A SCANNING ELECTRON MICROSCOPIC EVALUATION OF THREE COMMERCIALLY AVAILABLE DESENSITIZING TOOTHPASTES IN OCCLUDING EXPOSED DENTINAL TUBULES SIMULATING HYPERSENSITIVE DENTIN - AN IN-VITRO STUDY

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LIST OF ABBREVIATIONS

Sr. No.	Abbreviations	Full form
1	DH	Dentin hypersensitivity
2	n-HAp	Nano-hydroxyapatite
3	CSPS	Calcium Sodium Phosphosilicate
4	HCA	Hydroxycarbonate apatite
5	ppm	Parts per million
6	SEM	Scanning Electron Microscope
7	OSHA	Occupational Safety and Health Administration
8	CDC	Centre for Disease Control
9	SnF_2	Stannous fluoride
10	ml	Milliliter
11	min	Minute
12	conc.	Concentration
13	M	Molarity
14	hr	Hour
15	GDA	Glutardialdehyde
16	EDTA	Ethylene Diamine Tetra-acetic Acid
17	F	Fluoride
18	s	Second
19	$ZnCO_3$	Zinc carbonate
20	wt	Weight
21	nm	Nanometer
22	DP	Desensitizing Paste
23	Er:YAG	Erbium-doped Yttrium Aluminium Garnet
24	RPE	Red Propolis Extract
25	ACC	Arginine Calcium Carbonate
26	NaF	Sodium fluoride
27	CLSM	Confocal Laser Scanning Microscope
28	MIH	Molar incisor hypomineralization
29	g	Gram
30	ATR/FTIR	Attenuated Total Reflection Fourier-Transform Infrared
31	MTT	3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide
32	CEJ	Cemento-enamel junction
33	N	Newton
34	kV	KiloVolt
35	ANOVA	Analysis of Variance
36	N	Total number of samples
37	n	Number of specimens in each group
38	p-value	Probability of happening of an event
39	%	Percentage
40	SD	Standard Deviation
41	α	Alpha
42	β	Beta
43	λ	Lambda
44	Ω	Omega

45	i.e.	That is
46	x_i	Observation on each object
47	HSD	Honest Significant Difference
48	MS	Mean Square
49	GM	Grand Mean
50	TSS	Total Sum of Squares
51	SSB	Between group Sum of Squares
52	SSW	Within group Sum of Squares
53	Nd:YAG	Neodymium-doped Yttrium Aluminium Garnet
54	PDL	Periodontal ligament
55	EDXS	Energy-Dispersive X-ray Spectroscopy
56	CMC	Carboxymethyl cellulose
57	BGC	Bioactive glass-ceramic
58	ESEM	Environmental Scanning Electron Microscope
59	FE-SEM	Field Emission Scanning Electron Microscope
60	XRD	X-ray diffraction
61	HRTEM	High Resolution Transmission Electron Microscope
62	cm	Centimeter
63	μ	Micron
64	std.	Standard
65	DF	Degree of Freedom
66	μm	Micrometre
67	SPSS	Statistical Package for the Social Sciences
68	Ltd.	Limited
69	IBM corp.	International Business Machines Corporation
70	NaMFP	Sodium monofluorophosphate

Introduction

'Dentine hypersensitivity is an enigma, being frequently encountered yet ill understood'.

Johnson (1982)

A standout amongst the most normally confronted clinical issues is Dentin hypersensitivity (DH) and least satisfactorily treated chronic problem in dentistry.[1] "DH is characterized by a short, sharp pain arising from exposed dentin in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or disease" defined by **Canadian Advisory Board on DH (2003).**[2]

DH is an agonizing clinical condition. According to **Bartold PM (2006)**[3] prevalence rate of DH is reported to range between 4%-74%. The prevalent age group

for DH falls in the range of 20-40 years, with its peak at the end of third decade. **Sakalauskiene Z et al. (2011)**[4] accounted that there is higher incidence rate of DH in females as compared to males. **Balcheva G et al. (2017)**[5] determined that most affected site by DH are buccal cervical areas of permanent teeth. Most frequently affected teeth by DH are canines followed by first premolars, incisors, second premolars and molars.

DH represents its development in two phases: "lesion localization and lesion initiation". Lesion localization happens due to loss of enamel by means of attrision, abrasion, erosion or abfraction. Gingival recession is another reason for lesion localization which is generally due to tooth brush abrasion, pocket reduction surgery, tooth preparation for prosthesis, excessive flossing or secondary to periodontal diseases.[6,7] Lesion initiation requires removal of tubular plugs and smear layer which expose dentinal tubules and pulp to environment. Tubular plug and smear layer are composed of elements of protein and sediments derived from salivary calcium phosphates and seal the dentinal tubules discrepantly and temporarily.[7]

Pain experienced due to DH can be explained by various theories namely odontoblastic transduction theory, neural theory and hydrodynamic theory. There is no conclusive evidence to support the odontoblastic transduction and the neural theory.[1] The hydrodynamic theory was first proposed by **Gysi A et al. (1900)**[8] with confirmatory evidence provided by **Brannstrom M et al. (1964)**[9] remains the most widely accepted theory of DH. According to this theory, there is rapid shift in either direction of the fluid within the dentinal tubules on stimulus application which results in activation of sensory nerve fibers within the inner dentin surface as well as pulp.

The severity of DH depends on the width of the dentinal tubules. The rate of fluid flow directly dependents on the fourth power of the radius. If the tubular diameter doubles, there is increase in fluid flow by 16-fold. Sensitive teeth have two times wider tubules at the buccal cervical area compared to nonsensitive teeth.[10] The dentinal tubules are approximately 5µ wide at pulpodentinal junction, at middle dentin 1.2 µm and 1µ at dentinoenamel junction.[11]

Some commonly employed approaches in the treatment and prevention of DH depending upon **mechanism of action are:**

1. Nerve desensitization
2. Protein precipitation
3. **Dentinal tubular occlusion**
4. Dentin adhesive sealers
5. Lasers
6. Homeopathic medications

There are myriad of desensitizing agents and proprietary products like fluorides, potassium nitrate, oxalate containing compounds, dentin bonding agent, portland cement, propolis and lasers. The tooth treated with these agents **occludes the dentinal tubules** by deposition of precipitates thus resulting in decrease of dentinal permeability. Some other agents like sodium fluoride, stannous fluoride, calcium phosphate, calcium carbonate, potassium oxalate and bioactive glasses works on the same principle.[12]

According to **Grossman,** an ideal dentin desensitizing agent should be rapidly acting with long lasting effects, non-irritating, painless, easy to apply and should not

discolour the tooth.[13] Newer novel biomaterials like the **Nano-hydroxyapatite (n-HAp), Novamin** and **Pro-Argin** have been introduced in the treatment of DH work on the principle of dentinal tubule occlusion.

Nano-hydroxyapatite (n-HAp) is the most biocompatible materials expressed chemically as $Ca_{10}(PO_4)_6(OH)_2$ has gained wide acceptance in biomedicine and dentistry in recent years. They easily penetrate into dentin tubules and block fluid movement within the tubules when combined with various agents. **Roveri N et al. (2009)** stated that n-HAp is responsible for surface remineralization by forming a biomimetic apatite layer on enamel and dentin surface. This occurs due to the chemical and physical characteristics of innovative nanostructured hydroxyapatite particles which closely resembles enamel mineral constituents.[14]

Novamin/ Calcium Sodium Phosphosilicate (CSPS) is a bioactive glass and a highly biocompatible material. According to **Hench and Andersson (1993)** CSPS was originally developed as a bone regenerative material. Novamin when exposed to body fluids, it reacts and deposits hydroxycarbonate apatite (HCA), a chemically similar mineral to enamel and dentin.[15,16] In saliva, sodium ions from novamin particles immediately begins to exchange with hydrogen cations. This rapid exchange of ions allows calcium and phosphate ions to be released from the particle structure. There is transitory increase in pH which promotes the precipitation of calcium and phosphate from the particles and saliva to form a calcium phosphate layer on exposed dentin surface.[17]

Pro-Argin is an amino acid and calcium carbonate present naturally in saliva has been identified as an active ingredient with potential oral health benefits. Pro-

Argin technology is based on the interaction between arginine and calcium carbonate which infiltrate and block the dentinal tubules and prevent dentinal fluid flow, thus reducing DH.[18]

There is sparse information available in the literature review regarding efficacy of **n-HAp, Novamin and Pro-Argin** in occluding dentinal tubules in DH treatment hence, the current study is undertaken.

The null hypothesis was that there is no significant difference between effectiveness of n-HAp, Novamin and Pro-Argin containing desensitizing toothpaste in occluding exposed dentinal tubules.

Aim and Objectives

Aim

To **evaluate** and **compare** the effects of three different desensitizing toothpastes (Nano-hydroxyapatite, Novamin and Pro-Argin) on dentinal tubule occlusion using Scanning Electron Microscope (SEM).

Objectives

1. To **evaluate** the surface of **denuded tooth** under SEM.

2. To **evaluate** effectiveness of **regular toothpaste** in dentinal tubule occlusion using SEM.

3. To **evaluate** effectiveness of **Nano-hydroxyapatite** in dentinal tubule occlusion using SEM.

4. To **evaluate** effectiveness of **Novamin** in dentinal tubule occlusion using SEM.

5. To **evaluate** effectiveness of **Pro-Argin** in dentinal tubule occlusion using SEM.

6. To **compare** the effectiveness of **Nano-hydroxyapatite, Novamin, Pro-Argin** and **Regular toothpaste** in dentinal tubule occlusion using SEM.

Review of Literature

Dentin hypersensitivity (DH) is a common clinical condition and a prime concern amongst the patients. According to the hydrodynamic mechanism, exposed dentin with patent tubules allows the movement of dentinal tubule fluid leading to DH.[19] A viable treatment modality consists of desensitization by tubule occlusion, which decreases both dentin permeability and fluid movement thereby reducing dentin sensitivity.[20]

DH has been treated by number of agents which have been claimed to reduce pain by occluding dentinal tubules. Thus before proceeding further, it is important to review various desensitizing agents and methods employed to assess tubular occlusion given by various researchers.

Ellingsen JE and Rolla G et al. (1987)[21] conducted a study using SEM and electron microprobe analysis after using Stannous fluoride (SnF_2) on dentin surface. Human premolars extracted for orthodontic reasons were used as test specimens. Samples were divided in 3 groups depending on surface treatment: Group I- 100 ml of 2% SnF_2 solution for 1, 5, 10 or 60 min. Group II- 100 ml of SnF_2 solution with conc. of 1%, 2%, 5% or 10% for 5 min. Group III-100 ml of 2% SnF_2 solution for 5 min. Samples were rinsed with running distilled water for 1 min and immersed in 1M potassium hydroxide or distilled water for 15 hr. They concluded that the topical application of SnF_2 form a layer of tin and fluoride on dentin which provides mechanical and chemical protection. They also stated that this layer is responsible for caries resistance and reduction in DH.

Addy M et al. (1989)[22] studied the effect of different toothpastes with various abrasive agents on dentin surface using SEM and X-ray microanalysis. They reported that surface changes occurred on exposed dentin were produced by abrasives agents like, calcium carbonate, dicalcium phosphate, alumina and silica accumulated on dentin surface and resulted in narrowing of dentinal tubules.

Dijkman GE et al. (1994)[23] evaluated closure of dentinal tubules by glutardialdehyde (GDA) treatment using SEM analysis. 40 specimens were divided into 5 groups (n=8) : 1- Untreated; 2- GDA treated; 3- EDTA (Ethylenediamine tetraacetic acid) treated; 4- GDA treated + EDTA treated; 5- EDTA treated + GDA treated. They concluded that treatment with GDA fixed the smear layer part on the

dentin surface in such a way that at least 50% of the dentinal tubules remained closed after EDTA treatment.

