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Research Article

Effect of two different Materials; Microgel p(NIPAM) and Sodium Fluoride on the Depth and Degree of Occlusion of the Dentinal Tubules at Different Dentin Depths

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Abstract

Objectives: To evaluate the effect of two different desensitizing materials microgel p(NIPAM) and 5% sodium fluoride (NaF) on the degree of occlusion and depth of penetration of the dentinal tubules on superficial and deep dentin after immediate application and thermocycling using environmental scanning electron microscope.

Materials and Methods: 20 non- carious, non-restored molars were included. Each tooth was cut in order to obtain four quadrants. Specimens were divided into two groups according to aging. Then they were further sub-divided into two subgroups according to dentin depths. Further subdivision into four divisions according to the desensitizing material. The specimens were evaluated for the depth of penetration and degree of occlusion of each material using environmental scanning electron microscope. One-way ANOVA followed by Tukey post hoc test was used to compare between more than two groups in non-related samples. Independent sample t-test was used to compare between two groups in non-related samples.

Results: p(Nipam) showed the highest mean value without statistically significant difference with the varnish group regarding the degree of occlusion, either on immediate evaluation or after thermocycling.

Conclusion: Better occlusion of dentinal tubules was shown on immediate testing rather than after thermocycling regardless the dentin type, however, more depth of penetration was shown after thermocycling regardless the dentin type. All the desensitizing materials showed better occlusion and depth of penetration in superficial dentin than in deep dentin.

Clinical relevance: p(NIPAM) microgel is a promising material in the treatment of dentin hypersensitivity, however further in-vivo studies are needed.

Keywords

Dentin hypersensitivity, Varnish, Thermocycling, Degree of occlusion, Microgel p(NIPAM), Depth of penetration

Declaration of Conflicting Interest

Author Salma Basyouni declares that she has no conflict of interest. Author Nermeen Hamza declares that she has no conflict of interest. Author Reham Mohsen declares that she has no conflict of interest. Author Faten Kamel declares that she has no conflict of interest.

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Introduction

Dentin hypersensitivity could be a common oral finding which arise from exposed dentin in response to many stimuli as thermal, evaporative, tactile, osmotic, or chemical and has been characterized by intense pain for a brief length[1,2]. Brännström M[3] in 1963 proposed the hydrodynamic theory that is considered so far, the foremost acknowledged hypothesis utilized to explain the mechanisms of dentin hypersensitivity [1, 2, 4]. Agreeing to this hypothesis, open tubules of exposed dentine permit the movement of dentinal fluid within them, and therefore pulp nerves are indirectly stimulated[5]. Many factors play a role in the loss of tooth substance that could be chemical or mechanical, inducing the occurrence of dentin hypersensitivity. One of the common reasons for dentin hypersensitivity is dental erosion, it is proved to have a significant role in its onset, as dentinal tubule's openings can be easily opened and broadened by erosive acids[6,7,8]. Dentin hypersensitive-affected tooth could be differentiated from the non-sensitive tooth through many factors as width, patency status and the number of open tubules. Teeth affected by dentin hypersensitivity have a more noteworthy number of opened and sensitive tubules (8×) that are as well wider (2×), compared with non-sensitive teeth [9].

Though the aim of the current dentistry nowadays is the prevention, early mediation, and generally noninvasive treatments at the primary indications of dental illness, however, various treatment options aim to interfere with the mechanism of dentin hypersensitivity, either momentarily or forever. Basically, there are two main approaches known in dealing with dentin hypersensitivity either impede or diminish neural transmission and physically occlude or plug the open tubules [9,1,2]. A more effective treatment was shown with intratubular deposition that gave long-lasting results, as it decreases the fluid flow rate or completely seals the tubule lumen, while superficial occlusion of tubules has been proved to be removed easily by daily tooth brushing, or drinking of acidic beverages leading to short-term desensitizing effect [10,11] and therefore, depth of penetration of materials have been evaluated in the current study. The ability of desensitizing agents to resist the different challenges available in the oral environment that could be chemical or mechanical is highly related to their effectiveness. [6,12].

Fluoride varnishes proved to secure the dentin surface by the formation of calcium fluoride crystals [13]. However, due to the small diameter of those crystals (0.05mm) they might not be able to reduce the width of opened dentinal tubules of hypersensitive teeth and several applications might be mandatory. Therefore, blend of NaF with other chemicals such as tri calcium phosphate (TCP) have been proposed to overcome this limitation [14]; and so Clinpro White varnish has been used in the current study. p(NIPAM) microgel is a new material, that is remarkably affected by the environmental changes, that allowed them to significantly adjust their adaptation through altering their molecule size and surface charge density, and has been stated by P.Tempesti et al., in 2017 [15] that it represents a perfect case study for blocking of dentinal tubules. In addition, microgel's flexibility is considered a benefit; as it can be manufactured with explicit properties to outfit a certain application.

