Original Article

Effect of different root canal irrigants on surface roughness and microhardness of Biodentine combined with triple antibiotic paste: An *in vitro* study

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Abstract

Background: Biodentine is widely used for endodontic applications; recently, it has been incorporated with triple antibiotic paste (TAP). The effect of endodontic irrigants on the physical characteristics of this new combination needs to be studied.

Aims: The aim of the study was to evaluate the surface roughness and microhardness of Biodentine incorporated with TAP subjected to various endodontic irrigants.

Materials and Methods: Hundred cylindrical discs (6 mm × 3 mm) were prepared by mixing the Biodentine with TAP (3:1). The specimens were subjected to different irrigating solutions for 5 min in 5 groups ($n = 20$): Group 1: distilled water (control), Group 2: 2.5% sodium hypochlorite, Group 3: 17% ethylenediaminetetraacetic acid, Group 4: 2% chlorhexidine (CHX), and Group 5: 2% chitosan nanoparticles (CSNs). Half of the specimens in each group were subjected to surface roughness ($n = 10$) and another half to microhardness ($n = 10$). Surface roughness was measured using a surface roughness tester, and digital Vickers microhardness testing was performed on each specimen.

Statistical Analysis: One-way ANOVA and *post hoc* Tukey's tests ($P \leq 0.05$) were used.

Results: The highest microhardness was found with 2% CSN, whereas 2% CSN and 2% CHX had a minimal effect on the surface roughness of Biodentine incorporated with TAP ($P \leq 0.05$).

Conclusion: The root canal irrigant 2% CSN exhibited the highest microhardness and least surface roughness of modified Biodentine with TAP.

Keywords: Biodentine; microhardness; root canal irrigant; surface roughness; triple antibiotic paste

INTRODUCTION

In endodontics, calcium silicate cement (CSC) are considered a boon which has changed the prognosis of impossible cases. Owing to their inherent hydraulic characteristics, they set in the presence of blood and surrounding tissue fluids.[1] Tricalcium silicate-based cement Biodentine has been developed and used for various clinical applications such as mineral trioxide aggregate (MTA).[2] The powder components of Biodentine are zirconium oxide, calcium carbonate, and tricalcium silicate, and a liquid part containing calcium chloride acts as a water water-reducing agent as well as an accelerator. Biodentine has excellent sealing ability and sufficient

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strength and exhibits a relatively short setting time of 9–12 min.[3]

Teeth with root or furcal perforations/external resorption need to be repaired immediately using endodontic repair materials such as MTA or Biodentine to prevent bacterial contamination and periodontal tissue damage. Following the repair of the defects, these materials often encounter different root canal irrigants such as sodium hypochlorite (NaOCl) and 2% chlorhexidine (CHX) to disinfect the root canal system during further steps of root canal treatment or during retreatment if clinical situation demands.[4] Following a final flush with a chemical irrigant, some solutions may linger in the root canal space, affecting the biomaterial used to repair and modifying its characteristics such as surface roughness and microhardness.[5] The impact of irrigating solutions on MTA was studied by Smith *et al*.[6] and reported that MTA microhardness values decreased following contact with irrigation solutions.

Biodentine is primarily used for its biocompatibility and bioactivity in various endodontic applications; however, it does not possess antimicrobial properties. To achieve the utmost disinfection of the infected root canals with perforations requiring repair biomaterials, several antimicrobial agents have been combined with bioceramic materials such as Biodentine and MTA to boost their antibacterial activity. Recent literature shows that the incorporation of triple antibiotic paste (TAP) to Biodentine exhibits effective antibacterial activity against *Enterococcus faecalis* when utilized as root end-filling material.[7] It is reported that the incorporation of TAP with bioceramic materials such as MTA, Biodentine, and EndoSequence RRM significantly improved the calcium ion release potential compared to without TAP.[8] In addition, Biodentine combined with TAP exhibited alkaline pH and was dimensionally more stable compared to MTA with TAP.[9] However, the addition of any other materials to existing material affects the clinical performance of the new material. Since Biodentine is used for various endodontic applications such as pulp capping agent, an endodontic repair material for retro cavities, furcation repair, etc., It encounters different environmental conditions such as endodontic irrigants used during treatment or retreatment of root canals. Therefore, it should possess adequate surface characteristics and hardness/strength to withstand surrounding environment without disintegration for endodontic success.[10]

No previous research has investigated the influence of different irrigants on the surface characteristics of modified Biodentine incorporated with TAP; hence, this study was conducted to evaluate the surface roughness and microhardness of Biodentine incorporated with TAP subjecting to different root canal irrigants.

