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Dentistry Section

Effect of Calcium Hydroxide (CH), Triple Antibiotic Paste (TAP), Nano-sized CH and Nano-sized TAP as Intracanal Medicament on the Push-out Bond Strength of Biodentine: An In-vitro Study

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ABSTRACT

Introduction: Root perforations are one of the common procedural errors faced in endodontic procedures. The ideal root repair material must be capable of bonding to the dentin. Calcium Hydroxide (CH) and Triple Antibiotic Paste (TAP) are mostly used for root canal disinfection.

Aim: To evaluate the effect of calcium hydroxide, triple antibiotic paste, nano-sized calcium hydroxide and nano-sized triple antibiotic paste as intracanal medicament on the push-out bond strength of biodentine.

Materials and Methods: The present in-vitro study was conducted in Department of Conservative Dentistry and Endodontics, Sibar Institute of Dental Sciences, Guntur for a period of 1 month (March 2020). A total of fifty maxillary central incisors (n=50) were decoronated, instrumented with ProTaper rotary NiTi instrument followed by peesoreamers up to number 3, and were divided into five groups (n=10). Group I: CH, Group II: Nano CH, Group III: TAP, Group IV: Nano TAP and Group V: Control group. The respective medicaments were mixed with propylene glycol and placed to the working length. After one week, medicaments were removed using Passive Ultrasonic Irrigation (PUI). Later, samples were sectioned into slices of 2 mm thickness using hard tissue microtome, and biodentine root repair material was placed into lumen of the slices. All the samples were stored for one week at 37°C, then subjected to push-out bond strength test. Data were analysed using one-way ANOVA, Tuckey, Dunnets Post-hoc test (p-value <0.05)

Results: The highest push-out bond strength values were observed in the group IV (p-value <0.001) and least in the control group. Biodentine showed significantly higher resistance to displacement among the group II (p-value=0.431) and group IV (p-value=0.074) when compared with their conventional medicaments.

Conclusion: Nano Triple Antibiotic Paste and Nano Calcium Hydroxide enhanced the push-out bond strength values of biodentine when compared to their conventional intracanal medicaments.

Keywords: Calcium silicate cement, Endodontic procedure, Nanoparticles, Passive ultrasonic irrigation

INTRODUCTION

The primary rationale behind intracanal medicament is to kill the bacteria inside the root canal and to avert reinfection. Chemomechanical preparation cannot eliminate the bacteria residing deep in the dentinal tubules (Bystrom A and Sundqvist G) [1]. If the root canal is not appropriately dressed with antiseptic medicaments between the visits, the residual bacteria may increase in the same number as it was in the starting stage of the endodontic treatment. Thus the use of effective intracanal medication for disinfection of root canal is necessitated [2].

Nanotechnology provides the advantage of reducing the size of the particles as well as increasing the efficacy of materials with their specific physicochemical characteristics, such as ultrasmall sizes, increased surface area/mass ratio, bactericidal, increased chemical reactivity and bioavailability [3,4]. Nanoparticle size is <100 nm and can reach the complex anatomy of the root canal system. The use of nanoparticulate intracanal medicaments are proven as an effective way to eliminate resistant bacterial strains [4].

Several intracanal medicaments are used to disinfect the root canal during the inter appointment period. Calcium hydroxide is widely used in clinical procedures due to its high alkalinity, better antimicrobial properties, ability to denature the bacterial lipopolysaccharide and tissue dissolving property (Siqueira Jr JF and Lopes HP) [5]. However, it is ineffective in the elimination of Enterococcus fecalis, the most important bacteria in persistent infections and candidiasis infection. It also has disadvantages like prolonged treatment, alteration in the hardness, and demineralisation of dentine. Nano Calcium Hydroxide (NCH) particles are reported for higher levels of antimicrobial activity in comparison with conventional CH and deeper penetration into the dentinal tubules [6,7].