In the current study p(NIPAM) microgel has been compared with 5 % sodium fluoride varnish containing tri calcium phosphate regarding their effect in treating dentin hypersensitivity.

The morphological changes caused by the desensitizing agents was displayed using environmental electron microscope without the need to prepare any of the samples.

The aim of the study was to evaluate the effect of two different desensitizing materials; 5% sodium fluoride varnish with tri calcium phosphate and Microgel p(NIPAM) on the degree of occlusion and depth of penetration in the dentinal tubules on superficial and deep dentin upon immediate application and after thermocycling using environmental scanning electron microscope.

Materials and Methods

Materials

Clinpro White Varnish 5% sodium fluoride was used as a commercial control (3M), it was composed of 50 mg of sodium fluoride equivalent to 22.6 mg of fluoride ions (22,600 ppm of the fluoride ion), in an alcohol-based solution of modified rosins. Xylitol used as sweetener and Tri calcium phosphate.

For the synthesis of p(NIPAM) microgel, N-isopropylacrylamide (NIPAM) 97% (Sigma Aldrich), N, N'- methylenebisacrylamide 99% (Aldrich), potassium persulphate 98% (BDH Laboratory Suppliers), were used with no additional purification, and attained from commercial suppliers.

Methods

Synthesis of p(NIPAM)

800 mL of distilled water was used to dissolve 0.5 g of potassium per sulphate (initiator) in a 1 L reaction vessel. The reaction vessel was fixed with a three-necked lid and heated to 70°C with constant stirring. After mixing 5g NIPAM (monomer) and 0.5 g N, N'-methylenebisacrylamide (cross linker) in 200 mL of distilled water it was moved to the reaction vessel containing the initiator, where they were

stirred continuously for 6 hours at 70°C in an inert nitrogen atmosphere. The microgel dispersion was allowed to cool to room temperature once the reaction was completed. Fresh de-ionized water was used to dialyze the microgel for 7 days (changed daily), then centrifuged and freeze dried.

Teeth selection

In this study, a total of 20 non- carious, non-restored extracted molars free of caries, cracks, congenital anomalies or restorations were included, which was collected from the outpatient clinic of the dental school of Modern Science and Arts university (MSA), after collecting informed consent from the patients and the hospital. Age group 25 to 40 years, were selected for this study. The study was approved by the Ethics of Committee of Modern Science and Arts University (MSA) no. ETH 32.

Teeth preparation

Each tooth was cut in order to obtain four quadrants with total of 80 specimens. Teeth were decoronated 2mm below cemento-enamel junction (CEJ) using diamond disc (3M™ Diamond Cloth Disc 674W) under copious water cooling. They were then individually embedded in self-curing acrylic resin. A pencil and a graph paper were used to mark the middle part of the tooth in a mesio-distal direction, then it was cut along this mark in occluso-cervical direction parallel to the long axis of the tooth using abrasive disc under water coolant. The occlusal enamel was then removed using abrasive disc (3M FLAP disc 769F) 1 mm below dentino-enamel junction under copious water cooling, to expose a flat superficial dentin surface. They were furtherly cut 3mm below the superficial dentin in a mesio-distal direction using abrasive disc under water coolant as well to expose the flat deep dentin surface. Four surfaces of flat dentin discs with 3 mm width from each tooth were obtained with a total of 80 quadrants. The occlusal quadrants were marked from the apical side to identify the experimental superficial dentin surface. Finally, the apical quadrants were marked from the pulpal side to aid in identification of the experimental deep dentin surface. After that, the specimens were preserved in deionized water. Silicon carbide paper with a grit of 600 was used to polish all exposed dentin surfaces in all groups in order to create standardized smear layer. 50% citric acid (El Gomhoreya Company) was used to treat the specimens for 1 minute then rinsed thoroughly with distilled water to get the smear layer and smear plugs removed, in order to mimic hypersensitive dentin with opened dentinal tubules.

Teeth grouping

Specimens were divided into two groups according to aging into: (immediate mesial part), and (thermocycling distal part). Then they were further sub-divided into two subgroups according to dentin depths: (superficial dentin and deep dentin). Specimens were further subdivided into four divisions according to the desensitizing material where, control, Clinpro white varnish (5% NaF + TCP), Microgel p) NIPAM (, and Mix between the Clinpro white varnish and the p(NIPAM).

Environmental Scanning Electron Microscope (ESEM)

Occlusion of dentinal tubules was evaluated by detecting the morphological changes of the precipitate in the dentinal tubules using environmental scanning electron microscope (SEM) as well as the depth of penetration of the materials inside the dentinal tubules. Image analysis were done at 2500 x to show the diameter of the open tubules and at 1500 x to determine the depth of penetration of materials inside dentinal tubules. Scanning electron microscopy was performed by SEM/EDXA (JOEL JXA-840A, Electron probe micro-analyzer, Japan).

