration depth between the Biodentine and NeoMTA 2 materials. NeoPUTTY showed higher penetration depth than Biodentine and NeoMTA 2.

Conclusions This study reported that NeoPUTTY showed higher dentinal tubule penetration in primary teeth than NeoMTA 2 and Biodentine as pulpotomy materials.

Keywords Calcium silicate-based materials, Pulpotomy, Confocal laser scanning microscopy

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Introduction

Pulpotomy is an effective treatment for primary and young permanent teeth whose pulp is exposed via caries or trauma [1]. The procedure involves removing the infected or inflamed coronal pulp, preserving the radicular pulp with a suitable agent, and applying an appropriate coronal restoration [2]. Various pulpotomy agents, including formocresol, ferric sulfate, calcium hydroxide, mineral trioxide aggregate (MTA), and Biodentine (Septodont, Saint-Maur-des-Fossés, France), have been used [3, 4]. The American Academy of Pediatric Dentistry (AAPD) recommends MTA and formocresol for teeth expected to remain functional for at least 24 months [2, 5]. In 2022, the International Association of Pediatric Dentistry (IAPD) recommended MTA, Biodentine, and formocresol as pulpotomy agents, but noted the carcinogenic potential of formocresol [6].

MTA, a calcium silicate material, offers enhanced biocompatibility, good sealing, and the ability to promote pulp regeneration [7, 8]. It enhances odontoblast activity, promoting the formation of secondary dentin and hard tissue bridges (8,9).

Recently, new MTA products have been developed. NeoMTA Plus (NuSmile, Houston, Texas, USA), is composed mainly of tricalcium silicate and comes in a powder/liquid format [9]. NeoMTA 2 is its successor [10]. In 2020, NeoPUTTY (NuSmile), a pre-mixed version of NeoMTA Plus, entered the market in ready-to-use syringes, reducing preparation time [11]. Biodentine is made of calcium silicate, hardens in about 10 min, and offers comparable sealing capacity, biocompatibility, and hard-tissue formation [12].

The success of pulpotomy depends on factors such as the agent used, final restoration, pulp condition, base material, hemostasis method, and tooth isolation [13]. Ensuring a sealed coronal restoration is crucial to minimize microleakage. Deep penetration of pulpotomy agents into dentinal tubules is essential for a satisfactory seal. Studies have evaluated the penetration of calcium silicate materials in permanent teeth but none has investigated this in primary teeth [14, 15]. This study used confocal laser scanning microscopy (CLSM) to assess the penetration of NeoMTA 2, NeoPUTTY, and Biodentine into dentinal tubules. The null hypothesis was that the penetration would not differ among these materials.

Materials and methods

Sample selection and Preparation

This study was conducted after approval from the Scientific Research Ethics Committee of the University of Lokman Hekim (No: 2024/188). The sample size was calculated using the effect size of previous study with a power of 0.80 and a α of 0.05 in the G*Power 3.1.9.6. program [16]. The total minimum sample size was determined as 42 with = 14 per group. Forty-two primary molars extracted for reasons unrelated to the study were collected, and any debris and soft tissue were removed using a periodontal curette. Only lower second primary molars with intact pulp chambers, sound or minimally carious, without cracks or restorations, and exhibiting similar root lengths (≥ two-thirds of the original root length) and morphology were included in the study to provide standardization. Before the primary teeth were extracted, parents and children were informed in detail, and the parents signed an informed consent form. The samples were stored in distilled water containing 0.1% thymol crystals at room temperature until the beginning of the experimental procedures.

All teeth were embedded in acrylic in a mold so that the furcation area was visible. Cavity preparation was performed using round and fissure burs under water cooling. Biodentine and NeoMTA 2 were mixed according to the manufacturer's instructions. Then, 1 drop of 0.1% diluted rhodamine-B dye was added to Biodentine, NeoMTA 2, and pre-mixed NeoPUTTY. All samples were divided into 3 separate groups and the following materials were placed on the cavity floor. Subsequently, a sterile cotton pellet moistened with saline solution was placed inside the cavity and left for 5 min, after which the cavity was sealed with glass ionomer cement. (Fuji II, GC Corp., Tokyo, Japan)

Group 1: Biodentine.
Group 2: NeoMTA 2.
Group 3: NeoPUTTY.