MATERIALS AND METHODS

Institutional Ethical Clearance (Ref No: IEC/2022-23/05) was obtained before conducting this study and was performed accordingly.

Preparation of the experimental materials

Triple antibiotic paste

Metronidazole 400 mg tablets (Abbott Pvt. Ltd., Mumbai, India), ciprofloxacin 200 mg tablets (Cipla Pvt. Ltd., Mumbai, India), and minocycline 100 mg tablets (Fine Research Lab Chem Industries, Mumbai, India) were used. The sugar coatings of the tablets were removed with a surgical blade and were ground separately in a mortar and pestle and sieved to obtain fine powder particles. The obtained powder particles were then mixed in a 1:1:1 ratio. The carrier was prepared by mixing macrogol (Bayer Healthcare Pharmaceuticals, India) in a 1:1 ratio with propylene glycol (MP) (Smply Mount Pleasant, Tennessee). On a glass slab, the powder components were blended in a 5:1 ratio with the MP carrier.[11] Moreover, the final concentration of 1 mg/mL was used further.

Triple antibiotic paste incorporated with Biodentine

Biodentine (Septodont, Saint-Maur-des-Fosses, France) was manipulated by mixing the powder and liquid components in a 5:1 ratio in an amalgamator for 30 s. Further, Biodentine and TAP paste (1:1 ratio) were mixed on a sterilized blending pad using a plastic spatula vigorously for about 10 s, until a smooth mix was achieved.

Preparation of specimens

A total of 100 cylindrical specimens were prepared using a mold of 6 mm × 3 mm dimensions that were placed on a glass slab, and the abovementioned freshly prepared experimental material was placed into the mold with a carrier and compacted using a hand plugger to condense properly and excess material was removed. Another glass slab was placed on the mold for obtaining uniform surface for about 5 min and then the samples were wrapped with a wet cotton pellet and were left to set at a room temperature for 10 min. Later, the upper surface of each specimen was polished at room temperature using silicon carbide grinding papers (Laxmi Abrasives Pvt. Limited, Uttar Pradesh, India) to obtain smooth surface and was rinsed in distilled water (DW) for 1 min and air-dried for 5 s.[12] Specimens were arbitrarily assigned into 5 groups ($n = 20$) and subjected to 5 mL of root canal irrigants in a petri dish for 5 min as follows:

- Group 1: Distilled water (DW)
- Group 2: 2.5% Sodium hypochlorite (2.5% NaOCl)
- Group 3: 17% Ethylenediaminetetraacetic acid (17% EDTA)
- Group 4: 2% Chlorhexidine gluconate (2% CHX)
- Group 5: 2% chitosan nanoparticles (2% CSNs).

In each group, half of the specimens were subjected to surface roughness (n = 10) and another half to microhardness (n = 10).

Evaluation of surface roughness

The surface roughness was measured using a digital roughness tester (SR 300, Taylor Hobson, Leicester, England). On each specimen, the roughness value Ra (μm) was measured at three different predetermined locations at a distance of 0.8 × 3, and the average of three readings was recorded [Figure 1].

Evaluation of microhardness

The microhardness was measured using a digital Vickers microhardness tester (Fine Testing Instruments, West Bengal, India) using a diamond indenter with 500 g load for 30 s. On each specimen, three arbitrary indentations were made at different locations, no closer than 1 mm to the specimen's edge or other indentations, and the average of three readings was noted as a final value for each specimen [Figure 2].

Statistical analysis

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) software, version 23 (IBM-Statistics, Chicago, IL, USA). One-way ANOVA and multiple comparison between the groups were done using *post hoc* Tukey's test at $P \leq 0.05$ level of significance.

RESULTS

Analysis by one-way ANOVA showed that the highest surface roughness was found in the EDTA group followed by 2.5% NaOCl, 2% CHX, and 0.2% CSN and least in DW, whereas for microhardness, the highest values were found in the DW (control) and 0.2% CSN groups followed by the 2% CHX group, while the lowest value was seen in the 2.5% NaOCl and 17% EDTA groups [Table 1].