Statistical Analysis

In each test, the mean and standard deviation values were determined for each category. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine the ordinality of the data, and the results revealed parametric (normal) distribution. One-way ANOVA was used to compare more than two groups in unrelated samples, followed by a Tukey post hoc examination. To distinguish between two groups of unrelated samples, an independent sample t-test was used. $P \leq 0.05$ was selected as the significance standard. IBM® SPSS® Statistics Version 20 for Windows was used for statistical analysis.

Results

Degree of occlusion in superficial dentin upon immediate evaluation

The difference between the control superficial group and each of the varnish, p(NIPAM) and mix groups was statistically significant ($p<0.001$). Most of the tubules in the specimens treated with p(NIPAM), varnish and mix were partially or completely occluded, while no statistically significant difference was found between (varnish) and each of (p(NIPAM)) and (mix) groups where ($p=0.858$) and ($p=0.076$). However, a statistically significant difference was found between (p(NIPAM)) and (mix) groups where ($p=0.030$). Image analysis was done as well following the scanning by ESEM so as to measure the diameter of dentinal tubules and it showed that p(NIPAM) group had the highest percentage of dentinal occlusion i.e. the least dentinal tubule diameter, followed by the varnish and then the mix group **(Figure-1)**. However, the highest mean value was for the p(NIPAM) followed by the varnish, mix group and then control **(Table-1)**.