The samples were stored at 37 °C under 100% humidity in an incubator for 4 weeks. Subsequently, 2-mm-thick sections—including 1 mm of the crown and 1 mm of the root from the furcation region—were obtained under water cooling using a low-speed diamond cut-off wheel (NTI Diamond Disc Double Sided Handpiece D918-220, 22 mm, Henry Schein, New York, NY, USA) (Fig. 1).

CLSM analysis

Primary molar tooth sections were placed on a glass slide and examined using a CLSM (LSM 980, Zeiss, Germany) (Fig. 2). Samples were scanned with 0.10 µm step size under 543-nm laser lines and 2 × 5 magnification. Z-stack images of 2420 × 2420 pixels were recorded from the surface of the section to the depth, and images of 4 separate regions were obtained for each sample. All images were analyzed using ImageJ/Fiji software (National Institute of Health, USA). The penetration depth was measured by drawing a line tool from the material to the tooth perimeter (Fig. 3). In each image, 10 separate lines were drawn from the penetration areas, and the average was calculated.

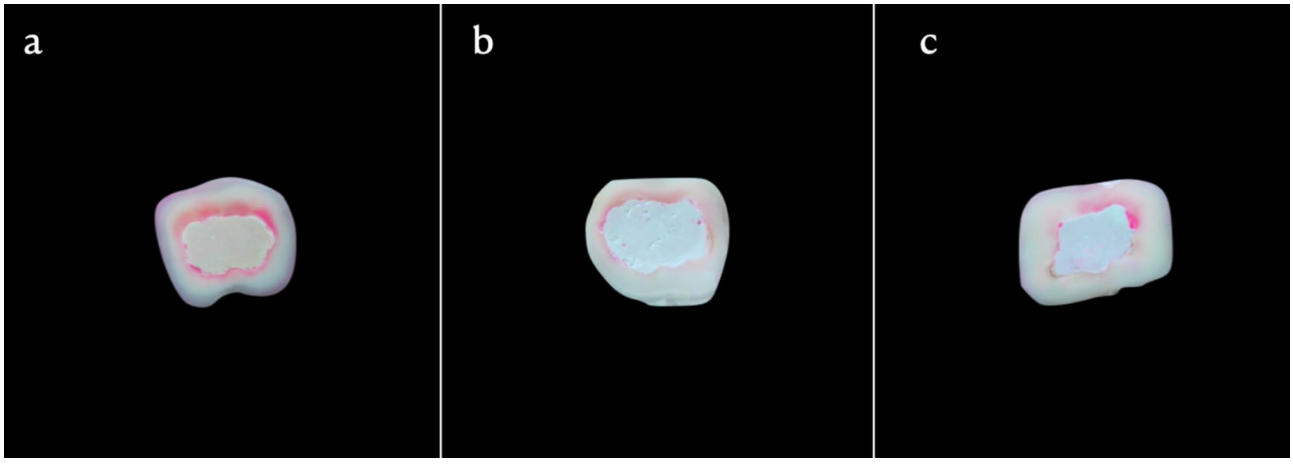

Fig. 1 The photography of dentin sections before CLSM analysis **a**; Biodentine, **b**; NeoMTA 2, **c**; NeoPUTTY

