Dental Research Journal

Original Article

Effect of indirect ultrasonic activation of modified bioceramic materials on the bond strength and tubular penetration in root canals

Bandaru Yamini[1], Praveen Kumar Gali[1], Bolla Nagesh[1], Sujana Varri[1], Roopadevi Garlapati[1], K. Madhu Kiran Naik[1]

[1]Department of Conservative Dentistry and Endodontics, Sibar Institute of Dental Sciences, Guntur, Andhra Pradesh, India

Received: 31-Jan-2019
Revised: 25-Dec-2019
Accepted: 03-May-2020
Published: 22-Jun-2021

Address for correspondence:
Dr. Praveen Kumar Gali, Department of Conservative Dentistry and Endodontics, Sibar Institute of Dental Sciences, Guntur - 522 509, Andhra Pradesh, India.
E-mail: galibds@gmail.com

ABSTRACT

Background: To maintain the integrity of the interface between root canal filling and radicular dentin an ideal endodontic biomaterial should have good adhesion. This study was aimed to evaluate the bond strength and tubular penetration of modified bioceramic materials by indirect ultrasonic activation.

Materials and Methods: In the present *in vitro* experimental study, 120 coronal root slices of 2 mm were prepared from mandibular premolars and randomly divided into six groups (n = 20) in accordance to placement techniques: Group I: Nano Biodentine-manual, Group II: $CaCl_2$ modified ProRootMTA-manual (MM), Group III: Biodentine-manual, Group IV: Nano Biodentine: Ultrasonic, Group V: $CaCl_2$ modified ProRootMTA-ultrasonic, and Group VI: Biodentine-ultrasonic (BDU). The samples were kept in humidifier for 4 days at 37°C and push out bond strength, sealer penetration were evaluated using an universal testing machine and confocal laser scanning microscope respectively. Data were subjected to statistical analysis using SPSS software by using One-way ANOVA for overall significance and Tukey's multiple *post hoc* test for intergroup comparison ($P < 0.05$).

Results: Highest push out bond strength and greater tubular penetration were observed with Group VI (BDU), while the lowest bond strength and tubular penetration were associated with Group II (MM).

Conclusion: Within the limitations of current study it was observed that Biodentine with indirect ultrasonic activation has resulted in highest pushout bond strength among all the study groups.

Key Words: Confocal laser scanning microscopy, mineral trioxide aggregate, nanomaterials, tricalcium silicates

INTRODUCTION

Endodontic materials should be resistant to dislocating forces such as functional pressure or condensation for good long term prognosis. Since past decade there has been a greater utilization of bioceramic materials in endodontics. Mineral trioxide aggregate (MTA), a calcium silicate based hydraulic cement (CSC) is widely used for perforation repair, root-end filling, pulpotomy, apexification and regenerative procedures. MTA has several desirable properties such as biocompatibility, superior sealing ability, and the ability to set in the presence of moisture and wet environment. However, MTA posses some notable shortcomings such as long

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Yamini B, Gali PK, Nagesh B, Varri S, Garlapati RD, Naik KM. Effect of indirect ultrasonic activation of modified bioceramic materials on the bond strength and tubular penetration in root canals. Dent Res J 2021;18:45.

setting time, poor immediate washout resistance and difficult handling property. In few recent studies, to improve the physicochemical properties of MTA, the addition of calcium chloride ($CaCl_2$) to MTA[1] with the concentration of 2%–15% has been proposed[1] to reduce the setting time of the material[2,3] with similar biocompatibility.[4]

Biodentine (Septodont, Saint Maur des Fosses, France) is a more recent CSC with improved physical properties and reduced setting time as compared to MTA.[1] Biodentine powder is mainly composed of tricalcium silicate, calcium carbonate (filler material) and zirconium oxide (radiopacifier), whilst the liquid consists of $CaCl_2$ (used as a setting accelerator) and a hydro soluble polymer (water-reducing/super plasticizing agent). This biomaterial is considered as a biocompatible and bioactive dentine substitute and has been indicated for coronal and radicular restorations.[1]

Nanotechnology can be beneficial in producing and constructing advanced biomaterials with exclusive biological, chemical and physical properties.[5] In present study Nano Biodentine (NBD) has been included to evaluate its adhesive properties compared to Biodentine.