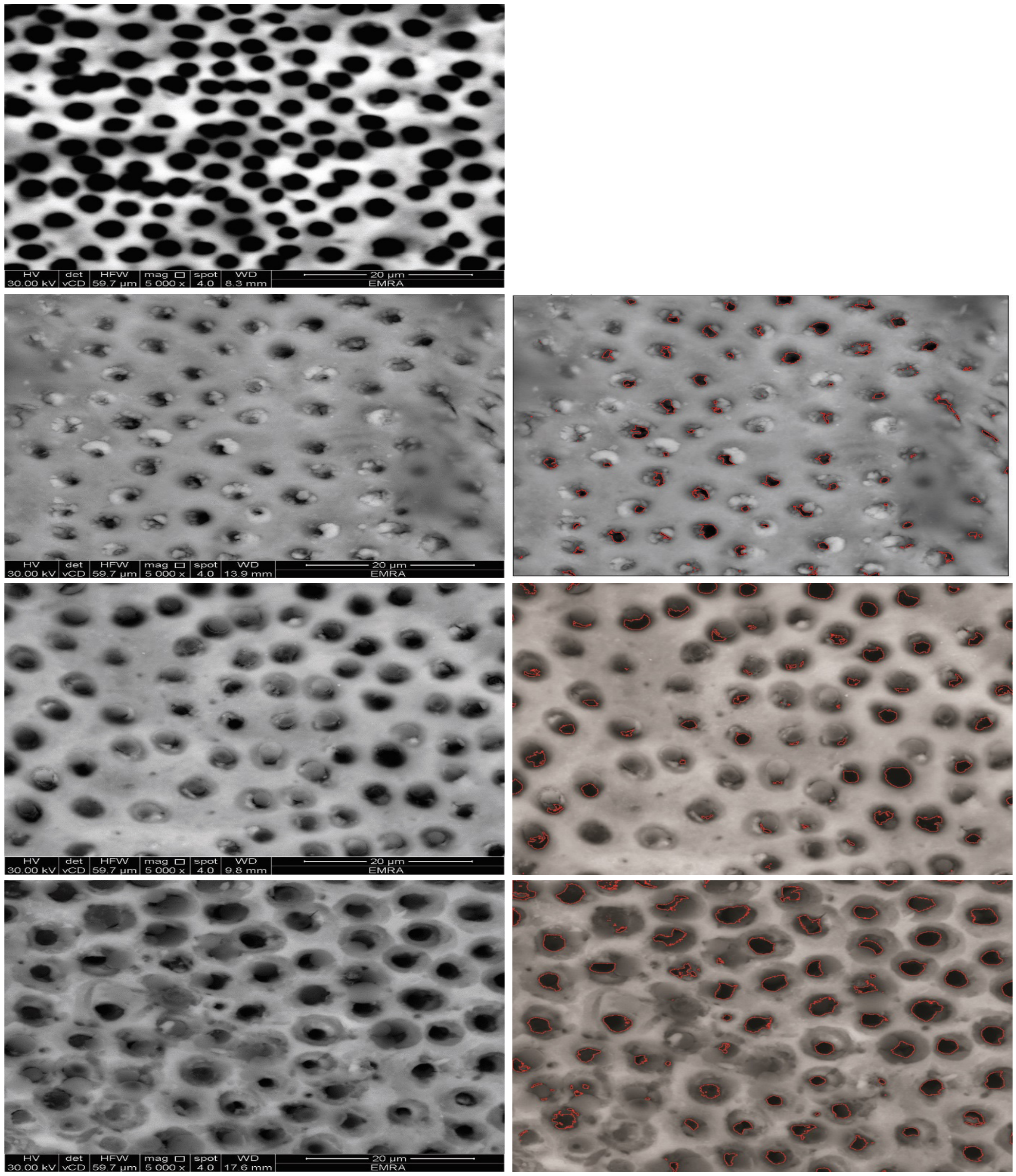

Figure 1 ESEM pictures with magnification 5000 X showing results of degree of occlusion after application of A) control group B) p(NIPAM), D) Varnish, F) Mix of varnish and p(NIPAM) on superficial dentin after immediate testing, and the image analysis with magnification 2500 X C)p(NIPAM), E) Varnish, G) Mix

Variables	Superficial dentin	Deep dentin
	Mean	Mean
p(NIPAM)	96.11 (1.22)	85.53 (3.28)
Varnish	95.59 (1.48)	91.58 (1.94)
Mix of varnish and p(NIPAM)	93.20 (1.90)	89.18 (4.83)
Control	69.64 (9.75)	63.28 (7.50)
p-value	<0.001*	<0.001*

**significant (p<0.05)   ns; non-significant (p>0.05)*

Table-1 The mean and standard deviation (SD) values of degree of occlusion of p(NIPAM), Varnish, Mix of varnish and p(NIPAM) and Control groups in Superficial dentin (control and experimental) and deep dentin upon immediate testing. SD is shown between brackets.

Degree of occlusion in deep dentin upon immediate evaluation

A statistical difference was shown between the control and each of (varnish), (p(NIPAM)) and (mix) groups where ($p<0.001$). However, there was no statistically significant difference between (varnish), (p(NIPAM)) and (mix) groups where ($p=0.057$), with the highest mean value for the varnish group while the control group showed the least mean value with most of the dentinal tubules were opened **(Table 1)**. The image analysis as well showed the least diameter of dentinal tubule for the varnish group followed by mix and then P(NiPAM) **(Figure-2)**.