The TAP is a mixture of ciprofloxacin, metronidazole, and minocycline. Initially, its use was recommended by Sato I et al., the proportions of the three antibiotics were recommended in a ratio of 1:1:1 by Hoshino E et al., 3:3:1 (Takushige T et al.,) and a 3 Mix MP paste using propylene glycol and macrogol as a vehicle in the ration 7:1; however, the ideal guideline to which to adhere remains a controversy [8-10]. Triple Antibiotic Paste (TAP) also has some of the demerits, like causing tooth discolouration due to the presence of minocycline [11,12]. Karczewski A et al., reported the synthesis of clindamycin modified triple antibiotic nanofibres. In this study, the preparation of nano TAP was done by particle reduction using a ball miller [13].

Endodontic treatment can be complicated and challenging. Some of the iatrogenic errors complicate the outcome of endodontic treatment. Of those mishaps, one of the most commonly encountered procedural errors is a perforation of the root, which affects the prognosis of the tooth. Ideally, root perforations should immediately be repaired with a biocompatible material to seal the communication with the periradicular tissues. An ideal perforation repair material should provide an adequate seal, be biocompatible, not affected by blood contamination, not be extruded during condensation, able to resist masticatory forces under function, bactericidal, induce bone formation and healing, radio-opaque, induce mineralisation, cementogenesis and easily manipulated for placement [14,15].

Calcium hydroxide, glass ionomer cement, Mineral Trioxide Aggregate (MTA), Hydroxyapatite, etc., are commonly used to manage perforation defects. Among these calcium silicate cements like MTA and Biodentine are emerging in contemporary practice because of their biomimetic properties and ability to withstand moist conditions. Biodentine (Septodont, Saint Maur des Fosses, France) has several advantages over MTA because of better handling properties, increased strength and hardness close to that of natural dentine, smaller particle size, increased micromechanical retention, ability to complete single appointment procedure [16-19].

Interim medicaments should be removed entirely from the root canals to maintain the sealing efficacy of root canal sealers and filling materials. The literature to date suggests that no method was able to remove the intracanal medicament placed in the root canals completely. The frequency of the ultrasonic device is in the range of 20-40 kHz. It has advanced fluid dynamics by the formation of cavitation and acoustic microstreaming that can remove the debris even from the complex anatomy of the root canal system (Goodman A et al.,) [20].

To date, many studies have been attempted to evaluate the effect of different intracanal medicaments on the bonding ability of calcium silicate-based cements [21-23]. None of them were based on the effect of nanoparticle ranged intracanal medicaments. The purpose of this study was to evaluate the effect of conventional TAP, CH, and their nano modified intracanal medicaments, i.e., nano TAP, NCH on the push-out bond strength of biodentine root repair material.

MATERIALS AND METHODS

This in-vitro study was conducted in March 2020 for a period of one month in the Department of Conservative Dentistry and Endodontics at Sibar Institute of Dental Sciences, Takkellapadu, Guntur, Andhra Pradesh, India. The study protocol was approved by the Institutional Ethical Committee (IEC) (Pr.228/IEC/SIBAR/2020).

Inclusion criteria: Maxillary central incisors that were freshly extracted for periodontal reasons also with complete root formation and single canal with <10° curvature, no visible caries, fracture, cracks, resorption, calcifications. The degree of curvature was calculated using the methodology described by Schneider SW [24]. Canal morphology was assessed by buccolingual and mesiodistal radiographs.

Exclusion criteria: Open apex tooth, teeth having more than one canal and >10° curvature, teeth that were having fracture, crack lines, resorptive defects and calcification.

Preparation of nano medicaments: Nano medicaments are prepared mechanically by particle size reduction attained through a ball miller (top-down approach) similar to Zhang W et al., [25]. The average particle size of nano modified intracanal medicaments were analysed using the dynamic laser scattering method as done by Che L et al., in Zetasizer nano Ver.7.12 (Malvern Instrument Co., Southborough, UK) equipped with a He-Ne laser of wavelength 632.8 nm [26].

