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An Invitro Confocal Study to Compare and Evaluate the Effect of Cryotreated Sodium Hypochlorite on Penetration Depth of Different Root Canal Sealers into the Dentinal Tubules.

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KEYWORDS

Bio C Sealer; BioRoot RCS; Cryotreated NaOCl; MTA Fillapex; Zinc Oxide Eugenol.

ABSTRACT:

Aim and objectives: To evaluate the effect of cryotreated NaOCl on the dentin penetrability of BioRoot RCS, MTA Fillapex, Bio C Sealer, and Zinc Oxide Eugenol sealer.

Method: Forty extracted human mandibular premolars were prepared using a protaper gold rotary system and irrigated with cryotreated 5.25% NaOCl (2.5°C), 17% EDTA, and normal saline. The teeth were divided into four groups based on the sealer used: BioRoot RCS, MTA Fillapex, Bio C Sealer, and ZnO Eugenol and the obturation was performed using the above. Sealers mixed with rhodamine dye, and penetration depth was analyzed using confocal laser scanning microscopy (CLSM). Statistical analysis was conducted using ANOVA and Tukey's post hoc test.

Results: BioRoot RCS exhibited the deepest penetration (310.00 ± 4.94 µm), followed by Bio C Sealer (265.10 ± 7.93 µm), MTA Fillapex (226.50 ± 8.50 µm), and ZnO Eugenol (192.70 ± 6.03 µm). Statistical analysis revealed significant differences among groups ($p < 0.0001$), with BioRoot RCS showing superior penetration compared to other sealers.

Conclusion: Cryotreated NaOCl enhances sealer penetration, with bioceramic sealers like BioRoot RCS showing the highest depth. These findings highlight the potential of cryotreated NaOCl in improving endodontic sealing and reducing bacterial infiltration.the need for routine screening and preventive supplementation strategies in antenatal care.

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Introduction

Successful endodontic treatment relies on elimination of pulp tissues, effective root canal disinfection,and subsequent obturation to achieve fluid tight seal. Complete canal cleaning, shaping, and adequate hermetic seal obtained during obturation are crucial to prevent the reinfection.[1] Root canal sealers and obturating materials play a vital role in sealing minute gaps,lateral canals,deltas,fins thereby helps to prevent the percolation of microbes and their byproducts into periapical area.Effective sealers must strongly adhere to root dentin, exhibit biocompatibility and possess antibacterial properties. Sealer penetration into dentinal tubules enhances seal longevity and minimizes the remaining bacterial infiltration.[2]

Effectiveness of sealer is influenced by its physical and chemical properties,interaction of sealer with the dentin as well as canal preparation technique.[3] Sodium hypochlorite (NaOCl) is commonly used for irrigation due to its antibacterial and tissue-dissolving properties, but its oxidative nature will degrade the collagen matrix of dentin and reduces the sealer adhesion. Recent studies have explored cryotreatment—cooling NaOCl—to enhance antibacterial efficacy while minimizing adverse effects on dentin integrity. Cryotreatment may improve NaOCl's efficiency, reduce oxidative dentin degradation, and enhance sealer penetration. It may also modify dentin's microstructure or sealer viscosity, facilitating deeper penetration.[4,5]

Confocal laser scanning microscopy (CLSM) is a powerful tool for assessing sealer penetration depth into dentinal tubules. Unlike traditional microscopy, CLSM provides high-resolution, three-dimensional imaging of the sealer-dentin interface.[6] This study aims to compare sealer penetration depth following cryotreated NaOCl irrigation using CLSM, contributing to optimized irrigation protocols and improved endodontic outcomes.

Method

Experimental design:

Forty single rooted human mandibular premolar extracted due to periodontal reasons are taken.The teeth which are free of caries and with single and straight canals were included and the teeth with craze lines and any developmental anomalies are excluded from the study.The samples were then divided into 4 groups based on the root canal endodontic sealers used.

Sample Preparation

The extracted teeth were cleaned and sterilized in an autoclave at 121 degree Celsius, 15 psi pressure for 15 minutes and then stored in distilled water till further use.

Each tooth were decoronated using diamond disks to standardize the tooth length 14mm.(Fig 1)

Working length was determined using 15 K file and confirmed radiographically. Root canal were prepared using protaper gold rotary file till F2(25/0.8).(Fig 2)During cleaning and shaping, all the teeth were then irrigated using cryotreated 5.25%NaOCl (2.5°C)(Fig 3), 17% EDTA and normal saline as an intermediate irrigant.

