COMPARATIVE EVALUATION OF PRO-ARGIN BASED DENTIFRICE AND CARBON DIOXIDE LASER IN THE TREATMENT OF DENTIN HYPERSENSITIVITY-AN IN VITRO STUDY

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BRANCH – II
PERIODONTICS

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
Chennai – 600 032

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CERTIFICATE

This is to certify that **Dr. PUSHPINDER SANDHU**, Post Graduate student (2010–2013) in the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 has done this dissertation titled **"COMPARATIVE EVALUATION OF PRO-ARGIN BASED DENTIFRICE AND CARBON DIOXIDE LASER IN THE TREATMENT OF DENTIN HYPERSENSITIVITY- AN IN VITRO STUDY"** under the direct guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University**, Chennai – 600 032 for **M.D.S.,** (Branch – II) **Periodontics** degree examination.

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ABSTRACT

Background: Dentin hypersensitivity is a common painful clinical condition affecting 72.5-98% of periodontal patients. It has been observed in approximately half of the patients following scaling and root planing. The current opinion based on Brannstrom hydrodynamic theory suggests dentin tubule occlusion as a logical treatment for dentin hypersensitivity.

Aim: To compare the dentinal tubule occluding ability of Pro-Argin based dentifrice and Carbon Dioxide Laser in the treatment of dentinal hypersensitivity.

Materials and Methods: Thirty single rooted human teeth extracted due to advanced periodontal disease were used in this study. The teeth were scaled and root planed. Thirty dentin discs were prepared and randomly divided into three groups. In group I, the specimens were stored in Distilled Water (DW); in group II, the specimens were brushed with Pro-Argin based dentifrice (PA); in group III, the specimens were irradiated with Carbon Dioxide Laser (CDL). All the specimens were fixed and observed under scanning electron microscope.

Results: The Carbon Dioxide Laser group showed the maximum number of completely occluded tubules followed by Pro-Argin and Distilled Water group. Pro-Argin group showed more number of partially occluded tubules than the other two groups. The reduction in tubule diameter was also highest in carbon dioxide laser group.

Conclusion: The Carbon dioxide laser proved to be most efficient in the occlusion of patent dentinal tubules. The partial tubule occlusion achieved by Pro-Argin is also valuable as decrease in the tubule diameter causes decrease in the pain due to dentinal hypersensitivity.

***Key words*:** Dentin hypersensitivity, Pro-Argin, Carbon Dioxide Laser, Tubule diameter, Desensitizing agents.

DECLARATION

TITLE OF DISSERTATION	"Comparative evaluation of Pro-Argin based dentifrice and Carbon Dioxide Laser in the treatment of Dentin Hypersensitivity- An In Vitro Study."
PLACE OF STUDY	Tamil Nadu Government Dental College & Hospital, Chennai-600003
DURATION OF THE COURSE	3 Years
NAME OF THE GUIDE	Dr.K.Malathi
HEAD OF THE DEPARTMENT	Dr.K.Malathi

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This agreement herein after the "Agreement" is entered into on this day ……………. between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai- 600 003 , (hereinafter referred to as, 'the college')

And

Mrs. Dr. K. MALATHI, aged 46 years working as **Professor & H.O.D.** in Department of Periodontics at the college, having residence address at 10/221, Subamathi Nivas, Plot No 96, 4th Cross street, Vimala Nagar, Medavakkam, Chennai - 100 (herein after referred to as the 'Principal Investigator')

And

Miss Dr. PUSHPINDER SANDHU, aged 28 years currently studying as final year **Post graduate student** in the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai -3 (herein after referred to as the 'PG and co- investigator') residing at HNO.6 Adarsh Colony, near Gurdev Hospital, Ferozepur road, Ludhiana, Punjab.

Whereas the 'PG student as part of her curriculum undertakes to research on **"COMPARATIVE EVALUATION OF PRO-ARGIN BASED DENTIFRICE AND CARBON DIOXIDE LASER IN THE TREATMENT OF DENTIN HYPERSENSTIVITY- AN IN VITRO STUDY"** for which purpose the Principal investigator shall act as Principal investigator and the College shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator

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Witnesses **Student Guide**

1.

2.

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LIST OF ABBREVIATIONS USED

ArF	Argon Fluoride
Cm	Centimeter
Ca(OH)2	Calcium hydroxide
CDL	Carbon Dioxide Laser
CPP-ACP	Casein phosphopeptide-Amorphous calcium phosphate
CDS	Cervical Dentine sensitivty
CEJ	Cementoenamel junction
COT	Completely occluded tubule
DW	Distilled water
DNO	Diameter of Not Occluded
DPO	Diameter of Partially Occluded
e.g.	For example
et al	And others
Exp.	Experimental
EDTA	Ethylene Diamine Tetra Acetic acid
Er:YAG	Erbium:Yttrium Aliminium Garnet
ErCr:YSGG	Erbium,Chromium:Yttrium Scandium Gallium Garnet
Fig.	Figure
F	Fluorine
GCM	GC tooth mousse
GaAs	Gallium Arsenide

Ga Al As	Gallium Aluminium Arsenide
H3 PO4	Orthophosphoric acid
HCl	Hydrochloric acid
HeNe	Helium Neon
HS	Highly significant
HSD	Highly Significant Difference
i.e.	That is
InGaAsP	Indium Gallium Arsenide Phosphorus
KCl	Potassium chloride
Kv	Kilovoltage
LASER	Light Amplification by Stimulated Emission of Radiation
mmol/l	Millimoles per liter
Min	Minutes
Ml	Milliliter
Mm	Millimeter
N	Newton
n / no.	Number
Nd:YAG	Neodymium: Yttrium Aluminium Garnet
NOT	Not Occluded Tubule
NS	Not significant
NaOCl	Sodium hypochlorite
Nm	Nanometer
POT	Partially occluded tubule
Ph	Potential of Hydrogen

P	Value Probability value
PA	Pro-Argin
SMF	Sodium Monofluorophosphate
S	Significant
SD	Standard Deviation
SEM	Scanning Electron Microscope
Sq.mm / mm2	Square millimetre
SPSS	Statistical Package for Social Sciences
µm	Micrometer
Wt/vol	Weight/Volume
Wt%	Weight percent
XeCl	Xenon Chloride

INTRODUCTION

Dentin hypersensitivity is one of the most frequently presenting symptoms in dental practice. It may range from mild discomfort to severe pain affecting the life style of the person. ***Dowell et al*** defined dentin hypersensitivity as a transient pain arising from exposed dentin, typically in response to chemical, thermal, tactile, or osmotic stimuli, which cannot be explained by any other dental defect or pathology**.**[30] The condition generally involves the facial surfaces of teeth near the cervical aspect and is very common in premolars and canines.[5] ***Uchida et al.*** stated that, this condition typically develops in patients with gingival recession, periodontal diseases, and loss of cementum following non-surgical periodontal therapy or after periodontal surgery. [111] In addition, certain other conditions like tooth erosion, dentin exposure as a result of a developmental anomaly, and improper brushing habits can predispose patients to cervical dentin exposure and pain.[13,67]

To be hypersensitive, dentin must be exposed and the exposed tubules must be open and patent to the pulp.[4,28] The processes of exposure and opening of tubules are complex and multifactorial. Nonetheless, current evidence,[4,28,32,33] suggests that gingival recession, resulting from abrasion or periodontal disease, is the primary route through which the underlying dentin becomes exposed, and acid erosion is an important factor in opening exposed dentin tubules. A systematic review reported that root sensitivity occurs in approximately half of patients following scaling and root planing.[113]

Once a patient has dentin hypersensitivity, any external stimulus, such as physical pressure or air movement, can cause discomfort to the patient The most widely accepted theory for dentin hypersensitivity is the hydrodynamic theory proposed by Brannstrom,[16] who suggested that pain may result from the movement of the dentinal fluid in the tubules provoked by external stimuli, such as temperature, physical or osmotic changes which, in turn, trigger nerve fibers within the pulp.

Approaches to control the condition falls in two broad categories: those that occlude the exposed dentinal tubules and those that reduce the sensitivity threshold of the pulp. The desensitizing agents have been used in different forms like dentifrices, mouthwash or topical gels with variable efficiency in reducing or eliminating the hypersensitivity.

Laser desensitization has been introduced as an effective tool for the treatment of hypersensitivity. The main advantage of laser treatment is the immediate effect in relieving pain. Amongst the various types of lasers, carbon dioxide laser has been used with promising results in occluding patent dentinal tubules.

Recently a new product based on the Pro-Argin Technology has been launched. It consists of 8.0% arginine, calcium carbonate, 1450 ppm fluoride and is reported to provide superior relief of dentin hypersensitivity compared to other leading dentifrices.

The purpose of this in vitro study is to compare microscopically the efficacy of Carbon Dioxide laser irradiation in occluding patent dentinal tubules with Pro-Argin technology based dentifrice after periodontal therapy.

AIM

To compare the dentinal tubule occluding ability of Pro-Argin based dentifrice and Carbon Dioxide Laser in the treatment of dentinal hypersensitivity.

OBJECTIVES OF THE STUDY

1. To evaluate the dentinal tubule occluding ability of Pro-Argin based dentifrice (PA) on patent dentinal tubules by scanning electron microscopy.

2. To evaluate the dentinal tubule occluding ability of Carbon Dioxide Laser (CDL) on patent dentinal tubules by scanning electron microscopy.

3. To compare the efficacy Pro-Argin based dentifrice and Carbon Dioxide Laser in occluding the dentinal tubules.

REVIEW OF LITERATURE

DENTAL ANATOMY

In a normal tooth, dentin is covered by enamel in the coronal part and by a thin protective layer of cementum in the radicular part. Dentin consists of many thousands of microscopic tubular structures called Dentinal Tubules that radiate outwards from the pulp towards the dentinal surface. They are typically 0.5 to 2 microns in diameter. Dentinal tubules contain plasma-like biological fluid. Each tubule contains a cytoplasmic cell process called Tomes fiber and an odontoblast that communicates with the pulp. Within them, there are two types of nerve fibers, myelinated (A-fibers) and unmyelinated (C-fibers). The A-fibers are responsible for dentin hypersensitivity, perceived as pain in response to all stimuli. Depending on the depth, about 30,000 tubules can be found in 1 mm^2 cross-section of dentin.[27] According to ***Absi EG***, the number of open dentinal tubules per surface area in the teeth with dentin hypersensitivity can be 8 times that of teeth non-responsive to stimuli.[2]

According to ***Narhi et al***,[82] A fibers are responsible for sensitivity of dentin, among them Aδ fibers play the most predominant role. C fibers respond to external irritant (eg. chemical agents) reaching the pulp in pulpitis. There are other intradental nerve units that have conduction velocities above the range of Aδ fibers and these fibers are classified as AD fibers and appear to respond in the same way as Aδ fibers.

DENTIN HYPERSENSITIVITY

Dentinal hypersensitivity is characterized by short sharp pain arising from the exposed dentin in response to stimulus typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or pathology.[30] The other terms as given by ***Addy M,2002***[4] are Dentin sensitivity, Dentin hypersensitivity, Cervical hypersensitivity/ sensitivity, Root hypersensitivity/ sensitivity**,** Cemental hypersensitivity/ sensitivity

Characteristics of hypersensitivity:

It is a dental clinical condition caused by the exposure of dentinal surface to the oral environment by the loss of either enamel or cementum. In simpler terms it can be defined as acute pain of short duration caused by opened dentinal tubules.[94] The pain varies from individual to individual and amongst teeth. It can range from discrete discomfort to extreme severity. The pain can be triggered by various thermal, chemical and mechanical stimuli. Out of thermal stimuli most common is the cold stimuli. The chemical stimuli can be in the form of acidic foods, sweet items and rarely salty foods. Mechanically the pain may be elicited on contact with fingernail, tooth brush bristles, atmospheric air in mouth breathers and the air from triple syringe.[47]

Clinically, sensitive dentin appears similar to non sensitive dentin and also the number of dentinal tubules per area is more.[12]

Mechanism of stimulus transmission across dentin:

Pashley[89] proposed 3 possible hypothesis for dentinal hypersensitivity as the cause:

1. Direct nerve ending theory.
2. Odontoblastic receptor theory or dentinal receptor theory.
3. Hydrodynamic theory.

Direct Nerve Ending Theory

According to ***Anderson et al***,[8] if dentin was directly innervated, then chemical stimuli to the exposed dentin surface should cause pain. But it was observed that application of an allogenic (pain inducing) substance such as potassium chloride, acetylcholine, 5- hydroxytrptamine and histamine failed to elicit a response, whereas when applied directly to exposed pulpal tissue, an immediate response was elicited. Similarly, topical anaesthetic solution when applied to the exposed sensitive dentin did not decrease the sensitivity.

Anderson[8] proposed two possible explanations:

1. There were no nerve elements in dentin. The pain was evoked due to the stimulation of receptor mechanisms in the pulp by disturbance transmitted through the tubules by non-neural means.
2. There were receptor mechanisms in the dentin that could be stimulated indirectly, but cannot be reached by direct stimulation from chemical agents because of some barrier diffusion in the tubules.

Recent auto radiography studies of intradental nerves have demonstrated that nerve fibers in the pulp/dentin border area are injured by dentinal stimulation, with 50% reduction in the number of innervated dentinal tubules and in some instances, loss of nerve fibers in dentin. These results suggest that the existence of nerve fibers in dentin is not necessary pre-requisite for its sensitivity.

Dentinal Receptor Mechanism Hypothesis

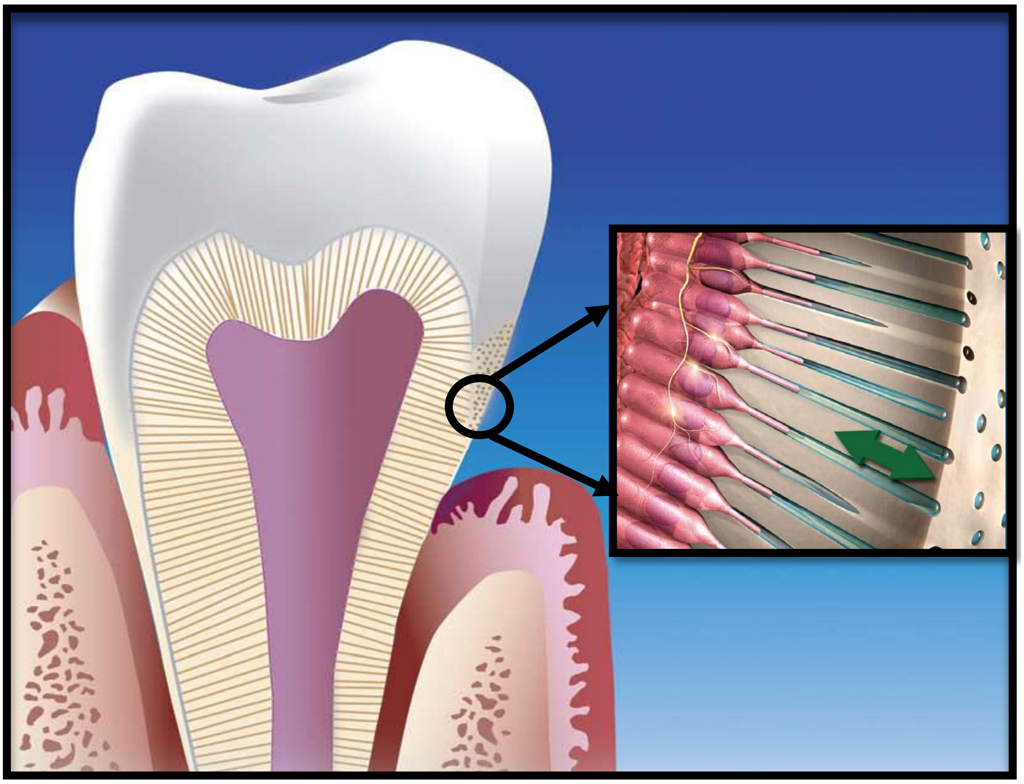
Proponents of dentinal receptor mechanism hypothesis have suggested that the odontoblast have a special sensory function. The terminal sensory nerve endings in close proximity to the odontoblast layer form a functional synapse that acts as an excitatory synapse. These specialized junctional complexes were concluded to be a unique type of '*neurosensitive complex'*.[10,34]

Various studies were conducted to establish the presence of any synaptic junction or special form of connection between the odontoblast processes and nerve endings. This theory is called **Transducer theory.** In order to substantiate this theory the presence of neurotransmitter substance such as acetylcholine should be demonstrated by evidence of acetyl cholinesterase activity in the dentin. ***Avery and co-workers***[12] have shown that odontoblast protoplasmic extensions were cholinesterase positive.***Tencate***[110] demonstrated cholinesterase activity in both myelinated and non-myelinated nerve fibers of pulp, but not close to odontoblasts or there processes. But it was concluded that if the transmission of impulses associated with dentinal hypersensitivity was via dentinal receptor mechanism, then there was no evidence to suggest that these impulses were mediated by cholinergic activity.

Several investigators like ***Kim***[62] ***and Berman***[14] have suggested that the nerve impulses in the pulp may be modulated by polypeptides such as plasma kinins and substance P, this is termed as **Modulation Theory.**

The Hydrodynamic Theory

Dentin is composed of hollow tubes containing a fluid or semi fluid material. ***Gysi***[50] proposed that the movement of fluid in dentinal canaliculi in either direction resulted in sensation of pain. ***Fish***[31] proposed that the fluid within the dentinal tubules is apparently extracellular. ***Stanley***[31] observed that free fluid makes up about 2% of enamel volume and 25% of dentinal volume. The diameter of these tubules was 2.5µm at the pulpal end and 0.9µm at the periphery. According to ***Brannstrom***[18] the conical shape of dentinal tubules together with the movement of fluid by the capillary action should obey the simple physical laws as liquid in gas capillary tubes *(Poiseuilles law).*



Photograph1- Illustration of Hydrodynamic theory

Brannstrom[18] suggested that if displacement of tubule contents was rapid, it could deform nerve fibers in the pulp or predentin or damage odontoblasts cells and both these effects appear capable of producing pain. More recently this definition has been redefined to state that minute fluid shifts, either dentinal fluid or tubule contents, across dentin in either direction, in response to tactile, thermal or osmotic stimuli, can stimulate mechanoreceptors in or near pulp which, in turn excite sensory nerves to cause pain.