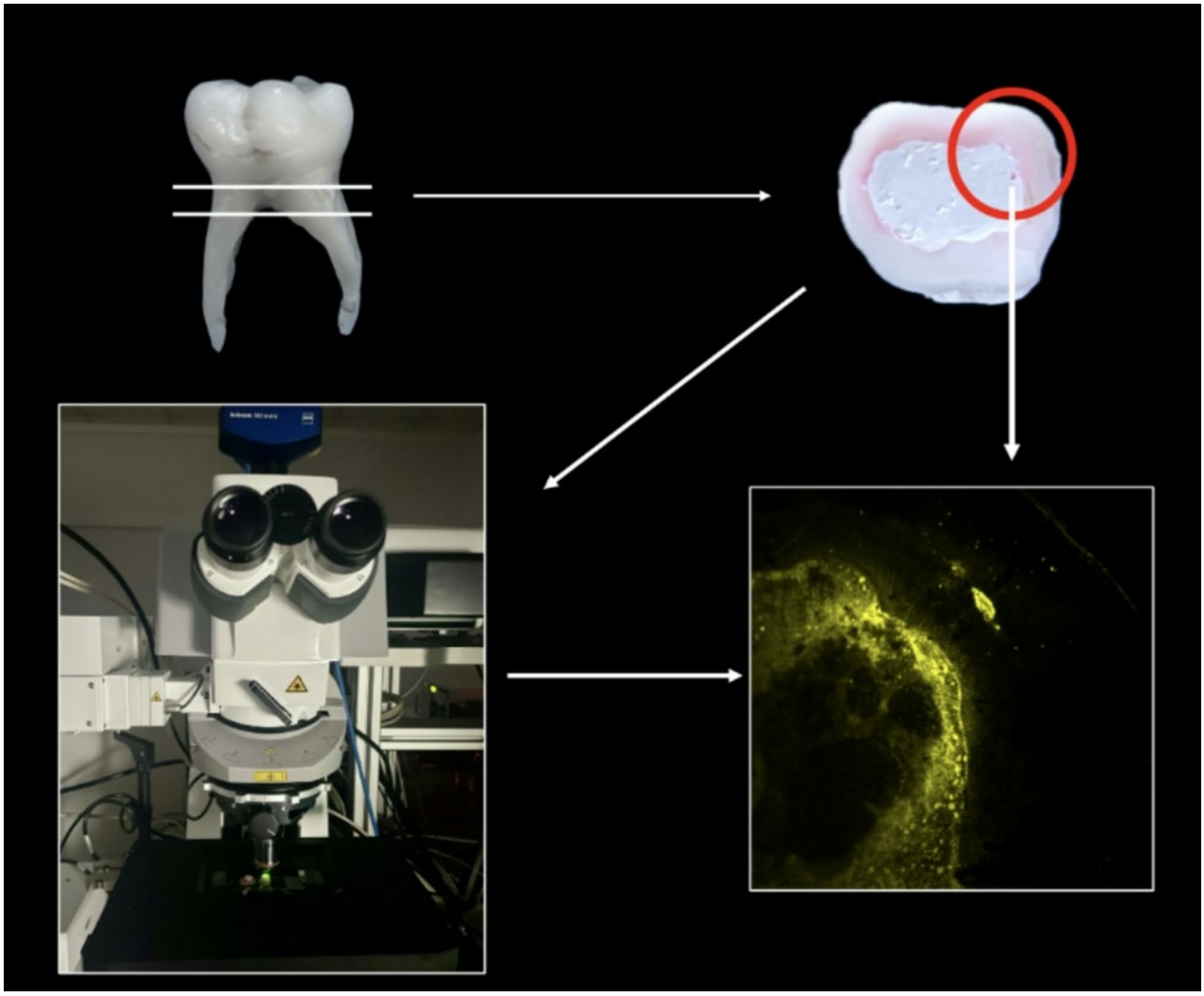

Fig. 2 The workflow presentation of the experimental design

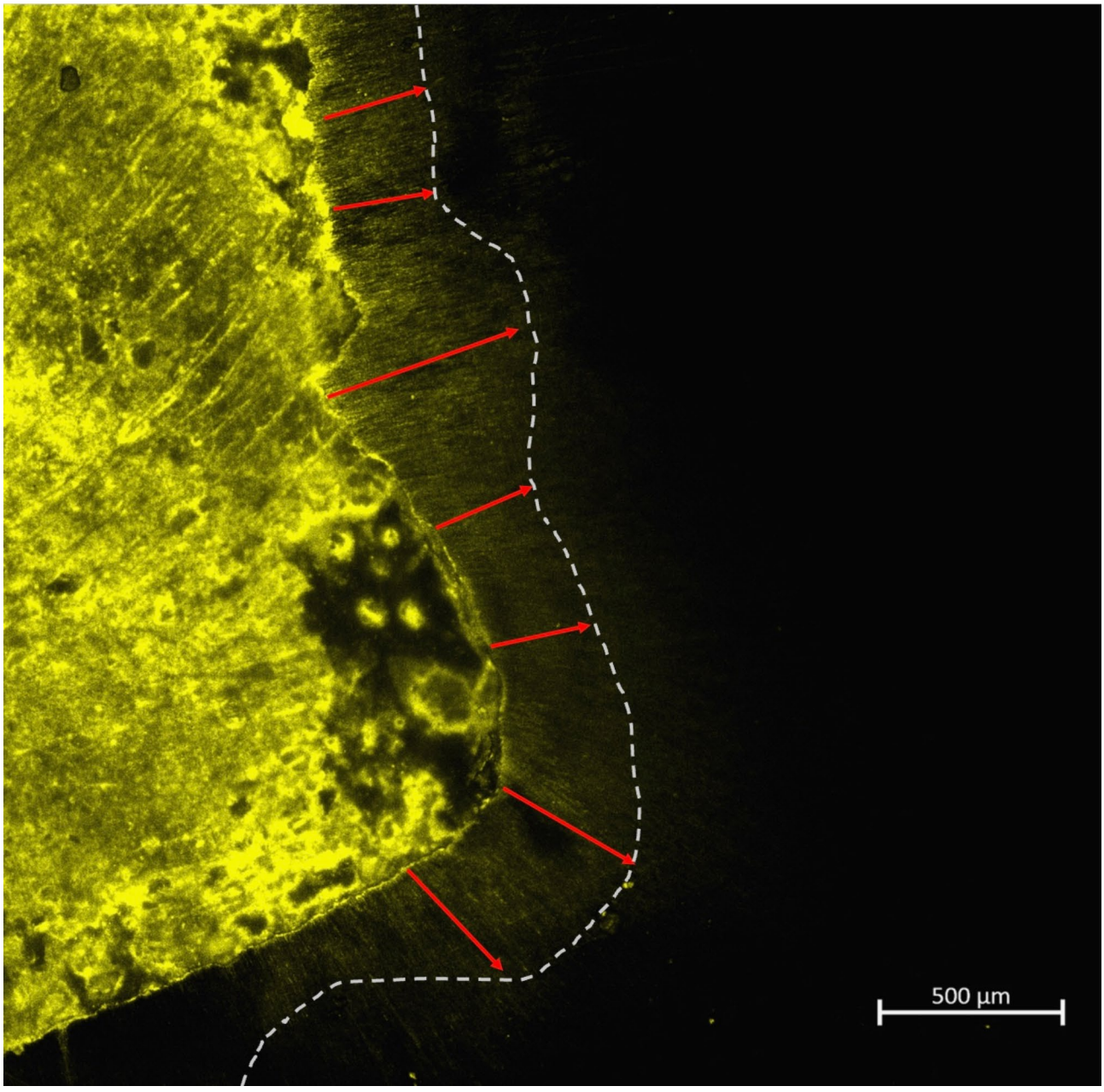

Fig. 3 The representative image shows the measurement of penetration depth. (Scale bar = 500 µm) Red arrows indicated penetration depth from the material margin to the tooth perimeter

Statistical analysis

All statistical analyses were performed using SPSS version 29.0.1. The normality of data distribution was assessed using the Shapiro-Wilk test, while homogeneity was evaluated using Levene's test. The penetration depth of the materials was analyzed using one-way ANOVA, followed by the Tukey and Bonferroni test for post-hoc comparisons between groups at a significance level of $p \leq 0.05$.

Results

There was no significant difference in penetration depth between the Biodentine and NeoMTA 2 materials. NeoPUTTY showed higher penetration depth than Biodentine and NeoMTA 2. Table 1 shows the average, minimum, and maximum values of penetration depth in micrometers. Representative CLSM images of each material group are presented in Fig. 4. All materials penetrated the anisotropic perimeter to varying depths according to CLSM analysis.

Table 1 The table shows the mean penetration depth of materials (μm). Different superscript letters indicated statistical difference at $p \leq 0.05$

Materials	Penetration depth (Mean± SD.)	Min.	Max.	*p*
Biodentine	348.59[a] ±59.72	236.27	476.44	0.002
NeoMTA Plus	342.55[a] ±134.08	259.98	479.02	
NeoPUTTY	462.21[b] ±60.55	233.42	681.97	

Discussion

The primary goal of pulpotomy is to preserve primary teeth with vital pulp until physiological exfoliation [17]. Treatment success depends on preventing microleakage resulting from the coronal restoration [18]. Penetration of pulpotomy materials into dentinal tubules is crucial. Calcium silicate-based materials promote apatite accumulation in tissue fluid, enhancing sealing ability and filling marginal pores between dentin and the material [19]. This interaction locks residual microorganisms inside the tubules, providing enhanced antimicrobial effects and sealing [20]. This study analyzed the dentinal penetration of Biodentine, NeoMTA 2, and NeoPUTTY using CLSM.

