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Jeppiar Nagar, Chennai



EFFECT OF DENTIN CONDITIONING, ACID ETCHING AND ADHESIVES ON SURFACE MICROSTRUCTURE OF BIODENTINE AT VARIOUS TIME INTERVALS AFTER MANIPULATION : AN INVITRO QUALITATIVE ANALYSIS

By

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Dissertation submitted to

Sathyabama Institute of Science & Technology
(Deemed to be University) Chennai, Tamil Nadu.

In partial fulfillment of the requirements for the degree of

Master of Dental Surgery

In the Speciality of

CONSERVATIVE DENTISTRY AND ENDODONTICS

Under the guidance of

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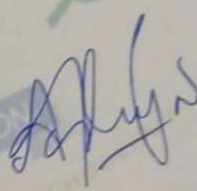
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INTRODUCTION

Pulp capping is a clinical procedure involving the placement of a biomaterial, which aims to maintain pulp vitality by stimulating tertiary dentin formation as a hard-tissue barrier. Over time, a number of materials have been developed to maintain pulp vitality as pulp capping agents. $\text{Ca}(\text{OH})_2$ was introduced to the dental profession in 1921 by Hermann and has been considered the "gold standard" of pulp capping materials for several decades. (1) Calcium hydroxide has excellent antibacterial properties. (2) A study found a 100% reduction in microorganisms associated with pulp infections after one-hour contact with calcium hydroxide. (3)

$\text{Ca}(\text{OH})_2$ is believed to affect pulp repair by several mechanisms of action. It possesses antibacterial properties, and this can minimize or eliminate bacterial penetration to the pulp. (2) Traditionally, it has been believed that calcium hydroxide's high pH irritates the pulp tissue, which stimulates repair via some unknown mechanism. (4) In recent years, this "unknown mechanism" may have been explained by the release of bioactive molecules.

However, $\text{Ca}(\text{OH})_2$ has a few drawbacks: insufficient adherence to dentinal walls, poor sealing ability, dissolution over time and multiple tunnel defects in the induced dentin bridges. (5)

Due to its high alkalinity, Ca(OH)_2 in direct contact with the pulp locally destroys a layer of pulp tissue and creates an uncontrolled necrotic zone. This necrotic layer induces an inflammatory reaction that persists in time or leads to intra-pulpal calcifications. Furthermore, the results of clinical studies showed success rates with Ca(OH)_2 pulp capping on carious exposures are highly variable, unpredictable, and often unsuccessful. (6), (7), (8), (9).

The vital pulp therapy procedures resulted in more predictable outcomes with the advent of hydraulic calcium silicate-based cements, which offer various impressive bio-inductive and bio-conductive properties, as these calcium silicate-based cement aid in the stimulation of pulp cell recruitment, their differentiation, and termination by upregulating various growth factors and thus promoting tertiary dentinogenesis. (10), (11)

Mineral trioxide aggregate (MTA) is a bioactive cement pioneered by Torabinejad et al. in the early 1990s for endodontic repair and as root-end filling material with favourable physical properties. (12), (13). The original MTA, ProRoot MTA Gray (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA), was marketed in 1998 and that composed of 75% Type I Portland cement, 20% bismuth oxide and 5% calcium sulfate dihydrate. The gray MTA, containing tetracalcium aluminoferrite, with a composition similar to that of the original type, is less popular for

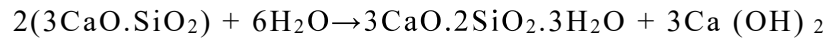
esthetic reasons as it caused discoloration of the tooth. ProRoot MTA White was introduced in 2002 and differs from its predecessor in composition, i.e., eliminating tetracalcium aluminoferrite and increasing calcium silicates. (14) MTA's advantages are its ability to set in moist environment, sealing ability, biocompatibility, bioactivity, and capacity to promote mineralized tissue formation.(15) MTA has proven to induce mineralization beneath exposed pulp and have the potential of maintaining pulp vitality. Hence, the indications for the use of MTA have expanded considerably from its original use, and it has become a superior substitute for Ca(OH)_2 in many other clinical applications, including direct and indirect pulp capping, perforation repairs in roots or in furcation and the apexification procedure. (16), (17), (18) Despite its high clinical advantages, there were also some limitations that prevented the clinicians from using it regularly. The major ones being its initial setting time of 45 minutes and a final setting time of up to 284 minutes, poor handling characteristics, discoloration of the remaining tooth structure and the presence of heavy metals in the powder that led to uncontrolled expansion and cytotoxicity of freshly mixed MTA. (19), (20), (21)

Biodentine was launched by Septodont in the year 2009 in the dental market as a 'dentin substitute'. Compared to other calcium-silicate based cements, this material presents two advantages: i) a faster setting time of about 12 minutes and ii) higher mechanical

properties. These physicochemical properties associated with the biological behavior suggest that it may be used as a permanent dentine substitute, making the material the first choice for vital pulp therapy procedures among clinicians. (22) Biodentine is an inorganic, non-metallic compound presented in a capsulated powder and liquid form. The powder composition is 80.1 wt % tricalcium silicate, dicalcium Silicate, 14.9 wt % calcium carbonate, 5 wt % zirconium oxide, and iron oxide as a colouring agent. The liquid contains calcium chloride, which acts as an accelerator, hydrosoluble polymer function as water reducing agent and water. (23)

A single capsule containing 0.7 grams of powder is mixed for 30 seconds in a mixing device at a 4000–4200 rpm speed with exactly five drops of liquid. Upon mixing the powder phase with the liquid phase, the following sequence of events occurs: Tricalcium silicate mixes with the water component and leads to a hydrated calcium silicate gel (C-S-H) structure and calcium hydroxide. The gel structure develops through nucleation and growth on the tricalcium silicate surface, and gradually fills the spaces between the tricalcium silicate grains. Crystallization of the C-S-H gel structure occurs through continuous hydration, resulting in the formation of CaCO_3 crystals between the unreacted grains. The crystals of CaCO_3 slowly fill in the porosities between the unreacted grains of cement over approximately two weeks until

finally reaching a maximum. This process makes the structure relatively impermeable to water and slows down the effects of further reactions. (24) The following formula summarises the complete hydration reaction:



C₃S

C-S-H

The calcium hydroxide produced as a by-product of hydration of Biodentine is leached out and causes necrosis when in contact with the pulp. The released hydroxyl ions increase the pH in the underlying pulp tissue leading to an anti-microbial effect. Calcium ions are involved in dentin-bridge formation as they stimulate dental pulp stem cells (DPSC) differentiation. Silicon ions promote mineralization. (25)

The outcomes of vital pulp therapy procedures rely on a bacterial tight seal provided by the coronal restoration.(26) The time of placement of a coronal restoration is paramount in vital pulp therapy procedures. The degree of pulpal inflammation is associated with the bacterial leakage around the restorations and its frequency varies according to the material used.(27) The pulp will have the highest probability for wound repair and survival if microleakage is minimized or eliminated. (28) The appropriate restorative material for each case must be carefully selected. The delivery should be executed with a high level of skill to provide the best likelihood of

a predictable seal against microleakage.(29) Effective definitive restorative materials that are most often used clinically are Glass Ionomer Cement (GIC), Bonded Amalgam and Composite resin restorations.(30), (31) Survival rates for composites are improving as there are tremendous advances in material technology and tooth isolation requirements. (32), (33)

Placement of these restoratives requires pre-treatment of the cavity with acids such as 10% polyacrylic acid prior to a GIC restoration and 37% phosphoric acid or self-etching primer or universal adhesive prior to composite resin restoration to achieve a better bonding with the tooth structure. GIC and the adhesive systems which bond composite resin restorations to the tooth, can simultaneously bond to calcium silicate-based cement by chemical, micro-mechanical or combination of both. Dental adhesives primarily bond through mechanical interlocking of the monomer that set within the created surface porosities. Besides, micromechanical bonding, the future lies most with self-etch adhesive that causes limited decalcification of dentin based on Adhesion-Decalcification (AD) concept in combination with incorporation of functional monomer like 10- Methacryloyloxydecyl dihydrogen phosphate (10-MDP) that bonds through its phosphate groups to the substrate.(34) Thereby, in this study ClearFil SE, Kuraray Noraitake, Tokyo, Japan- two step self-etch 6th generation adhesive and Single Bond Universal, 3M ESPE, St. Paul, MN, USA - 8th generation universal

adhesive both comprise of mildly acidic monomer (10 – MDP) in their composition, and they have a ph of 2.9.

Bondentine when used as a base in an acid-etched cavity before composite placement, leakage occurred at its interface to dentine which was more pronounced compared to that of unetched Bondentine.(22) It was found that bond strength of Bondentine to composite ranged from 9 MPa to 14 Mpa when different adhesive systems were used (35), (36), (37), (38). The estimated bond strength required to resist contraction forces sufficiently to constitute gap-free restoration margins ranges between 17 MPa to 20 Mpa.(38)

Based on the AD concept, polyalkenoic acid, which is a polymer with multitude of carboxylic functional group acts as chemical hands that grab individual calcium ions along the mineral substrate. The chemical bond along with the shallow hybridization explains the successful self-adhesion of GIC without any pre-treatments. (34) Therefore in an attempt was made in this study to evaluate the effect of GIC type II liquid (GC, Fuji II, GC, Europe) on the surface of Bondentine. The bond strength of GIC and Bondentine were found be ranging between 5 Mpa to 6 Mpa when an early bonding was carried out. (35), (30). The reason for variable bond strengths reported between Bondentine and Glass Ionomers or Composites is unclear. (39)

Most of the in-vitro and clinical studies conducted failed to report the time of placement of coronal restoration or the kind of pre-treatment of the cavity before receiving a definitive restoration(40), (41), (42) and to our knowledge, there are no studies reporting the effect of dentin conditioning (10% polyacrylic acid) pre-treatment on Biodentine. There remains inexplicit evidence on the restorative protocol, especially the surface pre-treatments on the Biodentine before the placement of the definitive restoration.

AIM AND OBJECTIVES

The aim of the study was

To qualitatively analyse the effect of dentin conditioner, glass ionomer type II liquid and combination as pre-treatment for GIC restorations and the effect of phosphoric acid etching, two step self-etch adhesive and universal adhesive in self etch mode as pre-treatment for composite resin restorations at 5 minutes, 12 minutes, 24 hours, and 7 days after manipulation using Scanning Electron Microscopy with X ray energy dispersive mode (SEM-EDX), X- ray diffractometry and micro Raman spectroscopy.

Objectives of the study were

- To analyse the surface morphology of Biodentine after subjecting to various pre-treatment at different time intervals using SEM-EDX
- To analyse the chemical composition of Biodentine after pre-treatment at different time intervals using XRD
- To analyse the surface chemical compositional changes after pre-treatment at different time interval using micro Raman spectroscopy.

NULL HYPOTHESIS

The study's null hypothesis is that the surface microstructure, and chemical composition of the Biodentine is not affected by the different pre-treatments at various time intervals after manipulation.

REVIEW OF LITERATURE

Camilleri et al. 2011 (43) investigated the hydration and characterization of Theracal and Biodentine used for pulp capping. Setting mechanism and characterization of set Biodentine and Theracal after immersion in HBSS for 28 days was investigated by SEM and XRD analysis. The bioactivity and surface microstructure of cements immersed in Hanks Balanced Salt Solution (HBSS) or water was also assessed by similar techniques together with leaching in solution investigated by ion chromatography (IC). The observations showed that presence of a resin matrix modifies the setting mechanism and calcium ion leaching of Theracal and Biodentine hydration leads to the formation of calcium hydroxide which was present in the material matrix and on the material surface and both materials leached calcium ions in solution.

Camilleri et al. 2011 (44) investigated and characterized the replacement of the Portland cement component in mineral trioxide aggregate (MTA) with tricalcium silicate cement which is manufactured using the sol-gel method from pure raw materials. Tricalcium silicate and Portland cement were characterized - SEM and EDX, and XRD with Rietveld refinement. In addition the hydration products of the material after 28 days of curing were evaluated by plotting atomic ratio plots from the EDX data. The cement leachate was evaluated for pH and chemical composition by

inductively coupled plasma. The observations showed that tricalcium silicate could prospectively replace the Portland cement component in MTA and on hydration both cements produced calcium silicate hydrate and calcium hydroxide. The calcium hydroxide was leached in solution with higher leaching in HBSS. The leaching of calcium hydroxide in solution resulted in an alkaline pH. The reaction of Calcium with the phosphorus present in HBSS resulting in the deposition of calcium phosphate on the cement surface.

Formosa et al. 2012 (30) the microstructure and surface characteristics of radiopaque tricalcium silicate cement exposed to different curing conditions - 100% humidity or immersed in either water or a simulated body fluid at 37 °C. The materials used were tricalcium silicate and Portland cements with and without the addition of bismuth oxide radiopacifier. The material characterization of the hydrated cements was done using SEM- EDX & XRD analyses and the surface morphology was assessed using optical profilometry. Testing was done on samples that cured at 100% humidity or immersed in either water or HBSS for 1 and 28 days at 37 °C. The leachate analysis was performed by XRF of the storage solution. The pH of the storage solution was assessed. The observations showed that tricalcium silicate has higher reaction rate. Cement immersion in HBSS led to deposition of calcium phosphate during the early stages following immersion extensive

calcification after 28 days. The pH of all storage solutions was alkaline. The immersion in distilled water resulted in a higher pH of the solution than when the cements were immersed in HBSS. Leachate analysis showed higher levels in tricalcium silicate.

Grech et al. 2012 (45) investigated the composition of materials and leachate of a hydrated prototype cement composed of tricalcium silicate and radiopacifier . The materials used were a prototype-radiopacified tricalcium silicate cement, Biodentine, Bioaggregate and IRM. The pH and calcium ion concentration of the leachate were investigated. The hydrated cements were characterized using SEM- EDX, XRD & FTIR. It was observed that Biodentine and Bioaggregate led to the formation of calcium silicate hydrate and calcium hydroxide, which was leached in solution. The hydrated materials were composed of a cementitious phase that was rich in Calcium and silicon and a radiopacifying material. Biodentine included calcium carbonate. FT-IR indicated the occurrence of a poorly crystalline calcium silicate hydrate. Biodentine showed the presence of calcium carbonate.

Raskin A et al. 2012 (46) evaluated the sealing efficacy of Biodentine at the cervical margins of approximal cavities placed in molars and compared the use of Biodentine in combination with resin-based adhesives and a resin composite, compared with a resin-modified glass-ionomer cement (Fuji II LC). The experimental

groups were G1 Biodentine, G2 Fuji II LC as a filling material, G3 Biodentine as a base + Optibond Solo Plus + silane + Filtek Z250, G4 as in G3 without silane, G5 - Biodentine as a base + Septobond SE + Filtek Z250, G6 - Fuji II LC as a base + Optibond Solo Plus + Filtek Z250. The teeth were thermocycled 2500x (5°C to 55°C), specimens were then sealed with a 1-mm window around the marginal interface. Samples were immersed in a 50% w/v silver nitrate solution and exposed to a photo-developing solution. The teeth were embedded in resin and sectioned through the restorations. The silver penetration was directly measured using a light microscope. They showed that dye penetration was higher at the enamel restoration interface of G5, at the dentin substitute/ resin composite Interface - G5 showed that the leakage were beyond the interface and reaching the cavity floor and a comparison of Enamel and Dentin. They concluded that Biodentine as dentin substitute in cervical lining restorations or as a restorative material in approximal cavities when the cervical extent is under the CEJ seems to perform well without any conditioning treatment due to the formation of hydroxyapatite crystals at the Biodentine surface.

Camilleri et al. 2013 (47) characterized and investigated the hydration of a tricalcium silicate-based proprietary brand cement - Biodentine and a laboratory manufactured -TCS-20-Z and compare their properties to MTA Angelus. The specific surface area and the particle size distribution of the un-hydrated cements and zirconium

oxide were analysed using gas adsorption method and SEM analysis of the un-hydrated cements and set materials were carried out for mineralogy and microstructure. The assessment of bioactivity and hydration was done using the SEM- EDX, X ray fluorescence spectroscopy, XRD, Rietveld refined X-ray diffraction and calorimetry. The radiopacity of the materials was investigated using ISO 6876 methods. The observations revealed that Biodentine showed the presence of calcium carbonate particles and MTA Angelus showed the presence of dicalcium silicate, tricalcium aluminate, calcium, aluminium and silicon oxides. TCS and MTA Angelus exhibited similar specific surface area while Biodentine had a greater specific surface area. It was also observed that the rate of reaction of tricalcium calcium silicate was higher for Biodentine attributed to the optimized particle size distribution, the presence of CaCO_3 and the use of CaCl_2 . Calcium hydroxide was produced as a by-product of reaction in all hydrated cements.

Grech et al. 2013 (48) investigated the physical properties of prototype radiopacified tricalcium silicate cement, Bioaggregate and Biodentine. IRM was used as a control. Radiopacity assessment was assessed with the thickness of aluminium. Anti-washout resistance - investigated using a novel basket-drop method fluid uptake, sorption and solubility - gravimetric method, setting time was assessed by indentation technique and compressive strength and micro-hardness of the test materials were investigated. The testing

was performed with immersion in HBSS. The observations showed that addition of admixtures to tricalcium silicate-based cements affects the physical properties of the materials. They concluded that Biodentine is the strongest material in relation to compression and surface properties.

Maria Giovanna Gandolfi et al. 2013 (49) evaluated the biointeractivity and apatite-forming ability and physical properties - porosity, water sorption, solubility, and setting time of Biodentine compared to ProRoot MTA as gold standard material. Biodentine and ProRoot MTA pastes were prepared and analyzed for calcium release and alkalinizing activity (3 hours–28 days), setting time, water sorption, porosity, solubility, surface microstructure and composition, and apatite-forming ability in simulated body fluid. The observations showed that Biodentine - biointeractive tricalcium silicate material with interesting chemical-physical properties and represents a fast-setting alternative to the conventional calcium silicate MTA-like cements and Biodentine showed higher release of free calcium ions, higher alkalinizing capability, and the formation of smaller calcium phosphate deposits, calcium carbonate - mineralizing cell activation. The large open pore volume and water sorption - broad wet biointeractive surface - release of the calcium and hydroxyl ions - formation of a calcium phosphate coating. The Biodentine showed a solubility value of 11.93%, whereas for ProRoot MTA it was 18.34 %

Mesut Enes Odabas et al. 2013 (50) measured the shear bond strength of different adhesive systems to Biodentine with different time intervals. Early and delayed bonding was carried out at 12 minutes and after 24 hours with etch-and-rinse adhesive system - Prime & Bond NT, 2-step self-etch adhesive system - Clearfil SE Bond; 1-step self-etch adhesive systems - Clearfil S³ Bond; control – where no adhesive treatment was done. The shear bond strength and fracture analysis (stereomicroscope) at 25x magnification was done after 24 hours of bonding. The results showed that highest bond strength value was obtained for two-step self-etch adhesive at a 24-hour period and failed cohesively within the Biodentine; lowest bond strength was obtained for etch-and-rinse adhesive at a 12-minute period and failed adhesively.

