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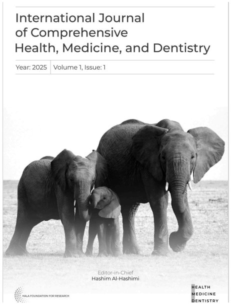

Original Article

Effect of Various Surface Treatments on Micromorphology of MTA Plus® and Biodentine® and Comparative Evaluation of Their Shear Bond Strength to Composite Resin- an invitro SEM analysis

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Abstract

Introduction: To evaluate the micromorphological changes due to different surface treatments on MTA Plus® and Biodentine® and comparison of their shear bond strength to composite resin with three different adhesive systems. Methods: Eighty acrylic blocks with a standardized central hole were prepared and filled with MTA Plus (Group A) and Biodentine (Group B). They were randomly divided into four subgroups each; A1, B1: Control groups (untreated samples); A2, B2: One-step self- etch adhesive; A3, B3: Two-step self-etch adhesive; A4, B4: Two-step etch and rinse adhesive. Surface treatment was performed using phosphoric acid or self-etch primer, and untreated surfaces were used as controls. The surface changes were observed under a scanning electron microscope. The specimens were bonded with the respective adhesive systems followed by composite resin restoration. The shear bond strength was tested using universal testing machine and the data was subjected to one-way ANOVA and Tukey's multiple comparison test. Results: Extensive loss of globular structure was seen in phosphoric acid treated group while only selective loss was observed in samples treated with self-etch primer. Treated surfaces of MTA Plus showed more internal pores and cracks as compared to treated Biodentine surfaces. The one-step self-etch adhesive system showed significantly higher shear bond strength when compared with other groups ($p < 0.05$). MTA Plus exhibited better shear bond strength as compared to Biodentine, which was statistically non-significant ($p > 0.05$). Conclusions: Either surface treatments cause alteration in micromorphology of MTA- Plus and Biodentine. One-step self-etch adhesives could be a preferred choice when bonding composite to Biodentine or MTA Plus.

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Introduction

The current concepts in endodontics and in management of deep carious lesions are based on pulp protection, its preservation and regeneration (Ford TR et al, 1996). Calcium hydroxide has been widely used for pulp protection due to its ability to form dentin bridge. However, due to its shortcomings such as high solubility, poor adaptation to dentin and formation of dentin bridge with multiple tunnel defects, various calcium silicate-based materials have been introduced (Nowicka A, 2013). Mineral trioxide aggregate (MTA) has been used as a predictable material for various procedures like direct pulp capping, pulpotomy, perforation repair and as a retrograde root-end filling material because of its desirable properties such as biocompatibility and setting in the wet environment (Parirokh M et al 2010) (Torabinejad M et al 2010). However, its long setting time, difficult handling and potential of discoloration do not allow its immediate bonding to the composite resin in cases of pulp capping and perforation repairs (Parirokh M et al, 2010). Hence various modifications have been made in MTA formulations to overcome these shortcomings. Originally, ProRoot MTA was introduced but was later modified to overcome the problem of discoloration. Hence White MTA evolved which differed from the gray MTA in the absence of iron. (Asgary S et al, 2005) The prolonged setting time was still a problem; hence MTA Angelus was introduced whose setting time was 10 minutes as compared to 165 minutes for ProRoot MTA (Torabinejad M et al, 1995). A newer modification of MTA has been developed and marketed as MTA Plus. It is claimed to have finer particle size with better anti-washout resistance and has been shown to set in relatively shorter duration (Formosa LM et al, 2013) (Camilleri J et al, 2013).

Other calcium silicate-based materials have also gained popularity since they resemble MTA in their properties. These include biodentine, bioaggregate, calcium-enriched mixture etc. Biodentine is a newer tricalcium silicate-based material which has been promoted as a “bioactive dentin substitute”. It has also been claimed to have better physical and biological properties than other calcium silicate-based materials (Grech L et al, 2013). It has shorter setting time, good sealing ability, biocompatibility and remineralization properties (Zhou HM et al, 2013). Thus, shorter setting times, better handling properties and potential to avoid discoloration of MTA Plus and biodentine may allow them to be used more predictably as dentin substitute when immediate bonding with composite resin is required.