Samples were then divided into 4 groups of 10 samples each on the basis of different sealers used, which are mixed with rhodamine dye were applied to root canals.(Fig 4)

- GROUP A- BioRoot RCS sealer was applied
- GROUP B - MTA Fillapex sealer was applied
- GROUP C - Bio C sealer was applied
- GROUP D - Zinc oxide eugenol sealer was applied

All the samples were then obturated using warm vertical compaction technique .Samples were then stored in 100% relative humidity at 37 degree celsius for 24 hours to ensure complete set of the sealer.All the samples were then sectioned in bucco-lingual direction and is viewed under confocal laser scanning microscope at 10X magnification.(Fig 5)

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Fig1.Sectioning to standardise the root length

Fig2.Biomechanical preparation

Fig3.Irrigation with preheated SodiumHypochlorite(2.5degree)

Fig4.Sealers mixed with Rhodamine dye

Fig5.Sectioned Sample

Statistical Analysis

The collected data underwent statistical analysis, with analysis of variance (ANOVA) employed to evaluate the sealing ability of different sealer types. A significance level of 0.05 was established for statistical comparisons. Tukey's post hoc test was utilized to assess pairwise differences among groups categorized based on the type of sealer used.

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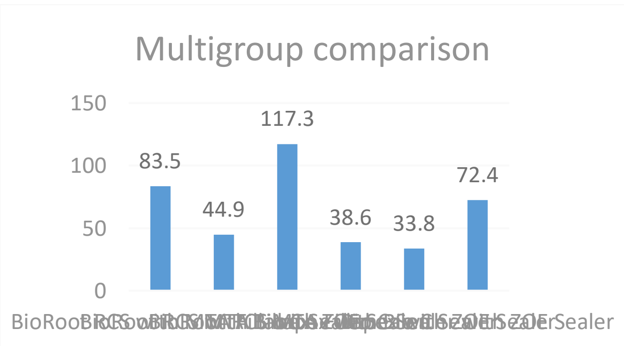
Results

This study evaluated the dentin penetrability of four sealers after cryotreated NaOCl irrigation. BioRoot RCS exhibited the greatest penetration (310.00 ± 4.94 µm), followed by Bio-C Sealer (265.10 ± 7.93 µm), MTA Fillapex (226.50 ± 8.50 µm), and ZOE Sealer (192.70 ± 6.03 µm). The mean penetration depth across all groups was 248.58 ± 44.81 µm. One-way ANOVA showed significant differences among the groups (F = 520.353, P < 0.0001).

	N	Mean ± SD	Std. Error	95% Confidence Interval for Mean		F Score	P Value
				Lower Bound	Upper Bound		
Bio root rcs	10	310.00 ±4.94	1.563	306.46	313.54	520.353	<0.0001*
Mta fillapex	10	226.50±8.50	2.688	220.42	232.58		
Bio c sealer	10	265.10±7.93	2.510	259.42	270.78		
Zoe sealer	10	192.70±6.03	1.909	188.38	197.02		
Total	40	248.58±44.81	7.086	234.24	262.91		

Tukey's post hoc test confirmed significant penetration differences between all groups (P < 0.0001). BioRoot RCS showed significantly higher penetration than MTA Fillapex, Bio-C Sealer, and ZOE Sealer. Bio-C Sealer exhibited superior penetration over MTA Fillapex, which in turn performed better than ZOE Sealer. The 95% confidence intervals did not include zero, reinforcing statistical significance at the 0.05 level.



Graphical representation of the inter-group comparison

The above graph presents the pairwise comparisons of mean differences between the tested groups using post hoc analysis.The mean penetration depth of BioRoot RCS are significantly higher when compared to all other groups. Specifically, the mean difference between BioRoot RCS and MTA Fillapex was 83.500 ± 3.132, with a P-value of <0.0001, indicating statistically significant. Similarly, BioRoot RCS showed a significant difference of 44.900 ± 3.132 (P < 0.0001) when compared to Bio-C Sealer and a difference of 117.300 ± 3.132 (P < 0.0001) when compared to ZOE Sealer. MTA Fillapex exhibited lower p-value which was statistically significant compared to Bio-C Sealer, with a mean difference of -38.600 ± 3.132 (P < 0.0001), confirming that Bio-C Sealer had higher mean values.However MTA Fillapex showed statistically significant when compared to ZOE Sealer, with a mean difference of

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33.800 ± 3.132 (P < 0.0001). All comparisons had a 95% confidence interval that did not include zero, confirming the statistical significance of the differences at the 0.05 level. These findings indicate significant variations among the tested groups, with BioRoot RCS exhibiting the highest values and ZOE Sealer the lowest.

Discussion

Endodontic treatment aims to irradicate microbes through biomechanical preparation with effective irrigation[7]. Anatomical complexities and biofilm adherence complicates successful disinfection.[8] Since 1936,NaOCl is widely used as an endodontic irrigating solution,which possesses strong antibacterial and tissue-dissolving properties[9]. The sodium hypochlorite with a concentration of 5.25%causes oxidative effects which can degrade collagen and weaken the dentin.[10] Heating NaOCl enhances efficacy but risks active chlorine loss and thermal dentin damage.[11,12] Cryotreatment (2.5°C) retains antimicrobial potency while minimizing structural degradation and reducing postoperative pain.[13,14,15]

Sealer penetration is essential for successful root canal therapy, as it ensures a fluid-tight seal and prevents bacterial ingress.[16] Cryotreated NaOCl enhances dentin permeability, facilitating deeper sealer penetration by effectively removing the smear layer. CLSM provides high-resolution imaging for evaluating the sealer penetration depth.

