Original Article

Advent of novel vehicle in regenerative endodontics and its effect on dentinal penetration of intracanal medicaments

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Abstract

Context: Regenerative endodontic procedures allow continued root development, but open apices limit mechanical debridement which implores the need for an effective and potent intracanal medicament to disinfect the canal space.

Aim: This study aimed to assess the effect of nanobubble (NB) water on dentinal penetration of intracanal medicaments in simulated immature teeth.

Materials and Methods: A total of 60 freshly extracted mandibular premolars were prepared to simulate blunderbuss canals. Canals were irrigated with 5.25% NaOCl and 17% ethylenediaminetetraacetic acid by a final rinse of saline. Teeth were divided into 3 groups with different medicaments which were further subdivided into two subgroups with saline and NB water as vehicle, respectively. The longitudinal sections of teeth were evaluated using stereomicroscopy.

Statistical Analysis Used: Two-way ANOVA and Tukey's post hoc test were used.

Results: The results indicated that samples with NB water as vehicle showed better penetration than their counterparts that were carried with saline. The highest penetration was seen in the middle third of the root.

Conclusions: It could be concluded that NB water enhanced the dentinal penetration of all antimicrobial agents and calcium hypochlorite with NB water showed maximum penetration depth.

Keywords: Dentin permeability; intracanal medicament; regenerative endodontics; vehicle

INTRODUCTION

Trauma or caries in immature teeth necessitates endodontic therapy, but open apices and thin dentinal walls pose a challenge and mandate a disparate treatment protocol, from routine root canal treatment which relies primarily on chemomechanical preparation for disinfection.[1] Regenerative endodontic procedures (REPs) have been advocated for these cases since it can allow thickening of the root canal walls and continued root development.[2] However, in regenerative endodontic therapy, open root apex provides no barrier for control of instrumentation and disinfection.[3] Hence, to achieve adequate disinfection of the root canal space without biomechanical preparation implores the need for effective irrigation and potent intracanal medicament to reach the doorsill of disinfection necessary for adequate healing.

A number of topical or systemic antibiotics have been employed to augment mechanical debridement. Systemic antibiotic therapies, however, have several significant drawbacks, such as selectivity of antimicrobial action, possible development of resistant bacteria, and risk

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for adverse host reactions. Hence, topical use of a broad-spectrum antiseptic agent with minimal or no potential for adverse reactions, which can be locally delivered to the site of infection, is preferable.[4]

Efficacy of the medicament lies remotely in their antimicrobial action which can be enhanced by improved dentinal penetration. The ability of the vehicle to enhance diffusion of the medicament through root dentin and the anatomical aberrations of root canal anatomy must be taken into consideration while choosing an appropriate vehicle. Nanobubbles (NBs) have recently been used in medicine for drug delivery by increasing the drug's penetration capacity without inducing systemic toxicity.[5] This study assessed the effect of NB water on dentinal penetration of intracanal medicaments in simulated immature teeth.

MATERIALS AND METHODS

The present study was approved by the institutional research and developmental committee and was carried out in two stages: (1) sample collection and preparation and (2) visualization under stereomicroscope.

A total of 60 freshly extracted mandibular premolars of similar shape and size were collected from the Department of Oral and Maxillofacial Surgery at the institute. Extracted teeth were then debrided of calculus, stains, and any remnants of periodontal tissue with ultrasonic scaler (UDS-P Ultrasonic Scaler, Woodpecker, China). The teeth collected were securely stored in a container with physiological saline (Saline Parenteral Pvt. Ltd., Aligarh, India). The coronal portion of the crowns were cut perpendicular to the long axis of the tooth, 14 mm from the apex with low-speed diamond disc (Isomet 2000 Precision saw, Buehler, USA) under copious water spray. Subsequently, 400 grit aluminum oxide abrasive paper was used to obtain flat surface, and the root canal length was standardized for all samples at 14 mm. After the removal of pulp tissue, the working length was determined by using a 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal, up to the apical foramen. The root canals were cleaned and shaped using the ProTaper gold rotary files (Dentsply Sirona Canada) in a sequential manner from S1 till F3, followed by preparation with peeso reamer (Dentsply Maillefer, Ballaigues, Switzerland), starting with peeso reamer #1 and finishing with peeso reamer #2. Canals were irrigated between files with 2 mL of 5.25% NaOCl (Septodent Healthcare India, Navi Mumbai, India) using a side venting needle (Vishal Dentocare, Ahmedabad, India), the needle was placed within the canal at the depth, 1 mm less than the working length. To eliminate the smear layer, 5 mL of 17% ethylenediaminetetraacetic acid (Smear Clear™, Kerr, California) for 3 min was used, followed by a final rinse of 2 mL distilled water. The canals were washed with saline solution and then each dried with absorbent paper points.