Arrais CA et al. (2003)[24] conducted a study to evaluate the tubule occluding ability of three commercial available dentifrices Sensodyne, Emoform and sorrisso by SEM. 50 cervical areas from buccal and lingual surfaces of third human molars were taken. Specimens were randomly divided into five groups (n=10): G1-no brushing; G2-brushing without dentifrice; G3-brushing with Sensodyne (Strontium chloride 10%, titanium dioxide, calcium carbonate); G4-brushing with Emoform (Potassium nitrate, calcium carbonate); G5-brushing with Sorriso (1500ppm Sodium monofluoro-phosphate, calcium carbonate, sodium lauryl sulfate, sodium silicate). Specimens were brushed for 4 min/day for 7 days. They reported that the use of all dentifrices occluded more dentinal tubules than no brushing and brushing without dentifrices groups.

Ahmed TR et al. (2005)[25] evaluated the use of digital image analysis along with the SEM in quantifying the effectiveness of a Butler Protect desensitizing agent (3% potassium oxalate) on measuring tubular permeability from micrographs of control and treated dentin surfaces. Dentin disc models were used to investigate the occluding potential of Butler Protect. They stated that there was only a little difference after a single application but, multiple application of Butler Protect demonstrated even greater decreases in tubular permeability.

Burwell AK et al. (2010)[15] determined the potential of remineralization of Novamin through a number of in vitro and in situ studies. Study 1 investigated the ability of novamin containing dentifrices to prevent demineralization of dentin surfaces. Study 2 explored the potential of these dentifrices to remineralize existing lesions on the root surface. Study 3 investigated the ability of novamin dentifrices to heal existing white-spot lesions on enamel. Finally, an in situ study 4 was designed to characterize the morphological changes on tooth surfaces that were subjected to different types of damage and then treated with novamin. They concluded from all four different studies that novamin adhered to an exposed dentin as well as enamel surface when incorporated into a dentifrice and reacts to form a mineralized layer. The layer formed was resistant to acid challenges and mechanically strong.

Shetty S et al. (2010)[26] evaluated the effectiveness of n-HAp as an in-office desensitizing agent. SEM analysis of 40 freshly extracted teeth was done to assess the dentinal tubular occlusion. Patients were divided into four groups (n=10). Group I and II were treated with n-HAp in two forms (dry sol gel and liquid precipitate form) and group III and IV were treated as a positive control group (distilled water) and a negative control group (no treatment) respectively. They reported that n-HAp showed completely obliterated dentinal tubules, whereas the other groups showed predominantly open or partially occluded tubules.

Wang Z et al. (2011)[27] evaluated the effectiveness of a novel bioactive glass-containing toothpaste on dentin permeability and remineralization under SEM after 7days. The dentin discs were randomly divided into three groups containing

specimens: Group 1 (Control) – EDTA etched specimens, Group 2 – EDTA-etched specimens brushed with distilled water and Group 3 – EDTA-etched specimens brushed with 1.0 g bioactive glass-containing toothpaste (Novamin). Attenuated total reflection Fourier transform infrared (ATR⁄FTIR) spectroscopy was performed to view the mineral variation on demineralized dentin. Qualitative information of elemental variation was detected by energy dispersive X-ray Spectroscopy (EDXS) analysis. They determined that novamin containing toothpaste significantly reduced dentin permeability and revealed increased mineral content.

Davies M et al. (2011)[28] compared the efficacy of a recently developed arginine-containing dentifrice with two traditional strontium-based products and fluoride to occlude dentinal tubules and effect of acid challenge under SEM. 200 wisdom teeth dentin specimens with patent tubules were divided into four groups. Group 1: Arginine with calcium carbonate while Group 2, Group 3 and Group 4: Strontium acetate hemihydrate, Strontium chloride hexahydrate and Fluoride containing product respectively. They determined that all four desensitizing pastes off ered good tubular occlusion. They also found that the specimens after subjecting to 0.3% citric acid challenge for 10 s, 30 s, 2 mins, 5 mins or 10 mins only the strontium acetate pastes retained the level of occlusion after 2 mins or 5mins.

Tschoppe P et al. (2011)[29] conducted an in-vitro study to compare the effects of n-HAp toothpaste on enamel and dentin subsurface lesions remineralization. 35 bovine incisors were used to prepare (n=70) enamel specimens from the labial aspects and dentin specimens (n=85) were derived from the cervical regions. 4 Groups

divided depending on remineralizing solution as follow: pure n-HAp, 20 wt% $ZnCO_3$/n-HAp, 24 wt% $ZnCO_3$/n-HAp and 0.14 wt% amine fluoride toothpastes respectively. Brushing procedures were performed with the respective toothpaste/storage solution slurry twice daily. They determined that the toothpastes containing n-HAp showed higher remineralizing effects compared to amine fluoride toothpastes on bovine dentin and enamel after second and fifth week of use.

Joshi S et al. (2013)[10] evaluated and compared NovaMin desensitizer and Gluma desensitizer (hydroxyethyl methacrylate and glutaraldehyde) on dentinal tubule occlusion under SEM. Twenty dentin specimens were allocated to each of 3 groups: Control, Gluma desensitizer, and NovaMin. They concluded that both the agents were effective in occluding dentinal tubules but NovaMin appeared more efficacious on initial application in occluding dentinal tubules completely.

Sadiasa A et al. (2013)[30] conducted an in-vitro study to evaluate the effectiveness of carboxymethyl cellulose (CMC) hydrogel in delivering hydroxyapatite (HAp) to dentinal tubules and reducing DH. Dentin discs samples were randomly grouped into four groups with each group treated with prepared hydrogel by mixing CMC/glycerol and distilled water/sorbitol then modified to contain 0%, 10%, 20% and 30% HAp respectively. Occlusion of the dentin tubules was observed by SEM before and after treatment. They concluded that blocking of the dentin tubules was markedly increased by the addition of HAp to the hydrogel samples that results in reduction or elimination of DH.

Dhillon P et al. (2014)[31] evaluated the efficacy of various desensitizing agents in reducing DH using SEM. 25 dentin blocks were obtained from the roots of the single rooted anterior teeth and randomly divided into five groups. Four test groups were diode laser (940 nm), desensitizing pastes containing Pro-Argin, Hydroxyapatite and Novamin respectively and fifth was control group. They found that all test groups showed an increase in the percentage of tubular occlusion at 0, 24, 72, 120 and 168 hrs. The percentage of occlusion was found to be highest for Novamin as compared to the other groups over a period of 7 days.

Tunar OL et al. (2014)[32] compared the effects of Er:YAG Laser, desensitizing paste (DP) containing 8% Arginine and Calcium Carbonate and their combinations on human dentin tubules using SEM analysis. 40 dentin specimens were obtained from freshly extracted impacted third molars and divided in four groups. Group I served as the control, Group II, Group III and Group IV recieved Er:YAG laser, a DP containing 8% arginine and calcium carbonate and DP+Er:YAG laser in combination respectively. They concluded that occlusion and narrowing of dentinal tubules was obtained in all treatment groups, but more effective tubule occlusion was observed in DP+Er:YAG laser group.

Khetawat S et al. (2015)[33] studied that hydroxyapatite particles are surface nanostructured having higher surface area therefore having higher reactivity. This property allows them to bind to enamel and dentin apatite forming a biomimetic coating on enamel and contrasting plaque formation. Hydroxyapatites prevent tooth

from decay, rebuild and occlude dentinal tubules resulting in eliminating hypersensitivity.

Zhong Y et al. (2015)[34] conducted an in-vitro study to assess the ability and efficacy of bioactive glass-ceramic (HX-BGC) to reduce dentin tubule permeability. 50 Dentine discs from human third molars were randomly divided into five groups each containing 10 specimens and were treated with different regimens: Group 1 - distilled water; Group 2 - Sensodyne Repair toothpaste (containing NovaMin); Group 3 – 7.5% HX-BGC toothpaste; Group 4 - control toothpaste (without HX-BGC) and Group 5 - HX-BGC powder. Specimens were treated for 20s/daily for 7 days by brushing with an electric toothbrush and were immersed in artificial saliva after daily treatment. Dentin morphology and surface deposits were observed by SEM after one day and 7 days of treatment. They concluded that the bioactive glass-ceramic material HX-BGC is highly effective in reducing dentin permeability by occluding open dentin tubules compared to Novamin.

Arnold WH et al. (2015)[35] investigated the effectiveness of various toothpastes on dentin tubule occlusion using qualitative and quantitative methods. Twelve dentin discs were used for each brushing experimental group. Samples were divided in 8 groups: **Positive control-**Elmex toothpaste, **Toothpaste 1-**Elmex Sensitive Professional (Pro-Argin, calcium carbonate), **Toothpaste 2-**Sensodyne Rapid (Strontium acetate), **Toothpaste 3-**Sensodyne Repair (Stannous fluoride), **Toothpaste 4-**BioRepair Sensitive (Zinc-carbonate hydroxyapatite), **Toothpaste 5-**Colgate Total Sensitive (New silica), **Toothpaste 6-**Dontodent Sensitive

(Tetrapotassium pyrophosphate, hydroxyapatite) and six dentin discs were brushed only with artificial saliva as the **negative control.** The brushing simulated a total brushing time of 1 year and occlusion of the dentin tubules was investigated using EDXS and SEM. They reported that after the application of toothpaste 1 a scattered thin layer of silicon covered the dentin surface. A clear thin layer of silicon covered the dentin surface and the open dentinal tubules were observed after the application of toothpaste 2. There was no clear silicon layer was observed after the application of toothpaste 3. Whereas, several occluded dentinal tubules were found after the application of toothpaste 4. However, neither a silicon layer on the dentin surface nor occluded dentin tubules were observed after the application of toothpastes 5 and 6. Also, they found that the occlusion is unstable and can be removed with acid erosion.

.**Chen CL et al. (2015)**[36] studied and compared the effectiveness of different desensitizing agents in dentinal tubule occlusion using SEM. Eighty dentin discs from extracted human molars were included and were randomly divided in four groups(n=20): Group 1-Red Propolis extract (RPE), Group 2-Novamin; Group 3-Arginine-calcium carbonate (ACC) and Group 4-Saline. They stated that ACC demonstrated more tubule occlusion followed by RPE and Novamin and saline. They also found that following 6% citric acid challenge RPE treated discs retained more tubule occlusion followed by ACC and Novamin respectively.

Amaechi BT et al. (2015)[37] conducted an in-situ study to compare dentin tubular occlusion by theobromine containing dentifrices with (Theodent-classic-F) and without (Theodent-classic) fluoride with 1,500 ppm fluoride toothpaste, Colgate-

Regular (Fluoride) and Sensodyne-5000-Nupro (Novamin). Each of the 80 subjects wore four intraoral appliances bearing dentin blocks for twice daily 7 days while using one of four test dentifrices (n=20/dentifrice). Treated blocks and their control blocks were examined under SEM. They concluded that Theobromine containing toothpastes have equal potential in occluding dentinal tubules within a shorter time period than Novamin-containing toothpaste but, at the end of 7 days all 3 pastes were equally effective in occluding dentinal tubules except Colgate regular toothpaste.

Kulal R et al. (2016)[38] evaluated and compared the effects of three different desensitizing agents containing n-HAp, Novamin and Pro-Argin on dentinal permeability and tubular occlusion using SEM. A total of 40 disc shaped dentin specimens were dissected from extracted premolars and divided into four groups. Group A (control group), Group B (15% n-HAp), Group C (5% Novamin) and Group D (8% Pro-Argin). Each disc was subjected to a pre-treatment and post-treatment SEM analysis after a period of seven days. They reported that efficacy of n-HAp toothpaste (98.1%) was greater compared to Novamin (83.1%) and Pro-Argin (69.1%) respectively.