Camilleri J et al. 2013 (22) compared Biodentine to glass ionomer and resin modified cements in an "open sandwich" restoration. The effect of etching with 35% phosphoric acid was investigated by assessment of surface topography, Fourier transform infrared (FT-IR) spectroscopy, surface micro-hardness and micro-leakage using tagged carboxylated-modified fluorescent microspheres and tracing the presence of these particles with a confocal microscope. The acid etching was done with phosphoric acid for 20 seconds. The observations showed that Calcium to silicon ratio of the etched Biodentine was lower than that of the un-etched surface. The chloride peak of the etched Biodentine was lower than that of the

un-etched Biodentine and exhibited significant amounts of leakage at the dentin – material interface. FTIR plots showed no changes in the peaks for Biodentine after etching. Microhardness of the etched and unetched Biodentine did not show a significant difference.

Kayahan MB et al. 2013 (51) evaluated the effect of acid etching on the compressive strength of calcium silicate–based cements namely ProRoot MTA, MTA Angelus, Calcium Enriched Mixture (CEM) cement, Biodentine. The samples were incubated at 37°C for a week before subjecting to acid etching. The acid etching was done with 37% phosphoric acid for 15 seconds after a week . The compressive strength of the samples was then calculated in megapascals using a universal testing machine. They concluded that after a 1-week interval, an acid etching process had no adverse effect on the compressive strength of Biodentine and ProRoot MTA.

Hashem DF et al. 2014 (36) compared in-vitro micro-shear bond strengths of resin composite to calcium silicate cement (Biodentine) vs. glass ionomer cement vs. resin modified glass ionomer cement (RM-GIC) using an adhesive in self-etch /total etch mode after aging three substrates - Biodentine, glass ionomer cement and RM-GIC and the bond of these substrates. They further characterizing their failure modes. Adhesive used in this study was scotch bond universal. The samples were divided two groups based on substrate aging and bond aging. The first group was aging the substrate,

which underwent early ($t = 0$ minute, 5 minute, 20 minute, 24 hour) or delayed ($t = 2$ weeks, 1 month, 3 months, 6 months) substrate aging before bonding and micro-shear bond strength was done at the end of 24 hours. The second group was the aging of the bond, in which the adhesive was applied after either early ($t = 5$ minutes) or delayed ($t = 2$ week) substrate aging and then bond strength analysis were done at the end of 2 weeks, 1 month, 3 months and 6 months. The failure modes were identified using stereomicroscope. SEM images of selected samples were analyzed. They concluded that Biodentine is weak in its early setting phase. Placing the overlying composite is best delayed for at least 2 weeks to allow adequate setting/maturation of the Biodentine to withstand sufficiently the contraction forces of the resin composite.

Cantek N K et al. 2014 (35) evaluated bond strength of methacrylate based (MB) composites, silorane-based (SB) composites, and glass ionomer cement (GIC) to Biodentine and mineral trioxide aggregate (MTA). Immediate bonding was done after 12 minutes of setting time to Biodentine. Shear bond strength was done after 24 hours. The adhesive used for methacrylate based composite is a 2-step total-etch adhesive system (All-Bond 2) and for Silorane-based composite is 2-step self-etching primer. They concluded that shear bond strength values of Biodentine was significantly increased for the methacrylate based composite compared to MTA. The pre-treatment of Biodentine surface prior to placement of GIC was not

clear. The observations showed that shear bond strength values of GIC that were used with Biodentine - 6.7 Mpa, were higher than their shear bond strength when bonded to MTA -5.3 Mpa.

Altunsoy et al. 2015 (31) investigated the shear bond strength of a self-adhering flowable composite and a flowable composite to MTA, Biodentine and CEM. The bonding was done after 72 hours storage at 37°C in 100% humidity and the adhesive used for flowable composite was Futurabond DC which is a one-step self-etch system. The shear bond strength was done after 24 hours storage at 37°C at 100% humidity. The observations showed that the Vertise Flow–CEM and X-tra base–MTA groups showed significantly higher SBS values than the group made of Vertise flow–Biodentine.

Hashem et al. 2015 (52) assessed the effectiveness of calcium silicate cement- Biodentine vs GIC; control group as indirect pulp capping materials in patients with reversible pulpitis and to compare the effectiveness of CBCT vs PA radiographs in detecting periapical (PA) changes at baseline (T0) and at 12 months (T12) postoperatively. 72 restorations (36 Biodentine, 36 Fuji IX) were placed randomly in 53 patients. CBCT/PA radiographs were taken at T0 and T12. Two calibrated examiners assessed the presence/absence and increase/decrease in the size of existing PA radiolucencies under standardized conditions. The definitive resin composite veneer restoration was placed 1 month after baseline in a

"closed sandwich" technique where achievable. A standardized bonding procedure was followed using a total etch adhesive for both groups, following the manufacturer's instructions. The follow-up was longitudinal at T1, T6, and T12 months (2 weeks) at which time PA radiographs/CBCT were repeated. CBCT demonstrated a statistically significant difference between the 2 materials. The majority of teeth with healing/healed lesions identified using CBCT had received Biodentine, whereas the majority of teeth with new/progressing lesions had received Fuji IX.

Cengiz E et al. 2016 (30) evaluated the micro-shear bond strength of tricalcium silicate based materials (Theracal LC and Biodentine) to different restorative materials. Immediate bonding was done after the initial setting time (12 minutes). The restoratives used for the study are Fuji IX, where no dentin conditioner used, Fuji II (RM GIC) where no dentin conditioner applied, Equia fil – followed by application of Equia coat and cured for 20 seconds, Vertise flow (self-adhering composite), Filtek bulk fill in which a Scotch bond universal was preceded by etching, Filtek Z250, where Prime and bond NT was used preceded by etching and a Clearfil SE (2 step self-etching adhesive) was used. The micro shear bond strength was tested after 24 hours and failure mode analysis by stereomicroscopy. The observations showed that self-etching adhesives increased the bond strength of Theracal LC whereas Etch and Rinse adhesives increased the bond strength of Biodentine, with a bond strength

value of 13.85 Mpa. It was postulated that with etch and rinse adhesives the retention occurs due to micromechanical interlocking. The bond strength of Biodentine to GIC were found to be range between 5 to 6 Mpa.

Hakan Çolak et al. 2016 (37) evaluated the effects of 3 different adhesives with different functional monomers, on the shear bond strength of Biodentine. The adhesives used were Clearfil S3 Bond, Adper Prompt L-Pop (3M/ESPE) and a total Etch System (Acid: Etch-37 (37%) w/BAC; Bisco, USA) with Prime Bond N&T. Early (9 mins), delayed (48 hours) bonding was done. The shear bond strength and fracture analysis using stereomicroscopy after 48 hours. They concluded that Biodentine exhibited better bond strength, if they are used with self-etch adhesive systems especially with Clearfil S³ Bond, which contains 10-MDP which more effective binds to calcium ions in the Biodentine.

Meraji N et al. 2017 (53) characterized the interface between dentin replacement materials - Biodentine, Theracal LC and Fuji IX and composite resin and to measure the shear bond strength after dynamic aging. The bonding strategies used were total-etch and bonding agent or a self-etch primer (AdheSe One F, Ivoclar) and composite resin or a glass ionomer cement. All combinations were thermocycled for 3000 cycles. The interface was characterized using scanning electron microscopy and elemental mapping. They

concluded that when Biodentine was overlayed with composite, there occurred an elemental migration of aluminium, barium, ytterbium, and fluorine and Calcium was shown to be stable and not to migrate. Biodentine also exhibited the lowest shear bond strength when compared with Theracal LC and GIC regardless of the bonding technique. The interface of the GIC and the Biodentine showed an evident wide open space, which was attributed to the absorption of water from the Biodentine surface leading to the incomplete hydration of Biodentine. (Dentin conditioner was not used prior in this study).

Aksoy S et al. 2017(54) compared shear bond strength of various adhesive systems to a Biodentine at different time intervals. Each group was divided into 7 subgroups of 10 units each. The adhesives used are Prime&Bond NT, Single Bond Universal (self-etch mode and etch and rinse mode), All-Bond Universal (self-etch mode and etch and rinse mode) and G-aenial Universal Bond (self-etch mode and etch-and-rinse mode). After the application of adhesive systems, compomer was applied over Biodentine. Shear bond strength and failure analysis with stereomicroscopy was done at different time interval - 12 minute, 24 hour, 48 hour and 96 hour. The observations showed that highest bond strength values were obtained with the G- aenial group, the reason may be due to the solvent (acetone) may be easily removed from the adhesion surface. Lower shear bond strength was detected in groups with Prime and

bond NT attributed to low molecular weight monomers like urethane dimethacrylate and dipenta erythritol penta acrylate monophosphate. Waiting period of at least 24 hours is recommended to obtain high shear bond strength values. It may be suggested that sufficient bonding performance may also be obtained without an acid etching procedure, when universal adhesive systems applied on Biodentine as these showed similar bond values in SE and ER modes.

Tulumbaci et al. 2017 (38) evaluated the shear bond strength of composite resin, compomer XP) and RM GIC to white MTA and Biodentine. The samples were stored for 72 hours at 37°C and 100% humidity. The adhesives used were Prime and Bond NT followed by application of phosphoric acid etchant and no pre-treatment was done for RM GIC. The micro shear bond strength was done after 24 hours. The observations showed that MTA has shown better shear bond strength to compomer and composite resin materials than Biodentine. RM-GIC showed lower bond strength values in both MTA and Biodentine groups. Biodentine showed more adhesive failure than white MTA group.

Katge et al. 2017 (55) compared Biodentine and MTA for direct pulp capping in young permanent molars by clinical and radiographic evaluation in 7- to 9- year-old children. In 50 patients, 29 patients with bilateral asymptomatic first permanent molars with carious involvement were selected. According to split mouth design,

these patients were then divided into 2 groups, Biodentine group (right side) and MTA group (left side). The pulp-capping procedure was performed by using Biodentine and MTA in 58 asymptomatic bilateral permanent molars with pulp exposure. Treatment outcome was assessed clinically through pulpal sensitivity tests as well as radiographically to evaluate dentin bridge formation. After 3 months, Biodentine was partially removed, leaving 1-mm layer of Biodentine intact. The permanent restoration was then done by using composite resin restorative material. The follow up was at baseline, 6 months and 12 months. On radiographic and clinical parameters , the study reported 100% success rate with both Biodentine and MTA at baseline and 6- and 12-month follow-up.

Claudia Brizuela et al. 2017 (56) conducted a randomised controlled trail that included 169 patients and the inclusion criterion was patients with 1 carious permanent tooth with pulpal exposure, a candidate for a direct pulp capping procedure. The patients were randomly allocated to one of the experimental groups (CH, Biodentine, or MTA). A layer of glass ionomer liner that had been photopolymerized for 20 seconds. Final restoration was done with direct resin restoration and occlusion was checked. Clinical follow-up examinations were performed at 1 week, 3 months, 6 months, and 1 year. Biodentine showed 100% success, has the advantage of easy handling, sets in 12 minutes, and does not cause discoloration of the tooth.

Bakhtiar et al. 2017 (57) investigated the clinical efficacy of TheraCal as compared with Biodentine and MTA for partial pulpotomy. In this clinical trial, partial pulpotomy was performed for 27 sound human maxillary and mandibular third molars scheduled for extraction. The teeth were randomly divided into 3 groups ($n = 9$) and underwent partial pulpotomy with TheraCal, Biodentine, and ProRoot MTA. The teeth were then restored with glass ionomer cement – after 7 days. Clinical and electric pulp tests were performed after 1 and 8 weeks. The teeth were radiographed and extracted at 8 weeks. Histologic sections were prepared and analyzed for pulp inflammation and dentinal bridge formation. Biodentine and MTA performed better than TheraCal when used as partial pulpotomy agent and presented the best clinical outcomes.

Parinyaprom et al. 2017 (58) compared the success rates of DPC by using either ProRoot MTA or Biodentine in the cariously exposed permanent teeth of 6- to 18-year-old patients. 59 cariously exposed permanent teeth, including teeth with diagnosis of normal pulp, reversible pulpitis, or irreversible pulpitis, early periapical involvement, and exposure size of up to 2.5 mm, were included. Each patient with only 1 cariously exposed tooth was randomly allocated to DPC with either ProRoot MTA ($n = 30$) or Biodentine ($n = 29$). Clinical and radiographic examinations were used to determine success. All teeth were restored with resin composite, amalgam, or an Stainless Steel Crown (SSC), depending on the

amount of tooth structure left. Number of composite restorations in Biodentine group - 40.7 (11/27). Patients were recalled every 6 months till 54 months with a mean follow up of Biodentine group – 19.6 ± 10.5 months. Biodentine was non-inferior to ProRoot MTA when used as a DPC material for cariously exposed permanent teeth of 6- to 18-year-old patients. Success rate in Biodentine group – were 96.4%.

Anastasiadis et al. 2018 (39) evaluated the effects on surface properties (morphology, roughness, microhardness and composition) and bond strength of the composite of 4 types of base materials – Equia – Fil, MTA angelus, Biodentine, IRM. The pre- treatments were done after storage of 4 days. Pre-treatments were done using phosphoric acid etching, two self-etch adhesives – Select one Prime & Bond NT and Clearfil S³ bond. The morphology was assessed using stereomicroscopy at 20x, roughness assessed using optical profiler at 2x20 magnification, hardness using Vickers diamond indenter, composition using ATR FTIR and bond strength using shear bond strength . The observations in the Biodentine group showed that after phosphoric acid etching, the chemical composition of Biodentine changed as the calcium sulphate was replaced with calcium phosphate and these insoluble uniform precipitates on the surface of Biodentine, increase in surface roughness was found, which increased the surface area for bonding to resin composite by 154%. After treating the surface of Biodentine with Select prime

and bond NT and Clearfil S³ surface, the roughness of Biodentine increased as the acid attacks the interparticle grain due to the presence of smaller particles size. The ATR FTIR – peak of CO₃ was shown to be larger. The P/Si peak after Select prime and bond NT – 0.07 and after Clearfil S³ – 0.18, this was attributed to the solubility of the salts formed. The MDP in case of Clearfil S3 were less soluble.

Palma PJ et al. 2018 (59) assessed the proper time to perform restorative procedures [immediately (12 min) or seven days] after placement of two CSC materials (ProRoot MTA or Biodentine), and to test the performance of two different restorative protocols (Prime & Bond NT, a resin-based flowable composite (SDR) versus a Fuji IX glass ionomer. The shear bond strength and stereomicroscopy analysis was done after 48 hours in 12 minutes group and after 7 days in the 7 days group. The adhesive used is Prime and bond NT Active. The observations showed that MTA/delayed SDR presented statistical differences compared to MTA/immediate SDR and no differences were observed regarding Biodentine performance (Biodentine/immediate SDR and Biodentine/delayed SDR).

Mohammad Hossein Nekoofar et al. 2018 (60) measured the micro-shear bond strength of a resin composite using several adhesive systems and a RM-GIC to different aged Biodentine specimens. Bonding were done at 12 min, one week and one month intervals.

The adhesives used were Adper Single Bond 2 and Clearfil SE Bond. The micro shear bond strength was done after 24 hours storage at 37°C. No pre-treatment was done for RM-GIC Group. The observations showed that aging to one week resulted in increased micro-shear bond strength in all groups except for the RM-GIC group, this can be attributed to the improved chemical reaction between hydrated calcium silicate gel (CSH gel) and hydration products such as calcite and the carboxyl group of poly acids in GIC.

Awawdeh L et al. 2018 (40) aimed to evaluate the clinical performance of Biodentine and white MTA in cariously exposed mature permanent teeth. Biodentine should set within 12 minutes according to the manufacturer; however, it occasionally took up to 30 minutes in this study, and the tooth was temporized with Intermediate Restorative Material (IRM). The final restoration material used was amalgam or a resin composite; the final restoration was performed during the first review appointment. Follow up period of 3 years, Biodentine and MTA have favorable and comparable success rates when used as direct pulp capping or pulpotomy material in permanent mature teeth with carious exposure.

Taha NA et al. 2018 (41) assessed the outcome of full pulpotomy using Biodentine in permanent teeth with carious exposures and symptoms indicative of irreversible pulpitis. 20 permanent molar teeth in 14 patients with carious pulp exposure were treated with Biodentine pulpotomy. Full pulpotomy was performed by amputating the exposed pulp to the level of the canal orifices, hemostasis was achieved with a cotton pellet moistened with 2.5% sodium hypochlorite and a 3-mm layer of Biodentine was placed as the pulpotomy agent. After 12 minutes of waiting for the initial setting, a layer of resin-modified glass ionomer liner (Vitrebond; 3M ESPE) was applied, and the tooth was restored with glass ionomer cement and a stainless steel crown, amalgam, or resin composite. Composite restorations – 5 /20 teeth. Clinical and radiographic evaluation was completed after 6 months and 1 year postoperatively and the follow up rate was 100%. Young permanent teeth with carious exposure can be treated successfully with full pulpotomy using Biodentine, and clinical signs and symptoms of irreversible pulpitis are not a contraindication. At 6 months- 100% clinical and radiographic success. At 12 months – 100% clinical and 95% radiographic success.

Ha HT et al. 2019 (61) investigated the influence of Biodentine maturation time on the shear bond strength to a resin-based composite restorative material and glass ionomer cement. The maturation time allowed before bonding were 12 minutes, 72 hours

and 2 weeks. The adhesive used was Optibond FL- 3 step E&R adhesive. No pre-treatment were done for GIC restoration. The shear bond strength was done 90 minutes after bonding. They concluded that Biodentine presents significantly lower shear bond strength to resin based composite than glass ionomer cement even after long-term maturation (2 weeks), bonding a resin-based composite restorative material on Biodentine with a maturation time of at least 72 hours will result in increase in shear bond strength and initially set Biodentine presents very low shear bond strength values. The mean shear bond strength values of Biodentine at 12 minutes, 72 hours and 2 weeks were found to be 2.8, 7.9 and 10.5 Mpa.

Shafiei F et al. 2019 (62) assessed the effect of acid-etching time on the compressive strength, microstructure and shear bond strength of All-Bond Universal adhesive to MTA-Angelus (AMTA) and Biodentine(BD) done after 72 hours. The acid etching were done at the time intervals of 5s, 10 s and 15s. The adhesive used was All Bond Universal (ABU) after 24 hours of setting time of the tricalcium based cements. The observations showed that Biodentine had significantly higher compressive strength compared to AMTA, regardless of acid etching/etching time. They concluded that 5-seconds etching have no effect on compressive strength and 10-s and 15-s etching significantly decreased compressive strength. SEM analysis showed that at 10-s and 15-s etched surfaces had the same

pattern as 5-s etched surfaces with lower numbers of dissolved crystals and shortening acid-etching time to 5 s provided an advantageous effect on resin bonding with ultra-mild ABU preventing the detrimental effect of 10-s and 15-s etching on the compressive strength of AMTA and BD. BD did not benefit from etching in terms of resin bonding.