Indirect Ultrasonic agitation has been proposed as an effective mode of increasing the packability and density of endodontic materials as well as the compressive strength of hydraulic cements.[6]

Till date, a little information is available on the bond strength and adhesive properties of $CaCl_2$ modified MTA and NBD to radicular canal dentin with the indirect ultrasonic activation. This current study evaluates the push out bond strength of $CaCl_2$ modified MTA and NBD with manual placement and indirect ultrasonic activation techniques.

MATERIALS AND METHODS

An *in vitro* experimental study was conducted on recently extracted human mandibular premolars with single root, extracted due to periodontal and orthodontic considerations between July and September 2018 at department of conservative dentistry and endodontics, SIBAR institute of dental sciences, Takkellapadu, Guntur, and Andhra Pradesh, India. Institutional ethical clearance was obtained. The sample size was calculated using Raosoft online sample size calculator and the power of study set was 80%. The criteria for teeth selection included the presence of single rooted teeth with canal curvature between 5° and 10°, the absence of any micro cracks on dentinal walls under stereomicroscope at 4X magnification, absence of internal and external resorption or calcification and with complete formation of root apex. Each tooth was radiographed both buccolingually and mesiodistally to detect any possible calcifications. After a thorough screening, a total of 120 samples were considered for the inclusion in this *in vitro* study.

Specimen preparation

The collected single rooted human mandibular premolars were decoronated at the cementoenamel junction with a diamond disc (Wuxi xiangsheng industrial and trading co.) mounted on a micro motor (NSK Ti-max Nao 95LS) under water coolant. A total of 120 samples were selected, which were sectioned perpendicular to the long axis at mid coronal third of root to attain dentin discs of 2mm thickness using Isomet precision cutter (Buehler Inc). While selecting, these discs were gauged with a customized acrylic gauge for a standardized internal diameter. The root sections were immersed in 17% EDTA (Vista Dental Products, U.S.A) followed by water rinse and then in 2.5% sodium hypochlorite (PrevestDenpro Dental Products, India) each for 1 min to remove the smear layer. They were then cleansed with distilled water in an ultrasonic bath. Later, the root canal surfaces of the samples were dried with a dehydrating solution using absorbent points (Hydrol, septodont).

Experimental procedure

The root sections were randomly assigned into 6 groups:

- Group I: Nano Biodentine-manual (NBDM) (n = 20)
- Group II: ProRoot MTA modified with $CaCl_2$-manual (MM) (n = 20)
- Group III: Biodentine-manual (BDM) (n = 20)
- Group IV: Nano Biodentine-ultrasonic placement (NBDU) (n = 20)
- Group V: ProRoot MTA modified with $CaCl_2$-ultrasonic placement (MU) (n = 20)
- Group VI: Biodentine ultrasonic placement (BDU) (n = 20).

Manipulation

MTA (DentsplyMaillefer, Ballaigues, Switzerland) and Biodentine (Septodont, Saint Maur des Fosses, France) were manipulated according to the manufacturer's instructions. To achieve uniformity while manipulation all the materials used in present

study has been mixed using a capsule attached to a mechanical activator (Ultramat-2). MTA and 5% Calcium chloride were mixed in a 3:1proportionas previously suggested.[1] Nano Biodentine (NBD), obtained by Ball milling of Biodentine in a zirconia ball-mill machine (Gold Belt Global) for 24 h. The particle size of final product has been evaluated and confirmed to be in the range of 2.3–5 nm by Dynamic light scattering (Department of Biochemistry, Osmania University, Hyderabad). To enable the condensation of the materials, the specimens were placed over a tinfoil, which was stabilized on a customized elastomeric block [Figure 1].

Measurement of setting time

All the materials were estimated for setting time as it might be the influencing factor on the bond strength, in plastic cylindrical templates by using stop watch/ tachometer till the disappearance of surface gloss.

Manual condensation

The materials were then delivered into the lumen of the root canal of each specimen in groups I (NBDM), II (MM) and III (BDM) with a MTA Endo gun (DentsplyMaillefer). The specimens were obturated using a stainless endodontic plugger of size #5 which is subjected to 3.22 MPa vertical compression which is frequently evaluated and corrected by a pressure sensitive device (Department of Mechanical Engineering, AcharyaNagarjuna University, Guntur).

