THE EFFECTIVENESS OF DIFFERENT MINERALIZING AGENTS IN REDUCING DENTINE PERMEABILITY - *AN IN VITRO STUDY*

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ABSTRACT

Title: The effectiveness of different mineralizing agents in reducing dentine permeability- *an In Vitro study*.

Objectives: To evaluate and compare the effectiveness of mineralizing agents in reducing the dentine permeability by tubule occlusion using fluid flow filtration device and SEM/EDX analysis.

Material and Methods: Seventy five (n= 75) dentine discs of 1±0.2 mm width were prepared from sound permanent human molars. Fifty (n=50) dentine specimens were randomly divided into five groups (n=10). Group 1: GC Tooth Mousse Plus (Reca Dent GC Corporation Tokyo, Japan), Group 2: Clinpro White Varnish (3M ESPE, USA), Group 3: Duraphat Varnish (Pharbil Waltrop GmbH, Germany), Group 4: Colgate sensitive Pro-Relief dentifrice (Colgate Palmolive, Thailand), Group 5: Biodentine (Septodont/UK). The manufacturer's instructions for the mixing and application procedures were followed accordingly. Dentine permeability was measured after Rx application at 10 minutes, and after artificial saliva immersion at 10 minutes and 7 days interval. Subsequently, all the samples were subjected to citric acid challenge for 3 minutes and the permeability measured. The quantitative measurements in the permeability were quantified using a fluid flow filtration device functioning at 100 cmH_2O (1.4 psi) pressure. Three repetitive measurements at 4 minutes duration were recorded. The average permeability values were expressed as percentage of maximum hydraulic conductance. Data were analyzed by two-way repeated measures ANOVA to determine if there are any significant differences between inter and intra treatment groups. The remaining dentine specimens (n=25) were used for SEM/EDX analyses to obtain qualitative results on dentine morphology and surface deposits.

Results: Each of these five mineralizing agents significantly reduced dentine permeability immediately after treatment application at 10 minutes and created precipitates on the treated dentine surfaces which reduced the diameter of dentin tubules. All the treatment agents increased the permeability values after 10 min and 7 days artificial saliva immersion except Clinpro white varnish and Biodentine which showed a decrease in permeability for the later immersion with the treated surface exhibiting partial modification. Clinpro white varnish exhibited significant resistance to acid challenge compared to other treatment agents under both SEM/EDX analysis and dentine permeability evaluation.

Conclusion: Clinpro white varnish and Biodentine are effective agents in reducing dentine sensitivity compared to others. The Clinpro white varnish has a supplementary effect of resisting the acid challenge.

ABSTRAK

Tajuk: keberkesanan ejen semula mineralizing berbeza dalam mengurangkan dentin permeability- kajian in Vitro**.**

Objektif: Untuk menilai dan membandingkan keberkesanan agen mineralizing dalam mengurangkan kebolehtelapan dentin oleh tubul stalemate menggunakan aliran cecair peranti hidrostatik dan analisis SEM / EDX.

Bahan dan Kaedah: Tujuh puluh lima (n=75) cakera dentin daripada 1 ± 0.2 mm lebar telah disediakan daripada gigi geraham kekal manusia yang kukuh. Lima puluh (n=50) spesimen dentin secara rawak dibahagikan kepada lima kumpulan (n = 10). Kumpulan 1: Rx dengan GC Gigi Mousse Plus (Reca Dent GC Corporation Tokyo , Jepun), Kumpulan 2 : Clinpro White Varnish (3M Espe , Amerika Syarikat) , Kumpulan 3 : Duraphat Varnish (Pharbil Waltrop GmbH, Jerman), Kumpulan 4 : Colgate sensitif Pro -Relief dentifris (Colgate Palmolive , Thailand), Kumpulan 5: Biodentine (Septodont / UK) arahandaripada pengilang bagu tatacara penggunaan diikuti dengan sewajarnya. Dentin kebolehtelapan diukur selepas permohonan Rx pada 10 minit, dan selepas direnadam dalam air liur tiruan selama 10 minit dan 1 minggu berselang seli. Selepas itu, semua sampel tertakluk kepada cabaran asid sitrik selama 3 minit dan kebolehtelapan telah diukur. Pengukuran kuantitatif dalam kealiran hidraulik (kebolehtelapan) telah diukur menggunakan peranti hidrostatik aliran bendalir yang berfungsi pada 100 cmH2O tekanan (1.4 psi). Tiga ukuran diambil berulang ulang selama 4 minit Tempoh dan direkodkan. Nilai kebolehtelapan purata telah dinyatakan sebagai peratusan kealiran hidraulik maksimum. Data diperolehi dengan dua cara iatu mengulangi langkah-langkah ANOVA untuk menentukan sama ada terdapat perbezaan yang signifikan antara kumpulan dan

antara dalam rawatan. Spesimen dentin baki (n = 25) telah digunakan untuk SEM / EDX analisis untuk mendapatkan keputusan kualitatif morfologi dentin dan deposit permukaan.

Keputusan: Setiap lima ejen mineralizing berkurangan dentin kebolehtelapan serta-merta selepas permohonan rawatan di 10 minit dan dicipta mendakan pada permukaan dentin dirawat yang mengurangkan diameter tubul dentin. Semua ejen rawatan meningkat nilai kebolehtelapan selepas 10 min dan 7 hari tiruan air liur rendaman kecuali Clinpro varnis putih dan Biodentine yang menunjukkan penurunan dalam kebolehtelapan untuk rendaman kemudian dengan permukaan yang dirawat mempamerkan pengubahsuaian separa. Clinpro varnis putih dipamerkan tentangan signifikan kepada cabaran asid berbanding dengan ejen rawatan lain di bawah kedua-dua SEM / EDX analisis dan dentin penilaian kebolehtelapan.

Kesimpulan: varnis putih Clinpro dan Biodentine adalah agen berkesan dalam mengurangkan sensitiviti dentin berbanding orang lain. The Clinpro varnis mempunyai kesan tambahan untuk menentang cabaran asid.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

1	Dentine Hypersensitivity	**DH**
2	Dentine Permeability/ Hydraulic conductance	**Lp**
3	Treatment	**Rx**
4	Scanning Electronic Microscopy	**SEM**
5	Element Sensitive Detector	**EDX**
6	Caesin Phospho Peptide- Amorphous Calcium Phosphate Fluoride	**CPP-ACPF**
7	Mineral Trioxide Aggregate	**MTA**
8	Polyethylene tubes	**PE tubes**
9	Cemento-enamel junction	**CEJ**
10	Calcium Silicate Cement	**CSC**
11	Artificial Saliva	**AS**
12	Silicon Carbide grit paper	**SiC grit paper**

CHAPTER 1: INTRODUCTION

1.1 Background

Dentine hypersensitivity (DH) is a common dilemma in an adult population with a prevalence that ranges from 8-57% (Ahmed et al., 2005). Dentine hypersensitivity has a multifactorial etiology and is described clinically as an acute pain caused by the stimulation of exposed dentine and its tubules allowing the movement of intra dentinal fluid (Gillam et al., 1997) to pulpal nerves by chemical, thermal, tactile, mechanical, evaporative or osmotic stimuli that cannot be ascribed to any form of dental defect or pathology (Addy, 1990; Holland et al., 1997; Markowitz & Pashley, 2008; West, 2007). The dentine exposure to the oral environment perhaps occurs corollary to gingival recession with root erosion, abrasion, attrition, surgical and non-surgical periodontal therapies (Ahmed et al., 2005; Arrais et al., 2004; Wara-aswapati et al., 2005).

The term Dentine sensitivity is used to depict the pain experienced by the patient in their formerly insensitive dentine. However, the term ‘‘hypersensitive dentine ‘‘ is ought to be used when clinical manifestations of sensitivity exaggerate in comparison to previous history of sensitivity (Pashley, 2013). Brannstrom et al proposed the hydrodynamic theory (Martin Brännström, 1986; Martin Brännström & Garberoglio, 1972) which is a universally accepted theory and provides the presumable explanation for the mechanism of DH in which the movement of the intra-dentinal fluid in response to a stimuli leads to the changes in intra pulpal pressure which as a result stimulates the nerve endings and hence pain occurs. Hence, a significant presumption of hydrodynamic theory is that the sensitive dentine is permeable (Martin Brännström & Garberoglio, 1972). It was determined by a histological study that an increase in the count and patency width of dentine tubules are related to the DH severity (Ishikawa, 1969). The movement of dentinal fluid within the tubule is proportional to the 4th power of tubule radius and the difference of pressure between the two ends of a tubule according to the Hagen Poiseuille equation (Wylie & Wilson, 1994). Thus, the current criteria of therapy is established on two phenomena based on the hydrodynamic theory that is occlusion of the patent dentinal tubules and cessation of the dentine neuronal activity (Gillam et al., 1997). According to this theory, any decrease in dentine fluid flow would eventually reduce dentine permeability. Pashley in conformity with this theory investigated that perhaps the dentine hypersensitivity would be reduced via the formation of crystalline aggregates within the tubule by a physiochemical process from dentinal fluids or salivary minerals, tertiary/sclerotic dentine formation, dentine tubules invasion by bacteria and plasma proteins adsorption (Pashley, 1986). Nevertheless, even though the two criteria have been reported to be efficacious in alleviating or reducing dentine hypersensitivity, there is a variation in the time it takes for the cure.

There are different therapies aimed for the dentine sensitivity treatment at present but the main challenge is to discover a material or any treatment which could best eliminate the pain and does not reoccur which is woefully not employed yet (Naylor et al., 2006). Different strategies have been approached by the dental professionals as a directed therapy of dentine hypersensitivity such as implementation of nerve desensitizers, anti-inflammatory drugs, ion/salts, protein precipitants, dentine sealants, laser, mucogingival grafts, and restorative materials (Bartold, 2006). Moreover, the amalgamation of various treatment modalities were carried out to have profound effects (Kumar & Mehta, 2005). Therapy is mainly concentrated on the mechanism of either occluding the dentinal tubules or alteration of the tubule contents.

Nevertheless, advancement has been made towards fulfilling the criteria of material that could deposit insoluble calcium and phosphate salts onto the tooth surface and further plug the dentinal tubules mechanically. This could be achieved by using mineralizing agents such as Casein Phospho Peptide-Amorpous Calcium Phosphate Fluoride - CPP-ACPF (GC Tooth Mousse Plus Recaldent), Tri-calcium Phosphate with fluoride (Clinpro White Varnish 3M ESPE), Arginine - calcium carbonate based dentifrice (Colgate sensitive Pro-Relief dentifrice) and Calcium silicate based cement such as Biodentine (Septodont). These minerals are bioactive and biocompatible and atudies have showed that these agents can form stable acid insoluble apatite precipitates (Gandolfi et al., 2011; Tay & Pashley, 2008).

Recently, insoluble calcium and phosphate salts are found to be used widely to remineralize subsurface enamel lesion and dentine sensitivity management which apparently could be a suitable material to occlude the dentine tubules (Lochaiwatana et al., 2015; Shetty et al., 2010; Suge et al., 2010). It was found by Pashley et al. (Pashley, O'Meara, et al., 1985) that fluoride varnish application reduced dentine permeability to about 20-50%. Similarly, a widely used in vivo mineralizing agent is Duraphat (Colgate) fluoride varnish and is found to be efficacious in reducing dentine sensitivity immediately on application over the exposed dentine (Gaffar, 1998). The fluoride ions facilitate the formation of fluorohydroxyapatite in the presence of calcium and phosphate ion which is now believed to be the crucial means of fluoride’s action (Shen et al., 2011).

A nanocomplex of the milk protein casein phosphopeptide (CPP) and amorpous calcium phosphate (ACP) has been used recently and have been reported to prevent teeth sensitivity by increasing the dentine tubule occlusion with re-mineralized dentin by maintenance of high concentration gradient of calcium phosphate on to the tooth surface (Oshiro et al., 2007; Rahiotis & Vougiouklakis, 2007; Reynolds, 1997) and is known to have great bioavailability. It has the potential to bind and maintain calcium and phosphate in a solution and also can bind to the plaque and enamel. Additionally, it is incapable of being dissolved and with a neutral pH forms a crystal precipitate. Therefore, when this calcium and phosphate binds with a protein, it becomes resistant to acidic environment and hence, decreases the demineralization (Reynolds, 1999). Moreover, a functionalized technology is the fluoridated tri calcium phosphate which is a bioactive mineral where the tri-calcium phosphate particles are ball milled with sodium lauryl sulphate. It works efficiently with fluoride ion that is stable and the product has the ability to remineralize the subsurface enamel lesions (Shen et al., 2011).

Furthermore, Colgate sensitive pro-relief dentifrice is a new saliva-based formulation that contains arginine and calcium carbonate (Kleinberg, 2002) and functions together to accelerate the natural process of occlusion by forming dentine like minerals within the dentine tubules thereby, covering the dentine surface entirely with a protective coating (Yang et al., 2014). Calcium silicate based cements such as ProRoot MTA (Dentsply, USA), MTA Angelus (Angelus, Brazil) and many others have been recommended for many clinical uses including retrograde filling, perforation repair, apexification, pulp-capping (Nair et al., 2008; Pace et al., 2008; Torabinejad & Chivian, 1999) and recently, have been proposed for dentine tubule occlusion. These cements are contemporary biologically active materials (Gandolfi, Taddei, et al., 2010; Taddei et al., 2009) and have been suggested to bio-mimic the dentine remineralization (Gandolfi, Gabriela, et al., 2008; Gandolfi et al., 2011; Gandolfi, Van Landuyt, et al., 2010; Tay et al., 2007). This new concept of making use of calcium-silicate based cement or silicate-based materials for dentine hypersensitivity was first put forward by Gandolfi (Gandolfi, Silvia, et al., 2008). Biodentine powder particle size are micrometrical ranging between 1.5 – 3 microns (Komabayashi & Spångberg, 2008) and due to its smaller particle size, it could easily enter the patent dentinal tubules and form precipitates to obturate the patent tubules.

Despite of the various therapeutic agents being used, dentine hypersensitivity usually reverts back as a result of abrasion caused by frequent tooth brushing, chemical erosion or mechanical displacement of the restorative material leading to temporary effects of desensitization (Kuroiwa et al., 1994; Wang et al., 2010).

It is significant that the desensitizing agents used should chemically react with the dental tissues by chemical means and attach to the tooth structure to a greater extent such that it decreases the chance of the dislodgement of obstructed dentinal tubules.

Therefore, the objective of our study was to evaluate the in vitro effectiveness of these calcium and phosphate based mineralizing agents in reduction of dentinal permeability. It has become necessary to have studies performed on comparative analysis aiming on its ability to physically plug the dentinal tubules effectively and decreasing its associated hydraulic conductance respectively.

1.2 Aim

To obtain an effective mineralizing agent that can best decrease the dentine permeability.

1.3 Objectives

- To evaluate and compare the effectiveness of commercially available mineralizing agents which are GC Tooth Mousse Plus, Clinpro White Varnish, Duraphat Varnish, Colgate sensitive Pro-relief dentifrice and Biodentine in reducing dentine permeability.
- To study and compare the ultrastructure of the layer deposited by these mineralizing agents on dentine.

CHAPTER 2: LITERATURE REVIEW

2.1 Dentine Hypersensitivity

Dentine hypersensitivity was frequently encountered yet was poorly understood and because of this reason, it was described as an enigma (Johnson et al., 1982). Dentine sensitivity or dentine hypersensitivity are the terms which have been used interchangeably to explain this condition clinically. However, the term “dentine” is often interchanged by a site location, for instance cervical hypersensitivity his often used explaining the same clinical issue. Lately, the term root dentine sensitivity or root dentine hypersensitivity has come into being (Von Troil et al., 2002). Sanz reported that root dentine sensitivity or root dentine hypersensitivity should be used in order to describe hypersensitivity resulting from periodontal disease since this would differentiate the condition from dentine hypersensitivity (Dowell & Addy, 1983; Sanz & Addy, 2002). A new definition was later suggested at an international workshop established on the design and clinical trials conduct for dentine hypersensitivity management which states that ‘Dentine hypersensitivity’ is described as a short, sharp pain due to exposed dentine, in response to an array of stimuli such as thermal, evaporative, tactile, osmotic or chemical which cannot be ascribed to any other form of dental defect or pathology (Holland et al., 1997). It was further recommended by the Canadian Advisory Board on Dentine Hypersensitivity 2003, that it would be more appropriate to replace ‘disease’ for ‘pathology’ in order to avoid dilemma with other conditions such as atypical odontalgia (Dowell et al., 1985). Different stimuli trigger the onset of pain such as thermal, mechanical or chemical. The most common complaint offered by the patients is from cold stimuli.

2.2 Structure of Dentine

2.2.1 Types of Dentine

Dentine is one of the living calcified tissues of a tooth which is formed from the dental papilla of the tooth germ. It is first formed as a layer of un-mineralized matrix known as pre dentin which has a thickness of 10 – 50 µm at variance and outlines the deeper most pulpal portion. It is composed of collagen mainly and resembles to the osteoid in bone. This pre-dentine with time gets mineralized into dentine when the mineralization front gets occupied with various non-collagenous matrix proteins (Nanci, 2008). Dentine constitutes of organic matter and water which is about 30% and inorganic matter which is 70% by weight. It mainly contains calcium phosphate in the form of small hydroxyapatite crystals; 20% is collagen fibres in form of a structure less matrix and the remainder is water. Dentine and enamel are united strongly at the scalloped border microscopically seen as dentinoenamel junction and the radicular portion of tooth is covered by cementum , the junction of which is less distinct because they overlap each other (Nanci, 2008). Dentine forms the bulk of a tooth and it is normally covered in the crown by enamel and in the root by cementum, hence it cannot be observed clinically in the mouth.

Primary dentine is the dentine which is formed during tooth formation. After the dentine formation takes place, odontoblast continues to be active even after tooth eruption into the oral cavity. Therefore, deposition of dentine continues throughout the life. Primary dentine forms the major portion of the tooth which outlines the pulp cavity and is known as **circumpulpal dentine.** The peripheral layer near by the enamel and cementum distinguishes from the remaining primary dentine in a way that there is a interstructual link between the collagen and non-collagenous matrix portion and also that it is mineralized generally termed as **mantle dentine** (Nanci, 2008). After primary dentine is formed, dentine continues to form slowly so eventually the size of the dental pulp gradually reduces. The dentine which forms as a result of this physiological change is called **secondary dentine** which has the same structure as that of the primary dentine. This dentine is formed when the root formation gets completed and continues to develop at a much slower rate by odontoblasts. It consists of a tubular structure, which is less regular and majority of it is continuous with the primary dentine. The dentinal tubules of secondary dentine usually undergoes sclerosis due to aging that is filled up with calcified material more often than primary dentine as reported by some evidence (Nanci, 2008). When a tooth is under threat from an external stimulus such as caries, attrition, abrasion and erosion etc., the odontoblasts will attempt to protect the pulp from the pathological disturbance by rapid dentine deposition. Tertiary dentine forms as a consequence of this pathology and its structure is irregular and disorganized**.** The stimulus is so strong that it kills the odontoblasts over that site thus causing the dentinal tubules deficient oft odontoblast processes form dead tracts.

2.2.2 Histology of Dentine

Microscopy reveals that the structural unit of dentine is the dentinal tubule. The odontoblast process end in the canaliculi - dentinal tubules which cross the dentine and augments throughout the dentine to the pulp from the dentinoenamel junction. This forms a network of diffusion for nutrients. The contour of the tubules represent the route taken by the odontoblast during dentine formation which takes a “S” shaped course from the pulpal surface to the outer surface of dentine. This pattern is less prominent in the incisal edges and cusp tips where the tubules perhaps run almost in straight course. The courses taken up results from the crowding of the path taken by the odontoblast as they move towards the center of pulp. An approximate of 30,000 - 40,000 tubules per square millimeter of dentine are present. Dentinal tubule contains an odontoblastic process which extends from an odontoblast. Moreover, it is reported that human cervical dentin has 19,000 tubules per mm^2, its superficial dentin and half way through it where the pulp is, the number of tubules increases to 30,000 tubules per $mm^{2.}$ Dentine tubules appear to be extended from the enamel-dentine junction to the pulp with an increase in its size from the periphery towards the pulp. Due to its presence, dentine appears to be permeable and pulpal tissues perhaps is in continuity with the mouth or restorative materials that further leads to dentine sensitivity or post-op sensitivities (Camps et al., 1997). The dentine which surrounds the odontoblastic processes is usually more mineralized than the other dentine. Dentinal tubules are tapered in morphology and measures around 2.5 micrometers in diameter near the pulp, 1.2 µm in the central portion of the dentine and 900 Nm near to the dentinoenamel junction. In the crown part of the molars and premolars, the number of tubules ranges from 59,000 to 76,000 per sq mm at the pulpal region. Dentine that surrounds the dentinal tubule is known as **peritubular dentine** which is highly mineralized whereas the dentine present in between the dentine tubules is known as **intertubular dentine,** which was formed by odontoblasts secretion and consists of a complex network of type 1 collagen fibers that is 50 to 100 nanometer in diameter and layered in by the apatite crystals.

Interglobular dentine represents the area of non-mineralized or hypomineralized dentine where the globular zones fail to mineralize in to a consistent mass in a mature dentine. This is usually found in human teeth of a person with vitamin deficiency or due to high levels of fluoride exposure during dentine formation which is frequently seen in circumpulpal dentine beneath the mantle dentine where the way of mineralization is fairly globular (Nanci, 2008).

Sclerotic dentine possesses the tubules that have been blocked with calcific content which occurs in various tubules within the same region and takes a glassy translucent appearance. This dentine is most common in the coronal mid-portion between the pulp and CEJ and apical radicular third .and tends to increase with age. It occurs due to physiological response and as a consequence of continued formation of peritubular dentin, tubular occlusion is achieved. Therefore, dentinal permeability is reduced and it protects the pulp from harmful stimuli (Nanci, 2008).

At a daily deposition rate of 4 micrometer, the organic matrix of primary dentine is formed in increments and is known as **incremental growth lines**. These incremental lines cross the dentinal tubules at right angle and form the normal straight pattern of dentine in a downward and root ward area. The 5 days increments is seen as **incremental lines of von Ebner** located at 20 micrometer apart from each other and the other incremental line is called as **contour lines of the Owen** which occurs as a result of secondary curvatures between the dentinal tubules (Nanci, 2008).

2.2.3 Innervations

Dentine-Pulp complex is rich in innervations where the nerves enter the pulp via the apical foramen alongside the afferent blood vessels and hence forms a neurovascular bundle following the same course after it is in the pulp chamber. Each nerve fiber further branches into terminal branches and forms an extensive plexus of nerves known as the **sub-odontoplastic plexus of Raschkow** in the coronal portion of tooth and no analogous plexus is found in the radicular portion of the tooth. The nerve bundles that makes an entrance to the pulp consists of the sensory afferent nerves, branch of trigeminal nerve and sympathetic branches from the superior cervical ganglion which has both mylineated and unmylineated axons. Under the electron microscopy study of premolars, the coronal dentine and pulp horns shows that there is a decrease in the abundance of nerves from predentin to the mineralizing front of the dentine (Nanci, 2008).

2.2.4 Physical Properties

Dentine is light yellowish in color usually evident in the teeth of young adults. However, teeth afflicted with pulpal disease or absence of pulp due to trauma and caries and some restorative materials such as amalgam and root canal sealers results in the discoloration of dentine which eventually darkens the clinical crown. Unlike enamel, it is elastic in nature which is significant for good teeth functioning and hence, more likely prevents fracture of the brittle enamel overlying it. It is slightly radiopaque in comparison to enamel (Fish, 1932; Schmidt & Keil, 1958).

2.2.5 Anatomical differences between sensitive and non-sensitive dentine

Sensitive dentine exhibits a greater number of dentinal tubules per unit area when compared to non-sensitive dentine which is around eight times as many tubules over the root surface (Absi et al., 1987). The channels are wider being two times greater in diameter than the average in non-sensitive dentine. Sensitive dentine also appears to have thinner smear layers and is less calcified when compared with the latter. Respectively, with greater number and wider dentinal tubules can lead to increased fluid permeability through dentine hence, an increased stimulus transmission and the pain response (Absi, Addy, & Adams, 1987).

2.3 Etiology of Dentine Hypersensitivity

Dentine is covered by enamel on the crown portion and cementum on the root surface anatomically so it is not exposed in the oral cavity. There are various causes which includes physiological, pathological and/or combination of both that would eventually lead to e

Enamel loss over the crown, as well as the cementum loss on root surface and eventually results in the dentinal tubules being exposed. Amongst all the causes, chronic periodontitis (Schluger, 1990), periodontal surgery (Glickman & Carranza, 1990), trauma inducing tooth brushing and mal habits of a patient holds significance in terms of dentine hypersensitivity. Gingival recession, periodontal diseases, cracked tooth syndrome, abrasion, erosion, and teeth fracture may lead to dentine hypersensitivity resulting in dentine exposure of dentin. This develops an environment where stimuli causes fluid movement within the tubules to activate nerve fibers, causing pain. Exposure of dentine occurs as a result of removal of cervical cementum during scaling/root planning procedure, restoration polishing, or vigorous tooth brushing by the patient especially after the intake of acidic drinks. Patients regurgitate due to bulimia that produces acid exposure, and subsequent brushing can lead to loss of tooth structure (Addy, 2002).