Carretero V et al. 2019 (63) evaluated shear bond strength between Biodentine and a composite resin, using different types of adhesives. Bonding to the substrate was done at 12 mins and 24 hours. The adhesives used were 3-step E&R adhesive - Optibond FL, 2-step etch-and-rinse -Solobond M and Universal adhesive - Scotchbond Universal in self etch mode. The shear bond strength and failure analysis done using stereomicroscopy was done after 24 hours of bonding. The observations showed that all adhesives obtained similar shear bond strength with Biodentine at 12 minutes and 24 hours of bonding, whereas the superior shear bond strength was observed after 24 hours following Biodentine placement.

Koutroulis et al. 2019 (64) analysed the rheological properties to determine the optimal water: powder ratio of tricalcium silicate-based prototype materials incorporating alternative radiopacifiers and fillers. Determination of how the proportion of water incorporated affected the physico-chemical behaviour of the materials. Endodontic cements replaced with 30% radiopacifier, and

additions of calcium phosphate and micro-silica were tested. The unmodified cements were mixed with a 0.35 water: powder ratio which served as control. At this water: powder ratio, unmodified Port-land cement without any addition had suitable consistency and characteristics. Assessment of material rheological properties enabled adjustment of the water: powder ratio in each material to provide comparable viscosity values to those of the pure cement. The flowability, phase analysis and calcium release were measured for both viscosity-matched and the standard 0.35 water: powder ratio blends. The prototype materials with the adjusted water: powder ratios were also characterized by SEM EDX and evaluated for radio-opacity. The observations showed that use of the 0.35 water: powder ratio is not appropriate - changing radiopacifier and incorporating additives. Zirconium oxide did not vary the water: powder ratio. Using the standard 0.35 ratio when the mixture had a low water demand resulted in higher flowability values and calcium release in solution. Micro-silica and Calcium phosphate altered the hydration of the materials. All materials were adequately radiopaque.

M. Hadis et al. 2019 (65) characterized the interface between dentine on the cavity floor and hydraulic calcium silicate and a glass ionomer cements to assess the material interaction with the dentine. The pre-treatments used were 20% polyacrylic acid and 17% EDTA applied for 60 seconds to freshly cut dentin -rinsed with

water for 10s -the dentine surface dried again for 10 seconds, followed by Biodentine was placed, were allowed to set for 20 minutes at 37 °C and 100% humidity after which the teeth were stored in Hank's balanced salt solution at 37° C for 30 days. The analysis used were Confocal (CM), AFM &SEM with elemental mapping. The observations showed that the migration of silicon into dentine and deposition of phosphorus at the interface. They concluded that dentine functionalization with EDTA enhanced the Biodentine interaction with the dentine and is recommended for clinical management.

Hashem et al. 2019 (52)compared Biodentine vs. glass ionomer cement as IPC materials in patients with reversible pulpitis after a 2-year follow-up. Evaluate the integrity of the overlying resin composite restorations using modified USPHS criteria and FDI criteria. Investigate the sensitivity of the modified USPHS criteria compared to the FDI criteria in the assessment of the restorations. 72 restorations (36 Biodentine, 36 Fuji IX) were placed randomly in 53 patients. Restorations were assessed using the modified USPHS and FDI criteria at T12 and T24. Definitive resin composite veneer restoration (N'Durance, Septodont, Louisville, KY, USA) was placed 1 month after baseline in a closed sandwich technique where clinically achievable. Periapical radiographs were taken at pre-treatment (T0), 12-month (T12), and 24-month (T24) review. Biodentine and Fuji IX were clinically effective - as IPC materials

in teeth with reversible pulpitis at T24. Resin composite restorations overlying both materials performed well at T24. Using the USPHS or FDI criteria is equally efficient at T24.

Uesrichai et al. 2019 (42) compared the outcome of partial pulpotomy using two cements, ProRoot MTA and Biodentine in permanent teeth of 6- to 18-year-old patients with signs and symptoms indicative of irreversible pulpitis. 69 permanent first molars with signs and symptoms indicative of irreversible pulpitis. All operators performed partial pulpotomy under a standardized protocol. Teeth were allocated, Pro-Root MTA (37 teeth) or Biodentine (32 teeth). To be categorized as having success, the evaluated tooth must have had both clinical and radiographic success. Biodentine was allowed to set for 12 minutes. Then, the tooth was restored with resin composite or stainless steel crown depending on the amount of tooth structure left. Composite restoration – One surface= 16/30 and Two surface = 10/30. Patients were recalled every 6 months and the mean follow up range of Biodentine was 32.7 ± 21.8 . Permanent teeth with signs and symptoms indicative of irreversible pulpitis in 6- to 18-year-old patients were successfully treated with partial pulpotomy using both cements. The Success rate in Biodentine group was 87% (26/30).

Bolhari B et al. 2020 (66) evaluated the interfacial characteristics of Biodentine, CEM Cement, and ProRoot MTA when restored with

different final restorative materials after different time intervals. Biodentine, CEM Cement and ProRoot MTA were layered with amalgam, composite resin or light cure glass ionomer cement. Layering was done either immediately, 24 or 72 hours after cement placement. The interface of cements with restorative materials was characterized using SEM and EDS after separation. Vickers surface microhardness test was also performed on the interface. The observations showed deposition of tin over the Biodentine with immediate layering of amalgam whereas in delayed layering with amalgam, showed some deposition of tin on Biodentine. Immediate placement of a composite resin on Biodentine showed calcium depletion accompanied by depletion of zirconium, barium phosphorus whereas delayed layering with composite created a rough surface with depletion of phosphorus depositions. Layering glass ionomer on CSCs also caused exposure to acidic pH due to the reaction between fluoroaluminosilicate glass with polyacrylic acid. They concluded that Biodentine was less affected when clinical conditions force immediate layering, use of Biodentine may be a better choice.

Singla MG et al. 2020 (67) compared the shear bond strength of Biodentine, Endocem mineral trioxide aggregate , and TheraCal LC to resin composite using a universal adhesive at their respective initial setting time and the specific modes of failure were analysed using a stereomicroscope at $\times 25$ magnification. The adhesives used

is Single Bond Universal, which was applied in a self-etch mode. The shear bond strength and failure analysis using stereomicroscopy was done after 24 hours. The observations showed that the failure mode was cohesive in type. Biodentine and Endocem MTA showed significantly lower shear bond strength values than TheraCal LC when bonded to composite after their initial setting time.

Paulo J. Palma et al. 2020 (68) evaluated the shear bond strength inherent to immediate and delayed definitive composite resin restorations, over three different biomaterials namely, Biodentine, TotalFill BC Putty, or PCM. The Early (12 minutes) and delayed (7 days) bonding were carried out. The two-step self-etch bonding system (Clearfil SE Bond) was used and restored using a flowable composite resin (SDR). Shear bond strength and fracture analysis were done after 48 hours. The observations showed that all tested biomaterials are the suitable alternatives which allow to perform restorative procedures immediately after pulp capping biomaterial placement. Biodentine and TotalFill BC Putty showed to have superior shear bond strength results when compared with PCM, within a delayed timeframe (7 days).

Ruba M. Mustafa. 2020 (69) investigated the shear bond strength between Biodentine and composite restoration at different maturation times of Biodentine aged in artificial saliva. The intervals for bonding were 12 minutes, 14 days and 28 days and adhesive used was total etch and rinse - Adper Single Bond 2

adhesive. The shear bond strength and fracture analysis using stereomicroscopy at 40x magnification was done after 30 days. The observations showed that shear bond strength of bonding composite to Biodentine was significantly reduced at the Biodentine primary setting phase or after prolonged time of exposure to the oral environment. They concluded that the best time for placement of an overlying composite restoration is at 14 days.

Abeulniel et al. 2020 (70) assessed the clinical and radiographic outcomes when using MTA and Biodentine as pulpotomy materials to maintain the vitality of traumatized immature anterior permanent teeth with pulp exposure. 50 traumatized immature anterior permanent teeth with exposed pulps were included in the study. Teeth were equally divided and randomly assigned to two groups MTA or Biodentine. After pulpotomy, pulp stumps were covered with MTA or Biodentine followed by a permanent restoration. A self-cure glass ionomer cement (GIC) was placed over the MTA before a final restoration of composite resin (Clearfil AP-X PLT., Kuraray) was done. Blinded clinical and radiographic evaluations were performed at baseline, immediate post-operative and after 6, 12 and 18 months. High success rate was observed when pulpotomy performed in young children in immature traumatized permanent incisors over an 18-month period, with comparable results when either MTA or Biodentine was used over the amputated pulp. Biodentine showed colour stability throughout the evaluation period.

MATERIALS AND METHODOLOGY

The study was approved by Institutional Review Board & Institutional Biosafety& Ethical Committee (IBEC), SIST. IRB number: 167/IRB-IBSEC/SIST

Study design: *In -vitro qualitative analysis*

MATERIALS USED: (Figure: 1 and 2) & (Table. No. 1)

1. Biodentine (Septodont, France)
2. 37% ortho phosphoric acid (D tech etching gel, D- tech technologies)
3. Glass ionomer type II liquid (Fuji II,GC Gold label, GC)
4. Dentin conditioner (10% polyacrylic acid , GC, Europe)
5. Clearfil SE (2 step self- etch adhesive, Kuraray, Tokyo, Japan)
6. Single bond universal adhesive (3M, ESPE, USA)
7. Resin acrylic blocks
8. X Ray Diffractometry (Rigaku, Tokyo, Japan)
9. Scanning Electron Microscope – X ray energy dispersive mode (Gemini SEM, Zeiss, Germany)
10. Micro Raman Spectroscopy (Renishaw plc, Wotton-under-Edge, UK)
11. Amalgamator (Smaco, China)
12. Microbrush (Oro, India)
13. Cotton gauze
14. Light cure unit (Coltolux, Coltene, Switzerland)

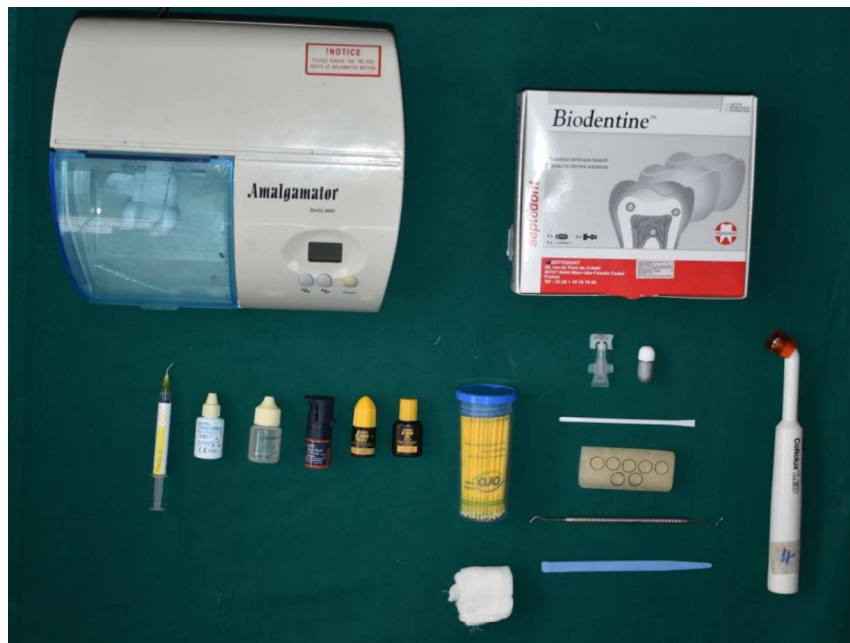


Figure 1: Armamentarium

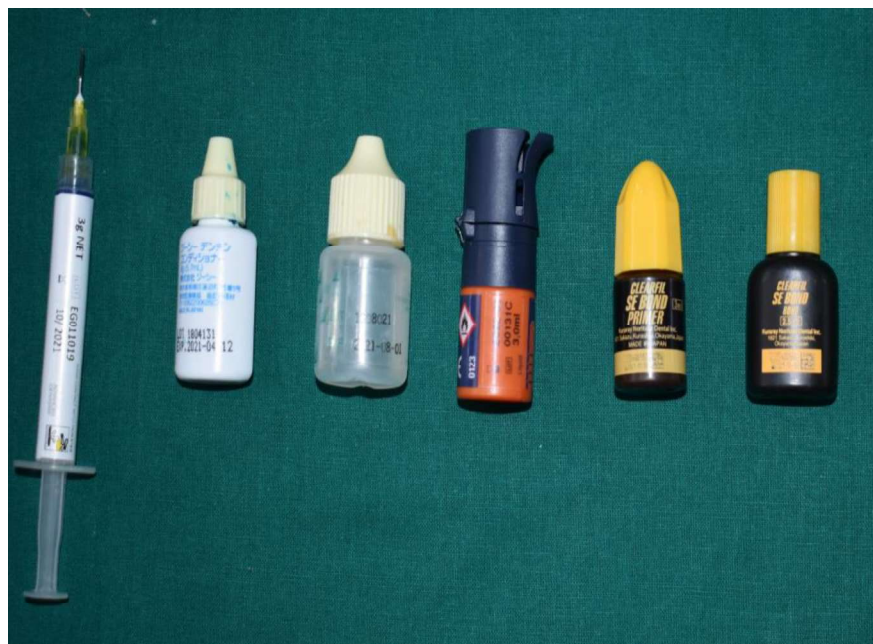
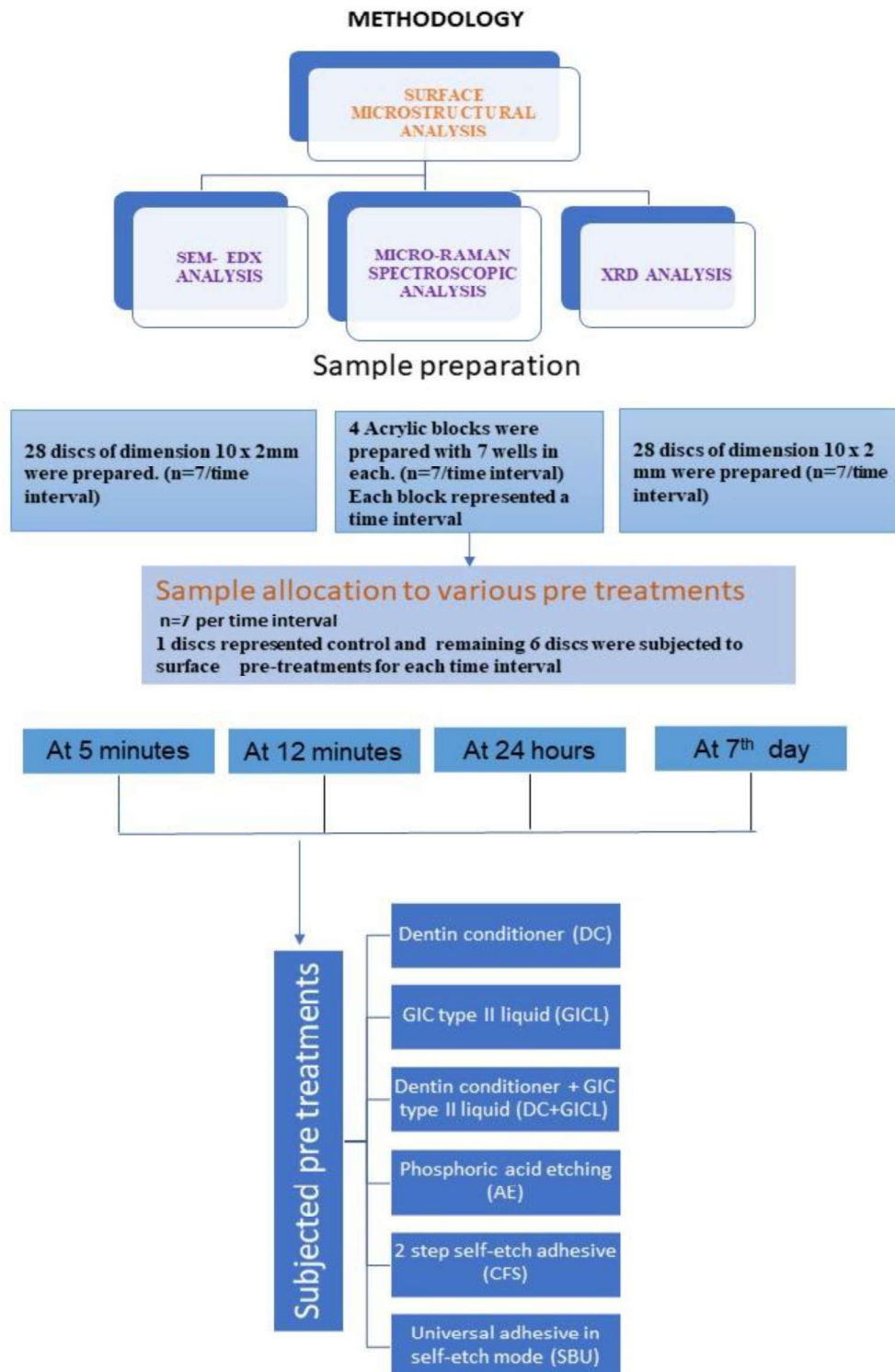


Figure 2: Batch number of the materials

Flowchart of the methodology carried out in the study



MATERIALS USED	MANUFACTURER	LOT NUMBER	PRINCIPLE INGREDIENTS	APPLICATION PROCEDURE
Biodentine	Septodont, France	B25878	Tri- calcium silicate	A capsule of Biodentine containing powder with mixed with five drops of liquid in an amalgamator at the speed of 4000-4200 rpm
Dentin conditioner (DC)	GC, Europe	1804131	10% polyacrylic acid	Applied dentin conditioner to the bonding surfaces for 20 seconds using a microbrush. Rinsed thoroughly with water for 20 seconds and blot dried.
GIC TYPE II LIQUID (GICL)	GC Fuji II, GC, Europe	1808021	Polybasic carboxylic acid 10-20%	A drop of liquid was dispensed into a well, a layer of liquid was applied using a microbrush on top of the surface.
Phosphoric acid etching (AE)	D tech etching gel, D- tech technologies	EG 011019	37% ortho phosphoric acid	A drop of acid etchant was dispensed into a well, applied on the surface using microbrush for 15 seconds and rinsed with water for 20 seconds and blot dried.

Clearfil SE (CFS)	Kuraray Noritake, Tokyo, Japan	Primer – 6Q0332 Bond – 6S0566	Primer: MDP, HEMA, dimethacrylate monomer, water, photoinitiator Bond: MDP, HEMA, dimethacrylate monomer, microfiller, photoinitiator	Primer was applied and left for 20 seconds and gently blown dry. Bond was applied and evened with mild air flow. Light cured for 10 seconds.
Single bond universal (SBU)	3M ESPE, St. Paul, MN, USA	00131C	BisGMA, a methacrylate functional copolymer dimethacrylates, HEMA, water, ethanol, colloidal filler, and photoinitiator	Single Bond universal was applied with rubbing motion using a microbrush for 20 seconds followed by a gentle air drying for 5 seconds and light cured for 10 seconds.

Table 1 – Overview of the materials used in the study. All the materials were used according to the

manufacturer's instructions.