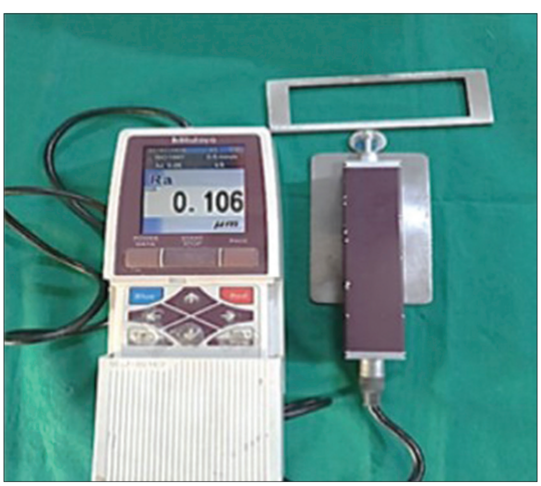

Figure 1: Specimen subjected to surface roughness testing

Multiple comparison between the groups by *post hoc* Tukey's test of surface roughness showed no significant difference between 2.5% NaOCl and 17% EDTA and between 2% CHX and 0.2% CSN ($P > 0.05$) and significant difference between other groups. For microhardness, significant difference was found between DW and 0.2% CSN and between 2.5% NaOCl and 17% EDTA ($P > 0.05$) and no significant difference was found between all other groups [Table 2].

DISCUSSION

Biodentine is the preferred material, especially for endodontic repair.[1-3] It is reported that root canal irrigants encounter biomaterials used for repair, affect the biomaterial, and modify its surface characteristics and microhardness.[5,6] A material's surface strength can be estimated from its surface microhardness, and Vickers hardness has been used as an indicator for evaluating the strength of calcium silicate-based materials.[13] On the other hand, the estimation of surface roughness indicates the ability of the material to be dimensional stable when subjected to different agents such as irrigants.[14] Hence, the parameters microhardness and surface roughness are of clinical importance, therefore, were tested in the present study for Biodentine modified with incorporating with TAP and subjected to different irrigating solutions.

Ratih *et al*.[15] demonstrated that a contact duration of 1 min using 0.5% CSN exhibited the greatest microhardness and lowest surface roughness when compared to 3 min as a final irrigant. According to Ersahan *et al*.,[12] 5 min of irrigation is widely regarded as adequate for posttreatment in the repair of root perforations using Biodentine. Therefore, in accordance with the above studies, in the present study, the experimental material was allowed to set for 10 min, and specimens of all groups were immersed in the experimental irrigating solutions for 5 min. The results of the current study showed that 0.2% CSN exhibited

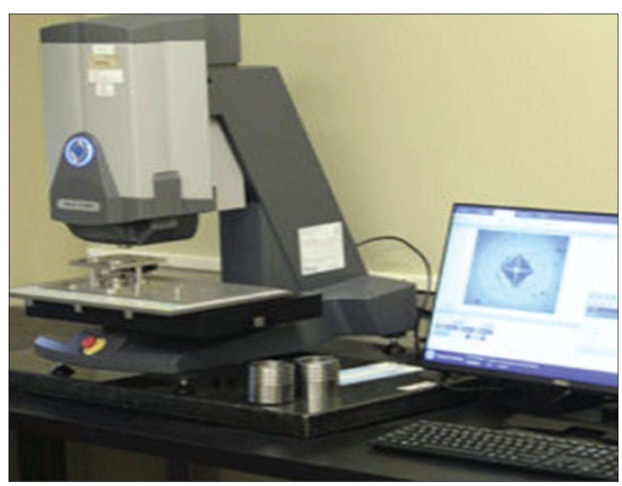

Figure 2: Specimen subjected to Vickers microhardness testing

Table 1: Comparison of surface roughness and microhardness of all groups by one-way ANOVA

Groups (n=10)	Surface roughness (Ra)		Microhardness (VHN)	
	Mean	SD	Mean	SD
Group 1 DW	1.194	0.033	87.0	1.194
Group 2 NaOCl	1.711	0.028	56.5	1.711
Group 3 17% EDTA	1.714	0.035	53.3	1.714
Group 4 2% CHX	1.497	0.037	77.8	1.497
Group 5 0.2% CSN	1.473	0.034	85.5	1.473
ANOVA	F=477.82		F=361.45	
P	P=0.000 (significant difference)		P=0.000 (significant difference)	

DW: Distilled water, NaOCl: Sodium hypochlorite, EDTA: Ethylenediaminetetraacetic acid, CHX: Chlorhexidine, CSNs: Chitosan nanoparticles, SD: Standard deviation, VHN: Vickers hardness

Table 2: Comparison of surface roughness and microhardness between all the groups by post hoc Tukey's test