2.3.1 Loss of Enamel

In spite of enamel being very rigid, around 95% of it is inorganic material in the form of hydroxyapatite crystals and it can still be removed. Loss of enamel over the crown causing dentine exposure can occur as a result of various causes. Tooth wear can be further classified as attrition, abrasion and erosion even though it usually occurs as a combination of these and with different proportional effects.

Attrition is a physical wear of tooth which occurs as a result of tooth-to-tooth contact for eg. an exaggerated occlusal function by parafunctional activities such as bruxism (Litonjua et al., 2003). .

Abrasion is a physical wear of tooth which occurs a result of foreign objects, for eg, vigorous brushing or habits causing injury (Addy, 2005). It is more prevalent in pipe smokers or tailors who tend to hold pins between their teeth and on incisal edge of the anterior tooth especially incisors are prone to abrasion. The cervical area on the buccal surfaces of teeth also has a high chance to develop abrasion; canines and premolars are more easily inflicted due to their anatomical position on the dental ridge (Addy, Absi, et al., 1987). .

Erosion is tooth wear which occurs as a result of acid dissolution of tooth material and doesn't involve bacteria. It can be result from acids of both intrinsic or extrinsic sources (Zero, 1996). Extrinsic acid is related to diet intake (Osborne et al., 1999) or is environmental. Intrinsic acid is related with health disorders such as bulimia and anorexia nervosa. Soft drinks, citrus fruit and wine are the main source for dietary acids. The palatal surfaces of upper incisors, occlusal and buccal surfaces of lower posterior teeth are the areas commonly affected in gastric reflux disease (Robb et al., 1995).

Abfraction occurs as a result of micro-fracture of tooth at the cervical area due to occlusal loads and has been hypothesized as a causative factor for tooth wear (Braem et al., 1992). Abfraction is believed to involve unusual occlusal stress resulting in excessive stress at the cervical region of the tooth which would eventually weaken the cervical region of tooth surface.

2.3.2 Loss of cementum

Cementum is a soft and thin layer which covers the dentine on radicular portion. Cementum is made up of 50% inorganic matter and can easily get denuded after being exposed due to gingival recession.

Gingival recession is defined as the apical migration of the gingival margin that results in the exposure of the cemento-enamel junction and the radicular surface. The etiology is multi-factorial for gingival recession and is not caused by a single factor. The chances of developing gingival recession is found to be much more in patients who does vigorous brushing, especially in those with high standard of plaque control (Serino et al., 1994) and also with increased frequency of tooth brushing (Khocht et al., 1993). Studies revealed that gingival recession could also result from periodontal therapy (Drisko et al., 2000; Lindhe et al., 1982).

Periodontal disease is defined as the disease of the peridontium which results in the loss of connective tissue attachment and destruction of peridontium (Schluger, 1990). Clinical finding indicates that gingival recession would result in a much greater area of dentine exposure, due to that cementum being easily removed in comparison to that resulting from loss of enamel at the cervical region. The prevalence of dentine hypersensitivity is found to be much higher in patients with periodontitis probably because of high risk and greater exposure of root resulting from the destruction of peridontium (Shiau, 2012)

2.3.3 Other Causes

Several studies have found that the dentine hypersensitivity occurs as a result of reversible pulpitis which increases immediately after in-office bleaching with hydrogen peroxide (Browning & Swift, 2007; Martin et al., 2013) and this transient sensitivity would cease within a week. Often external tooth whitening such as hydrogen peroxide or carbamide peroxide when used may cause the hydrogen peroxide to infiltrate through the enamel, dentine and into the pulp. Eventually, the glutathione peroxidase and catalase in the pulp doesn’t have enough time to inactivate the hydrogen peroxide hence, may cause sensitivity. Moreover, bleaching agents are hypertonic and so draws water from the pulp through the dentin and enamel by osmotic means to the whitening agent which further stimulates intradental nerves (Swift, 2005). Some periodontists believe that plaque begins and prolongs the dentine hypersensitivity (Bender 1986) while others do not agree to it The control of plaque both by mechanical and chemical means plays a significant role in preventing the exposure of dentinal tubules and hence attributes to the mechanism of natural repair of dentine hypersensitivity thereby, causes a decrease in the patency of dentinal tubules (Suge et al., 2006).

2.4 Etiopathogenesis

The occurrence of Dentine hypersensitivity is possible when two criteria are fulfilled namely, **lesion localization** where the dentine exposure takes place and the tubules are ought to be opened from pulpal region to its surface for the l**esion initiation** to occur (Orchardson & Gillam, 2006).

Lesion localization results in the loss of the hard tissue (enamel) exposing the dentine or loss of soft tissue due to gingival recession following tooth brushing abrasion, gum grafts, crown preparation, over flossing or subsequent to periodontitis. DH is only possible after the exposure of peripheral dentine tubule endings. The loss of enamel as a result of abrasion or erosion together with the dietary acid action predisposes to tubule patency (Dababneh et al., 1999; Shiau, 2012). However, since not all dentine is exposed , for the Lesion initiation to occur (Absi et al., 1987; Rimondini et al., 1995) , lesion localization needs to be present which would lead to the removal of smear layer and thus exhibits the patent dentinal tubules. Frequent exposure of these areas to abrasion or challenge to diet acid has been known to increase the softening process of the remaining tooth (Tarbet et al., 1980). The etiopathogenesis of DH has been summarized in Figure 2.1.

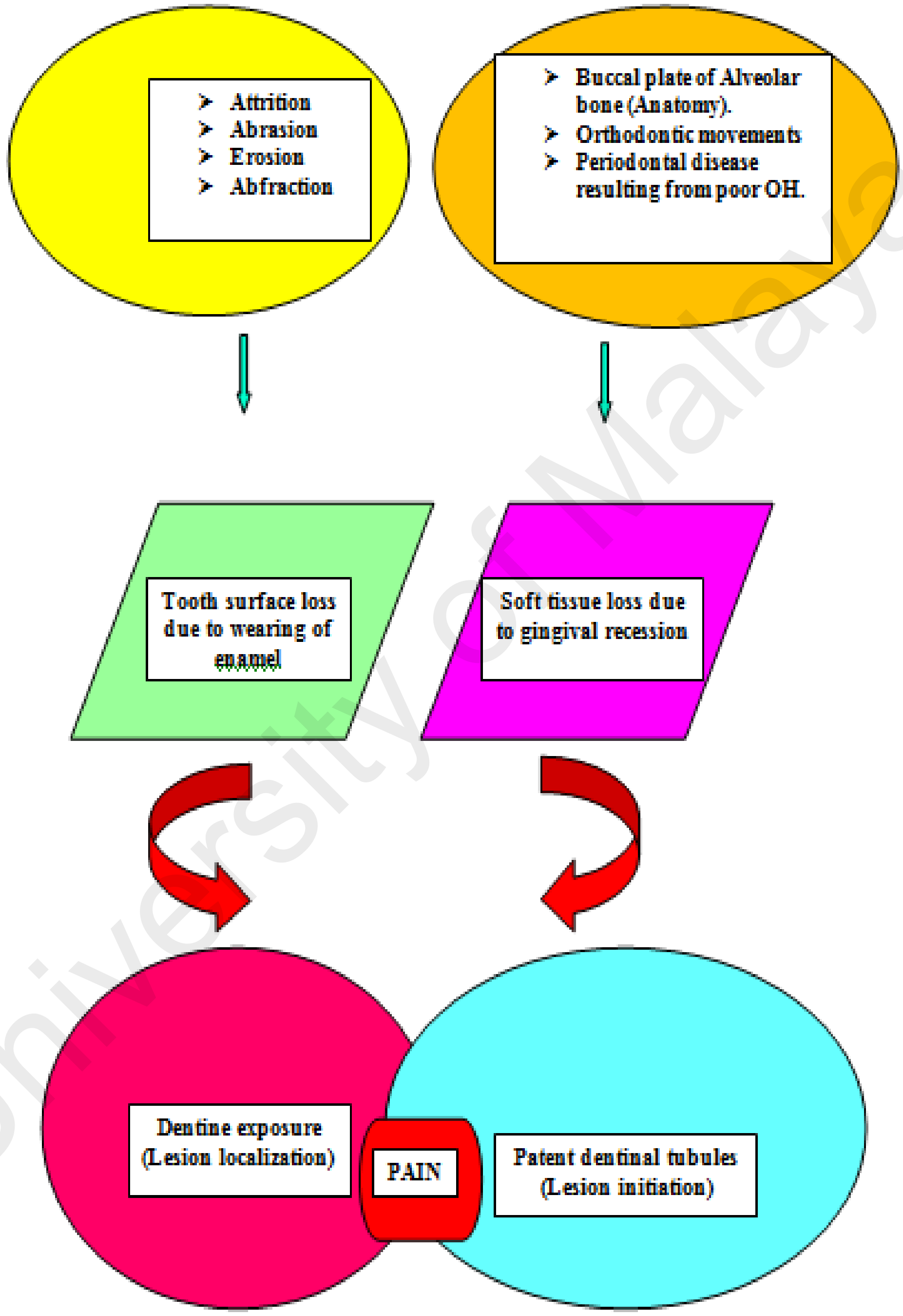


Figure 2.1 Etiopathogenesis of Dentine Hypersensitivity

2.5 Mechanism of Dentine Hypersensitivity

The etiopathogenesis hence, becomes the predisposing factor for the DH to occur and therefore, an external stimuli is needed to elicit its mechanism.

Dentine has fluid filled tubules that extends throughout its entire thickness from the pulp to the dentine junction with the enamel and cementum. Most of the tubules stem at their superficial periphery and widens as it approach the pulp. The pulp side of the tubule contains the odontoblast, a dentine forming unit, that produces a incessant cell layer at dentine and pulp junction. A cytoplasmic process devoid of organelles, from the odontoblast extends into the tubule across one third of the distance between pulp and dentinoenamel junction. The neuron fiber develops a plexus of Raschkow which lies deep beneath the odontoblast layer. The unmylineated axons from the plexus passes over the odontoblast layer while some axons makes an entry to the dentine tubules. Innervations of dentine tubules are more commonly found underneath the pulp horns and hence, when dentine gets exposed to pain provoking stimuli such as air currents, it induces fluid shift in the tubules which as a result activates the intra-dental neurons and causes sensitivity.

The finding that the tubules are filled with minerals over the non sensitive dentine surfaces denotes that there exists some sort of natural phenomena of desensitization. However. the reason why this phenomenon does not exist elsewhere is unknown.

It is significant for the dental professionals to have an insight out understanding about the process and mechanism of how external stimuli originated from the dentine tubules is transferred through the dentine both for an appropriate management of dentine hypersensitivity as well as to educate patients adequately about it. Normally, when dentine is not exposed, the enamel and cementum offers resistance to the pressure of pulp that is about 15 cmH_2O (1.47 kPa) which is higher than the surrounding oral pressure (Ciucchi et al., 1995). However, when this enclosure of enamel and cementum on dentine are destructed, leakage of the dentinal fluid occurs out of the dentine into the fetur oris.

Normally, the presence of smear layer is held responsible for the 86% of resistance to the dentinal fluid flow through dentine (Pashley et al., 1978) and plugs the patent dentinal tubules far better than that of resin tags in resin-bonded dentine (Carrilho et al., 2007). The internal diameter of dentinal tubules is 1µm approximately but narrows to functional diameter less than 0.1µm and merely contains fibers, $CaSO_4$ etc. The pores of the tubules are very much similar to those micro pores used to filter and remove microorganisms from the solution (Michelich et al., 1978). The problem occurs when these smear layers become inhabitant of micro-organisms and forms biofilm which further produces organic acids to dissolve the smear layers within 7-10 days (Martin Brännström, 1982; Kerns et al., 1991; Pashley, 1991). Eventually, with this loss, the dentine becomes prone to sensitivity.

Usually, it is the consequence of a localized pulpal inflammation where the presence of bacterial antigens within the pulp results in the release of inflammatory host mediators such as histamines, bradykinin, prostaglandins, neuropeptides etc.. These mediators directs pulp fibroblasts to divide rapidly as to repair the collagen in the pulp connective tissue that is ruined by polymorphonuclear cells derived from matrix metalloproteineases. The nerve growth factor expressed by these fibroblasts stimulates the pulpal neurons nearby to undergo multiplication that is nerve sprouting and thus, resulting in an increased innervations than is normally present. The receptors within the cell membranes of these sprouting neurons identify the bacterial antigens and inflammatory signals and in turn activates further formation of receptive sodium- channel proteins (Henry et al., 2009). Moreover, if the sensitive dentine is covered by plaque, the products of microorganism perhaps diffuses into the pulp where it triggers a mild inflammatory response. The pulpal neurons take up the signaling molecules that are eventually directed centrally to trigeminal ganglion via retrograde axoplasmic transport and induce an altered expression of gene of various proteins that include sodium channel subsets having lower thresholds (Gold et al., 2003). This perhaps could be a significant step in the conversion of sensitive to hypersensitive dentine. This feat of bacterial toxins on the neuron excitability gives an explanation of how teeth being treated for periodontitis accounting for high plaque scores were found to be more sensitive than reasonably plaque free teeth (Wallace & Bissada, 1990).

The neuronal fibers that give response to dentine stimulation have low electrical thresholds and conduction of velocities in the range A-β and A-δ (Närhi et al., 1991). The acute localized pain with sensitive teeth complain is usually symptomatic of activation of A fibers whereas C fibers in teeth are more responsive to direct mechanical stimulation of pulp, toxic heat and application of KCl, bradykinin etc to an exposed pulp.

Hence, bacterial products such as endotoxins and endotoxins from the saliva an plaque diffuses and make its way to pulp through fluid filled dentine tubules and disperse within the pulp. Pulpal inflammation, eminent pulp pressures, sprouting of neurons and more sensitive neurons etc are accountable for continued DH in a set of population who previously had developed dentine sensitivity and hence the sensitive dentine become

Although the mechanism of how pain passes through the dentine is not yet completely or clearly understood. So far, several theories have been proposed in order to explain the emergence of dentine hypersensitivity.

2.5.1 Direct Innervation Theory

This states that the nerve endings are present throughout the entire thickness of dentine and towards dentinoenamel junction, mechanical stimulation of which will generate an action potential (Irvine, 1988). Nevertheless, this theory had flaws due to insufficient concrete ground regarding the presence of nerve meshwork in external dentine. Moreover, the ultra structural studies done reveals that the plexus of Raschkow and intratubular nerves do not develop until the eruption of tooth which contradicts the fact newly erupted tooth is sensitive (Orchardson & Cadden, 2001).

2.5.2 Odontoblast Transduction Theory

This theory states that odontoblasts acts as a receptor and carries the impulses to the nerve endings (Rapp et al., 1968). Bulk of the studies has revealed that odontoblasts are matrix forming cells and no role has been played by it in carrying impulses. Furthermore, no synapses were found between the nerve terminal and odontoblasts (Pashley, 1988).

2.5.3 Hydrodynamic Theory

This theory was put forward by Brannstrom in 1964, which postulates that the dentine sensitivity is the outcome of the force of the fluid in motion (Martin Brännström & Åström, 1964). Hence, the presence and the movement of fluid form the basis of this theory. Furthermore, this theory is persistent with the presence of patent dentinal tubules in hypersensitive dentine. The nerve endings present at the end of the dentine or dentinopulpal complex is activated by outward fluid movement (Pashley, 1988). This response of A- delta intra dentinal afferent fibers relies on the magnitude of stimuli, mainly the stimuli such as cooling, evaporation, drying and masking with the chemical content which causes outward fluid flow results in more pain (Chidchuangchai et al., 2007). It was found that an outward fluid flow of 2-4mm/sec in the dentinal tubules is created in response to a pain-producing stimuli (Berggren & Brännström, 1965)

A study performed by Brannstrom reported that probing or air blasts had the potential to evoke a rapid outward fluid flow within the dentine tubules (Martin Brännström & Johnson, 1970). A Study by Matthews also reported that the cold stimuli were able to cause dentinal fluid to flow away from the pulp more rapidly and in intense way than that of the inward fluid flow resulting from heat stimulation (Matthews, 1970). Generally very little evidence could be found that contradicts the hydrodynamic theory. Hence, it is the universally accepted theory for explaining dentine hypersensitivity mechanism till date.

2.6 Clinical Features of Dentine Hypersensitivity

Dentine hypersensitivity has clinical features which have been well documented in various reviews (Irvine, 1988; Orchardson & Collins, 1987). Patients' complaint varies from mild discomfort to severe pain. It can affect any teeth of different individuals as the pain perception is linked with one's pain threshold and emotional as well as the nature of the stimulation. The pain may arise either from only one tooth to several teeth, a quadrant, a whole dental arch or even the full mouth. Majority of the patients would describe the pain which is rapid in onset, sharp/dull and continuous/intermittent (Gillam & Newman, 1993). Patients also presented a wide range of pain-inducing conditions which includes thermal, osmotic, chemical, physical or a combination of these. Thermal stimuli include hot and cold food or drink, and warm or cold air blast whereas osmotic stimuli includes commonly sweet foods and drinks. Chemical stimuli includes acidic food or drinks and mechanical stimuli includes tooth brushing. Amongst all of the above mentioned external stimuli, cold stimuli has been reported as the most common stimuli that triggers the dentine hypersensitivity pain (Rees et al., 2003). Symptoms elicited from carious exposure of dentine surfaces, pulpitis or cracked tooth are similar to cervical dentine hypersensitivity. Thus, it definitely requires excellent differential diagnosis.

2.7 Clinical diagnosis of Dentine Hypersensitivity

A proper clinical diagnosis should be affirmed which is the key way to an appropriate management of dentine sensitivity and therefore, must be performed by exclusion criteria (Shiau, 2012). Henceforth, it is necessary to opt out the symptoms that mimic the dentine hypersensitivity and should be identified and treated essentially before ending up to any diagnosis Those conditions include fractured or cracked teeth/cusps, faulty restorations or margins or marginal leakage, post-op sensitivity or bleaching sensitivity, periodontal disease and pulpitis (Addy & Dowell, 1983; Bartold, 2006). Moreover, a detailed and thorough history on diet , oral hygiene, clinical, radiographic and other diagnostic tests such as percussion, palpation and pulpal vitality tests should be performed (Shiau, 2012) assisting the clinician to track down the symptoms during the treatment procedure.

2.8 Prevalence of Dentine Hypersensitivity

Indefinite studies presented the prevalence of dentine hypersensitivity ranging from 2.8% to 74%. Dentine hypersensitivity is a very common problem in adult population and it has been reported that about more than 40% of the world population suffers or has experienced dentine hypersensitivity pain (Graham et al., 2003). The prevalence rate reported by West and her colleagues (West, 2007) for dentin hypersensitivity was about 42% in Europe. In western Europe and United States, the incidence ranges from 3-73% in the adult population (Chabanski et al., 1996; Clayton et al., 2002; Rees, 2000).

Reportedly, the prevalence of DH amongst the Chinese adult residents in Shanghai was about 16- 34% (Ye et al., 2012). These findings were similar to the study which included Chinese adults (Wang et al., 2012). The prevalence of dentine hypersensitivity was found to be ranging from 4 to 74% as reported by Rees (Rees et al., 2003). This large variation in the prevalence is associated to the population that was studied as well as the methodology used in the study. For instance, some studies preferred questionnaires to collect data to record the subjective response of subjects while others had used cold, evaporation or physical touch tests to trigger the exposed dentine (Dababneh et al., 1999). The incidence of DH is found to be much more in patient survey forms than in clinical investigations which denotes incidence of only 15% (Fischer et al., 1992; Flynn et al., 1985)**.** The prevalence of DH appeared usually higher in studies which included subjects consulting periodontal specialist clinics. A high prevalence of dentine hypersensitivity in subjects attending periodontal clinics is because there is a greater risk of root exposure resulting from overzealous brushing, connective tissue attachment loss and gingival recession subsequent to periodontal therapy (Chabanski et al., 1997). Study including patients with bulimia nervosa reported that almost half of them had dentine hypersensitivity (Spigset, 1991). The occurrence of DH is more in females than in males (Addy, 1990; Gillam et al., 1999; Kehua et al., 2009) merely due to overall health concern and increased awareness of oral hygiene (Absi et al., 1987; Addy, 1990) targeting the age group of 20-50 years with the crest at 30 and 40 years though it can affect any age (Flynn et al., 1985).The rate of occurrence was found to be mostly in between the third and fourth decade with following reductions in its incidence as an outcome of aging (Bartold, 2006)**.** Generally, the teeth which are involved are the canines and the premolars of both the jaws (Addy, Mostafa, et al., 1987).

Sites of predilection are the canines and first premolars the most and molars the least (Absi et al., 1987; Dababneh et al., 1999; Kawasaki et al., 2001). Regarding the intra oral distribution, teeth surfaces, more than about 90%, that exhibit hypersensitivity are the buccal and the labial aspects (Rees & Addy, 2002) with buccal aspect of the cervical areas more commonly affected. The main cause which is widely attributed to it is gingival recession which is found to be 47%, abrasion being 25% and erosion is estimated to be about 3%. Surveys also revealed that 76 % found the prevalence of erosion and tooth wear was being increased where as 74.5% believed the prevalence of gingival recession was increased (Strassler et al., 2008). It is much more exhibited in patients inflicted with periodontal conditions with a range from 60-90% where a rise was observed in the early days soon after the scaling and root planning or periodontal surgery(Markowitz & Pashley, 2008). Additionally, a substantial reduction could be seen by the end of 8 weeks after the therapy through the time period which varies from months to more than 30 years. Although, patients with little tooth sensitivity do not usually consult a dentist which makes it more challenging to find its incidence in masses than in hospitals and clinics (Taani & Awartani, 2002). Lately, there has been an increase in the number of younger adults suffering from dentine hypersensitivity that is probably due to the lifestyle modifications such as traumatic tooth brushing and acidic diets (Bamise et al., 2010).

2.9 Assessment of Dentine Hypersensitivity

2.9.1 Clinical Assessment

Various forms of assessment methods include patient questionnaires and subjective response to external stimuli is assessed. Stimuli undertaken for both the stimulus-based and response-based assessment can be classified into five main groups namely**,** chemical osmotic, electrical, mechanical, tactile and thermal.

According to the hydrodynamic theory, any disturbance in the dentinal fluid flow within the dentine tubule results in the activation of pulpal nociceptors thus leading to pain (M Brännström & Johnson, 1978). The most pain provoking stimuli is cold, evaporative and osmotic that causes an increased outward fluid flow within the dentine tubules resulting in fluid shear forces over the mechanoreceptor neurons at the central end of tubules. This in turn results in the activation of intradentinal Aδ neurons at the pulp-dentine interface hence, causing pain. The factual physiological stimuli is inward or outward fluid shifts. It has been revealed by in vivo studies that the pulp neuronal response was found to be directly proportional to the fluid flow rate (Vieira & Santiago, 2009).

Chemical stimulus assessment includes the application of hypertonic solutions such as sodium chloride, glucose and calcium chloride over the exposed dentine surface. **Osmotic** stimuli also includes the application of hypertonic solutions and therefore, it is important to understand the osmolality of a solution so as to determine if the solution is hypertonic or isotonic. An example would be the application of undiluted tooth pastes on the exposed dentine tubules which by its hypertonic nature induces pain resulting in DH due to outward fluid flow from the tubules. However, these highly concentrated tooth pastes will become less hypertonic after the contact with saliva during brushing. Another example for these type of solutions used in dental procedures are bleaching gels and acid etching agents for composite restorations. Even though these stimuli are short lived, special emphasis must be given for the manufacturing of dental products as isotonic as possible (Mantzourani & Sharma, 2013)

Tactile stimulus assessment is brought about by dragging a hand-held periodontal probe (Orchardson & Collins, 1987) over the exposed dentine surface of root. Others include Yeaple and Electronic pressure-sensitive instrument were also used by some authors (Fu et al., 2010).

Thermal stimulus as reported by several studies, cold air blast was used for dentine hypersensitivity assessment (Gillam et al., 1992; Uchida et al., 1980). Cold stimuli results in a fluid flow shift away from the pulp and produces a much rapid pulp neuronal response and more intense pain in comparison to the heat which results from an inward flow. This explains the reason why cold stimuli perhaps seems to be the strngest pain provoking stimuli in people with DH (Irwin & McCusker, 1996).

Electrical stimulus as reported by several studies, electric pulp tester was used to assess dentine hypersensitivity (Kleinberg et al., 1990; Tarbet et al., 1980)**.** A thermo-electrical device was used to assess dentine hypersensitivity to generate **thermal and electrical** stimulus (Smith & Ash, 1964)**.** A number of scales have been introduced which compares and records subjective degree of response to pain of an individual for the recording of dentine hypersensitivity response which are **Verbal Rating Scale (VRS)** (Keele, 1948)**, Schiff Cold Air Sensitivity Scale** (Schiff et al., 1993)**, Visual Analog Scale (VAS)** (Huskisson, 1974) & **Numeric Rating Scale (NRS)** (Farrar et al., 2001)

2.9.2 An in Vitro assessment of dentine permeability

There are various techniques which have been acquired to determine the efficacy of mineralizing agents on dentine permeability reduction.

Split-chamber device used for dentine permeability test

In several vitro studies, experimental design includes the dentine discs being mounted into several types of split chamber devices (Outhwaite et al., 1974).These dentine permeability tests have been used extensively to depict and perform the screening analysis of various therapeutic agents for hypersensitive teeth. Different techniques include the use of mapping of regional variations in dentine permeability via electro-chemical microscopy. This technique makes use of a split chamber that applies pressure towards the pulp side of dentine disc where when the fluid flows through a disc, reacts with the electrochemical probe that further scans the exterior surface of the disc which eventually produces electric current (Unwin et al., 1997). Additionally, animal models could also provide the oral characteristics such as saliva, oral biofilm, temperature and diet (S. C. S. Pinto et al., 2010).