A total of 81 Biodentine samples were prepared and used for various analysis.

1. SCANNING ELECTRON MICROSCOPY WITH X RAY

ENERGY DISPERSIVE MODE (SEM-EDX)ANALYSIS:

28 discs of Biodentine were totally prepared for SEM-EDX analysis. Biodentine was mixed according to the manufacturer's instructions in an amalgamator and filled into a stainless steel mold of dimension 10mmx2mm. The Biodentine was over-filled and compressed with a glass slab to remove the excess from the surface. All the discs were subjected to surface treatment as given below:

A. Control - No pre-treatment

B. Dentin Conditioner (DC) - (10% polyacrylic acid) was applied on the surface of prepared Biodentine disc with the help of a microbrush, after 20 seconds, it was rinsed off with water and blot dried. (figure 4a, 4b)

C. Glass ionomer type II liquid (GICL) - A thin layer of GIC type 2 liquid was applied on the surface of the prepared Biodentine disc with the help of a microbrush. (figure 5)

D. Dentin Conditioner followed by Glass Ionomer type II liquid (DC+GICL) - Dentin conditioner was applied on the surface of prepared Biodentine disc with the help of microbrush for 20 seconds and it was rinsed off with water and blot dried; followed by application of a thin layer of GIC type 2 liquid. (Figure 6a, 6b)

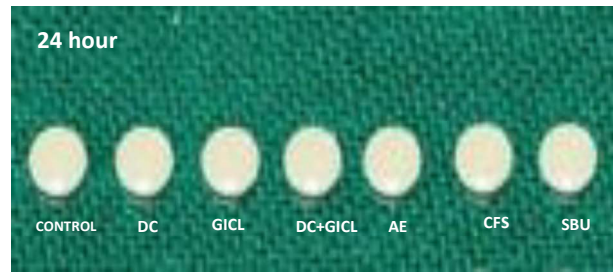


Figure 3: Representation of sample preparation for SEM analysis. 7 discs of Biodentine prepared for SEM-EDX analysis (24 hours)

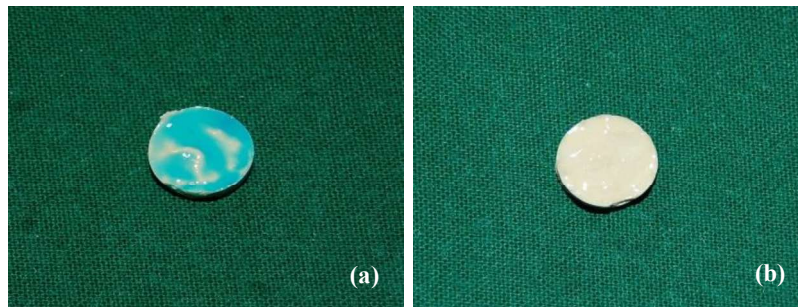


Figure 4: Pre- treatment with Dentin Conditioner (a), after blot drying (b)



Figure 5: Pre-treatment with GIC type II liquid

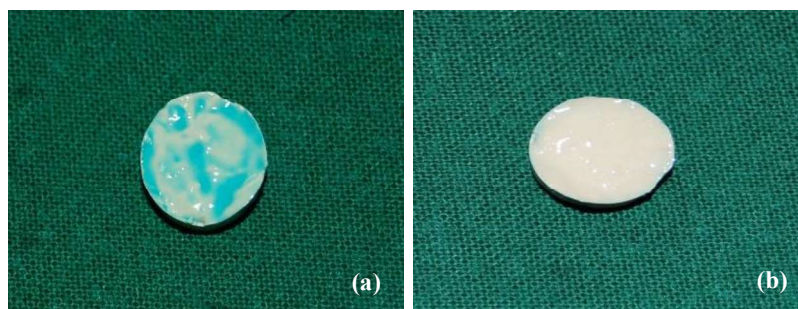
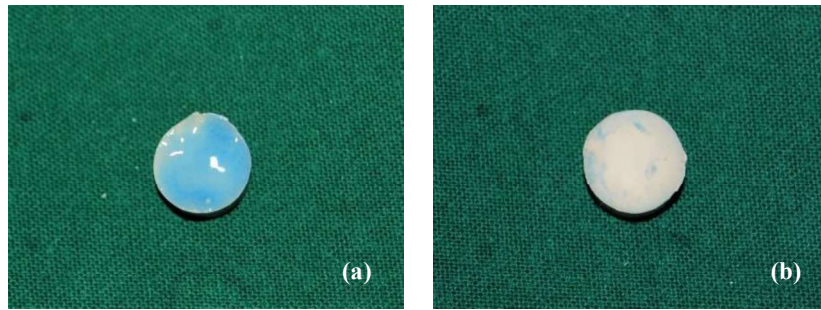


Figure 6: Dentin Conditioner (a) +GIC type II liquid (b) pre-treatment

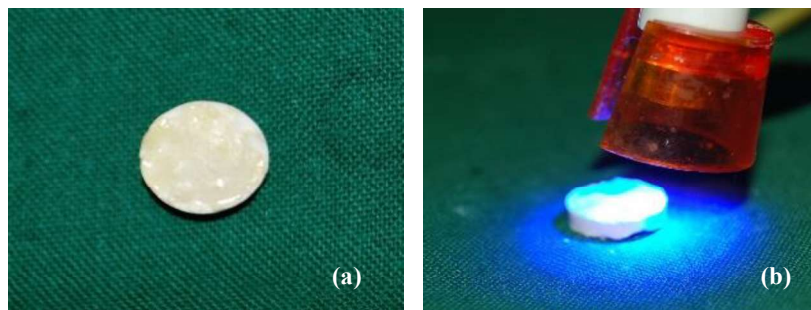
E. **Phosphoric Acid Etching (AE)** - 37% ortho phosphoric acid was applied on the surface of prepared Biodentine disc with the help of a microbrush, left for 20 seconds and then, rinsed off with water for 15 second and blot dried. (Figure 7a, 7b)

F. **ClearFil SE (CFS)** - Primer was applied with a microbrush on the surface of prepared Biodentine disc and left for 20 seconds and gently blown dry. Bond was applied with a microbrush and evened out with mild air flow followed by light curing for 10 seconds. (Figure 8 a, 8b)

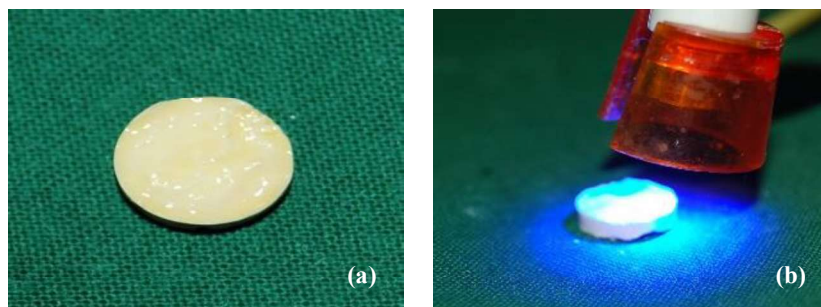
G. **Single Bond Universal (SBU)** – SBU was applied on the surface of prepared Biodentine disc with rubbing motion using a microbrush for 20 seconds followed by gentle air drying for 5 seconds and light cured for 10 seconds. (Figure 9a, 9b)



**Figure 7: Pre-treatment with 37% ortho phosphoric acid
(a), after blot drying (b).**



**Figure 8: Pre-treatment with Clearfil SE
(a) followed by light curing for 10 seconds (b).**



**Figure 9: Single Bond Universal pre-treatment
(a) followed by light curing (b).**

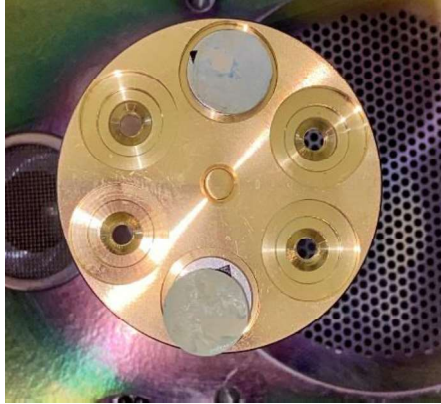


Figure 10: Sample placed on the aluminum stub for SEM- EDX analysis



Figure 11: Scanning electron microscopy with X-ray energy dispersive mode. (Gemini SEM, Zeiss, Germany)

Following each of the surface pre-treatments performed at the designated time interval, the Biodentine discs were vacuum desiccated and attached to aluminum stubs (figure 10), carbon coated and viewed under the scanning electron microscope at a power setting of 10 kV. The pre-treated surfaces were first scanned at a lower magnification and then scanning electron micrographs were obtained at a higher magnification followed by X-ray energy dispersive analysis (EDX) (figure 11) of the different phases at five random spots on the surface of the sample were analyzed.(22)

2. X RAY DIFFRACTOMETER ANALYSIS (XRD)

28 discs of Biodentine with the dimension 10mm x 2mm were prepared as mentioned above. From these 28 discs, 4 discs served as control whereas the remaining 24 discs were surface pre-treated as mentioned earlier. (Figure 4-9) The control and surface pre-treated samples at 5 minute, 12 minute, 24 hour and 7th day were crushed using a mortar and pestle for analysis (Figure 13). All samples were subjected to phase analysis using X-ray diffraction. The diffractometer used Cu Ka radiation at 40 mA and 45 kV. Samples were presented in powder form (figure 14) and the detector was set to rotate between 5° and 45°, at a sampling width of 0.05°, and a scan speed of 2°/min (figure 15).(22) The data were acquired in the form of graphs which represented the crystalline phases of the samples.



Figure 12: Representation of sample preparation for XRD analysis. 7 discs of Biodentine prepared for XRD analysis (24 hours)

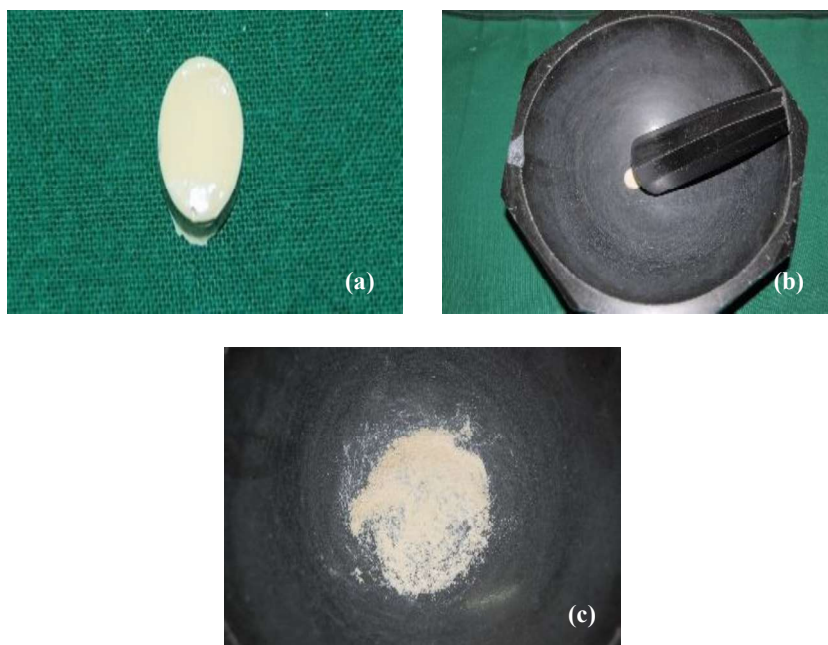


Figure 13: Prepared and pre-treated disc (a), powdered in motor and pestle (b,c)



Figure 14: Feeding the powdered samples into XRD



Figure 15: X – ray diffractometer (XRD) (Rigaku, Tokyo, Japan)

3. **MICRO-RAMAN SPECTROSCOPY ANALYSIS:**

4 acrylic blocks one for every tested time interval was prepared with 7 wells in each and polished (figure 16). Each well measured 10x2 mm. Biodentine was mixed according to the manufacturer's instructions with an amalgamator, the wells were over-filled and subsequently compressed with a glass slab to remove the excess from the surface. Each block had one Biodentine filled well which served as control and the remaining wells were subjected to surface pre-treatments as mentioned earlier. After surface pre-treatments, the samples were subjected to micro Raman spectroscopy analysis. A water immersion x60/1.2NA objective lens Plan Apo VC was used with a 532-nm laser source (25 mW line illumination) a 600 lines/mm diffraction grating. The Raman spectra were obtained on the surface of samples (figure 17). (71) The data were acquired in the form of graphs which represented the functional groups present on the surface.

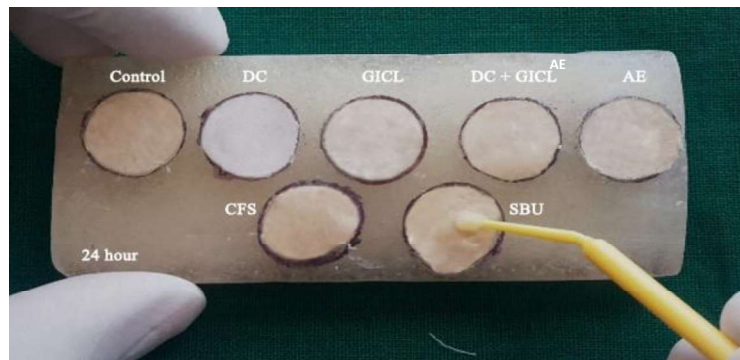


Figure 16: Wells in acrylic block filled with Biodentine for pre-treatment at 24 hours



Figure 17: Micro Raman Spectroscope

RESULTS

SCANNING ELECTRON MICROSCOPY ANALYSIS – SURFACE CHARACTERISATION

Scanning electron micrographs obtained at 1000x and 10,000x were used to analyse the surface morphology of the control and pre-treated Biodentine samples at different time intervals.

At 5 minute

The SEM analysis and the EDX could not be performed immediately, at 5 minutes from the time of manipulation and pre-treatment, due to technical limitations. Hence, the 5 minute pre-treatment samples were analysed after 12 minute (figure 18 A). The surface microstructure of the DC pre-treatment revealed irregular agglomerations (figure 18 B). The GICL pre-treated samples (figure 18 C) and DC+ GICL pre-treatment combination (figure 18 D) revealed irregular surface agglomerations with polymer coating. Phosphoric AE pre-treatment when viewed at 1000x revealed, cracks and craters like surface defects with loss of the agglomerations and eroded surface morphology (figure 18 E). When analysed at higher magnification 10,000x thin flake like residues were observed. The CFS pre-treatment revealed homogenous surface with sparsely located surface defects covered by a layer of polymeric coating (figure 18 F). The SBU pre-treatment revealed homogenous grainy appearance covered by a uniform polymeric coating (figure 18 G).

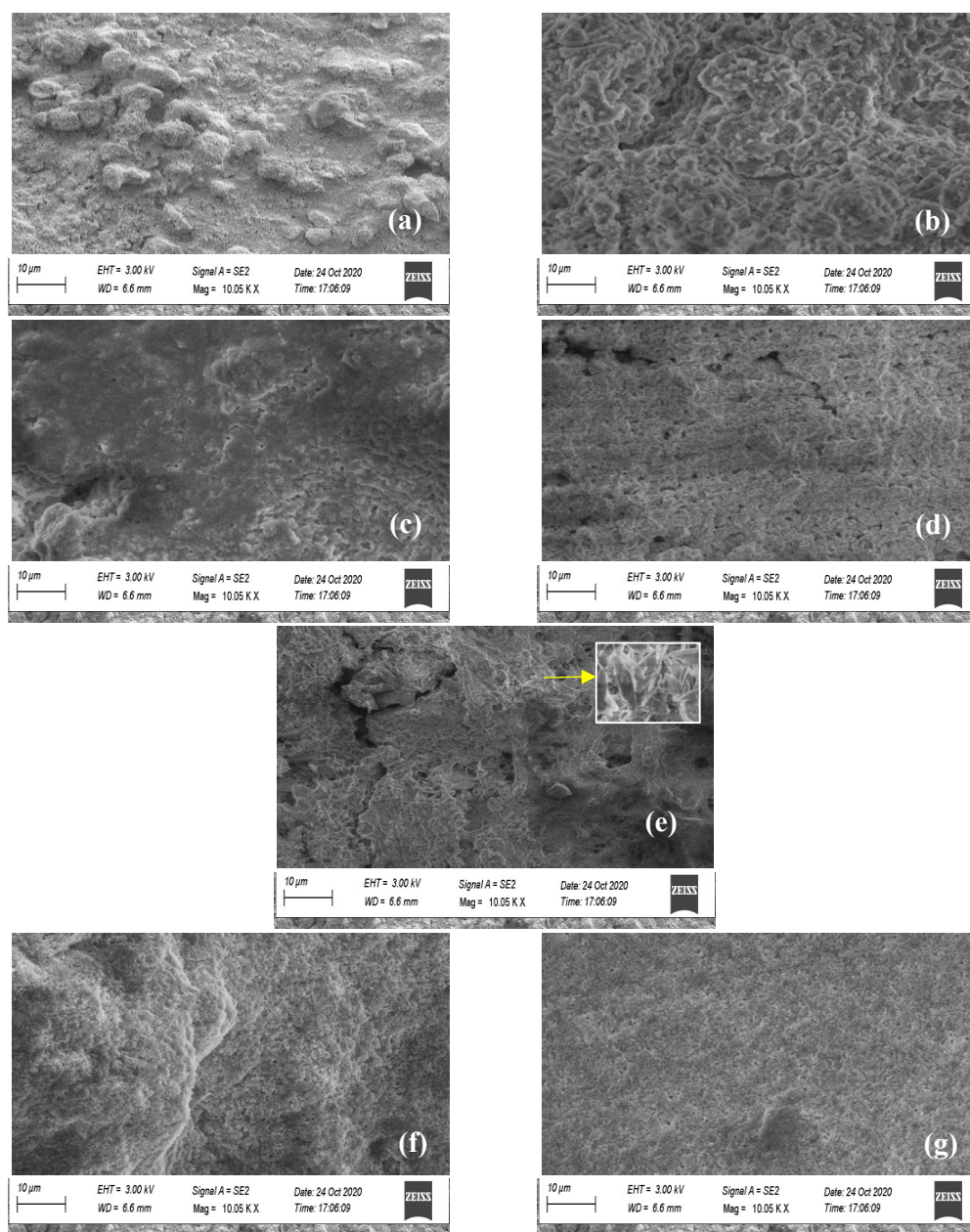


Figure 18: SEM images for 5 minutes (a) – control (12 minutes) revealed agglomerations on the surface; (b) the DC pre-treatment revealed irregular agglomerations; (c) the GICL and (d) the DC and GICL combination pre-treatment revealed irregular agglomerations with polymer coating; (e) Phosphoric AE pre-treatment revealed loss of agglomerations with eroded surface. The CFS pre-treatment revealed surface irregularities with a polymer coating (f). The SBU pre-treatment revealed homogenous grainy appearance with a uniform polymeric coating (g).