Stereomicroscopy

To examine the ability of NB water in enhancing the delivery of medicaments into dentinal tubules, the root canal was filled with intracanal medicaments using lentulospiral and condensed with finger pluggers, dissolved in saline or NB water (Nano Bubbles Tech Pvt. Ltd., Chandigarh, India) according to the group allocation as follows:

Group A: ($n = 20$) Intracanal used is calcium hydroxide ($Ca(OH)_2$). It is further subdivided into

- A1: ($n = 10$) $Ca(OH)_2$ with saline as vehicle (in the ratio of 1:1)
- A2: ($n = 10$) $Ca(OH)_2$ with NB water as vehicle (in the ratio of 1:1).

Group B: ($n = 20$) triple antibiotic paste (TAP) used as intracanal medicament. It is further subdivided into

- B1: ($n = 10$) TAP with saline as vehicle (in the ratio of 1:1)
- B2: ($n = 10$) TAP with NB water as vehicle (in the ratio of 1:1).

Group C: ($n = 20$) Intracanal used is calcium hypochlorite ($Ca(OCl)_2$). It is further subdivided into

- C1: ($n = 10$) $Ca(OCl)_2$ with saline as vehicle (in the ratio of 1:1)
- C2: ($n = 10$) $Ca(OCl)_2$ with NB water as vehicle (in the ratio of 1:1).

Intracanal medicaments were mixed with methylene blue before packing into canals. The specimens were then stored for 7 days at 37°C, in 100% humidity, to simulate physiological conditions. Canals were then washed with saline, roots were longitudinally sliced into 300 μm thickness by Zege microtome (SP1600, Leica), and the maximum dentinal penetration of the medicaments was observed using stereomicroscope (MZFI III, Leica), with specimens placed over grid paper/coordinate paper to measure the penetration depth.

The results were tabulated and statistically analyzed using two-way ANOVA considering the dentinal penetration of medicaments as dependent variable and type of vehicle as independent variable, and Tukey's *post hoc* test was used for multiple comparisons to determine whether any significant differences are present. $P < 0.05$ was considered to be statistically significant. Analysis was performed on Statistical Package for the Social Sciences 23 (IBM Corporation, Chicago).

RESULTS

Stereomicroscopic analysis was done to analyze the dentinal penetration of the intracanal medicaments.

Group A2, B2, and C2 showed enhanced penetration of the medicaments in comparison with A1, B1, and C1, respectively [Figures 1 and 2] [Table 1].