Dye diffusion used for dentine permeability test

Dye diffusion is an alternative method to analyze the effect of de-sensitizing agents on dentine permeability (S. C. S. Pinto et al., 2010). Fabio reported the use of dye infiltration test with 0.5% basic fuchsin for the in vitro evaluation of permeability In another study, Evans dye was reported to be applied to the dentine specimen and dentine permeability was analyzed by taking photographs by an image analysis software where each image was calibrated separately with a standardized scale (µm) where Three readings (upper, middle and lower parts) were taken for each image denoting the depth of the dye infiltration (S. C. S. Pinto et al., 2010).

Lasers

He-Ne laser or diode type laser, low output devices have been evaluated earlier for as prospective management for DH and were employed at a power 6mW. Efficacy varied from 5% to 100% but the mechanism by which it operates remains unclear whereas some investigations reported it to be its facilitative analgesic effect by decreasing neuronal impulse (Wakabayashi et al., 1993).

Dentin resistance was also found to be increased following the application of CO_2 laser irradiation (Fayad et al., 1996) where its effects was perhaps attributed to laser energy which evaporates the tubular fluid leaving it air filled and hence resistance increased. It was reported that CO_2 laser with 1W for duration- 5-10 s of irradiation could cure cervical DH without affecting the pulp adversely (Zhang et al., 1998).Various laser have been employed in the past such as Nd: YAG laser that functions at an energy output of 30 mJ. It melts the dentinal surface occluding the patent dentinal tubules (Lan et al., 1999) and has been recommended as a well functioning tool. Moreover, Er: YAG laser has also been reported to be have desensitizing effects as to occlude the open dentine tubules by insoluble salts (Corrêa Aranha et al., 2005). In spite of the fact that such promising aforementioned outcome could be produced for the management of DH, the occlusive extent in t the dentine tubules was not investigated.

2.9.2.1 Fluid Filtration device

Pashley's fluid filtration device is the most common way to study dentine permeability and employs the application of pressure at one end and forces the fluid to passout through the dentine on the other end. This Pashley's model has been used for the in vitro evaluation of different de-sensitzing agents in several studies (Gandolfi, 2008; Pashley et al., 1988; Sauro et al., 2006). This apparatus was previously used by Youngson (Youngson et al., 1999). This device operates based on the Darcy law, which measures the flow of fluid within the dentine tubules. The important variables which can affect the transmission of fluid flow has been defined by the Poiseuille-Hagen law are applied pressure, diameter of the dentine tubules, thickness of the fluid and tubules length which inturn depends on dentine thickness. However, while doing such experiments, various variables are characteristic of the tooth itself and so couldn't be altered for instance the size and the number of tubules (Outhwaite et al., 1976), regional varations of dentine permeability (Pashley et al., 1987) and microhardness of dentine (Pashley, Okabe, et al., 1985). The other variables are in control of the reseacrher and have been standardized correctly. Two significant variables that is the applied pressure and the time for application have not been standardized clearly in the literature (Camps et al., 1997)

Dentine Specimen Used

The dentine disc which is used in the study should be in close proximity to dentine pulp without the exposure of pulp horn, its thickness must be less as much as possible and the smear layer must be taken off (Fogel et al., 1988; Pashley, Michelich, et al., 1981; Tagami et al., 1990). Additionally, the dentine surface which is exposed should be as large as possible (Reeder et al., 1978) and should be covered with a drop of water (Goodis et al., 1990). The specimen used should be taken from a freshly extracted tooth or cryo preserved (Camps et al., 1994). The justification to use dentine disc for the experiment because it is easy to use, can be reproduced, its flat surface allows for the elemental analysis and perhaps could be correlated with the fluid flow investigations. Alongside, dentine disc can also be analyzed in a transverse plane to study the dentinal tubules which exhibits variations in its morphology, its diameter, orientation and its density throughout the entire tooth (Mordan et al., 1997). Likewise, the smear layer composition is also interlinked to its cut area (Eick et al., 1970). Therefore, this turns out to be the choice and make it as suitable control for the evaluation and a significant factor in the experimental set up.

2.9.2.2 In Vitro Evaluation by Scanning Electron Microscopy and EDX

A Scanning electronic microscopy (SEM) employs a focused high energy electronic beam to produce various signals at the solid specimen's surface. These signals arrived from the electron-specimen interaction exhibits an extensive information about the specimen that is surface topography, chemical composition, its complexity and distinct structures. The magnification of SEM imaging ranges from 20x to 30,000x. SEM are imperative tools indicated for both research and routine investigations in material sciences. Moreover, Scanning electron microscopy has been widely used to assess the tubule occluding mineralizing agents which evaluates the depth and density of occlusion of treatment effects and is performed in conjunction with the permeability evaluations that is used to assess the treatment effects in response to an evoked stimuli that provides a critical in vitro evaluation of all the experimental de-sensitizing agents (Markowitz & Pashley, 2008). This SEM is also equipped with an Energy Dispersive X-ray which does the elemental analysis on the entire dentine surface following to treatment applications.

2.10 Management of dentine hypersensitivity

The objective of the targeted treatment should first be shifted to the prevention of the factors prevailing to the condition which leads to the 'localization' of lesion through abrasion/erosion/ &/or gingival recession (West, 2008) with proper counseling of dietary intake and oral hygiene practices. Treatment strategies of dentine hypersensitivity falls under two categories (Mantzourani & Sharma, 2013; Shiau, 2012).

- To retard or lessen the neural signal.
- By occlusion of the patent tubules.

The second strategy includes a wide spectrum of treatment modalities ranging from the usage of salts/ions/proteins to the restorative materials such as dentine sealants, use of soft tissue grafting and with more advanced usage of lasers. All done in order occlude/seal the tubules (Shiau, 2012). On a broader spectrum, the treatment largely depends on the level of patients' pain threshold level. Gillam et al. reported that for a large percentage of people, dentin hypersensitivity was not a serious problem and therefore, they did not consult a dentist for its treatment (Gillam et al., 1999). Few other studies also quoted that 25% of people experiencing dentin hypersensitivity would manage the problem by using at-home desensitizing product (Gillam et al., 1999; Taani & Awartani, 2002) whereas 17% of the patients consulted for a professional therapy (Gillam et al., 1999; Taani & Awartani, 2002).

It was suggested by Grossman that the ideal agent or therapy for dentine hypersensitivity should not affect aesthetics, feasible to apply, rapid in action, does not irritate the pulp, doesn't cause pain and have long term effect (Grossman, 1935).The main therapeutic strategies used in the management of dentine hypersensitivity is summarized in Figure 2.2

Management strategies of Dentine Hypersensitivity
1 Impeding Neural Transmission: Potassium Nitrate
2 Physically Blocking/ Covering Dentine Tubules
2.1Tubule pluggers
2.1.1 Ions/Salts such as Strontium salts, Casein phosphopeptides Calcium, Phosphate, Oxalates, Fluorides etc.
2.1.2 Protein precipitates such as Glutaraldehyde, Formaldehyde etc
2.2 Dentine Sealers
2.2.1 Glass ionomer cements
2.2.2 Composite resins
2.2.3 Dentine adhesive agents/ Sealants
2.2.4 Varnishes
2.3 Fluoride iontophoresis
2.4 Crown / Restorative placement
2.5 Mucogingival & soft tissue grafting/ surgery
2.6 Lasers: Nd:YAG & CO_2 Lasers

Figure 2.2 Main management strategies used for Dentine Hypersensitivity

2.10.1 Mineralizing agents used in the study

Dental professionals have been using a wide variety of agents and treatments in the dental office to manage the dentine hypersensitivity.

Casein Phospho Peptide-Amorpous Calcium Phosphate Fluoride (CPP-ACPF)

A nano complex of the milk protein casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP) has been initially developed to decrease enamel caries (Reynolds, 1997). It has been reported to prevent teeth sensitivity by increasing the dentine tubule occlusion with re-mineralized dentin by maintenance of high concentration of calcium phosphate on to the tooth surface and is known to have great bioavailability (Rahiotis & Vougiouklakis, 2007). It has the potential to bind and maintain calcium and phosphate in a solution and also can bind to the plaque and enamel. Additionally, it is incapable of being dissolved and with a neutral pH forms a crystal precipitate and thereby, could plug the dentine tubule (Walsh, 2000). Therefore, when this calcium and phosphate binds with a protein, it becomes resistant to acidic environment and hence, decreases the demineralization (Reynolds, 1999). The CPP-ACPF is available as a commercial product such as GC tooth mousse or MI paste plus which with the availability of casein phosphopeptides is believed to vehicle the bio-available calcium, phosphate and fluoride ions to the tooth (Bröchner et al., 2011). Therefore, this product has been clinically used to remineralize molars that were hypoplastic / under-developed or any white spot lesions such as enamel opacities and mild fluorosis and neutralizes the acidic attack from the interior and exterior origin when it comes to erosion (Piekarz et al., 2008). It has been widely used to plug the exposed dentinal tubules. The casein, originally is a bovine milk phosphor-protein derived from nature and functions via the interaction with calcium and phosphate. Nevertheless, keeping in mind all the studies both clinical and laboratory done earlier, efficacy has been evident when it comes to re-mineralization of enamel (Al-Batayneh, 2009).The fluoride in it is known to facilitate the deposition of fluorohydroxy apatite whilst the calcium and phosphate being present and hence, is an accepted mode of fluorides' action (Cate, 1999; Lynch et al., 2004).

Tri-Calcium Phosphate with Fluoride

Tri-calcium phosphate is a functional product where it forms complexes with sodium lauryl phosphate and was introduced in the market in 2009 (Karlinsey et al., 2011; Pitchika et al., 2013). The formulation of Clinpro white varnish contains an advanced technology of tri-calcium phosphate and fluoride that provides protection to teeth and aids in relieving dentine hypersensitivity. It has been proven by the clinical studies that it could flow much more easily due to its low viscosity allowing improved delivery inter proximally and across tooth surfaces in comparison to other varnish contenders and delivers more fluoride. It is tolerant to both saliva and moisture and sets promptly in its presence. It is pertinent to use on both enamel and dentine. Calcium Phosphate has been intensifying in the field of dentine hypersensitivity management. It is both a biocompatible, bioactive agent being the major constituent of human bone and teeth.

Hence, it is suitable to be used for the dentine tubule occlusion and renders immediate termination of dentine hypersensitivity (Shetty et al., 2010; Suge et al., 2010). Fluoride ion are known to accelerate the formation of fluoroapatite or fluorhydroxyapatite in the presence of calcium and phosphate ions (Lynch et al., 2004) amongst which fluoroapatite was reported to be more effective in comparison to hydroxyapatite followed by CaF_2 due to its potential to saturate an artificial saliva solution (Suge et al., 2008).

Fluoride

Products contain fluorides with a concentration ranging from (3,000- 22,500 ppm) is reported to be effectual in alleviating dentine hypersensitivity (Erdemir et al., 2010; Greenhill & Pashley, 1981; Prabhakar et al., 2013)**.** Tooth pastes and varnishes containing fluoride have been employed as both for home-use and in-office agents for alleviating the pain and distress caused by dentine hypersensitivity for about decades although the process by which it functions is not yet completely understood. Fluoride is thought to decrease the dentine solubility so the equilibrium over the dentine surface would shift towards non-sensitivity (Addy & Urquhart, 1992).

Fluoride is also found to block the dentinal tubules by precipitate formation on the dentine therapy, decreasing dentine hypersensitivity yet therapy with acidulated phosphate fluoride gel in the vitro studies of hydraulic conductance across dentine samples failed to plug the dentinal tubules (Greenhill & Pashley, 1981). It also noted that topical fluoride gel (1.23% NaF) is found to be effective in reducing post-operative sensitivity linked with bleaching procedures (Armênio et al., 2008; Haywood, 2002). Fluoride products such as sodium fluoride varnish for in-office use contains high concentration of fluoride (22,600 ppm F) that has the potential to be toxic if not used appropriately. The application procedure when indicated for full mouth is a time consuming process and is usually applied locally. According to a clinical study, a single application of fluoride varnish could provide both rapid and a long lasting effect in decreasing dentine hypersensitivity for about 24 weeks (Ritter et al., 2006). Although, post operative care after the in office application is perhaps not very well accepted by some patients where they are instructed to refrain from eating for about 4 hours with an exception of food that doesn't require chewing. Additionally, both brushing and flossing is instructed to be withheld the day of application and to avoid removing the varnish layer so that the agent is effective (Gangarosa, 1994). It is found by Pashley that the application of varnish resulted in a decrease of dentine permeability by 20-50% (Pashley, O'Meara, et al., 1985). The fluoride varnish is available in the market with several brand names, one of which is Duraphat. It has been found to be efficient in decreasing dentine hypersensitivity and has an immediate effect when applied to the surface of exposed dentin (Gaffar, 1998). It dries rapidly when in contact with saliva, and it is easy to apply and a single application was found to increase enamel fluoride by 77% with proven safety, efficacy and broad range of clinical uses. It has been reported that sodium fluoride is known to decrease hypersensitivity by occlusion of dentine tubules via the process of crystallization thereby, decreasing the fluid flow towards pulp (Gaffar, 1998).

Fluoride facilitates re-mineralization and it also has been found that when tooth is challenged with caries, the application of fluoride varnish results in the release of fluoride ion from the reservoir and remineralizes the tooth which has been de-mineralized. Furthermore, the Calcium fluoride acts as a source of fluoride readily available subsequently to acid challenge. It has been reported that a single Duraphat varnish application is found to be a source of calcium fluoride that could give active fluoride protection for months (Affairs, 2006; Grobler et al., 1983; Helfenstein & Steiner, 1994; Moberg et al., 2004; Weintraub et al., 2006).

Arginine and calcium carbonate based agent

Arginine is a positively charged amino acid that is found naturally in saliva. This functions on the basic natural principle of occluding tubules by salivary glycol protein. Arginine works in conjunction with calcium carbonate so that it stimulates the natural phenomena of dentine tubules by obliteration with dentine like deposits within the tubules. The calcium and phosphate are carried by saliva in close proximity to dentinal tubule thereby forms precipitates of both salivary glycol proteins with calcium and phosphate preferably favorable under alkaline environment with a physiological pH 6.5 – 7.5. A saliva-based composition that is arginine and calcium carbonate complex has been developed after extensive research on natural desensitizing properties of saliva and have been formulated in order to alleviate the symptoms of dentine hypersensitivity (Kleinberg, 2002). The compound is believed to mimic the natural mechanism of desensitization by saliva. Dentifrices containing arginine when applied topically also showed immediate response of relief from dentine sensitivity.

However, it is also noted that like any other treatment, this also failed to resist an acid challenge. It was also reported by several studies that a single application of an 8% arginine and calcium carbonate containing prophylaxis paste had the ability to produce an increased level of immediate relief from dentine hypersensitivity in comparison to a fluoride free prophylaxis paste (Collins et al., 2013; Tsai et al., 2012). Moreover, it doesn't cause pain and is feasible to use. It is generally accepted by the patients and does not require post operative care.

Calcium Silicate based Cements

Since the early 1990s Mineral Trioxide Aggregate (MTA) which is basically Calcium Silicate based Cements (CSC) was investigated for endodontic applications and then given approval for endodontic use by the U.S. Food and Drug Administration in 1998 (Roberts et al., 2008). It is used in the treatment of a variety of root canal perforations, pulpotomy and treatment of vital pulp (Torabinejad & Chivian, 1999). In addition, it has the ability to produce an apical barrier in teeth with necrotic pulp and open apex.

MTA powder consists of fine hydrophilic particles. The principle compounds of this material are tri-calcium silicate, di-calcium silicate, tri-calcium aluminate, tri-calcium oxide, and silicate oxide. Radiopacifier (bismuth oxide) is added to make MTA radiopaque and visible in the radiographs. In the last few years, there are relatively new types of CSCs have been developed, aiming to improve some MTA disadvantages such as the difficulty in handling properties (Johnson, 1999) and long setting time (Torabinejad *et al.* 1995).

In fact, chemical analysis of elements present in MTA and Portland cement (PC) showed that PC have similar chemical elements as MTA except that bismuth is found in MTA (Estrela et al., 2000; Funteas et al., 2003; Islam et al., 2006). For this reason, PC has been studied extensively as alternative to MTA (Gonçalves et al., 2010). Although the PC could claimed as a MTA substitute, it is important to emphasize that PC and MTA are not the same materials (Islam et al., 2006).

Recently, a new bioactive cement (CSC), Biodentine (Septodont, UK) was launched in a dental market, 2010 as a dentine substitute. Biodentine is stated to be used as a dentine restorative material in addition to endodontic indications similar to those of MTA. Biodentine consists of a powder in a capsule and liquid in a pipette. The powder mainly the principal component of PCs, as well as calcium carbonate. zirconium dioxide serves as radiopacifier. The powder is mixed with the liquid in a capsule in the triturator for 30 seconds. Once mixed, Biodentine is claimed to be set in approximately 12 minutes (Septodont, UK). The consistency of Biodentine is similar to that of phosphate cement (Dammaschke, 2010).

CSCs have been proposed for dentine tubule occlusion (Nair et al., 2008; Pace et al., 2008; Torabinejad & Chivian, 1999). This new concept of making use of CSCs or silicate-based materials for dentine hypersensitivity was first put forward by Gandolfi (Gandolfi, Silvia, et al., 2008). These materials need water or other fluids to facilitate its manipulation, trigger setting and to achieve an insoluble bioactive cements having various mineralizing characteristics as recently reported by various authors (Gandolfi et al., 2011; Tay & Pashley, 2008). Seventy to eighty percentage of the powder particle size are micrometrical ranging between 1.5 – 3 microns size t (Komabayashi & Spångberg, 2008). Owing to its smaller particle size, it could easily enter the patent dentinal tubules.

These CSCs are hydraulic materials and when hydrated, the silicate stages of PC undergoes a sequence of physio-chemical reactions and hence, in large quantity forms a nano-porous gel of naturally occurring form of calcium hydroxide/ Portlandite and causes a quick release of calcium hydroxide into the storage media that eventually increases its pH until 12 (Gandolfi, Taddei, et al., 2010; Girão et al., 2007; Taddei et al., 2009). Later calcium hydroxide dissolves forming precipitates such as estringite and calcite over the dentine surface and within the dentine tubules (Gandolfi, Ciapetti, et al., 2010; Gandolfi et al., 2011). Eventually, the precipitates which formed were held responsible to occlude the exposed dentine tubules and decrease the dentine permeability (Gandolfi, Silvia, et al., 2008).

These cements when mixed with water forms a soft plastic paste that could be applied over the dentine surface. In the presence of water, this cement was capable to set and form a stable solid compound (Camilleri & Pitt Ford, 2006). Calcium-silicate based cement are contemporary biologically active materials and have been suggested to bio mimic the dentine remineralization owing to their ability of forming apatite when immersed in counterfeit bodily fluids such as blood, saliva and Hank's Balanced Salt Solution (HBSS), etc. The results reported by Gandolfi et al. reported that the calcium silicate formulations provides protection to root dentine surface and thus plays a significant role in the management of dentine hypersensitivity (Gandolfi et al., 2012) . It has been reported by a study that when calcium-silicate based cement hardens, it releases calcium hydroxide that reacts with a fluid containing phosphate to form calcium deficient apatite through an amorphous calcium phosphate stage (Gandolfi, Silvia, et al., 2008).

Moreover, as reported by a recent study, calcium silicate cements are found to be able to plug dentine tubules with acid resistant and stable calcium-phosphate plugs. It has been reported that calcium silicate based cements when undergoes hydration results in the calcium hydroxide formation in 1-7 days which is a complex and dynamic reaction (Gandolfi, Ciapetti, et al., 2010).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study design

Laboratory based experimental study.

Sample size calculation

The sample size was calculated using a G power: sample size calculator. The effect size was set as 1.3, α was 0.5 and power was 80%. The required sample size was 5 per group. But in this study we used 10 samples per group to have higher precision of measurements (Gandolfi et al., 2012; Wang et al., 2010).

Pilot study

The pilot study was carried out on 2 samples from each group samples in order to check for the stability of the fluid filtration system and to ensure there was no leakage of the tubes and from the specimens. It was observed during most of the cases after the dentine specimen was fit onto the fluid filtration device that the air bubble moved quickly while during the first reading whereas less in the second and third instance. The first reading was observed to be considerably larger than the subsequent reading. Therefore, it was decided to elapse 1 minute during the main study before the air bubble was altered for the first reading. The most probable reason for this could be the early movement of fluid into the tubules or perhaps an air bubble within the pulp chamber (Elgalaid et al., 2004) or could be since the pressure applied by a clamp was released, the fluid moved faster toward the dentine and so the air bubble. The pressure of 100 cmH_20 was maintained throughout the experiment to make sure that there wasn't any leakage from specimens and tubes of the system. The constant check helped us out to overcome any errors when encountered while performing the experiment (Maryam, 2008). Thus, this aided us to be over cautious while performing the main study and helped us improvise which not only validated the results but also much reliable results were produced throughout the main study.

3.2 Inclusion Criteria

- All un-restored permanent molars which are sound or with enamel caries.
- Teeth with no pulp exposure
- Teeth with no pulp stones.

3.3 Exclusion Criteria

- Grossly Carious Teeth.
- Anterior Teeth and Premolars
- Deciduous teeth

3.4 Collection of Teeth

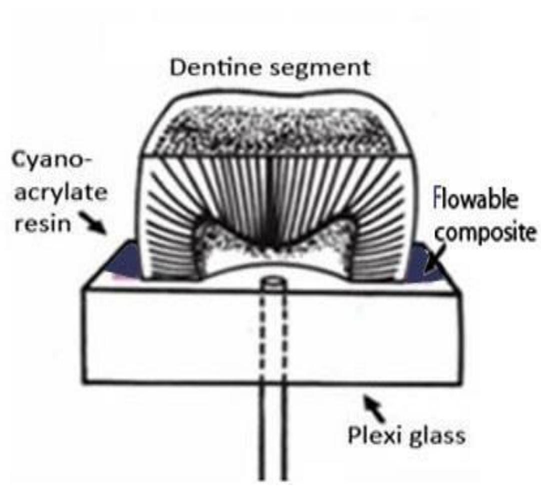
Seventy five non-carious, un restored permanent human molars were collected from clinics and multi health centers. The teeth were cleaned thoroughly and stored in 1% T chloramine solution. Ethical clearance for the study was approved by Dental ethics committee, Faculty of Dentistry in University of Malaya before the commencement of study (Ethics Committee / IRB Reference Number: DF RD1402/0002(L)). The teeth collected were used within 3 to 5 months following the extraction.

3.5 Dentine Specimen Preparation

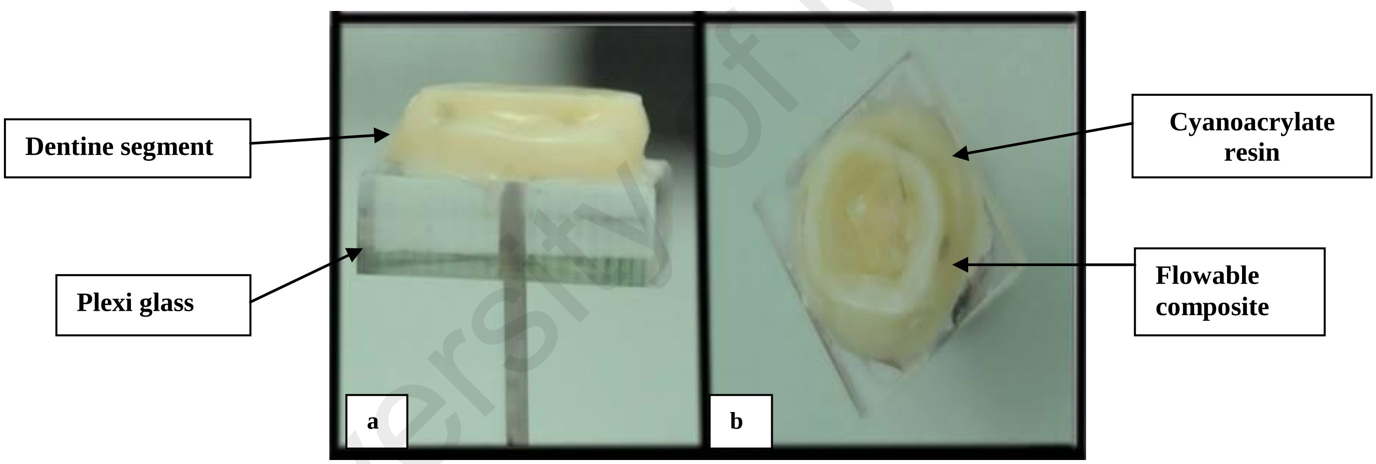
Dentine discs (n=75) were obtained from caries free extracted human molars extracted for surgical reasons and were prepared by sectioning each molar horizontally with a slow speed sawing diamond disc (Micracut125 Metkon, University of Malaya) under constant water cooling. The coronal and radicular portion were removed from each tooth 1mm below the occlusal pit and 2 mm below the CEJ (Elgalaid et al., 2004) to obtain a dentine segment of minimal thickness 1± 0.2 mm (Gandolfi, Silvia, et al., 2008; Mordan et al., 1997; D. H. Pashley, 1986; Pereira et al., 2005; Prati, 1994). The pulp tissue was removed with barbed broach and care was taken not to damage the pre-dentine surface and the inner wall of the pulp chamber.