At 12 minute

The control sample revealed homogenous surface with a few agglomerations (figure 19 A). DC surface pre -treatment revealed that these huge agglomerations were replaced by a grainy morphology (figure 19 B). GICL surface pre - treatment revealed a homogenous polymer coating with isolated cracks (figure 19 C). DC and GICL combination surface pre-treatment revealed agglomerations on the surface interspersed with the polymer coating (figure 19 D). Phosphoric AE pre-treatment revealed a frosty appearance with multiple surface defects. At a higher magnification of 10,000x, the surface revealed particles of unreacted powder projecting from an eroded matrix with complete loss of grainy morphology. Crumbled surface leading to crater like defects occupied by raw flake like structures were also observed at this magnification (figure 19 E). CSF surface pre-treatment revealed a homogenous underlying morphology coated with a discontinuous polymeric layer with multiple voids (figure 19 F). SBU pre-treatment revealed a uniform polymer coating (figure 19 G).

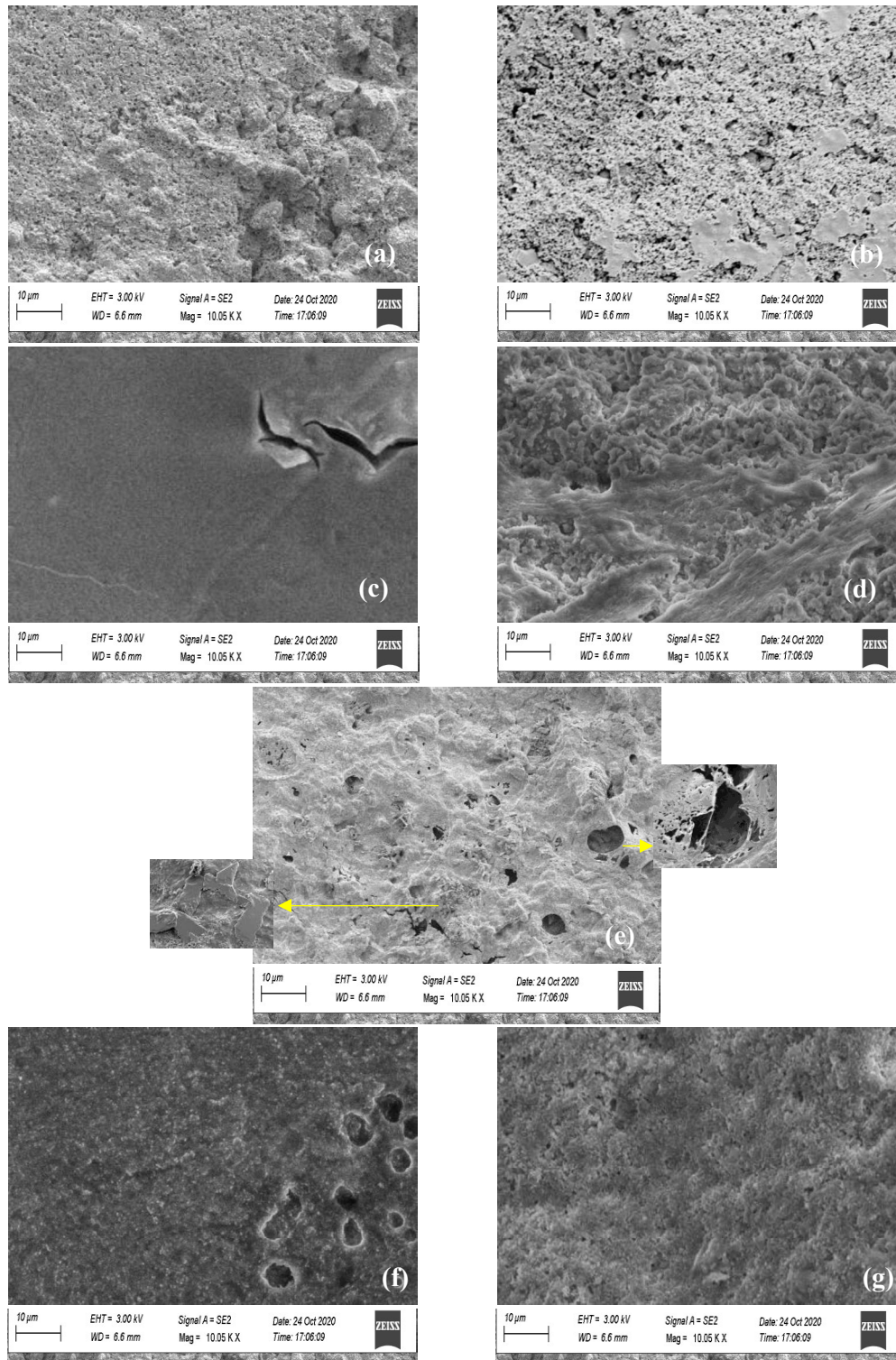


Figure 19: SEM images for 12 minutes time interval (a) – control revealed homogenous surface with few agglomerations; (b) the DC pre-treatment revealed replacement of the agglomerations with a grainy morphology (c) the GICL pre-treatment revealed homogenous polymer coated surface with isolated cracks; (d) the DC and GICL combination pre-treatment revealed irregular agglomerations interspersed with polymer coating; (e) Phosphoric AE pre-treatment revealed the particles of unreacted powder projecting from the eroded matrix with complete loss of grainy morphology (f) CSF surface pre-treatment revealed a homogenous underlying morphology coated with a discontinuous polymeric layer with multiple voids (g) SBU pre-treatment revealed a uniform polymer coating.

At 24 hour

The control sample revealed needle like grainy surface morphology (figure 20 A). DC pre-treatment resulted in partial loss of grainy morphology interspersed with smooth eroded surfaces (figure 20 B). GICL pre-treatment (figure 20C) revealed uniform polymeric coating, but with few cracks. DC+GICL pre-treatment showed a uniform polymeric coating on the surface (figure 20D). Phosphoric AE pre-treatment showed damaged needle like grains with raw flake like structures on the surface with no evidence of micro porosities (figure 20 E). Both CFS (figure 20 F) and SBU pre-treatment (figure 20 G) revealed an uniform surface roughness with irregular areas of polymer coating.

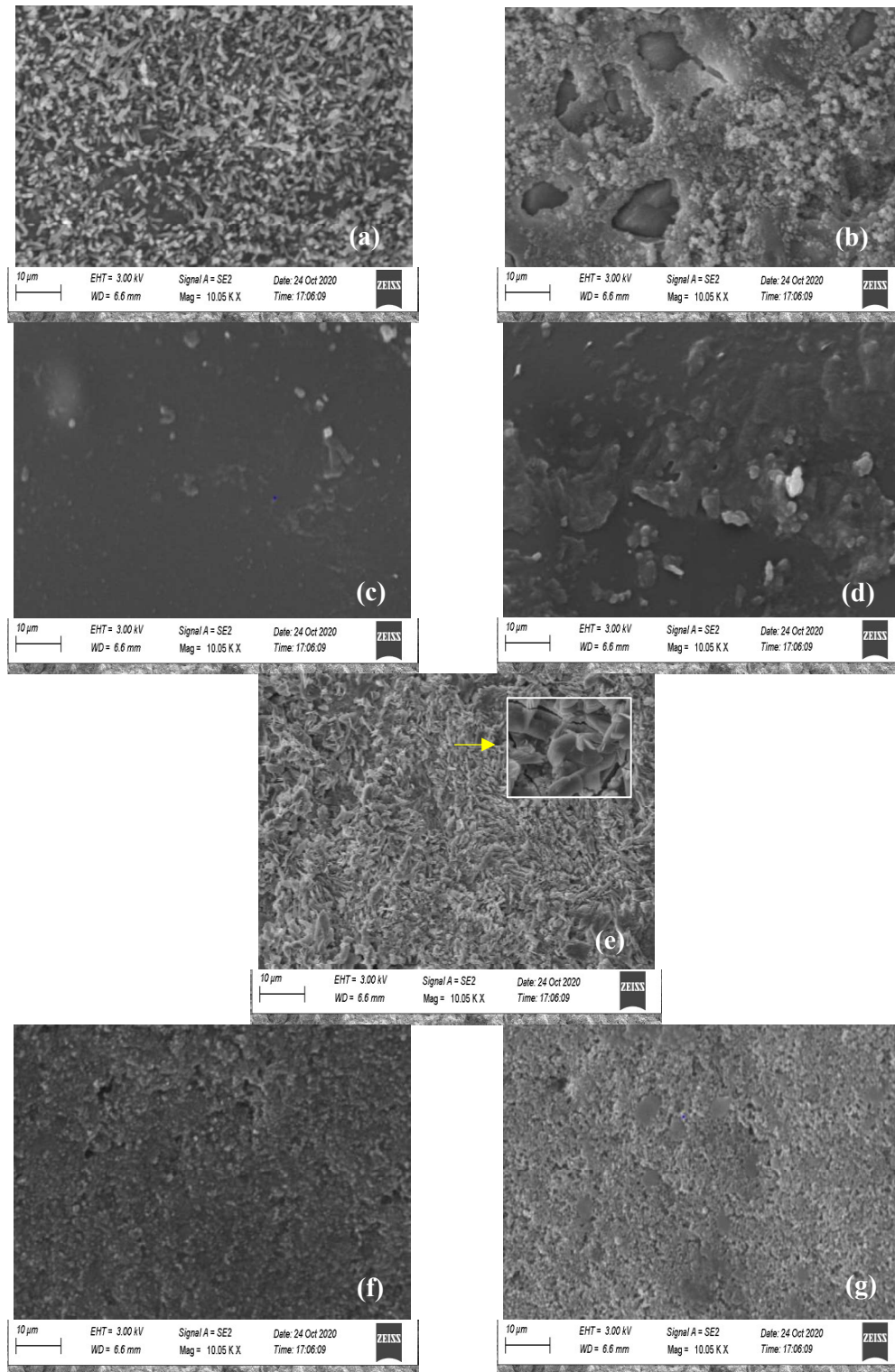


Figure 20: SEM images for 24 hour (a) – control revealed needle like grainy morphology; (b) the DC pre-treatment revealed partial loss of grainy morphology with smooth eroded surface; (c) GICL pre-treatment revealed uniform polymeric coating, but with few cracks (d) DC+GICL pre-treatment showed a uniform polymer coated surface. (e) AE pre-treatment revealed the damaged needle like grainy structure with flake like surface defects and absence of micro porosities (f) the CFS pre-treatment and (g) SBU pre-treatment revealed surface roughness with irregular areas of polymer coating.

At 7th day

The control sample revealed partially coalesced smooth morphology with less grainy matrix amidst them (figure 21 A). DC pre-treated sample revealed also revealed similar surface morphology to that of control with no evidence of acid pre-treatment (figure 21 B). GICL pre-treated sample revealed underlying surface morphology similar to that of control with polymeric coating showing isolated cracks (figure 21 C). DC+GICL pre-treatment showed complete loss of surface morphology with a smooth polymeric coating (figure 21 D). Phosphoric AE showed surface irregularities in the form of well-defined micro-porosities widespread on the surface (figure 21 E). The CFS pre-treatment showed a smooth surface (figure 21 F) and SBU pre-treatment revealed non continuous polymer coating with large surface defects in the form of voids (figure 21 G).

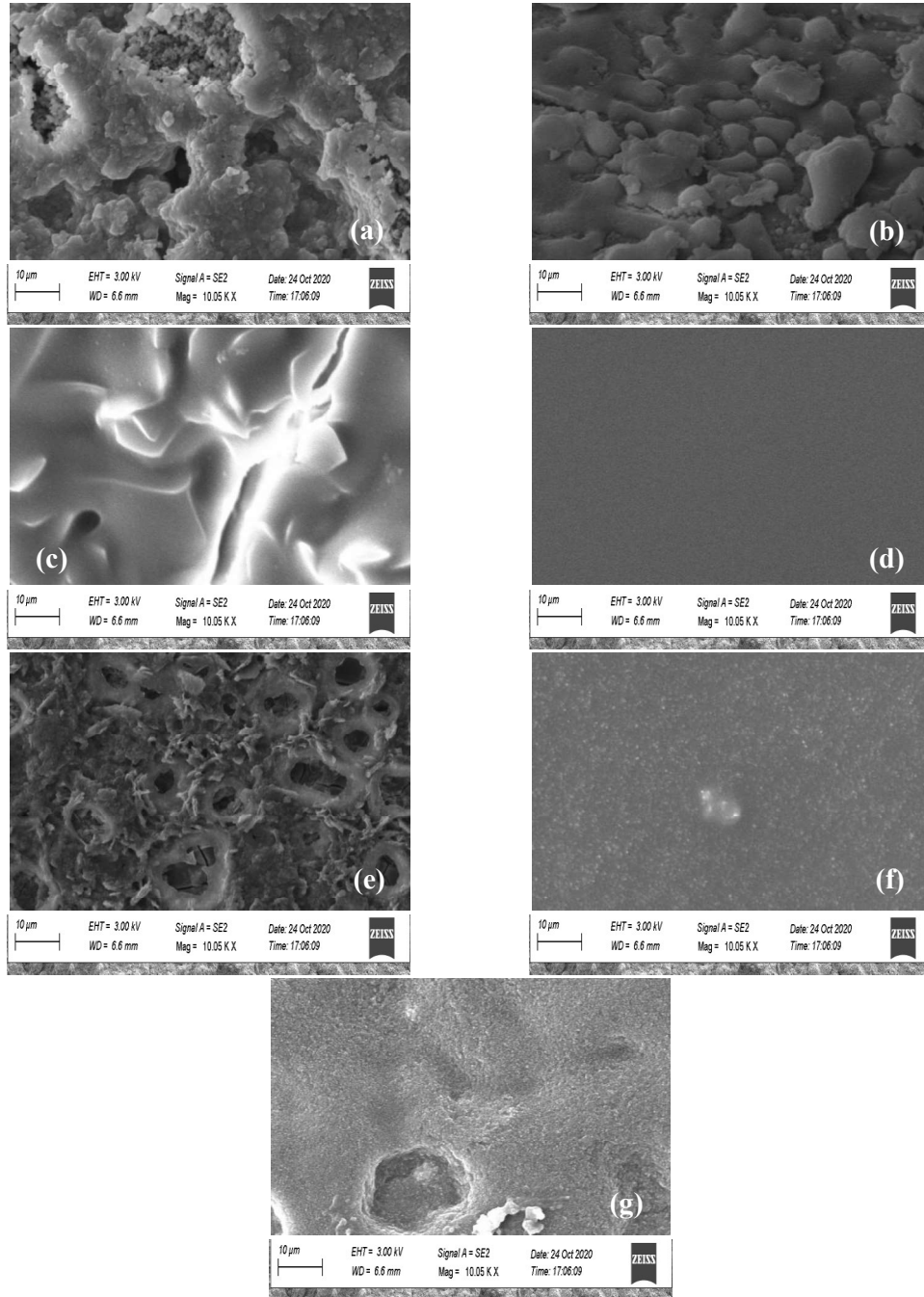
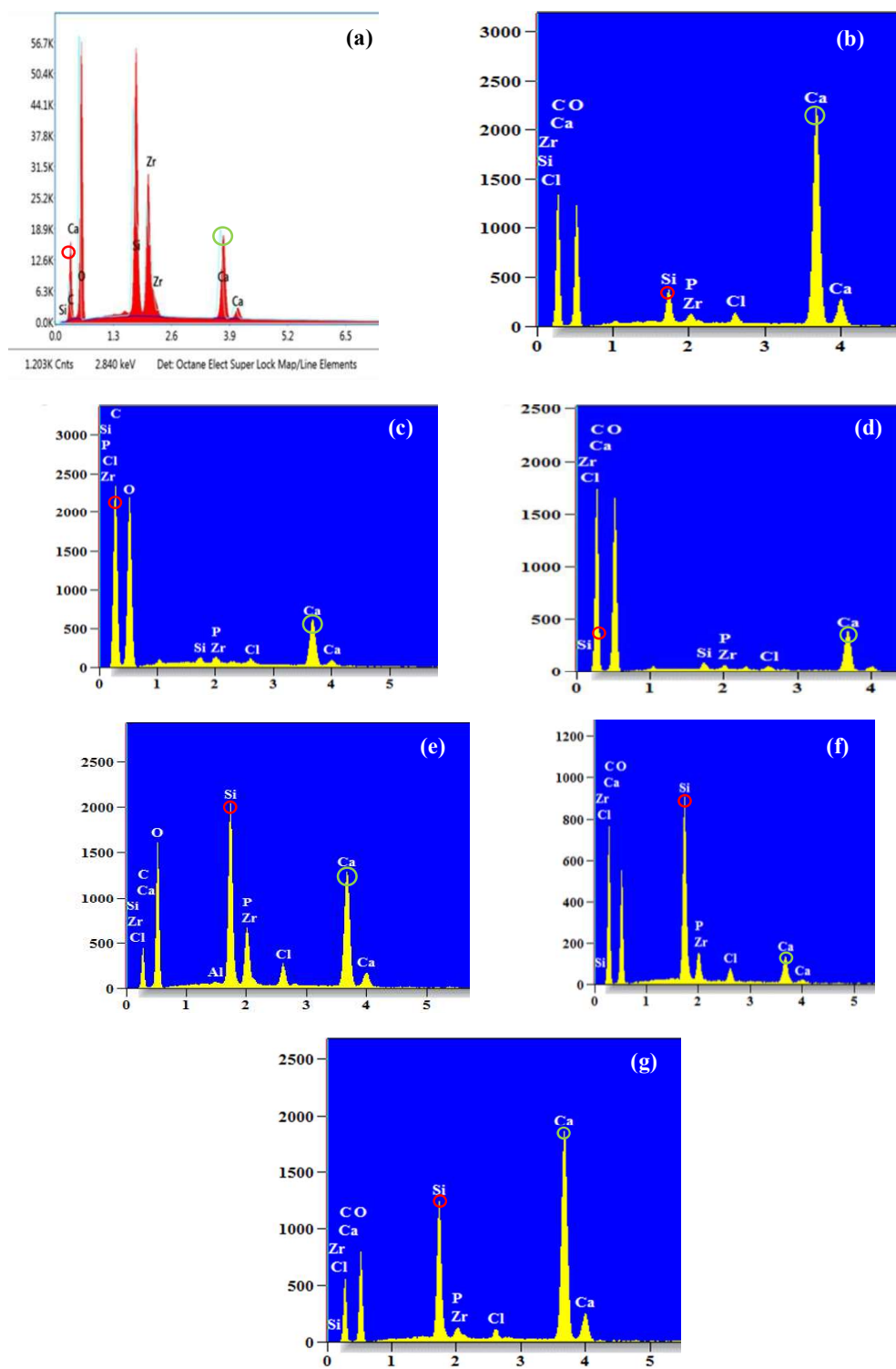


Figure 21: SEM images for 7th day (a) – control revealed partially coalesced homogenous microstructure with less grainy matrix amidst them (b) the DC pre-treatment revealed surface agglomerations with complete loss of grainy structure (c) the GICL pre-treatment revealed polymeric residues with surface cracks and irregularities ; (d) the combination of DC and GICL pre-treatment revealed loss of surface morphology with smooth polymeric coating; (e) AE pre-treatment revealed surface irregularities in the form of well-defined micro-porosities widespread on the surface; (f) the CFS pre-treatment revealed a smooth surface morphology; (g) the SBU pre-treatment revealed non continuous polymer coating with large surface defects in the form of voids.