The bonding of these bioactive materials to composite resin is as important as their bonding with underlying dentin surface for successful outcome of various procedures such as vital pulp therapy and perforation repairs. Various types of adhesive systems have evolved to allow for more predictable bonding of composite resin with underlying dentin or dentin substitutes. The conventional two-step etch and rinse systems involve the application of phosphoric acid prior to the adhesive application. Recently, two step and one step self-etch adhesive systems have also been introduced which differ in their pH, nature of solvent and the filler content. (Atash R et al, 2005) These factors may affect the surface micromorphology of bioceramic materials and hence may have an influence on their shear bond strength to composite resin (Shin JH et al, 2014). There are several studies on the bond strengths of composite resin with MTA using different adhesive systems (Bayrak S et al, 2009) (Altunsoy M et al, 2015); however not many studies have been reported on the surface interaction of different adhesive systems with biodentine and MTA Plus and their effect on shear bond strengths with composite resin.

The aim of this study was to evaluate the shear bond strengths of biodentine and MTA Plus to composite resin with different adhesive systems and to analyze the micromorphologic changes because of various surface treatments on biodentine and MTA Plus using scanning electron microscope.

Materials and Methods

Sample preparation

Eighty cylindrical acrylic blocks with a central hole of 4 mm diameter and 2 mm height were prepared and randomly divided into two groups (n=40). MTA Plus and biodentine were mixed according to the manufacturer's instructions (Table 1). The holes in the acrylic blocks were filled with the respective materials using a spatula and were covered with wet cotton pellets and temporary filling material (Cavit; 3M ESPE, St. Paul, MN, USA) for initial setting. The specimens were stored at 37 degrees Celsius at 100% humidity till initial setting time for each material, which is <1hr for MTA Plus and approximately 12 mins for Biodentine (Table 1-*SDS sheets) After initial set, the temporary material was removed, and the surfaces of materials were neither rinsed nor polished.

The two groups were randomly divided into three subgroups each based upon the type of adhesive system used for bonding composite (n=10). The three commercial adhesive systems namely Tetric-N-Bond Universal (TBU) (Ivoclar Vivadent, Lichenstein Asia), Clearfil SE Bond (CSE) (Kuraray, Osaka, Japan), Adper Single Bond 2 (ASB) (3M ESPE, St. Paul, MN, USA), were applied according to the manufacturers' instructions (Table 1). The fourth subgroup in each group was left untreated (control, n=10). A plastic tube with an internal diameter of 2 mm and 2 mm height was applied to bonded specimen prior to curing the adhesive and then light cured for 30 seconds. The plastic tube was filled with resin composite (Filtek Z350, 3M ESPE, St. Paul, MN) and light cured (Monitex, Taiwan) for 40 seconds. The tube was left in place throughout the testing process. The specimens were stored in 100% humidity for 24 hours at 37degrees Celsius.

Table 1:

MATERIAL	MANUFACTURER	COMPOSITION	APPLICATION TECHNIQUE
MTA Plus* (Group A)	Prevest Denpro, Jammu, India for Avalon Biomed Inc., Bradenton, FL, USA	Powder: tricalcium silicate, dicalcium silicate, bismuth oxide, tricalcium aluminate, calcium sulphate, finely ground Portland Liquid: sterilized/ distilled water/ anti wash-out gel	Mix in a water/ cement ratio of 0.35 into a putty like consistency
Biodentine** (Group B)	Septodont, Saint Maur-des- Fossees Cedex, France	Powder: tricalcium silicate, dicalcium silicate, calcium carbonate and oxide filler, iron oxide shade, zirconium oxide	Mixing premeasured unit dose capsules of liquid and powder in a high speed amalgamator for 30 seconds