3.6 Specimen Attachment

The dentine disc was then mounted to an acrylic plexi glass section (YSME Sdn Bhd, Malaysia) which was cut into size 1.5cm x 1.5cm x 0.5cm. . It was then roughened on the top surface to make it frosted in appearance, rather than perfectly clear and smooth. This helped the plexi glass to adhere to the specimen attached. A small hole was drilled through the middle of the plexi glass section which was slightly smaller than the diameter of an 18 gauge stainless steel tube (<1.24 mm). These 18 gauge stainless steel tubes were cut into sections of length 1.5 cm using a cutting disc and the rough margins on the ends were smoothened. A cyanoacrylate resin was applied alongside the stainless tube and a mallet was used to drive the cut piece of it through the prepared hole of the plexi glass section so that 1-2 mm of the stainless steel tube is extruded from the top surface of the plexi glass. This would prevent inadvertent sealing of the stainless steel tube by the cyanoacrylate resin when the specimen is attached to it. An endodontic file was used to remove the debris inside the stainless steel tubing. Later, the root portion of the specimen was affixed to the prepared surface of the plexi glass with cyanoacrylate resin. The tight seal and leakage was further prevented by the application of flowable composite around the interface between the disc and the adhesive agent (3M ESPE FiltekZ350XT, USA) (Figure 3.1).



Diagrammatic representation of specimen attachment



Photographic representation of a) Front view & b) Top view of specimen attachment

Figure 3.1 Specimen Attachment to Plexi glass.

3.7 Fluid Filtration device

The schematic drawing of the capillary tube set up with its respective attachment to dentine specimen and the microcapillary tube via polyethylene tubes (PE160 & 90) is shown in Figure 3.2.

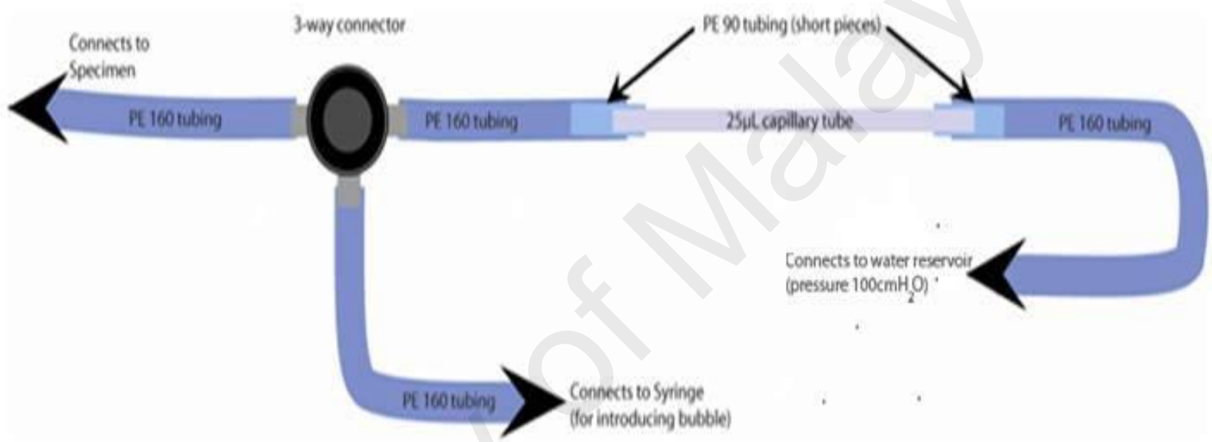


Figure 3.2 Schematic drawing of the capillary tube set up

3.7.1 Armamentarium used to construct Fluid Filtration System

- Polyethylene 160 tube (Warner instruments, USA)
- Polyethylene 90 tube (Warner instruments, USA)
- 25μl micro- capillary tube (Fischer Scientific, USA)
- 3 way connector (Quest Precision Engineering Sdn Bhd, Kuala Lumpur, Malaysia)
- Gillmonts syringe (Fischer Scientific, USA)
- 25-30 cm^3 empty syringe barrel.
- 18 gauge blunt disposable needles (YSME Sdn Bhd, Malaysia)
- Wooden ruler (2 meters length)

- Retort stand (Graces scientific Sdn Bhd, Malaysia)
- Metallic ruler (15 cm)
- Hemostats
- Rubber bands.

In Figure 3.3, right end of the device had 25-30cm^3 empty syringe barrel as a water reservoir taped to wooden ruler using rubber band, which was 2 meter in length and held by retort stand (Figure 3.5). The upper meniscus of the water in reservoir was positioned 100 cm above the dentine specimen to maintain the pressure 1.4 psi. Furthermore, it was connected to PE 160 tube via 18 gauge blunt needle. This tube was inserted into short piece of PE 90 tube and further attached to one end of 25ul capillary tube which lies between the reservoir and the specimen. The other end of tube was attached in a similar way and was connected the right exit of a 3 way connector. The left end of the device was connected to dentine specimen with PE 160 tube, which was attached to the left exit of the connector. The bottom exit of the connector united with the Gillmonts syringe with its 18 gauge blunt needle by the same PE tube as is shown in Figure 3.4. The purpose of the syringe was to insert, withdraw and control the position of air bubble into the tube by turning the syringe's dial in clock wise and anticlockwise direction. Insertion of more than a bubble was avoided. Moreover, the capillary tube was positioned parallel with a metallic ruler that was graduated in 0.5mm increments and measured in millimeters. It helped to record the movements of an inserted bubble during experiment. The fluid movements toward the tubing were controlled by multiple haemostats whose ends were covered by rubber sheaths to prevent the damage to PE tubing during clamping as shown in Figure 3.6 & 3.7.

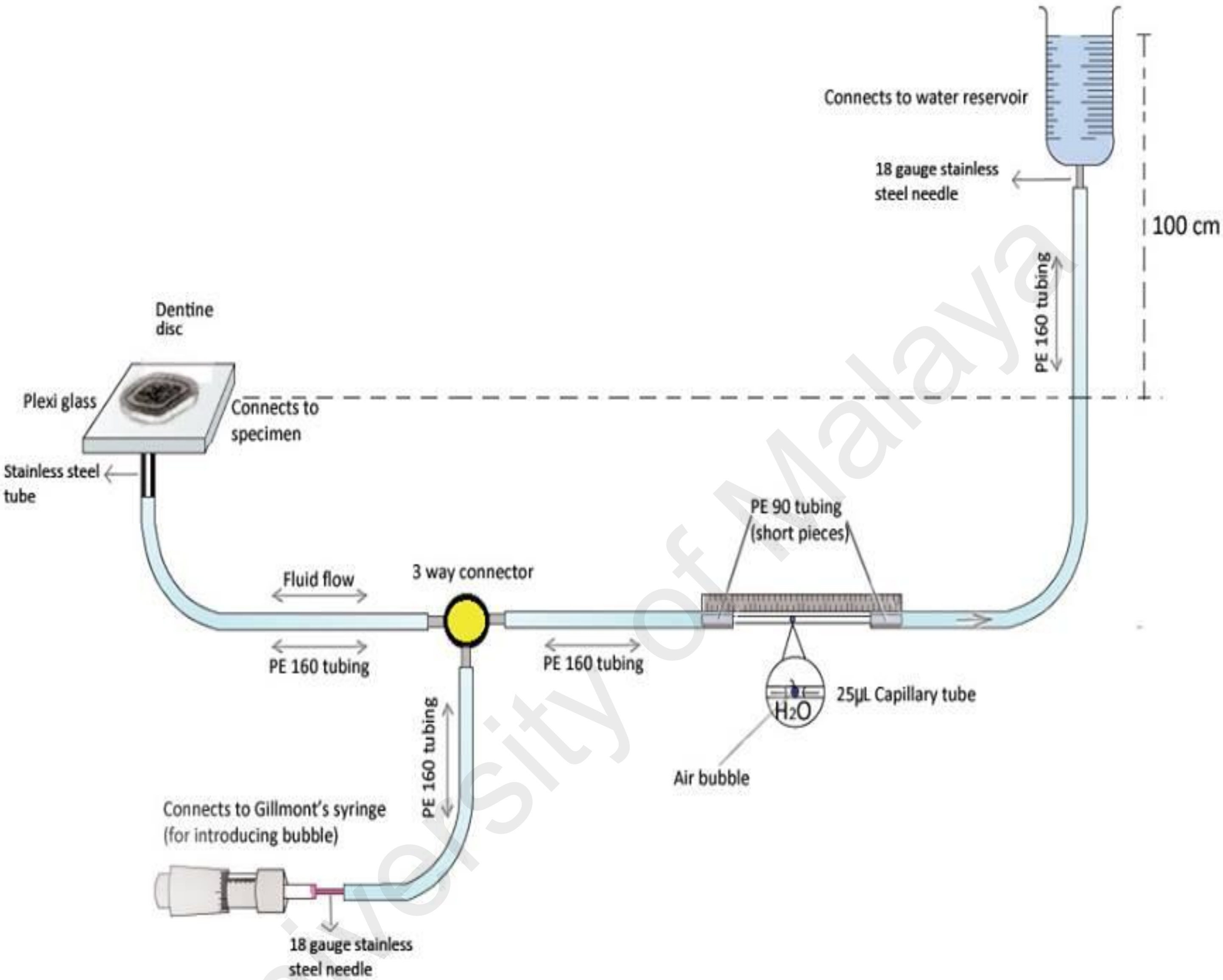


Figure 3.3 Experimental set up of ''Fluid Filtration device''.

3.7.2 Design of the Fluid Filtration device

The photographic representation of the Fluid filtration device along with its respective description is shown in Figure 3.4, 3.5, 3.6 & 3.7.

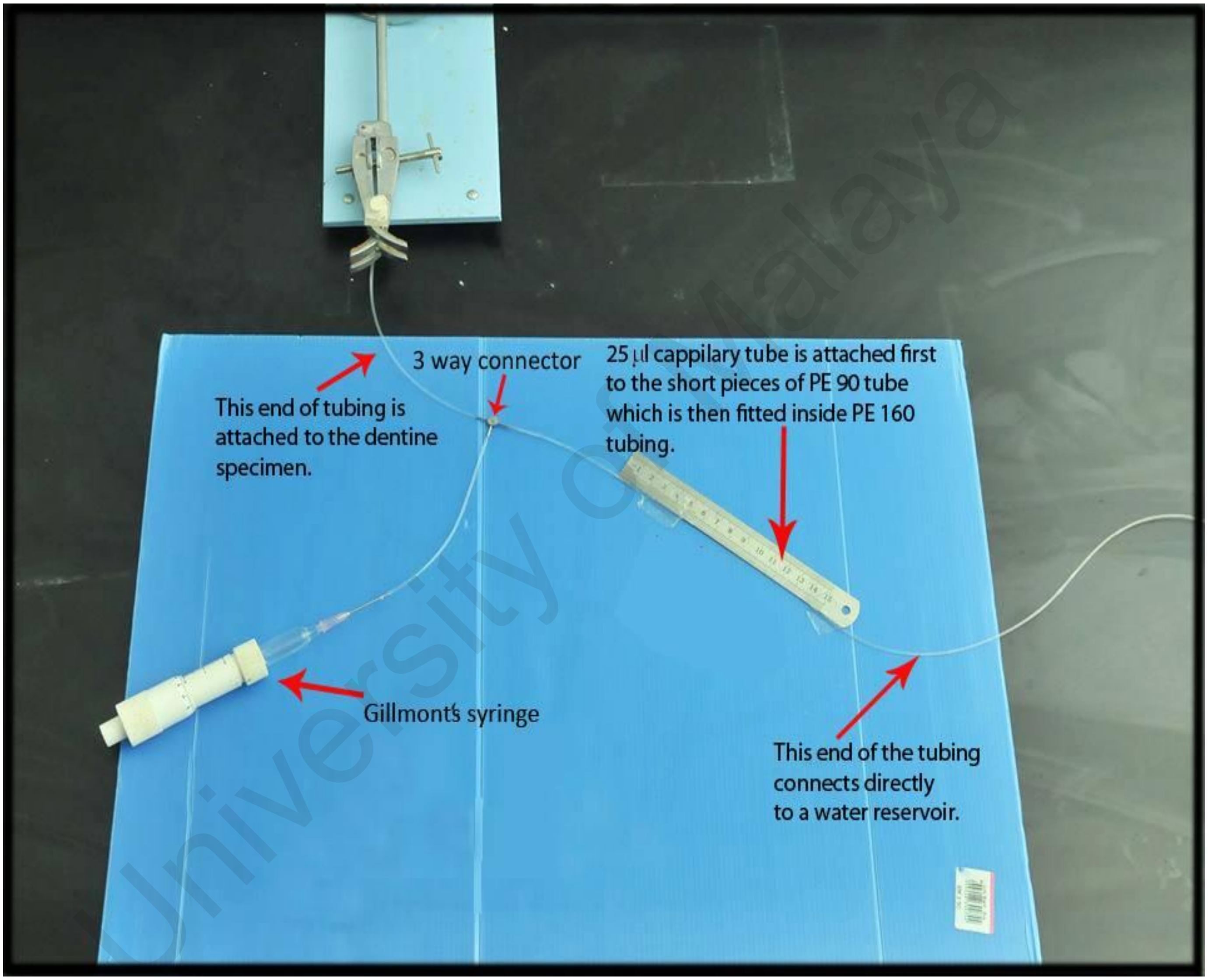


Figure 3.4 Closer photographic view of the tubes with its respective attachment

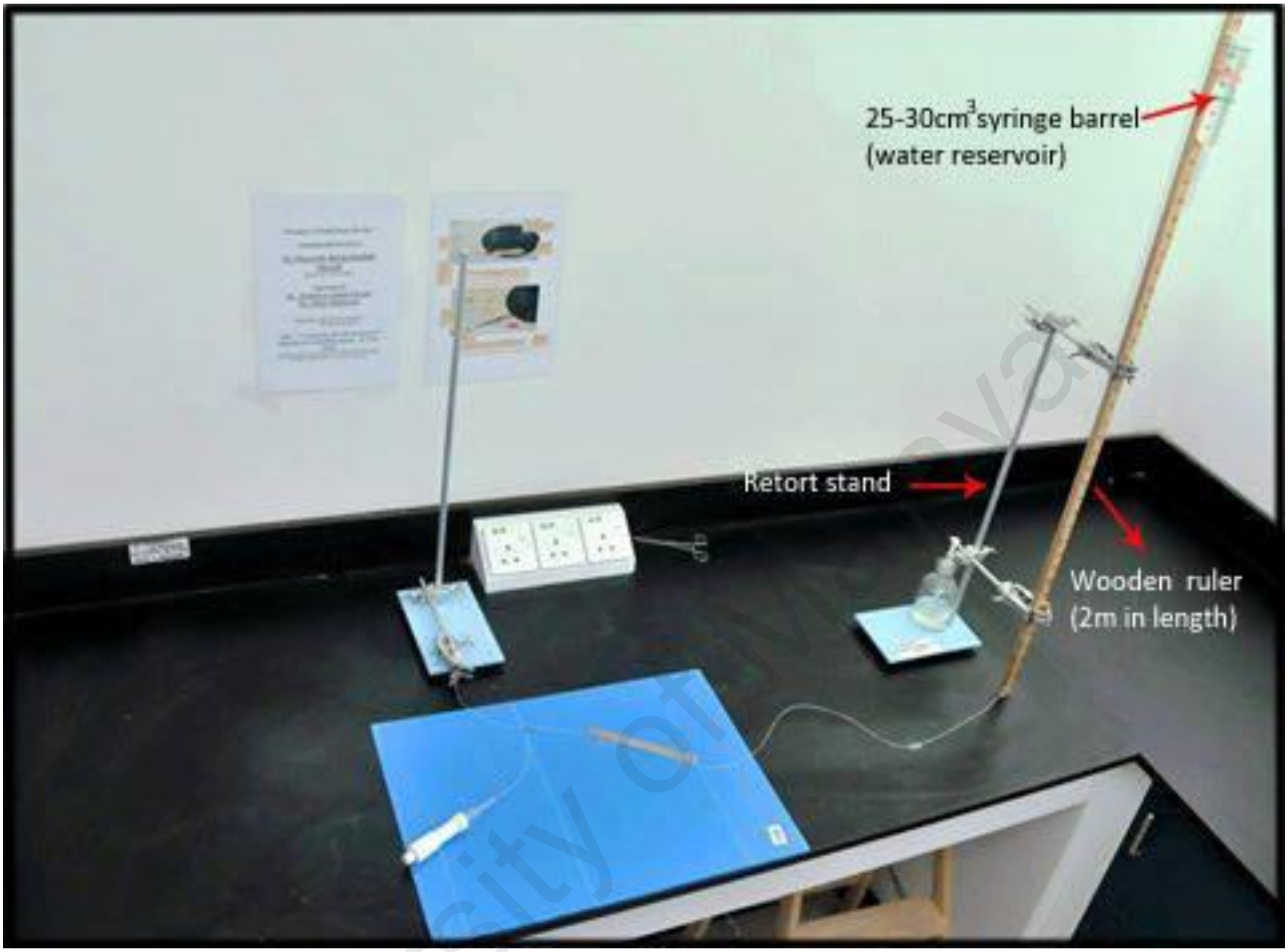
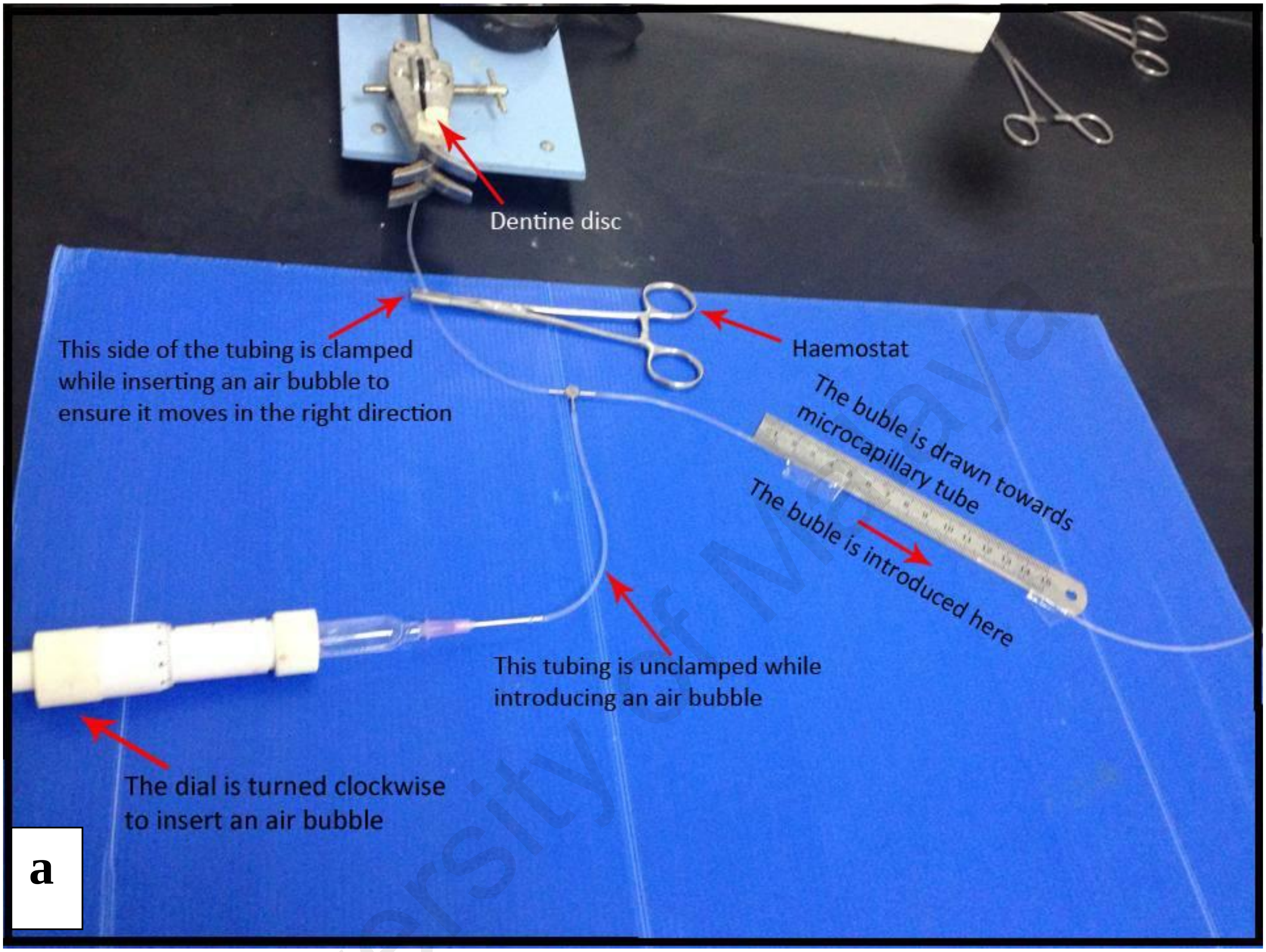


Figure 3.5 Complete photographic representation of the Fluid filtration device.


Dentine disc
This side of the tubing is clamped while inserting an air bubble to ensure it moves in the right direction
Haemostat
The buble is drawn towards microcapillary tube
The buble is introduced here
This tubing is unclamped while introducing an air bubble
The dial is turned clockwise to insert an air bubble
a

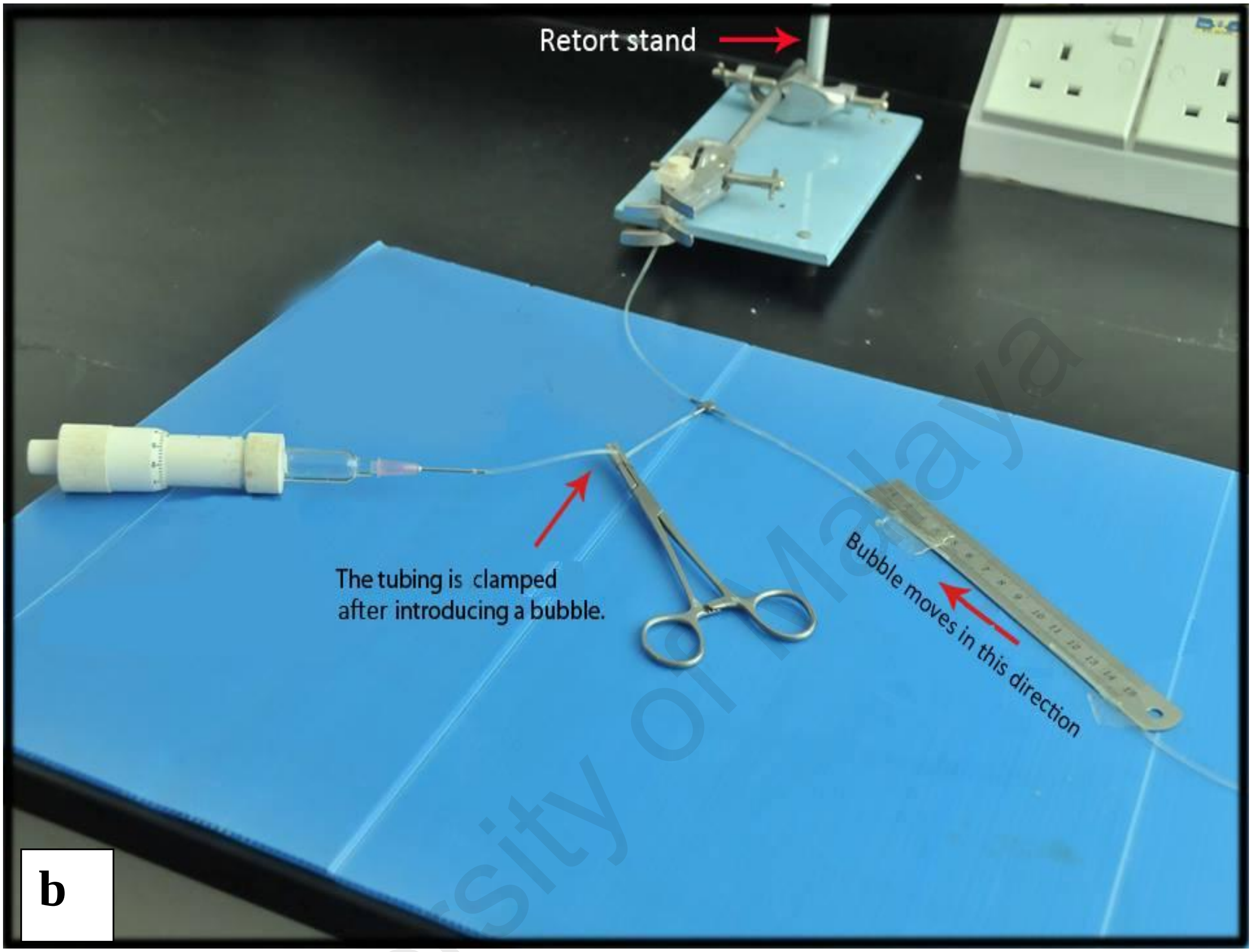


Figure 3.6 Steps of introduction of an air bubble into the fluid filtration device

Figure 3.7 Front photographic view of the fluid filtration device

3.7.3 Dentine Permeability Measurement

At the beginning, the reservoir was filled with distilled water to the required level and it was ensured it was flown through all the tubes including dentine specimen without any leakage. It was also added frequently during the experiment to maintain the pressure (1.4psi). An air bubble was introduced by detaching the end of the PE tube connecting the bottom exit of the 3 way connector and it was flipped with finger to remove the fluid from that area and replaced it with air. Later, it was reconnected and eventually an air bubble was formed. A small air bubble within the tube formed was moved towards the capillary tube using Gillmont's syringe (Figure 3.6).The initial position of the bubble inside the capillary tube was recorded. The entry of the bubble in the direction of the dentine specimen was prevented by clamping PE tube using haemostat between the specimen and the connector. However, during the measurement of the permeability PE tube was unclamped in that area and whereas the area between the connector and Gillmonts syringe was clamped (Figure 3.7). Thus, allowing the fluid to move towards the specimen (Figure 3.7).