EDX ANALYSIS

The elements present in the microstructure were determined using X ray energy dispersive mode. The elemental data were acquired in the form of graph.

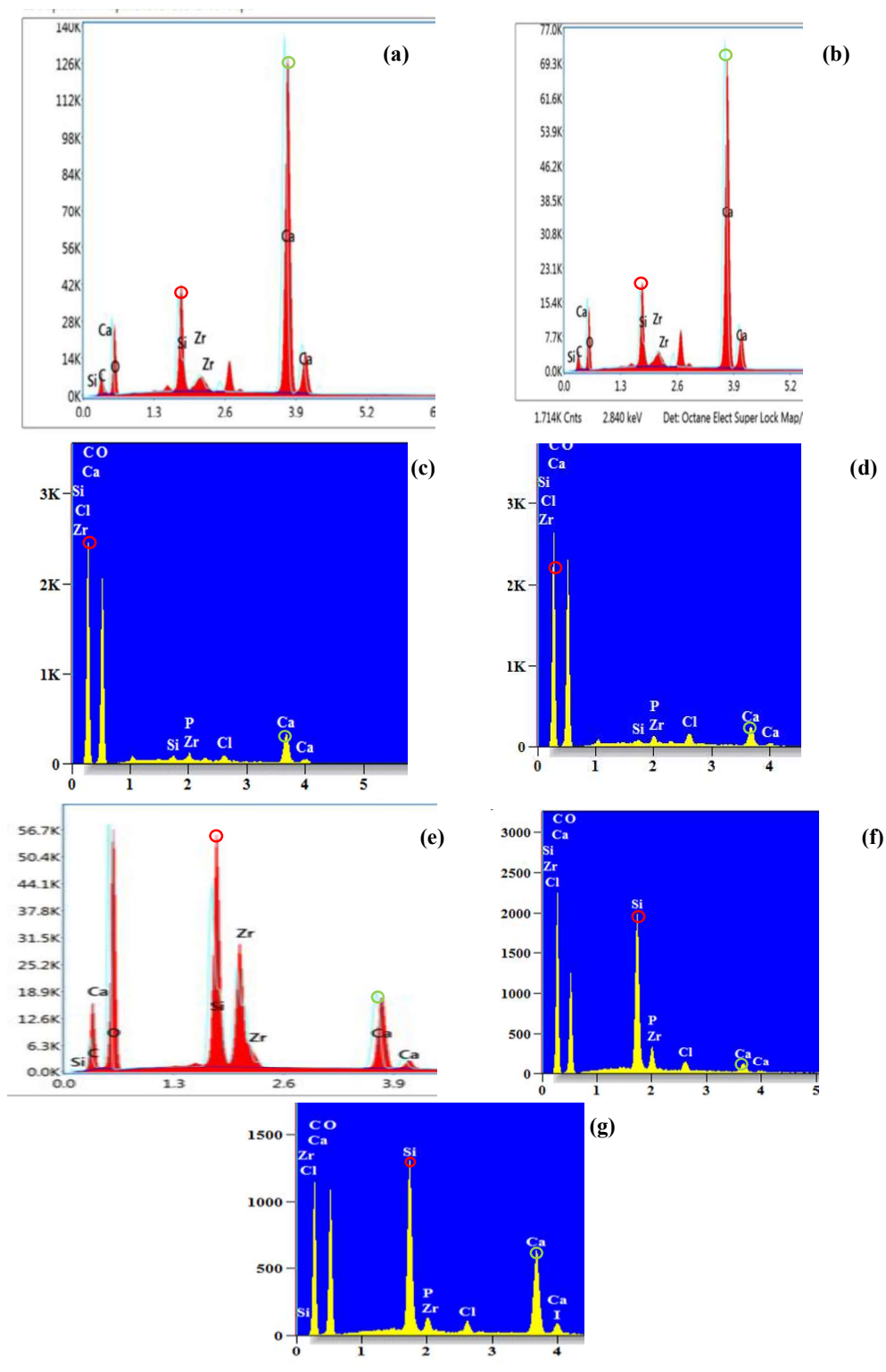
EDX analysis for all the control samples except 5 minute, revealed peaks for carbon, oxygen, chloride interspersed in the matrix of calcium and silicon, for all tested times. The calcium to silicon ratio was 8.3 for the 12 minute control sample (graph 2a), which further increased to 9.8 for the 24 hour control (graph 3a) and declined to 5.7 at 7th day (graph 4a). DC, GICL and a combination pre-treatment of both showed variable calcium to silicon ratio at all time intervals, with DC pre-treatment showing a highest of 19 at 5 minutes and DC+GICL pre-treatment showing the lowest of 0.53 at 7 days (graph 2 b-d, 3 b-d, 4 b-d). AE, CFS, and SBU pre-treatment revealed a drastic reduction in the calcium to silicon ratio at all time intervals when compared with the respective control samples, with phosphoric AE pre-treatment showing the lowest of 0.2 at 5 minutes and highest of 2.6 at 24 hour (graph 1 e-g, 2 e-g, 3e-g, 4 e-g).



Graph 1 shows EDX of the 5 minutes time interval of control (a), DC (b), GICL (c), DC+GICL (d), AE (e), CFS (f), SBU (g).

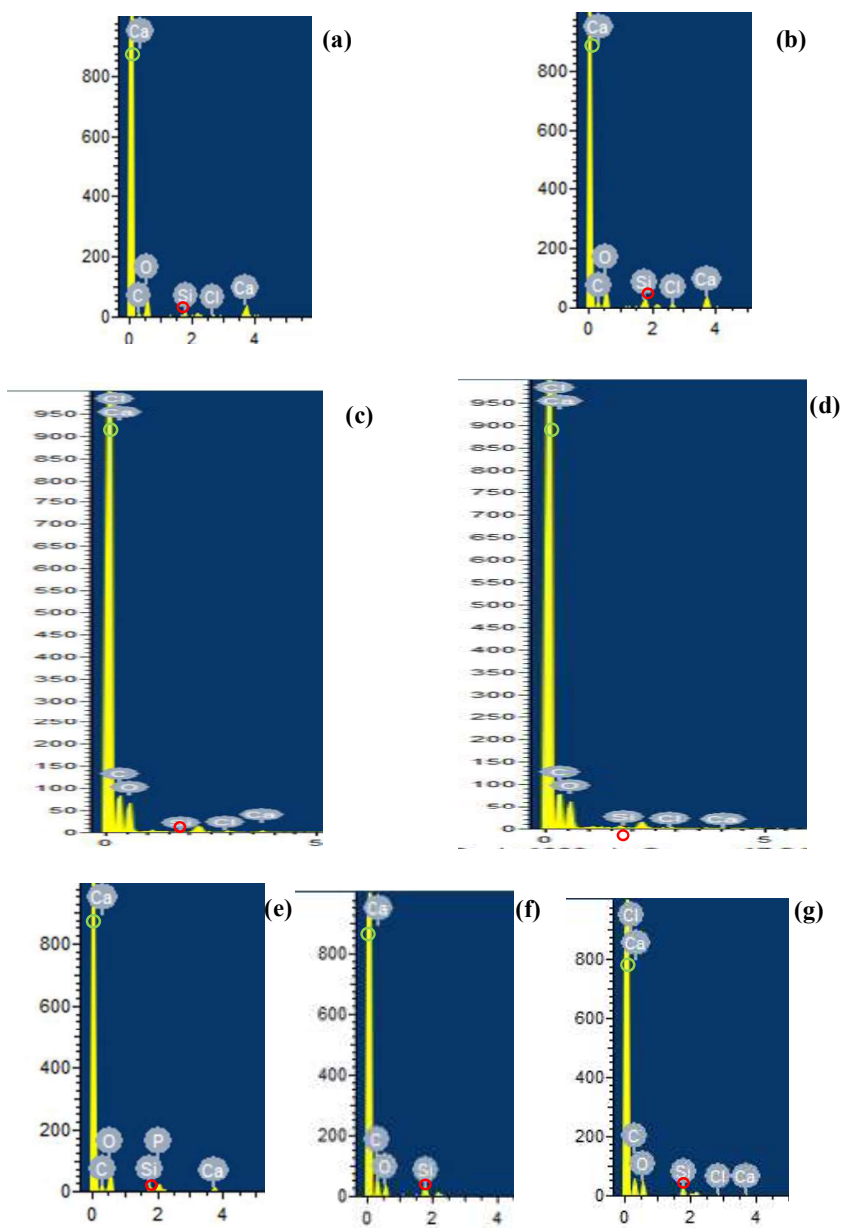
○ Represents calcium

○ Represents silicon



Graph 2 shows the EDX of 12 minutes time interval of control (a), DC(b), GICL(c), DC+GICL(d), AE(e), CFS(f), SBU(G).In the control sample the calcium to silicon ratio was observed to be 8.3 (a).

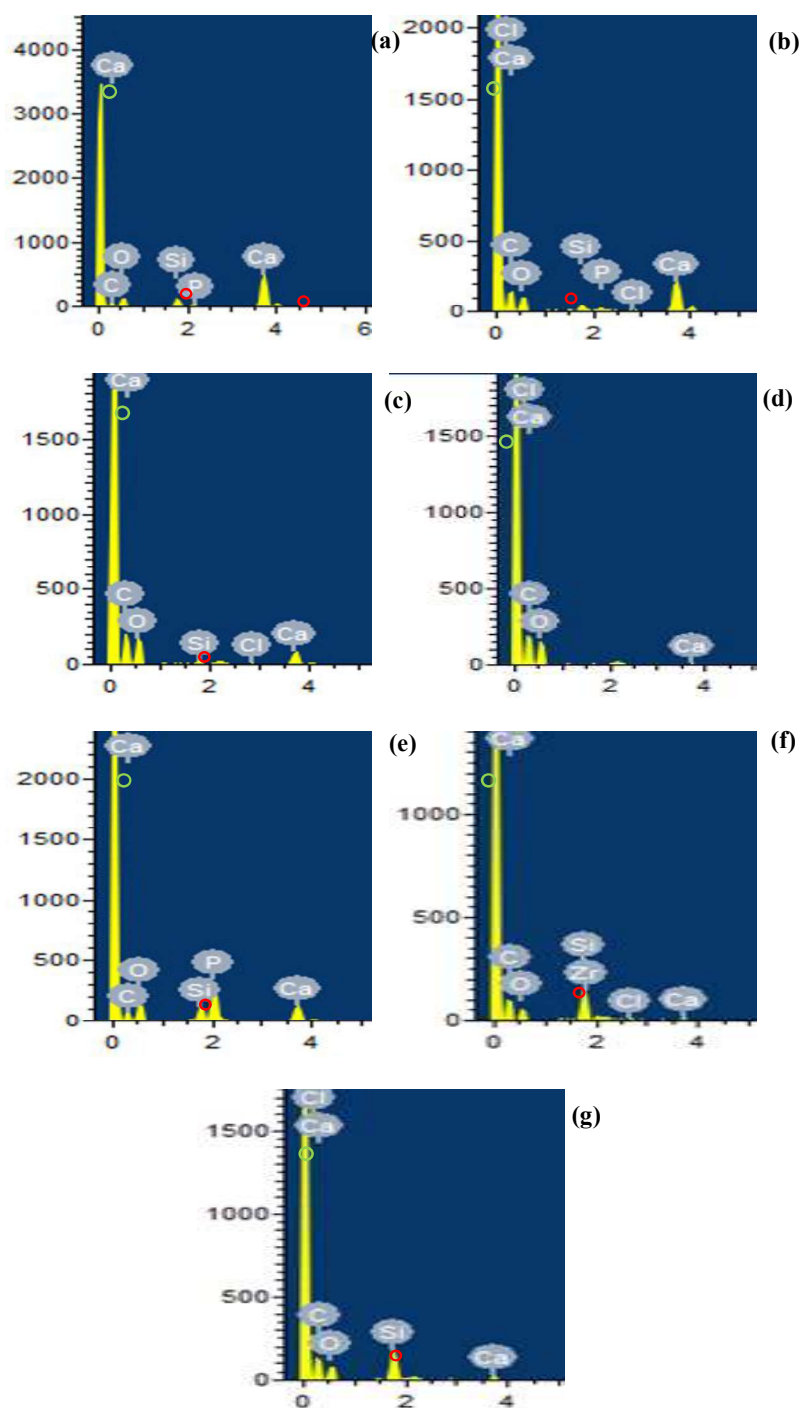
- Represents calcium
- Represents silicon



Graph 3 shows the EDX of 24 hour time interval of control (a), DC (b), GICL (c), DC+GICL (d), AE(e), CFS (f), SBU (g). In the

○ Represents calcium

○ Represents silicon



Graph 4 shows EDX of the 7 days time interval of control (a), DC (b), GICL (c), DC+GICL (d), AE(e), CFS (f), SBU (g).In the control sample the calcium to silicon ratio was observed to be 5.7 (a).

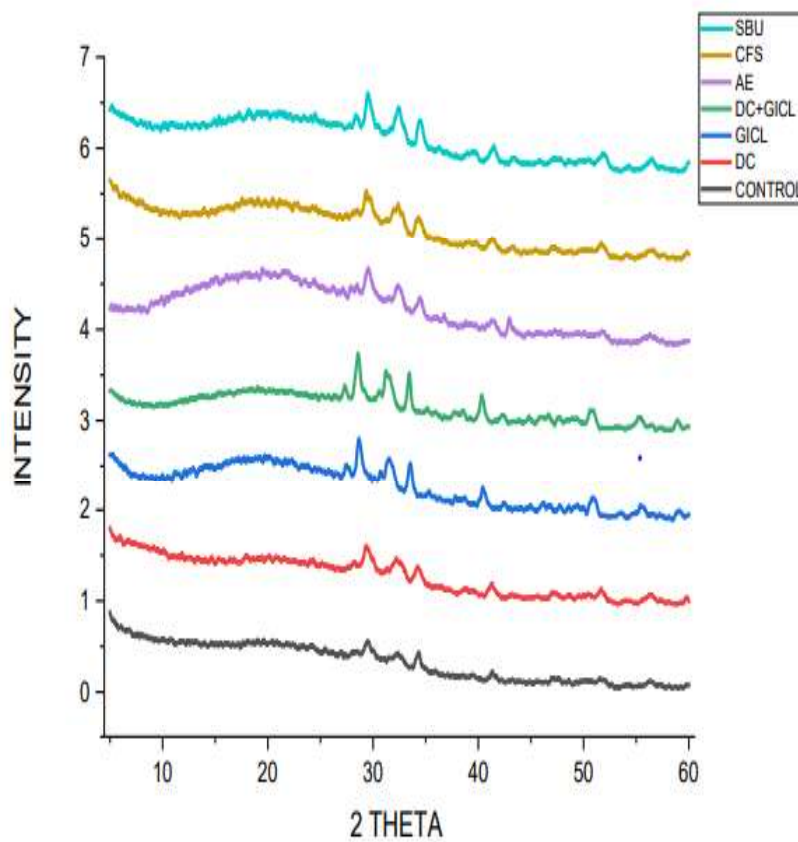
○ Represents calcium
○ Represents silicon

XRD ANALYSIS

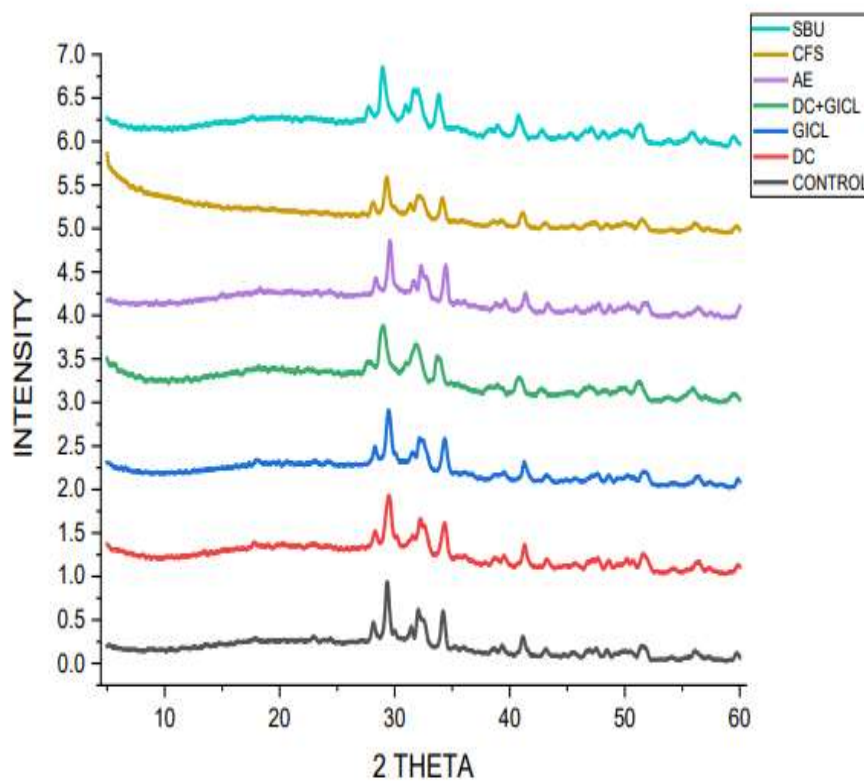
The data was acquired in the form of graphs with peaks that represented the crystalline phases in the samples. All the samples matched with JCPDS-ICDD (Joint Committee on Powder Diffraction Standards – International Centre for Diffraction Data) # 31-0301, which confirmed the tricalcium silicate presence, a triclinic phase with a lattice parameter of $a = 14.013 \text{ \AA}$, $b = 14.210 \text{ \AA}$ and $c = 25.100 \text{ \AA}$ with a space group of $p1(1).(72)$ The diffraction peaks observed at 29.51° , 32.06° , 32.32° , 32.70° , and 34.73° 2θ can be respectively indexed as (401), (009), (044), (322), (445) planes of Biodentine. The peaks at 29.51° 2θ represents calcium carbonate, 32.06° 2θ , 32.32° 2θ , 32.7° 2θ represents calcium silicates, and 34.73° 2θ represents the calcium hydroxide. All the control and pre-treated Biodentine samples showed peaks for calcium carbonate(CC), calcium silicate(CS) and calcium hydroxide(CH) at all tested times.

At 5 minute, (Graph 5) the control sample exhibited sharp peaks for CC, CS, and CH crystalline phases and a broad peak around around the diffraction angle 20° 2θ indicating the presence of amorphous phase. The surface pre-treatment with DC, GICL and DC+GICL showed reduction in the intensities of all the crystalline peaks when compared to the control. The sharpness of the major peak at 32.06° 2θ indicating the crystallinity of set Biodentine was low at 5 minutes, both in the control sample and the pre treatment samples alike.

