Original Article

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Dentistry Section

Comparative Evaluation of Various Root Canal Irrigants on the Marginal Integrity of Furcal Perforation Repair Material: An In-vitro Study

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ABSTRACT

Introduction: Furcal perforations can occur during access cavity preparation while locating the canal orifices. This must be sealed immediately. After the repair of furcal perforation, endodontic treatment should be performed with various irrigants to clean the root canal system. This procedure causes unavoidable contact of endodontic irrigants with the site of furcal repair.

Aim: To evaluate the effect of root canal irrigants on the marginal integrity of furcal perforation repair material using protein leakage assessment.

Materials and Methods: This in-vitro study was conducted from June 2021 to September 2021 at Kalinga Institute of Dental Sciences, KIIT Deemed to be University, Bhubaneswar, Odisha, India. A total of 90 extracted mandibular molars with intact furcation were used. Access cavities were prepared. Based on the repair materials, samples were randomly divided into two groups. An artificial perforation of diameter 2 mm was made in the furcation area. The specimens were divided according to the furcation perforation repair materials used: Biodentine, Endosequence (n=45 each). Perforations were filled with Biodentine, Endosequence. They were then subdivided into three subgroups, each containing samples of (n=15) according to the irrigating solutions used. Each group was irrigated with 0.2% Chitosan, Chloroquick, and 5.25% Sodium Hypochlorite (NaOCl) for two minutes, respectively. Protein {Bovine Serum Albumin (BSA)} microleakage was checked by preparing apparatus having upper and lower chamber. Protein leakage through the furcation repair material into the lower chamber was assessed by Ultraviolet (UV) visible spectrophotometry. The microleakage was assessed with a reagent Coomassie Brilliant BlueG-250 daily for 60 days. Intergroup comparisons were made using one way Analysis of Variance (ANOVA) test. The multi group comparisons were made using Tukey Honestly Significant Difference (HSD) tests.

Results: A 0.2% Chitosan showed more protein leakage than Chloroquick over a period of 60 days ($p<0.001$), as compared to the baseline, 30 days values with both the furcation repair materials. A 5.25% NaOCl irrigated samples exhibited highest protein leakage among all the irrigants.

Conclusion: Biodentine has a better sealing ability than Endosequence BC sealer. Chloroquick proved to be the better irrigant as compared to Chitosan and Sodium Hypochlorite (NaOCl) in terms of affecting sealing of furcation repair materials.

Keywords: Biodentine, Chitosan, Chloroquick, Endosequence BC, Protein leakage

INTRODUCTION

Iatrogenic complication is a common endodontic accident that can occur during the preparation of access cavity, difficulty while locating canal orifices especially when there is extensive caries and altered tooth anatomy and while locating an extra canal. Furcal perforations are an artificial interaction between the endodontic space and the periradicular tissue. This must be sealed immediately with repair material to avoid resorption of alveolar bone, microleakage, periodontal ligament injury and to prevent any periapical infection [1]. An ideal repair material for perforation must induce formation of bone and cementum, should be biocompatible with the host, non carcinogenic, non toxic, easily available and inexpensive [1].

Biodentine (Septodont, USA) a calcium-silicate based material exhibits biocompatibility, bioactivity, and has induction potential for bone formation. They are highly antibacterial and resistant to washout [2-4]. Biodentine showed less microleakage than Mineral Trioxide Aggregate (MTA), making it a viable alternative to MTA for filling furcal perforations in primary molars [5]. Endosequence BC (Brassler, USA) is non toxic, non resorbable and hydrophilic in nature which is in favour of an ideal repair material for repair of furcation perforation [6,7]. Jeevani E et al., evaluated Biodentine, Endosequence BC and MM-MTA for their sealing abilities on furcation perforations using UV-spectrophotometry. They concluded that Endosequence BC was more effective than other root materials [8]. Various irrigants have to be used to clean the root canal system after the repair of furcal perforation. Recently, irrigants like chitosan and Chloroquick are being used [9]. Chitosan a natural polysaccharide, shows a good amount of biocompatibility, bioadhesion, biodegradability, hydrophilicity and lacks toxicity [10]. Mathew SP et al., compared and evaluated the removal of smear layer with Ethylenediaminetetraacetic (EDTA) acid and Chitosan, Chitosan group caused least alteration in surface structure and Calcium/Phosphorus (Ca/P) ratio of root dentine [11]. Chloroquick (innovationsendo, India), which is a one-step irrigating solution containing 5% NaOCl and 18% Etidronicacid exhibit antimicrobial property and dissolution activity, helping in removal of smear layer [12]. After the repair of furcation perforation, the effect of different irrigants on the sealing ability of these materials needs to be assessed.