Specimen Preparation

Fifty maxillary central incisors (n=50) that were under inclusion criteria were selected and disinfected in 0.1% thymol solution for 24 hours and stored in saline until use. All the samples were decoronated upto cementoenamel junction using a diamond disc and the apical patencies was checked with a 10 k file (Mani.Inc., Tochigi, Japan). The working lengths were confirmed with radiographs. Root canals of all samples were prepared to the working length by using Protaper (Dentsply Maillefer, Ballaigues, Switzerland) NiTi rotary system to a size F3 using 17% Ethylenediaminetetraacetic acid (EDTA) (Desmear, Anabond Stedman Pharma Research Ltd, India). The canals were irrigated with 2 mL of 3% Sodium hypochlorite (NaOCl) (Prime Dental Products Pvt., Ltd.,) after each instrumentation. The samples were then prepared with peesoreamer (Mani, Inc. Ltd Japan) upto a size of number 3 to attain a standardised internal diameter of 1.3 mm. Final irrigation was done with 3% of NaOCl, followed by 17% EDTA to remove the smear layer. The samples were finally irrigated with 2 mL of saline to avoid harmful effects of NaOCl and EDTA and dried with paper points. The samples (n=50) were divided into five groups, as follows:

Group I: Calcium Hydroxide paste (CH) (n=10)

Group II: Nano Calcium Hydroxide paste (NCH) (n=10)

Group III: Triple Antibiotic Paste (n=10)

Group IV: Nano Triple Antibiotic Paste (n=10)

Group V: Control group (n=10)

The respective medicaments in Groups I-IV will be mixed with propylene glycol and placed to working length using a lentulospiral (Dentsply, Konstanz, Germany). No medicament was placed in the control group. The coronal orifices were sealed with a cotton pellet and temporary filling material (Cavit; 3M ESPE, Seefeld, Germany), and the samples were stored for one week in an incubator at 37°C and 100% relative humidity. After one week, the medicaments from group I-IV were removed from the root canals using Passive Ultrasonic Irrigation (PUI). A 15 U file was used, which was kept 2 mm short of working length and agitated for 30 seconds duration at a power setting of 6. Distilled water was used as an irrigant in PUI followed by 5 mL of NaOCl irrigation and a final flush of 5 mL of distilled water. The samples were sectioned perpendicular to the long axis to obtain 2 mm slices from the middle third of the root from each sample using hard tissue microtome (Isomet; Beuhler Ltd., NY, USA) so that from each group a total of 10 slices were taken from 10 samples.

The biodentine root repair material was mixed according to the manufacturer's instructions, i.e., liquid from a single-dose container was emptied into a powder-containing capsule and mixed for 30 seconds at 4,000-4,200 rpm. It was incrementally placed into canal spaces of dentine slices being placed on a glass slab and condensed with a hand plugger. Excess material was removed from the surface of samples with a plastic instrument. The specimens were stored in an incubator at 37°C in 100% relative humidity for one week.

Push-Out Test

After one week, the samples were subjected to push-out bond strength analysis using Instron AutoGraph AG15 universal testing machine (Shimadzu, Japan) [Table/Fig-1]. Samples were placed on a custom made metal slab with a central hole. It was aligned with the centre of the test sample, which allowed the stainless steel stylus of the machine to pass freely once the bond between the test material and tooth was broken. The plunger was placed such that it had only minimin clearance with the sample to ensure contact with test material only. A stainless steel needle with 1 mm diameter was used to apply the compressive load with downward pressure on the surface of test material at a speed of 1 mm/min. The maximum force at the time of dislodgement was recorded in Newtons. The push-out bond strength was calculated in MPa by using the formula

Push-out Bond strength (MPa)=debonding force (N)/surface area (2p×r×h) in mm^2, where 'p' is the constant (3.14), 'r' is the root canal radius, and 'h' is the thickness of the root dentine slice in millimetres [27].

[Table/Fig-1]: Push-out test.