Kunam D et al. (2016)[39] conducted an in-vitro study and evaluated the degree of dentinal tubular occlusion and depth of penetration of n-HAp derived from chicken eggshell powder with and without addition of 2% sodium fluoride (NaF) using SEM and Confocal Laser Scanning Microscope (CLSM). Seventy dentin discs were obtained from freshly extracted maxillary and mandibular molars. Groups were divided into four depending on the experimental agents used as follow: Group 1: (n=10) Untreated (control), Group 2: (n=20) NaF, Group 3: (n=20) n-HAp and Group

4: (n=20) combination of n-HAp and 2%NaF. They concluded that dentin disc treated with the combination of n-HAp and 2% NaF reported complete dentinal tubular occlusion and significantly greater depth of penetration than n-HAp and 2% NaF alone.

Pathan AB et al. (2016)[40] evaluated the ability of three desensitizing agents on dentinal tubule obliteration and their durability under SEM. 60 dentin specimens were obtained from 30 extracted sound maxillary first premolars and divided in 4 groups with 15 specimens in each group: Group 1- samples were immersed in artificial saliva, Group 2- Vivasens, Group 3-VOCO Admira Protect and Group 4-Neo Active Apatite suspension (n-Hap). These agents were applied on specimen for 10-20 sec and examined under SEM showed that all three desensitizing agents were responsible for dentinal tubule occlusion. Then samples were brushed for 1 week and 1 month followed by SEM analysis to evaluate the durability. They reported that Admira protect group showed best results in occluding a higher number of dentinal tubules and showed best durability followed by Vivasens and Neo Active Apatite suspension.

Shah S et al. (2017)[41] evaluated the efficacy of NovaMin and Pro-Argin containing desensitizing dentifrices on occlusion of dentinal Tubules under SEM. Seventy extracted human permanent molars were divided into four groups: Group 1–distilled water (control) (n=10); Group 2–SHY-NM toothpaste (n=20); Group 3–Sensitive Pro-Relief toothpaste (n=20); Group 4– Thermoseal toothpaste (n=20). Each dentin disc was brushed for 2min once daily for seven consecutive days. They found

that NovaMin-containing toothpaste showed the highest tubular occlusion (95.58%) followed by Sensitive Pro-Relief (89.90%) and Thermoseal (86.12%).

Yilmaz NA et al. (2017)[42] studied the efficacy and durability of five different desensitizers on tubule occlusion and dentin permeability reduction under SEM. 100 dentin discs were divided in 5 groups depending on use of desensitizing agents: 1.Gluma Desensitizer Power gel, 2.Bifluorid 12, 3.Gluma Self Etch Bond, 4.D/Sense Crystal and 5.Nupro Sensodyne Prophylaxis Paste with Novamin. Samples were examined after desensitizing agent treatments and following post treatments of 6% citric acid challenge for 1 min or immersion in artificial saliva for 24 hours. They reported that all the agents significantly reduced dentin permeability by changing the morphology of the dentin surface but, following post-treatments there was some reduction in permeability values.

Cunha SR et al. (2017)[43] conducted a study to evaluate the association between Nd:YAG laser and two desensitizing dentifrices containing 15% NovaMin or 8% arginine for treatments of DH. 80 samples were randomized into eight groups (n=10): Laser (L), Laser+ Photoabsorber (LP), Arginine (A), Arginine+Laser (AL), Arginine+Laser+Photoabsorber (ALP), NovaMin (N), NovaMin+Laser (NL) and NovaMin+Laser+Photoabsorber (NLP). Specimens were then analyzed with an environmental scanning electron microscope (ESEM) to ensure open dentin tubules. They concluded that none of the group presented better tubule occlusion than NovaMin by itself. Arginine presented improved tubule occlusion only when treatment associated with Nd:YAG laser.

Ma Q et al. (2017)[44] compared the dentinal tubule occluding efficacy of two different methods of using a nano-scaled bioactive glass (BG) containing desensitising agent. Citric acid treated dentin discs were randomly divided into 7 groups (n=8). Group A1, A2 and A3: samples coated with BG desensitising paste; Group B1, B2 and B3: samples coated with BG desensitising paste and covered with matched transparent trays and control group (Group C): samples treated with deionised water. Field Emission Scanning Electron Microscopy (FE-SEM) was used to capture topographical images and elemental compositions of dentine discs were identified using EDXS. They concluded that the application of transparent trays in combination with nano-scaled BG-containing desensitising paste could increase the dentinal tubule occluding capacity and also shorten the treatment time.

Bekes K et al. (2017)[45] compared the efficacy desensitizing products containing 8% arginine and calcium carbonate in reducing hypersensitivity. Nineteen children with at least one molar incisor hypomineralization (MIH)-affected molar with hypersensitivity were included. Hypersensitivity was assessed with an evaporative (air) and a tactile stimulus. Each child received a single in-office treatment with a desensitizing paste containing 8% arginine and calcium carbonate with 1450 ppm fluoride followed by 8 weeks of brushing twice daily for at least 2 min with a desensitizing toothpaste containing 8% arginine and calcium carbonate with 1450 ppm fluoride (Elmex Sensitive Professional toothpaste). Additionally, the corresponding mouthwash was used. They suggested that 8% arginine and calcium carbonate were able to reduce hypersensitivity successfully during 8-week trial.

Hiller KA et al. (2018)[46] conducted an in-vitro study to evaluate the effect of toothpastes with different active ingredients on dentin permeability on multiple applications and several thermal ageing cycles in the presence or absence of human saliva. Dentin samples were divided into 3 groups: hydroxyapatite containing toothpaste (BR), potassium nitrate (SP) and an arginine and calcium carbonate (EH) containing toothpaste respectively. Dentin permeability was measured as hydraulic conductance using capillary flow system (Flodec, Geneva) after application and results were expressed in percentage. They reported that without saliva, the ranking (best first) of dentin permeability was BR (61%) <SP(87%)< EH(118%), with saliva EH(63%) < SP(72%) < BR(88%). BR reduced dentin permeability significantly more in absence of saliva whereas with saliva EH was superior. Repeated material application decreased permeability while thermal ageing increased dentin permeability.

Baglar S et al. (2018)[47] conducted an in-vitro study and evaluated the capacity of pure n-HAp and 1%, 2%, and 3% F^- doped n-HAp on dentinal tubular occlusion and cytotoxicity effect of experimental agents. 40 dentin specimens were randomly divided into five groups: Group 1- no treatment, Group 2-10% pure n-HAp and Group 3, 4, 5 specimens treated with 1%, 2%, and 3% F^- doped 10% n-HAp respectively. To evaluate the effectiveness of the materials pH, FTIR Spectroscopy and SEM analysis were performed. To determine cytotoxicity of the materials, MTT assay was used. They stated that the pure n-HAp and 1% F^- doped n-HAp were most biocompatible and most successful in tubular occlusion compared to other study groups.

Katakam D et al. (2019)[48] compared and evaluated dentinal tubular occlusion property of current desensitizing agents using SEM. 30 human premolars extracted for orthodontic reasons were collected and cut longitudinally to obtained flat surface with exposed dentinal tubules. The teeth were randomly divided into three groups according to the dentin surface treatments: Group A- Aclaim (n-HAp) toothpaste, Group B- Colgate Sensitive Pro-relief (Pro-Argin) toothpaste and Group C- Clinpro (NaF with functionalized tricalcium phosphate) toothpaste. Brushing technique was performed for 1 week twice daily. They reported that occlusion of dentinal tubules by Group B was comparatively superior compared to Group A and Group C respectively.

Onwubu SC et al. (2019)[49] evaluated the occluding efficacy of nanohydroxyapatite synthesized from the eggshell waste (EnHAp) in the treatment of DH. 25 simulated dentin specimens were randomly assigned to five groups: Group 1- untreated, Group 2- Eggshell powder alone, Group 3- Calcined eggshell powder, Group 4- Colgate Sensitive treated EnHAp and Group 5- Calcium dihydrogen phosphate (n=5). The purity and phase change were studied using FTIR and X-ray diffraction (XRD). Field emission scanning electron microscopy (FE-SEM) and high-resolution transmission electron microscopy (HRTEM) were used to observe the morphology of EnHAp. They determined that the application of EnHAp resulted in an efficient occlusion of dentin tubules and an irregular rod structure with a particle size of 65 nm.

Materials and Method

One hundred and five freshly extracted human mature permanent maxillary premolars under inclusion criteria were included in the study. All the extracted teeth were collected, cleaned and disinfected as per the recommendation and guidelines given by OSHA and CDC. (2003 report 17).[50] Teeth were stored in 10% formalin at room temperature.

Approval from Institutional Ethics Committee was taken for the study.

Method of Selection of Study Subject

Inclusion Criteria:

1. Extracted teeth of patients with age (18-25years) for orthodontic or periodontal purpose.

Exclusion Criteria:

1. Teeth with caries, abrasion and erosion
2. Restored teeth
3. Fracture
4. Endodontically treated teeth
5. Developmental anomalies

Armamentarium:

Instruments and Equipment:

- Straight probe (GDC, India) (PLATE–I)
- Explorer (GDC, India) (PLATE–I)
- Tweezer (GDC, India) (PLATE–I)
- Cotton holder and waste receiver (GDC, India) (PLATE–I)
- Williams graduated probe (GDC, India) (PLATE–I)
- L-Mould (PLATE–I)
- High speed airotor (NSK, Japan) (PLATE–I)
- FG-245 plain cut tungsten carbide Fissure bur (SS white, India) (PLATE–I)
- Digital vernier caliper (Workzone hand tools, Germany) (PLATE II)
- 2.5 ml 24 gauge needle syringe (Dispovan, India) (PLATE-II)

- Oral B Cross Action Toothbrush (Procter and Gamble, USA) (PLATE-II)
- Customized jig for brushing (PLATE-II)
- Scanning Electron Microscope (Carl Zeiss Evo 18, Jena, Germany) (PLATE-II)
- SCILABS software (5.5.2) (Rungis, France)

Materials:

- Spacer wax (MAARC, India) (PLATE–III)
- Polyvinyl siloxane putty impession material (Aquasil Dentsply, Germany) (PLATE–III)
- Auto polymerized acrylic resin (DPI RR cold cure, India) (PLATE–III)
- Emery paper grit-600 (CUMI, India) (PLATE–III)
- Distilled water (Horse brand, India) (PLATE–III)
- 30% phosphoric acid (PLATE-IV)
- Artificial Saliva (ICPA Wet Mouth, India) (PLATE-IV)
- α- Regular toothpaste (PLATE-IV)
- β- Toothpaste containing n-HAp (PLATE-IV)
- λ- Toothpaste containing Novamin (PLATE-IV)
- Ω- Toothpaste containing Pro-Argin (PLATE-IV)

Sample Preparation

Root portion of each sample from cemento-enamel junction (CEJ) to root apex was encircled with thin sheet of spacer wax. Further, the teeth were mounted in cold-cure acrylic resin 1 mm apical to the CEJ using L-shaped mould of dimensions 1.5 × 1.5 cm. The teeth were then removed from the acrylic resin block after setting of material. The wax was replaced with polyvinyl siloxane putty material followed by remounting the teeth into the acrylic blocks. Standardized class V cavity was prepared using FG-245 plain cut tungsten carbide Fissure bur (SS white, India). Cavities were prepared with high speed airotor (NSK, Japan) under a continuous water spray having standardized dimensions of 2 mm height, 4 mm mesiodistal width and pulpal depth of 2 mm.[51] Cavity was placed 1 mm coronal to CEJ. A digital vernier caliper (Workzone hand tools, Germany) was used to measure the widths of the prepared cavity design. The depth of the cavity was measured using a williams graduated probe (GDC).

The exposed dentin surface was polished with emery paper (CUMI, India) and washed with distilled water. Each specimen then etched with 30% phosphoric acid for 15 minute to remove any smear layer produced during class V cavity preparation and to expose the dentin tubules to simulate DH. All the samples were stored in distilled water. A total of 105 prepared samples were allocated using a computer assisted randomization technique into five groups according to the surface treatment.