There are no studies in the literature reporting the effect of Chitosan and Chloroquick on the sealing of furcation repair materials. Thus, this study evaluated the effect of three root canal irrigants (0.2% Chitosan, Chloroquick and 5.25% NaOCl) on the marginal integrity of Biodentine and Endosequence BC used as furcation perforation repair materials using UV Spectrophotometer. It was hypothesised that above three irrigants will not affect the marginal integrity of Biodentine and Endosequence BC.

MATERIALS AND METHODS

This in-vitro study was conducted from June 2021 to September 2021 at Kalinga Institute of Dental Sciences, KIIT University,

Bhubaneswar, Odisha, India, after taking Institutional Ethical Committee (IEC) approval (letter no-KIIT/KIMS/IEC/177/2019).

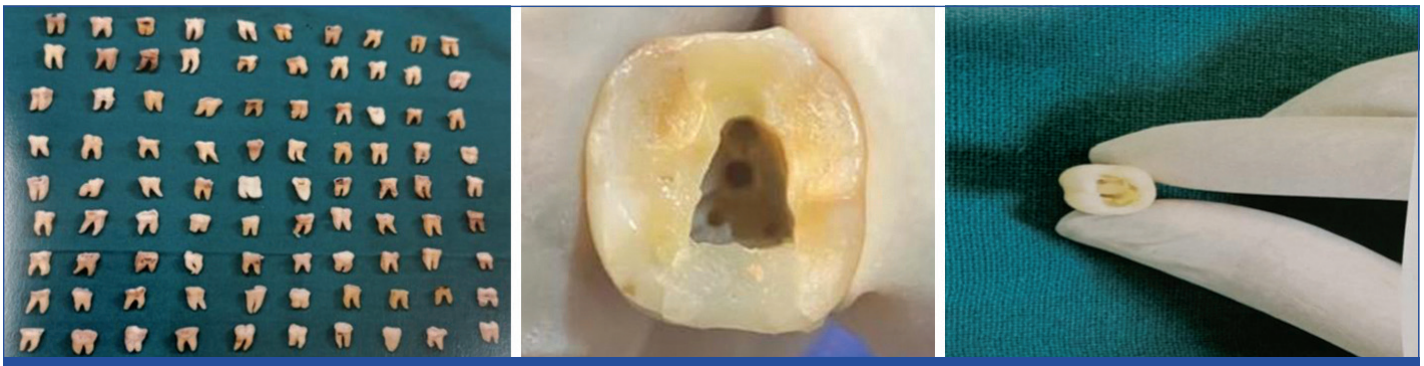
Inclusion criteria: Ninety extracted (periodontally compromised) multi-rooted non carious permanent mandibular molars with intact furcation, non fused and well developed roots [Table/Fig-1a] were included in the study.

Exclusion criteria: Grossly, decayed teeth, teeth with fractured crowns, root canal treated teeth and teeth with fractured root were excluded from the study.