		Liquid: calcium chloride, hydrosoluble polymer as a water reducing agent	
Filtek Z350***	3M ESPE, St. Paul, MN, USA	BIS-GMA, BIS-EMA , UDMA with small amounts of TEGDMA. • The filler contains a combination of a non-agglomerated/non-aggregated, nanosilica filler, and loosely bound agglomerated zirconia/silica nanocluster	Place in increment of 2mm and light cure for 20 seconds
Tetric-N-Bond Universal ****(pH=2.5-3) (TBU) (Subgroup A2, B2)	Ivoclar Vivadent, Lichenstein Asia	Methacrylates MCAP, D3MA, HEMA, Bis-GMA,MDP,Water, Ethanol,Highly dispersed silicon dioxide ,Initiators and Stabilisers	One-step self etch system 1. Dry the surface 2. Apply and scrub the adhesive for 20 seconds 3. Gentle air stream 4. Light cure for 10 seconds
Clearfil SE Bond *****(pH=2.0) (CSE) (Subgroup A3, B3)	Kuraray, Osaka, Japan	Primer: MDP, HEMA, photoinitiator, dimethacrylate resin, water Adhesive: MDP, HEMA, photoinitiator, dimethacrylate resin, BisGMA, colloidal silica	Two-step self etch system 1. Dry the surface 2. Apply primer for 20seconds 3. Gentle air stream 4. Apply the adhesive 5. Light cure for 10 seconds
Adper Single Bond 2 ******(ASB) (Subgroup A3, B3)	3M ESPE, St. Paul, MN, USA	Etchant: 35% Phosphoric acid (pH= 0.6) Adhesive: bis-GMA, HEMA, dimethacrylates, silica nanofiller, ethanol, water, photoinitiator,	Two step etch and rinse system 1. Apply etchant for 15 seconds

		methacrylate functional copolymer of polyacrylic and polyitaconic acids	2. Rinse with water for 10 seconds and blot dry 3. Apply 2 consecutive coats of adhesive for 15 seconds with gentle scrubbing 4. Gentle air stream for 5 seconds 5. Light cure for 10 seconds

Materials used in this study

TBU- Tetric-N-Bond Universal; CSE- Clearfil SE Bond; ASB- Adper Single Bond 2; HEMA- hydroxy ethyl methacrylate; D3MA- decandiol dimethacrylate; bis-GMA- bisphenol A- glycidyl dimethacrylate; MCAP- methacrylated carboxylic acid polymer; UDMA- diurethane dimethacrylate; TEGDMA- triethylene glycol dimethacrylate; MDP- 10-methacryloyloxydecyldihydrogenphosphate; bis-EMA- bisphenol A- polyethylene glycol diether dimethacrylate; SEP- self-etch primer

*www.AvalonBiomed.com/mta_plus/

**www.septodontusa.com/product/biodentine/

***filtek technical product file, 3M ESPE

****Safety data sheet/ivoclar vivadent/tetric-n-bond-en/

*****SDS at kuraraydental.com

******dentalsafetydatasheets.com/3m-espe-adper-single-bond-2/

Shear Bond Strength Testing

The specimens were mounted in the Universal testing machine. A knife-edged loading head was placed at the biodentine/ MTA Plus and composite interface and stressed in shear at a rate of 1 mm/min. The maximum load at failure was recorded and converted to Megapascals (MPa) by dividing the peak load at failure by the specimen surface area.

Shear bond strength (MPa) = $F\ (N)/\pi\ r^2$

Scanning Electron Microscopic Analysis

New samples were again prepared as described previously for both biodentine and MTA Plus and stored at 37 degrees Celsius at 100 % humidity till initial setting time. The samples were then randomly divided into three subgroups each according to the surface treatment done. The control group- untreated samples; phosphoric acid-treated group- 37% phosphoric acid was applied to the samples for 15 seconds, followed by rinsing with water for 15 seconds and then air-dried; self-etch primer group- self-etch primer (SEP (CSE/ TBU)) was applied to the samples for 15 seconds, followed by rinsing with water for 15 seconds and then air-dried. The specimens were then gold sputtered and observed under a scanning electron microscope. Photomicrographs of biodentine and MTA Plus specimens were recorded at 600 X and 1000 X magnification.