Subsequently, the movement of an air bubble within the capillary tube was noted for 5 minutes. The first minute's reading was discarded because the device was allowed to reach its steady state. Apparently, the next four minutes readings were recorded. Three repeated measurements were recorded in the same way for each specimen and its average values were considered. The linear movement of a bubble was observed against a calibrated ruler incremented and measured in mm and converted into volume displacement (hydraulic conductance, Lp) as shown by the formula below. The dentine permeability (Lp) was calculated by dividing the fluid flow (µL) by surface area of dentine exposed (cm^{-2}) and hydrostatic pressure cm H_2O/psi.

$$\text{Dentine permeability, Lp} = \frac{Q}{P(SA)} \quad \text{(Pereira et al., 2005)}$$

Where,

Lp is permeability of dentine

Q is filtration rate in µl / min^1

SA is surface area of the dentin in cm^2,

P is hydrostatic pressure difference across dentine in cm H_2O / psi

An example of the sample calculation is as follows:

1) The bubble movement was observed in 4 minutes = x mm /4 min = mm / min
2) The capillary tube factor was calculated by dividing 25 µl capillary tube which was 65 mm in length = 0.385 µl/ mm
3) The fluid flow was multiplied by the capillary tube factor : x mm/ min x 0.385 µl/ mm = x µl / min
4) It was divided by the pressure (1.4 psi = 100 cmH_2O).
5) It was divided by the surface area (1 cm^2)
6) Later, it was multiplied by 100 to get the relative fluid flow percentage %

   The Lp values of each dentine specimen were expressed as percentage (LpT%) of maximum dentine permeability of the fluid flow across acid etched dentine disc of the same specimen (obtained after the treatment with acid etchant i.e, Lp = 100%). Hence, each dentine specimen represented as its own control.

3.7.4 Experimental Design

The quantitative changes in the dentine permeability (Lp) through the dentine disc specimens induced by the treatments applied were quantified by fluid filtration device in vitro working at 100 cm H_2O pressure (1.422psi) (Elgalaid et al., 2007). Figure 3.10 shows the summary of the experimental design for dentine permeability measurement of different treatments (LpT). Ten samples for each group were allocated by random sampling (n =50) for groups 1-5. The fluid flow through the dentine samples was measured after each of the following stages:

The upper dentine surface was wet sanded with 600 silicon carbide abrasive paper for 30 seconds (Gandolfi et al., 2012; Wang et al., 2010) to create a standard smear layer. This established a minimum permeability value (LpT1) for the first treatment. The dentine surface of the specimens were then treated with acid etchant that is 37% Ortho phosphoric acid (3M ESPE Scotch bond etchant) for 60 seconds (Elgalaid et al., 2007; Lochaiwatana et al., 2015) and washed with distilled water. This established a maximum permeability value for the second treatment (LpT2). Subsequently**,** dentine specimens were subjected to five different mineralizing agents by following the manufacturer's instructions for 10 minutes each and its value was measured (LpT3). The composition of the materials that were used is summarized in Table 3.1 and the mineralizing agents used in the study are presented in Figure 3.9. The description of the treatment groups are as follows

1. Group 1 : GC Tooth Mousse Plus (n=10)
2. Group 2 : Clinpro White Varnish (n=10)
3. Group 3 : Duraphat Varnish (n=10)
4. Group 4 : Colgate Sensitive Pro- Relief dentifrice (n=10)
5. Group 5 : Biodentine (n=10)

Fourth (LpT4) and the fifth (LpT5) values were obtained by immersing the treated specimens in artificial saliva 37^{o}C for 10 minuteis and 7 days. The composition of artificial saliva (Alfatech Sdn Bhd, Malaysia) that was used is summarized in Table 3.2 (Wang et al., 2011). The final value (LpT6) was recorded by challenging the dentine specimens to 0.02 M Citric acid solution (pH 2.5) for 3 minutes.

Group 1 GC Tooth Mousse Plus

Group 2 Clinpro White Varnish

Group 3 Duraphat Varnish

Group 4 Colgate sensitive Pro-relief Dentifrice

Group 5 Biodentine

Figure 3.8 Mineralizing agents used in the study

Mineralizing agents	Manufacturer	Active Ingredients	Manufacturer's instructions
GC Tooth Mousse Plus	Reca Dent GC Corporation Tokyo, Japan.	Casein Phospho Peptide – Amorphous Calcium Phosphate Fluoride	• Squeeze a small amount of GC Tooth Mousse plus onto your hand or a cotton tip, swab could be used for application • Apply directly onto the tooth surfaces and leave undisturbed for 3 minutes. • Avoid eating or drinking for 30 minutes following application.
Clinpro White Varnish	3M ESPE, USA	Tri-calcium Phosphate , 5% sodium fluoride	• Peel the foil pouch open to expose the Clinpro white varnish unit- dose package &applicator brush. • Open unit- dosage package & dispense contents onto dispensing guide sticker. • Dispense entire contents onto the shaded inner circle and use dosage guidelines to determine the amount (0.25, 0.40 & 0.50 ml). • Use the applicator brush ti thoroughly mix & while mixing keep the material evenly distributed inside the inner circle of the dosing guide.
Duraphat Varnish	Pharbil Waltrop GmbH. Waltrop, Germany.	5% Sodium Fluoride (22,600ppm)	• Isolate the tooth with cotton rolls & dry with compressed air or with cotton gauze before application since varnish sets in the presence of moisture. • Mix the varnish well and apply it onto the teeth surfaces with a small disposable brush or applicator. • Varnish should be applied as a thin film. • Instruct patients to avoid eating for 2-4 hrs after the application
Colgate sensitive Pro-Relief dentifrice	Colgate –Palmolive, Thailand	Arginine 8%,Sodium Monofluorophosphate and Calcium carbonate	• Apply 1 inch strip of the product to a gentle tooth brush. • Make sure to brush all sensitive areas of the teeth. • The product can be applied to the sensitive tooth with finger tip & gently massage for 1 min.
Biodentine	Septodont / UK	Tricalcium Silicate and Calcium Chloride	• Take a capsule & gently tap it on a hard surface to loosen the powder. Open a capsule. Detach a single dose container of liquid & gently tap in a capsule to force all the liquid down to the container. • Twist cap to open & pour 5 drops from the single dose container into the capsule . • Close the capsule and place on a mixing device such as Rotomix at the speed of 4000-4200 rev/min & mix for 30 seconds • Open the capsule & check materials consistency. • Collect Biodentine with the instrument supplied in the box.

Table 3.1 Active ingredients & manufacturer's instructions of the mineralizing agents applied

Table 3.2 Composition of artificial Saliva

Artificial saliva composition	Quantity (g)
Calcium Chloride Dihydrate Reagent Granula, ($CaCL_{2}$)	500
Potassium Chloride Reagent (KCl)	500
Potassium Phosphate Monobasic Reagent (KH_2PO_4)	500
Tris Hydroxymethylaminomethane primary (Tris)	25

3.8 SEM/EDX Analysis

Dentine discs (n=25) were prepared following the specimen preparation method used for dentine permeability measurement. Five dentine discs amongst those were selected randomly, prepared and processed for the qualitative analysis of each treatment group for acid etching (60 seconds), application of testing agents for 10mins, after artificial saliva immersion for 10 minutes and 7 days interval and citric acid challenge for 3 minutes. Each specimen was air dried in a sun-dry cabinet at a constant room temperature 37^{o}C and sputter coated with Gold in a vacuum evaporator (Figure 3.12) (Polaron Q150RS, Hi-Tech Instruments Sdn Bhd, Malaysia).

The scanning electron microscope (Quanta FEG 250, Crest Co., Holland) with a low vacuum operating mode was used to examine the specimens at 10Kv and subjected to morphological analysis (Figure 3.13, 3.14). The SEM images of each specimen were captured using the magnification 3500x and 7500x.This SEM is also equipped with an energy x-ray dispersive spectrometer- EDX (Oxford x-Max using software Inca, Crest Co., Holland) which was used for elemental and chemical analysis of dentine samples. All the samples were analyzed at 20Kv. This was done to see the tubule occlusion, crystal precipitate, its pattern and eventually with the citric acid challenge, change in the crystal pattern. The experimental design for the SEM/EDX evaluations for different treatments is summarized in Figure 3.10 where as the experimental design for both the dentine permeability and SEM/EDX evaluation is summarized in Figure 3.11.

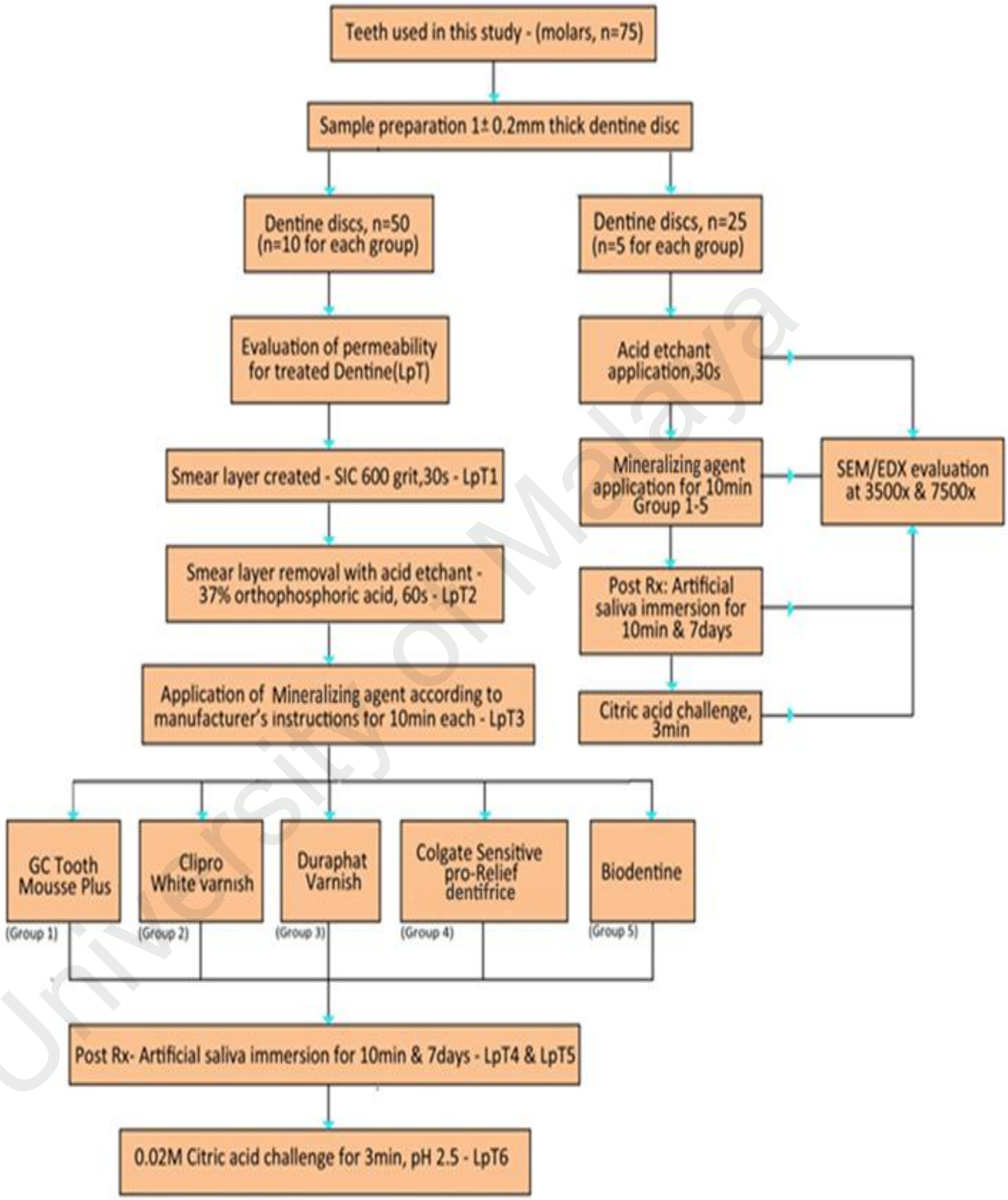


Figure 3.9 Summary of the experimental design

Figure 3.10 Sun-dry cabinet - to dry out the wet dentine specimen after each treatment session to avoid charging during the SEM imaging

Figure 3.11 Scanning Electronic Microscope (University Malaya) used for the imaging of dentine specimens at high resolution magnification of 3500x and 7500x.

Figure 3.12 Sample placement over the table top of SEM

3.9 Statistical Analysis

Statistical analysis was performed using SPSS version 20 for Windows. The permeability data (LpT1, LpT2, LpT3, LpT5, LpT6) were transformed into the percentages. Means and standard deviations of Lp values were calculated for each group. The Homogeneity of variance was assessed by using Levene's test ($p > 0.05$).Two- way repeated ANOVA procedure was used to evaluate inter treatment and intra treatment effects. Post hoc multiple comparisons were used to identify significant difference using Bonferroni test. All p values were set 0.05.

CHAPTER 4: RESULTS

4.1 Dentine Permeability Evaluation

4.1.1 Quantitative evaluation of permeability for treated dentine

The descriptive statistics for the overall Lp values are shown in table 4.1. The skewness values are all within ±1. Hence, all the distributions are symmetrical. The distributions are fairly normal.

Table 4.1 Descriptive statistics for LpT stages overall for all treatment groups

	LpT2 Acid Etchant	LpT3 Treatment Applied	LpT4 Artficial Saliva immersion10 min	LpT5 Artficial Saliva immersion 1week	LpT6 CitricAcid immersion 3min
Mean	100	30.88	36.42	37.07	53.34
Std. Deviation	0	15.59	14.17	16.09	18.86
Skewness		-.054	-.375	.031	-.333

Less than 1 in magnitude ➔ symmetrical

A part of the results from two way ANOVA repeated measures is presented in Table 4.2. The p-values for Lp stages and Lp stages *treatment interactions were less than 0.05. Hence, the means for at least one pair of Lp stages differ significantly. There is a significant interaction between Lp stages and treatment.

Table 4.2 Results for Intra-groups and group interaction

Effect	Type III Sum of Squares	Df	Mean Square	F	Sig.
Lp	14059.60	1.95	7210.20	51.60	.000
Lp*Treatment	4861.01	7.80	623.20	4.40	.000

The results testing the differences between the LpT stages and Treatment groups are shown in Table 4.3. The p-value is also less than 0.05. Hence, one pair of group differs significantly.

Table 4.3 Difference of Inter-treatment groups

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Intercept	310968.6	1	310968.6	547.2	.000
Treatment	9200.8	4	2300.2	4.0	.007
Error	25571.4	45	568.2		

Treatment Agent	n	Mean	Std. Dev	P
GC tooth mousse +	10	32.77	15.80	0.981
3M Clinpro Varnish	10	28.40	13.45	
Duraphat	10	30.71	17.87	
Colgate sensitive pro relief Arginine 8%	10	31.84	13.11	
Bio dentine	10	30.70	19.74	

At baseline LpT3, no statistically differences were observed ($P>0.05$)

The values expressed as percentage for means ± standard deviations (n=10 per group) for each Lp stages across five treatments groups. All treatments showed a decrease in dentine permeability. The changes in the dentine permeability produced by the application of different treatments are presented in Table 4.4.

Permeability, LpT2 after acid etchant treatment increased the permeability to a maximum level arbitrary equal to 100%. Each acid etched dentine represented its own control of that specimen (Pashley et al., 1996; Prati, 1994).

The application of GC Tooth mousse plus, Clinpro white varnish, Duraphat varnish, Colgate sensitive pro-relief dentifrice and Biodentine after acid etching resulted in significant reduction of permeability ($P<0.05$) compared to its maximum acid etched value (LpT2 to LpT3). No statistically significant differences were observed amongst all the treatment groups. The immersion of treated specimens in artificial saliva for 10 minutes (LpT4) was responsible for a partial increase in permeability for all treatments (Table 4.4). However, there were no significant differences for all the treatments from LpT3 to LpT4 ($p > 0.05$). There were no significant differences at LpT4 between the treatment groups. Moreover, immersion of treated specimens in artificial saliva for 7 days resulted in further increase in mean dentine permeability values except for Clinpro white varnish and Biodentine which showed decrease in permeability but there were no significant differences ($p>0.05$) . However, the mean values for Clinpro white varnish at LpT5 were significantly different compared to GC Tooth mousse plus ($p=0.002$)), Duraphat varnish ($p=0.003$), Colgate sensitive pro-relief dentifrice ($p=0.010$) but not for Biodentine ($p=0.248$). The exposure of treated dentine specimens to citric acid after artificial saliva immersion for 7 days was responsible for an increase of dentine permeability values of all treatment groups. There was a statistically significant increase in permeability observed for GC Tooth mousse plus, Duraphat varnish and Biodentine from LpT5 to LpT6 ($p<0.05$) but not for other treatment groups (Clinpro white varnish and Colgate sensitive pro-relief dentifrice) as presented in Figure 4.2. However, Clinpro white varnish showed statistically significant differences at LpT6 compared to other treatment groups ($p < 0.05$).

Table 4.4 Permeability (Lp) data after treatments at different Lp stages

Treatments (LpT%)	**GC Tooth Mousse Plus (Group=1)**	**Clinpro White Varnish (Group-2)**	**Duraphat Varnish (Group-3)**	**Colgate Sensitive Pro-relief dentifrice (Group 4)**	**Biodentine Group 5**
Smear Layer LpT1	29.5±14.1	26.8±13.1	26.8±17.2	20.6±13.3	19.9±15.3
Acid etchant application LpT2	100±0	100±0	100±0	100±0	100±0
Treatment application LpT3	32.8±15.8	28.4±13.4	30.7±17.9	31.8±13.1	30.7±19.7
Artificial saliva immersion-10minutes LpT4	37.6±15.9	30.2±10.8	40.6±16.3	37.6±14.3	36.2±13.7
Artificial saliva immersion-1week LpT5	44.7±11.3	20.2*±7.6	44.5±19.6	41.7±14.8	34.4±12.1
Citric acid challenge-3minutes LpT6	66.2±7.5	27.5 *±11.7	60.7±14.7	58.0±16.8	54.3±15.4

] denotes the significant differences within the group where as

(*) shows the significant difference between the group at different stages.

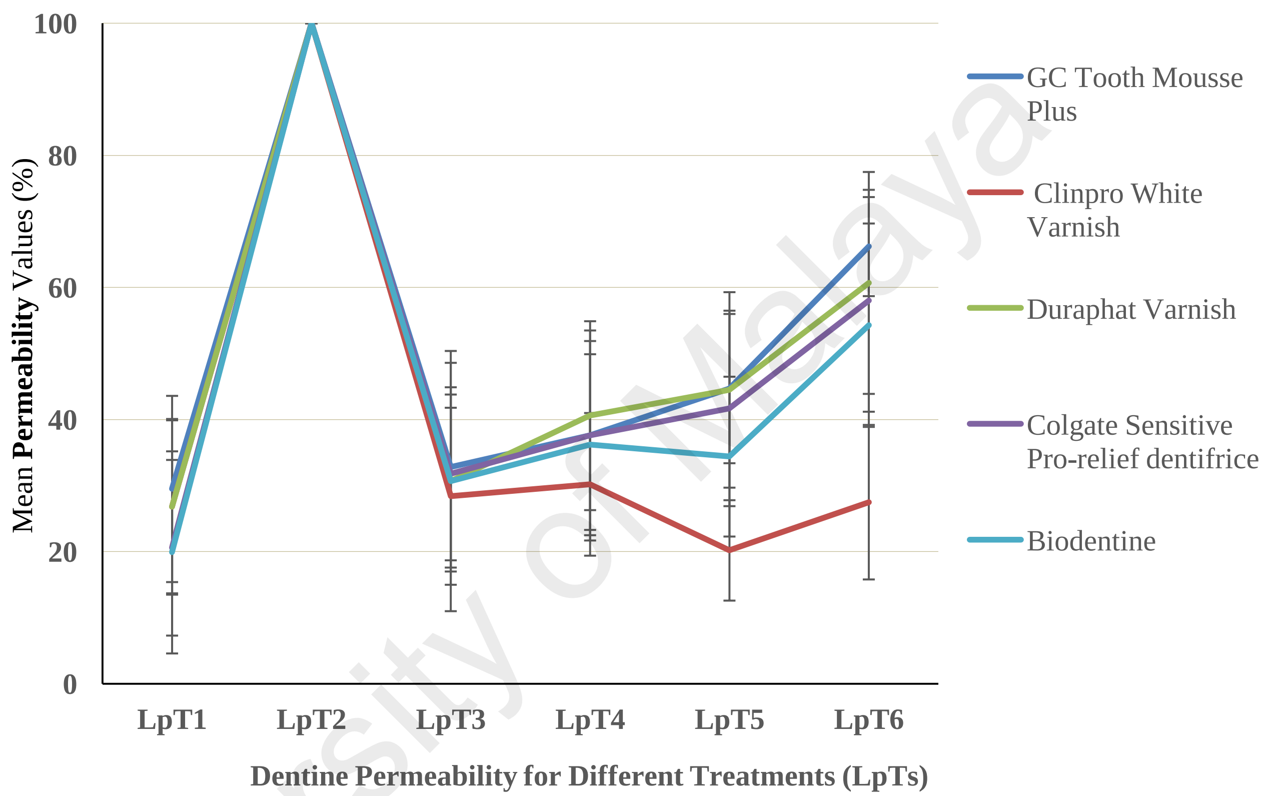


Figure 4.1 Mean permeability values for Mineralizing agents

The reduction in permeability of all groups 1-5 following all the LpT stages is presented in the form of a Line graph (Figure 4.1). However, there is a decline of peak at LpT5 with 80% reduction in permeability for Clinpro white varnish.

The average permeability values are seen to increase from LpT5 to LpT6 in Figure 4.2. However, The LpT5 values shows that Clinpro white varnish had significantly reduced permeability much more in comparison compared to other groups except for Biodentine ($p=0.248$) whereas the LpT6 values for Clinpro white varnish shows significant reduction in the permeability compared to all the other treatment groups $p < 0.001$.

4.1.2 Dentine morphological evaluation by SEM/EDX analysis

The process of mineralization with occlusion of dentinal tubules were investigated further by using scanning electron microscopy and element sensitive detector to record qualitatively the elemental composition that is Ca, P/O and other elements within the treated dentine specimen which is in accordance with the experimental protocol (Tsuda et al., 1996).

Dentine discs were subjected to SEM/EDX analysis immediately after acid etching which led to the removal of smear layer and exposure of dentine tubules. Each of these applied treatments on etched dentine specimens produced morphological modifications which completely covered the entire dentine surface masking all open dentine tubules created by Etchant.

4.2.2.1Acid etched dentine

Dentine discs (n=5) of each group were subjected to SEM/EDX analysis immediately after acid etching for 60 seconds which led to the removal of smear layer and exposure of dentine tubules (Figure 4.2 i & ii).

4.2.2.2 GC Tooth Mousse Plus

SEM analysis of acid etched dentine samples treated by application of GC Tooth Mousse demonstrated layers of granular deposits with a cobble stone appearance. The surface appeared to be homogeneously covered occluding the dentinal tubular orifice. Only few orifices of dentinal tubules were visible (Figure 4.3 a i & ii). After 10 minutes of artificial saliva immersion, the debris was washed away from the dentine and the surface appears to be uncovered by deposits with open tubule orifices (Figure 4.4 a i & ii). After 7 days of immersion in artificial saliva, fine debris appears to be observable on inter tubular and various areas illustrate tubules closed by an amorphous material and a partially distorted homogenous matrix was evident (Figure 4.5a i & ii). The subsequent challenge to citric acid solution further eroded away the deposits leading to an increased exposure of dentinal tubules (Figure 4.6 a i & ii).

EDX analysis after the GC Tooth Mousse Plus treatment revealed the prevalent content of Ca (Calcium), P (Phosphorus), Si (Silicon), Ti (Titanium) and traces of Na (Sodium) were present (Figure 4.7a). The concentration content of Ca (Calcium) and P (Phosphorus) were lowered after being immersed in artificial saliva for 10 minutes (Figure 4.8a). However, following immersion in artificial saliva for 7 days, EDX detected high peaks of Ca (Calcium) and P (Phosphorus) (Figure 4.9a) but with eventual exposure to citric acid for 3 minutes, abrupt decrease in the content of Ca (Calcium) and P (Phosphorus) was observed with traces of Si (Silicon) and Cl (Chlorine) (Figure 4.10a).

4.2.2.3 Clinpro White Varnish

The application of Clinpro White varnish on dentine surface resulted in dentine tubule occlusion covering the entire dentine surface. A fine hexagonal crystal layer was observed which plugged the dentinal surface at treatment application (Figure 4.3b i & ii). Following the immersion in artificial saliva for 10 minutes, a completely transformed morphology appeared with a transition from a crystalline structure to a much smoother surface with few vacant tubules (Fig. 4.4b i & ii). The Artificial saliva immersion for 7 days demonstrated a homogenous surface with less partially blocked tubular orifice (Fig. 4.5b i & ii). A much smoother and uniform morphology was exhibited after the citric acid challenge for 3 minutes with few unoccupied dentinal tubules. The crystal like structures were rarely evident (Fig. 4.6b i & ii).