Indirect ultrasonic activation

After condensation of each specimen in Groups IV (NBDU), V (MU) and VI (BDU) with hand plugger, the end of the plugger remained in contact with the material while it is indirectly activated for 5 seconds with ultrasonic tip CPR 1 (Dentsply, Tulsa, United States) attached to a P5 NewtronXS™ unit (SATELEC, 4th Gen. Aceton, North America) set on its medium power setting i.e., with frequency value adjusted at 5 [Figure 2]. Excess unset material was removed gently from the surface of the specimens using the scalpel blade. The samples were wrapped in wet pieces of gauze and kept in incubator at 37° and 100% humidity for 96 h to ensure complete setting of the material.

Push out test

Ten samples from each group were evaluated for bond strength of the materials using an Universal Testing Machine (Instron Universal Testing Machine, Model 8801). The samples were mounted on a metal slab with a 1.5-mm central aperture. A cylindrical stainless steel plunger of 1-mm diameter and operating at a speed of 1 mm/min^{-1} was used to apply force on materials inside root slices [Figure 3]. The load applied to the material at the time of displacement was recorded in Newton and later converted to megapascals (MPa).

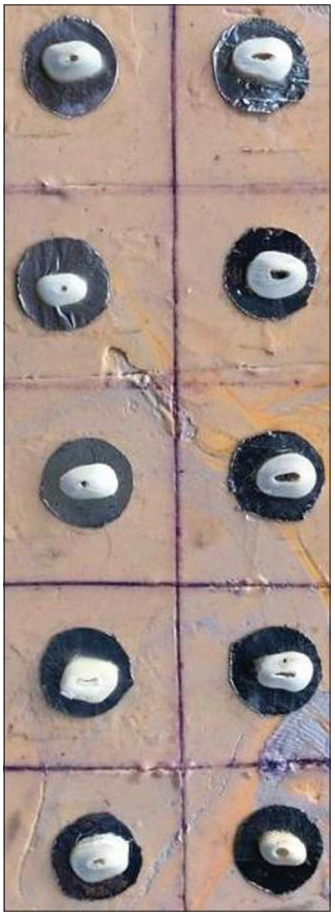

Figure 1: Customized elastomeric block.

Figure 2: Indirect ultrasonic activation against hand plugger remained in contact with the material.

Evaluation of mode of failure

Mode of failure was evaluated by observing under stereomicroscope at 10 X magnification. Mode of failures was identified as cohesive (within either of the filling material, dentin), adhesive (between filling material and dentin) and mixed (both cohesive and adhesive failures) [Figure 4].

Evaluation under confocal laser scanning microscope

Remaining ten samples from each group were evaluated under confocal laser scanning microscope (LSM 880, Zeiss Microscopes, Germany) to assess the extension of root filling material into dentinal tubules [Figure 5]. In order to facilitate analysis, the filling materials were labeled with Rhodamine B (Sigma Aldrich, St. Louis, MO, USA) to an approximate concentration of 0.1%. Samples were examined at 2.5 X (total magnification of 50 X) and 10 X (total magnification of 100 X) magnifications to evaluate the penetration as well as maximum depth of penetration in micrometers and images were analyzed by Ziess LSM 880 image examiner software (ZEN Blue version). For depth of penetration the point of deepest penetration has been considered and recorded in micrometers [Table 1].

Statistical analysis

Mean pushout bondstrength values [Table 2] are obtained and subjected to statistical analysis using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA test was used for evaluation of overall significance ($P < 0.05$) [Table 3] and Pair wise comparison of the groups by Tukey's multiple *post hoc* test [Table 4].

RESULTS

Box plot diagram represents distribution of push out bond strength values with standard deviation [Figure 6]. Push out bond strength was higher in Group VI (BDU), and least with Group II (MM). Intergroup comparison revealed a Statistically significant differenceamong most of the groups ($P < 0.05$) except between Group II versus III, between Group III versus V and between Group I versus II, III, IV, V. Mean push out bond strength values had suggested the bond strength values in the following order:

Group VI (BDU) > Group IV (NBDU) > Group I (NBDM) > Group V (MU) > Group III (BDM) > Group II (MM).