All toothpastes were blinded with labels i.e., α, β, λ and Ω for Group B, Group C, Group D and Group E respectively in order to prevent identity revelation of products to operator.

Distribution of Study Groups:

Control Groups

Group A (Negative control):- No treatment (n = 5)

Group B (Positive control):- α-Regular toothpaste (n = 25)

Experimental Groups

Group C:- β- Toothpaste containing n-HAp (n = 25)

Group D:**-** λ- Toothpaste containing Novamin (n = 25)

Group E:**- Ω**- Toothpaste containing Pro-Argin (n = 25)

The samples of group A were subjected to a pre-treatment SEM analysis. Pea-size amount of each paste was used by mixing with 1 ml of artificial saliva (ICPA Wet Mouth, India). Each treatment group (Group B, Group C, Group D, Group E) samples were brushed for **2 min twice daily for 2 months**[45] at **0.9 N approximately** load[52] using **Oral B Cross Action Toothbrush by customized jig machine**. In between, the samples were stored in artificial saliva (ICPA Wet Mouth, India). Subsequent to the treatment, the samples were dried and prepared for analysis by SEM.

After drying, the samples were mounted onto aluminium stubs and subsequently coated with a thin layer of gold/palladium in a sputter coater. The surface of the samples were scanned and examined using SEM at **1000 X and 5 kV.** Photographs of the samples were obtained from the camera which was fixed to the SEM machine (Carl Zeiss Evo 18, Germany).

Method of Measurement and Data Collection:

The mean value of total number of open dentinal tubules was recorded from 10μm field of negative control group samples and this was assumed constant for all the remaining group samples. This assumption was made because after treatment in many cases the individual open dentinal tubule will be no longer visible. Dentinal tubules were classified as open, partially occluded and completely occluded dentinal tubules. In partially occluded tubules, particles were observed within the tubule which results in significant reduction in the diameter of dentinal tubule compared to the negative control group. The number of completely occluded tubules were calculated as the total number of dentinal tubules minus the number of open tubules and partially occluded tubules.[53] Counting of tubules was done using **SCILABS software (5.5.2)** (Rungis, France). The collected data was tabulated using an excel sheet (Microsoft Office 2010). The comparison of mean number of dentinal tubules was performed statistically using one-way analysis of variance (ANOVA). The paired comparison between groups was carried out using Tukey's post-hoc test. The statistical analysis was carried out using SPSS version 20.0 (International Business Machines Corporation) for each type of dentinal tubules and the inferences were obtained. The statistical significance was tested at 5% level.

Algorithm for Methodology

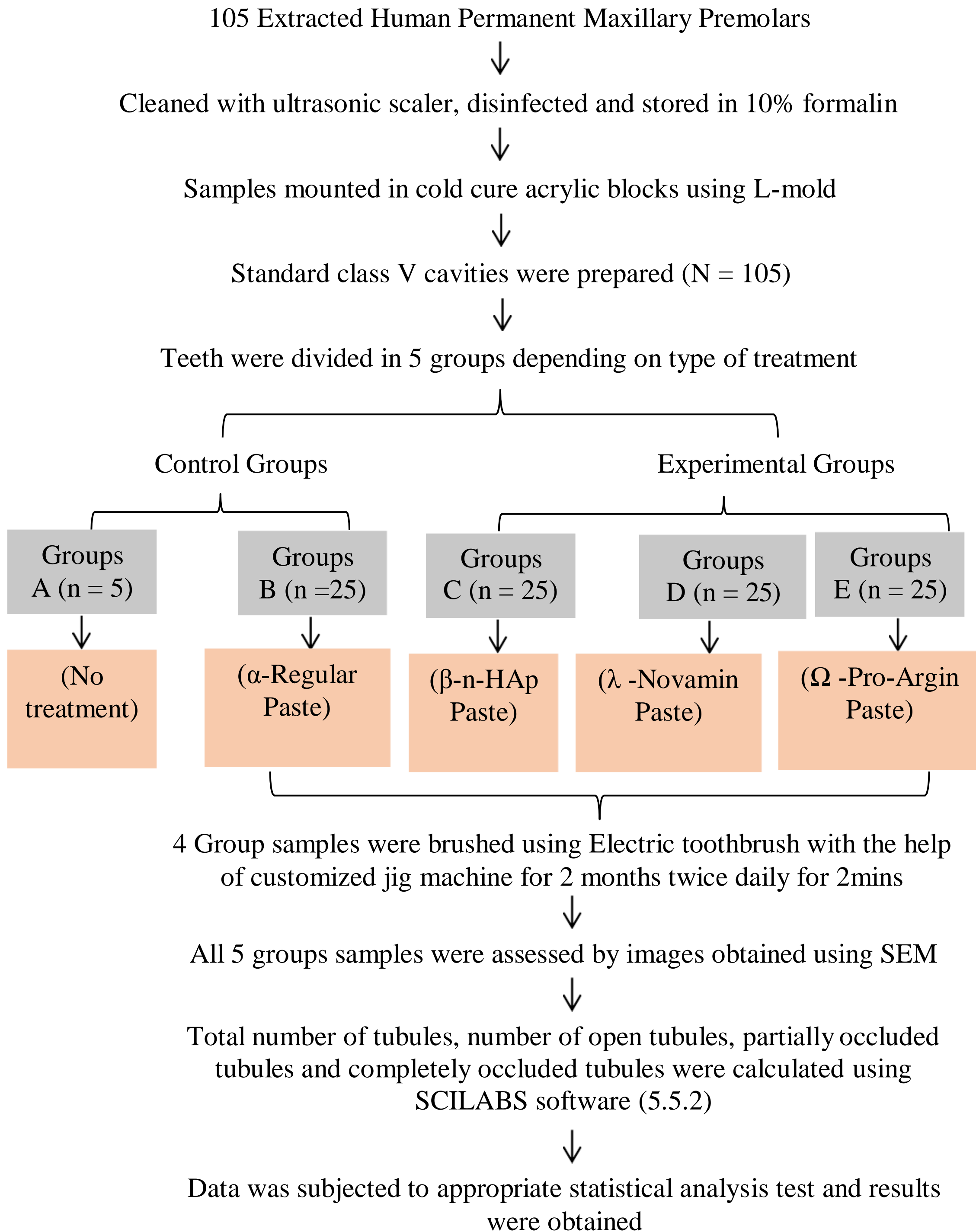


PLATE I

ARMAMENTARIUM

Hand instruments
(GDC, India)

Cotton holder and waste receiver
(GDC, India)

Williams graduated probe
(GDC, India)

L-Mould

High speed airotor
(NSK, Japan)

FG-245 plain cut tungsten carbide fissure bur
(SS white, India)

PLATE II

ARMAMENTARIUM

Digital vernier caliper
(Workzone hand tools, Germany)

2.5ml 24 gauge needle syringe
(Dispovan, India)

Oral B Cross Action Toothbrush
(Procter and Gamble, USA)

Customized jig for brushing

Scanning Electron Microscope
(Carl zeiss Evo 18, Jena, Germany)

PLATE III

MATERIALS

Spacer wax
(MAARC, India)

Polyvinyl siloxane putty impression material
(Aquasil Densply, Germany)

Auto polymerized acrylic resin
(DPI RR Cold Cure, India)

Emery paper Grit-600
(CUMI, India)

Distilled water
(Horse brand,India)

PLATE IV

MATERIALS

30% Phosphoric acid

Artificial Saliva
(ICPA Wet Mouth, India)

α-Regular toothpaste

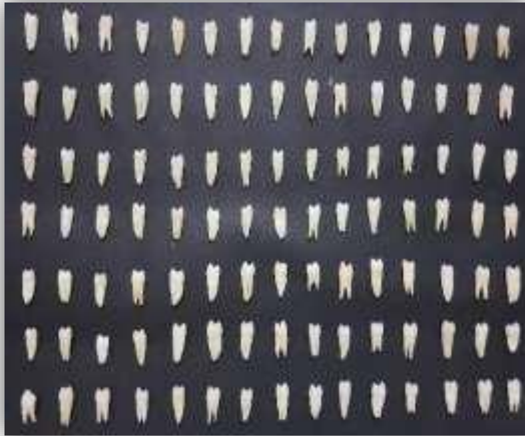
β -Toothpaste containing n-HAp

λ - Toothpaste containing Novamin

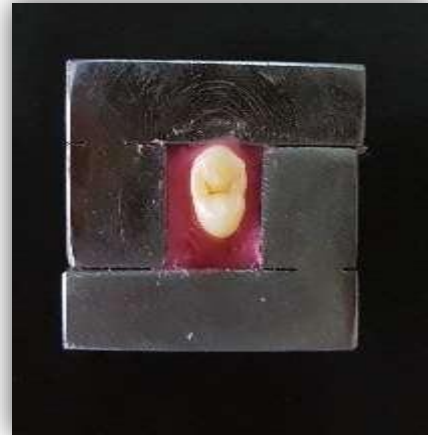
Ω -Toothpaste containing Pro-Argin

PLATE V

METHODOLOGY



Sample size (N=105 teeth)



Sample preparation

Prepared sample

Tooth preparation
(Class V cavity)

Standard Class V cavity

PLATE VI

METHODOLOGY

Height =2mm

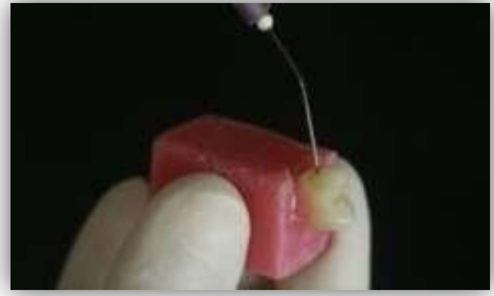
Mesiodistal width=4mm

Pulpal depth=2mm

PLATE VII

METHODOLOGY

Sample polished using emery paper
(CUMI, India)



Sample washed with distilled water

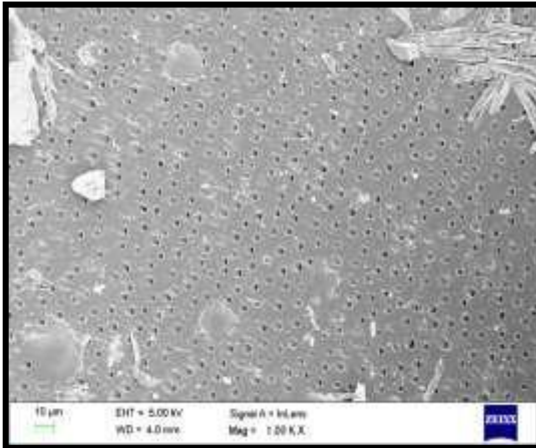
Sample etched with 30% phosphoric acid

Sample washed with distilled water

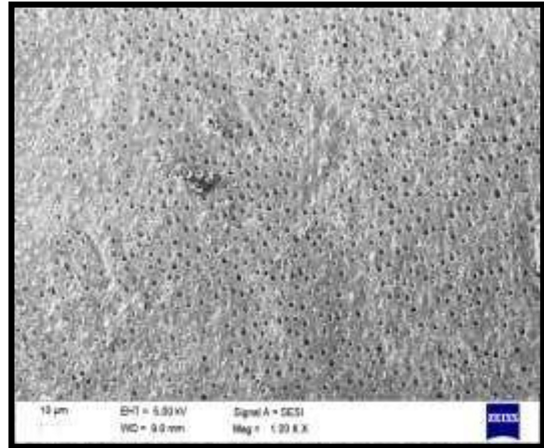
Sample while brushing

PLATE VIII

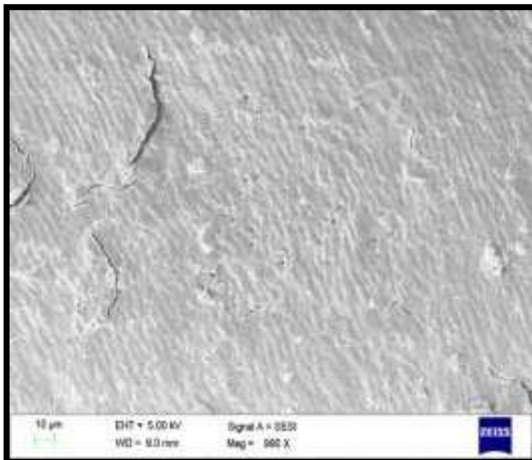
SCANNING ELECTRON MICROSCOPE IMAGES



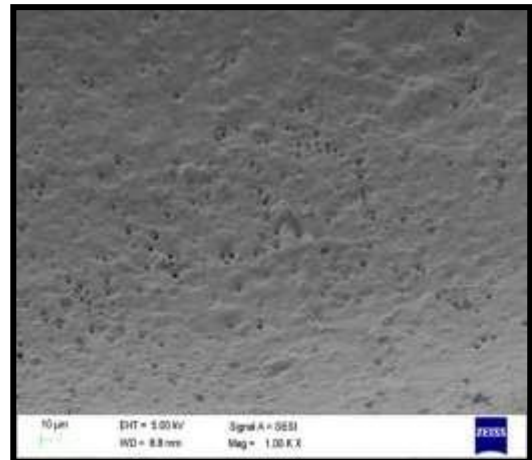
Group A
(No treatment)