Modified Hydrodynamic Theory

Several investigators like ***Narhi***,[82] ***Kim***,[62] ***Anderson,***[8] ***Scott,***[8] ***Bilotto***[8] have used a neurophysiological model to evaluate dentin sensitivity. The results from these studies suggested that the application of various chemical solutions (in particular potassium containing compounds) to dentin resulted in raising the intratubular potassium content, which in turn rendered the intradental nerves less excitable to further stimuli by depolarizing the nerve fiber membrane. On the basis of these studies, ***Kim and Markowitz***[74] proposed an alternative mechanism namely ***Direct Ionic Diffusion*** i.e., desensitization of dentin by blocking nerve activity

Topical desensitizing agents have been classified on the basis of their chemical and physical properties. The chemical agents are Corticosteroids, Silver nitrate, ferric oxalate, potassium nitrate, sodium citrate and the physical agents are resins, sealants, soft tissue grafts and lasers.[29]

Grossman[49] described the criteria for an ideal desensitizing agent about half a century ago as follows. The desensitizing agent must be Non-irritating to the pulp, painless on application, easy to apply, rapid in action, effective for long time, non-staining and cost effective.

Micheleih V, Pashley DH, Whitford GM. (1978),[79] evaluated the functional versus anatomical tubule radius and observed that the functional radii of the dentin discs ranged from 5 to 40% of the anatomic radii. This difference was due to the fact that SEM visualizes only the surface while the functional techniques measure the radii within dentin tubules. It was concluded that the functional methods like filtration and surface tension are preferred to study the dynamics of fluid flow through dentin.

Dowell P, Addy M. (1983),[31] reviewed the literature and stated that the nerve fibres only penetrate a limited distance along the dentinal tubules. Therefore, it is evident that the stimulation of pulp nerve fibres occurs by hydrodynamic mechanism. It was concluded that occlusion of dentinal tubules would appear an essential prerequisite for an effective desensitising agent.

Carrigan PJ, Morse DR, Furst LA et al (1984),[23] examined the human dentinal tubules according to the age of the subject and location on the tooth using scanning electron microscopy. They concluded that the number of dentinal tubules decreased with increasing age and apical location. This may account for the marked sensitivity and increased bacterial penetration of coronal dentin when compared to apical dentin.

Dowell P, Addy M, Dummer P. (1985),[30] defined dentin hypersensitivity as short, sharp pain arising from exposed dentin in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical, which cannot be ascribed to any other dental defect or pathology.

Absi EG, Addy M, Adams D. (1987),[2] examined caries-free non-sensitive and hypersensitive teeth with exposed cervical root areas by scanning electron microscopy. It was observed that hypersensitive teeth showed highly significantly increased numbers of tubules per unit area (approximately 8X), the tubule diameters were also significantly wider (approximately 2 X) in hypersensitive compared to nonsensitive teeth.

Pashley DH, Tao L, Boyd L, et al. (1988),[88] conducted a study to analyse the substructure of smear layers using SEM. It was observed that the layers are composed, in part, of aggregates of globular subunits approx. 0.05-0.1 micron in diameter.

Addy M (1990),[5] stated that the management of hypersensitivity requires the determination of etiologic factors and predisposing influences, and where possible, their control or modification. In particular, the presence of erosive elements should be identified and separated from abrasive influences such as tooth brushing.

Fischer C, Fischer R G, Wennberg A. (1992), [38] stated that the prevalence of hypersensitivity was higher among females than males aged 20-49. Incisors and premolars had the highest prevalence of dentin hypersensitivity to air and probe stimuli, while molars had the lowest.

Wen CR, Caffesse RG, Morrison EC. et al (1992),[118] stated that root-planed, non-acid treated specimens had an amorphous, irregular surface which corresponded to a smear layer.

Chabanski MB, Gillam DG, Bulman JS. et al (1997),[24] stated that the prevalence of Cervical Dentin Sensitivity ranged between 72.5 and 98% of patients. Cold stimulation was perceived to be the dominant pain-producing stimulus. They suggested that previous periodontal treatment and/or periodontal disease may play a role in the aetiology of CDS.

Mordan NJ, Barber PM, Gillam DG. (1997),[80] stated that the dentin disc is a good, reliable model for in vitro screening and testing of potential desensitizing agents, provided controls are applied.

Gillam DG, Mordan NJ, Newman HN. (1997),[45] concluded that the use of Pashley Dentin Disc model to determine surface characteristics, and reductions in dentin permeability through tubule by narrowing or occlusion, provides a useful screening method for evaluating potential desensitizing agents.

Liu H C, Lan W H, Hsieh C C. (1998),[71] stated that premolars and molars were the most common teeth sensitive to the air and probe stimuli, while the incisors were the least sensitive ones.

Dababneh RH, Khouri AT, Addy M. (1999),[29] concluded that therapeutic intervention by desensitising agents may provide only partial pain relief and recurrence is common. There is no gold standard agent to compare the efficacy of new treatments available.

Schilke R, Lisson JA, Baub, et al, (2000),[102] compared the number and diameter of bovine permanent central incisor and human deciduous and third molars and suggested that if standardized preparations are used, bovine incisor crown dentin is a suitable substitute for human molar dentin in adhesion studies.

Isik, Tarim, Hafez, Yalcin (2000),[58] demonstrated that the use of tetracycline HCl solution between 50 mg/ml and 150 mg/ml showed a statistically significant opening of dentin tubules. All tetracycline HCl groups at 1, 3, and 5 minutes showed smear layer removal from the dentin surface.

Addy M (2002),[4] stated that dentin hypersensitivity is a tooth-wear phenomenon characterised predominantly by erosion. Abrasion caused by dentifrice appears to be a secondary aggravating factor, gingival recession probably accounts for most dentin exposure at the gingival margin. It was concluded that the management strategies should take into account aetiological and predisposing factors, rather than treatment alone.

Drisko C. (2002),[32] stated that removal of cementum resulting in the exposure of dentinal tubules leads to dentin hypersensitivity. It may occur either due to incorrect oral hygiene techniques by patient or instrumentation of the root surface by dental professionals in the management of periodontal diseases.

Von Troil B, Needleman I, Sanz M. (2002),[113] did a systematic review to evaluate the prevalence of root sensitivity following periodontal therapy. It was observed that the prevalence of root sensitivity was 9–23% before and 54–55% after periodontal therapy. An increase in the intensity of root sensitivity occurred 1–3weeks following therapy, after which it decreased. The authors concluded that root sensitivity occurs in approximately half of the patients following subgingival scaling and root planing.

Kleinberg I. (2002),[63] reviewed the role of saliva and evaluated the effectiveness of an arginine bicarbonate/calcium carbonate complex called SensiStat in the treatment of dentin hypersensitivity. They stated that saliva with its remineralizing ability can gradually plug open tubules by supplying calcium and phosphate ions and by forming a perceptible aggregate consisting of calcium phosphate in combination with salivary glycoproteins.

Schellenberg U, Krey G, Bosshardt D et al (2002),[100] observed that in premolars the coronal dentin showed significantly higher tubule density than the radicular dentin and the number of tubules present on the vestibular/oral walls was significantly higher than those on the mesial/distal walls.

Pereira JC, Martineli AC, Tung MS. (2002),[90] determined a methodological sequence in vitro which may allow the reproduction of dentin for SEM analysis, after the use of different desensitizing agents and concluded that the comparison of the photomicrographs of dentin discs with their respective impressions made of Aquasil ULV and epoxy resin replicas can reproduce the characteristics of the dentin surface treated with desensitizing agents.

Haznedaroglu F. (2003),[51] concluded that lower concentrations of citric acid with lower pH values removed smear layer more efficiently than the ones with higher pH values. However, more destruction of peritubular dentin was observed at higher concentrations with low pH values.

Carda C, Peydro A. (2006),[21] observed the ultrastructural patterns of human dentinal tubules, odontoblasts processes and nerve fibres and stated that the distribution of the dentinal tubules is homogeneous; containing a principal odontoblastic prolongation in the regions of the inner dentin. The nervous fibres appeared accompanying 30-70% of the odontoblastic prolongations and their synapsis-like relation with the odontotoblastic processes was demonstrated.

Suge T, Kawasaki A, Ishikawa K (2006),[109] evaluated the effects of plaque control on the patency of dentinal tubules and observed that in the plaque control group, some of the dentinal tubules were occluded with calcium and phosphorus precipitate and their diameter also decreased in contrast to the non-plaque control group.

Drisko C. (2007),[33] stated that the successful treatment of patients with mild sensitivity and minimal recession can be accomplished by correcting destructive oral hygiene habits and use of a desensitising dentifrice. Moderate to severe dentin hypersensitivity in the presence of gingival recession ≥1 mm usually requires surgical root coverage procedure with or without daily use of desensitising toothpastes and/or professional application of desensitising agents, dentin bonding materials, or cervical restorations.

Hegde MN, Bhalla N. (2009),[52] carried out a cross-sectional study over a period of one calendar month in southern India to establish the prevalence of dentin hypersensitivity. The overall prevalence of dentin hypersensitivity was found to be 26%.

Cummins D. (2009),[28] reviewed the various approaches to relieve sensitivity by professional and home-use products, with emphasis on the clinical evidence for the efficacy of desensitizing toothpaste, and introduced a new innovative dentifrice technology containing 8% arginine, calcium carbonate, and 1450 ppm fluoride.

Lopes MB, Sinhoreti MA, Gonini Júnior A et al (2009),[72] compared the tubular dimensions and distribution in human and bovine dentin and concluded that superficial dentin tubule diameter was significantly smaller than deep dentin and middle dentin tubule diameters, which did not differ significantly from each other. The number of tubules per square millimetre, regardless of the region, was significantly greater in human dentin than in bovine dentin.

STUDIES ON DENTIFRICE

Uchida A. (1980),[111] evaluated 10% strontium chloride dentifrice in the treatment of dentin hypersensitivity in patients following periodontal surgery concluded that 10% strontium chloride hexahydrate dentifrice was effective in reducing dentin hypersensitivity to a point below the preoperative level.

Pashley DH, O'Meara JA, Kepler EE et al (1984),[87] measured the fluid flow (i.e., hydraulic conductance) across dentin discs before and after brushing the discs with desensitizing dentifrices and concluded that dentifrice containing oxalate as the active ingredient was far more effective.

McFall WT Jr, Morgan WC Jr. (1985),[77] compared a dentifrice containing 0.8% sodium monofluorophosphate and 1.3% formalin with the control and observed that the reduction in response to mechanical stimuli was not statistically significant reduction in comparison to thermal stimulus at both 14 and 28 days.

Ong G, Strahan JD. (1989),[84] compared the effectiveness of a dentifrice with 2% dibasic sodium citrate in poloxamer 407 with a control dentifrice containing 0.76% sodium monofluorophosphate in decreasing dentin hypersensitivity and concluded that the test dentifrice was not significantly more effective than the control in decreasing dentinal hypersensitivity.

Addy M, Mostafa P. (1989),[3] measured the availability and uptake of fluoride and metal ions contained in commercial and test toothpastes and also observed the effects of exposure of dentin sections to these toothpastes, and abrasive only pastes in particular by scanning electron microscopy and X-ray microanalysis. It was observed that levels of fluoride and metal ions on the dentin surface had increased. It was concluded that more attention should be given to the therapeutic potential of toothpaste ingredients, particularly abrasives, to produce benefit by occlusion of dentinal tubules.

Kerns DG, Pashley DH, Van Dyke TE. et al (1991),[61] longitudinally evaluated the occlusion of dentinal tubules by the application of potassium oxalate and scaling and root planing and concluded that the creation of a smear layer or application of oxalates to occlude dentinal tubules to reduce sensitivity is relatively short-lived.

Chesters R, Kaufman HW, Wolff MS (1992),[26] compared a potassium citrate-SMFP, potassium nitrate-SMFP containing dentifrice with a dentifrice containing SMFP alone but no potassium salt and concluded that the potassium citrate-SMFP dentifrice was significantly more effective than the others in reducing sensitivity after 3 weeks.

Olsson S, Oilo, Adamezak E. (1993),[83] evaluated the structure of dentin at three different levels, near enamel, central, and deep dentin and concluded that the discs from the deeper layers of dentin showed an increasing number of tubules with an increasing diameter and discs from the occlusal part of the tooth showed a higher number and area percentage of tubule.

Suge T, Ishikawa, Kawasaki A. et al (1995),[107] evaluated the effect of fluoride on the precipitate formed by the Calcium Phosphate Precipitate (CPP) and on its capacity to occlude dentinal tubules and concluded that dentin permeability was reduced to 15% by the CPP treatment regardless of the NaF concentration but the Ca/P molar ratio of the precipitate was higher in the presence of NaF.

Gillam DG, Bulman JS, Jackson RJ (1996),[43] compared the efficacy of strontium acetate/sodium fluoride (SrAcNaF) with potassium chloride/sodium monofluorophosphate (KCl/MFP) and a fluoride dentifrice containing sodium fluoride/sodium monofluorophosphate (NaMFP). It was concluded that fluoride-containing dentifrice was as effective as the 2 desensitizing dentifrices in alleviating cervical hypersensitivity over time.

Holland G R, Narhi M N, Addy M. et al (1997),[54] laid down the guidelines for clinical trials on dental hypersensitivity in a consensus report and recommended a double-blind randomized parallel groups design. Subject selection is based on a clinical diagnosis of dentin hypersensitivity and multiple sites can be scored. The vestibular surfaces of incisors, cuspids and bicuspids are preferred. Tactile, cold and evaporative air stimuli should be applied with control and the range of sensitivity is recorded. Most trials should last for 8 weeks. Follow-up evaluation is required and at least 2 independent trials should be conducted before a product receives approval.

Reynolds EC. (1998),[95] proposed that anticariogenic action of CPP-ACP is due to its ability to localize Amorphous Calcium Phosphate in dental plaque, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization.

West, Addy, Hughes. (1998),[122] examined the quantitative and qualitative effects of toothpastes, their solid and liquid phases and detergents on dentin and acrylic and concluded that toothpastes, solid phase, liquid phase and detergents have the potential to abrade or erode dentin to a variable degree which appeared to plateau around 2μm and result in tubule exposure.

Schiff T, Dos Santos M, Laffi S (1998),[101] evaluated the effect of a dentifrice containing 5.0% potassium nitrate, 1500 ppm sodium monofluorophosphate in a precipitated calcium carbonate (PCC) base and placebo dentifrice without potassium nitrate and concluded that after four and eight weeks use the potassium nitrate/PCC dentifrice group demonstrated statistically significant improvements as compared to the placebo dentifrice.

Morris MF, Davis RD, Richardson BW. (1999),[81] compared the clinical effect of oxalate-containing pre-polymerized resin suspension (Pain-Free), a 0.7% fluoride solution (DentinBloc), and a distilled water placebo and concluded that pre-polymerized resin suspension (Pain-Free), the fluoride containing solution (DentinBloc), both decreased dentin sensitivity in contrast to placebo.

Gillam DG, Khan N, Mordan NJ. et al (1999),[44] compared the surface effects and tubule penetration of the sensodyne sealant, ALL-BOND 2, ONE-Step, Butler Protect, Oxa-gel by scanning electron microscopy. The author concluded that the three products were more effective than either Butler Protect (potassium oxalate) or Oxa-gel (potassium oxalate in a gel) as there was a marked decrease in both the level of coverage and tubule occlusion.

Jain P, Reinhardt JW, Krell KV. (2000),[59] determined the effect of dentin desensitizing agents (oxalates and glutaraldehyde) and dentin bonding agents, with and without resin-based composite (RBC) on dentin permeability and morphology of the dentin surface The treatment modalities were: Sensodyne Dentin

Desensitizer, Gluma Dentin Desensitizer, All-Bond DS (primers without etching), Etch+Primers (All-Bond 2 system), Etch+Primers+Adhesive and Etch+Primers+Adhesive+RBC (AElite Flo). The greatest reduction in permeability was caused by All-Bond DS followed by Sensodyne Dentin Desensitizer and Etch+Primers+Adhesive+RBC.

West NX, Hughes JA, Addy M. (2002),[119] examined the surface morphological changes of etched and unetched dentin in vitro due to tooth brushing with and without desensitizing toothpastes and concluded that artificial silica based paste was significantly better for occluding the dentin tubules.

Arrais CAG, Micheloni CD, Giannini M et al (2003),[11] evaluated the tubule occluding ability of three commercially available dentifrices (Sensodyne, Emoform and Sorriso) by SEM and concluded that the three tested dentifrices produced increased dentinal occlusion as compared to controls but equivalent occlusion among each other.

Geiger S, Matalon S, Blasbalg J (2003),[42] compared the effect of ACP in test group and potassium chloride in placebo group and concluded that both the experimental and placebo treatments resulted in a reduction in hypersensitivity but the ACP treatment group showed a much more rapid reduction in hypersensitivity over time.

Kowalczyk A, Botulinski B, Jaworska M. (2006),[67] evaluated the efficiency of GC Tooth Mousse in the treatment of dentin hypersensitivity and concluded that GC Tooth Mousse preparation, based on Recaldent technology provides short-term therapeutic effect in treating hypersensitivity of dentin.