Table 1: Mean depth of penetration of bioceramic materials

Groups (n=10)	Mean depth of penetration (µm)	P
Group I (NBDM)	130.8727	<0.001
Group II (MM)	70.2865	<0.001
Group III (BDM)	190.4952	<0.001
Group IV (NBDU)	382.2743	<0.001
Group V (MU)	256.4572	<0.001
Group VI (BDU)	310.5746	<0.001

One way ANOVA was done to compare the mean depth of penetration between the groups. BDM: Biodentine manual, NBDM: Nano BDM, MTA: Mineral trioxide aggregate, MM: ProRootMTA-manual, MU: ProRootMTA-ultrasonic, BDU: Biodentine-ultrasonic, NBDU: Nano BDU

Figure 3: Universal Testing Machine containing cylindrical stainless steel plunger of 1-mm diameter and operating at a speed of 1 mm/min^{-1} used to apply force on materials, inside root slices.

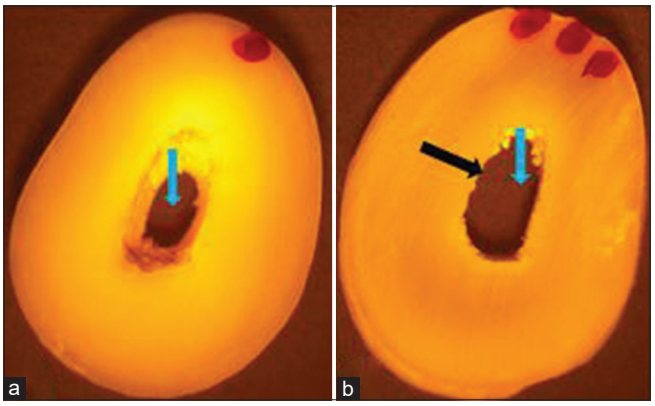

Figure 4: Mode of failures under stereomicroscope. Inspection of samples under a stereomicroscope at ×10: (a) Cohesive failure showing fracture within the obturating material. (b) Mixed mode of failure with cohesive failure observed within the material at some areas and black arrow indicates the adhesive failures between obturating material and tooth interface.

Table 2: Mean, standard deviation, standard error and confidence intervals for bond strength (Mpa) in six groups (1, 2, 3, 4, 5, 6)

Groups	Minimum	Maximum	Mean	SD	SE	95% CI for mean	
						Lower bound	Upper bound
Group 1	6.63	56.09	24.25	16.89	5.34	12.17	36.33
Group 2	6.97	24.88	14.14	5.04	1.59	10.53	17.75
Group 3	18.82	25.48	21.74	2.43	0.77	20.01	23.48
Group 4	14.70	64.22	37.12	14.76	4.67	26.56	47.68
Group 5	2.25	45.65	22.62	13.01	4.11	13.32	31.93
Group 6	70.84	74.96	73.47	1.29	0.41	72.54	74.39

SD: Standard deviation, SE: Standard error, CI: Confidence interval

Table 3: Comparison of six groups (1, 2, 3, 4, 5, 6) with bond strength (Mpa) by one way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*F*	*P*
Between groups	5	23,174.22	4634.84	39.4355	0.0001*
Within groups	54	6346.61	117.53		
Total	59	29,520.82			

*$P<0.05$

Table 4: Pairwise comparison of six groups (1, 2, 3, 4, 5, 6) with bond strength (Mpa) by Tukeys multiple *post hoc* procedures

Groups	Mean difference	SE	*P*	95% CI	
				Lower bound	Upper bound
Group 1-Group 2	10.11	4.85	0.3100	−4.21	24.44
Group 1-Group 3	2.51	4.85	0.9950	−11.81	16.84
Group 1-Group 4	−12.87	4.85	0.1020	−27.19	1.46
Group 1-Group 5	1.63	4.85	0.9990	−12.69	15.96
Group 1-Group 6	−49.21	4.85	0.0001*	−63.54	−34.89
Group 2-Group 3	−7.60	4.85	0.6230	−21.93	6.72
Group 2-Group 4	−22.98	4.85	0.0001*	−37.31	−8.66
Group 2-Group 5	−8.48	4.85	0.5060	−22.81	5.84
Group 2-Group 6	−59.33	4.85	0.0001*	−73.65	−45.00
Group 3-Group 4	−15.38	4.85	0.0290*	−29.70	−1.05
Group 3-Group 5	−0.88	4.85	1.0000	−15.20	13.45
Group 3-Group 6	−51.72	4.85	0.0001*	−66.05	−37.40
Group 4-Group 5	14.50	4.85	0.0460*	0.18	28.82
Group 4-Group 6	−36.34	4.85	0.0001*	−50.67	−22.02
Group 5-Group 6	−50.84	4.85	0.0001*	−65.17	−36.52