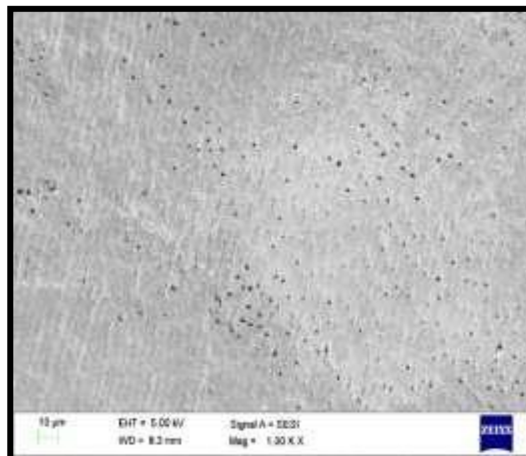
Group B
(α-Regular toothpaste)



Group C
(β -Toothpaste containing n-HAp)



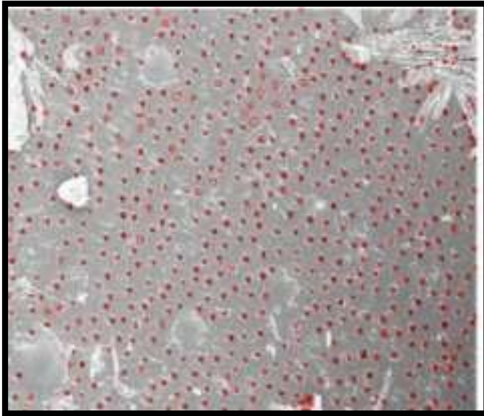
Group D
(λ -Toothpaste containing Novamin)



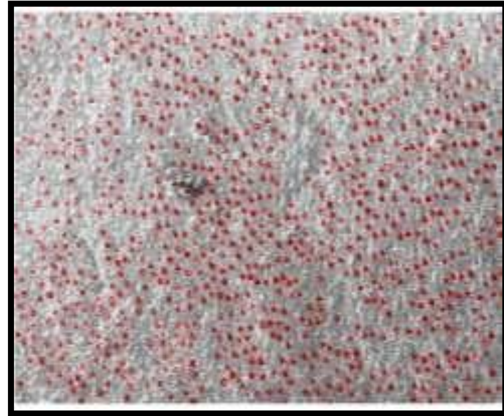
Group E
(Ω- Toothpaste containing Pro-Argin)

PLATE IX

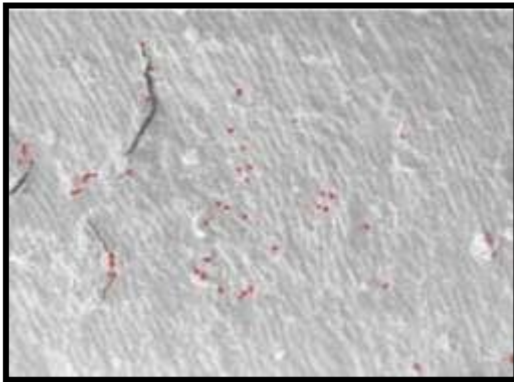
IMAGE ANALYSIS USING SCILABS



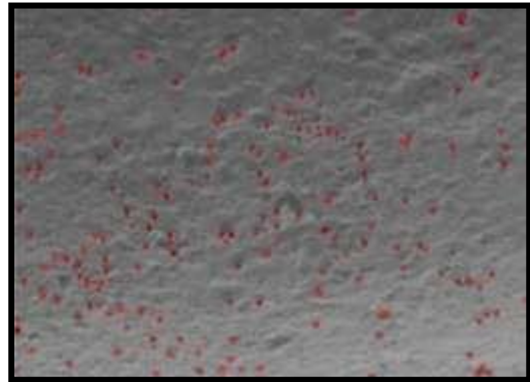
Group A
(No treatment)



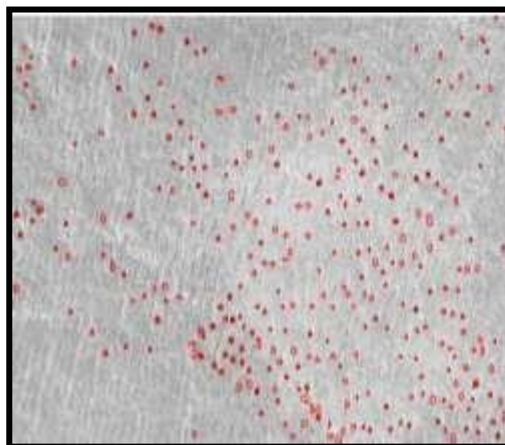
Group B
(α-Regular toothpaste)



Group C
(β-Toothpaste containing n-HAp)



Group D
(λ-Toothpaste containing Novamin)



Group E
(Ω-Toothpaste containing Pro-Argin)

Result

This in-vitro study was carried out to evaluate and compare the effects of three different desensitizing toothpastes (Nano-hydroxyapatite, Novamin and Pro-Argin) on dentinal tubule occlusion using SEM. A total of 105 samples were prepared and divided using a computer assisted randomization technique into five groups as follow:

Control Groups

Group A (Negative control): No treatment (n = 5)

Group B (Positive control): α-Regular toothpaste (n = 25)

Experimental Groups

Group C: β- Toothpaste containing n-HAp (n = 25)

Group D: λ- Toothpaste containing Novamin (n = 25)

Group E: Ω- Toothpaste containing Pro-Argin (n = 25)

The samples of group A were subjected to a pre-treatment SEM analysis. Each treatment group (Group B, Group C, Group D, Group E) samples were brushed for 2 mins twice daily for 2 months using Oral B Cross Action Toothbrush by customized jig machine. Subsequent to the treatment, the samples were dried and prepared for analysis by SEM. The counting of total number of dentinal tubules, open dentinal tubules, partially occluded dentinal tubules and completely occluded dentinal tubules from the obtained images was done using SCILABS software (5.5.2) (Rungis, France) and reported data was evaluated for statistical analysis.

Statistical Analysis:

The data of total number, open, partially occluded and completely occluded dentinal tubules was obtained and summarized in terms of mean, standard deviation, median and range for each study group. The comparison of mean number of dentinal tubules was performed statistically using one-way analysis of variance (ANOVA). The paired comparison between groups was carried out using Tukey's post-hoc test. The statistical analysis was carried out using SPSS version 20.0 (IBM Corporation) for each type of dentinal tubules and the inferences were obtained. The statistical significance was tested at 5% level.

The description of methods and formulations used in the study are as below: If $x_1, x_2, \ldots x_n$ are the observations on random variable X, then

A. **Sample mean** for a set of observations is given by

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

B. **Standard deviation** for a set of observations in given by

$$s = \sqrt{\frac{1}{(n-1)}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

where x_i = observation on each object

n = number of objects

C. **Median**: It is the middle value of a set of values when arranged in the increasing order of magnitude.

D. **Range** is the difference between maximum and minimum value of the variable.

E. **One-way Analysis of variance**

Analysis of variance (ANOVA) is used to test the significance of difference in the mean of three or more groups. The basic assumption is that the variable of interest is normally distributed in the population under study.

Method:

Here the interest is to test the null hypothesis that the population means are same, i.e.

$$H_0 : \mu_1 = \mu_2 = \ldots \mu_m$$

against the alternative H_1 that they are not same.

Some of the statistics computed to test the hypothesis are as below:

i. **Grand mean**: It is the mean of set of all observations in the studied groups and is given by:

$$\bar{x}_{GM} = \frac{1}{N}\sum_{i=1}^{N} x_i$$

ii. **Total sum of squares:** It is the sum of squares of each observation from the grand mean and is given by:

$$TSS = \sum_{i=1}^{N} (x_i - \bar{x}_{GM})^2$$

Total sums of squares is the sum of two components i.e., variation between groups and within groups.

iii. **Between group sum of squares**

$$SSB = \sum_{j=1}^{m} n_j (\bar{x}_j - \bar{x}_{GM})^2$$

iv. **Within group sum of squares**

$$SSW = \sum_{j=1}^{m}\sum_{i=1}^{n} (x_{ij} - \bar{x}_j)^2$$

The mean sum of squares is obtained by dividing the above sum of squares with the respective degrees of freedom, i.e. N-1, p-1 and p (n-1).

v. **F-statistic:** It is the ratio of between and within mean sum of squares

$$F = \frac{MS_{Between}}{MS_{Within}}$$

If the p-value based on F-statistic is greater than 0.05, H_0 is accepted, otherwise H_1 is accepted.

vi. Tukey's post-hoc test

After performing ANOVA, if alternative hypothesis H_1 is accepted, then the subsequent interest is to determine the pair wise significance of difference in the means of study groups. This could be carried using Tukey's post-hoc test. The difference between the means of all groups are determined and compared with this critical difference called the honest significant difference (HSD). It is given by:

$$HSD = q\sqrt{\frac{MS_{within}}{n}}$$

where, q is the studentized range statistic derived from the tables, n is the sample size and the mean square value is from the ANOVA analysis. If the critical difference exceeds the absolute difference between any two sample means, then the corresponding means differ significantly.

Overall Results:

Descriptive statistics for number of **open dentinal tubules** across different study groups have been presented in **Table 1.**

In Group A, the mean number of open dentinal tubules was maximum i.e. 583, SD: 20.48, with a median of 587 and ranging between 555-610.

In Group B, the mean number of open dentinal tubules was 292, SD: 63.55, with a median of 295 and with range of 177-389.

In Group C, the mean number open dentinal tubules was 1, SD: 2.47, with a median of 0 and with range of 0-9.

In Group D, the mean number open dentinal tubules was 2, SD: 3.18, with median of 0 and with range of 0-11.

In Group E, the mean number open dentinal tubules was 9, SD: 19.93, with median of 0 and with range of 0-87.

Table 2 gives comparison of mean number of open dentinal tubules across all the five study groups using one-way analysis of variance (ANOVA). It was evident from the table that the mean number of open dentinal tubules differed significantly across all the groups ($p < 0.0001$). Hence, the pair-wise analysis was carried out using Tukey's post-hoc test with results showed in **Table 3.**

Table 3 shows that the pairwise comparison of mean difference of open dentinal tubules between Group A and all other groups was statistically significant with $p < 0.0001$. Similar was the observation for Group B. However, the mean differences between Groups C, Group D and Group E were statistically insignificant ($p > 0.05$).

Figure 1 with column chart showing mean number of open dentinal tubules across different study groups.

Descriptive statistics for number of **partially occluded dentinal tubules** across Group B (Positive control) and all other experimental groups provided in **Table 4.**

In Group B, the mean number of partially occluded dentinal tubules was 72.68, SD: 20.49, with a median of 68 and ranging between 46-120.