Statistical Analysis

The data of shear bond strength was subjected to one way analysis of variance and Tukey's multiple post-hoc test. The p-value was set at < 0.05 for significant difference.

Results

Shear Bond Strength Test

The shear bond strength values for biodentine and MTA Plus with different adhesive systems are shown in Table 2. Groups with different letters on the superscript are significantly different from each other (p< 0.05).

Table: 2

Groups	Subgroup 1	Subgroup 2	Subgroup 3	Subgroup 4
Group A (MTA Plus)	A1- TBU	A2- CSE	A3-ASB	A4- Untreated
	18.7 ± 2.99^{c}	11.6 ± 1.32^{a}	12.8 ± 1.29^{a}	8.1 ± 1.60^{b}
Group B (Biodentine)	B1- TBU	B2-CSE	B3-ASB	B4-Untreated
	17.9 ± 0.99^{c}	10.7 ± 1.32^{a}	11.5 ± 1.25^{a}	7.86 ± 1.60^{b}

Mean and standard deviations of shear bond strength values of adhesive systems to biodentine and MTA Plus

Groups with same letters on the vertical are not significantly different from each other (p>0.05)

When comparing the different adhesive systems, TBU showed significantly higher shear bond strength as compared with CSE and ASB. Shear bond strengths were relatively higher for MTA Plus subgroups but were not significantly different from biodentine subgroups. Etch and rinse adhesive system (ASB) exhibited relatively higher bond strength values in comparison with two-step self- etch system (CSE), though there was no significant difference.

Scanning Electron Microscopic Analysis

There were distinct differences in the surface micromorphology of MTA Plus and biodentine after the mentioned surface treatments as depicted in Figures 1, 2 and 3. SEM images of untreated surface of MTA Plus exhibited intense, large globular structures which appear to agglomerate together loosely (Fig. 1a) while untreated biodentine surfaces showed a dense microstructure and a crystalline appearance (Fig. 1b). The surface structure of biodentine was less porous when compared with MTA Plus. The phosphoric acid-treated MTA Plus and biodentine surfaces showed remarkable loss of globular structure (Fig. 2a, 2b) but with deeper internal pores in MTA Plus surfaces (Fig. 2a). Cracks could be observed on MTA

Plus surface treated with phosphoric acid but not in biodentine. In SEP–treated group, there was only selective loss of globular structure in MTA Plus (Fig. 3a) while biodentine exhibited hexagonal structures due to partially eroded crystalline structure (Fig. 3b).