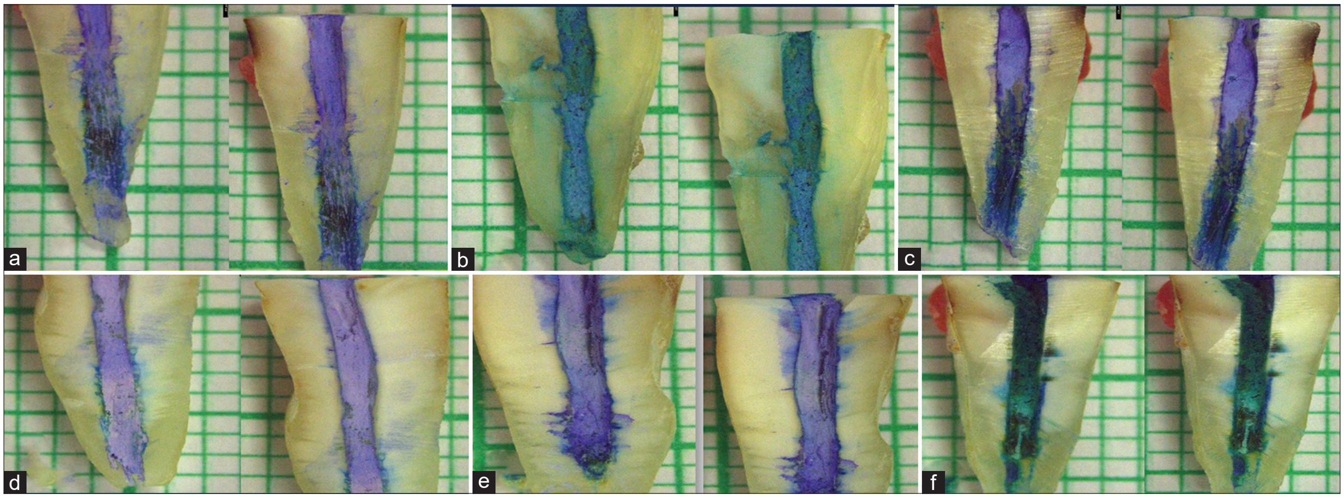

Figure 1: Representative images of groups under stereomicroscope showing dentinal penetration of intracanal medicament (a) Group A1: Calcium hydroxide ($Ca(OH)_2$) + Saline (b) Group A2: $Ca(OH)_2$ + nanobubble water (NBW) (c) Group B1: Triple antibiotic paste (TAP) + Saline (d) Group B2: TAP + NBW (e) Group C1: Calcium hypochlorite ($Ca(OCl)_2$) + Saline (f): Group C2: $Ca(OCl)_2$ + NBW

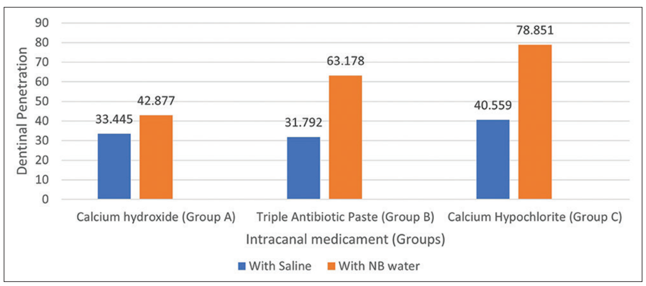

Figure 2: Mean values of dentinal penetration of the intracanal medicaments with saline and nanobubble water

Table 1: Intergroup comparison of the dentinal penetration of the intracanal medicaments with saline and nanobubble water

Medicament	Groups	*n*	Mean	SD	SEM	*P*
$Ca(OH)_2$	A1	10	33.4450	2.75275	0.87050	0.000*
	A2	10	42.8770	2.93360	0.92769	
TAP	B1	10	31.7920	3.03034	0.95828	0.000*
	B2	10	63.1780	4.08515	1.29184	
$Ca(OCl)_2$	C1	10	40.5590	2.93489	0.92809	0.000*
	C2	10	78.8510	3.45705	1.09321	

*$P<0.05$ is statistically significant, $P<0.01$ is statistically highly significant. SD: Standard deviation, SEM: Standard error of mean, TAP: Triple antibiotic paste, $Ca(OH)_2$: Calcium hydroxide, $Ca(OCl)_2$: Calcium hypochlorite

DISCUSSION

Dental trauma can lead to pulp necrosis, which represents a clinical challenge in immature permanent teeth. Because of the incompletion of immature roots, a subsequent fracture and loss of tooth can occur. In this clinical scenario, the purpose of the endodontic treatment is not only to prevent and heal the apical periodontitis but also to promote apical closure with root development and constitute the functional competence of the pulp tissue. To achieve this goal, the biologically recommended treatment approach is regeneration that allows the thickening of the dentinal walls and lengthening of immature permanent roots.[6,7] Most recent studies have recommended the use of intracanal medicaments for forming an environment suitable for revascularization.[8-10]

An intracanal medicament should penetrate deeply and densely through the dentinal tubules for its antimicrobial activity and blockage effect to prevent reinfection.[11] The present study investigated the penetration of three advisably less cytotoxic intracanal medicaments[12] advocated for use during REPs, $Ca(OH)_2$, TAP, and $Ca(OCl)_2$, using a novel vehicle, NB water to assess its effect on the dentinal penetration of the agents.