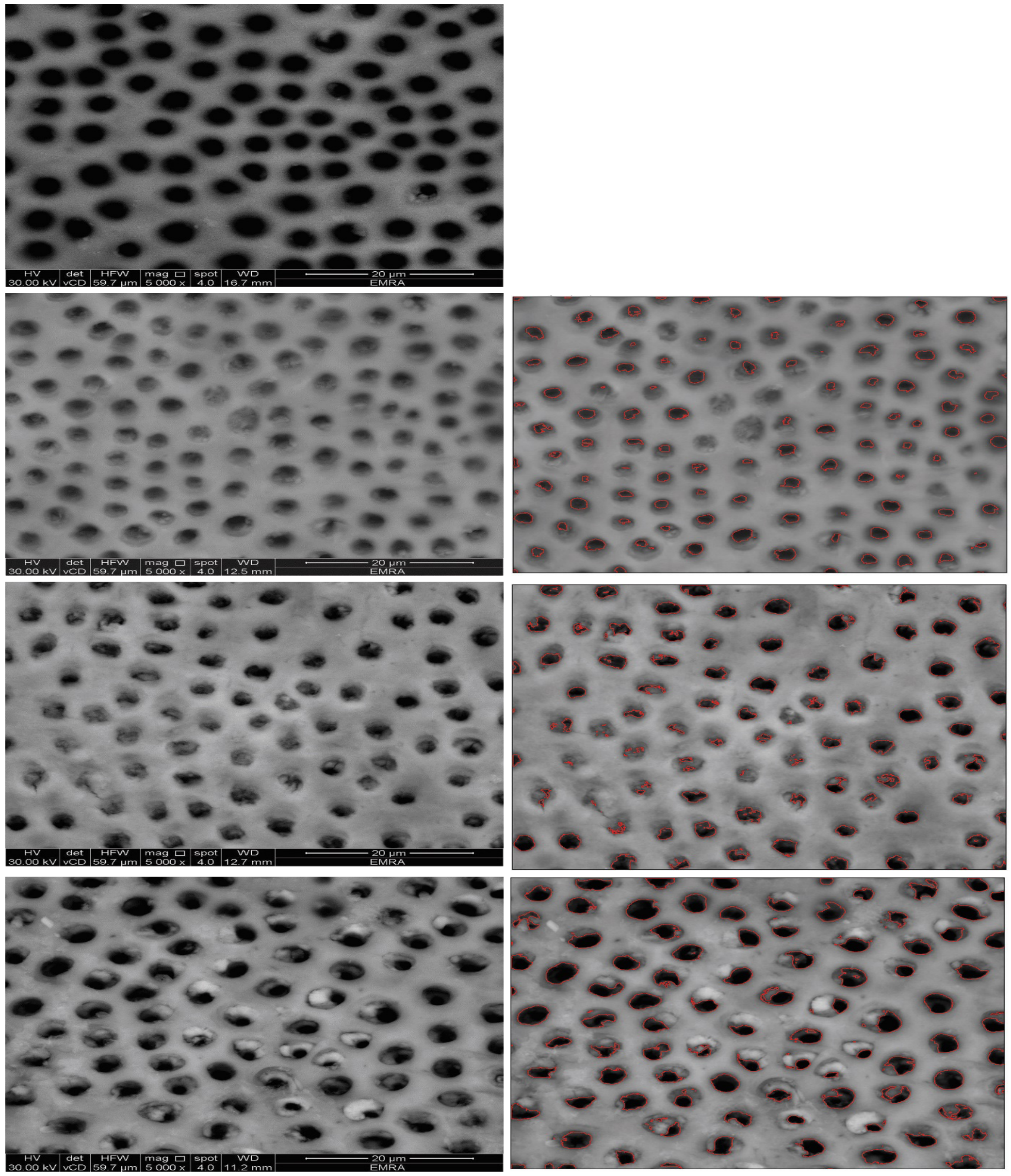

Figure-2 Microscopic pictures with magnification 5000x showing results of degree of occlusion after application of A) control B) varnish, D)mix, F) p(NIPAM) on deep dentin, after immediate evaluation and the image analysis with magnification 2500 x C) varnish, E) Mix of varnish and p(NIPAM), G) p(NIPAM).

Degree of occlusion in superficial dentin after thermocycling

A statistically significant difference was found between the control group and each of the varnish, p(NIPAM) and mix groups where ($p<0.001$). No statistically significant difference was found between (varnish) and each of (p(NIPAM)) and (mix) groups where ($p=0.050$) and ($p=0.888$). However, a statistically significant difference was found between (p(NIPAM)) and (Mix) groups where ($p=0.022$). p(NIPAM) group showed the highest mean value followed by the varnish, then mix and lastly the control group **(Table 2)**. ESEM showed the highest degree of occlusion for the p(NIPAM) group, and the image analysis showing the least tubular diameter for the p(NIPAM) group **(Figure-3)**.

Variables	Superficial dentin	Deep dentin
	Mean	Mean
p(NIPAM)	92.18 (3.17)	81.28 (7.28)
Varnish	87.80 (1.79)	86.34 (3.90)
Mix of varnish and p(NIPAM)	87.03 (2.64)	81.57 (4.81)
Control	76.66 (3.48)	66.07 (2.69)
p-value	<0.001*	<0.001*

**; significant (p<0.05)   ns; non-significant (p>0.05)*

Table-2 The mean and standard deviation (SD) values of degree of occlusion of p(NIPAM), Varnish, Mix and Control in Superficial dentin (Control and experimental) and deep dentin after thermocycling. SD is shown between brackets