Sauro S, Gandolfi MG, Prati C, Mongiorgi R. (2006),[99] evaluated the dentinal permeability and morphology after a single exposure to phytocomplex substances (extracted from rhubarb, spinach and mint) containing oxalates with experimental paste containing 5% potassium oxalate, Elmex and Sensodyne and found that phytocomplex treatments reduced dentinal permeability by occluding dentinal tubules through formation of calcium oxalate crystals. It was concluded that phytocomplexes extracted from rhubarb and spinach, used in different formulations, would be effective as topical treatment of dentinal hypersensitivity.

Lee BS, Kang SH, Wang YL. et al (2007),[69] evaluated dentin bonding agents for DP-bioglass particles with HNO3,NaOH, and H3PO4 as catalysts, One Coat Bond and Seal & Protect and concluded that the best sealing performance of tubular occlusion was rendered by DP-bioglass catalyzed with HNO3 and was considered to exhibit the greatest potential in treating dentin hypersensitivity.

Gandolfi MG, Silvia F, Gasparotto G et al (2008),[40] compared the in vitro effectiveness of a calcium silicate paste based on Portland cement (DSC) with GC Tooth Mousse, D/Sense Crystal, and By Sealant and Dentosan S on dentin permeability and dentin morphology. It was concluded that test treatment and both oxalate-based products (D/Sense Crystal and By Sealant) significantly decreased dentin permeability and created crystals and precipitates on the dentin surface that reduced the diameter of dentinal tubules.

R Docimo, L Montesani, P Maturo et al (2009),[92] conducted a eight week trial clinical trial to evaluate the effectiveness of 8% arginine, calcium carbonate and 1450ppm fluoride with sodium fluoride and concluded that the test arginine, calcim carbonate, 1450ppm of fluoride provided significant reduction in the dentin hypersensitivity than the other group.

F Ayad, N Ayad, E Delgado et al (2009),[35] conducted a 3 day clinical trial to compare the instant relief of dentin hypersensitivity by 8% arginine, calcium carbonate and 1450ppm of fluoride with potassium nitrate and concluded that arginine based dentifrice provide significant instant relief than potassium nitrate.

Irene Petrou Rod Heu Mike Stranick Stacey Lavender (2009),[56] studied the mechanism of action of 8% arginine and calcium carbonate containing dentifrice. The authors concluded that arginine and calcium carbonate work together to accelerate the natural mechanisms of occlusion to deposit a dentin-like mineral, containing calcium and phosphate, within the dentin tubules and in a protective layer on the dentin surface

Komabayashi T, Imai Y, Ahn C et al (2010),[65] evaluated the effect of calcium and fluoride phosphate solutions treatment on dentin permeability (Lp) and concluded that an average decrease of 34% Lp occurred and the reaction products covered the entire dentin disc surface.

Walsh LJ. (2010),[114] compared the therapeutic effect of 10% CPP-ACP crème (GC Tooth Mousse) applied topically each night before retiring in conjunction with a conventional dentifrice twice daily, to the twice daily use of an established potassium nitrate dentifrice and concluded that both CPP-ACP crème and the potassium nitrate dentifrice gave similar clinically useful reductions in CDS.

Pinto SCS, Pochapski MT, Wambier DS (2010),[91] assessed the influence of the topical application of, 2% potassium nitrate plus 2% sodium fluoride gel; 5% sodium fluoride varnish; 3% hydroxyethylcellulose gel on dentin permeability and dentinal tubule occlusion and concluded that 2% nitrate potassium plus 2% sodium fluoride gel and 5% fluoride varnish caused partial occlusion causing decrease in dentin permeability.

Stacey Ann Lavender, Irene Petrou, BS, Rodman Heu, MS et al (2010),[106] studied the mode of action of desensitizing dentifrice containing 8.0% arginine, calcium carbonate and1450 ppm fluoride. They concluded that the positively charged arginine is attracted to the negatively charged dentin surface where it helps attract and adhere calcium carbonate to the dentin surface and deep into the tubules.

Xiong ZH, Xia L, Mei L, et al (2011),[123] evaluated Novamin, Pro- Argin, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and 75% NaF glycerine and concluded that Novamin may have better immediate occluding effects than other desensitizing dentifrices.

West NX, Macdonald EL, Jones SB et al (2011),[120] compared novamin 5% and 8% pro-argin based toothpaste and concluded that novamin 5% showed better dentin tubule occlusion and retention abilities in an oral environment which is under constant dietary acid challenge conditions than the 8% arginine based toothpaste technology.

Markowitz K. (2012),[73] described a new mouthwash-based desensitizing technology and concluded that the new desensitizing mouthwash, based on the Pro-Argin™ mouthwash technology effectively reduced sensitivity symptoms and can be used alone or as a adjunct to the use of the arginine-containing dentifrice in the home treatment of tooth sensitivity.

Wang Z, Jiang T, Sauro S, et al (2012),[116] examined in vitro dentinal tubule occlusion and mineral precipitation ability Sensodyne, Freshmint and Colgate Sensitive and observed that the dentinal tubules were partially occluded and the intensity of phosphate peaked after 1 week, but the dentinal tubules opened and the phosphate peak declined after acid etching.

LASERS

The term *Laser* is an acronym for "**Light Amplification by Stimulated Emission of Radiation**".

Historically, Leon Goldman in 1964 reported the laser application on a healthy, living human tooth for the first time. Since then laser surgeries have revolutionized dentistry in the advent of systems specifically designed for the dentist. Stated best by Apfelberg in 1987, lasers are a "new and different scalpel", (optical Knife or light scalpel).Food and Drug Administration, USA approved the use of lasers for soft tissue surgeries in January 1990 and for periodontal surgeries in June 1999.

For a laser to have a biological effect, the energy must be absorbed. The degree of absorption in the tissues will vary as a function of wavelength and optical characteristics of the target tissue. If the peak emission of the laser matches the absorption spectrum of one or more components of the target tissue, a predictable and specific interactive effect will occur.

Types of Lasers

Clinical classification

Two categories of lasers are used in medicine and dentistry.

1. *Soft lasers* –provide cold low energy at wave length believed to stimulate circulation and cellular activity. Eg .He-Ne, Gallium- Arsenide, Ga-Al- Arsenide lasers.

2. *Hard lasers* – provide high energy at wavelength to cut, coagulate and vaporize the tissues. Eg . Nd-YAG laser, CO2 laser.

TABLE 1 – COMMERCIALLY AVAILABLE LASERS

LASER TYPE	COMMERCIALLY AVAILABLE
EXCIMER LASER	Argon Flouride (ArF) Xenon Chloride (XeCl)
GAS LASER	Argon Helium Neon (He Ne) Carbon Dioxide (CO_2)
DIODE LASER	Indium Gallium Arsenide Phosphorus (In Gl As P) Gallium Aluminium Arsenide (Ga Al As) Gallium Arsenide (Ga As)
SOLID LASER	Neodymium:Yag Laser (Nd:YAG) Erbium group of lasers Er:YAG ErCr:YSGG

The advent of dental lasers has raised another possibility for the treatment for DH, and has become the object of research interest in recent decades. The effect of Laser phototherapy in the treatment of dentinal hypersensitivity depends of certain variables, like

1. Type of laser : low or high-power laser
2. Wavelength
3. Exposure parameters: current, voltage and time of exposure

Various types of lasers have been used but CO2, Nd:YAG and Er:YAG lasers have been regarded suitable for the management of DH. The mechanism of action of lasers in the treatment of dentinal hypersensitivity is related to the increase in tooth surface temperature resulting in complete or partial closure of dentinal tubules after recrystallization of dentinal surface.

Several other mechanisms have also been proposed to explain the decrease in pain after Laser Phototherapy in Dentinal Hypersensitivity. Some authors have attributed the positive effects to the formation of tertiary dentin and the reduction in sensory nerve activity. Although the neurophysiological mechanism is not conclusive, it is postulated that LPT mediates an analgesic effect by the depolarization of C-fibre afferents. This interference in the polarity of cell membrane by increasing the amplitude of action potential blocks the transmission of pain stimuli in hypersensitive dentin.[22]

According to the histological studies conducted by ***Melcer J,Ferreira AN***,[22] the formation of hard tissue is a reactive response of the dental pulp to laser irradiation. ***Matsui et al.***[22] showed that hydroxyl generated by laser irradiation activates cell signalling molecules such as G-protein, which promotes the formation of hard tissue by human dental pulp cells.

Russ P. Read, J. Craig Baumgartner, Stephen M. Clark.(1995),[97] evaluated the effects of carbon dioxide laser on human root dentin and concluded that cracks and fissure were observed in the specimens and the laser delivery tip should be modified.

Hsin- Cheng Liu, Chun-Pin Lin, Wan-Hong Lan (1997),[55] evaluated the sealing depth of Nd:YAG laser on human dentinal tubules and observed the sealing depth of 4μm in the center and 3μm at the margin of the lased surface. The authors concluded that Nd:YAG laser meets all the criteria of an ideal desensitizing agent and can be used in the treatment of dentinal hypersensitivity.

Chengfei Zhang, Koukichi Matsumoto, Yuichi Kimura et al (1998),[25] studied the effects of carbon dioxide laser in treatment of cervical dentinal hypersensitivity and observed that all the teeth remained vital and the patients were immediately free of sensitive pain. The authors concluded that carbon dioxide laser is useful in the treatment of cervical dentinal hypersensitivity without thermal damage to the pulp.

Wan-Hong Lan, Kau-Wu Chen, Jiiang-Huei Jeng et al (2000),[117] compared the morphological changes on the dentin surface after Nd:YAG, CO_2 laser irradiation. both the laser types and smear layer have a significant influence on the morphological changes of dentin surfaces irradiated by lasers.

Lier BB, Rösing CK, Aass AM Gjermo P.(2002),[70] evaluated the effect of Nd:YAG laser on dentin hypersensitivity and concluded that the result was insignificant and the effect lasted for 16 weeks.

Antonio Pinheiro, Fatima Zanin, Luc Louis Maurice Weckx (2004),[9] evaluated the effectiveness of 660nm wavelength red diode laser and 830nm wavelength infra red diode laser and concluded that 660nm red diode laser was more effective in providing both immediate and late desensitization as compared to 830 nm infrared diode laser.

Reza Birang, Jamshid Poursamimi, Norbert Gutknecht. et al (2007),[96] compared the effects of Nd:YAG and Er:YAG laser in dentin hypersensitivity treatment and concluded that both the lasers produced acceptable therapeutic effect but Nd:YAG laser is more effective in occluding the dentinal tubules than Er:YAG laser.

Cankat Kara, Recep Orbak (2009),[20] compared Nd:YAG laser and fluoride varnish for the treatment of dentinal hypersensitivity and concluded that Nd:YAG is a suitable tool in the immediate successful treatment of dentinal hypersensitivity added to the advantage of shorter treatment time.

Snezana Pesevska, Marija Nakova, Kiro Ivanovski (2010),[105] compared the effectiveness of low level laser irradiation and topical fluoride application in the treatment of dentinal hypersensitivity following scaling and root planing. The authors concluded low level biostimulative lasers can be successfully used following scaling and root planing to avoid dentinal hypersensitivity.

A.S. Bakry, H. Takahashi, M. Otsuki (2011),[1] examined the micro-morphological features and chemical changes that occurs on the dentinal surface after 45S5 bioglass application before and after the carbon dioxide laser treatment. The study was based on the hypothesis that carbon dioxide laser irradiation modifies the calcium-phosphate interaction layer formed on the dentinal surface. It was concluded that 45S5 bioglass could effectively occlude the dentinal tubule orifice with calcium-phosphate crystals and the carbon dioxide laser application improved the mechanical organization of calcium-phosphate crystals.

Ana Cristina Cury Camargo Romano, Ana Cecilia Corrêa Aranha, Bruno Lopes da Silveira et al (2011),[7] evaluated carbon dioxide laser irradiation associated with calcium hydroxide in the treatment of dentinal hypersensitivity. It was observed that the groups treated with calcium hydroxide alone exhibited higher scores whereas carbon dioxide laser group showed cracks, fusion, recrystallization and carbonization. A temperature rise of 1.5°C was noted. The authors concluded that carbon dioxide laser is safe and results in dentinal tubular occlusion by partial fusion and resolidification.

Zahi Badran & Hervé Boutigny & Xavier Struillou et al (2011),[125] examined the microscopically occluding effects of the Er:YAG laser on exposed dentinal tubules**.** The authors concluded that Er:YAG laser irradiation provided better long-term desensitizing effects than topical application of fluoride paste.

Fabrizio Sgolastra, Ambra Petrucci, Roberto Gatto (2011),[36] reviewed the effectiveness of lasers in treatment of dentin hypersensitivity and concluded that laser therapy can reduce dentin hypersensitivity related pain, but the evidence for its effectiveness is weak, and the possibility of a placebo effect must be considered.

Ana Cecilia Corrêa Aranha & Carlos de Paula Eduardo (2012),[6] analyzed the dentin permeability and morphology of exposed dentin surface after irradiation with Er:YAG, ErCr:YSGG lasers using different parameters. It was concluded that Er:YAG laser proved superior to ErCr:YSGG in decreasing dentin permeability but none of the lasers could completely seal the dentinal tubules.

Meral Arslan Malkoç & Müjde Sevimay (2012),[78] evaluated the mineral content of dentin prepared using three different desensitizing agents and the Nd:YAG laser. The result showed that the resin based agent occluded the dentinal tubules, the glutaraldehyde-containing agent increased the Ca/P ratio, and Nd:YAG laser irradiation decreased the Ca/P ratio. The mean percentages by weight of Ca, Mg, K, Na and P were not affected by Nd:YAG laser irradiation or any of the desensitizing agents.

MATERIALS AND METHODS

This in vitro study consists of thirty extracted periodontally compromised single- rooted teeth, suitable to the selection criteria, obtained from the Department of Oral Maxillofacial Surgery, Tamil Nadu Government Dental College and Hospital.

INCLUSION CRITERIA

- Thirty single rooted permanent human teeth extracted because of extensive loss of periodontal supporting tissues were used in this study.
- Evidence of calculus deposits.

EXCLUSION CRITERIA

- History of periodontal therapy within past 6 months.
- Fluorosis.
- Absence of caries or filling materials.
- Teeth with developmental anomalies such as concrescence, fusion, dentinogenesis imperfecta, enamel hypoplasia.

PROCEDURAL STEPS

Sampling

Step-1: The extracted teeth were immediately washed in running tap water to remove blood, saliva, and soft tissue debris by light scrubbing with a sterile scrub brush.

Step-2: The specimens were stored in Thymol solution at 4°C for not more than one month until required for the experiment.

Dentin disc specimen preparation

Step-3: Dentin discs of 2mm thickness were prepared from the radicular portion of the tooth 2mm below the level of cemento-enamel junction using double sided diamond disk operated in a water cooled airotor (NSK, Japan).

Step-4: The dentin discs were polished with sequential grades of silicon carbide paper of various grit sizes (viz 400, 600, 800, and 1000) to create a standard smear layer.

Step-5: After allocating the dentin specimens to three groups they were then etched by immersing in 6% citric acid for 2 minutes to simulate dentin hypersensitivity condition.

Step-6: The specimens were subjected to the experiment and were stored in artificial saliva during the experimental period.

Experimental groups and treatments

30 dentin specimens were taken and divided into three groups.

***Group I*:** Specimens brushed with Distilled Water (DW).

***Group II*:** Specimens brushed with dentifrice containing 8.0% Arginine, calcium carbonate and 1450 ppm fluoride **(**PA)

***Group III*:** Specimens irradiated with Carbon Dioxide Laser 0.5W (CDL)

In group I, the specimens were rinsed in distilled water after brushing session of 2 minutes and stored in a closed container containing artificial saliva. The artificial saliva was prepared and it contained: Distilled water (700 ml), Ca(OH)2(1.56 Mm/l), KCl (150.00 mM/l), HCl (36.00 mM/l), H3 PO4 (0.88 mM/l), buffer (99.7 mM/l), pH 7.2.

In group II, the specimens were brushed with Pro-Argin based dentifrice using a powered toothbrush operating at 6800 strokes per minute for two minutes and the specimens were stored in artificial saliva for 15 minutes.

In group III, the specimens were irradiated with pulsed carbon dioxide laser for 5 sec/cycle at the output of 0.5W. The cycle was repeated 6 times and the specimens were stored in artificial saliva for 15 minutes.

Preparation for scanning electron microscopic examination (SEM)

Step-7: The specimens were fixed in freshly prepared 4% glutaraldehyde solution (pH 7.2) at room temperature for 2½ hours and washed thrice with DPBS for 10 minutes each.

Step-8: The specimens were then dehydrated in graded series of aqueous ethanol (50, 70, 80, 95 and 100%) for 10 minutes at each concentration.

Step-9: The specimens were then dried in a dessicator for 48 hours.

Step-10: The specimens were then sputter coated with 200 A° of gold using a sputter coater (Hitachi E-1010 Ion Sputter).

Step-11: The specimens were mounted in scanning electron microscope stubs and examined in Scanning Electron Microscope (Hitachi S-3400 N) operating at an accelerated voltage of 15-20 kV.

Step-12: All specimens were viewed at a standard magnification of 1000x. The numbers of tubules completely occluded, partially occluded and not occluded were counted. The diameter of tubules was also measured. The data collected was statistically analyzed.

STUDY PARAMETERS:

I. Number and percentage of tubules Completely Occluded (CO) per unit area.

II. Number and percentage of tubules Partially Occluded (PO) per unit area.

III. Number and percentage of tubules Not Occluded (NO) per unit area.

IV. Diameter of Partially Occluded (PO) and Not Occluded (NO) tubules.

CALCULATION OF NUMBER OF TUBULES:

The number of tubules was calculated manually by counting the number of tubules in the photomicrograph. To facilitate the counting, a nine square grid was applied on the photomicrograph using Corel Draw version 5 software. Checking for complete and partial occlusion of the tubules and patent tubules (no occlusion) was done by magnifying the photomicrograph one and half times the original magnification.