The present investigation demonstrates that the chemical nature of a sealer exerts a decisive influence on its ability to penetrate dentinal tubules. BioRoot RCS achieved a mean penetration depth of (310 ± 4.94 µm), outperforming Bio-C Sealer (265.10 ± 7.93 µm), MTA Fillapex (226.50 ± 8.50 µm), and zinc-oxide–eugenol (ZnOE) (192.70 ± 6.03 µm). This graded performance mirrors earlier reports in which calcium-silicate sealers surpassed resin- and eugenol-based counterparts in intratubular diffusion. The superiority of BioRoot RCS can be attributed to a combination of intrinsic hydrophilicity, reduced film thickness, and ultrafine particle size, each of which promotes intimate wetting of moist dentine and capillary-driven ingress.[17] Moreover, its sustained calcium-ion release fosters the precipitation of a calcium-phosphate layer, creating a mineral infiltration zone that strengthens mechanical interlocking and chemical bonding with the dentinal wall.[18] Collectively, these features confirm that BioRoot RCS establishes a biologically active and mechanically robust interface, thereby setting a contemporary benchmark for obturation quality.

Conversely, ZnOE exhibited the shallowest penetration—a finding that underscores the limitations of this long-standing material in achieving a fluid-tight seal. The Fluid within the dentinal tubules repel eugenol due to its hydrophobic nature which limits the adhesion of zinc oxide–eugenol (ZOE)–based sealer to the dentinal walls. This limitation compromises their interfacial adaptation to dentin, increasing the risk of microleakage and potentially reducing long-term sealing efficacy..[19,20] In addition, volumetric shrinkage of 0.3–1 % during setting along with relatively large particle size of zinc oxide, generates voids and obstructs entry into narrow tubules, compromising interfacial integrity when strict dryness is not attainable.[21] These deficiencies invite reconsideration of ZnOE for clinical situations where deep tubular penetration and long-term sealability are critical.

The intermediate performance of MTA Fillapex and Bio-C Sealer illuminates the complex relation between rheology and bioactivity. MTA Fillapex benefits from a fine particle distribution and slight hygroscopic expansion that minimise interfacial gaps[22].However, its lower calcium release yields only a thin mineral-infiltration zone, thereby limiting its depth of diffusion.[23] Bio-C Sealer, on the other hand, releases more calcium and displays higher solubility than MTA Fillapex, enabling the formation of a longer and thicker apatite layer.[24]Nevertheless, its marginally greater viscosity and film thickness constrain full exploitation of expanded tubules, positioning its penetration between BioRoot RCS and MTA Fillapex. These observations confirm that incremental improvements in flow and ion-exchange properties translate into clinically meaningful gains in adaptation and seal durability.

A notable adjunct in this study was the utilisation of cryotreated sodium hypochlorite (NaOCl) at 2.5 °C during irrigation. Thermal modulation of NaOCl is well recognised for altering its physicochemical behaviour; while heating accelerates tissue dissolution, it concomitantly destabilises the solution, elevates chlorine loss, and introduces thermal stresses in radicular dentine.

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By contrast, cooling NaOCl preserves available chlorine, enhances smear-layer removal, and sustains antimicrobial potency, all while maintaining its analgesic benefits. The present findings align with those of De-Deus et al. demonstrating that cryotreated NaOCl significantly increases dentinal-tubule permeability, thereby facilitating deeper sealer ingress across all material classes.[25]Enhanced organic matrix dissolution at lower temperature augments tubule patency, while reduced vasodilatory signalling mitigates postoperative pain and inflammation—an outcome consistent with the vasoconstrictive reduction in leukocyte extravasation and endothelial dysfunction reported in cryotherapy literature.[26,27]

Taken together, these data underscore two clinically important inferences.First, hydrophilic, calcium-silicate sealers—particularly BioRoot RCS—should be preferred when maximal tubular penetration and a biologically favourable interface are desired. Second, integrating cryotherapy into the irrigation sequence constitutes a simple yet powerful strategy to potentiate the performance of any sealer by enlarging dentinal-tubule access and minimising postoperative sequelae. The synergy between a cooled NaOCl protocol and a bioactive sealer offers an evidence-based pathway to achieve a hermetic, long-lasting seal and, consequently, to enhance the predictability of endodontic outcomes.

Conclusion

Cryotreated NaOCl significantly enhances sealer penetration into dentinal tubules, improving root canal treatment outcomes. BioRoot RCS exhibited the greatest penetration, followed by Bio-C Sealer, MTA Fillapex, and ZOE Sealer. Cryotreatment enhances sealer performance by improving dentin permeability and optimizing smear layer removal.

Previous studies highlight cryotreated NaOCl's benefits, including superior antibacterial efficacy and reduced cytotoxicity. Further research is needed to assess its long-term effects on dentin properties. Understanding these interactions is crucial for integrating cryotreated NaOCl into endodontic practice, optimizing irrigation protocols, and improving patient outcomes.

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