In Group C, the mean number of partially occluded dentinal tubules was 36.04, SD: 49.63, with a median of 17 and range of 0-187.

In Group D, the mean number of partially occluded dentinal tubules was 79.68, SD: 74.21, with a median of 81 and range of 0-221.

In Group E, the mean number of partially occluded dentinal tubules was 143.80, SD: 67.36, with a median of 148 and range of 21-283.

Table 5 gives the comparison of mean number of partially occluded dentinal tubules across Group B (Positive control) and all other experimental groups using ANOVA. It was evident from the table that the mean number of partially occluded dentinal tubules differed significantly across all the groups with $p < 0.0001$. Hence, the pair-wise analysis was carried out using Tukey's post-hoc test with results shown in **Table 6.**

Table 6 shows pairwise comparison of mean number of partially occluded dentinal tubules across Group B (Positive control) and all other experimental groups. The mean difference between Groups B and Group E was statistically significant with $p < 0.0001$. Further, the mean difference between Groups C and Group D was statistically significant with p-value of 0.039. Whereas, the mean difference between Group C and Group E was statistically significant with $p < 0.0001$. The mean difference between Groups D and Group E was also significant with p-value of 0.001. However, the mean difference of Group B was statistically insignificant from Group C and Group D ($p > 0.05$)

Figure 2 with column chart showing mean number of partially occluded dentinal tubules across Group B (Positive control) and all other experimental groups.

Descriptive statistics for number of **completely occluded dentinal tubules** across Group B (Positive control) and all other experimental groups provided in **Table 7.**

In Group B, the mean number of completely occluded tubules was 218.76, SD: 62.46, with a median of 210 and ranging between 113-348.

In Group C, the mean number of completely occluded dentinal tubules was 546, SD: 49.96, with a median of 566 and range of 396-583.

In Group D, the mean number of completely occluded dentinal tubules was 501.8, SD: 75.59, with a median of 499 and range of 351-583.

In Group E, the mean number of completely occluded dentinal tubules was 430.44, SD: 72.66, with median of 435 and range of 277-562.

Table 8 gives the comparison of mean number of completely occluded dentinal tubules across Group B (Positive control) and all other experimental groups using ANOVA. It was evident from the table that the mean number of completely occluded dentinal tubules differed significantly across all the groups with $p < 0.0001$. Hence, the pair-wise analysis was carried out using Tukey's post-hoc test with results shown in **Table 9.**

Table 9 shows pairwise comparison of mean number of completely occluded dentinal tubules across Group B (Positive control) and all other experimental groups. The mean difference of completely occluded dentinal tubules between Group B (Positive control) from all other experimental groups was statistically significant with $p < 0.0001$. Further, the mean difference of Groups C differed significantly from Groups D and Group E with $p < 0.0001$. The mean difference between Groups D and Group E was also statistically significant with a p-value of 0.001.

Figure 3 with column chart showing mean number of completely occluded dentinal tubules across Group B (Positive control) and all other experimental groups.

Discussion

Dentin hypersensitivity is a relatively common problem experienced in clinical dental practice.[54] It is characterized by a short, sharp pain arising from exposed dentin which cannot be explained to any other form of dental defect or disease.[55] This condition may occur due to non-carious lesions like attrition, abrasion, erosion, abfraction or fracture of teeth which are responsible for removal of the enamel or cementum that covers the dentin surface.[3] It can be triggered by an external stimulus, such as a thermal (cold), tactile (tooth brushing or use of dental instruments), osmotic (sweet) or evaporative (air blast).[56]

The treatment of DH is quite challenging. It emphasizes on the use of a material that chemically reacts, physically occludes and adheres densely to dentinal tubules for significantly reducing the possibility of reopening the occluded tubules

and prevent its recurrence.[41] A wide array of treatment modalities like chemically desensitizing the nerve ending, forming protein precipitate and physically blocking the exposed dentinal tubules are suggested depending on their mechanism of action. According to the use, desensitizing agents are broadly classified as At-home or In-office. The desensitizing agents like potassium nitrate, potassium chloride or potassium citrate are responsible to block neural transmission at the pulpal tissues by chemically depolarizing the nerve synapse. While, agents like glutaraldehyde, silver nitrate, zinc chloride and strontium chloride hexahydrate are effective in reducing DH by forming protein precipitate. Other agents like potassium oxalate, strontium chloride, stannous fluoride, sodium fluoride, calcium phosphate, calcium sodium phosphosilicate, nanohydroxyapatite, bioactive glasses (novamin), arginine and calcium carbonate are responsible for physically occluding the dentinal tubules.[12] At-home agents appear to be the most rational, non-invasive and feasible treatment option for mild to moderate DH and are most commonly used by patients.[41] The only drawback of this approach is that it is a time-consuming process.[12]

The in-office desensitizing approaches available are lasers (eg. Nd-YAG, Erbium- YAG) and dentin adhesive sealers namely fluoride varnishes, oxalic acid and resin, glass ionomer cement, composites, and dentin bonding agents.[12] Due to the variety of currently available over-the-counter products, there exists confusion among patients regarding the selection of best amongst available options as no consensus is prevalent for the same.

The currently available literature reveals paucity of studies conducted to evaluate and compare the effectiveness of recently marketed n-HAp, Novamin and

Pro-Argin containing toothpaste in reducing DH, hence the current experimental in-vitro study was conducted.

Rees JS et al. (2004)[57] stated that maxillary premolars are the most commonly affected teeth by DH followed by the upper first molars while incisors being the least affected. **Balcheva G et al. (2017)**[5] found that the most affected site by DH is a buccal cervical area of permanent teeth. Therefore, in the present study extracted human maxillary premolars were selected.

Kulal R et al. (2016) [38] conducted an in-vitro study to evaluate and compare the effects of desensitizing agents containing n-HAp, Novamin and Pro-Argin on dentinal permeability and tubular occlusion using SEM. They reported that n-HAp had 98.1% tubule occlusion, while Pro-Argin showed 69.1% tubule occlusion. This difference was statistically significant. Assuming that similar differences could be obtained in the proposed study, the estimated sample size is 25 per group and 5 in the negative control group that can provide the effect with 95% confidence interval and 80% power of z-test for proportions.

The formula used was:

$$n = \frac{(z_{\alpha/2} + z_{\beta})^2 * (p_1(1-p_1) + p_2(1-p_2))}{(p1 - p2)^2}$$

Where $z\alpha/2$ is the critical value of standard normal distribution for 95% confidence level (i.e. α =0.05), z_{β} is the critical value for 80% power (β =0.2), p_1 and p_2 are the expected proportions.

In total 105 extracted human permanent maxillary premolars were collected, cleaned, disinfected and stored in 10% formalin as per the recommendation and guidelines given by OSHA and CDC(2003 report 17).[50] This was done to prevent samples from getting dehydrated.

The mechanical forces generated while tooth brushing are transmitted from the tooth to the bone but before that these forces are absorbed by periodontal ligament (PDL).[58] To replicate PDL in current study, a thin layer of wax sheet was applied on the root surface of each tooth and mounted in acrylic resin block 1 mm apical to the CEJ[59] using a brass L-mould. The tooth was removed from the acrylic block after the setting of acrylic material. **Sengun A et al. (2008)[59]and Shafiei F et al. (2014)[60]** suggested the use of a polyvinyl siloxane to simulate the PDL. In correspondence to this, the wax was replaced by application of light body addition silicone (polyvinyl siloxane) elastomeric impression material and teeth were remounted back to the acrylic block.

Standardized class V cavity was prepared to expose dentin in cervical 1/3rd of teeth. Cavities were prepared with standardized dimensions of 2mm height, 4mm

mesiodistal width and pulpal depth of 2mm[51] using FG-245 plain cut tungsten carbide Fissure bur (SS white, India) on buccal surface 1mm coronal to CEJ. [38,61] A digital vernier caliper (Workzone hand tools, Germany) was used to measure the widths of the prepared cavity design.[62,63] The depth of the cavity was measured using williams graduated probe (GDC).

The exposed dentin surface was polished with emery paper and washed with distilled water. Each sample then etched with 30% phosphoric acid for 15 minutes and thoroughly washed using distilled water. This was done to remove the smear layer produced during class V cavity preparation and to replicate the clinical condition of DH. All the samples were stored in distilled water after treating with 30% phosphoric acid.[38]

A total of 105 prepared samples were allocated by a computer-assisted randomization technique into five groups.

Control Groups:

Group A (Negative control): No treatment (n=5)

Group B (Positive control): α-Regular toothpaste (n=25)

Experimental Groups:

Group C: β- Toothpaste containing n-HAp (n=25)

Group D: λ- Toothpaste containing Novamin (n=25)

Group E: Ω- Toothpaste containing Pro-Argin (n=25)

In the current study, each group samples were brushed with pea-size amount of allocated toothpaste by mixing with 1 ml of artificial saliva (ICPA Wet Mouth, India).[28] **Bekes K et al. (2018)[45]** in their study on DH with 8% arginine reported that brushing for 2 min twice daily for 2 months significantly reduced DH; hence, in the current study similar time duration was considered. **Wiegand A et al. (2013)[52]** concluded that sonic toothbrush (0.9±0.2 N) and manual toothbrush (1.6±0.3 N) exerted significantly equivalent load while tooth brushing. Hence, in the present study samples were brushed at 0.9 N load using a customized jig with powered Oral B Cross Action toothbrush exerting up to 48,800 movements/min.[64] The special jig was designed to maintain the pressure and consistency of brushing strokes in all samples (Plate II). [29,65]

In current study to evaluate the efficacy of different desensitizing agents, the quantification of dentinal tubular occlusion was performed. Literature suggests various in-vitro methods to assess the efficacy of desensitizing products like SEM, CLSM, Electron Microprobe Analysis and Fourier-transform infrared spectroscopy (FTIR). In the current study, SEM analysis was used as it is non-destructive approach and can provide three dimensional and high-resolution images. It gives topographical, morphological and compositional data which is represented in digital form, easy to operate and work faster.[36,66] The surface of the samples were scanned at 1000X and 5kV. In present study, mean value of the total number of open dentinal tubules was recorded from 10µm field of negative control group samples (Group A) and this was considered constant for all the remaining group samples. This assumption was made because after treatment in many cases the individual open dentinal tubule will be no longer visible. Dentinal tubules were classified as open, partially occluded and

completely occluded dentinal tubules. In partially occluded tubules, particles were observed within the tubule which results in significant reduction in the diameter of dentinal tubule compared to the negative control group. The number of completely occluded dentinal tubules were calculated as the total number of dentinal tubules minus the number of open dentinal tubules and partially occluded dentinal tubules.[53] Those tubules were calculated in each image of all of the samples using SCILABS software (5.2.2) (Rungis, France). This was done to avoid subjective human error occurring in case of manual counting. The collected data was tabulated using an excel sheet (Microsoft Office 2010).

The comparison of the mean number of dentinal tubules was performed statistically using one-way analysis of variance (ANOVA). The paired comparison between groups was carried out using Tukey's post-hoc test. The statistical analysis was carried out using SPSS version 20.0 (IBM Corp.) for each type of dentinal tubules and the inferences were obtained. The statistical significance was tested at 5% level. The overall results are discussed under the following section.

In the present study, no toothpaste was used in **Group A**. **583** mean number of open dentinal tubules (Table 1) was recorded from SEM image of **10µm** field of Group A samples and was considered standard for all other groups.