*$P<0.05$. SE: Standard error, CI: Confidence interval

DISCUSSION

In the present study, the dentin discs of 2 mm thickness were used for the purpose of push out test measurement. Over estimation of the bond strength may occur if thickness of discs is Increased due to increase the area of friction.[7] The chemical and physical properties play a major role in the clinical outcome of root canal filling materials which can be affected by factors such as mixing and placement techniques, delivery system, exposure to various clinical environments, storage conditions and the ratio of the constituent components.[8] To fill voids and spaces between the obturant and the tooth structure and to form a fluid tight seal, the root canal filling materials are used with the semisolid or solid core filling material to provide the required adhesion.[9] In contemporary endodontics the concept of monoblock has increased the potential of creating good adhesion in root canal walls.[10,11] In the present study all the materials have only one interface that extends circumferentially between the material and the root canal wall which is a primary monoblock.

Hydraulic cements are finely ground materials (powders), yielding complex hydrated products when mixed with water or specific water based component. Any interference in hydration reaction might influence the biological, chemical, and physical properties of the resulting productwith altered clinical performance.[12]

The conventional Proroot MTA and Bio dentine have been modified in present study by the addition of $CaCl_2$ and Nanoparticularization respectively.

The addition of $CaCl_2$ at the concentrations of 2%–15% improves physicochemical properties of MTA and shortens the setting time. However, the shortcomings of this modified material include reduced expansion, coherence which might influence the bond strength with radicular dentin. However, considering the advantages of addition of calcium chloride such as shortened setting time and increased biomineralization,[13] altering the mode of placement

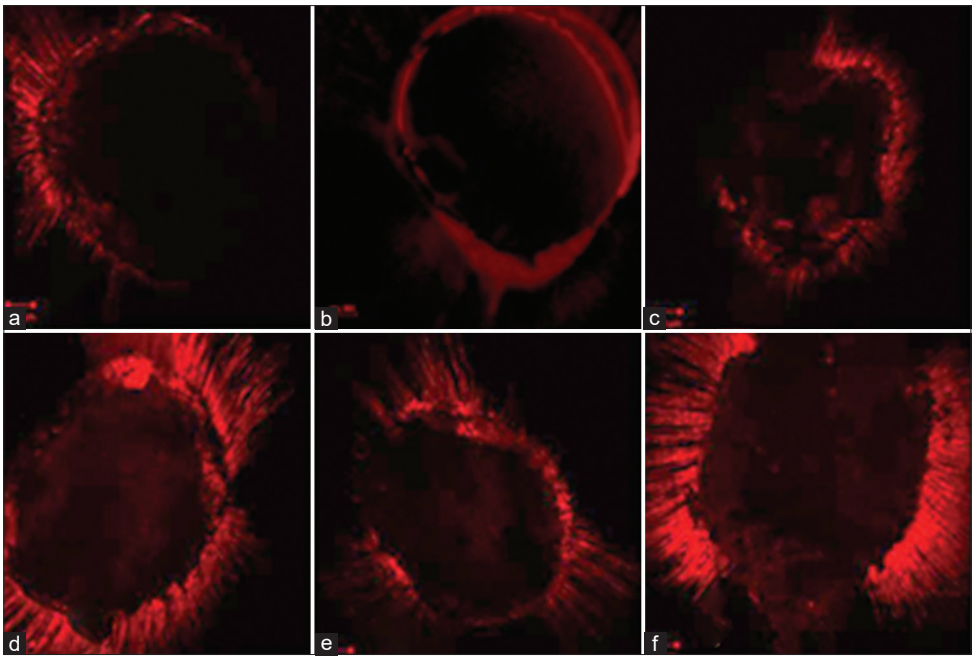

Figure 5: Tubular penetration under confocal laser scanning microscope. Images showing lateral extension of bioceramic material in the root sections when observed under Confocal Laser scanning microscope (LSM 880, zeiss microscopes, Germany) under 10X zoom (total magnification of 100X). (a) NBDM; (b) MM; (c) BDM; (d) NBDU; (e) MU; (f) BDU. BDM: Biodentine-manual, NBDM: Nanobiodentine manual, MTA: Mineral trioxide aggregate, MM: ProRootMTA-manual, MU: ProRootMTA-ultrasonic, BDU: Biodentine-ultrasonic, NBDU: Nano biodentine-ultrasonic.