MEASUREMENT OF DIAMETER OF TUBULES:

The diameter of the tubules was measured by using a SEM connected to computer device. Two tubules in each grid of the photomicrograph which were partially occluded and not occluded were chosen and diameter was measured. So a total of eighteen tubules [PO×9, NO×9] in each specimen were measured for diameter. Only those tubules that showed an almost circular lumen were selected. The measurement of the smallest diameter across the tubule orifice minimized the error caused by tubules cut obliquely. The measurements were recorded for each tubule present in the photomicrograph from digital display of the numerical data in microns for tubule diameter.

GRADING OF TUBULE PATENCY ***(West et al, 1998)***

Patency of tubules was graded as follows:

Grade A: Smear layer with some tubules just apparent.

Grade B: Less than or equal to 10 tubules visible with majority occluded.

Grade C: Greater than 10 tubules visible with majority occluded.

Grade D: Less than or equal to 10 tubules visible with majority patent.

Grade E: Greater than 10 tubules visible with the majority patent.

ARMAMENTARIUM

Specimen preparation

- Freshly extracted periodontally compromised single rooted teeth
- Graceys curettes no 1&2, 3&4
- Ultrasonic scaler (EMS)
- Airotor handpiece (NSK Co. Japan)
- Flexible diamond disk
- Measuring scale
- Tweezer
- Micromotor with straight handpiece
- Distilled water
- Thymol crystals
- Artificial saliva

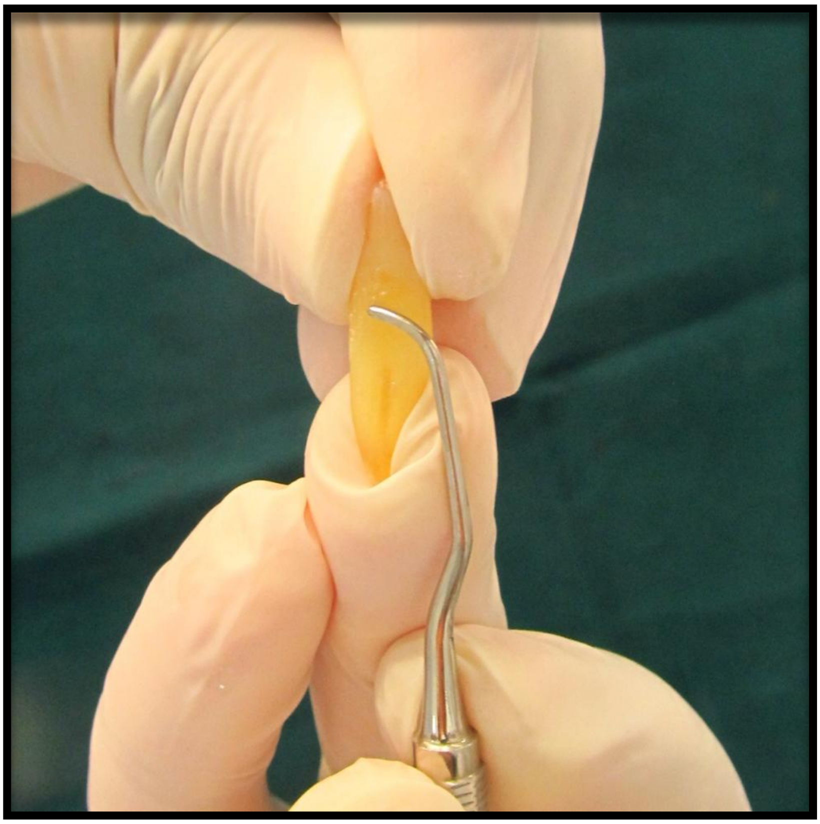
Test agents

- Pro-Argin based dentifrice
- Carbon dioxide Laser Unit(PC015-C MIKRO SURGICAL LASER SYSTEM)
- Laser protection eyewear

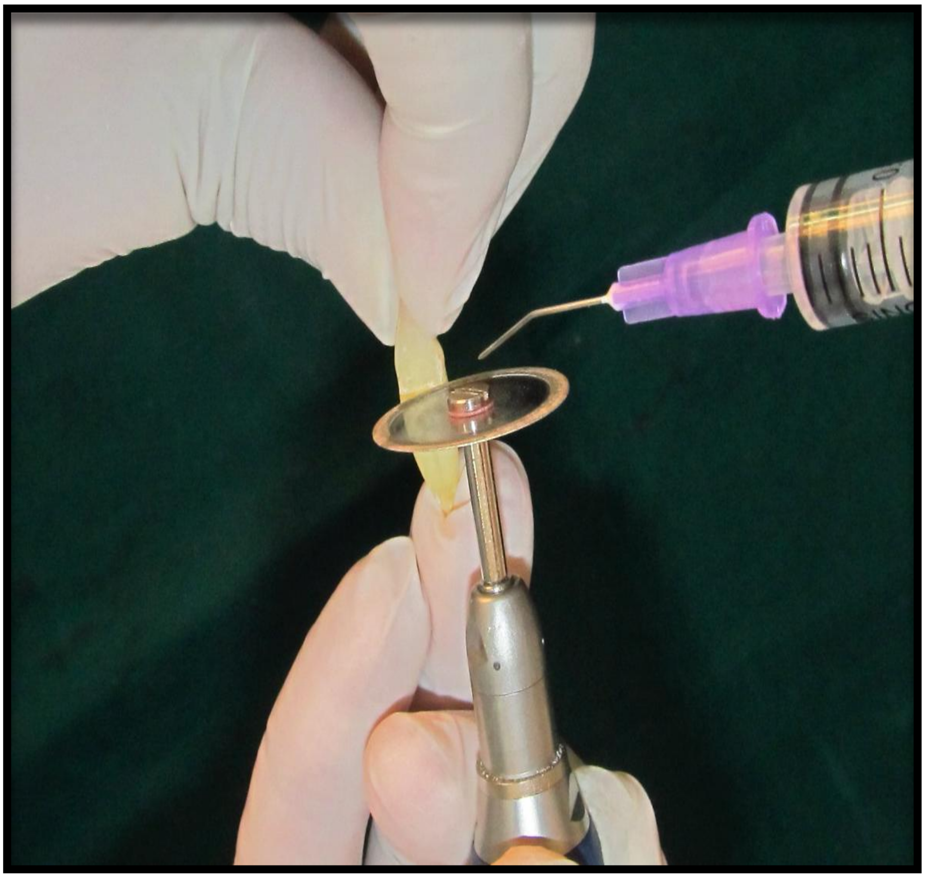
Scanning Electron Microscopic Examination

- 4% Glutaraldehyde (pH 7.2)
- Phosphate buffered saline (PBS) pH 7.2
- Aqueous ethanol (50, 70, 85, 95 &100%)
- Gold sputtering unit (Hitachi E-1010 Ion Sputter)
- Scanning Electron Microscope(Hitachi S-3400 N)

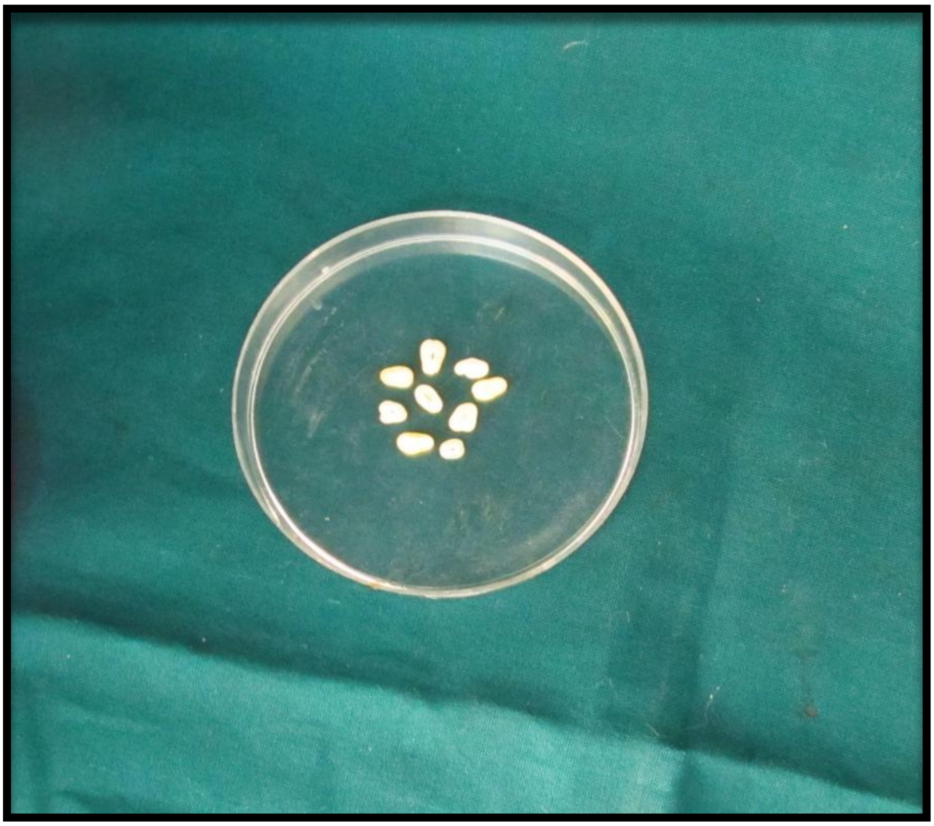
PHOTOGRAPH 2- ARMAMENTARIUM FOR SPECIMEN PREPARATION



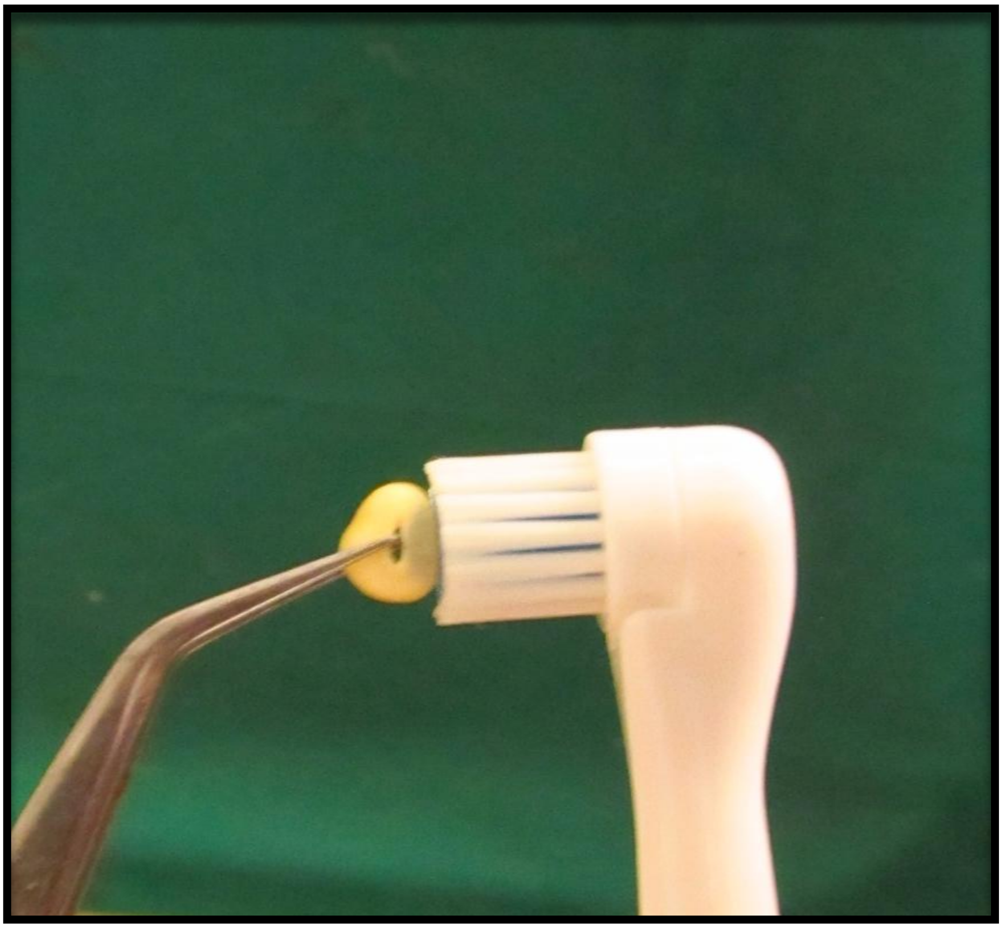
PHOTOGRAPH 3- ROOT PLANING OF THE TOOTH SPECIMEN



PHOTOGRAPH 4- SECTIONING OF THE TOOTH USING DIAMOND DISC

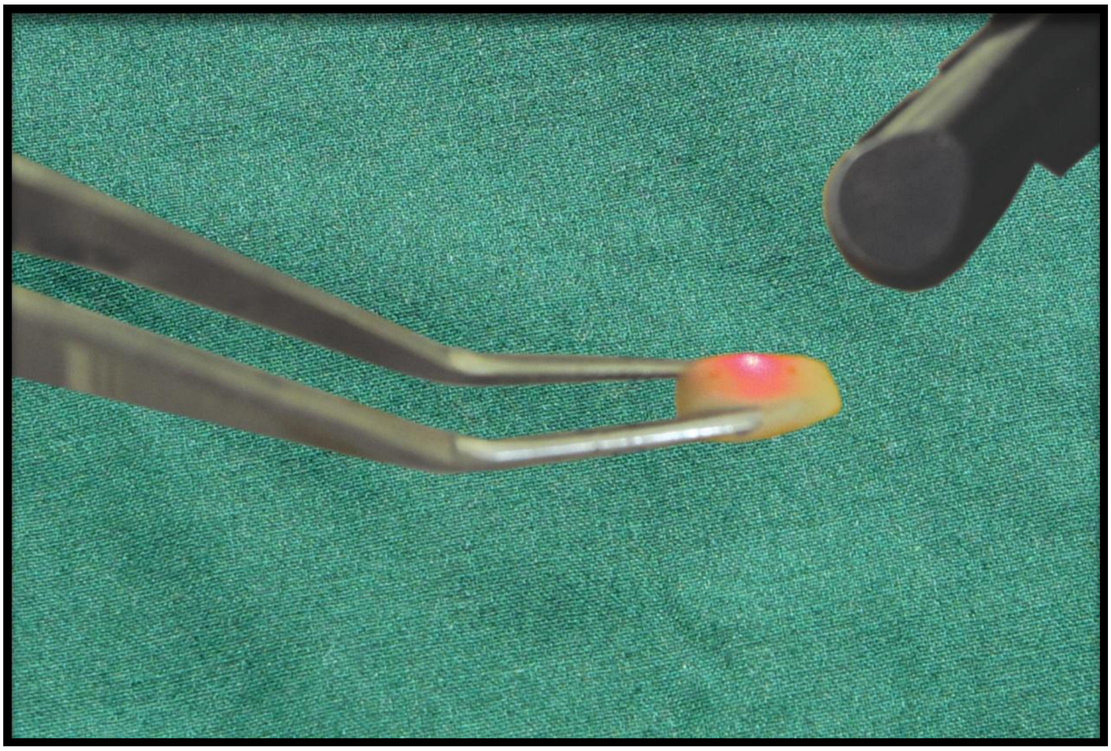


PHOTOGRAPH 5- PREPARED DENTIN DISCS

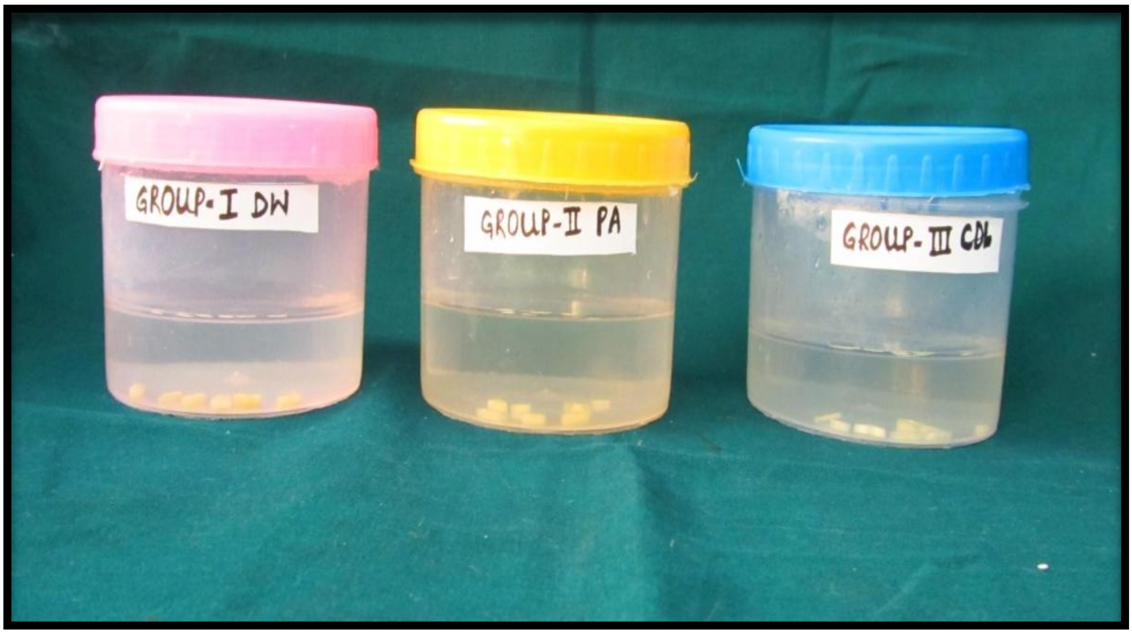


PHOTOGRAPH 6 - BRUSHING OF TEETH WITH PRO-ARGIN BASED DENTIFRICE

PHOTOGRAPH 7 - CARBON DIOXIDE LASER UNIT



PHOTOGRAPH 8 - SPECIMENS SUBJECTED TO CARBON DIOXIDE LASER



PHOTOGRAH 9 - SPECIMENS STORED IN ARTIFICIAL SALIVA

PHOTOGRAPH 10 - FIXING OF SPECIMENS FOR SCANNING ELECTRON MICROSCOPE EVALUATION

PHOTOGRAPH 11- DESSICATOR FOR DEHYDRATING THE SPECIMENS

PHOTOGRAPH 12- GOLD SPUTTERING UNIT (HITACHI E-1010 ION SPUTTER)

PHOTOGRAPH 13- SCANNING ELECTRON MICROSCOPE

STATISTICAL ANALYSIS

The data obtained was statistically analysed using SPSS (version 17) statistical analysis software.