The CLSM was chosen for its ability to evaluate the depth of material penetration and the quality of sealer tags [21]. Unlike SEM or stereomicroscopy, CLSM enables three-dimensional evaluation of dye penetration beneath the specimen surface without requiring surface preparation that may cause artifacts [22]. However, a major limitation of CLSM is that it cannot directly visualize non-fluorescent materials. Therefore, the use of fluorescent dyes, such as Rhodamine-B, is necessary to visualize the penetration of materials [18]. Samples were prepared based on previous studies, and 0.1% rhodamine-B was used as a fluorescent agent to visualize penetration [14–16]. It has been reported that the presence of small amounts of Rhodamine-B does not affect the physical properties of the materials [14, 16, 23]. Furthermore, to ensure the consistency and reliability of our results, all groups were processed using the same standardized technique, and a relatively large sample size was employed to minimize variability.

Although there is ongoing debate regarding the nature of tubular infiltration, many studies have used the term 'penetration' to describe the presence of mineral tags and intratubular crystalline structures formed by calcium silicate cement [24]. This process is considered part of a broader interfacial biomineralization phenomenon. Despite their porous microstructure, calcium silicate-based cements have demonstrated good adaptation and tubular interaction in previous SEM and CLSM studies [25, 26]. In our study, although full circumferential spread was not observed, CLSM images confirmed material penetration into the dentinal tubules of primary teeth, supporting the potential for intratubular interaction. Based on previous studies, the penetration depth was measured to determine dentinal tubule penetration [14–16, 27].

We found that NeoPUTTY showed greater penetration depth than Biodentine and NeoMTA 2, with no significant difference between the latter two. Thus, the null hypothesis was rejected.

The diameter of dentinal tubules and particle size of materials affect penetration ability. Previous studies have reported various penetration depths for calcium silicate materials [25]. A previous study investigated the penetration of ProRoot MTA as a retrograde filling material by SEM. The results indicated that no tubular penetration was observed at any level [28]. However, subsequent research has demonstrated the penetration of calcium silicate-based materials. Another study reported that Biodentine showed higher bond strength values than MTA. This was attributed to the smaller particle size and uniform Biodentine content, which allowed for better penetration of the cement into the dentinal tubule [29]. On the other hand, Akbulut et al. revealed a higher penetration of the BIOfactor MTA into root canal dentin, with no discernible difference between the MTA Angelus and

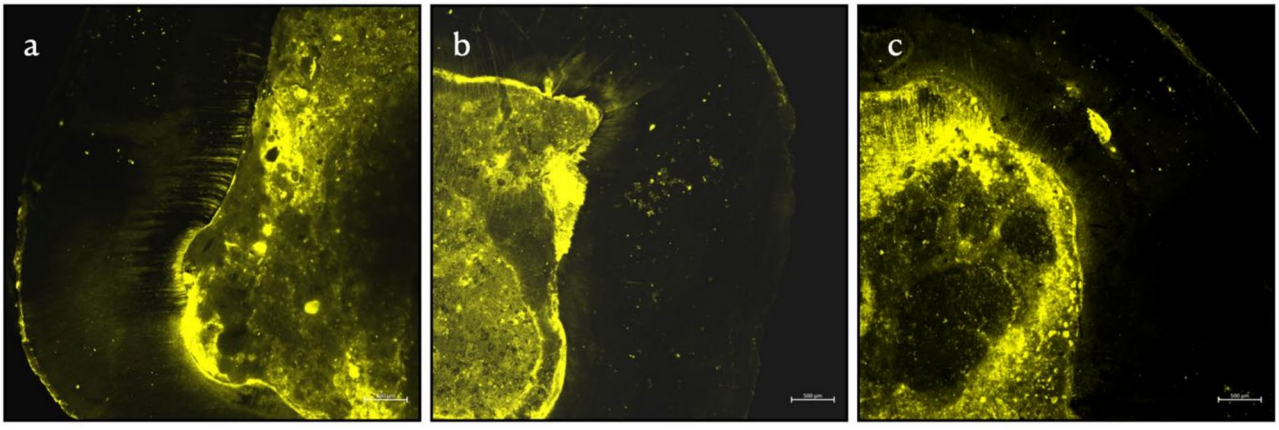

Fig. 4 The CLSM images represent quarter-section of samples (Scale bar = 500 μm). **a**; Biodentine, **b**; NeoMTA 2, **c**; NeoPUTTY

Biodentine [15]. Another study found that NeoMTA Plus and Biodentine could penetrate the root dentinal tubules of permanent teeth to a similar extent, regardless of the particle size of the materials [14].