Group B was treated with regular toothpaste. The mean number of open **(292)**, partially occluded **(72.68)** and completely occluded tubules **(218.76)** were obtained with this group (Table 1, 4, 7 respectively). Regular toothpaste composed of calcium carbonate, silica, sodium silicate, sodium monofluorophosphate (NaMFP) (1000ppm) and sodium lauryl sulfate. According to **Addy M et al. (1989)** abrasive

agents from regular toothpaste such as calcium carbonate, silica and sodium silicate might be responsible for partial or complete occlusion of dentinal tubules by continuous deposition on exposed dentin surface.[22,67]

In **Group C (n-HAp)** the mean number of open **(1)**, partially occluded **(36.04)** and completely occluded tubules **(546)** were reported (Table 1, 4, 7 respectively). Higher number of dentinal tubule occlusion in Group C could be attributed to the properties of n-HAp particles. These particles are biomimetic mineral composed of calcium and phosphate ions which deposit in demineralized dentin and it is similar to inorganic component of teeth.[68] n-HAp particles having strong surface bioactivity and biocompatibility that induces remineralization actively.[14] The smaller size and reactivity of n-HAp is responsible for deposition of particles in dentinal tubules.[69] **Kunam D et al. (2016)** evaluated the pattern of occlusion after treatment of dentin discs with n-HAp slurry under SEM and observed that n-HAp showed higher number of dentinal tubule occlusion which resulted in partial coverage of dentin surface with precipitate.[39]

In **Group D (Novamin)** the mean number of open **(2)**, partially occluded **(79.68)** and completely occluded tubules **(501.80)** were obtained (Table 1, 4, 7 respectively). The result obtained can be justified as, novamin particles are made up of calcium sodium phosphosilicate with 25% sodium, 25% calcium, 6-8% phosphate and silica.[70] Novamin has a strong affinity to bind to collagen; thus when it comes in contact with dentin having more collagen, more novamin binds the exposed dentinal surfaces thus physically occluding the dentinal tubules.[71] However, the main drawback of using novamin is that it takes longer duration for apatite formation and

dentinal tubule occlusion.[72] **Shah S et al. (2017)** in their in-vitro study evaluated the efficacy of Novamin and Pro-Argin containing desensitizing dentifrices on occlusion of dentinal tubules. They stated that Novamin containing toothpaste showed uniform and maximum tubular occlusion (95.58%) whereas, pro-argin showed mean tubular occlusion (89.90%) under SEM.[41]

In **Group E (Pro-Argin)** the mean number of open **(9)**, partially occluded **(143.80)** and completely occluded tubules (**430.44)** were reported (Table 1, 4, 7 respectively). This is in accordance to the mechanism where, arginine physically adsorbs on the surface of the calcium carbonate resulting in formation of positively charged agglomerate and readily binds to the negatively charged exposed dentin surfaces. Pro-Argin is an amino acid that is positively charged at physiologic pH of 6.5-7.5.[73] The results might have obtained because, pro-argin paste had a lower concentration of NaMFP (1.440ppm) and it can produce free fluoride ions only when hydrolyzed by salivary alkaline phosphatase. The enzymatic and microbiological effects of human saliva could not be possible in present study since artificial saliva was used.[43] In the study conducted by **Cunha SR et al. (2017)** arginine containing desensitizing paste reported highest number of open dentinal tubules (99) compared to Novamin containing desensitizing paste (90) and Laser (87) when observed under SEM.[43]

Group wise comparison of mean number of open dentinal tubules:

The comparison of mean number of open dentinal tubules across different study groups i.e. Group A (No treatment) **583** > Group B (Regular toothpaste) **292** > Group E (Pro-Argin) **9** > Group D (Novamin) **2** > Group C (n-HAp) **1** differed significantly

(p <0.0001) (Table 1, 2). The pairwise comparison of mean difference of open dentinal tubules between Group A (No treatment) and all other groups was statistically significant with p < 0.0001. Similar was the observation for Group B (Regular toothpaste). However, the mean differences between Groups C (n-HAp), Group D (Novamin) and Group E (Pro-Argin) was statistically insignificant with p > 0.05 (Table 3).

Group wise comparison of mean number of partially occluded dentinal tubules:

The comparison of mean number of partially occluded dentinal tubules across different study groups i.e. Group E (Pro-Argin) **143.80 >** Group D (Novamin) **79.68 >** Group B (Regular toothpaste) **72.68** > Group C (n-HAp) **36.04** differed significantly (p < 0.0001) (Table 4, 5). The pairwise comparison of mean difference of partially occluded tubules between Groups B (Regular toothpaste) and Group E (Pro-Argin) was statistically significant with p < 0.0001. Further, the mean difference between Groups C (n-HAp) and Group D (Novamin) was statistically significant with p-value of 0.039. Similarly, the mean difference between Group C (n-HAp) and Group E (Pro-Argin) was statistically significant with p < 0.0001. The mean difference between Groups D (Novamin) and Group E (Pro-Argin) was also significant with a p-value of 0.001. However, the mean difference of Group B (Regular toothpaste) was statistically insignificant from Group C (n-HAp) and Group D (Novamin) with p > 0.05 (Table 6).

Group wise comparison of mean number of completely occluded dentinal tubules:

The comparison of mean number of completely occluded dentinal tubules across different study groups i.e. Group C (n-HAp) **546 >** Group D (Novamin) **501.80** > Group E (Pro-Argin) **430.44** >Group B (Regular toothpaste) **218.76** was statistically significant with $p < 0.0001$ (Table 7, 8). The pairwise comparison of mean difference of completely occluded tubules between Group B (Regular toothpaste) from all other experimental groups was statistically significant with $p < 0.0001$. Further, the mean difference of Groups C (n-HAp) differed significantly from Groups D (Novamin) and Group E (Pro-Argin) with $p < 0.0001$. The mean difference between Groups D (Novamin) and Group E (Pro-Argin) was also statistically significant with a p-value of 0.001 (Table 9).

To summarize the results of present study, Group C (n-HAp) reported maximum dentinal tubular occlusion followed by Group D (Novamin), Group E (Pro-Argin) and Group B (Regular toothpaste) respectively.

The findings of our study are in correspondence with the following studies. According to **Kulal L et al. (2016)**[38] 15% n-HAp crystals were more effective as compared to the 5% Novamin and 8% Pro-Argin desensitizing agents by achieving 98.1% tubule occlusion after 7days of treatment. **Baglar S et al. (2018)** in their in-vitro study reported that n-HAp was able to occlude all the dentinal tubules completely when viewed under SEM.[47] n-HAp was found to be better than other toothpastes due to its excellent bioactive properties and formation of biomimetic apatite layer on exposed dentin surface with striking similarities to dental hard tissues.[14,69]

Thus the null hypothesis of the present study that there is no significant difference between the effectiveness of Nanohydroxyapatite, Novamin and Pro-Argin containing desensitizing toothpaste in occluding exposed dentinal tubules was rejected.

Further long term in-vitro and clinical trials are necessary to validate the outcome of these new products as an efficacious desensitizing agents.

Therefore, within the limitations of the present study, it can be concluded that n-HAp containing desensitizing toothpaste proved to be most effective in occluding dentinal tubules followed by Novamin, Pro-Argin and Regular toothpaste respectively.

Limitations

1. In-vitro studies cannot simulate the dynamics of oral conditions in toto therefore results can be extrapolated to vivo conditions within limitation.

2. Desensitizing toothpaste may need more time period to show results therefore long term studies need to be carried out.

3. Scanning electron microscope could only characterize the morphology of samples in dehydrated state.

Summary and Conclusion

Dentin Hypersensitivity is one of the most serious dental issues encountered in clinical dentistry associated with an exaggerating response to stimulus on exposed dentinal tubules. In most instances, this condition can be managed by patients through wide range of home care desensitizing products containing calcium carbonate, strontium, oxalates, fluorides, sodium monofluorophosphate, etc.; whereas some contain novel agents like Nano-hydroxyapatite, Novamin and Pro-Argin.

The aim of the present study was to evaluate and compare the effects of Nano-hydroxyapatite, Novamin and Pro-Argin containing desensitizing toothpastes on dentinal tubule occlusion using SEM.

105 freshly extracted human permanent maxillary premolars fulfilling the inclusion criteria were selected for the study. All samples were mounted in cold-cure

acrylic resin. A standardized class V cavity was placed on buccal surface of tooth 1mm coronal to CEJ. Dentin surface was then polished with emery paper and washed with distilled water. 30% phosphoric acid was then used for 15 mins to simulate hypersensitive dentin by removing smear layer and thoroughly washed with distilled water. All the samples were stored in distilled water after treating with 30% phosphoric acid.

Depending upon the desensitizing toothpastes used, the samples were allocated by computer assisted randomization technique into 5 groups as follows:

Control Groups:

Group A (Negative control):- No treatment (n=5)

Group B (Positive control):- α-Regular toothpaste (n=25)

Experimental Groups:

Group C:- β- Toothpaste containing n-HAp (n=25)

Group D:- λ- Toothpaste containing Novamin (n=25)

Group E:- Ω- Toothpaste containing Pro-Argin (n=25)

All the samples excluding Group A were brushed for 2mins twice daily for 2months with respective desensitizing toothpaste using powered electric toothbrush on customized jig. Subsequent to the treatment, all the samples were dried and analyzed using SEM. The data obtained from images was recorded using SCILABS software and analyzed.

The results obtained indicated that there was highly significant difference in dentinal tubule occlusion between Group B (Regular toothpaste) and other experimental groups ($P < 0.0001$). The inter group comparison revealed maximum tubular occlusion with Group C (n-HAp) followed by Group D (Novamin), Group E (Pro-Argin) and Group B (Regular toothpaste) respectively.

Within the limitations of the study, following conclusion can be drawn-

1. The number of dentinal tubules occluded in all experimental groups were significantly higher when compared to regular toothpaste.

2. n-HAp containing toothpaste reported highest efficacy in occluding dentinal tubules followed by Novamin, Pro-Argin and Regular toothpaste at the end of 2 months.

Taking into consideration the finding of present study, it can be concluded that n-HAp novel biomaterial is potential treatment modality for dentin hypersensitivity.

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Tables and Graphs

Table 1: Descriptive statistics for number of open dentinal tubules across different study groups

OPEN DENTINAL TUBULES	Groups				
	Group A (n=5)	Group B (n=25)	Group C (n=25)	Group D (n=25)	Group E (n=25)
Mean	**583**	**292**	**1**	**2**	**9**
SD	20.48	63.55	2.47	3.18	19.93
Median	587	295	0	0	0
Minimum	555	177	0	0	0
Maximum	610	389	9	11	87

SD: Standard deviation

Table 2 : Comparison of mean number of open dentinal tubules across different study groups

Source of variation	Sum of Squares	DF	Mean Square	F-statistics	P-value*
Between Groups	2779622.937	4	694905.734	640.290	< 0.0001
Within Groups	108529.920	100	1085.299		
Total	2888152.857	104			

*Using one-way ANOVA

Table 3: Pairwise comparison of mean number of open dentinal tubules across different study groups using Tukey's post-hoc test

Paired groups		Mean difference	Std. error	P-value	95% confidence interval	
					Lower bound	Upper bound
Group A	Group B	291.44	16.139	< 0.0001	246.60	336.28
	Group C	582.04	16.139	< 0.0001	537.20	626.88
	Group D	581.48	16.139	< 0.0001	536.64	626.32
	Group E	574.24	16.139	< 0.0001	529.40	619.08
Group B	Group C	290.6	9.318	< 0.0001	264.71	316.49
	Group D	290.04	9.318	< 0.0001	264.15	315.93
	Group E	282.8	9.318	< 0.0001	256.91	308.69
Group C	Group D	-0.560	9.318	1.000	-26.45	25.33
	Group E	-7.800	9.318	0.918	-33.69	18.09
Group D	Group E	-7.240	9.318	0.937	-33.13	18.65

Table 4: Descriptive statistics for number of partially occluded dentinal tubules across Group B (Positive control) and all other experimental groups

PARTIALLY OCCLUDED DENTINAL TUBULES	Group			
	Group B (n=25)	Group C (n=25)	Group D (n=25)	Group E (n=25)
Mean	**72.68**	**36.04**	**79.68**	**143.80**
Standard Deviation	20.49	49.63	74.21	67.36
Median	68	17	81	148
Minimum	46	0	0	21
Maximum	120	187	221	283