STATISTICAL ANALYSIS

Data were entered in MS-Excel and analysed in Statistical Package for the Social Sciences (SPSS) version 21.0. Shapiro Wilk test was applied to find normality. Descriptive statistics were represented by Mean with Standard Deviation (SD). A 95% confidence intervals were calculated. One-way ANOVA, Tuckey, Dunnets post-hoc test were applied to find significance. p-value <0.05 was considered statistically significant.

RESULTS

[Table/Fig-2] represents the mean values and standard deviation of push-out bond strength (MPa) of all groups. The mean highest push-out bond strength values were observed in group IV i.e., 12.33 MPa (p-value <0.001) and least in the group V i.e., 3.32 MPa. Biodentine showed significantly higher resistance to displacement among the group II (p-value=0.431) and group IV (p-value=0.074) when compared with their conventional medicaments [Table/Fig-3].

Group	Minimum	Maximum	Mean	SD	Median	IQR	p-value
Group I	2.34	12.03	6.54	3.13	6.25	4.28	
Group II	6.09	13.13	8.65	2.39	7.81	3.95	
Group III	5.63	15.78	9.03	2.88	7.88	3.18	<0.001
Group IV	6.88	15.01	12.33	2.95	12.97	4.09	
Group V	3.25	3.41	3.32	0.07	3.31	0.12	

[Table/Fig-2]: Mean push-out bond strength values and Standard Deviations (SD) of all test groups.
(One-way ANOVA,Tuckey, Dunnet post-hoc test were applied to find significance. p-value <0.05 was considered statistically significant)

Intergroup comparison		p-value
Group I	II	0.431
	III	0.269
	IV	**<0.001**
	V	0.292
Group II	I	0.431
	III	0.998
	IV	0.036
	V	**0.017**
Group III	I	0.269
	II	0.998
	IV	0.074
	V	**0.009**
Group IV	I	**<0.001**
	II	**0.036**
	III	0.074
	V	**<0.001**
Group V	I	0.292
	II	**0.017**
	III	**0.009**
	IV	**<0.001**

[Table/Fig-3]: Table showing intergroup comparisions between the experimental groups and p-value.
(One-way ANOVA,Tuckey, Dunnet post-hoc test were applied to find significance. p-value <0.05 was considered statistically significant)

DISCUSSION

Root repair materials should adhere well to root canal dentine and offer significant resistance during the function in the oral cavity. There are various bond strength tests like tensile, shear, push-out bond strength to study the adhesive property of root repair materials to the dentine. In the present study, the push-out bond strength test was used, as it is a reliable, efficient, and practical method [27-29].

Chou K et al.,and Berkhoff JA et al., reported that removing intracanal medicaments entirely is a challenge because of their penetration and binding to root canal dentine [30,31]. Calt S and Serper A reported that the residual intracanal medicament affects the penetration of obturating material into the root canal dentine [32]. Margelos J et al., confirmed the interaction of residual intracanal medicaments with zinc oxide based sealers affecting the quality of the sealer and Ozturk TY et al., reported formation of more voids during the marginal adaptation of biodentine with root canal wall [33,34].

In the present study, PUI with distilled water, NaOCl as irrigants using stainless steel 15 U file keeping 2 mm short of working length was performed to remove the intracanal medicaments. This method enables better acoustic streaming of the irrigants producing micro cavitation bubbles within the root canals (Van der Sluis LW et al.,) [35]. The effect of intracanal medicaments on the bond strength of root repair materials are critical because they affect the penetration ability of calcium silicate-based cements into the root canal dentine and formation of tag like structures and mineral infiltration zone by hindering calcium silicon ion exchange with the dentine [16].