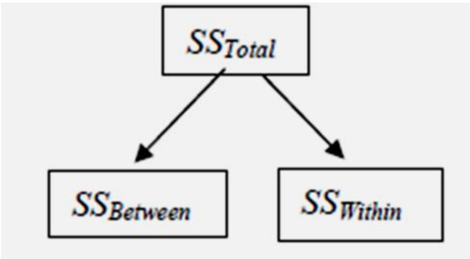
One Way Analysis of Variance (One-Way ANOVA) was used in the present study to compare the tubule occlusion and tubule diameter. It is a way to test the equality of three or more means at one time by using variances. . Sample variances are calculated as *SS/df* and these sample variances are called Mean Squares (*MS*)

A p-value of 0.05 or less was considered for statistical significance.

Formulae used in Analysis

Null Hypothesis states that

H0: μ1 = μ2 = μ3 = . . . = μa



$$\boxed{SS_{Total} = \sum X^2 - \frac{\left(\sum X\right)^2}{N}} \quad \boxed{df_{Total} = N - 1}$$

$$\boxed{SS_{Between} = \frac{\left(\sum X_1\right)^2}{n_1} + \frac{\left(\sum X_2\right)^2}{n_2} + \ldots + \frac{\left(\sum X_a\right)^2}{n_a} - \frac{\left(\sum X\right)^2}{N}} \quad \boxed{df_{Between} = a - 1}$$

$$\boxed{SS_{Within} = SS_{Total} - SS_{Between}} \quad \boxed{df_{Within} = N - a}$$

$$\boxed{s^2 = \frac{SS}{df} = MS} \quad \boxed{F = \frac{MS_{Between}}{MS_{Within}}}$$

SS – Sum of Squares

MS – Mean Squares

N - Total no of observations in the experiment

a - No of groups

$n1$ - no of observations in group 1

F - Observed Variance Ratio

tCrit is the critical value from a *t*-table using the *df* of the error term from the ANOVA table. F > *tCrit at 5%value means that the difference between the groups is significant at the 5% value.*

Turkey HSD Post hoc tests are designed for situations in which a significant omnibus F-test has been obtained with a factor that consists of three or more means and additional exploration of the differences among means is needed to provide specific information on which means are significantly different from each other.

HSD =$\sqrt{MS}within/n$

n - Number of values we are dealing with in each group (not total n).

MS- Mean Square value from the ANOVA that is already computed

RESULTS

A total of 30 dentin specimens were taken for this study. The specimens were divided into 3 treatment groups. Group I Distilled Water (DW) (n=10), group II Pro Argin group (PA) (n=10) and group III Carbon Dioxide Laser (CDL) (n=10). The mean number of tubular occlusion, diameter of tubules and grade of tubular patency were evaluated.

Table II shows the Master chart-I of tubule occlusion in group I, II and III.

Table III shows the Master chart-II of the diameter of tubules in group I, II and III.

COMPARISON OF MEAN OF DENTINAL TUBULES OCCLUDED (Table-IV & V, Figure- 1, 2 &3)

In DW group, a mean of 6.00 ± 2.789 tubules were completely occluded, 26.30 ± 14.150 were partially occluded, and 286.140 ± 28.356 were not occluded. In PA group, a mean of 232.00 ± 13.333 tubules were completely occluded, 61.10 ± 8.034 were partially occluded and 23.10 ± 4.954 were not occluded. In CDL group, a mean of 285.30 ± 22.637 tubules were completely occluded, 21.50 ± 7.184 were partially occluded, and 7.80 ± 1.932 were not occluded.

Intergroup comparisons analysed by One Way ANOVA test for mean of Completely Occluded Tubules (COT), Partially Occluded Tubules (POT), and Not Occluded Tubules (NOT) showed a highly significant difference ($p=0.000$) between the three groups.

For the mean of Completely Occluded tubules (COT), Post Hoc test, showed highly significant differences between DW group – PA group (p=0.000), PA group – CDL group (p=0.000), DW group – CDL group (p=0.000).

For the mean of Partially Occluded Tubules (POT), Post Hoc test, showed a highly significant difference between DW group – PA group (P=0.000), PA group – CDL group (p=0.000) and no significant difference between DW group – CDL group (p=0.556)

For mean of Not Occluded Tubules (NOT), Post Hoc test, showed highly significant differences in DW group – PA group (p=0.000), PA group – CDL group (p=0.000), DW group – CDL group (p=0.000).

COMPARISON OF DIAMETER OF DENTINAL TUBULES (Table-VI & VII, Figure 4 &5)

In DW group, the mean diameter of tubules was 2.42 ± 0.25 μm for Partially Occluded Tubule (POT) and 2.96 ± 0.08 μm for Not Occluded Tubules (NOT). In PA group, the mean diameter of tubules was 1.73 ± 0.58 μm for Partially Occluded Tubules (POT) and 3.01 ± 0.90 μm for Not Occluded Tubules (NOT). In CDL group, the mean diameter of tubules was 1.22 ± 0.35 μm for Partially Occluded Tubules (POT) and 3.03 ± 0.10 μm for Not Occluded Tubules (NOT).

For diameter of Partially Occluded tubules (POT), on intergroup comparison by One Way ANOVA test there was a highly significant difference between three groups (p=0.000). By Post Hoc test, significant difference was seen between DW group – PA group (p=0.003), PA group – CDL group (p=0.028) and also in DW group - CDL group (p=0.005).

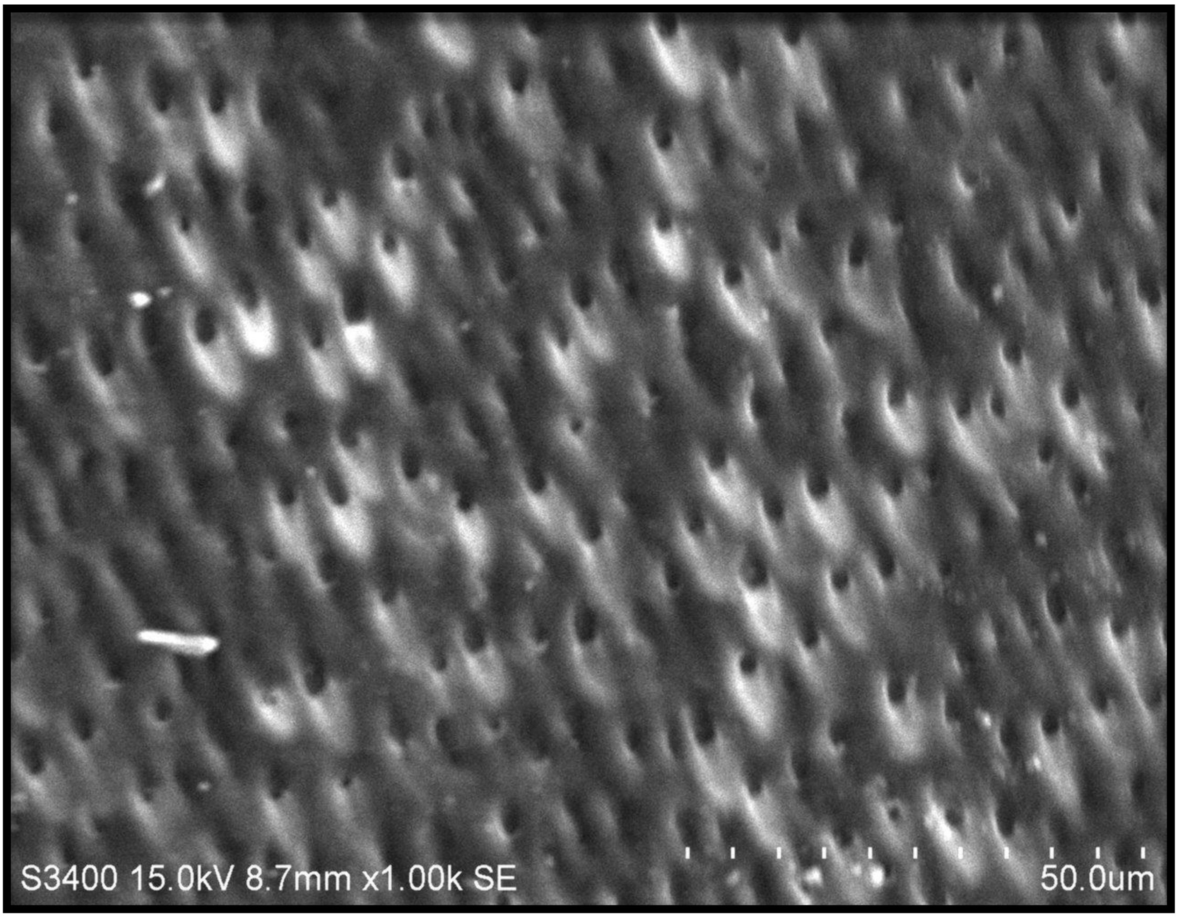
For diameter of Not Occluded tubules, on intergroup comparison by One Way ANOVA test, there was no significant difference between three group ($p=0.94$).

Table VIII- shows the overall comparison of dentinal tubule occlusion and diameter between the group I, II and III.

GRADING OF TUBULE PATENCY (Table-IX)

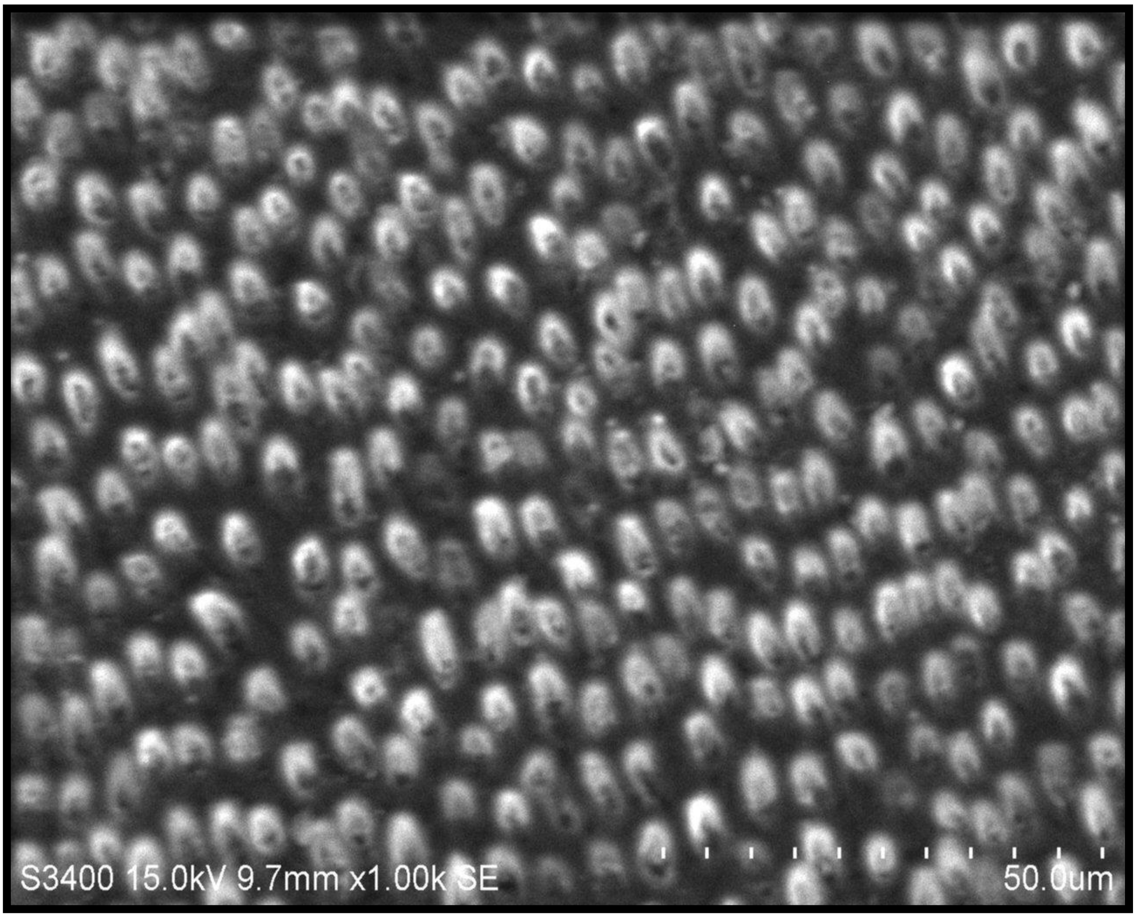
The patency of tubules in each photomicrograph were graded A to E according to ***West et al***. In DW group, all photomicrographs showed greater than 10 tubules visible with the majority patent (Grade E), in PA group majority (80%) of the photomicrographs showed greater than 10 tubules visible with the majority patent (Grade E), and in CDL group, all photomicrographs showed greater than 10 tubules visible with majority occluded (Grade C).

PHOTOMICROGRAPH-1



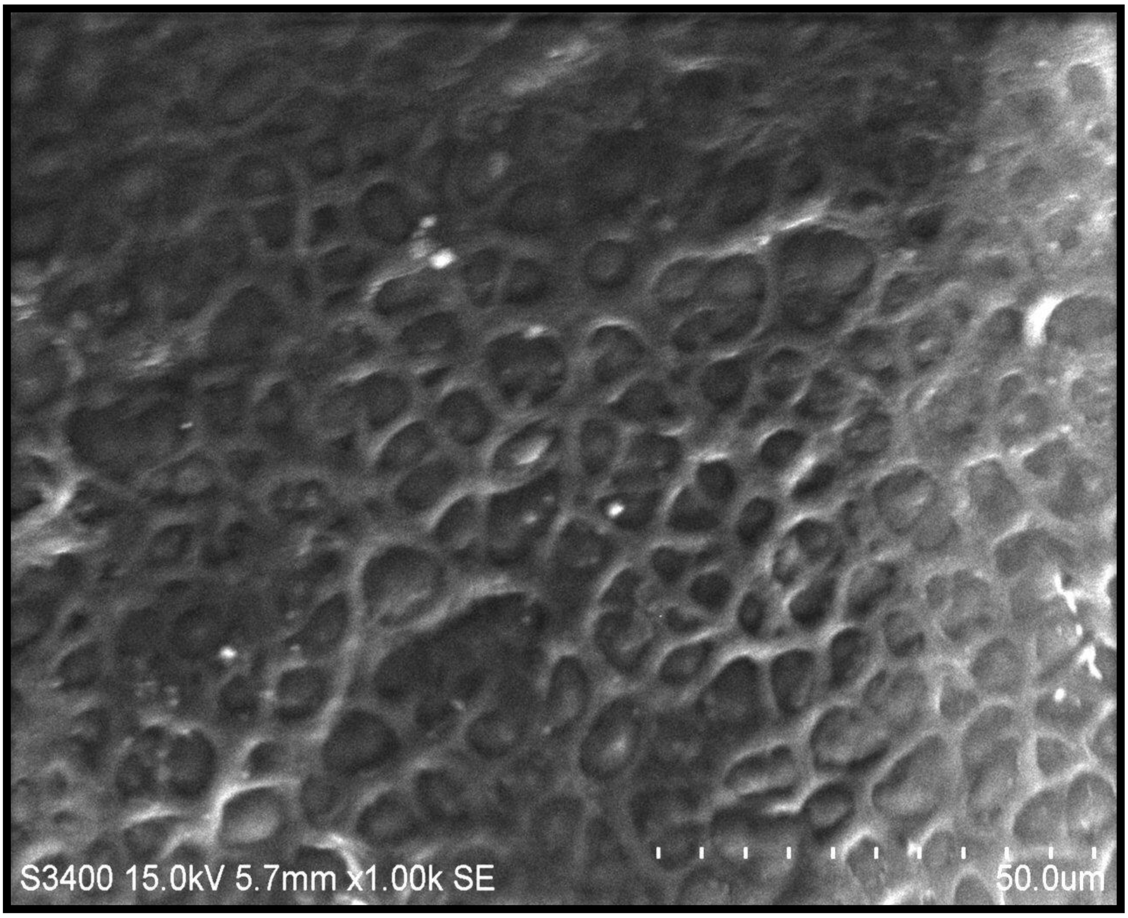
Photomicrograph of specimens treated with Distilled Water