EDX analysis revealed high content of Ca (Calcium), P (Phosphorus) and traces of Na (Sodium) and Cl (Chlorine) on treatment application (Figure 4.7b). High peaks of Ca

(Calcium) and P (Phosphorus) were observed following immersion in artificial saliva for 10 minutes (Figure 4.8b) which were consistent even after the 7 days of immersion in artificial saliva (Figure 4.9b). However, the peaks of Ca (Calcium) and P (Phosphorus) still maintain the same level even after the citric acid challenge (Figure 4.10b).

4.2.2.4 Duraphat Varnish

Duraphat varnish treatment application created a finely crystalline surface masking all the dentine tubule orifices. No open tubule orifices were observed (Figure 4.3c i & ii). A shift of morphology was observed to an amorphous aggregate with few patent tubules at 10 minutes of immersion in artificial saliva (Figure 4.4c i & ii). However, after 7 days of immersion in artificial saliva, the surface appeared smooth with few opened tubules were observed (Figure 4.5c i & ii). Subsequently, the citric acid challenge for 3 minutes further led to the removal of tubular plugs and exposed the orifices (Figure 4.6c i & ii)

EDX analysis after the treatment application on dentine specimens demonstrated low level of Ca (Calcium) and traces of Mg (Magnesium), Na (Sodium), F (Fluoride) and Si (Silicon) (Figure 4.7c). An increased content of Ca (Calcium) and emergence of P (Phosphorus) were observed after immersion in artificial saliva for 10 minutes (Figure 4.8c) which were consistent at 7 days immersion in artificial saliva with the presence of new element K (Potassium) (Figure 4.9c). However, with the citric acid immersion for 3 minutes, a significant decrease in the content of Ca (Calcium) and P (Phosphorus) was observed without any traces of K (Potassium) (Figure 4.10c).

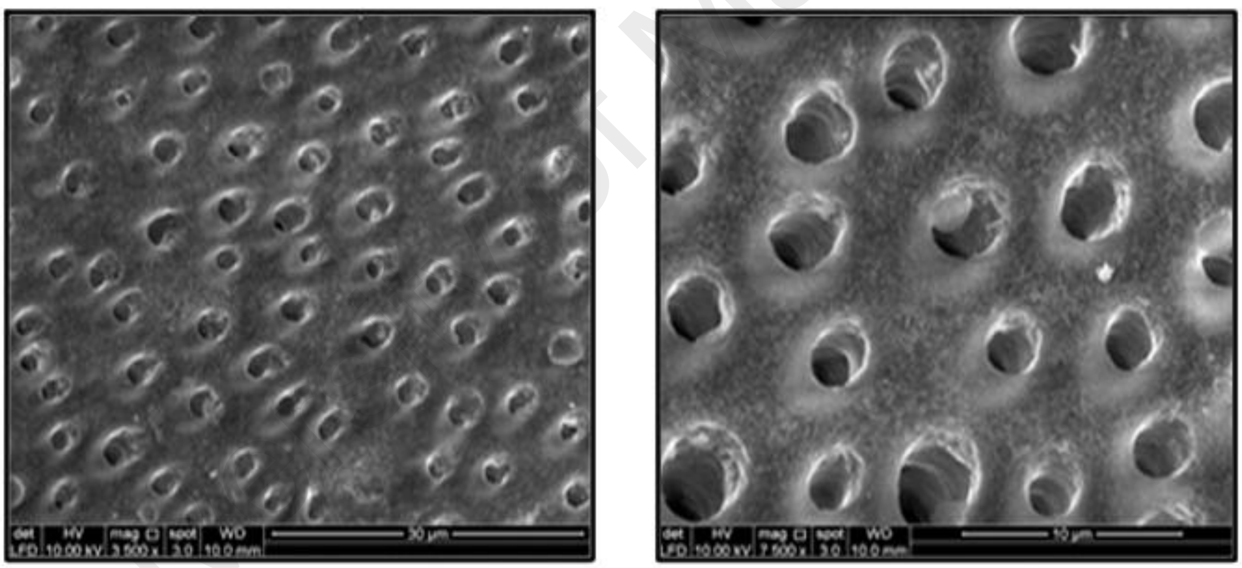
4.2.2.5 Colgate Sensitive Pro-Relief dentifrice

Colgate Sensitive Pro-relief dentifrice treatment application produced a layer of finely coarse granular precipitates masking the dentine surface but only few open tubules were apparent (Figure 4.3d i & ii). After the immersion of treated dentine specimen in artificial saliva for 10 minutes, there was a change observed in its morphology with some vacant tubules (Figure 4.4d i & ii). The following immersion in artificial saliva for 7 days resulted in drastic change of its morphology and a much hazy and smooth surface was observed. Sparse tubules were obliterated by the precipitates (Figure 4.5d i & ii). Further with the citric acid challenge for 3 minutes, the surface appeared to be washed out and revealed the inter tubular dentine and few tubules were partially plugged (Figure 4.6d i & ii).

EDX analysis after the treatment applied to the dentine surface revealed high content of Ca (Calcium), Ti (Titanium) and traces of Na (Sodium) (Figure 4.7d). The following immersion in artificial saliva after 10 minutes, showed moderate decrease in Ca (Calcium) with the appearance of P (Phosphorous) (Figure 4.8d). However, the Ca (Calcium) and P (Phosphorous) peaks were increased after the immersion in artificial saliva for 7days with the evidence of K (Potassium) appearance. (Figure 4.9d). Inevitably, there were not much changes to the content of Ca (Calcium), P (Phosphorus) and K (Potassium) after the exposure to citric acid for 3 minutes (Figure 4.10d).

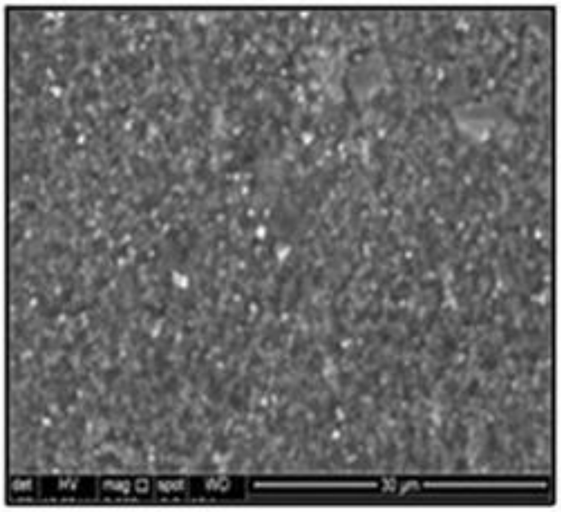
4.2.2.6 Biodentine

Biodentine applied to the dentine surface demonstrated different size and shape of calcium silicate particles covering the entire surface of dentine leaving few vacant orifice of dentinal tubule (Figure 4.3e i & ii). Following the immersion in artificial saliva for 10 minutes, the morphology of the particles were changed with few open tubule orifices (Figure 4.4e i & ii). However, following the artificial saliva immersion for 7 days, the dentine surface appeared to have a globular morphology with a further slight increase in the number of the exposed dentinal tubule (Figure 4.5e i & ii).The subsequent citric acid challenge to the dentine specimen for 3 minutes resulted in an increase in the number of vacant dentinal tubules (Figure 4.6e i & ii). EDX analysis on treatment application demonstrated high content of Ca (Calcium), Si (Silicon) and Cl (Chlorine) (Figure 4.7e) and the Ca (Calcium) peak was moderately decreased after the immersion in artificial saliva for 10 minutes with the appearance of P (Phosphorous) (Figure 4.8e). However, there was an increase in the Ca (Calcium) content when immersed in artificial saliva for 7 days (Figure 4.9e) and the Ca (Calcium) remain unchanged even after being challenged to citric acid with the presence of Si (Silicon)(Figure 4.10e).

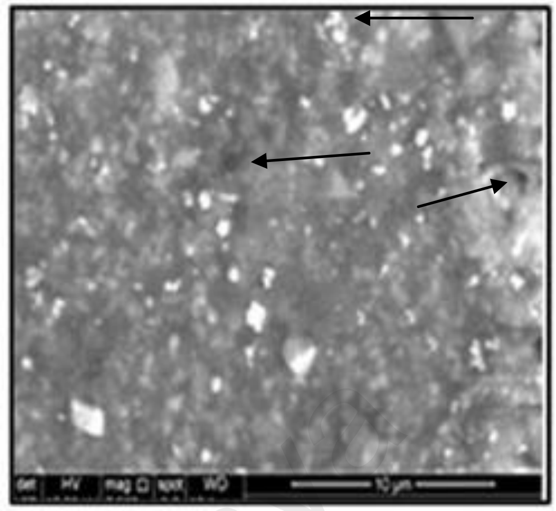


i. Acid etched dentine (3500x) **ii. Acid etched dentine (7500x)**

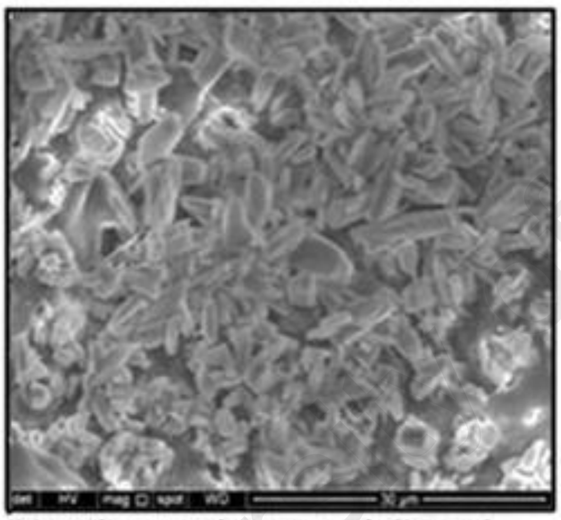
Figure 4.2 SEM Images of Acid etched dentine specimen at 3500x and 7500x.



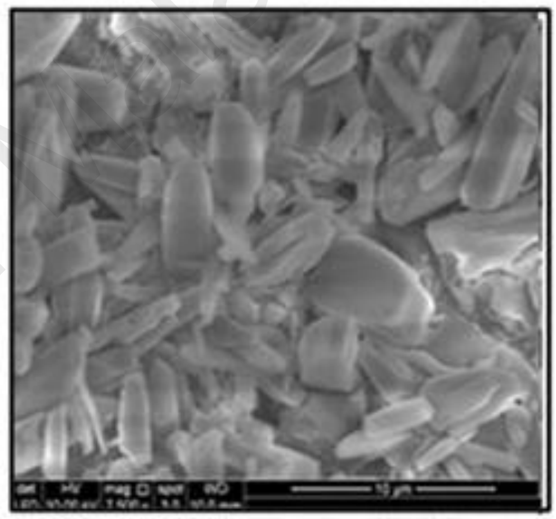
(a) i. GC Tooth Mousse Plus (3500x)



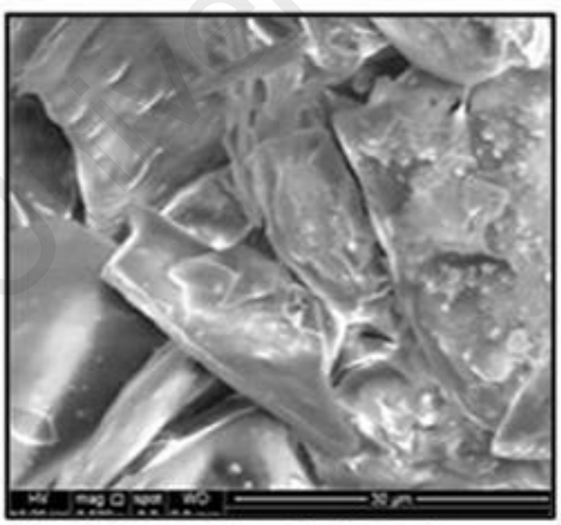
ii. GC Tooth Mousse Plus (7500x)



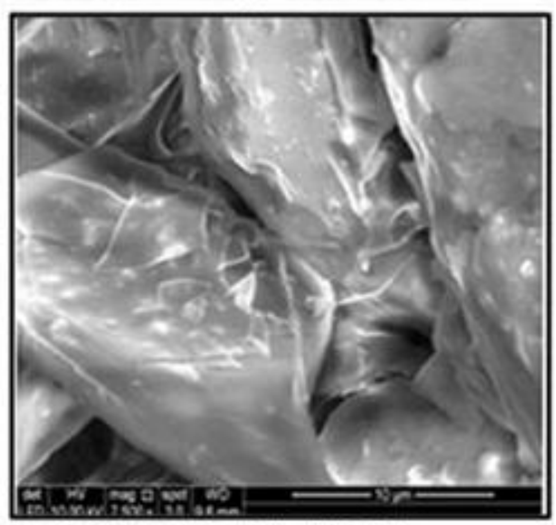
(b) i. Clinpro White Varnish (3500x)



ii. Clinpro White Varnish (7500x)



(c) i. Duraphat Varnish (3500x)



ii. Duraphat Varnish (7500x)

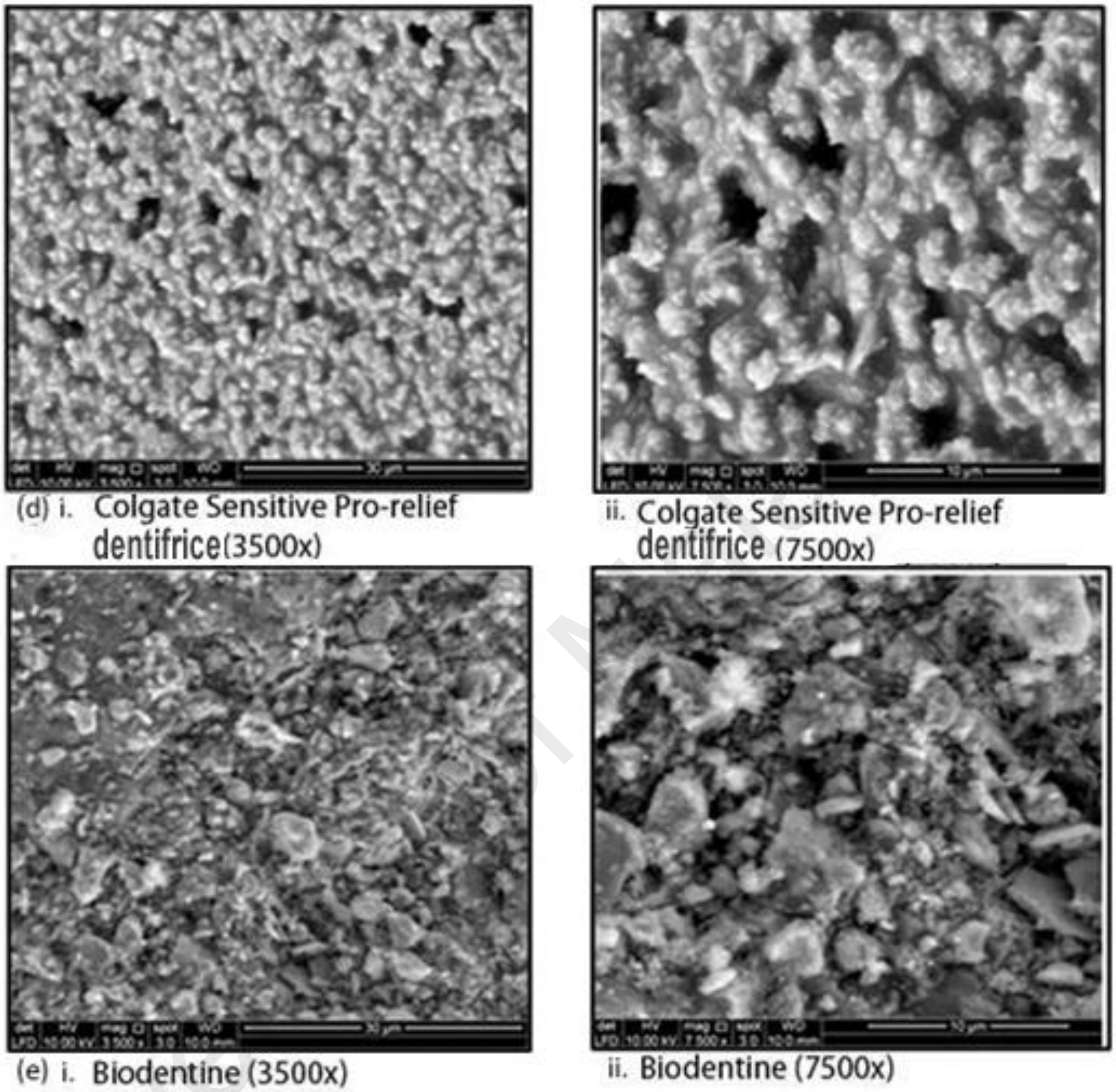
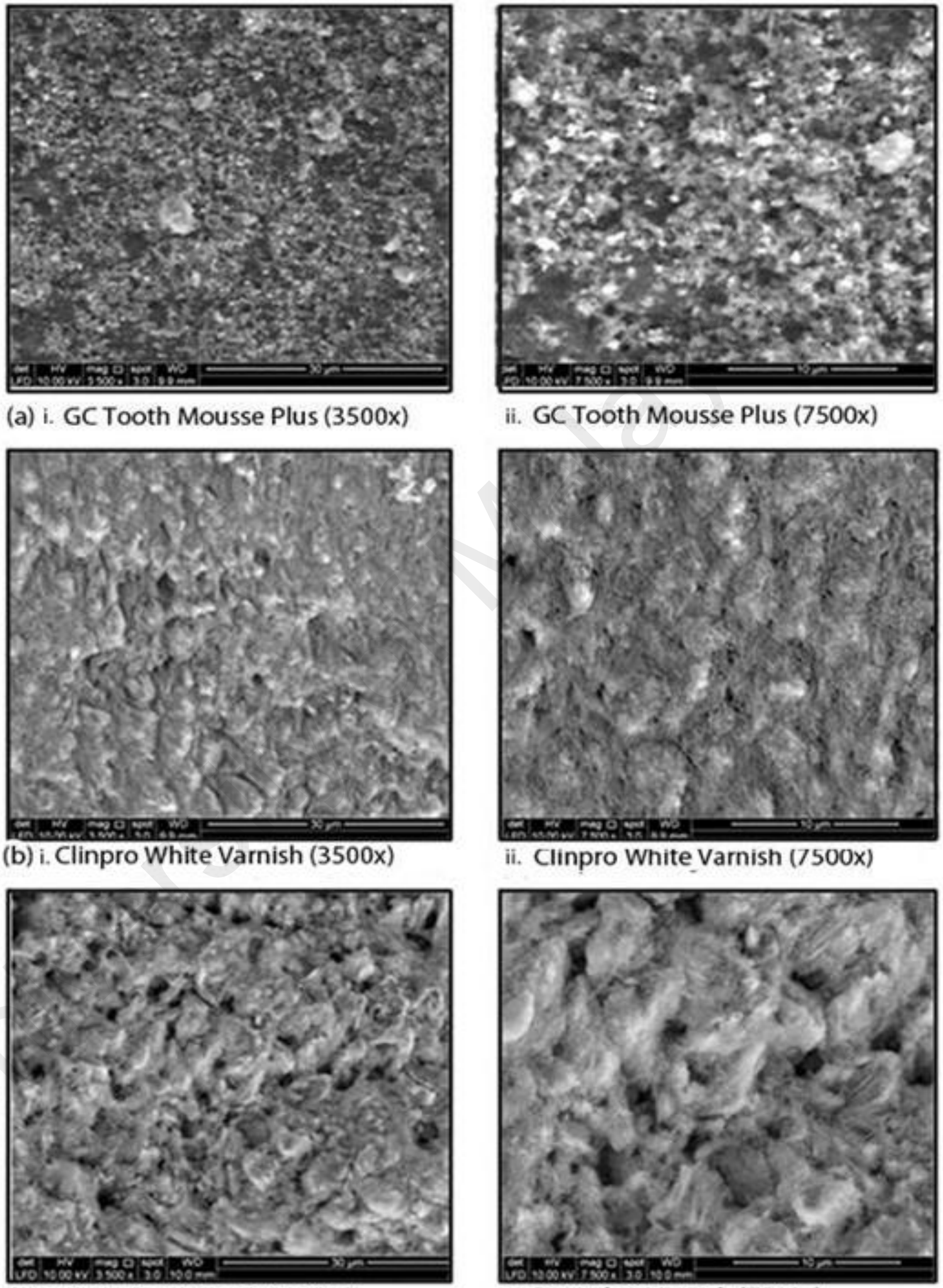


Figure 4.3 SEM images of dentine specimens after 10 minutes of treatment application at 3500x and 7500x.



(a) i. GC Tooth Mousse Plus (3500x) ii. GC Tooth Mousse Plus (7500x)

(b) i. Clinpro White Varnish (3500x) ii. Clinpro White Varnish (7500x)

(c) i. Duraphat Varnish (3500x) ii. Duraphat Varnish (7500x)

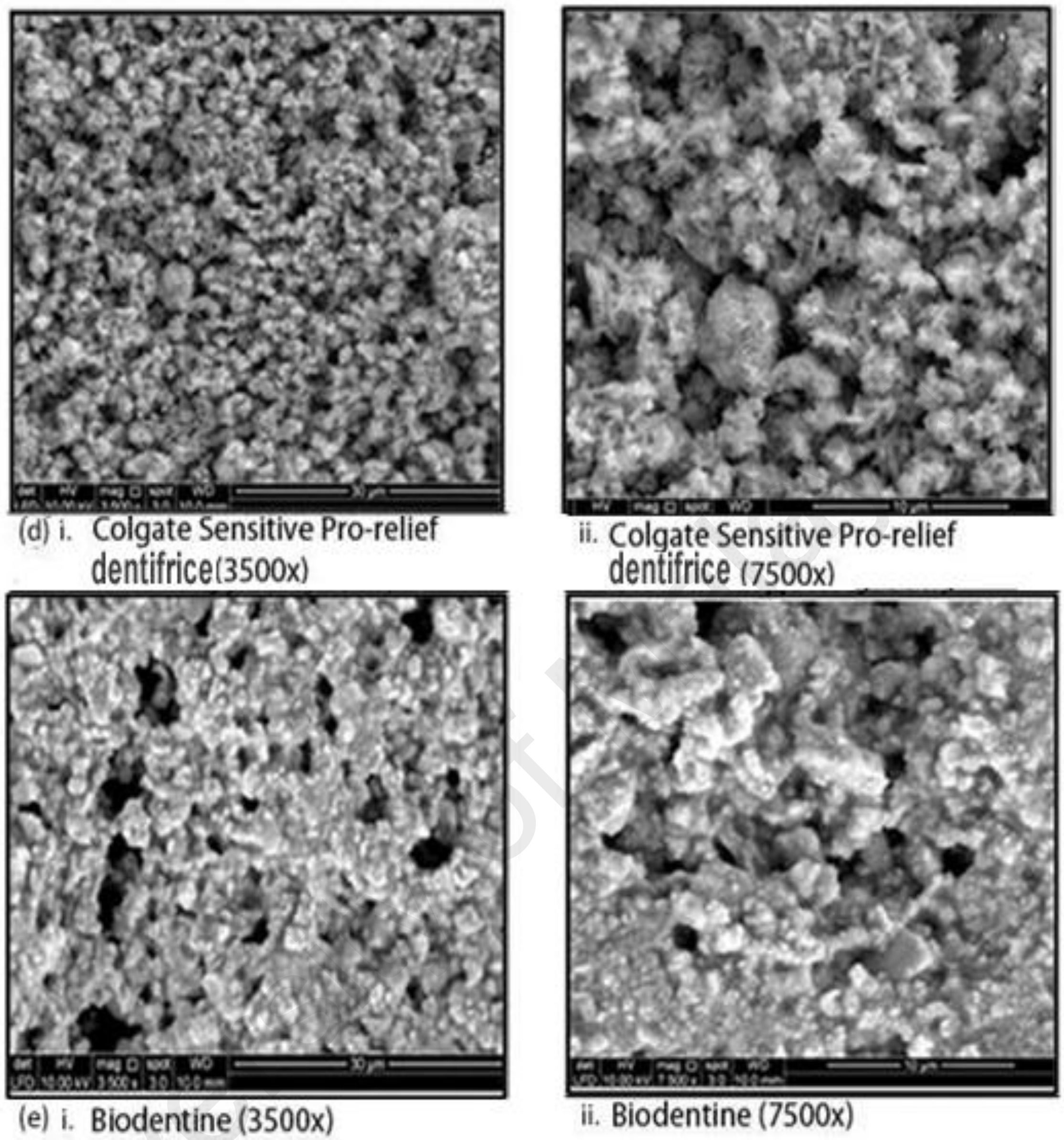
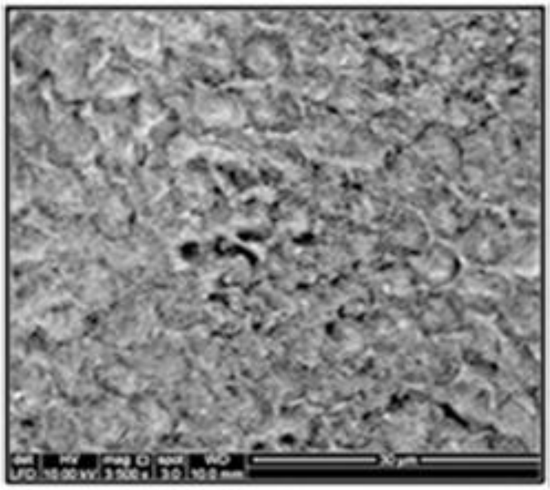
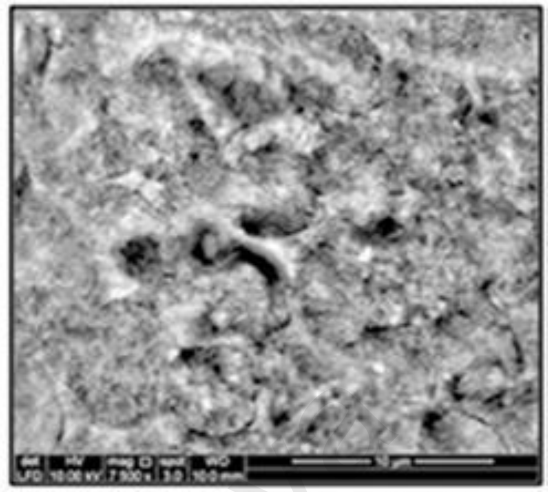


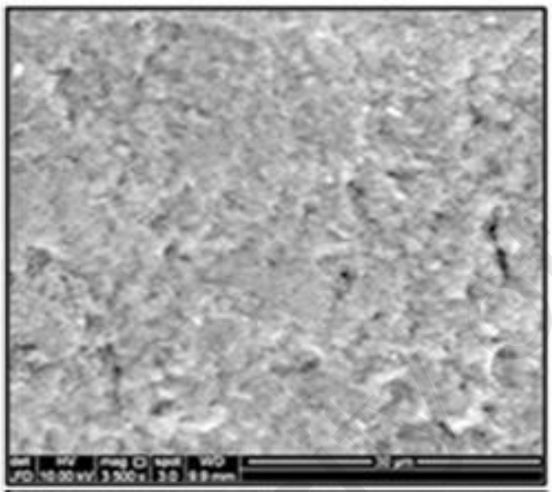
Figure 4.4 SEM images of treated dentine specimens immersed for 10 minutes in artificial saliva immersion at 3500x and 7500x.