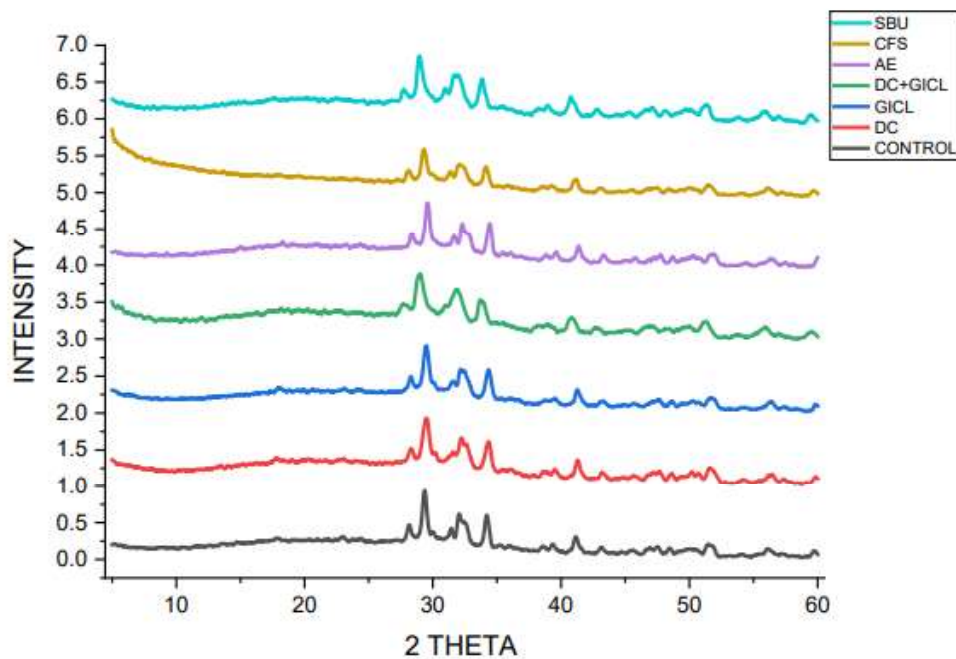
After the attainment of the initial setting at 12 minutes (graph 6), the control sample showed sharp peaks for CC, CS and CH. The same was observed for DC pre-treatment sample and a marked reduction in the peak intensities was observed after phosphoric AE pre-treatment followed by DC+GICL pre-treatment in the 12 minute samples. CSF and the SBU pre-treatments showed sharp peaks but with lesser intensity when compared to the control. A broad peak around the diffraction angle 2θ could be observed in the control and all pre-treatment samples at 12 minute. However, it was worth noting that the intensity of this peak was more prominent after phosphoric AE pre-treatment. The control and all pre treated samples at 24 hour (graph 7) showed similar peak intensities for CC, CS and CH and the presence of the broad peak at the diffraction angle 2θ was also evident. However, the peak intensities for the crystalline phases were less prominent than those observed in the 12 minute control sample. The XRD of the control and pre treated samples at 7th day (graph 8) showed the absence of the broad peak at the diffraction angle of 2θ , and all the peak which appeared for CC, CS and CH were found to be sharper and more intense when compared to the other tested times.



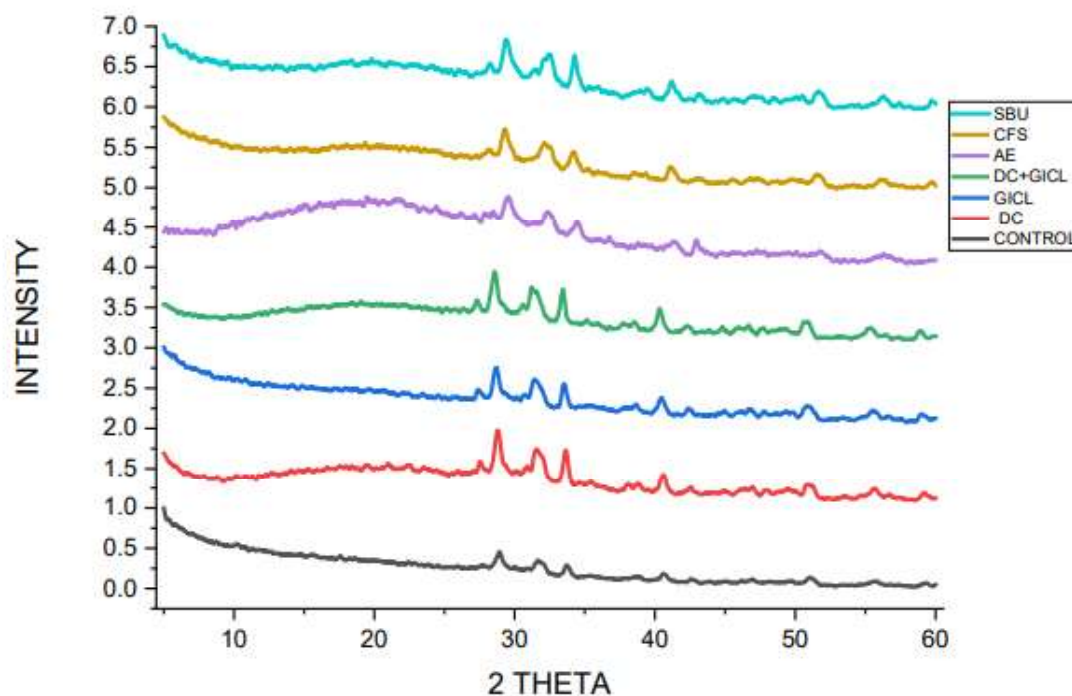
Graph 5 shows the XRD of 5 minute control, DC, GICL, DC+GICL, AE, CFS, SBU . Major peak at 32.06 2 θ showed reduced intensity at 5 minute in the control sample. The same was noted in all the pre-treatment samples.



Graph 6 shows the XRD of 12 minute control, DC, GICL, DC+GICL, AE, CFS, SBU. There was a marked reduction in the peak intensities after the AE pre-treatment followed by DC+GCL pre-treatment (d). CSF and the SBU pre-treatments showed sharp peaks but with lesser intensity when compared to the control at 12 minutes



Graph 7 shows the XRD of 24 hour - control, DC, GICL, DC+GICL, AE, CFS, SBU. Peak intensities for the crystalline phases (32.06 20) were less prominent than those observed in the 12 minute control sample



Graph 8 shows XRD of 7 day - control, DC, GICL, DC+GICL, AE, CFS, SBU. The absence of the broad peak at the diffraction angle of 20° , and all the peak which appeared for calcium carbonate, calcium silicate and calcium silicate were found to be sharp and more intense when compared to the other time intervals.

MICRO RAMAN SPECTROSCOPY ANALYSIS

The Raman spectra for the control sample at 5 minutes (Graph 9) showed peaks for zirconium oxide (ZO), calcium silicate (CS) and calcium carbonate (calcite) and the hydroxyl (OH-) functional groups. The peak position for zirconium oxide were at 480 cm^{-1} , all peaks in the region of 820 cm^{-1} , 860 cm^{-1} , and 883 cm^{-1} corresponded to the symmetric stretching of Si – O and symmetric stretching of SiO_4 , for calcite it was found to be at 1086 cm^{-1} , and

for hydroxyl group it was at 1600 cm^{-1} (graph 9) . The surface pre-treatment with DC at 5 minute showed peak intensities and the peak positions similar to that of the control. The GICL surface pre-treatment showed the same peaks for zirconium oxide, silicate, and calcite; all the peaks were found to be shorter than the control. New peaks appeared at 1480 cm^{-1} which was indicative of the symmetrical stretching of C-O group as the reaction by-product of carboxylic acid with calcium in Biodentine. The DC+GCL surface pre-treatment showed peaks indicative of zirconium oxide, silicate and calcite, but the peak intensity fell drastically and were less defined when compared to that of the control. New peaks appeared at 1315 cm^{-1} , 1485 cm^{-1} and at 1700 cm^{-1} which could be the result of reaction by-products. The peak at 1315 cm^{-1} indicated the hydroxyl group. The peak at 1485 cm^{-1} indicated the symmetrical stretching of CO_2 . Peak at 1700 cm^{-1} indicated the C=O stretching. AE, CSF and SBU pre treated samples showed peak positions for zirconium oxide, silicates and calcite. The peaks for calcite and silicate were ill defined compared to the control. Peak at 1512 cm^{-1} indicated OH- group and peaks appeared at 1785 cm^{-1} and 1934 cm^{-1} indicated phosphate functional group residues in the phosphoric AE pre- treatment sample. The surface pre-treatment with CFS showed new peaks at 1658 cm^{-1} indicative of hydroxyl group and at 1768 cm^{-1} indicative of phosphate ester groups formed as a reaction by-product. Pre-treatment with SBU showed reduction in all the

peaks and short and sharp peak appeared at 1485 cm^{-1} and at 1500 cm^{-1}

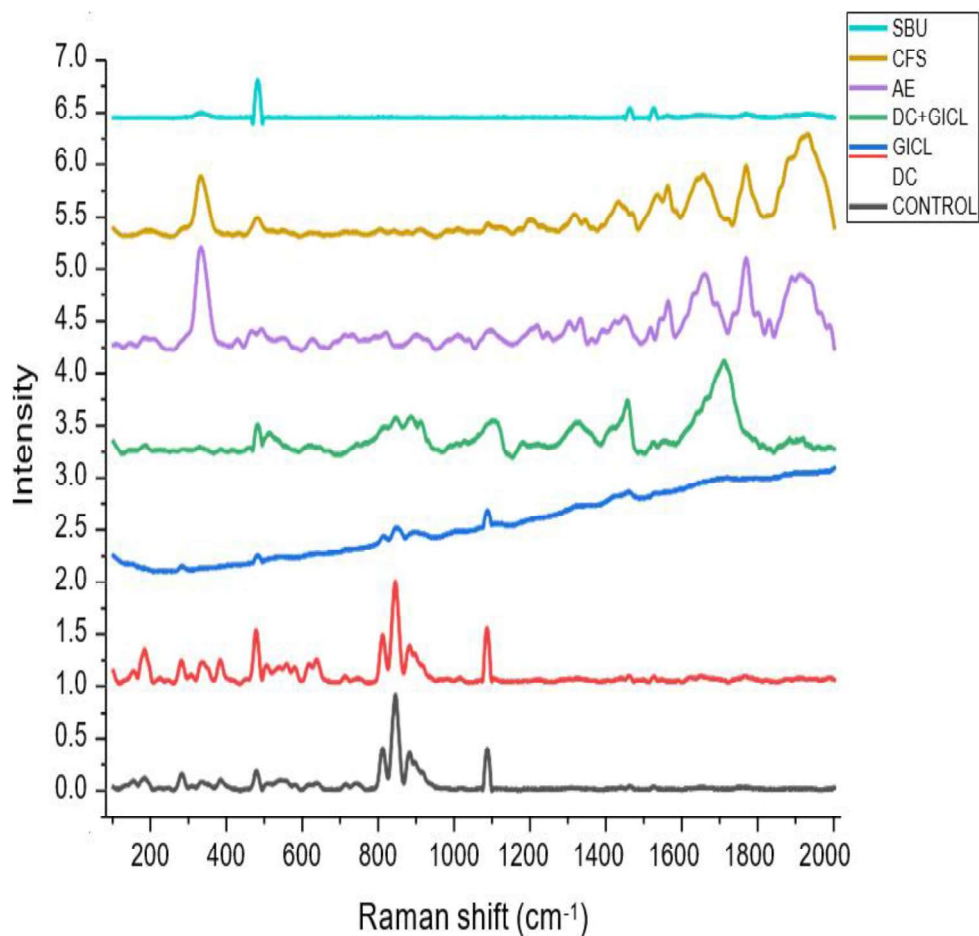
The Raman spectra at 12 minutes (graph 10) for the control and the DC pre-treatment samples showed peak intensities and peak positions similar to the 5 minutes control sample. The 12 minute GICL and DC+GICL surface pre-treatments showed Raman spectra similar to the 5 minute GCL and DC+GICL surface pre-treatment respectively. The phosphoric AE pre-treatment showed peaks for zirconium oxide, silicate, calcite. However, the peak intensities were lower for silicates, calcites when compared to the control. A sharp peak appeared at 1512 cm^{-1} indicative of the OH^- group. New peaks that appeared at 1785 cm^{-1} and 1934 cm^{-1} were indicative of phosphate functional group residues. The CFS and SBU pre-treatments showed similar peaks for zirconium oxide, silicates, calcites. However, the peaks depicting silicate and calcite were ill defined. New peaks appeared at 1658 cm^{-1} and at 1768 cm^{-1} similar to corresponding pre-treatments at 5 minute.

The Raman spectra for the control sample at 24 hour (graph 11) showed peaks for zirconium oxide, silicate, calcite and hydroxyl group. The only silicate peak intensity which was detected in the 24 hour control was at 860 cm^{-1} . All the peaks appeared to be shorter but sharper when compared to the control at 5 minute and 12 minute. The DC pre-treatment showed peak intensities and peak

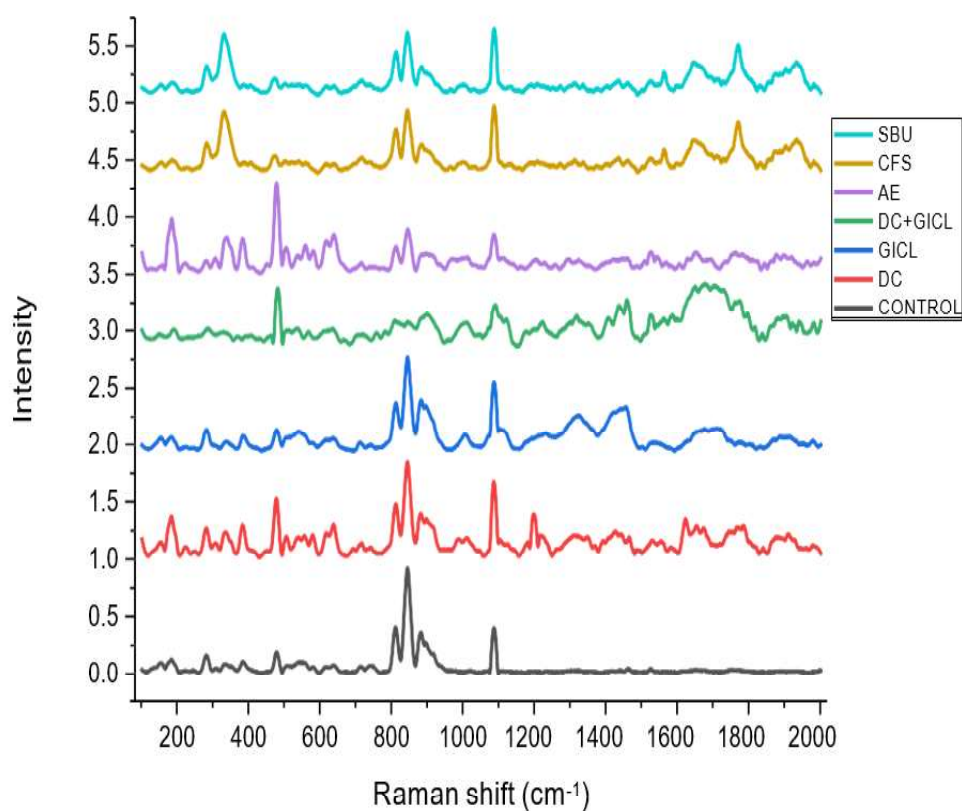
positions similar to the control at 24 hour. The GICL pre treated sample showed short peaks for zirconium oxide, silicates and calcites when compared to the corresponding surface pre-treatment at 5 minute and 12 minute respectively. Whereas, the new peaks observed at 1700 cm^{-1} were found to be similar to the 5 minute and 12 minute GICL pre-treatment. The DC+GICL pre-treatment showed ill defined peaks for zirconium oxide, silicates, calcite and the hydroxyl group. New peaks appeared at 1315 cm^{-1} , 1485 cm^{-1} and at 1700 cm^{-1} which could be representative of the reaction by products. The phosphoric AE exhibited ill defined silicate peaks and the calcite peaks were shorter when compared to the control at 24 hours. New broad peaks appeared at 1785 cm^{-1} and 1934 cm^{-1} indicative of the phosphate functional group residues. The CFS pre-treatment revealed reduced peak intensities for silicate and calcite, when compared to the control. New peaks that were not evident in the control appeared at 1347 cm^{-1} , 1387 cm^{-1} , indicative of the presence of calcium hydroxide. Similar peaks that appeared after pre treatment at 1658 cm^{-1} (hydroxyl) and at 1785 cm^{-1} (phosphate ester) were comparable to the pre-treatment at 5 minute and 12 minute.

The Raman spectra of the control sample at 7th day (graph 12) showed peak positions for silicate and calcite at 860 cm^{-1} and at 1086 cm^{-1} . The peak intensities were lower when compared to the 5 minute, 12 minute and 24 hour control. New peaks appeared at

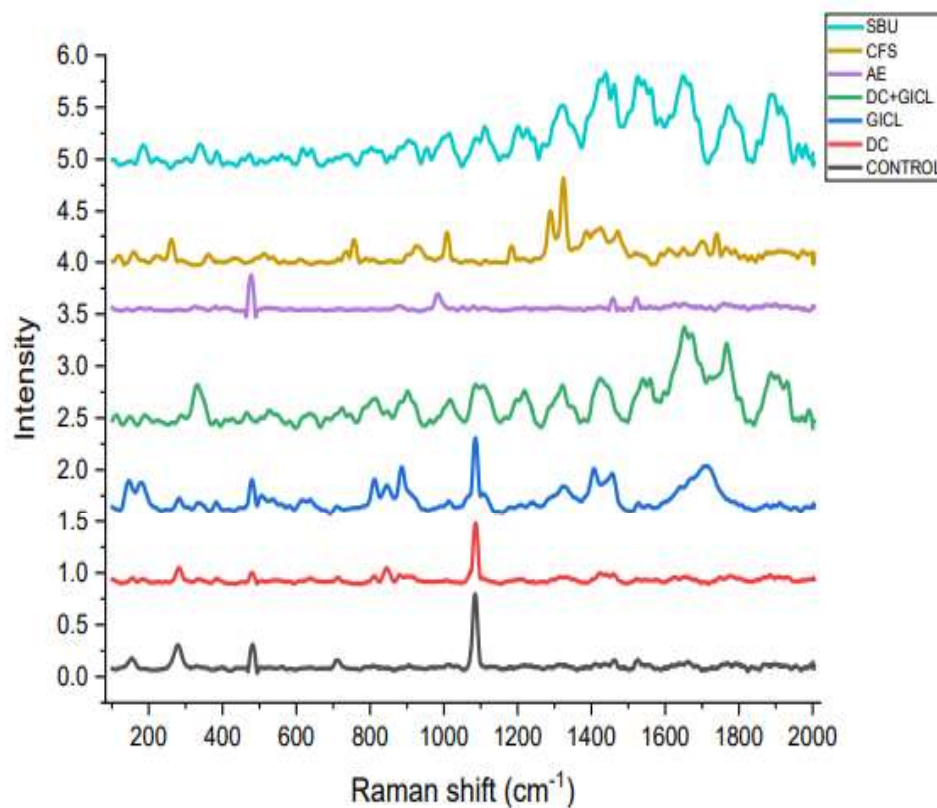
1347, 1380 and 1512 cm^{-1} which indicated the presence of calcium hydroxide at the 7th day. All the pre-treatment samples at the 7th day showed similar peak positions and peak intensities to that of the control sample. However, the reaction by-products that appeared in DC, GICL and DC+GICL, CFS, and SBU pre-treated samples were ill-defined compared to the other tested times. The phosphoric AE and CFS pre-treated samples at 7th day showed sharp peaks for calcium hydroxide at 1347 cm^{-1} and at 1380 cm^{-1} when compared to the control at 7th day.