Table 5: Comparison of mean number of partially occluded dentinal tubules across Group B (Positive control) and all other experimental groups

Source of variation	Sum of Squares	DF	Mean Square	F-statistic	P-value*
Between Groups	150484.910	3	50161.637	15.521	< 0.0001
Within Groups	310267.840	96	3231.957		
Total	460752.750	99			

*Using one-way ANOVA

Table 6: Pairwise comparison of mean number of partially occluded dentinal tubules across Group B (Positive control) and all other experimental groups using Tukey's post-hoc test

Paired groups		Mean difference	P-value	95% confidence interval	
				Lower bound	Upper bound
Group B	Group C	36.640	.110	-5.40	78.68
	Group D	-7.000	.972	-49.04	35.04
	Group E	-71.12	< 0.0001	-113.16	-29.08
Group C	Group D	-43.64	.039	-85.68	-1.60
	Group E	-107.76	< 0.0001	-149.80	-65.72
Group D	Group E	-64.12	.001	-106.16	-22.08

Table 7: Descriptive statistics for completely occluded dentinal tubules across Group B (Positive control) and all other experimental groups

COMPLETELY OCCLUDED DENTINAL TUBULES	Group			
	Group B (n=25)	Group C (n=25)	Group D (n=25)	Group E (n=25)
Mean	**218.76**	**546.00**	**501.80**	**430.44**
Standard Deviation	62.46	49.96	75.59	72.66
Median	210	566	499	435
Minimum	113	396	351	277
Maximum	348	583	583	562

Table 8: Comparison of mean number of completely occluded dentinal tubules across Group B (Positive control) and all other experimental groups

Source of variation	Sum of Squares	DF	Mean Square	F-statistic	P-value*
Between Groups	1577538.030	3	525846.010	120.952	< 0.0001
Within Groups	417366.720	96	4347.570		
Total	1994904.750	99			

*Using one-way ANOVA

Table 9: Pairwise comparison of mean number of completely occluded dentinal tubules across Group B (Positive control) and all other experimental groups using Tukey's post-hoc test

Paired groups		Mean difference	P-value	95% confidence interval	
				Lower bound	Upper bound
Group B	Group C	-327.24	< 0.0001	-376.00	-278.48
	Group D	-283.04	< 0.0001	-331.80	-234.28
	Group E	-211.68	< 0.0001	-260.44	-162.92
Group C	Group D	44.200	< 0.0001	-4.56	92.96
	Group E	115.56	< 0.0001	66.80	164.32
Group D	Group E	71.36	.001	22.60	120.12

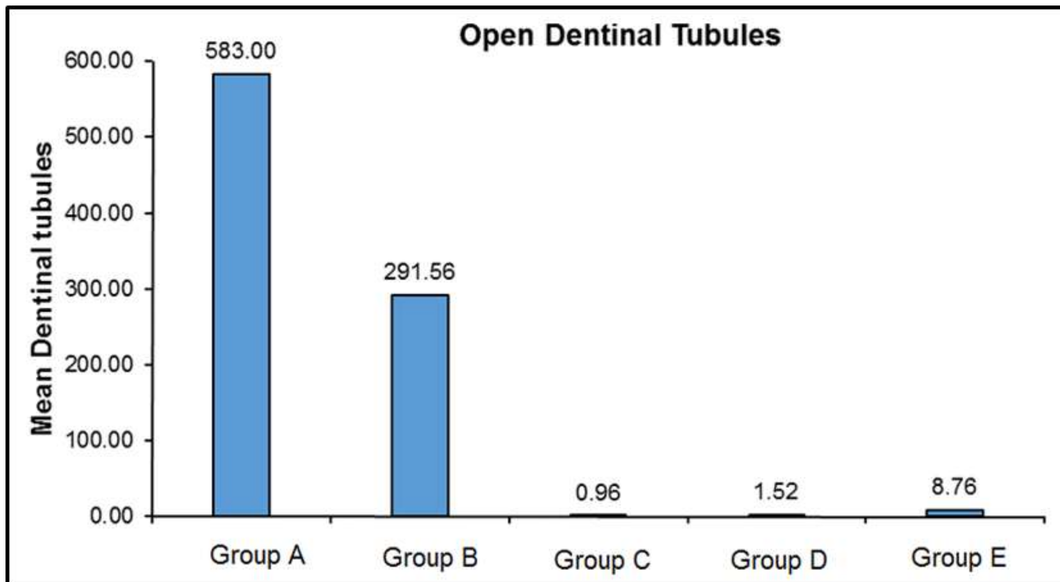

Figure 1: Column chart showing mean number of open dentinal tubules across different study groups

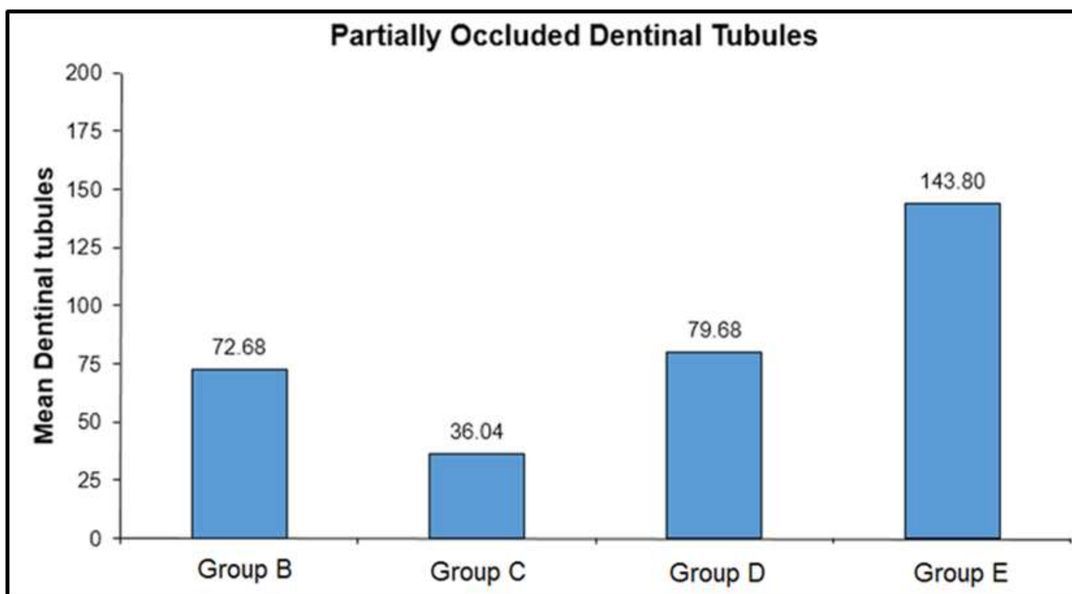

Figure 2: Column chart showing mean number of partially occluded dentinal tubules across Group B (Positive control) and all other experimental groups

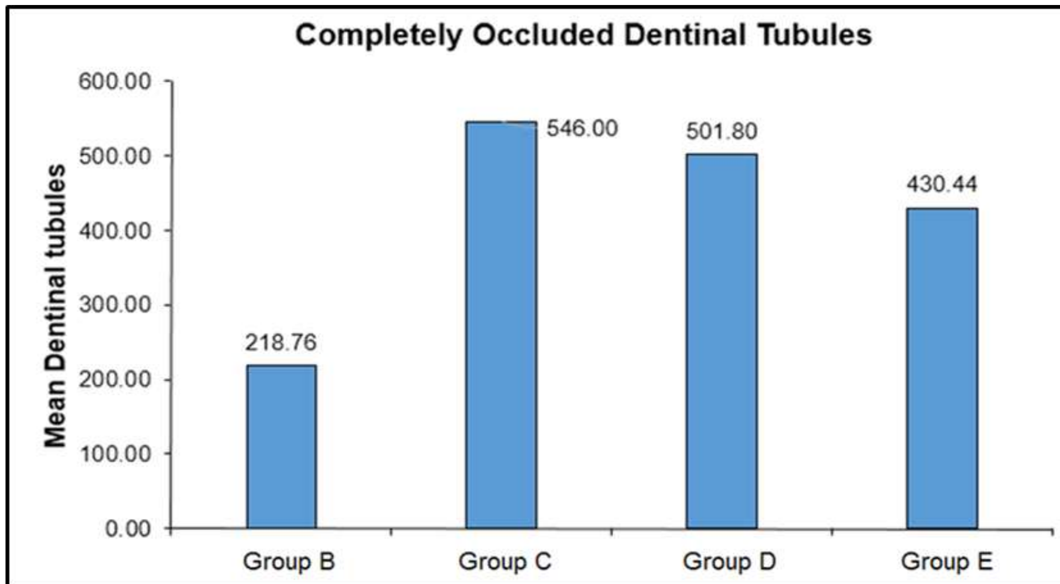

Figure 3: Column chart showing mean number of completely occluded dentinal tubules across Group B (Positive control) and all other experimental groups

MASTER CHART

Group A(Negative control)-No treatment (n=5)

Dental Tubules	Sample Number						
	1	2	3	4	5		
Total Number of Dentinal Tubules	610	587	555	590	573	**MEAN**	583
Open Dentinal Tubules	610	587	555	590	573	**MEAN**	583
Partially Occluded Dentinal Tubules	0	0	0	0	0		
Completely Occluded Dentinal Tubules	0	0	0	0	0		

Group B(Positive control) - α-Regular toothpaste (n=25)

Dental Tubules	Sample Number																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Total Number of Dentinal Tubules	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583
Open Dentinal Tubules	250	220	285	389	295	219	214	224	177	250	308	384	382	389	270	378	338	345	329	298	215	317	299	258	256
Partially Occluded Dentinal Tubules	63	80	75	81	78	85	72	60	58	120	81	52	67	81	119	63	58	62	52	46	68	59	51	68	118
Completely Occluded Dentinal Tubules	270	283	223	113	210	279	297	299	348	213	194	147	134	113	194	142	187	176	202	239	300	207	233	257	209

Group C:- β- toothpaste containing Nano-hydroxyapatite (n=25)

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Dental Tubules	Sample Number																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Total Number of Dentinal Tubules	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583
Open Dentinal Tubules	0	0	0	0	0	0	0	0	0	0	2	0	0	9	6	7	0	0	0	0	0	0	0	0	0
Partially Occluded Dentinal Tubules	0	0	0	0	0	0	0	0	40	52	39	104	132	63	42	41	35	39	110	187	17	0	0	0	0
Completely Occluded Dentinal Tubules	583	583	583	583	583	583	583	583	543	531	542	479	451	511	535	535	548	544	473	396	566	583	583	583	583

Group D:- λ- toothpaste containing Novamin (n=25)

Dental Tubules	Sample Number																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Total Number of Dentinal Tubules	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583
Open Dentinal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	7	3	2	6	0	0	0	0	0	0	0	9	11
Partially Occluded Dentinal Tubules	4	7	8	42	7	0	197	97	128	119	82	108	78	81	129	87	20	21	0	0	0	221	180	155	221
Completely Occluded Dentinal Tubules	579	576	575	541	576	583	386	486	455	464	501	475	498	499	452	490	563	562	583	583	583	362	403	419	351

Group E:- Ω- toothpaste containing Proargin (n=25)

Dental Tubules	Sample Number																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Total Number of Dentinal Tubules	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583	583
Open Dentinal Tubules	18	5	7	3	0	4	0	6	0	0	0	0	0	0	0	0	0	11	9	6	0	0	7	87	56
Partially Occluded Dentinal Tubules	161	72	108	130	78	175	51	52	283	105	148	170	236	96	148	254	21	151	178	183	120	104	201	120	250
Completely Occluded Dentinal Tubules	404	506	468	450	505	404	532	525	300	478	435	413	347	487	435	329	562	421	396	394	463	479	375	376	277