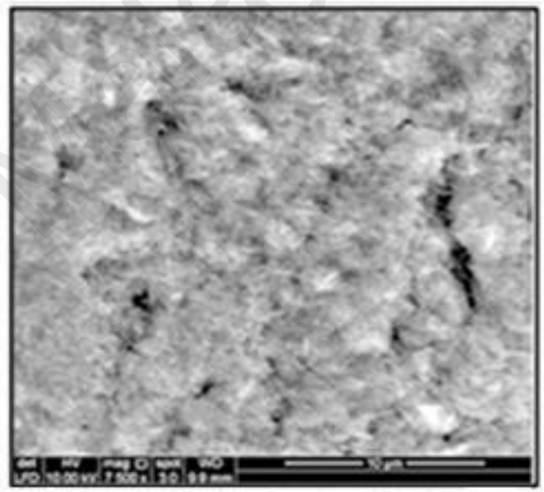
(a) i. GC Tooth Mousse Plus (3500x)



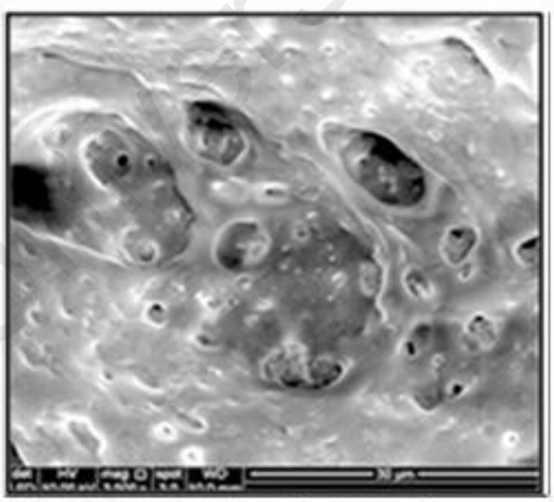
ii. GC Tooth Mousse Plus (7500x)



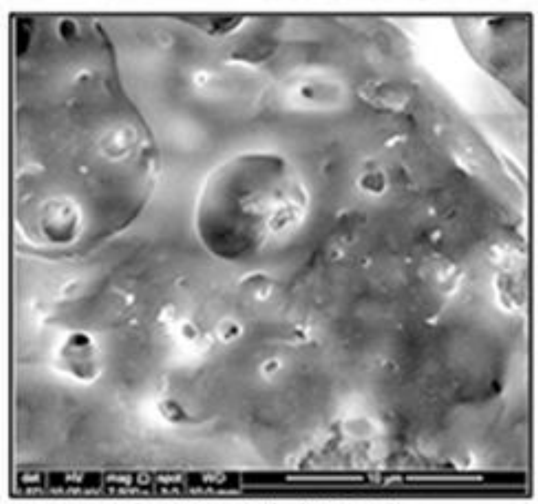
(b) i. Clinpro White Varnish (3500x)



ii. Clinpro White Varnish (7500x)



(c) i. Duraphat Varnish (3500x)



ii. Duraphat Varnish (7500x)

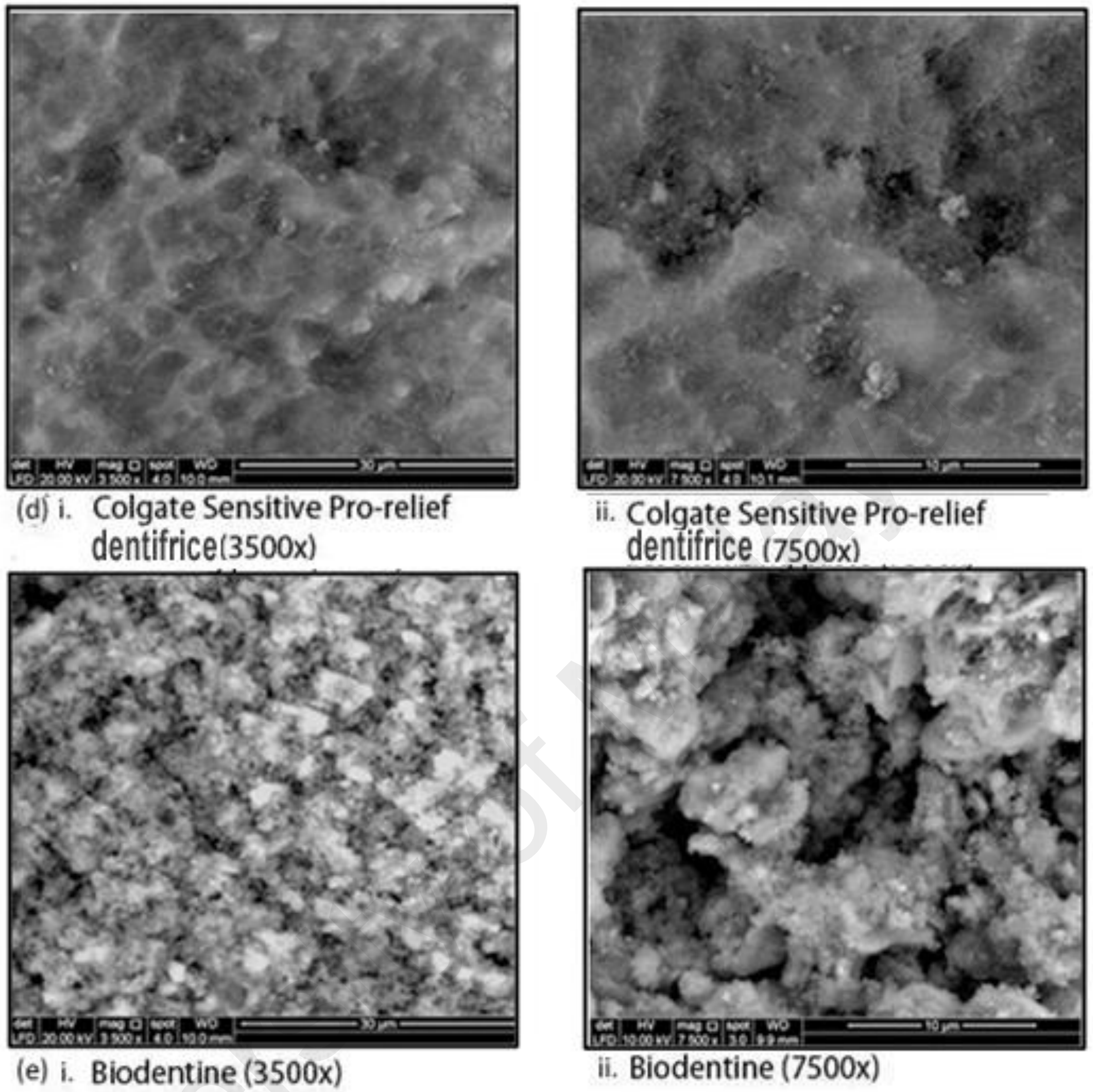
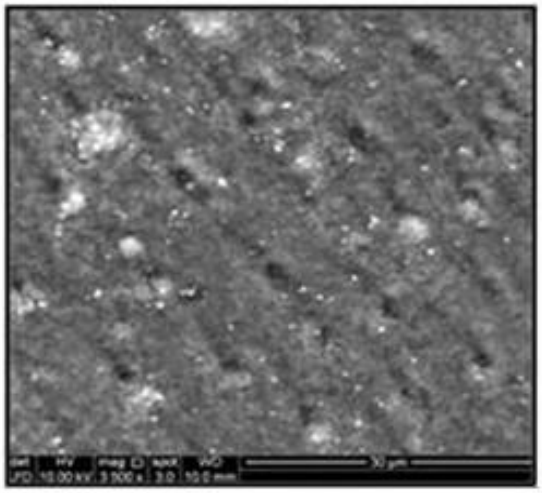
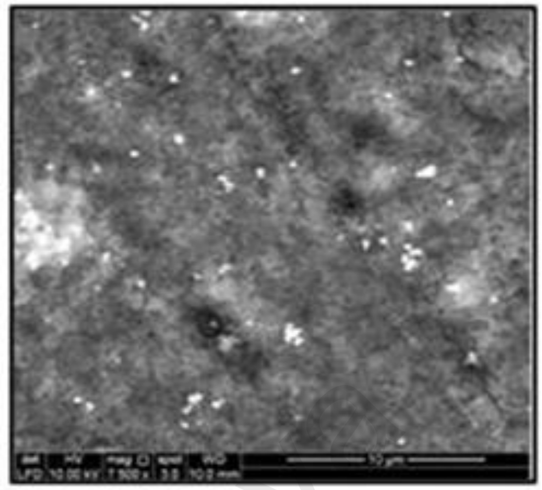


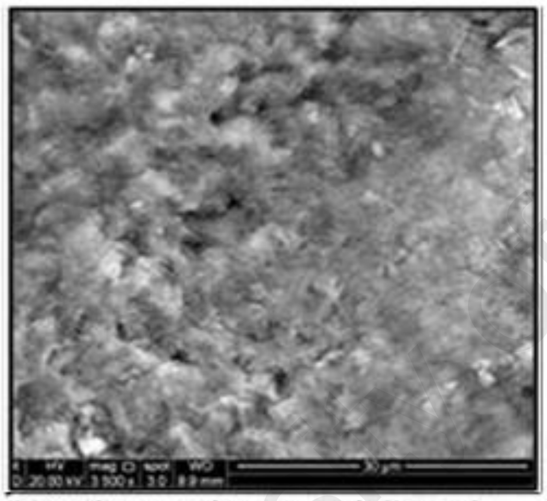
Figure 4.5 SEM images of treated dentine specimens immersed in artificial saliva for 7 days at 3500x and 7500x



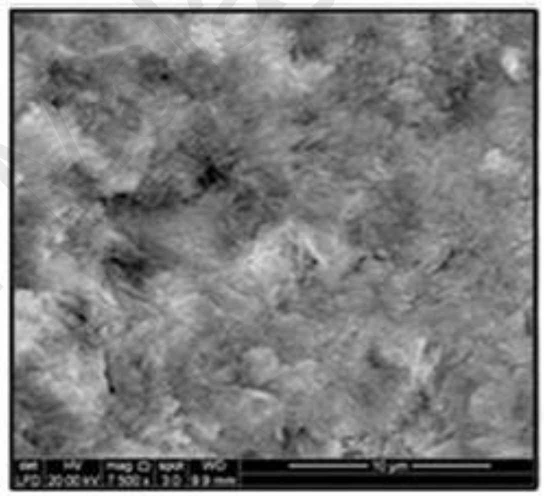
(a) i. GC Tooth Mousse Plus (3500x)



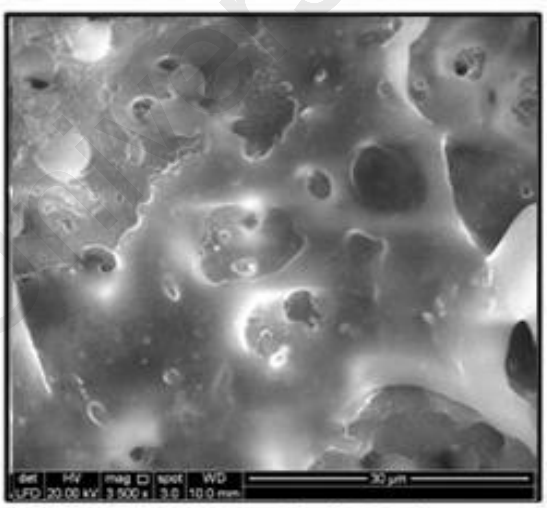
ii. GC Tooth Mousse Plus (7500x)



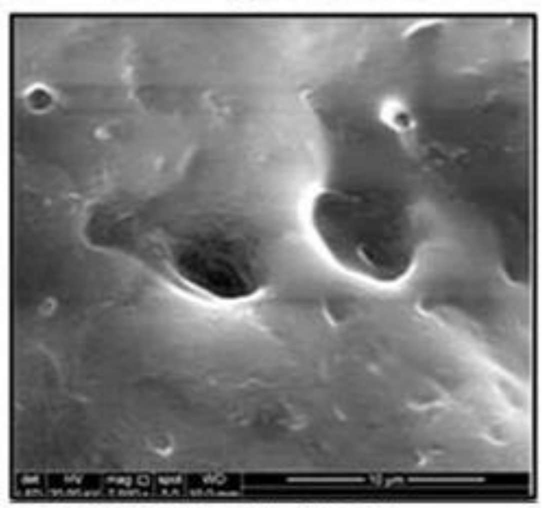
(b) i. Clinpro White Varnish (3500x)



ii. Clinpro White Varnish (7500x)



(c) i. Duraphat Varnish (3500x)



ii. Duraphat Varnish (7500x)

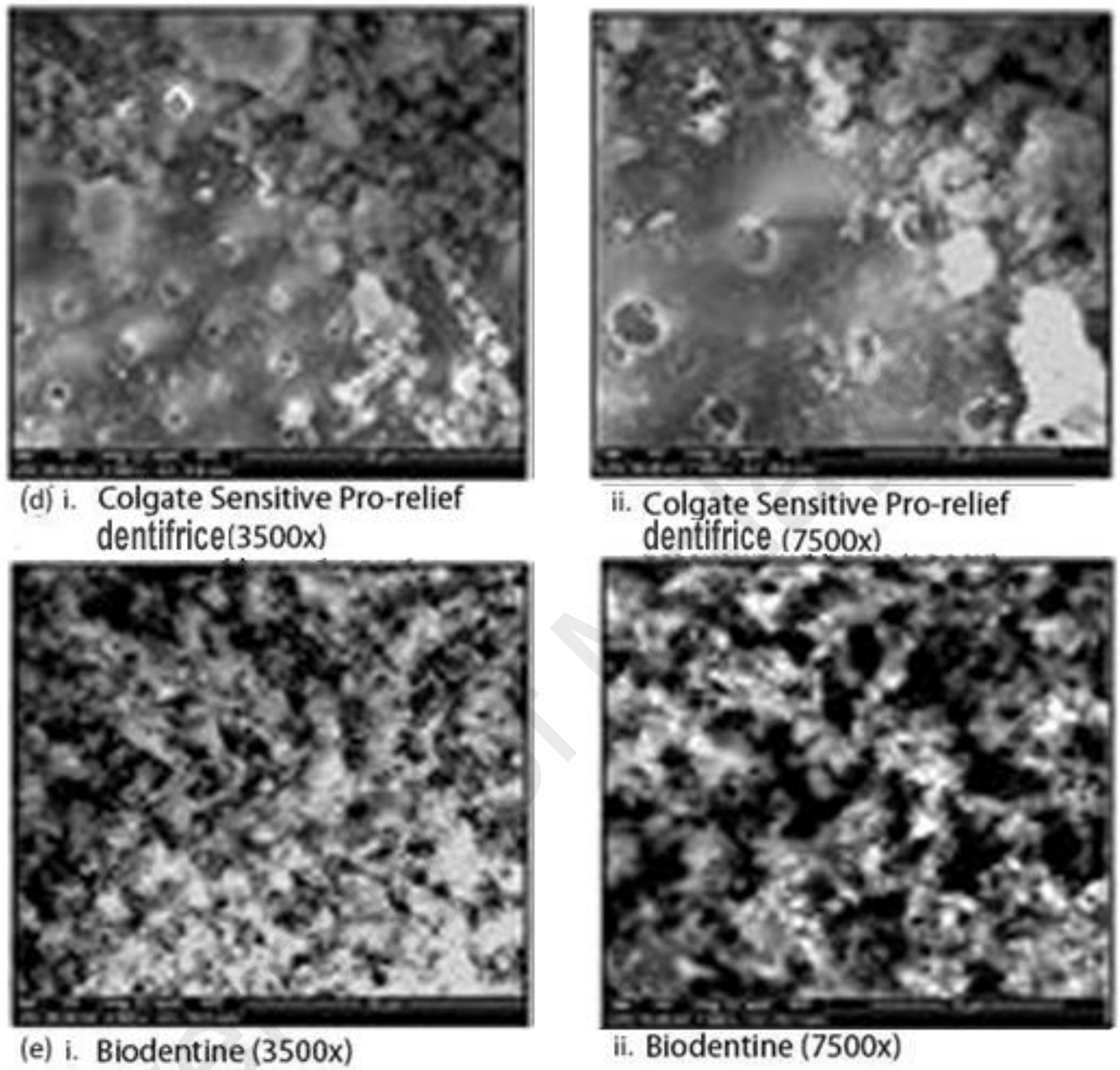


Figure 4.6 SEM images of treated dentine specimens after 3 minutes of citric acid challenge at 3500x and 7500x

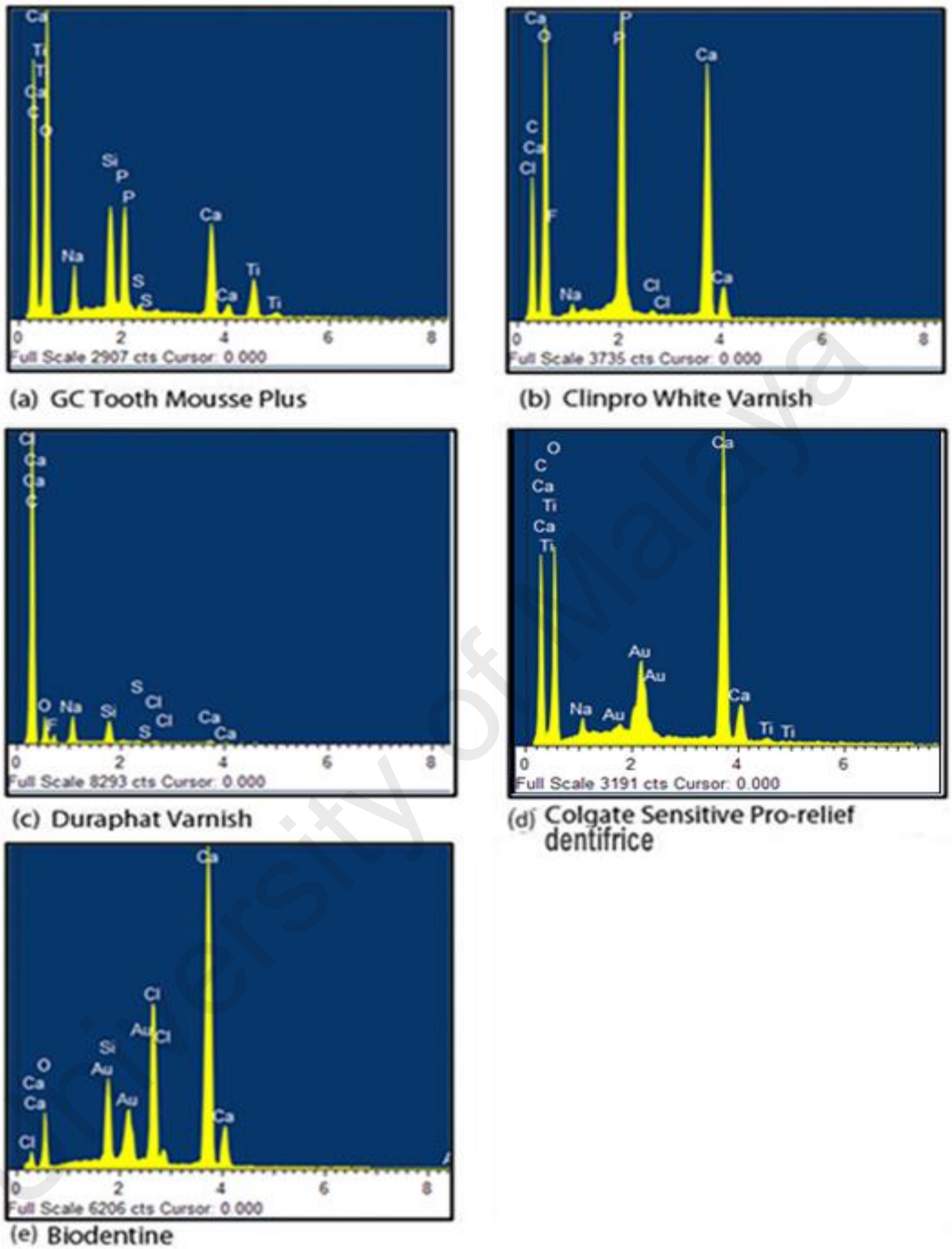


Figure 4.7 EDX spectra of dentine specimen after 10 minutes of treatment application

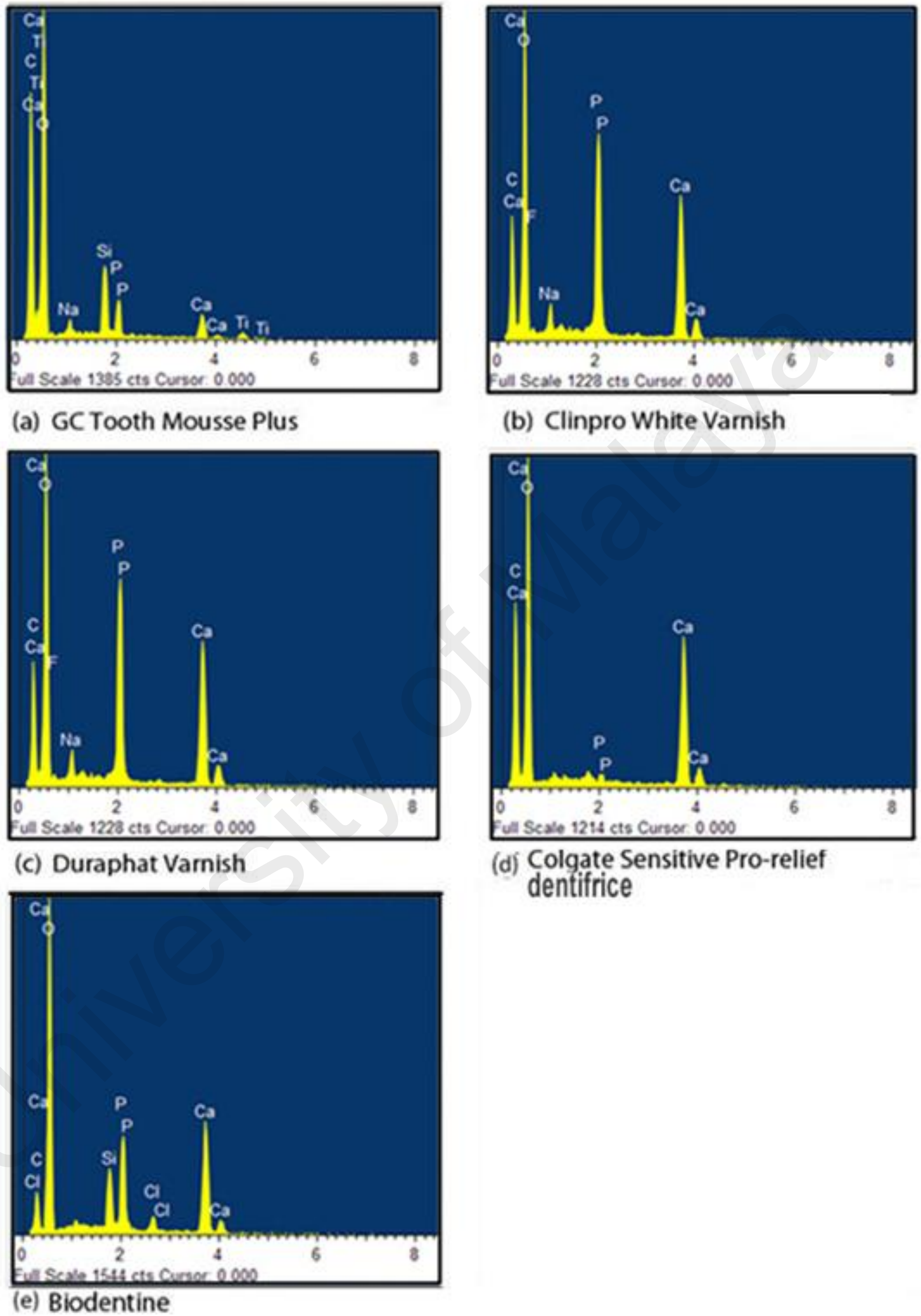


Figure 4.8 EDX spectra of treated dentine specimen after being immersed for 10 minutes in artificial saliva.