Groups	Comparison	Surface roughness		Microhardness	
		Difference	P	Difference	P
Group 1 DW	Group 2 2.5% NaOCl	−0.5170	0.000 (significant difference)	30.50	0.000 (significant difference)
	Group 3 17% EDTA	−0.5200	0.000 (significant difference)	33.70	0.000 (significant difference)
	Group 4 2% CHX	−0.3030	0.000 (significant difference)	9.20	0.000 (significant difference)
	Group 5 0.2% CSN	−0.2790	0.000 (significant difference)	1.50	0.603 (NS)
Group 2 NaOCl	Group 3 17% EDTA	−0.0030	0.987 (NS)	2.10	0.255 (NS)
	Group 4 2% CHX	0.2140	0.000 (significant difference)	−21.30	0.000 (significant difference)
	Group 5 0.2% CSN	0.2380	0.000 (significant difference)	−29.00	0.000 (significant difference)
Group 3 EDTA solution	Group 4 2% CHX	0.2170	0.000 (significant difference)	−24.50	0.000 (significant difference)
	Group 5 0.2% CSN	0.2410	0.000 (significant difference)	−32.20	0.000 (significant difference)
Group 4 CHX	Group 5 0.2% CSN	0.0240	0.563 (NS)	−5.70	0.007 (significant difference)
Group 5 0.2% CSN	-	-	-	-	-

DW: Distilled water, NaOCl: Sodium hypochlorite, EDTA: Ethylenediaminetetraacetic acid, CHX: Chlorhexidine, CSNs: Chitosan nanoparticles, SD: Standard deviation, NS: No significant difference

the highest microhardness and lowest surface roughness compared to all other groups at 5-min exposure time.

Literature suggests that lower pH environments affect the physical and chemical properties of biomaterials. Even though 2% CHX is not an acid, its effect on MTA showed that a decrease in surface hardness, sealing ability, and setting time was slowed down.[16] This could be the reason for decreased surface roughness and microhardness for CHX in the present study compared to CSN.

The surface roughness and microhardness of Biodentine after subjecting to different root canal irrigants were studied by Ersahan *et al*.,[12] who reported that the microhardness of Biodentine specimens exposed to NaOCl and CHX for 5 min was lower than EDTA and no significant differences were found between the irrigants for surface roughness of Biodentine. Contrary to the above study findings, the present study results showed lower microhardness and higher surface roughness for specimens treated with EDTA and NaOCl compared to CHX. Similar to the present study, Antonijević *et al*.[17] showed that the microhardness of Biodentine was reduced after being treated with 17% EDTA. This is because 17% EDTA has a strong chelating impact, which normally affects the microhardness of Biodentine over an extended period which is also supported by Wang *et al*.,[18] who reported that solutions with acidic pH such as EDTA weaken the microhardness of MTA. According to Deepthi *et al*.,[19] the microhardness values of CSC such as Biodentine and MTA were reduced in an acidic environment, resulting in a more porous and less crystalline microstructures of these cement. The disagreement between the results of the present study and other studies may be related to differences in the tests employed and the contact time of irrigants.

It is reported that being a weak chelating agent, the effect of CSN on root dentin is less compared to EDTA. Furthermore, it immobilizes the covalent bonds with dentinal collagen leading to the remineralization of root dentin.[20] This phenomenon occurs due to the fact that the phosphate groups attract the calcium ions, causing crystal nucleation and the formation of a calcium phosphate layer,[21] whereas EDTA, on the other hand, is incapable of remineralizing demineralized dentin.[22] Hence, this could be the reason for the highest surface hardness and less roughness of CNS compared to EDTA. Similar to the present study, the effect of EDTA showed lower pushout strength of Biodentine compared to CSN.[23] Lee *et al*.[24] showed that 17% EDTA has adverse effects on the hydration, microhardness, and cell adhesion of CSC.

It is very difficult to say the exact time of irrigation because the effectiveness of an irrigant depends on various factors, such as concentration, composition, temperature, pH, and surrounding environment. However, in the present study, a 5-min application time for all the study irrigants was found to cause fewer adverse effects on root dentin which is in accordance with Ersahan *et al*.[12]

Since no studies were reported regarding the effect of root canal irrigants on the biodentine modifed with TAP, therefore, from the findings of the present study, it can be hypothesized that modified Biodentine with TAP exhibits sufficient microhardness and minimal surface roughness when subjected to 0.2% CSN nanoparticles over other irrigants. The clinical significance of this study was to determine the effect of different irrigants on the microhardness and surface roughness of modified Biodentine with TAP. This helps to estimate which irrigant exhibits drastic effects on this new experimental material. Since this is an *in vitro* study, certain limitations include factors such as small sample size, methods of evaluation, exposure time, and concentration of materials used, and it cannot simulate actual clinical conditions; hence, other *in vivo* studies and *in vitro* studies using other study designs should also be considered in future.

CONCLUSION

Exposure to all the study irrigation solutions, except 0.2% CSN had an adverse effect on surface roughness and microhardness of modified Biodentine with TAP. Therefore, in clinical situations, such as perforation repair with this modified Biodentine with TAP, the use of 0.2% CSN may be favored over other irrigants.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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