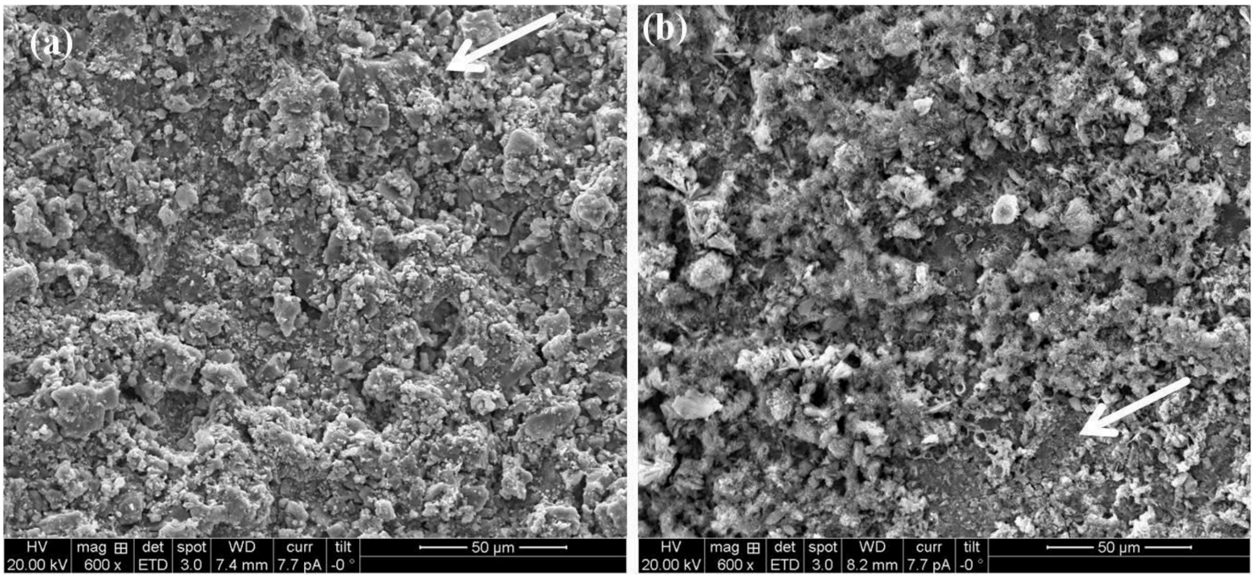

Figure 1: Scanning electron microscopic micrographs of untreated MTA Plus and Biodentine surfaces (a) Untreated MTA Plus surface with dense globular microstructure; bright white particles representing radiopacifier phase (arrowheads) (600x) (b) Untreated biodentine surface with less intense globular microstructure; needle-like crystalline appearance (arrowheads) (600x)

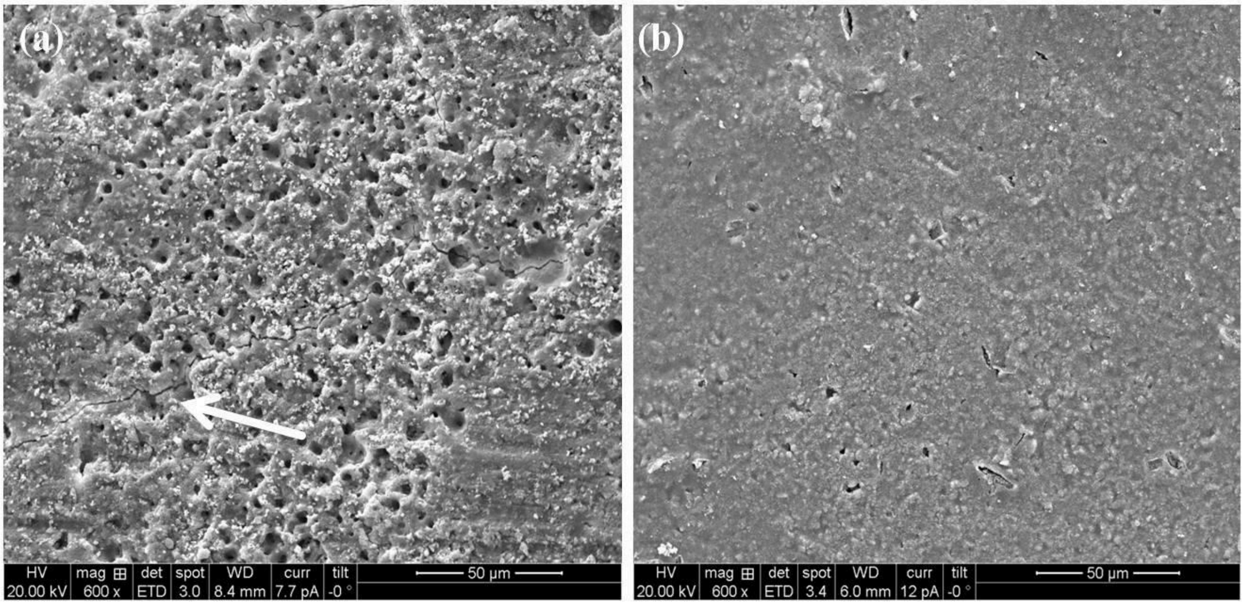

Figure 2: Scanning electron microscopic micrographs of phosphoric acid- treated MTA Plus and Biodentine surfaces (a) MTA Plus - Severe loss of globular structure with formation of deep pores and cracks (arrowheads) (600x) (b) Biodentine- Loss of globular structure with formation of pores; loss of crystalline appearance (600x)