PHTOMICROGRAPH-2



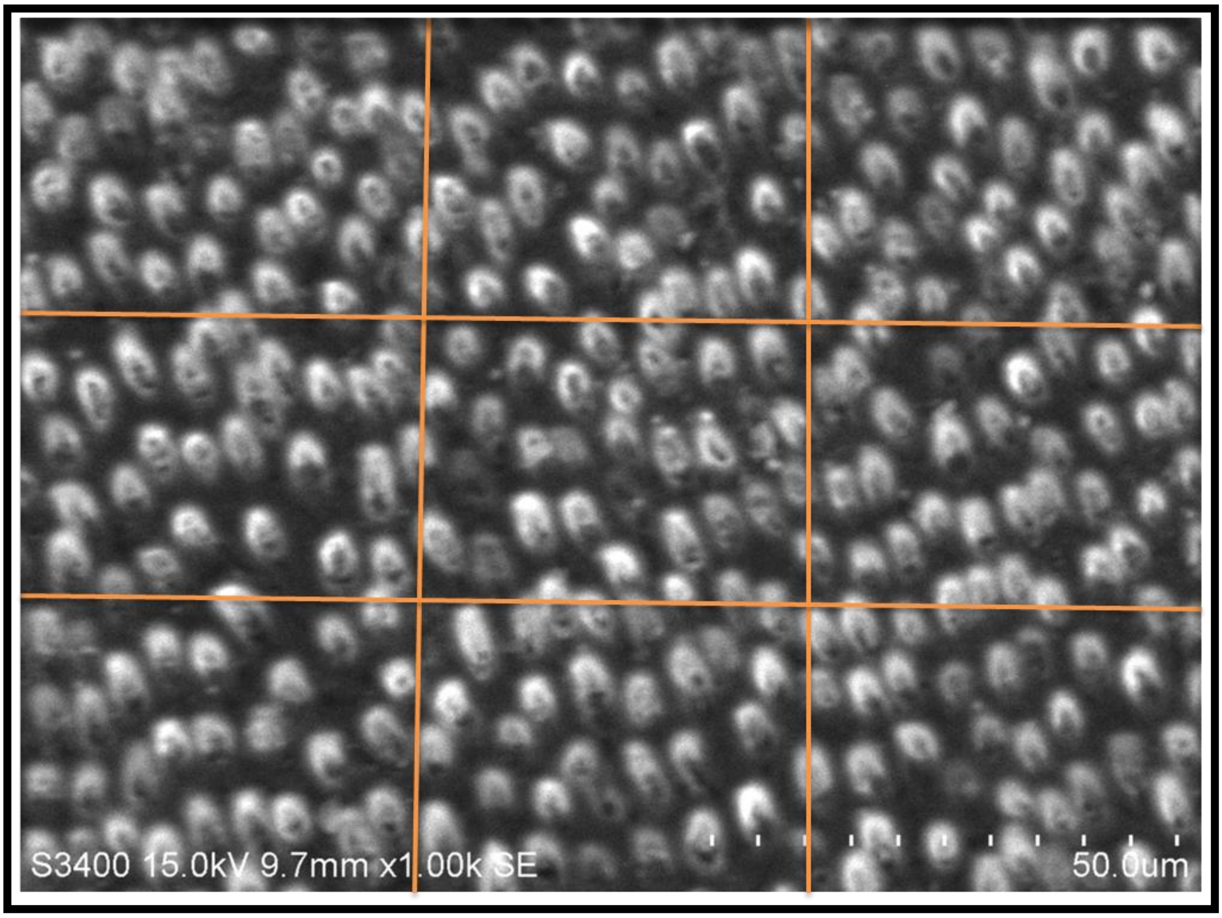
Photomicrograph of specimens treated with Pro-Argin based Dentifrice

PHOTOMICROGRAPH-3



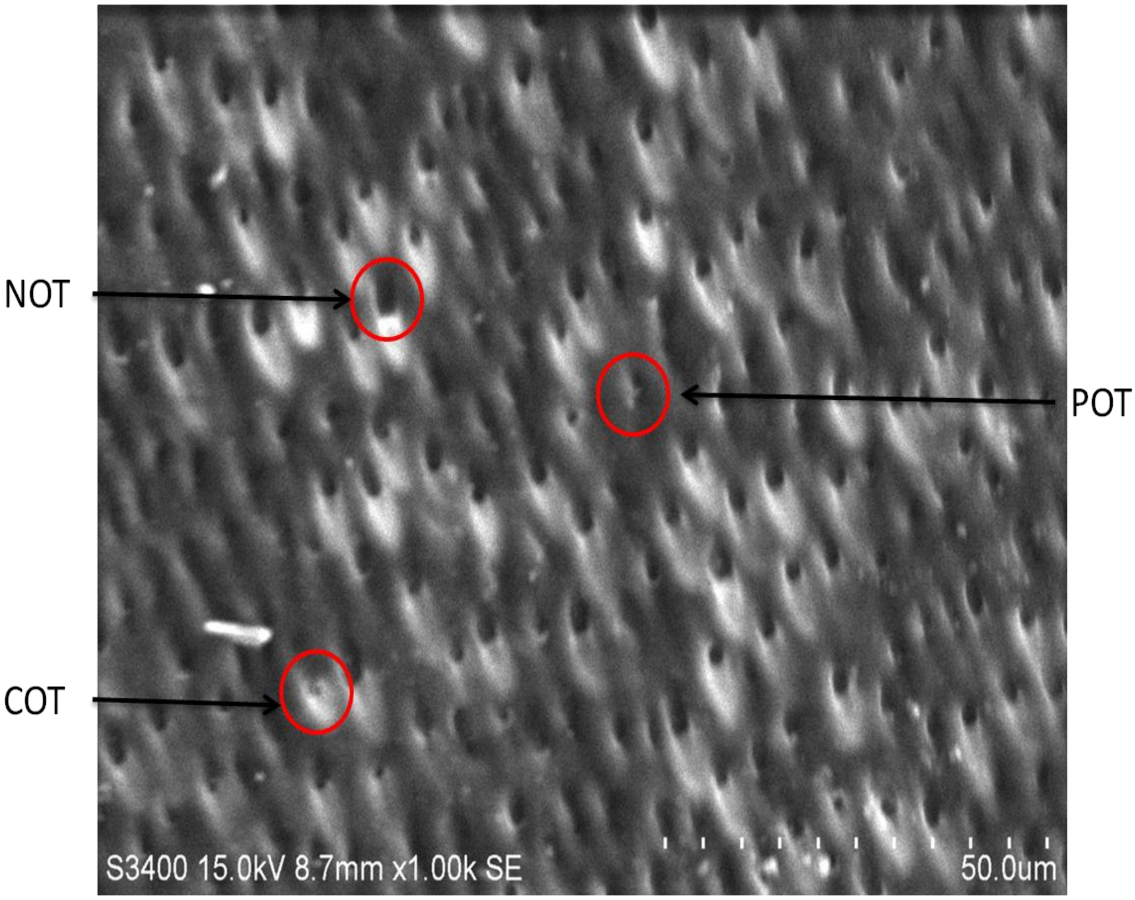
Photomicrograph of specimens irradiated with Carbon Dioxide Laser

PHOTOMICROGRAPH-4



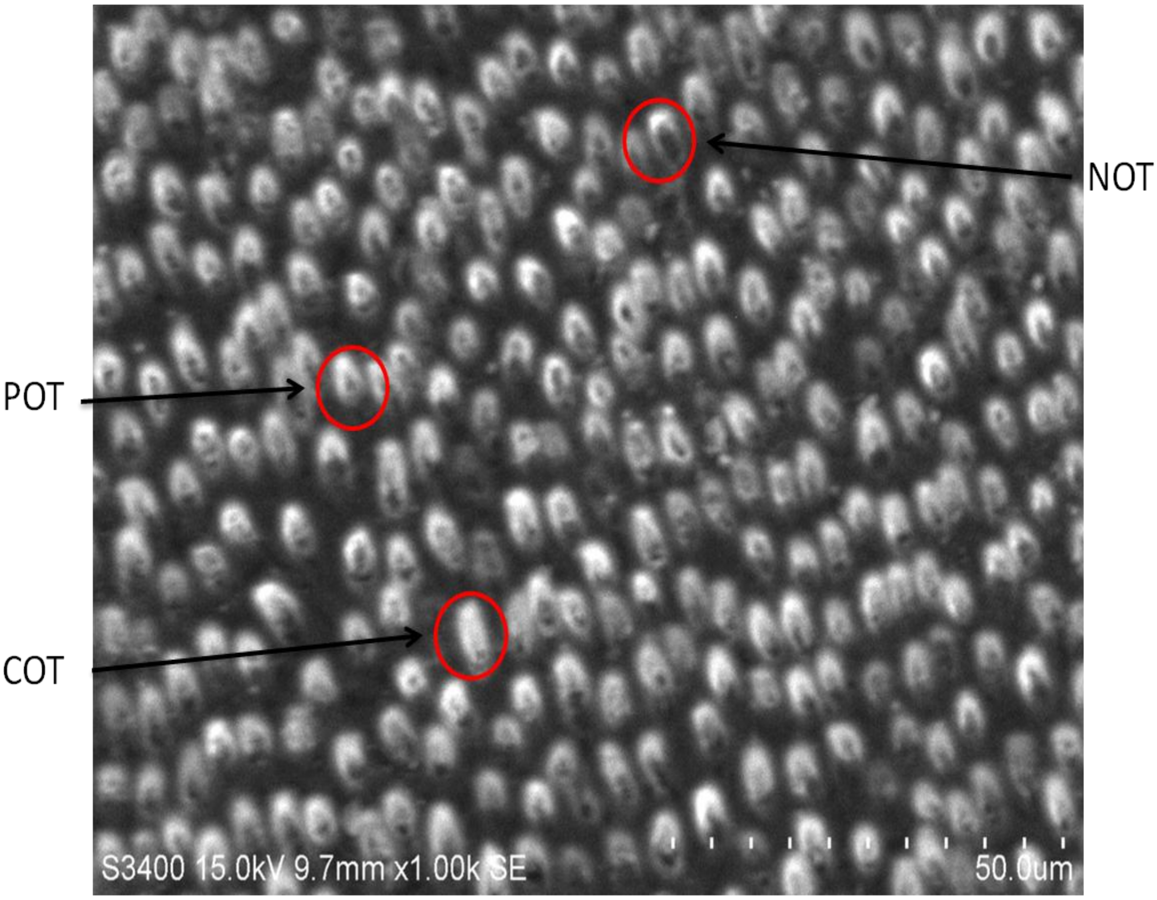
Photomicrograph showing application of grid lines

PHOTOMIICROGRAPH-5



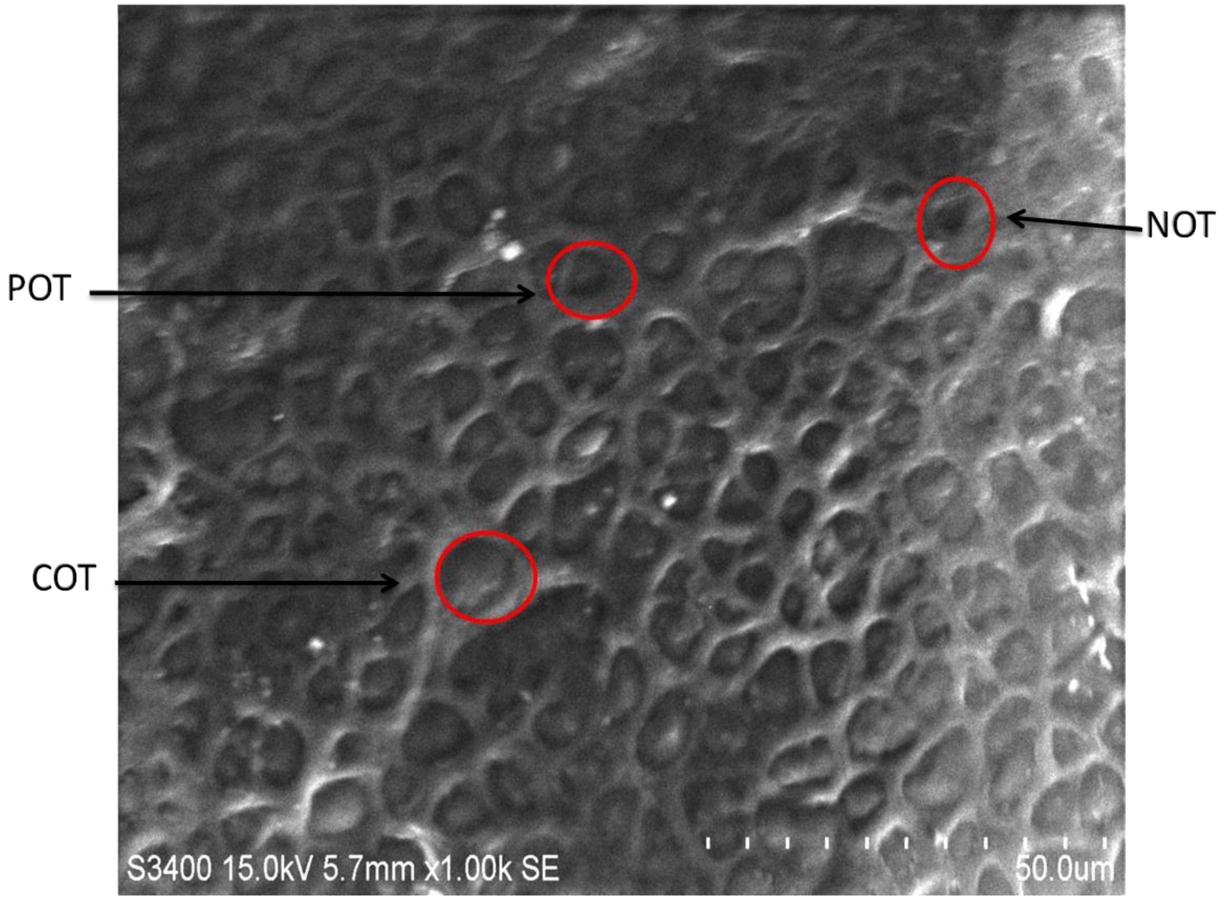
Photomicrograph showing Completely Occluded Tubule (COT), Partially Occluded Tubule (POT) and Not Occluded Tubule (NOT) in specimen belonging to Distilled Water

PHOTOMICROGRAPH-6



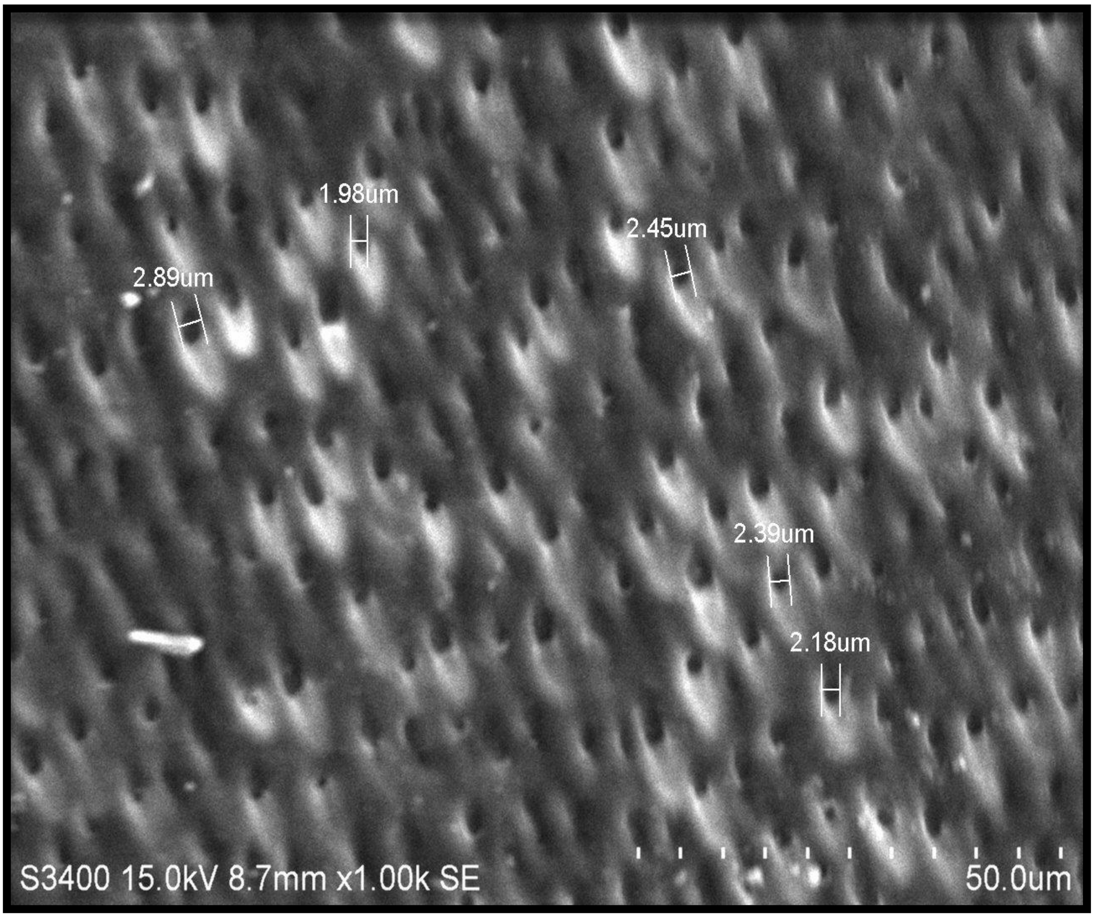
Photomicrograph showing Completely Occluded Tubule (COT), Partially Occluded Tubule (POT) and Not Occluded Tubule (NOT) in specimen belonging to Pro- Argin group