Study Procedure

Access cavities were prepared and canal orifices were located. Cyanoacrylate resin was used to seal the root tips (FeviKwik, Pidilite, India). Orifices of the root canal were sealed with cavit (3M ESPE). To ensure each perforation was centered between the roots, a black marker pen was used to mark the location of the defect. An artificial perforation of diameter 2 mm using an ISO 014 round diamond high speed bur with water coolant was made [Table/Fig-1b]. Two coats of nail varnish were applied to the tooth's exterior surface. In addition, the perforations sites were rinsed with water using an air/water syringe and dried using oil-free air. The specimens were divided according to the furcation perforation repair materials into two groups of 45 teeth each. Perforations (N=45) were filled with Biodentine (Septodont, Saint-Maur-des Fosses Cedex, France). The powder was mixed with liquid according to the manufacturer's instructions and was packed in the perforations using a plastic filling instrument. Endosequnce BC is premixed, condensable putty that comes in a syringable form and was placed directly into the perforations made on the sample.

Access cavities were restored with Intermediate Restorative Material (IRM) (Dentsply) and left for 72 hour at 37ºC in an incubator, to allow the setting of repair material. Before assessing the leakage, temporary filling material was removed from the cavities of the samples, and the setting of the Biodentine and Endosequence BC were checked with an explorer [Table/Fig-1c].



[Table/Fig-1]: a) Ninety teeth samples, b) Prepared artificial perforation, c) Image showing set furcation repair material.

Biodentine and Endosequence BC samples were subdivided into three subgroups, each containing samples of (n=15) according to the irrigating solutions used. Group 1 was irrigated with 10 mL of 0.2% Chitosan (Thahira chemicals, Kerela, India), group 2 was irrigated with 10 mL of Chloroquick Innovationsendo, India) and group 3 was irrigated with 10 mL of 5.25% NaOCl (Percan-Septodont Healthcare India Pvt., Ltd.,) for two minutes, respectively [11,12]. Chitosan acetate solution was prepared at a concentration of 0.2% by mixing 0.2 gm of chitosan powder (Thahira chemicals, Kerela, India), diluted in 100 mL of 1% acetic acid. The pH level of the Chitosan acetate solution was adjusted to 3.2 with NaOH. This solution was stirred for 1 hour with a magnetic stirrer [11].

Protein leakage test: For preparing the apparatus for assessing leakage [Table/Fig-2], the bottom of 5 mL Eppendorf tube® (Tarson) was prepared by slicing it with the help of a carborundum disk so as to snugly fit the crown of the tooth which was inserted and tightly sealed with cyanoacrylate paste and the remaining gap was sealed with modelling wax. Another Eppendorf tube of 5 mL was attached below around the cervical portion of the tooth and closed off with cyanoacrylate paste. Both the Eppendorf tubes were sealed together in the center with modelling wax. The upper Eppendorf tube was filled with 3 mL of 1 gm/1 mL BSA solution (Sigma-Aldrich). The lower Eppendorf tube was filled with 2 mL of distilled water. The apparatus was arranged for each experimental group and stored at 37°C in an incubator for seven days. The distilled water (OrganoLaboratories New Delhi, India) in the lower chamber, and freshly prepared 1gm/1 mL of BSA in the upper chamber was refilled everyday using a pipette during the experiment for 60 days. Leakage at the end of first day is considered as baseline. At the bottom of the Eppendorf tube, a hole of 1 mm diameter was prepared to replenish the solution followed by sealing of the hole with parafilm every time after replenishing the solution to prevent its contamination.