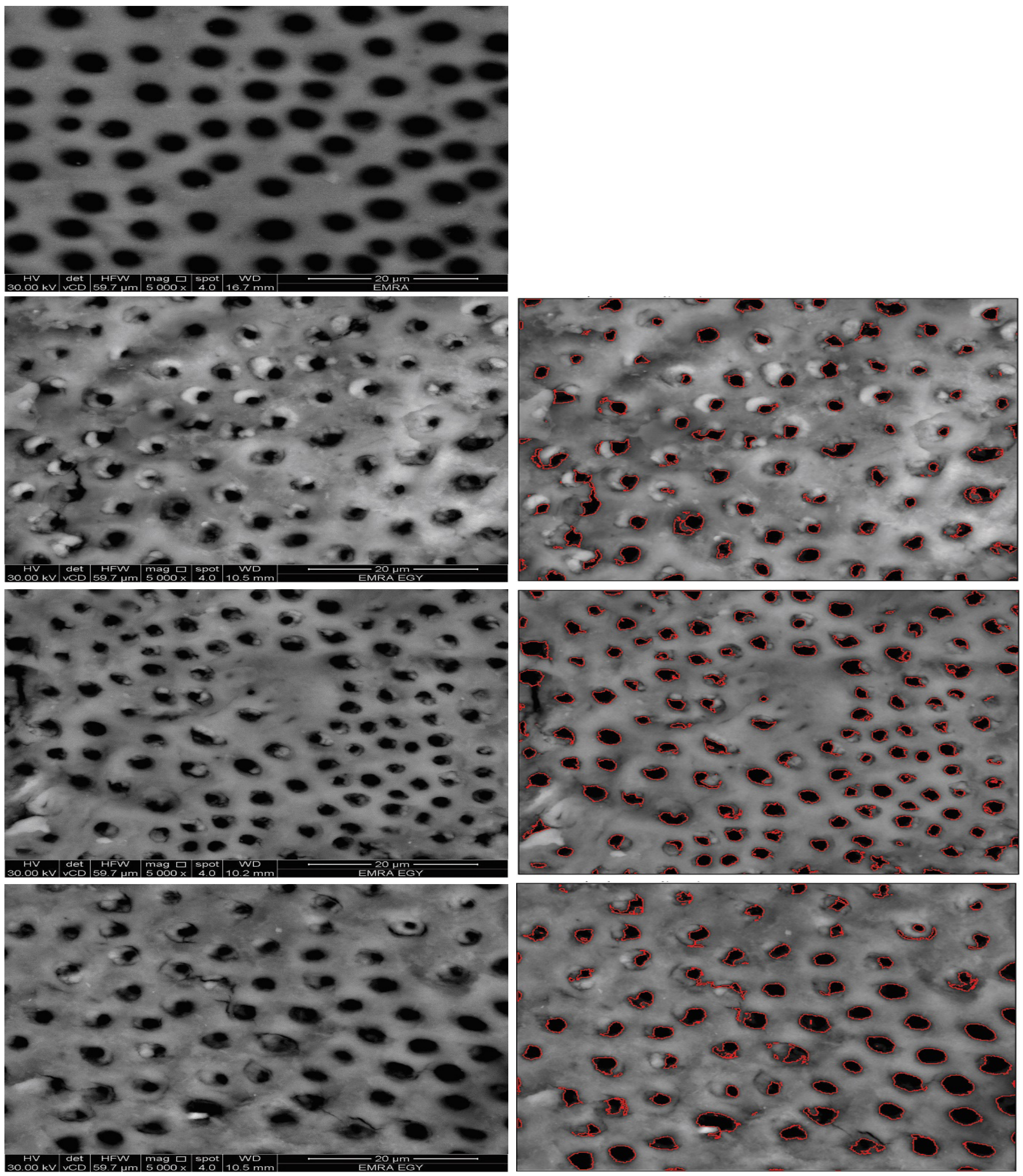

Figure-3 Microscopic pictures with magnification 5000x showing results of degree of occlusion after application of A) control B) p(NIPAM), D) varnish, F) mix on superficial dentin, after thermocycling evaluation and the image analysis with magnification 2500 x C) p(NIPAM), E) varnish, G) Mix

Degree of occlusion in deep dentin after thermocycling

Meanwhile, in deep dentin; no statistically significant difference was found between any of the materials used either the varnish, p(NIPAM) or the mix on the effect of degree of occlusion after thermocycling where (p=0.302). However, the varnish group showed the highest mean value followed by mix group, p(NIPAM) group and the least was the control group that showed a statistically significant difference with other materials (p<0.001) **(Table 2)**. The image analysis showed the highest percentage of occlusion for the varnish **(Figure-4)**.