PHOTOMICROGRAPH-7



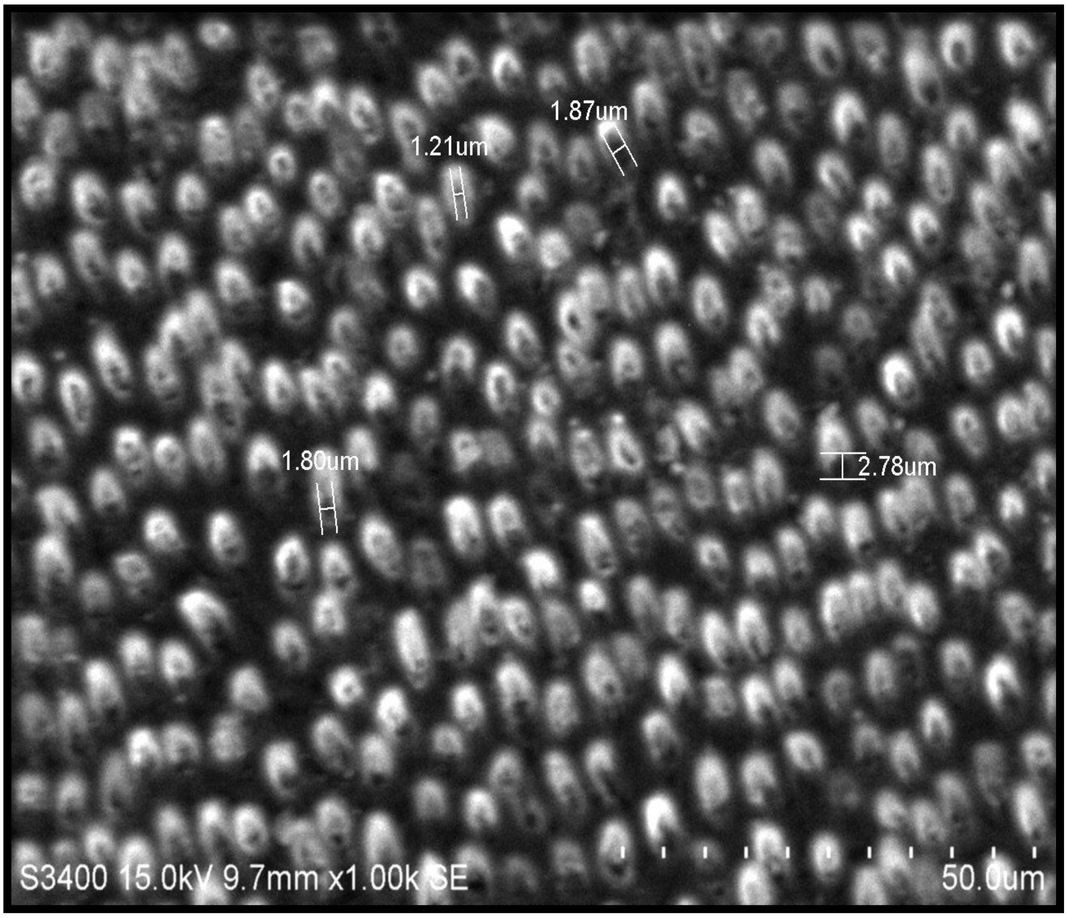
Photomicrograph showing Completely Occluded Tubule (COT), Partially Occluded Tubule (POT) and Not Occluded Tubule (NOT) in specimen belonging to Carbon Dioxide Laser group

PHOTOMICROGRAPH-8



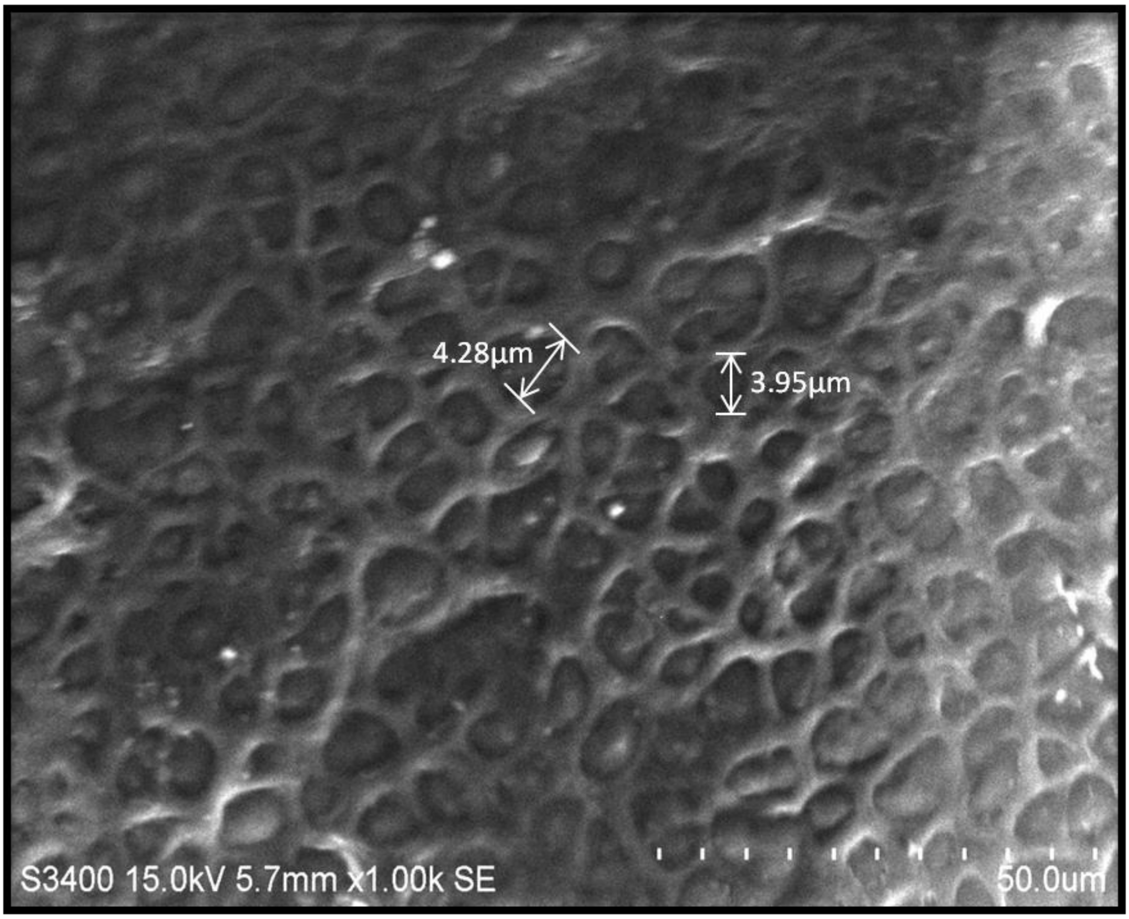
Photomicrograph showing diameter of Partially Occluded and Not Occluded tubules in specimen belonging to Distilled Water group

PHOTOMICROGRAPH-9



Photomicrograph showing diameter of Partially Occluded and Not Occluded tubules in specimen belonging to Pro-Argin group.

PHOTOMICROGRAPH-10



Photomicrograph showing diameter of Partially Occluded and Not Occluded tubules in specimen belonging to Carbon Dioxide Laser group.

TABLE-II

MASTERCHART- I

	GROUP 1				GROUP 2				GROUP 3			
S.n	**NO. of tubules**	**COT**	**POT**	**NOT**	**No. of tubules**	**COT**	**POT**	**NOT**	**No. of tubules**	**COT**	**POT**	**NOT**
1	285	6	63	216	326	240	60	26	359	324	36	9
2	316	3	18	295	335	239	68	30	296	275	23	8
3	352	13	24	315	346	245	73	32	327	287	29	11
4	334	5	33	296	314	232	59	23	315	284	25	6
5	298	7	22	269	326	242	63	21	263	233	20	10
6	326	5	16	305	293	202	68	23	317	295	15	7
7	319	6	25	288	309	239	51	19	306	288	11	7
8	338	7	24	307	299	235	46	18	309	283	17	9
9	306	4	26	276	315	230	63	22	323	298	20	5
10	313	4	12	297	303	216	60	17	311	286	19	6

TABLE-III

MASTERCHART-II

	Group 1		Group 2		Group 3	
S. n	**Diameter of POT**	**Diameter of NOT**	**Diameter of POT**	**Diameter of NOT**	**Diameter of POT**	**Diameter of NOT**
1	2.89	3.01	1.21	2.91	1.42	3.14
2	2.45	2.98	1.8	3.01	1.55	3.1
3	2.39	3.01	2.78	3.15	1.79	2.93
4	2.18	2.79	1.39	3.07	1.12	2.94
5	2.37	3.01	1.38	2.96	0.82	3.17
6	1.98	2.92	1.38	3.11	0.98	3.09
7	2.43	3.07	0.98	2.95	0.71	2.93
8	2.52	2.88	1.87	3.02	1.58	2.88
9	2.32	2.98	2.53	3.06	1.12	3.11
10	2.69	3	2.05	2.86	1.13	3.01

TABLE-IV: COMPARISON OF NUMBER OF TUBULE OCCLUSION IN THREE GROUPS

TREATMENT GROUPS	PARTICULARS	COMPLETELY OCCLUDED	PARTIALLY OCCLUDED	NOT OCCLUDED
Group- I Distilled Water (DW)	n Mean ± SD	10 6.00±2.78	10 26.30±14.15	10 286.40±28.35
Group-II Pro- Argin (PA)	n Mean ± SD	10 232.00±13.33	10 61.10±8.03	10 23.10±4.95
Group- III Carbon Dioxide Laser (CDL)	n Mean ± SD	10 285.30±22.63	10 21.50±7.18	10 7.80±1.93
	p- Value*	0.000 (HS)	0.000 (HS)	0.000 (HS)

*One Way ANOVA, P<0.001: Highly significant, P<0.05: Significant,

P>0.05: Not significant

TABLE-V: COMPARISON OF NUMBER OF TUBULE OCCLUSION BETWEEN THREE GROUPS

	Treatment group	p-value# for Completely Occluded tubules	p-value# for Partially Occluded tubules	p-value# for Not Occluded tubules
Comparison of tubule occlusion between Groups	Group-I – II	0.000 (HS)	0.000 (HS)	0.000 (HS)
	Group II – III	0.000 (HS)	0.00 (HS)	0.119 (NS)
	Group I – III	0.000 (HS)	0.556 (NS)	0.000 (HS)

Post Hoc test, P<0.001: Highly significant, P<0.05: Significant,

P>0.05: Not significant

TABLE-VI: COMPARISON OF THE DIAMETER OF PATENT TUBULES IN THE THREE GROUPS

Treatment groups	Partially Occluded	Not Occluded
	Mean ± SD range µm	Mean ± SD range µm
Group I **Distilled Water (DW)**	2.42 ± 0.25	2.96 ± 0 .08
Group II **Pro-Argin (PA)**	1.73 ± 0.58	3.01 ± 0.09
Group III **Carbon Dioxide Laser (CDL)**	1.22 ± 0.35	3.03 ± 0.10
p-value*	0.000	

*One Way ANOVA test, P<0.001: Highly significant, P<0.05: Significant,

P>0.05: Not significant

TABLE-VII: COMPARISON OF DIAMETER OF PARTIALLY OCCLUDED TUBULES BETWEEN THREE GROUPS

	Treatment groups	p- value#
Diameter of Partially Occluded Tubules between the groups	DW-PA	0.003 (S)
	PA-CDL	0.028 (S)
	DW-CDL	0.000 (HS)

Post Hoc test, P<0.001: Highly significant, P<0.05: Significant,

P>0.05: Not significant

TABLE-VIII- OVERALL COMPARISON OF NUMBER OF TUBULE OCCLUSION AND DIAMETER OF TUBULES BETWEEN THE THREE GROUPS

TUBULE OCCLUSION	MEAN NUMBER OF TUBULES	MEAN DIAMETER OF TUBULES
COMPLETELY OCCLUDED TUBULES	CDL>PA>DW	NA
PARTIALLY OCCLUDED TUBULES	PA>DW>CDL	DW>PA>CDL
NOT OCCLUDED TUBULES	DW>PA>CDL	CDL>PA>DW

TABLE- IX: GRADING OF TUBULE PATENCY (West et al, 1998)

SAMPLE	DW Group	PA Group	CDL Group
1	E	E	C
2	E	E	C
3	E	E	C
4	E	C	C
5	E	E	C
6	E	E	C
7	E	E	C
8	E	E	C
9	E	C	C
10	E	E	C

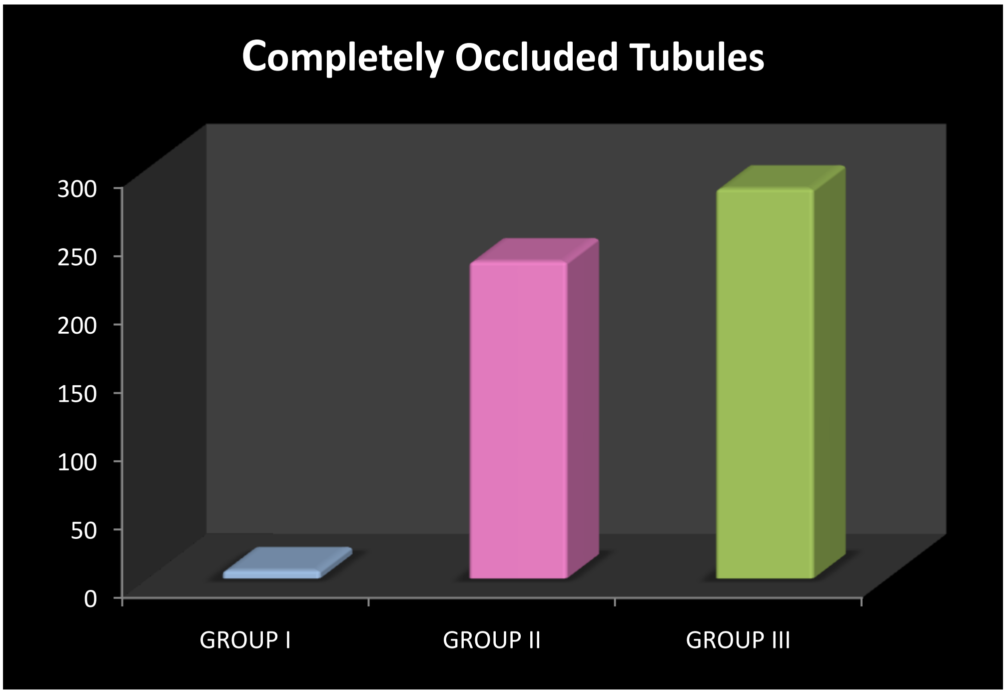


Figure 1- Comparison of number of Completely Occluded tubules between the three groups

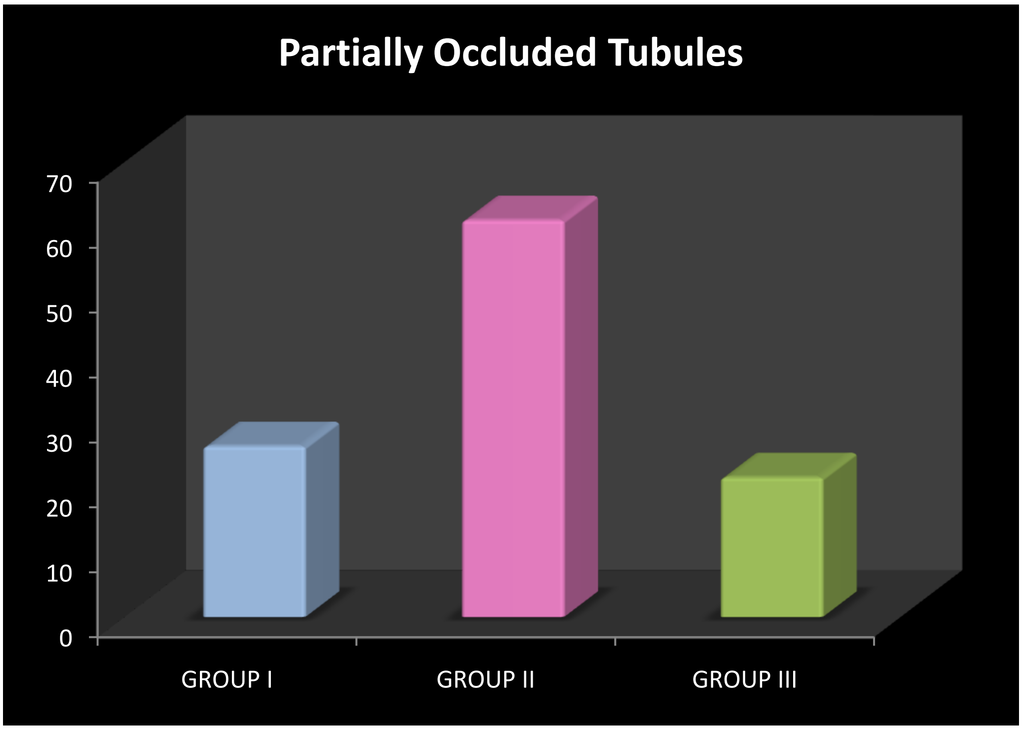


Figure 2- Comparison of number of Partially Occluded tubules between the three group