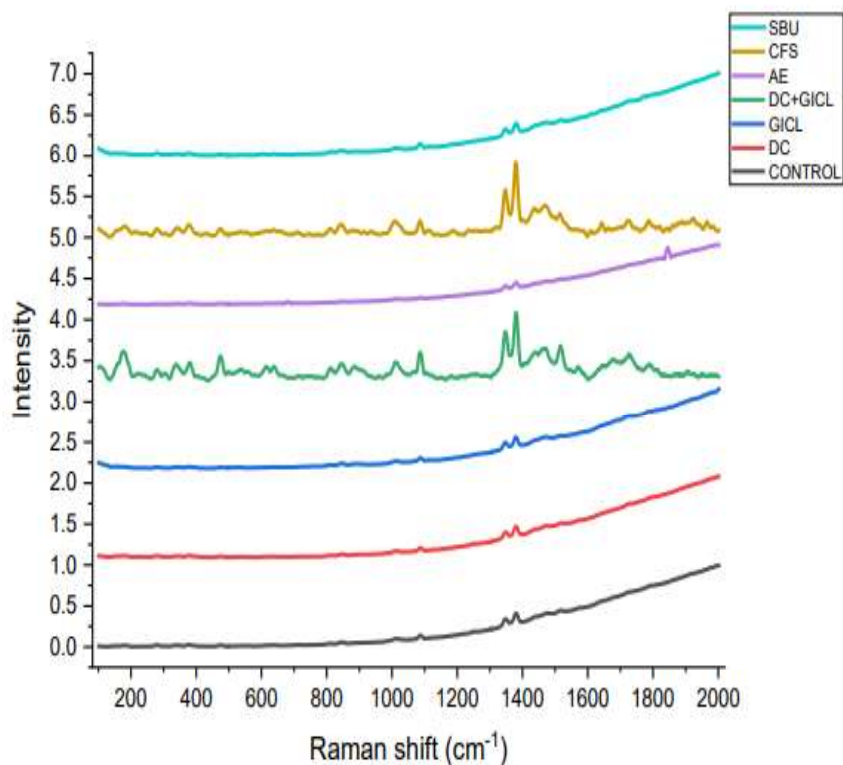
Graph 9 shows micro Raman spectra of 5 minute - control, DC, GICL, DC+GICL, AE, CFS, SBU. Control sample at 5 minutes showed peaks for zirconium oxide (ZO), calcium silicate (CS) and calcium carbonate (calcite) and the hydroxyl (OH-) functional groups. In DC+GICL, AE reduction in the all the peaks were observed. New peaks appeared at 1315 cm^{-1} , 1485 cm^{-1} and at 1700 cm^{-1} in (d). In AE, new peaks appeared at 1512 cm^{-1} , 1785 cm^{-1} and 1934 cm^{-1} . In CFS new peaks appeared at 1658 cm^{-1} at 1768 cm^{-1} . In SBU reduction in all the peaks were observed and short and sharp peaks appeared at 1485 cm^{-1} and 1500 cm^{-1} .



Graph 10 shows micro Raman spectra of 12 minute - control, DC, GICL, DC+GICL, AE, CFS, SBU. In AE the peak intensities were lower for silicates, calcites when compared to the control. Peaks appeared at 1512 cm^{-1} , 1685 cm^{-1} , 1785 cm^{-1} and 1934 cm^{-1} . In CFS and SBU peaks depicting silicate and calcite were ill defined. New peaks appeared at 1658 cm^{-1} and at 1768 cm^{-1} .



Graph 11 shows micro Raman spectra of 24 hour - control, DC, GICL, DC+GICL, AE, CFS, SBU. All the peaks appeared to be shorter but sharper when compared to the control at 5 minutes and 12 minutes. In DC+GICL, ill defined peaks appeared with new peaks at 1315 cm^{-1} , 1485 cm^{-1} and at 1700 cm^{-1} . In AE, very short peaks appeared for silicate and calcite. SBU showed reduced peak intensities for silicate and calcite. New peaks appeared at 1347 cm^{-1} , 1387 cm^{-1} , 1658 cm^{-1} and 1785 cm^{-1} .



Graph 12 shows micro Raman spectra of 7 day - control, DC, GICL, DC+GICL, AE, CFS, SBU. All the pre-treatment samples at the 7th day showed similar peak positions and peak intensities to that of the control sample except DC+ GICL and CFS. DC+GICL and CFS pre-treatments showed sharper peaks at 1347 cm^{-1} and 1380 cm^{-1} .

DISCUSSION

Various possible influential factors affecting the success rate of vital pulp therapy have been considered in studies such as the maxilla versus mandible, tooth position, capping material, exposure site and the time span before placement of the restoration.(73), (74)

The placement of a permanent, well-sealed restoration at the time of pulp capping is crucial to clinical success (75). According to the European Society of Endodontology (ESE) position statement, a hydraulic calcium silicate or a glass ionomer cement should be placed over the deep dentine when pulp is not exposed and hydraulic calcium silicate material should be placed directly onto the exposed pulp in pulp capping, partial pulpotomy or full pulpotomy prior to a definitive restoration. (76) Placement of a permanent restoration in the same session as pulp capping with calcium silicate based cements is advantageous for both the patient and the clinician. Nevertheless, the influence of immediate bonding of restorative materials on the properties of calcium silicate based cements should also be considered (51), (77), (78).

Biodentine is a calcium silicate based cement which offers many advantageous properties over other calcium silicate based cements. It has a faster initial setting time of 12 minutes and better handling characteristics as compared to MTA (23). Even though it takes over a month to attain its full maturation (24), the manufacturers claim that it can be successfully overlaid with a definitive restorative material after its initial setting time. However studies on the timing of placement of a definitive restoration over Biodentine have produced variable results. When Biodentine was overlaid with glass ionomers at both early and delayed time interval, it was observed that the shear bond strength were low (5 –

6 Mpa). (30), (35) On contrary, a study by Neekofar et al showed that aging the Biodentine to one month increased the bond strength between Biodentine and GIC (28.59 Mpa). (60) Several studies have demonstrated a poor early bond strength when composite resin was bonded to Biodentine with 3 step Etch and Rinse adhesive system as compared to that of mild self-etch adhesive systems.(36), (31), (54) Increased bond strength were observed when mild self-etch adhesives were used for early (after 12 minutes) and delayed bonding (after 24 hours and 7 days) to Biodentine.(50), (63), (69). As a part of a self-etch adhesive, the monomer bonds through the phosphate group to the calcium ions. The formation of a self-assembled layered structure developed on the surface, is termed as nano-layering. (34) As an inference from the above studies, it was evident that the delayed bonding to the Biodentine after a period of 7 to 14 days increased the bond strength to composite. Clinical trials on Biodentine pulp capping revealed lack of standardised protocols regarding the time and technique for placement of definitive coronal restoration (40), (41), (79), (55) The literature not only remains unclear on the timing and type of the definitive restoration, but is also split on the appropriate type of surface pre-treatments. In this study pre-treatment of the Biodentine surface with dentin conditioner (10% polyacrylic acid), glass ionomer liquid (20% polyacrylic acid) and a combination of both along with phosphoric acid etching, two step self-etch adhesive and a universal adhesive in the self-etch mode were considered.

In the clinical scenario, clinician places a definitive restoration on Biodentine either immediately or during the second visit, after hours of setting. When the clinician decides to place the restoration in the same visit, more often than not the clinician might not allow sufficient time for the initial setting of the material before subjecting it to pre-treatment. With an intent to simulate the clinical condition, in this study, the analysis was carried out after 5 minutes, 12 minutes, 24 hours, and 7 days, from the manipulation of Biodentine.

Thermocycling has been used in various studies for ageing of the specimen. Few studies have demonstrated that thermocycling can have a detrimental effect on the specimen by inducing thermal shock between the components of the specimen, thus creating microcracking. This might make it difficult to ascertain whether the surface changes occurred due to the pre-treatment or thermocycling. (80), (81), (82) Hence in this study, aging of Biodentine samples through thermocycling was not followed. The prepared samples were stored in an incubator at 37°C and 100 percent humidity until being subjected to pre-treatment at 24 hours and 7th day.

In order to study the surface morphology and characterise the elements present on the surface of Biodentine, scanning electron microscopy with X ray energy dispersive mode was used, X ray

diffractometer was used to analyse the crystallography and micro Raman spectroscopy was employed to characterise the chemical composition on the surface of the Biodentine after subjecting it to various pre-treatments at 5 minute, 12 minute, 24 hours and 7th day.

Scanning electron microscopy coupled with X-ray energy dispersive mode (SEM-EDX) is a non-destructive analytical method for high resolution surface imaging and quantitative identification of elements present on the surface. Surface imaging with SEM at magnifications of 1000X, 10,000X, at specified time intervals after all surface pre-treatments suggested varying degrees of disruption on the Biodentine surface compared to the controls. At 5 minute and 12 minute, all the surface pre-treatments either inflicted etching, porosities or erosion of the Biodentine samples. Bolhari et al. (66) suggested that these surface disruptions may have occurred due to calcium and zirconium depletion, and the same was confirmed from the results of EDX analysis in this study. All surface pre-treatments for glass ionomer restoration showed only a mild surface disruption at all the times tested, whereas the surface pre-treatments for composite resin restoration, inflicted more pronounced surface disruption at 5 minute and 12 minute. Phosphoric acid etching before 12 minutes caused erosion of the matrix, which was confirmed by EDX that showed a considerable reduction in calcium to silicon ratio compared to the control. This reduction in calcium to silicon ratio could drastically affect the formation of calcium

silicate hydrate gel that might in-turn adversely impact the surface hardness. At 24 hour, defects such as cracks, craters and crumbled surfaces that appeared with phosphoric acid etching seemed to be inconsistent with intended effect. Our findings are in agreement, correlating with the results of previous bond strength studies of Biodentine to composite that used ER adhesives.(36), (54), (31) But Cengiz et al. reported higher bond strength of Biodentine to composite resin after immediate layering using ER adhesive and attributed the same to micro-mechanical bonding. (30) The present study showed conflicting evidence where surface pre-treatment with phosphoric acid etching at 24 hours or prior resulted in poor surface characteristics unsuited for micro-mechanical bonding. It was only on the 7th day, phosphoric AE pre-treatment produced microporosities uniformly spread across the Biodentine surface. The surface damages with other pre-treatments were minimal at 24 hours and the negligible at the 7th day. This showed that Biodentine was relatively resistant to surface damage if pre-treatment was carried out after 24 hours of initial setting. And furthermore, reflects that among the surface pre-treatments for composite resin restoration, phosphoric acid etching was the most deleterious when performed at or prior to 24 hour.

The elemental analysis (EDX) of the control samples was in line with previous studies (22), (66). Additionally, it also revealed that control samples of Biodentine showed the presence of calcium

silicate hydrate gel with calcium carbonate interspersed between them. The control samples at 12 minute demonstrated a high calcium to silicon ratio which could be attributed to the presence of unreacted calcium silicate hydrate gel. At 24 hours, the SEM analysis showed needle like crystal growth, which was indicative of the initiation of crystallisation process, confirmed with EDX depicting low calcium to silicon ratio. The ongoing maturation process was demonstrated by a further reduction in calcium to silicon ratio on the 7th day. This was possibly the result of calcium carbonate formation over the calcium silicate hydrate (CSH) gel.. Previous literature has shown that calcium carbonate and CSH gel, in set Biodentine is responsible for its hardness (83), (84). Hence, it is assumed that at the 7th day, the Biodentine samples were least affected by any of the surface pre-treatment. Dentin conditioner, GIC liquid, and a combination of both agents increased the calcium to silicon ratio indicating a possible leaching of calcium ions from the surface of Biodentine. This finding was in line with a previous study .(66) In the present study, it was also observed that calcium to silicon ratio was drastically reduced after pre-treatment with CFS and SBU at all time intervals, which could be attributed to the interaction of calcium ions in the Biodentine with 10 – MDP present in these adhesives. (85).

X-ray diffraction (XRD) can be used to analyse and characterise the crystal phases of Biodentine. This technique

recognises specific diffraction peaks that are particular to various crystalline phases present in the material. XRD analysis revealed peaks for calcium carbonate, calcium silicate and calcium hydroxide. These peaks are indicative of the tricalcium silicate based materials. In the present study, these peaks were consistently detected in all the samples analysed at different time intervals irrespective of the surface pre-treatment. Thus, confirming no change in the chemical composition within Biodentine samples irrespective of the nature of surface pre-treatment.

Raman spectroscopy involves a non-invasive analytic approach for chemical identification and quantification.(86) It can probe specimens under near in vivo conditions, as well as in a solid and aqueous state. (87) Although specific sample preparation was not needed, the heat induced by the focused laser may damage the specimen's surface up to a certain depth.(88) In this study, the power setting of the laser was reduce to 50 mV in order to avoid any thermal damage to the samples. The micro Raman spectroscopic analysis of the control samples, at 5 minute and 12 minute revealed sharp peaks with a high intensity indicative of unreacted calcium silicate hydrate gel and calcite. At 24 hour, these peaks were present but at a lower intensity indicating the progress in the setting reaction. The peak intensity of silicates and calcites were further reduced with the appearance of calcium hydroxide peaks at the 7th day. This was representative of the slow, sustained and continuing

phase of the setting reaction. This finding was in line with FT-IR characterisation done by Alotaibi et al (2018). (110)

There was a significant reduction in the peaks for calcite and silicates when the Biodentine surface were pre-treated with DC, GICL, DC+GICL, AE, CSF, and SBU at 5 minute, 12 minute and 24 hour compared to their respective controls. When the surface was pre-treated with CFS and SBU adhesives, at all the tested times, peaks appeared at 1768 cm⁻¹ indicating presence of reaction by-products characterised by ester bonds. This finding is in line with a previous study by Anastasiadis et al 2018 who also demonstrated the formation of the ester groups as result of chemical interaction between 10- MDP molecules and calcium in Biodentine (68). Interestingly, when the surface was pre-treated with AE, at 7th day, no silicate peaks, very short and broad calcite peaks: and short and sharp calcium hydroxide peaks were observed. Camilleri et al., had investigated the effect of acid etching on Biodentine using FT-IR, their observations showed no change in the FT-IR peaks between the etched and un-etched Biodentine. Their finding was in contradiction with the micro Raman findings of the present study, but it is important to note that the authors failed to report the time of pre-treatment in their study. (22) Although, the SEM – EDX, Raman spectroscopic analyses showed considerable changes in the surface microstructure, the chemical composition within the sample remained stable. The XRD analysis of Biodentine after various

surface pre-treatments revealed no change in the chemical composition. Hence the null hypothesis was partially rejected.

From the results of the present study, it can be assumed that 37% ortho phosphoric acid etching is highly detrimental to the surface of Biodentine until 7 days from mixing. On the contrary, it is believed by some that the surface irregularities/defects, may play a major role in micromechanical bonding to the over-layering composite resin restoration (60). Few studies have demonstrated higher bond strength of Biodentine to composite when delayed layering was done.(61), (59). Few others in the literature also suggest poor bond strength between Biodentine and composite resin irrespective of the time of over-layering (36),(54) .

Considering the results of the present study, it is worth mentioning that all kinds of pre-treatments used in this study inflicted surface changes on Biodentine at all time intervals. Though Biodentine was chemically stable, loss of crystal structure on the pre-treated surface was evident. Correlating the findings in this study with the available literature, it is advisable to delay over-layering of any restoration on Biodentine after a vital pulp therapy procedure until 7th day from placement. In case of requirement of an immediate over-lay, glass ionomer or composite resin restoration with mildly acidic self-etch adhesives either one step or two step may be the choice for definitive restoration. The evidence on the

quality of bond between Biodentine and composites is still sparse. Studies reported premature cohesive failure within Biodentine when early over-layering with composite was performed which suggests that the Biodentine may be a weak substrate for micromechanical bonding during the initial phase of setting and might not be able to resist the stress of polymerization shrinkage when early bonding is attempted. (36),(37), (38),(54) . Further research is needed to evaluate the effect of various surface pre-treatment on physical and interfacial properties of Biodentine.

Few limitations of this study: As the samples could not be sufficiently sputter coated due to the initial hydration in the 5 minute sample, it posed difficulties in scanning the surface. Hence, the pre-treated samples at 5 minute could be subjected to SEM – EDX analysis only after 12 minutes. Secondly, the retention of certain pre-treatment agents such as GICL, CFS, and SBU on the surface of the sample hindered the thorough analysis of their effect on the underlying surface of Biodentine.

SUMMARY

Biodentine is a tricalcium silicate-based endodontic material which is used for direct/indirect pulp capping. These treatments' success depends on immediate bacteria tight coronal seal, and many times the placement of the permanent restoration is delayed until the pulpal status is stable. The most recommended immediate

restorations over calcium silicate-based cement after capping procedures are Glass Ionomer Cement (GIC) and composite resin restorations. GIC and composite placement require pre-treatment of the cavity with 10% polyacrylic acid and 37% orthophosphoric acid or self-etch adhesive or universal adhesive for efficient bonding. The study aimed to qualitatively analyse the effect of dentin conditioning, acid etching, 2 step self -etch adhesive and universal adhesive on the surface microstructure of Biodentine at 5 minutes, 12 minutes, 24 hours and 7 days of manipulation.

The effect of these pre-treatments on the surface microstructure of the Biodentine was assessed using qualitative analysis – Scanning Electron Microscope (SEM) in X ray energy dispersive mode (EDX), micro Raman spectroscopy and X-Ray Diffraction (XRD). The results of the study showed that the dentin conditioner pre-treatment did not affect the surface morphology, whereas the dentin conditioner and GIC liquid combination showed chemical compositional changes on the surface. The acid etching surface pre-treatment till the 24 hours caused detrimental effects on the surface microstructure. The mild self- adhesives and the combination of dentin conditioner and glass ionomer liquid type II caused least effect on the surface microstructure at all the tested times.

CONCLUSION

Within the limitation of this study, it is concluded that Biodentine showed surface microstructural changes after various surface pre-treatments at all time intervals tested.

- None of the surface pre – treatments tested adversely affected the surface microstructure or composition at 7th day.
- Phosphoric acid etching was the only surface pre-treatment that had adverse effect on the surface microstructure and composition at all the time intervals tested before the 7th day.
- None of the surface pre-treatments induced any change in the composition during the setting and maturation of Biodentine.

The effect of these surface pre- treatments must be further correlated with interfacial analysis and bond strength studies to make clinically relevant recommendations for placement of definitive restorations.

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