The present study is few among the first to test NB water technologies for disinfection in endodontics, especially pertaining to the enhanced penetration of intracanal medicaments used in regenerative endodontic treatment. The current irrigation protocols used in endodontic disinfection are limited in penetration and disinfection of the dentinal tubules.[13,14]

$Ca(OH)_2$ and TAP are commonly used intracanal medicaments in REPs. $Ca(OH)_2$ is highly basic in nature with a pH of 12.5–12.8 exhibiting favorable antibacterial properties. Although TAP has superior antimicrobial properties compared with that of $Ca(OH)_2$ when used at high concentration, higher cytotoxicity, antibiotic resistance, and discoloration have been reported with TAP when used at concentrations ≥1 mg/mL. $Ca(OCl)_2$ has been previously evaluated as an endodontic irrigant with a better antimicrobial properties and tissue dissolution capacity and also exhibits chemical stability.[15] Alfadda *et al*.[15] reported that the use of $Ca(OCl)_2$ was effective against *Enterococcus faecalis* as an irrigation solution, similar to sodium hypochlorite. $Ca(OCl)_2$ can be prepared as an intracanal medicament because of its granular structure.

The potency of NB water was observed and found to enhance dentinal penetration of intracanal medicaments into dentinal tubules, indicating that NB technologies may offer synergism and be an advantageous addition to the field of endodontics, especially in the area of disinfection. This study showed that medicaments penetrated deeper with NB water than with saline [Figure 2].

The hypotheses of the mechanism of action of NB are three-fold: (1) NB coalesce on the surface of solid materials into microbubbles, which then acts as a wedge, physically dislodging the body it is adhered to. It is also known as the "jack up phenomenon."[16] (2) NB contains pressurized air, and when it ruptures, it creates pressure waves to remove fine particles on the surface of solid materials.[17] Both factors might implicate its role in the removal of the smear layer, allowing better tubular penetration of the intracanal medicaments. (3) NB can decrease the surface tension of the liquid and increase the wettability, thereby improving the dentinal penetration of medicaments.[18]

The three-fold proposed mechanism of action is suggested to better clear the debris plugs and drive the medicaments deeper into the tubules. NB water with intracanal medicaments was also able to travel deep into the dentinal tubules as compared to medicaments dissolved in saline. The use of a stereozoom microscope was deemed suitable to visually confirm the depth of dentinal penetration.

The results also showed that the penetration of $Ca(OCl)_2$ was maximum, closely followed by TAP and with a bigger margin, the $Ca(OH)_2$, regardless of the vehicle used, although the latter two are currently recommended medicaments for regenerative treatments.[15] This corresponds to the studies conducted by Pereira *et al*.[19] and Adl *et al*.[20] which examined the antibacterial effect up to 200 mm in discrete studies and showed a more effective antibacterial effect of TAP compared with $Ca(OH)_2$, which was associated with the use of propylene glycol, which improved the dentinal penetration due to its low surface tension.

CONCLUSIONS

The exploration of NB water in the realm of regenerative endodontics holds significant promise, offering a novel approach to enhance the effectiveness of intracanal medicaments and irrigants. While $Ca(OCl)_2$ showed the maximum penetration depth when compared with TAP and $Ca(OH)_2$, all the intracanal medicaments showed better penetration with NB water than with saline.

By leveraging the unique properties of NBs, such as enhanced penetration, this innovative approach has the potential to elevate the standard of care in endodontic therapy. However, further research is warranted to validate the hypotheses of the mechanism of action. *In vivo* studies and clinical trials are necessary to corroborate the findings of *in vitro* assessments and also to ascertain the clinical relevance of NB water in the context of regenerative endodontics.

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Conflicts of interest

There are no conflicts of interest.

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