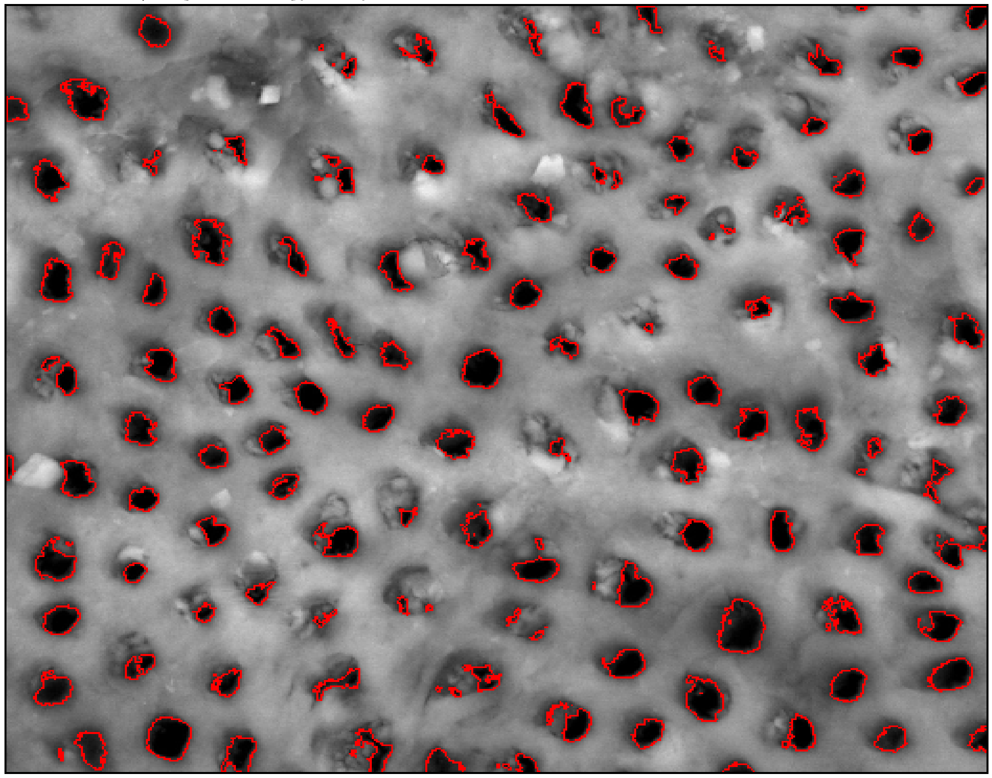

Figure-4 Diameter of the remaining opened dentinal tubules after application of Varnish on deep dentin after thermocycling using image J analysis

Relation between the evaluation of degree of occlusion immediately and after thermocycling

Comparing the results before and after thermocycling **(Figure-5)** it can be concluded that degree of occlusion was higher before thermocycling in either superficial or deep dentin.

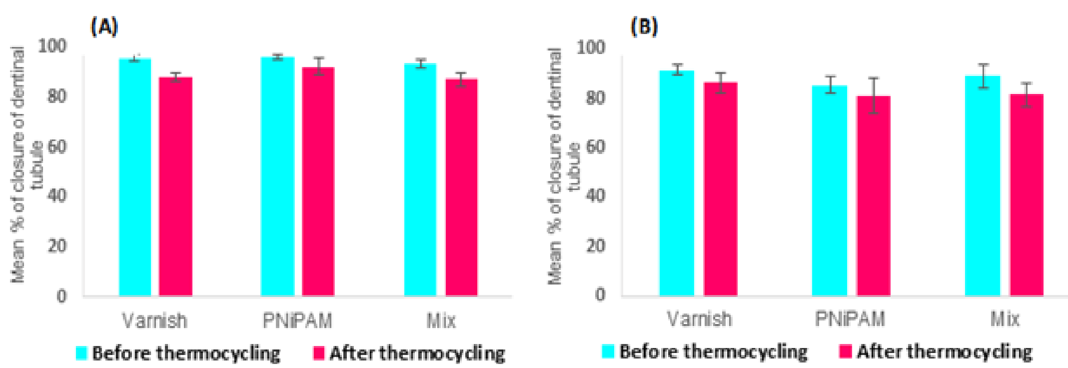

Figure-5 Representing effect of thermocycling on degree of occlusion for different materials (Varnish, p(NIPAM) and Mix) in A) superficial dentin, B) deep dentin.

Depth of penetration in superficial dentin upon immediate evaluation

There was a statistically significant difference between (mix) and each of (varnish) and (p(NIPAM)) groups where (p<0.001). However, the (varnish) and p(NIPAM) groups showed no statistically significant difference between them where (p=0.128). The highest mean value was for the varnish group followed by p(NIPAM) and then mix group **(Table-3), (Figure-6)**

Variables	Superficial dentin	Deep dentin
	Mean	Mean
Varnish	47.94 (3.29)	24.63 (1.81)
p(NIPAM)	43.63 (3.54)	19.07 (1.554)
Mix	29.75 (2.78)	16.29 (0.86)
p-value	<0.001*	<0.001*

**; significant (p<0.05)   ns; non-significant (p>0.05)*

Table-3 The mean and standard deviation (SD) values of depth of penetration of different materials (Varnish, p(NIPAM) and Mix) in Superficial dentin and deep dentin upon immediate testing. SD is shown between brackets.

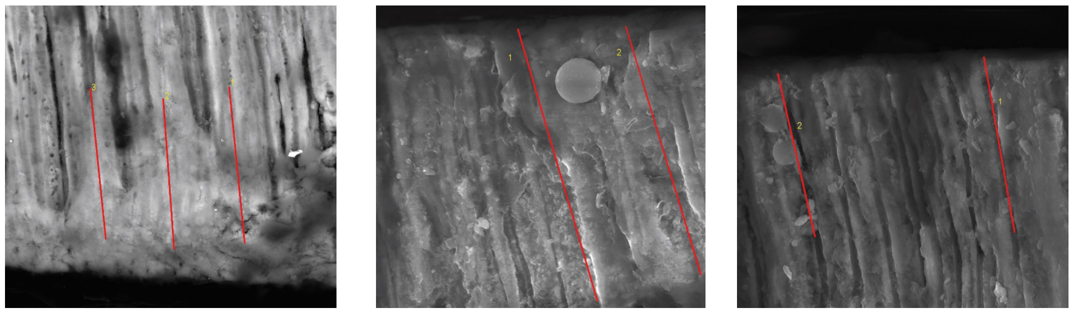

Figure-6 Image analysis representing the degree of penetration of a) Varnish, b) p(NIPAM) and c) Mix on superficial dentin using ESEM with magnification 1500 x after immediate testing.