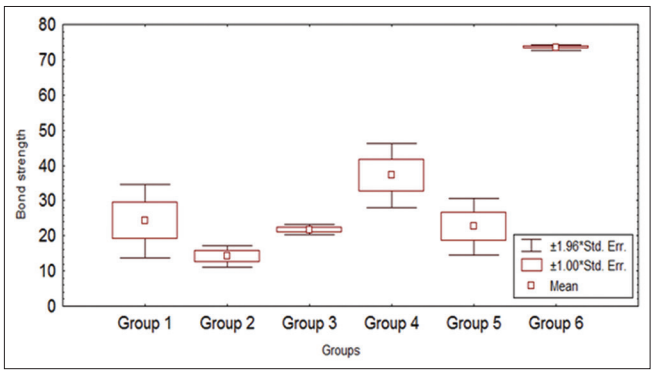

Figure 6: Box plot diagram represents distribution of push out bond strength values with standard deviation.

might aid in compensating the reduced bond strength with root canal dentin. Being a viable tool in endodontics, indirect ultrasonic agitation reduces the size of cement particles and consequently increases the total reactive surface by de-clustering the particles that are clogged to each other[14] resulting in increased flow, setting, and compaction of the material.[15] In the current study, Ultrasonic activation of $CaCl_2$ modified MTA resulted in better bond strength even superior to conventional MTA in accordance with the previous study. Besides ultrasonic placement, presence of nanoparticles also has a positive impact on the penetration of material into the dentinal tubules. There are other factors that might be able to influence the capacity of dentinal tubule penetration of the endodontic filling material: The surface activity of the sealers, the contact angle between sealer and the dentinal walls, the diameter of the opened dentinal tubules and the employed obturation technique.[16]

Materials with Nano particles have better workability, fluidity and positive influence on hydration process resulting in efficient filling of root canal. In present study, NBD demonstrated increased chemical reactivity is due to higher solubility and surface area as observed by shortened setting time compared to conventional Biodentine. This is substantiated by a fact that a given mass of material in nanoparticle form is much more reactive than the same mass of material made up of larger particles.[17] It was demonstrated that the more the material is soluble, the higher OH^- and Ca^{2+} release which is correlated to the higher solubility recorded for BD. This had led to more Ca^{2+} from nanomaterials.[18] Setting accelerator effect of the nanoparticles acts as seeds and stimulates the nucleation of calcium silicate, accelerating the hydration process (sowing effect), also helps in accelerating the setting time and hydration process.[19]

In the present study, the indirect ultrasonic mode of placement had a better bond strength value than

manual with a statistically significant difference. It was observed that Group VI (BDU), has shown highest bond strength values among all other groups. The effective combination of the unaltered hydration which enables the extension of material into the dentinal tubules with indirect ultrasonic activation has resulted in this superior bond strength. However the tubular penetration is lower than that of nanobiodentine with ultrasonic activation which might be due to less particle size of nanobiodentine than Biodentine thus enabling greater penetration. The reduced bond strength of NanoBiodentine compared to Biodentine could be due to the altered density, particle scattering leading to altered hydration characteristics. In the present study, the mean push out bond strength of Group III (BDM) and Group V (MU) had shown similar results with no statistically significant difference, suggestive of ultrasonic activation of MTA could achieve similar results in terms of bond strength even though there is difference in tubular penetration. Except for Groups IV (NBU) and VI (BDU) tubular penetration values are proportionate to push out bond strength values in all the groups of the present study. The greater tubular penetration of Group IV (NBDU) might be due to smaller particle size of nanobiodentine which is further enhanced by indirect ultrasonic activation. However, as previously described it altered the hydration properties resulting in lower bond strength. It was observed that majority of the failures associated with all the groups are of cohesive nature which suggests that the interface between filled material and root dentin was more durable than core material itself. At present the available literature is not sufficient to ensure the long term performance of the bioceramic materials used in the present study. Further research is needed to evaluate the clinical longevity of these modified bioceramic materials.