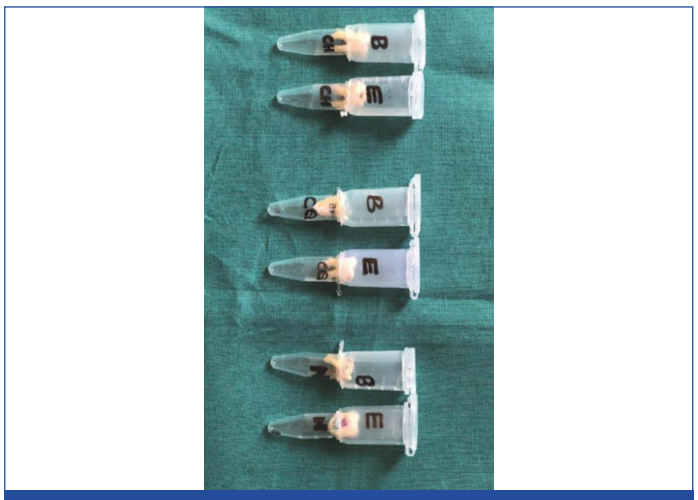
Ultraviolet (UV)- visible spectrophotometry: Protein microleakage through the furcation repair material into the lower chamber was assessed by two different examiners by UV-visible spectrophotometry. The microleakage was assessed with a reagent Coomassie Brilliant BlueG-250 (Sigma-Aldrich) daily for 60 days [Table/Fig-3]. The colour change of the protein reagent indicated leakage [Table/Fig-4]. UV Visible Spectrophotometer (Agilent Technologies, India) within a range of 465-595 nm was used to quantify protein concentration.

STATISTICAL ANALYSIS

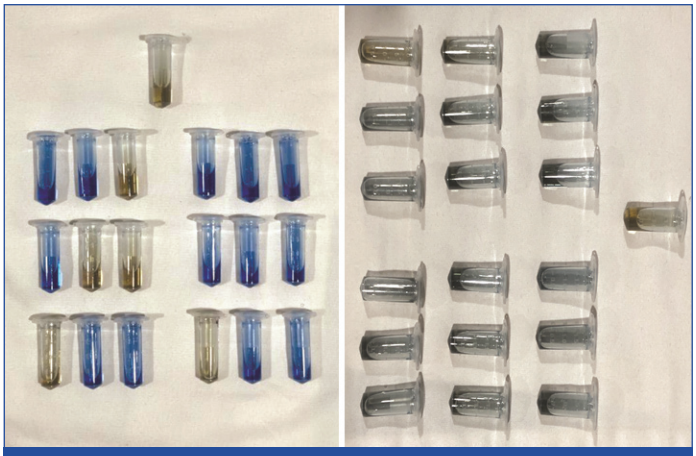
Statistical analysis was done by using SPSS software 25 (Armonk, NY:IBM Corp). Intergroup comparisons were made using one way Analysis of Variance (ANOVA) test. The multi group comparisons were made using Tukey Honestly Significant Difference (HSD) tests. Level of significance was set at (p-value <0.05).

RESULTS

Chloroquick exhibited least protein leakage as compared to 0.2% Chitosan and 5.25% NaOCl at a duration of 60 days (p<0.001)



[Table/Fig-2]: Models prepared for evaluation of protein leakage test.



[Table/Fig-3]: Addition of Coomassie Brilliant Blue in the lower chamber.
[Table/Fig-4]: Colour change indicating protein leakage. (Images from left to right)

[Table/Fig-5]. A 0.2% Chitosan showed more protein leakage as compared to Chloroquick over a period of 60 days (statistically significant p<0.001) as compared to baseline, 30 days (statistically non significant p=0.186, p=0.1) values with both the furcation repair materials [Table/Fig-2]. A 5.25% NaOCl irrigated samples exhibited highest protein leakage among all the irrigants over a duration of 60 days [Table/Fig-6].

Groups	Subgroups	Baseline	30 days	60 days	p-value
BD	Chitosan	0.090±0.006	0.166±0.018	0.221±0.007	<0.001
	Chloroquick	0.092±0.011	0.125±0.260	0.056±0.006	<0.001
	Sodium hypoclorite	0.260±0.027	0.437±0.058	0.511±0.011	<0.001
	p-value	<0.001	0.004*	<0.001	
ENSQ	Chitosan	0.250±0.024	0.284±0.0230	0.382±0.020	<0.001
	Chloroquick	0.232±0.024	0.182±0.014	0.255±0.021	<0.001
	Sodium hypoclorite	0.640±0.032	0.536±0.039	0.707±0.076	<0.001
	p-value	0.05	0.005	0.001	

[Table/Fig-5]: Mean protein leakage assessment (mg/mL).
BD: Biodentine; ENSQ: Endosequence BC; *p-value <0.05 considered significant

DISCUSSION

The findings of this study showed that irrigating Biodentine and Endosequence BC sealed perforations with all three irrigants have some detrimental effect on the seal provided by these repair materials. So the hypothesis was rejected.