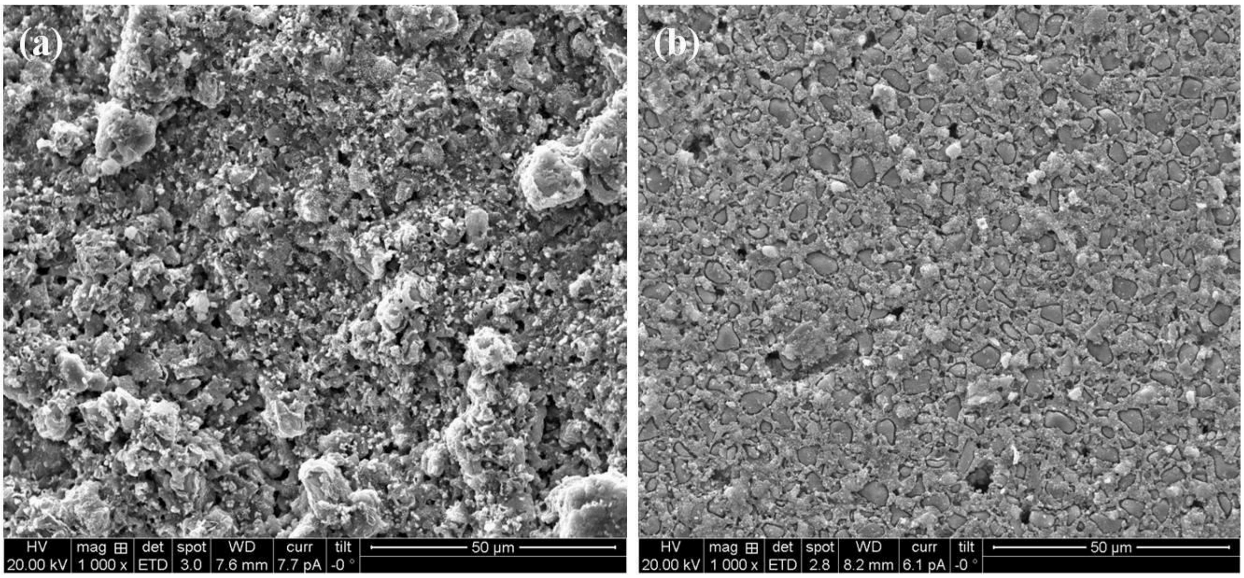

Figure 3: Scanning electron microscopic micrographs of self-etch primer- treated MTA Plus and Biodentine surfaces (a) MTA Plus- Selective loss of superficial globular structure with formation of porous surface (1000x) (b) Biodentine- Selective loss of crystalline appearance with formation of hexagonal microstructure (1000x)

Discussion

MTA Plus and biodentine are the newer calcium silicate materials which have been introduced to overcome the disadvantages of mineral trioxide aggregate (Formosa LM et al, 2013) (Camilleri J et al, 2013) (Zhou HM et al, 2013). These materials have also exhibited suitable bioactivity and biocompatibility and hence their use has extensively increased in clinical situations such as vital pulp therapy, perforation repairs where immediate bonding with a resin-based restorative material is required (Cornélio AL et al, 2015) (Tran XV et al, 2012). There are not many studies that have investigated the shear bond strengths of MTA Plus and biodentine with composite resin (Altunsoy M et al, 2015) (Odabas ME et al, 2013). Most of the published data has investigated the shear bond strengths of MTA and its other variants (Shin JH et al, 2014) (Bayrak S et al, 2009) (Altunsoy M et al, 2015) (Neelakantan P et al, 2012). There have been reports which stated that the morphologic changes due to surface treatments with phosphoric acid or self-etch primer affect the bond strengths of MTA (Shin JH et al, 2014). However, the effect of these surface treatments on MTA Plus and biodentine remains unclear.