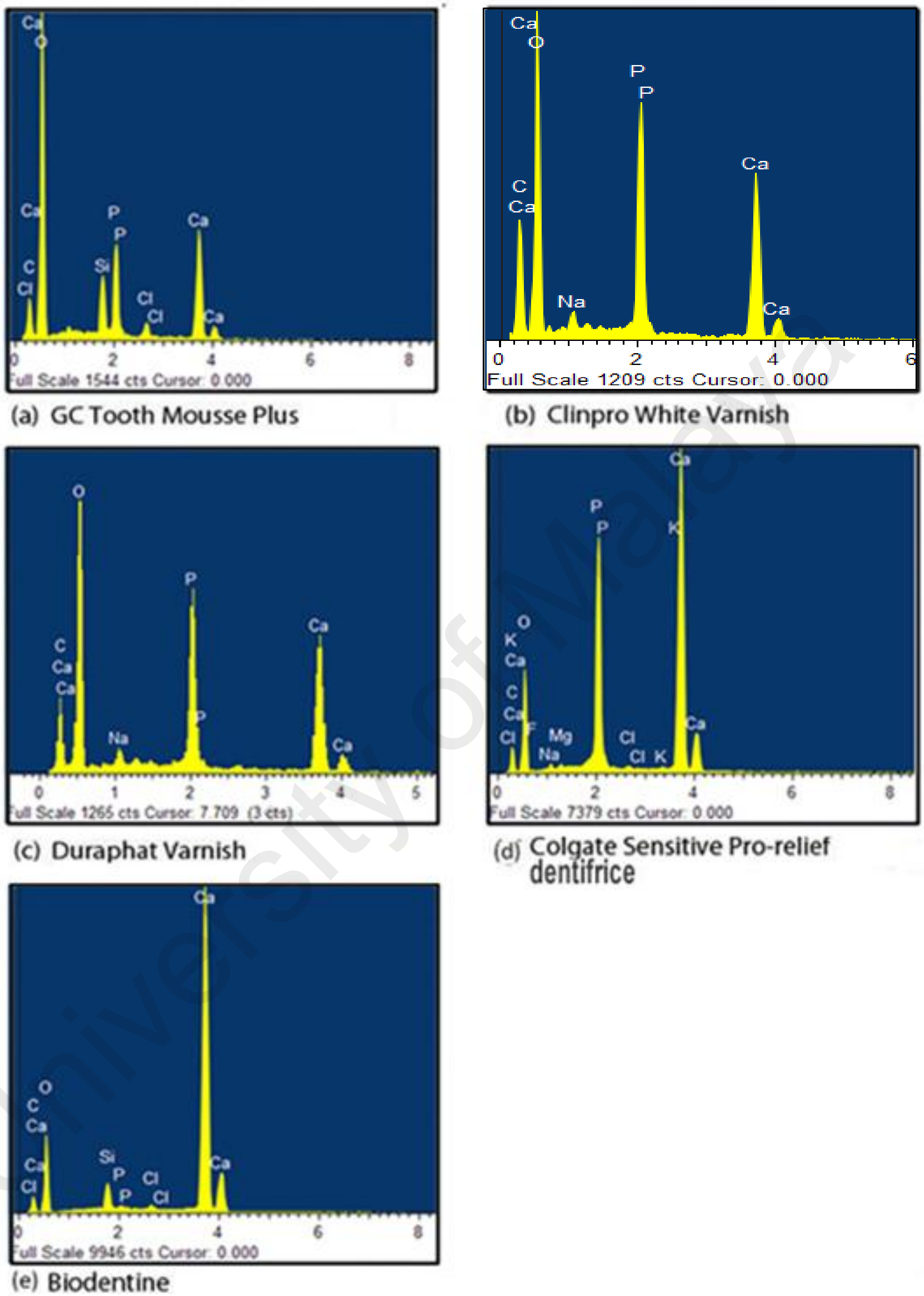


Figure 4.9 EDX spectra of treated dentine specimens immersed in artificial saliva for 7 days.

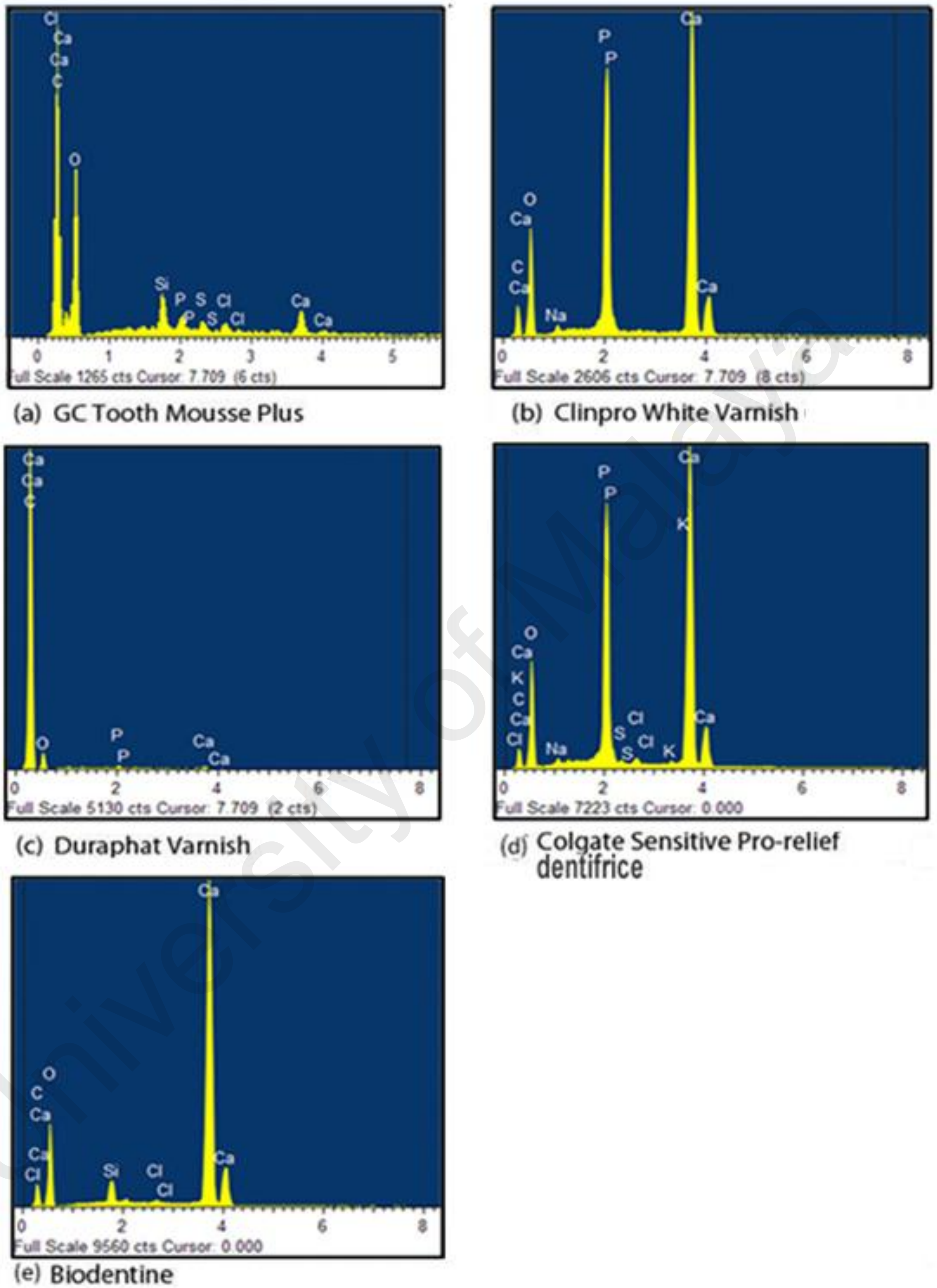


Figure 4.10 EDX spectra of treated dentine specimens after 3 minutes of citric acid challenge.

CHAPTER 5: DISCUSSION

The hydrodynamic theory is a universally accepted theory that best explains the mechanism of dentine hypersensitivity and hence, any material that results in a decrease of dentine permeability by occluding tubules would eventually be capable to reduce the symptoms of dentine hypersensitivity clinically (Markowitz & Pashley, 2008; Wang et al., 2010). The assessment of apatite and/or crystal mineral precipitate deposition in stages when immersed in virtual body fluids is a generally accepted means to ascertain the bioactivity of a component to remineralization (Bohner & Lemaitre, 2009). A key factor essential to decrease hypersensitivity is the hydraulic conductance method which provides the quantitative data illustrating the potential of occluding precipitate to impede the outward fluid flow through dentine tubules.

Therefore, the present study was designed to investigate the bio-active characteristics of potential mineralizing agents to decrease dentine permeability over 10 minutes & 7 days of artificial saliva immersion with following citric acid immersion for 3 minutes through tubule occlusion. This fluid filtration device has been used for the in vitro assessment of mineralizing agents by dentine permeability in various studies (Gandolfi, Silvia, et al., 2008; Prati, 1994; Prati et al., 2003; Prati et al., 2002; Sauro et al., 2006; Suge et al., 2002) which was developed by Pashley and his colleagues (Merchant et al., 1977).

The interstitial fluid pressure of the normal pulp is approximately 15-20 cm H_2O (1.47 – 1.96 kPa). Several researchers have proposed varous fliteration pressures and most of them have used a higher pressure as the higher the pressure is, the higher would be the displacement of an air bubble with in the tube. Although, pressures higher than the physiological pressures have been used in different research studies (Pashley et al., 1988;

Pashley & Galloway, 1985; Richardson et al., 1990; Youngson et al., 1999). As reported by Jean Camps, the use of high pressure is not recommended as it underestimates the Lp by causing disturbance to the intra tubular contents. The applied pressure is a variable which is not clearly standardized and have been reported with no justification and comparison to the physiological pulpal pressure. Various pressures such as 20cm H_2O, 70 cm H_2O and other higher pressures have been used till now but a pressure of 100cmH_2O/ 1.4psi(9.65 kPa) was used since after the application of any mineralizing agents, permeability is decreased and a low pressure used simulating the physiological interstitial pressure could not trace the permeability values greater than zero. A study for repeatability of dentine permeability measurement done by Elgalaid et al. showed that higher mean permeability value was achieved using 100cm H_2O pressure compared to 14 cmH_2O pressure. Therefore, the author suggested using 100cm H_2O pressure for this fluid filtration device measuring the dentine permeability (Elgalaid et al., 2008). Moreover, the efficacy of the study could be improvised as the time for evaluating each specimen would be reduced using a high pressure and would allow the researcher to increase the number of samples thus improving the power of study (Elgalaid et al., 2008). However, it has been reported in a study that with lower pressures, the intratubular contents is not altered and moreover, the chances of the hydraulic conductance being calucated is closer to to the reality unlike much higher pressures which would overestimate the dentine permeability (Camps et al., 1997).

Various types of fluid for the fluid filtration device such as water and saline of known viscosity were used (Goodis et al., 1993; Hansen et al., 1992). Additionally, Ringer's solution ,horse serum , saturated thymol solution (0.12%) , Bovine serum (Charoenlarp et al., 2007; Elgalaid et al., 2007; Krejci et al., 1993; Özok et al., 2002) were also used.

SEM/EDX analysis was used to assess and analyze surface morphological features sequential to product application, following immersion in artificial saliva (at 10 minutes &7 days) and after citric acid challenge to identify its potential to form stable acid-resistant crystal precipitates (Gandolfi, Silvia, et al., 2008; Prati et al., 2003; Prati et al., 2002).

The age and gender of people from whom molars were obtained was unknown and so there could have been difference in the quality of dentine from one tooth to another. Nevertheless, compensation was made by making use of the relative fluid filtration percentage so that each tooth acted as its own control (Drake et al., 1994). There was a difficulty in obtaining freshly extracted molars and therefore, teeth had to be stored in 1% T Chloramine solution for few months prior to use,

The dentine disc was used as it is a functional model for the in vitro evaluation of the applied mineralizing agents, is reproducible and can be readily used with a wide range of experimental agents. It provides a smooth flat surface for elemental analysis and thus could be consistent with fluid flow research (Mordan et al., 1997).

Minimum permeability was obtained by creating a smear layer using 600 SiC grit paper. The result of the study showed that the mean reduction in the permeability by smear layer was up to 70%. It is in agreement with the data reported by Pashley et al. as in which the permeability was decreased around 72 -80 % due to cavity preparation and root surface instrumentations (Pashley et al., 1978). Moreover, when dentine is cut with any hand or rotary instrumentation, it is likely to get covered with amorphous coats of smear layer on dentine surface containing mineral particulates and denatured collagen and hence, reducing DH (Kazemi et al., 1999; Pashley, 1984). It was reported that the smear layer removal with acid results in a significant increase in Lp from 5 to 40 times (Pashley, Kehl, et al., 1981; Reeder et al., 1978)

Achieving maximum dentine permeability by acid etching for 60 seconds was performed as a standardized procedure to remove the smear layer in order to ensure that the dentine tubules of all dentine discs were patent at all aspects there by, allowing the maximum permeability values to be measured and simulating the real situation of tubules during dentine hypersensitivity (Kim et al., 2013). The results of the study revealed that with the treatment of acid etchant (37% ortho phosphoric acid), dentine tubules were entirely open. Each acid etched dentine represented its own control of that specimen.

Furthermore, citric acid is a common constituent of citrus fruits, fruit juices and acidic carbonated beverages. Therefore, it was used as a final treatment to analyze the efficacy of these mineralizing agents to resist acidic exposure as it is quite a common practice of the population to consume these acidic substances which affects teeth and its restorative materials. Many studies had investigated the effects of restorative material in resisting the acid challenge (Gandolfi, 2008; Wiegand et al., 2007).

Dentine specimens when treated with different mineralizing agents demonstrated a decrease in permeability (LpT3%) which indicates that these materials used may perhaps aid in the occluding effect by the abrasives of the applied agents and a thick smear layer formation which might be due to increased abrasive action. It is essential to consider the significant decrease in the permeability LpT3% to nearly 70% after 10 minutes application of mineralizing agents. This would perhaps be due to its partice size. However, there was a slight increase in the permeability LpT4 % after the artificial saliva immersion at 10 minutes and a considerable increase in LpT5% after 7 days and therefore, the results of our study depicts the fact that the mineral precipitates formed could not withstand the 100cm H_2O pressure in the permeaibility test and most of them were flushed away and out of the dentine tubules. However, Clinpro white varnish and Biodentine showed a decrease in permeability at LpT5% comparatively. Following the citric acid challenge, there was a substantial increase in the permeability at LpT6% except for Clinpro white varnish.

GC Tooth mousse plus is a water based agent that contains a peptide nanocomplex of milk protein casein phosphopeptide (CPP) and an amorpous calcium phosphate (ACP) with fluoride has been reported to prevent teeth sensitivity by increasing the dentine tubule obstruction with remineralized dentin and maintaining high concentration gradient of calcium phosphate on to the tooth surface (Oshiro et al., 2007; Rahiotis & Vougiouklakis, 2007; Reynolds, 1997). The efficacy of GC Tooth Mousse was evaluated earlier by Hongye Yang et al. but that formulation did not contain Fluoride (Yang et al., 2014), However, CPP-ACPF was used in this study with added fluoride in the formulation and perhaps could be the reason for the formation of granular precipitates and enhanced mineralization. This is supported by the EDX analysis as the Ca (Calcium) and P (Phosphorus) content were found to be high on treatment application which remained consistent even after 7 days of artificial saliva immersion. This perhaps could be also by the precipitation of these ions form CPP-ACPF. However, other technique such as XRD is needed to determine whether remineralization has occurred. The crystal precipitates that formed over the dentine surface possibly could be created from the saliva and thereby, reduced dentine permeability (Suge et al., 2008). Perhaps, the hydroxyapatite binds with the peptide compound thus being a confined source of biologically available calcium and phosphate for dentine tubule occlusion and dentine/enamel remineralization (Geiger et al., 2002). Findings reported by Gandolfi et al on permeability reduction after treatment application was 66 ± 11 (%) which is double the value presented in this study that is 32.8 ± 15.8 (%) (Gandolfi, 2008). The permeability reduction was more in this study since the fluoride may have an advanced effect and the time of application was 10 minutes. However, the results suggest that the precipitates formed on dentine surface were not as effective and it could not resist the citric acid challenge. Hence, its effect may require several applications over a long duration in order to be clinically useful in decreasing dentine hypersensitivity. The EDX analysis on treatment with GC Tooth mousse plus also exhibited the presence of Si and Ti owing to its presence as silicon dioxide and titanium dioxide in the composition.

Clinpro white varnish is a functionalized tri-calcium phosphate technology which is formulated with sodium fluoride (fTCP) and is known to remineralize enamel sub surface lesions. The results of this study depicts a significant reduction in permeability and corresponds well with SEM evaluation of dentinal tubule orifice obstruction in dentin discs treated with this agent where the precipitates formed were found to be resistant to both artificial saliva immersion and citric acid challenge. Moreover, the Calcium and Phosphorus peaks were high throughout the treatment stages which is perhaps the reason the precipitates remained acid insoluble.

It has been reported in a study that the more the negative solubility constant (Ksp) is, the lower the solubility of the ion. The solubility constant of Tricalcium phosphate is reported to be 2.7×10^{-33} at 25℃ (Zhou et al., 2016) which clearly denotes the reason it was capable to withstand both the artificial saliva immersion and citric acid challenge according to our results . Moreover, SEM analysis also revealed the tubular plugs masked the entire dentine surface and few patent dentine tubule orifices were observed despite the challenges of artificial saliva immersion and citric acid challenge which remained consistent even though the morphological modification was observed. Overall, this is an effective agent in reducing dentin permeability via a tubule occlusion mechanism. The ability of the agent to reduce dentin permeability makes it to be potentially useful as a clinical dentin mineralizing agent which has to be documented in further future clinical studies. It seems to be a promising agent in the management of DH. Furthermore, the Solubility constant for ACP is 1×10^{-25}.

Calcium Carbonate is 4.68×10^{-9} and CaF_2 is 1.4×10^{-10} which explains the reason these precipitates were not resistant to artificial saliva immersion and citric acid challenge

Duraphat which contains sodium fluoride has been previously used in a number of studies and is well known therapeutic agent of dentine hypersensitivity. It is one of the most common desensitzer used for DH and is known to reduce DH by occlusion of tubules via crystallization and decreasing dentinal fluid flow to the pulp (Gaffar, 1998). Therefore, Duraphat varnish was included in our study as a positive control that contains sodium fluoride 5% (22,600 ppm). In this study, the results demonstrated that immediately after treatment application, permeability was reduced significantly but there was an increase in permeability after artificial saliva immersion which suggests perhaps that the artificial saliva medium was aggressive causing the precipitates to be removed as evident by SEM analysis or dissolved as indicated by decreased dentine Lp throughout the treatment stages. A study by Hasen in 1992 showed that the application of Sodium Fluoride varnish had relieved more than fifty percent of teeth with dentine hypersensitivity (Hansen, 1992). . However, an in vitro study demonstrated that electrical tooth brushing wiped out the sodium fluoride varnish and resulted in short term effect on dentinal hypersensitivity by reducing the diameter of the exposed dentinal tubule orifices (Lan et al., 1999). The results show that the application of fluoride may possibly result in the formation of a crystalline barrier by a CaF_2 precipitants within the dentine tubules which slowly dissolves in the salivary medium and possibly explains the reason of the patent dentin tubule orifices and a transition from is crystalline coating covering the dentine surface to plugged out tubules observed in SEM analysis. Moreover, results also show the preventive effect of Duraphat against acid challenge but there was not that significant that is 60.7 ± 14.7 and almost similar findings were reported by Pinto et al. that is 65.00 ± 20.

Colgate sensitive Pro-relief dentifrice contains Arginine and calcium carbonate that functions in conjunction to speed up the natural obliteration mechanism of dentine tubules by dentine like deposits containing calcium phosphate (Petrou et al., 2009). The in vitro results of this study depicts that this treatment reduced dentine permeability and induced effective occlusion performance by decreasing dentine permeability up to 31.8 ± 13.1 which is in agreement with the a study finding that is 31 ± 16 (Pinto et al., 2014). High calcium peaks were observed at treatment application which possibly would result in the formation of calcium containing coating covering the entire dentinal surface and occluding dentine tubules and almost similar findings were reported by Yang et al. (Yang et al., 2014). Following the immersion in artificial saliva in 10 minutes, calcium peak decreased which might be dissolved but however, after 7 days of immersion in AS, the Ca peak appeared to be high with the emergence of Phosphorus and Potassium which was probably because these ions might have reacted with the salivary minerals and the emergence of potassium is a positive outcome as its increased concentration in the form of precipitates not only plugs the dentine tubule but also could result in desensitization of the nerve endings thus, relieving sensitivity. Moreover, the route of desensitization by Arg-$CaCO_3$ aggregate is closely related to the natural desensitization offered by saliva and hence, is commonly investigated (Cummins, 2010). Surprisingly, the Calcium, Phosphorus and Potassium remained consistent even after being exposed to citric acid which would be possibly by the alkaline environment created by the Arg-$CaCO_3$ agglomerate where the calcium and phosphate ions can form precipitate and block the dentine tubules further.

Biodentine is a calcium based silicate cement and has been proposed by a study that it could occlude dentinal tubules with stable and acid resistant calcium phosphate deposits (Gandolfi, Silvia, et al., 2008). These are the recent cotemporary biomaterials that have been proposed for dentine hypersensitivity (Taddei et al., 2009). The most significant outcome of this study was the use of this agent which created a low permeability amongst the groups by plugging tubules. SEM analysis exhibited the formation of homogenous surface that occluded dentine tubules. Follwowing the 10 minutes immersion in artificial saliva, the morphology changed from its distinct microscopic aggregates to a much more void free particulates which was much smoother and unifrom masking all dentine tubules. Moreover, citric acid challenge did not remove the tubular plugs which perhaps could be due to the powder size of CSC powder (0.5µm) which led to the penetration into the dentinal tubule orifices or due to the low solubility of CSC in acidic environment. The results found in this study are consistent with those found by Gandolfi et al. (Gandolfi, Silvia, et al., 2008). It has been reported by several investigators that the hydration of CSC cement allows the formation of apatite crystals in the presence of biological fluids containing phosphates (Sarkar et al., 2005; Tay et al., 2007). The presence of high contents of Calcium after Biodentine application on SEM analysis revealed the presence of a surface coating most likely produced by its hydration. This coating formed by the Biodentine had the ability to resist both salivary immersion and acid challenge and similar findings were reported by Gandolfi et al. (Gandolfi, Silvia, et al., 2008). Further clinical studies are in progress to determine its clinical efficacy.

In the present study both Biodentine and Clinpro Varnish were able to occlude dentine tubules even after 7 day immersion of artificial saliva and final immersion in citric acid for 3 minutes. However, a long term acid resistance of the biomaterial should be further investigated in future studies.

One of the key characteristics of the mineralizing agents is to have a long term de-sensitizing effect which could withstand various challenges. On the whole, all the agents were able to occlude exposed dentine tubules in the presence of a simulated oral environment. It was demonstrated by the results of our study that further immersion in artificial saliva resulted in the formation of precipitates which is one of the chief mechanism of dentine hypersensitivity relief. Hence, saliva perhaps was responsible for both the flushing away and allocation of crystalline precipitates over the dentinal surface. This experimental study was designed in a way that each specimen was subjected to different conditions thus simulating the oral environment.

CHAPTER 6: CONCLUSION

- The decrease in dentine permeability reduction immediately after treatment application and crystal precipitate formation over the dentinal surface together with the resistance to acid challenge and artificial saliva immersion indicated that these five mineralizing agents included in our study may be useful products for dentine hypersensitivity treatment.
- Clinpro white varnish compared to other mineralizing agents represents an excellent approach to occlude dentine tubules and decrease dentine permeability even after the artificial saliva immersion and exhibited strong acid resistant capacity.
- Biodentine was found to be a promising agent to occlude dentinal tubules..

6.1 Recommendations and Suggestion for future work

- The measuring time, applied pressure and the air bubble size should be standardized so that the comparison would be better between different experimentations.
- It is obvious that in vitro experimentation is the ideal method of a biological system investigation and that it does highlight limited aspects of any natural mechanism. However, further in vivo studies and clinical trials are recommended for future studies.
- Long term acid resistance should be tested for the materials included in our study.
- Sample size could be increased and should be exposed to artificial saliva medium for long duration.
- Modification of the fluid filtration device such as the digital reading or flow dec device could be used

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