To eliminate smear layer from root canal produced during biomechanical preparation, a combination of NaOCl with tissue dissolving properties and a strong chelating agent such as EDTA acid is recommended [12,13]. A study conducted by Tay FR et al., concluded that the application of strong chelating agents like EDTA for more than 1 minute and 1 mL of volume to be associated with dentinal erosion [14]. For the complete removal of the smear layer, NaOCl should be used with other chelating agents like Chitosan, Etidronic acid, or HEBP (1-Hydroxyethylidene-1, 1-Bisphosphonate) which can eliminate the inorganic phase of the smear layer [15-18]. The effect of different irrigating solutions on the sealing ability of perforation repair materials needs to be assessed.

Over a period of 60 days Chitosan showed more protein leakage with Biodentine and Endosequence BC as compared to baseline value (p<0.001), which was statistically significant. The reason could be due to the acidic ph of Chitosan (3.2) [18]. When Chitosan was compared to Chloroquick (with Biodentine samples), Chitosan showed more protein leakage (p<0.001). During the setting reaction of Biodentine, the alkaline effect produced causes organic tissue dissloution out of the dentinal tublues thereby enhancing the micromechanical adhesion. An alkaline environment is formed between Biodentine and the tooth creating a way for the dentin substitute mass to enter the exposed opening of dentin canaliculi allowing Biodentine to be keyed to dentine, resulting in a steady anchorage with a bacteria tight-seal effect [7]. Chitosan has a ph of 3.2 which is acidic in nature would have interfered with the bonding of Biodentine to dentine resulting in more protein leakage compared to Chloroquick (p<0.001).

Previous studies (Mathew SP et al., Silva PV et al.,) proved that Chitosan removed the smear layer effectively as compared to EDTA [11,19]. Chitosan, at a low concentration, is capable of removing the smear layer from the surface of dentin by chelation.

Multiple Comparisons-Tukey HSD							
						95% Confidence interval	
Dependent variable	(I) Group	(J) Group	Mean difference (I-J)	Std. Error	p-value	Lower bound	Upper bound
BD baseline	1	2	0.033	0.006	<0.001	0.018371	0.048
		3	-0.170	0.006	<0.001	-0.185	-0.155
	2	3	-0.204	0.006	<0.001	-0.218	-0.188
ENSQ baseline	1	2	-0.018	0.009	0.186	-0.041	0.006
		3	-0.407	0.009	<0.001	-0.431	-0.383
	2	3	-0.387	0.009	<0.001	-0.414	-0.365
BD 30 days	1	2	-0.071	0.006	0.05	-0.194	0.079
		3	-0.312	0.056	<0.001	-0.449	-0.175
	2	3	-0.256	0.056	<0.001	-0.392	-0.118
ENSQ 30 days	1	2	0.118	0.059	0.100	0.093	0.141
		3	-0.252	0.009	<0.001	-0.276	-0.228
	2	3	-0.370	0.009	<0.001	-0.394	-0.345
BD 60 days	1	2	0.128	0.004	<0.001	0.119	0.137
		3	-0.290	0.004	<0.001	-0.299	-0.281
	2	3	-0.418	0.004	<0.001	-0.427	-0.409
ENSQ 60 days	1	2	0.127	0.017	<0.001	0.085	0.169
		3	-0.325	0.017	<0.001	-0.367	-0.283
	2	3	-0.452	0.017	<0.001	-0.494	-0.410