In our study, the untreated surface of MTA Plus exhibited intense, large globular structures which appear to agglomerate together loosely representing more reaction by-product (Fig. 1a). The composition was not tested in this study, however based on study by Camilleri (Camilleri J, 2015), it could be deduced that these large globular deposits were of calcium phosphate. This may be due to the fine particle size in MTA Plus that enable more reaction product formation in lesser time (Guven Y et al, 2015). As mentioned by Camilleri et al (Camilleri J et al, 2013), the specific surface area of MTA Plus was found to be 1.5366 while that of biodentine and ProRoot MTA were 2.811 and 0.9822 m^2/g respectively. (Camilleri J et al, 2013b) The unetched initial set biodentine appeared as a dense microstructure with crystalline appearance but with less microporosity. It exhibited some needle-like structures at the periphery of globular masses (Fig. 1b). Biodentine has been reported to be denser and less porous than MTA (Camilleri J et al, 2013b). This is in agreement with the study by DeSouza et al (De Souza ET et al,

2013) who found the mean porosity percentage for Biodentine as 7.09+/- 1.87 while that of MTA as 6.65+/- 1.93. This set biodentine consists of 5 microns round particles embedded in a calcium silicate hydrate matrix, the porosity is almost filled by calcium silicate hydrate and calcium hydroxide (Camilleri J, 2013) (Rajasekharan S et al, 2014). The untreated surfaces of both MTA Plus biodentine and exhibited bright white particles as the radiopacifier phase which showed more contrast in the microphotographs. As mentioned by Camilleri, these may be clusters rich in bismuth and oxygen in MTA Plus while biodentine may exhibit zirconium and oxygen (Camilleri J, 2015).

Alterations in surface micromorphology of MTA Plus and biodentine after application of 37% phosphoric acid or self-etch primer were observed under a scanning electron microscope. The phosphoric acid treated samples included surface conditioning by ASB while self-etch primer treated samples included surface conditioning by TBU and CSE. In this study, the surfaces of materials were rinsed with water even after application of self-etch primer to allow recognition of respective tested bioceramic materials under SEM.

MTA Plus surface treated with phosphoric acid exhibited erosion of the globular structure (Fig. 2a). There was loss of globular masses with formation of deep pores and cracks which may suggest more loss of calcium from the hydrated matrix. This is possibly due to the more destructive nature because of low pH of phosphoric acid (Table 1). In agreement with our findings, similar alterations in micromorphology were reported after phosphoric acid treatment on MTA surface (Shin JH et al, 2014). The SEP-treated MTA Plus surface also exhibited loss of globular structure but less intense than that observed on phosphoric acid treated surface. A regular rough, porous surface was observed with no cracks (Fig. 3a) which could be a possible reason for a better shear bond strength obtained in our study with one-step self-etch adhesive.

Biodentine surface treated with phosphoric acid exhibited loss of globular masses like MTA Plus. The surface was however less porous without any crack formation (Fig. 2b). This might be due to more acid-resistant surface of Biodentine (Laurent P et al, 2005) and a possible explanation for MTA Plus showing relatively more shear bond strengths than biodentine after phosphoric acid treatment. The SEP treated biodentine surface exhibited hexagonal structures throughout the surface due to selective loss of matrix reinforcing its acid-resistant nature (Fig. 3b). The surface treatment with a weaker acid in SEP causes less alteration in MTA Plus and biodentine structure while creating a uniformly porous and slightly remaining crystalline structure that provided a better bond with the composite resin.

The highest bond strength was observed with TBU (one-step self-etch) as compared with other adhesives. This could be attributed to the higher pH of TBU (Table 1) as compared with CSE and ASB(https:/www.asia.ivoclarvivadent.com/),(https:/www.multimedia.3m.com/),(https:/www.kuraray-dental.eu/). The solvent in TBU is both ethanol and water while CSE contains water only leading to incomplete polymerization of monomers in CSE and a better wettability with TBU (https:/www.asia.ivoclarvivadent.com/), (https://www. multimedia.3m.com/), (Jacobsen T et al, 1995). The water/ethanol solvents and the integrated micro-fillers used in TBU enhance its penetration into the pores created after mild etching. This finding corresponds to the results of the study by Carvalho et al (Carvalho CN et al, 2015) and Mortazavi et al (Mortazavi V et al, 2012) who found better bond strengths with filled adhesive systems. The presence of highly dispersed silica in TBU may be the reason for its better shear bond strength. Further, the TBU matrix is based on a combination of monomers of hydrophilic (HEMA), hydrophobic (D3MA) and intermediate (bis-GMA) nature. This combination of properties allows it to reliably bridge the gap between the hydrophilic substrate and the hydrophobic resin restorative, enabling better bond strengths. The presence of many carboxylic acid groups along a polymeric chain in MCAP allows multiple bonds to the substrate (https://www.asia.ivoclarvivadent.com/).