In this study, the push-out bond strength values of Biodentine were higher in the nano TAP (group IV), followed by TAP (group III) when compared to other groups. Nano medicament groups showed significantly higher bond strength values when compared to their conventional counterparts. The results of the present study were in accordance with Tulumbaci F et al.,who reported that the binding of residual minocycline to the calcium via chelation might have increased the push-out bond strength values of TAP [36]. On the contrary, Shaheen NA et al., found that intracanal medicaments have no significant effect on the bond strength values of biodentine. TAP causes excessive demineralisation of the dentinal surface, which might be the reason for the significant difference in the TAP group causing better penetration of the biodentine in our study [37]. The residues of intracanal medicaments shown to decrease the micromechanical interlocking properties of these cements. In the present study, the least mean pushout bond strength in the control group (saline) might be due to the interaction of residual NaOCl with the biodentine affecting the adhesion of calcium silicate based biodentine [38]. Pereira AC et al., found no significant effect of intracanal medicament on bond strength of biodentine when compared with chlorhexidine, TAP, distilled water and control groups [39]. However, they detected small amounts of phosphorous ions in the triple antibiotic group at their interface. The higher bond strength of biodentine was due to the small particle size and high flowability of biodentine, which might have improved the penetration of the material into the dentinal tubules, increasing bond strength when compared to MTA group in their study. The powder of biodentine is composed of tricalcium silicate, calcium carbonate, zirconium oxide and the liquid part contains water and calcium chloride as a setting accelerator and a hydrosoluble polymer as water reducing agent, which improves the physical and handling properties of this material [40].

In the present study, the NCH (group II) had shown better push-out bond strength values when compared with the CH (group I); however, these were inferior when compared to group III and IV. Propylene glycol was used as a vehicle in the present study, which might have negatively affected the carbonation reaction of CH with the environment, study by Cruz EV et al., [41]. The results of the present study are in accordance with Guiotti FA et al., who reported that CH remnants reduces the bond strengths of calcium silicate-based cements [42]. Yassen GH et al., reported that disruption of dentinal collagen occurs due to the high alkaline pH of CH [43]. This affects the bond between hydroxyapatite and collagen fibrils of dentine, which in turn decreases the push-out bond strength of biodentine. It might be the possible reason for the lesser push-out bond strength values of CH when compared to nano and conventional TAP. The study done by Nagas E et al., reported a higher bond strength of biodentine with CH group when compared with TAP [23]. It was due to the smaller particle size of biodentine and better penetration ability.

The possible reason for better push-out bond strength values of nano modified medicaments in the present study when compared with the conventional groups might be due to the size of nano medicaments less than 100 nm. Nano medicaments might be easily dislodged on the ultrasonic activation leading to better bonding with the exposed dentinal tubules in the root canal wall. So lesser remnants cause better exposure of dentinal surface, enhancing the tag like structures of biodentine which might have increased resistance to dislodgement study by Aggarwal V et al., [15].

The difference in bond strengths of biodentine might also be due to experimenting maxillary incisors, usage of the viscous vehicle for the medicaments, and experimental design, affecting the bond strength values of biodentine when compared with previous studies by Alsubait S et al., Nagas E et al., Tulumbaci F et al., [21,23,36].

Limitation(s)

Although it is not always possible to generalise the results of in-vitro studies in the clinical scenarios. In the present study, the authors have considered the application of ultrasonics in comparing the effect of residual intracanal medicaments on push-out bond strength of biodentine. Further studies are necessary to substantiate the findings of this research and also the removal efficacy of ultrasonic agitation on nano-sized intracanal medicaments and highlight their importance in endodontic treatment.

CONCLUSION(S)

Within the limitations of the present study it can be concluded that nano-sized medicaments can be efficiently used as intracanal medicaments as alternative to conventional medicaments because of improvement in the push-out bond strength values of biodentine. CH, NCH, TAP and nano TAP have enhanced the push-out bond strength values of biodentine. Nano TAP had shown highest mean pushout bond strength values when compared to other groups. NCH and nano TAP had significantly improved the push-out bond strength values when compared to CH and nano TAP respectively.

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- Manual Googling: Nov 24, 2021
- iThenticate Software: Dec 28, 2021 (4%)

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- Was informed consent obtained from the subjects involved in the study? NA
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