[Table/Fig-6]: Comparisons between all the groups at baseline, 30 days and 60 days. Group 1 (Chitosan), Group 2 (chloroquick), Group 3 (sodium hypochlorite).
p-value <0.05 considered significant; BD: Biodentine; ENSQ: Endosequence BC

This smear layer produced by chelation could have penetrated into the interfacial layer, which might have interfered with the chemical adhesion between the repair material and dentine [18]. For Endosequence BC samples both Chloroquick and Chitosan showed similar result over a period of 30 days (p=0.1) which was statistically non significant, but over a period of 60 days, Chloroquick irrigated samples showed less protein leakage as compared to Chitosan (p<0.001) which was statistically significant. The novel Chloroquick solution is a mix of Hydroxy Ethyl Bis Phosphonate and NaOCl, the importance of combination is that the NaOCl does not surrender its biological, antibacterial, and tissue dissolving properties [20,21], whereas the reduction and elimination of the inorganic element are done with the help of Hydroxyethylidene Bisphosphonate (HEBP).

Cobankara FK et al., conducted a study to evaluate the effects of various chelating agents on the mineral content of root canal dentin and concluded that Hydroxy Ethyl Bis Phosphonate had a soft and weak effect on Ca/P ratio as compared to 17% EDTA acid, 10% Citric acid, 2.25% Peracetic acid [22]. Another study conducted by Dineshkumar MK et al., concluded that HEBP treated root dentin showed the highest microhardness which could be due to the larger inter-tubular dentin area available for hybridisation and the partial depletion of surface calcium [23]. Chloroquick follows soft chelating irrigation protocol because of the better opening of dentinal tubules which were covered by the smear layer [12,24,25]. This might have resulted in better bonding, less leakage of furcation repair materials as compared to Chitosan. Over a period of 60 days Chloroquick irrigated Biodentine, Endosequence BC samples showed less protein leakage compared to baseline (p<0.001) which was statistically significant.

Over a period of 60 days, NaOCl irrigated samples showed highest microleakage as compared to its baseline values with both the furcation repair materials (p<0.001) which was statistically significant. As compared to Chloroquick and Chitosan, NaOCl irrigated samples showed higher leakage (p<0.001). The reason could be due to the dissolution of collagen fibrils from dentin caused by break down of the carbon atoms bond and disorganisation of the collagen's primary structure [19]. This disorganisation could have resulted in more leakage of NaOCl irrigated sample. Though Chlorquick contain 5% hypochlochlorite it showed less microleakage, the reason could be soft chelating irrigation protocol as explained earlier. Endosequence BC showed more leakage as compared to Biodentine with all the irrigants. This in accordance with the studies by Hirschberg CS et al., Kakani AK and Veeramachaneni C, [26,27]. But, in contrast to the study by, Lagisetti AK et al., and Jeevani E et al., they proposed that the deeper penetration of Endosequence BC is owed to its nanoparticle size, thus rendering a fluid-tight seal, while its putty consistency allows for improved adaptation to dentinal walls and superior handling [6,8]. On the other hand, Biodentine displayed superior adhesion to dentinal tubules due to the formation of tag-like structures within the dentinal tubules leading to a micromechanical anchorage [27]. In the present study, the probable cause of high protein leakage may be the viscosity of Endosequence BC which might have prevented the material to flow adequately into the dentinal tubules and seal the perforation.

Limitation(s)

Despite of the promising result this in-vitro study could not simulate clinical situation completely so further clinical studies need to be conducted to evaluate the sealing ability of furcation repair material.

CONCLUSION(S)

Chloroquick proved to be the better irrigant as compared to Chitosan and Sodium Hypochlorite (NaOCl) in terms of affecting sealing of furcation repair materials. Hence, it can be concluded that Chloroquick can be a better alternative to remove smear layer during biomechanical preparation in the clinical scenario of perforation repair.

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