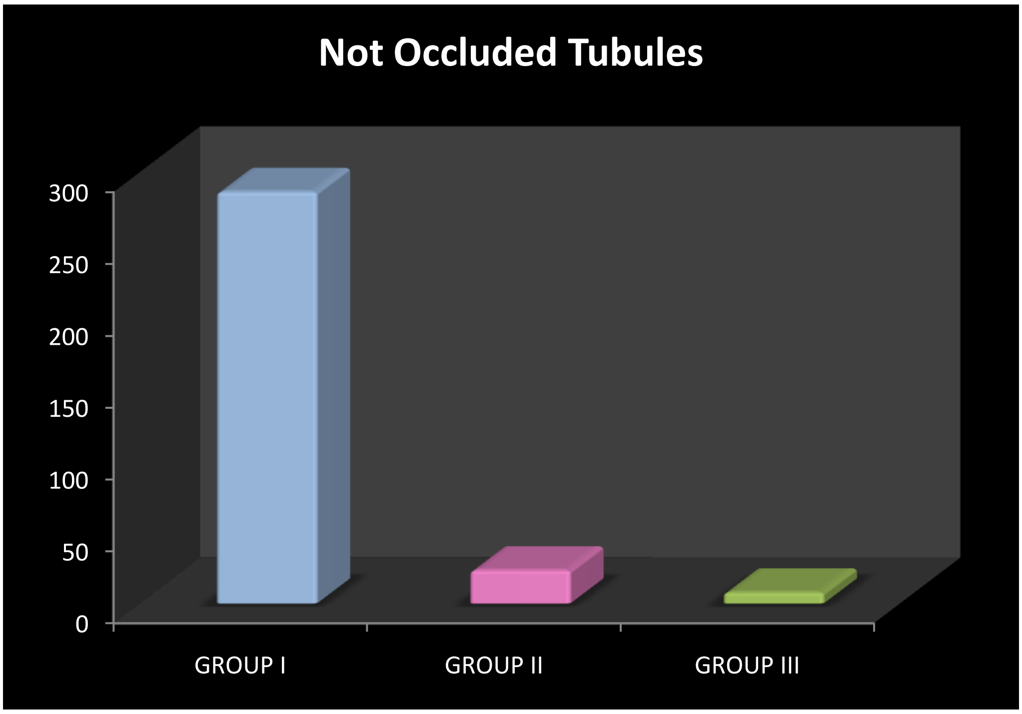


Figure 3- Comparison of number of Not Occluded tubules between the three groups

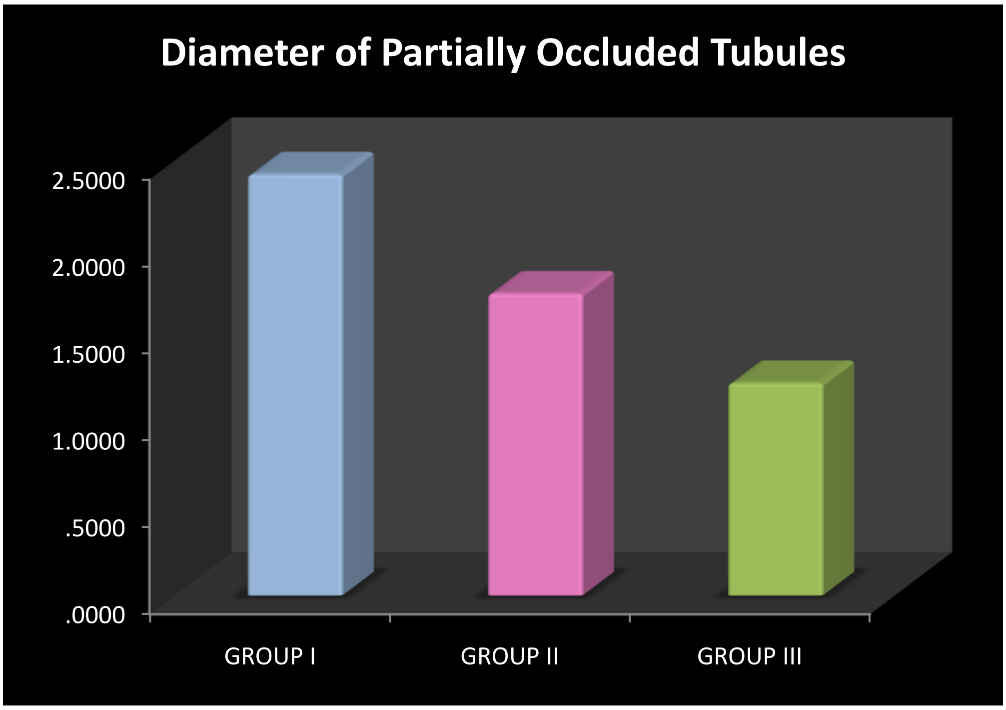


Figure 4- Comparison of diameter (in µm) of Partially Occluded tubules between the three groups

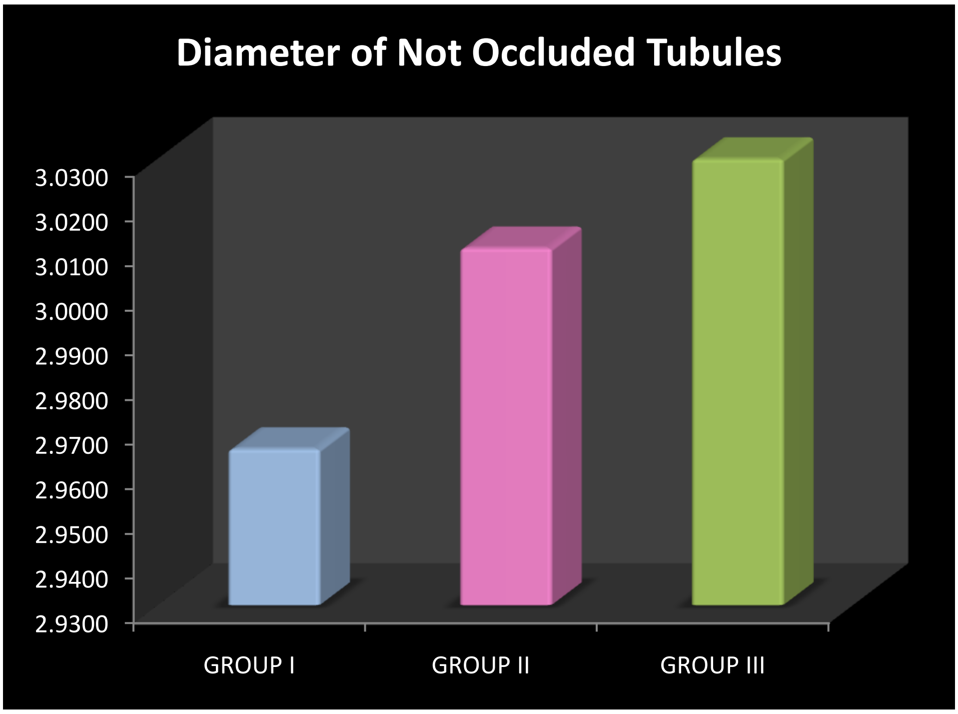


Figure 5-Comparison of diameter (in µm) of Not Occluded tubules between the three groups

DISCUSSION

Dentin hypersensitivity is characterised by short, sharp pain arising from exposed dentin in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical, which cannot be ascribed to any other dental defect or pathology.[30] This definition was adopted in the International Workshop on Dentin Hypersensitivity.[54]

Discomfort from dentin hypersensitivity is a common finding in adult population, with the available prevalence data ranging from 8–57%.[38,71] It was found to be much higher in periodontal patients, ranging between 72.5–98%.[24] In a systematic review conducted by ***Von Troil et al,***[113] it was concluded that root sensitivity occurs in approximately half of the patients following scaling and root planing.

Several hypotheses have been proposed for dentin hypersensitivity, among which perhaps the most widely accepted is the hydrodynamic mechanism which was first proposed by ***Gysi***[50] in the year 1900 and later studied heavily and correlated by ***Brannstrom***.[31] ***Brannstrom***[17] theorised that according to Hagen-Poseuille's equation of fluid dynamics, the contained fluid would obey the same physical laws as liquids in glass capillaries. It means that the movement of dentinal fluid in a tubule is proportional to the fourth power of tubular radius and the pressure between the two ends of the tubule. Therefore, doubling the tubule diameter results in sixteen fold increase in fluid flow and vice versa.[79] With this information the treatment by occluding the dentinal tubules with various desensitizing agents sounds plausible.

Historically, several chemical and physical agents have been studied. These agents include fluoride containing solutions/compounds,[81] oxalates,[99] potassium chloride or strontium chloride,[87] potassium nitrate,[104] amorphous calcium phosphates,[42] resin-based bonding agents and lasers.[59]

Recently, the advent of latest Pro- Argin technology has given a new direction to the treatment of dentin hypersensitivity. It has been stated that arginine provides far better benefits than other desensitizing dentifrices and one of the reason for this could be the natural occurrence of arginine in the saliva. At physiological pH arginine and calcium carbonate interact and bind to negatively charged dentin surface and dentinal tubules to plug and seal them. In the present study, a dentifrice containing 8% arginine along with calcium carbonate and 1450 ppm of fluoride was evaluated for its efficacy to occlude patent dentinal tubules.[106]

For the laser to be effective, it should have wavelength equal to the target tissue so that the radiations are absorbed well, with very little transmission to the surrounding tissues. The carbon dioxide laser satisfies this criterion as it operates in infra-red region and enamel, dentin and cementum also have absorption in this region due to the presence of hydroxyapatite crystals. This implies that the radiation from Carbon Dioxide Laser will be effectively absorbed by the dental hard tissue with minimal effect on the surrounding tissues. The present study, we have utilized Carbon Dioxide Laser to evaluate its effect on patent dentinal tubules.[97]

In the present study, single rooted teeth were used for dentin disc preparation, in analogous to the study by ***Komabayashi T et al***[66] who have also utilized single rooted teeth. Several studies have been done using incisors,[23] canines,[66] premolars,[10] and molars[72] for studying dentin permeability, numerical density of tubules and tubule diameter.

According to the study by ***Vieira AP et al,***[112] dental fluorosis affects the dentin structure and dentin fluoride level is correlated with dental fluorosis, dentin tubule size and density. Therefore, in the present study, teeth with fluorosis were excluded.

In this study, the extracted teeth were stored in 0.5% thymol similar to the study by ***Wang Z et al***.[115] However, other storage media such as 5% phosphate buffered saline solution,[102] 10% formalin,[11] Hank's balanced salt solution[66] and 0.1% sodium cacaodylate buffer[80,44] had also been used in various studies.

Dentin disc has been used extensively by ***Pashley DH et al.***[87] as a model for assessing the surface deposition, tubule-occluding effects of desensitizing agents as well as the effect of these agents on fluid flow through dentin (hydraulic conductance).[48,3,64] Therefore, we have also used dentin disc for evaluating the test agents. Moreover, the dentin disc would appear to be the method of choice because it is easy to use, reproducible and provides a flat surface for elemental analysis.

In the present study, the specimens were scaled and root planed using EMS scaler and area specific Gracey curette number 1/2, 3/4 in an apicocoronal direction. After root debridement, the teeth was sectioned 2mm below the cementoenamel junction, with double-sided diamond disks under saline irrigation at low speed and this is similar to ***Carlos de Paula Eduardo et al.***[22] who used steel disks with water irrigation.

In this study, dentin disc thickness of 2 mm was made in cross section from the radicular portion of the teeth in accordance to ***Russ P. Read et al***.[97] Some authors in previous studies have used dentin disc thickness of 3mm, [11] 1.5mm longitudinal section,[107] 1.0 mm cross section for studying hypersensitivity[102,80] to as low as 0.5 mm[107] for dentin permeability measurement.

In the present study, 30 dentin specimens were prepared and divided into three groups with 10 specimens in each group (n=10). The sample size in each group was in line with the study by ***Arrais et al***.[11]

In preparing the samples, we employed the polishing technique previously used by ***Komabayashi et al***,[66] using silicon carbide papers of different grit sizes which were intended to render the dentin surface as smooth as possible and to create a standard smear layer.

In this study acid, etching was done to remove any residual smear layer and to simulate dentin hypersensitivity by using 6% citric acid for 2 minutes this is in accordance to ***Haznedaroglu et al***.[51] who concluded that low concentrations are better in smear layer removal. In previous studies, other chemicals that have been used are EDTA,[10] 37% phosphoric acid.[72]

It has been proposed that saliva can solubilise materials adhering to teeth and contains calcium and phosphate ions that can interact with tooth surfaces.[40] Therefore, it is essential to evaluate that whether desensitising agents could occlude dentinal tubules effectively under the circumstances similar to the oral environment. Thus, in this study the specimens were stored in artificial saliva, the composition of which was similar to the one used by ***Arrais et al,***[11] after each experimental session.

It is well known that brushing is the usual mode of application of toothpastes. ***Wang Z et al***[115] had brushed the specimens using medium bristle toothbrush applied to the dentin surface at an inclination of about 90° under constant loading for 300 strokes per minute for 2 minutes. In the present study, brushing was performed with an electric toothbrush which vibrated at 6800 strokes per minute for 2 min. In an attempt to standardize the brushing force, brushing was done by the same individual for all the specimens as was done by ***McAndrew et al.***[76]

The most common method used to evaluate number and diameter of mineralized or demineralized dentin sections is a parametric estimation of SEM microphotographs.[76,61,118] It can be done with different magnifications ranging from 200x,[10] 500x,[102] 1000x,[44,11,122,65] 1500x,[83] 2000x,[44,66] 2500x,[83] 5000x[51] up to 15000x.[102,21] In this study, a magnification of 1000x was used for calculating both number and diameter similar to previous studies as mentioned.

In this study, we had utilized Corel draw version 5 software to apply a nine square grid over the photomicrograph to facilitate counting of tubules it is similar to the study by ***McAndrew et al***,[76] in which they applied a 12 square grid over the photomicrograph. Diameter of tubules was measured using SEM connected to a computer-assisted image analyzer.

The result of the present study was expressed in mean. This type of expression was used in a study by ***Carlos de Paula Eduardo et al.***[22]

In the present study, the mean diameter of tubules not occluded/patent ranged from 1.22 ± 0.35µm to 3.03 ± 0.10 µm, which is in excellent agreement with ***Lopes et al***.[72] They had used human molars and after etching with 37 % phosphoric acid for 20 seconds observed that the diameter of dentinal tubules at superficial to deep dentin ranged from 2.42 ± 0.56 µm to 2.99 ± 0.44 µm. Though in the current study, single rooted teeth were utilized but the results corroborates with their result.

In the present study, the mean number of tubules Completely Occluded (CO) was shown to be highest in CDL group followed by PA and DW group. In CDL group, a mean of 285.30 ± 22.63 of tubules were Completely Occluded and 21.50 ± 7.18 tubules were Partially Occluded. The surface of the specimens showed fusion, recrystallization with the formation of fusion pores and carbonization. The results are in line with the work of the ***A. C. C. C. Romano et al***, [7] who also observed fusion, carbonization and more than 50% of dentinal tubules blocked when dentin disc specimens were irradiated with carbon dioxide laser at 0.5W.

In this study, the mean of tubules Completely Occluded in PA group was 232 ± 13.33, Partially Occluded was 61.61 ± 8.034. The results are in contrary to those achieved by ***Stacey Ann Lavender et al***,[106] who observed complete obliteration of all the tubules. The reason for this may be related to the use of thin dentin discs of 800µm. Secondly, the method of dentifrice application was also different as they had applied the dentifrice using camel hair brush and then left the specimens undisturbed for 15 minute after which they were stirred in PBS. The cycle was repeated 5 times. But we had followed the method used by ***Wang Z et al.***[115]

Distilled water was used consistently as negative control in previous studies for comparing desensitizing agents. In the present study, DW group showed the highest number of patent tubules followed by PA and CDL groups. The mean of tubules completely and partially occluded were 6.00 ± 2.79 and 26.30 ± 14.15

respectively in DW group. These findings are in agreement with a study by ***Arrais et al.***[11]

In the present study, the CDL group showed a highest reduction in tubule diameter from 3.03 ± 0.10 μm to 1.22 ± 0.35 μm as compared to PA and DW groups. The PA group showed a reduction in tubule diameter from 3.01 ± 0.09 μm to 1.73 ± 0.58 μm. Since, there are no previous studies in the literature to our knowledge that have evaluated the reduction in tubule diameter for these agents; therefore, the data from the present study could not be compared.

In the present study, an overall interpretation showed that CDL group showed maximum number of Completely Occluded tubules and a maximum reduction in tubule diameter followed by PA and DW group.

West et al,[122] devised a grading system to record the surface characteristics of dentin specimens analysed by SEM. The photomicrographs were graded as follows; A-smear layer with some tubules just apparent; B- less than or equal to 10 tubules visible with the majority occluded; C- greater than 10 tubules visible with the majority occluded; D- less than or equal to 10 tubules visible with the majority patent; and E-greater than 10 tubules visible with the majority patent.

In the present study, an attempt was made to grade the photomicrographs according to ***West et al.*** All photomicrographs belonging to DW group fell under grade E (greater than 10 tubules visible with the majority patent), and 80% of photomicrographs in PA fell under grade E (greater than 10 tubules visible with the majority patent) and all photomicrographs in CDL group were under grade C (greater than 10 tubules visible with the majority occluded).

SUMMARY AND CONCLUSION

In the present study, 30 teeth extracted due to advanced periodontal disease were included. These teeth were cleaned, scaling and root planing was performed. 30 dentin disc of 2mm thickness were prepared and divided randomly into the 3 groups; Group1 – Distilled Water (DW), Group II- Pro - Argin (PA), Group III- Carbon Dioxide Laser (CDL). In DW group, the specimens were kept in distilled water; in PA group, dentin discs were brushed with Pro-Argin based dentifrice; In CDL group, the dentin discs were irradiated with carbon dioxide laser at 0.5W. After the treatment the test specimens were stored in artificial saliva and later fixed for SEM analysis. The specimens were viewed at 100x to 2000x. The SEM microphotographs were assessed for number of completely occluded, partially occluded and not occluded dentinal tubules. Also the diameter of the partially occluded and not occluded tubules was measured. The following result was obtained from the data acquired and the statistical analysis performed:

1. Carbon Dioxide Laser (CDL) group showed maximum number of completely occluded tubules followed by Pro-Argin (PA) group and Distilled Water (DW) group.
2. Pro-Argin (PA) group showed more number of partially occluded tubules as compared to Carbon Dioxide Laser (CDL) group and Distilled Water (DW) group.
3. The reduction in tubule diameter was also highest in Carbon Dioxide Laser (CDL) group followed by Pro-Argin (PA) group.

Within the limitations of the present in vitro study, it can be concluded that carbon dioxide laser is more efficient in completely occluding patent dentinal tubules. The partial occlusion seen in PA group should also be considered valuable as decrease in the diameter of the tubules will also decrease the pain due to dentin hypersensitivity. More clinical trials and SEM studies are recommended to compare the efficacy of pro-argin based dentifrice and carbon dioxide laser with different desensitizing agents flooding into the market.

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