The results of our study are similar to the recent study by Neelakantan et al (Neelakantan P et al, 2012) which exhibited better shear bond strength with one-step self-etch adhesive as compared with other adhesives. This was explained by the author due to the higher pH of the adhesive and the ethanol and water present as solvents which might have increased the wettability of the adhesive. However, our results are contrary to the study by Altunsoy et al (Altunsoy M et al, 2015) who found lower bond strength of composite to biodentine with one-step self-etch adhesive. This could however be due to surface alteration of biodentine by polishing with 400-grit sandpaper before surface treatment with SEP.

The values of shear bond strengths obtained with etch and rinse system (ASB) were slightly higher than with two step self-etch system (CSE), though were statistically not significant (p>0.05). This could be due to the composition of the CSE which is a water-based adhesive. This water may get trapped in the material and its vapor pressure may be lowered due to the presence of high amount of HEMA, thereby preventing its removal before polymerization (Pashley EL et al, 1998). We also speculate that etch and rinse system may cause better surface alteration as compared with CSE that had resulted in relatively higher bond strength values with ASB.

Based upon the findings of our study, we assume that the bond strength of calcium silicate materials to composite is significantly affected by the alterations in the surface micromorphology according to various types of surface treatments. We have evaluated a newer material MTA Plus which has a greater rate of reaction as compared with other MTA cements. This has been investigated by various authors who found a high portlandite peak in MTA Plus, suggesting greater rate of reaction due to finer particle size of the cement (Camilleri J et al, 2013) (Guven Y et al, 2014). This was compared with biodentine that has a shorter initial set time (http://www.septodontusa.com), (Bachoo IK et al, 2013). The bond strengths of MTA Plus and biodentine were found to be comparable in our study. MTA Plus exhibited relatively higher bond strength values as compared with biodentine which however was statistically non-significant (p>0.05). This can be due to the formation of more porous structure in MTA Plus after surface treatment that enabled greater mechanical interlocking with the composite. Further, MTA Plus gel enables the powder to set faster and be wash-out resistant within 5 minutes (MTA-Plus Directions for use 35).

When compared with MTA, various studies have reported less shear bond strength when composite resin is bonded with freshly placed MTA (Tunc ES et al, 2008) (Atabek D et al, 2012). This has been suggested due to the inadvertent effects on setting time and risk of dissolving MTA after etching or irrigation. While other studies have evaluated SBS with Biodentine using different adhesives and types of composites (Abdullah HA et al, 2022) (Kumar V et al, 2023) (Raina A et al, 2020) (Tulumbaci F et al, 2017). Both MTA Plus and biodentine have shorter setting time and less vulnerable to water changes due to calcium chloride accelerator and decreased liquid content in Biodentine and a finer particle size with an anti-wash out gel in MTA Plus. This enables higher bond strength values with composite on freshly placed cements in vital pulp therapy procedures etc. Further study needs to be done to analyze the effect of duration of application time of various adhesives on these bioceramic materials with respect to their micro-structural changes and the shear bond strength to composite resin.

Conclusion

Within the limitations of this study, we found that either surface treatment resulted in altered micromorphology of MTA Plus and biodentine. One-step self-etch adhesives could be a preferred choice when bonding composite to biodentine or MTA Plus after their immediate placement in procedures such as vital pulp therapy, perforation repairs and retrograde root end fillings to overcome the problems of lower shear bond strengths with MTA due to its lower anti-wash-out resistance.

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Both the authors have contributed equally to the work.

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