In addition, primary teeth have been shown to present higher dentinal tubule density compared to permanent teeth, and the tubule diameter increases closer to the pulp chamber. In deep dentin, near the pulpal floor, tubule diameters can reach up to 4.28 µm [30]. This anatomical feature may allow enhanced penetration of calcium silicate-based materials in pulpotomy applications, particularly in the context of primary teeth.

Although data on NeoPUTTY penetration are limited, this material is known for inducing mineralization activity [31]. Its pre-mixed formulation reduces preparation errors, making it user-friendly and improving the success rate [11, 32]. NeoMTA 2 is manually mixed by combining the powder and liquid on a glass plate with a metal spatula, while Biodentine is mixed by agitating a capsule containing the powder and liquid. These methods require technical precision to achieve a homogeneous mixture [33].

Setting time, which determines the transition from a liquid to a hardened state, is crucial for maintaining seal integrity, preventing microleakage, and allowing the material to withstand mechanical stress. An excessively prolonged setting time can lead to clinical challenges, as the material may fail to retain its shape and endure stress. It can also prevent the placement of the restoration within the same clinical session, potentially compromising the sealing ability of the material [34, 35].

Although the biological properties of calcium silicate-based materials—such as their regenerative capacity and biocompatibility with pulp tissue—have been extensively documented, there is limited information regarding their interaction with dentin, particularly in primary teeth [36]. This study was the first to evaluate the penetration of NeoPUTTY, Biodentine, and NeoMTA 2 into primary teeth. NeoPUTTY showed the best penetration and is also easy to use, making it advantageous for pulpotomy, particularly in pediatric dentistry.

The pulpotomy materials have clinical relevance, as the structural and histological differences between primary and permanent teeth may influence the behavior of the materials at the dentin interface [37]. Determining whether calcium silicate-based materials form a distinct interfacial layer or penetrate the dentinal tubules may provide valuable insight into their sealing ability, potential for preventing bacterial microleakage, and long-term clinical outcomes in pediatric endodontic treatments [38].

The limitation of this study is that an in vitro setting may not fully replicate clinical conditions, where factors such as the movement of tubular fluids could influence the penetration of materials. Nevertheless, both Rhodamine-B and calcium silicate-based materials exhibit hydrophilic properties. This raises the possibility that Rhodamine-B may dissociate from the material matrix and passively diffuse into the dentinal tubules, potentially leading to an overestimation of material penetration when assessed by CLSM. Therefore, while CLSM is a powerful tool for assessing the spatial distribution of fluorescent markers, its findings must be interpreted with caution, especially when evaluating hydrophilic materials labeled with hydrophilic dyes [22, 39]. Although we exclusively used extracted lower, primary, second molar teeth, variation in dentinal tubule density may still exist and thus be considered a limitation. Further research is needed to better understand the penetration of calcium silicate-containing materials into primary teeth. Future studies could investigate the penetration of materials into primary dentinal tubules and their interfacial properties using various techniques.

Conclusion

Calcium silicate-based materials are indispensable with penetration ability in pulpotomy treatment. This study reported that NeoPUTTY showed higher dentinal tubule penetration in primary teeth than NeoMTA 2 and Biodentine as pulpotomy materials.

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Author contributions
M.O.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing review & editing. A.O.: Project administration, Formal analysis, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Data availability
Data availability is upon request to the corresponding author.

Declarations

Ethics approval and consent to participate
The experimental procedures were approved by the Scientific Research Ethics Committee of the University of Lokman Hekim (No:2024/188). Also, the study protocol was conducted in accordance with the ethical principles for medical research (involving human participants, including research using identifiable human material or data) of World Medical Association (WMA) Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Consent to participate
Written informed consent was obtained from all individual participants included in the study.

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