CONCLUSION

Within the practical limitations of the current *in vitro* study, it can be concluded that indirect ultrasonic activation has resulted in superior interfacial bond strength and tubular penetration than the manual compaction. A combination of Biodentine with indirect ultrasonic activation has resulted in superior bond strength compared to all other groups. On the other hand, manual condensation of MTA has demonstrated the lowest bond strength values. Having greater tubular penetration, Nanobiodentine appears to be a promising root canal filling material.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

REFERENCES

1. Küçükkaya Eren S, Aksel H, Serper A. Effect of placement technique on the push-out bond strength of calcium-silicate based cements. Dent Mater J 2016;35:742-7.
2. Ber BS, Hatton JF, Stewart GP. Chemical modification of proroot MTA to improve handling characteristics and decrease setting time. J Endod 2007;33:1231-4.
3. Wiltbank KB, Schwartz SA, Schindler WG. Effect of selected accelerants on the physical properties of mineral trioxide aggregate and Portland cement. J Endod 2007;33:1235-8.
4. Jafarnia B, Jiang J, He J, Wang YH, Safavi KE, Zhu Q. Evaluation of cytotoxicity of MTA employing various additives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:739-44.
5. Sharaan M, Fayyad D. Nanoparticles: Do they affect the physicochemical properties of biomaterials of biodentine and bioactive glass? ENDO (LondEngl) 2017;11:1-8.
6. Basturk FB, Nekoofar MH, Günday M, Dummer PM. The effect of various mixing and placement techniques on the compressive strength of mineral trioxide aggregate. J Endod 2013;39:111-4.
7. Paulson L, Ballal NV, Bhagat A. Effect of root dentin conditioning on the pushout bond strength of biodentine. J Endod 2018;44:1186-90.
8. Nekoofar MH, Aseeley Z, Dummer PM. The effect of various mixing techniques on the surface microhardness of mineral trioxide aggregate. Int Endod J 2010;43:312-20.
9. Rajput JS, Jain RL, Pathak A. An evaluation of sealing ability of endodontic materials as root canal sealers. J Indian Soc Pedod Prev Dent 2004;22:1-7.
10. Belli S, Eraslan O, Eskitascioglu G, Karbhari V. Monoblocks in root canals: A finite elemental stress analysis study. Int Endod J 2011;44:817-26.
11. Sophia T, Deepak BS, Deepa J, Mallikarjun GK. The concept of monobloc in Endodontic – A review. CODS J Dent 2014;6:83-7.
12. Darvell BW, Wu RC. "MTA"-an hydraulic silicate cement: Review update and setting reaction. Dent Mater 2011;27:407-22.
13. Reyes-Carmona JF, Felippe MS, Felippe WT. The biomineralization ability of mineral trioxide aggregate and Portland cement on dentin enhances the push-out strength. J Endod 2010;36:286-91.
14. Yeung P, Liewehr FR, Moon PC. A quantitative comparison of the fill density of MTA produced by two placement techniques. J Endod 2006;32:456-9.
15. Matt GD, Thorpe JR, Strother JM, McClanahan SB. Comparative study of white and gray mineral trioxide aggregate (MTA) simulating a one- or two-step apical barrier technique. J Endod 2004;30:876-9.
16. Shah K. Microbial influence on the development of periapical

disease-literature review. J Dent Med Sci 2015;14:48-56.
17. Mitra SB, Wu D, Holmes BN. An application of nanotechnology in advanced dental materials. J Am Dent Assoc 2003;134:1382-90.
18. Saghiri MA, Asatourian A, Orangi J, Lotfi M, Soukup JW, Garcia-Godoy F, *et al*. Effect of particle size on calcium release and elevation of pH of endodontic cements. Dent Traumatol 2015;31:196-201.
19. Camiletti J, Soliman AM, Nehdi ML. Effect of nano calcium carbonate on early-age properties of ultra-high performance concrete. Mag Concrete Res 2013;65:297-307.