Depth of penetration in deep dentin upon immediate evaluation

Furthermore, in deep dentin on immediate evaluation, there was a statistically significant difference between (varnish) and each of (p(NIPAM)) and (mix) groups where ($p<0.001$). Also, a statistically significant difference was found between (p(NIPAM)) and (mix) groups where ($p=0.027$) but yet the varnish showed the highest degree of penetration followed by p(NIPAM) and then mix group **(Table 3), (Figure. 7)**.

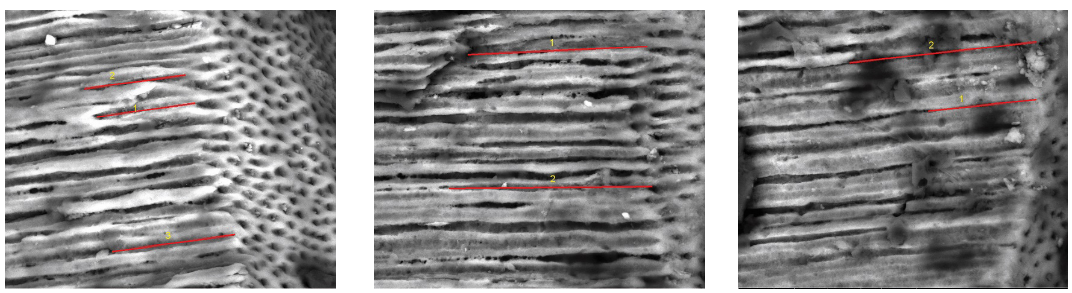

Figure-7 Image analysis representing results of degree of penetration of a) Varnish, b) p (NIPAM) and c) Mix on deep dentin using ESEM with magnification 1500 x after immediate testing.

Depth of penetration in superficial dentin after thermocycling

A statistically significant difference was found between (Mix) and each of (Varnish) and (p(NIPAM)) groups where ($p<0.001$). Meanwhile, no statistically significant difference was found between (p(NIPAM)) and (Varnish) groups where ($p=0.747$). However, the highest mean value was for the varnish group followed by the p(NIPAM) group then the mix group **(Table 4)**.

Depth of penetration in deep dentin after thermocycling

There was no statistically significant difference between (Varnish), (p(NIPAM)) and (Mix) groups where ($p=0.434$), with the highest mean value for the varnish group followed by the p(NIPAM) and then the mix group **(Table 4)**.

Relation between immediate evaluation and after thermocycling in depth of penetration

However, the depth of penetration of materials increased after thermocycling than before thermocycling either in superficial or deep dentin **(Figure-8)**.

Variables	Superficial dentin	Deep dentin
	Mean	Mean
Varnish	67.83 (4)	42.33 (4.30)
p(NIPAM)	65.42 (3.72)	40.07 (3.31)
Mix	45.23 (7.09)	39.23 (3.70)
p-value	<0.001*	0.434ns

**; significant (p<0.05)   ns; non-significant (p>0.05)*

Table-4 The mean, standard deviation (SD) values of depth of penetration of different materials (Varnish, p(NIPAM), and Mix) in Superficial dentin and deep dentin after thermocycling. SD is shown between brackets.

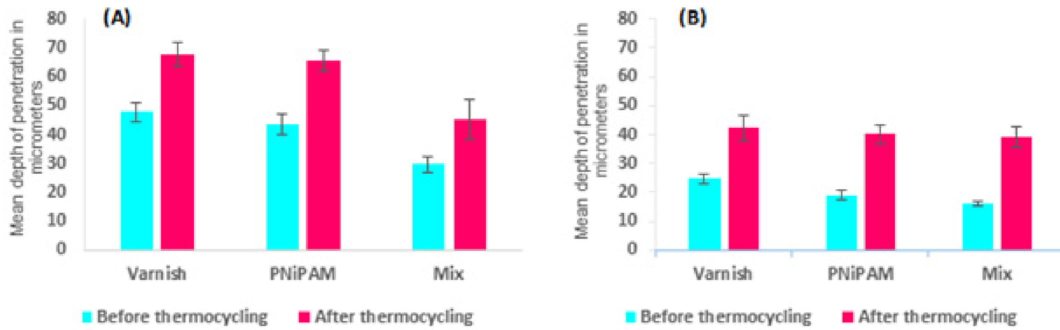

Figure-8 Representing effect of thermocycling on depth of penetration for different materials (Varnish, p(NIPAM) and Mix) in A) superficial dentin, B) deep dentin.

Relation between superficial and deep dentin

Moreover, all materials showed better penetration and occlusion of dentinal tubules in superficial dentin rather than deep dentin regardless aging **(Figure- 9,10)**.

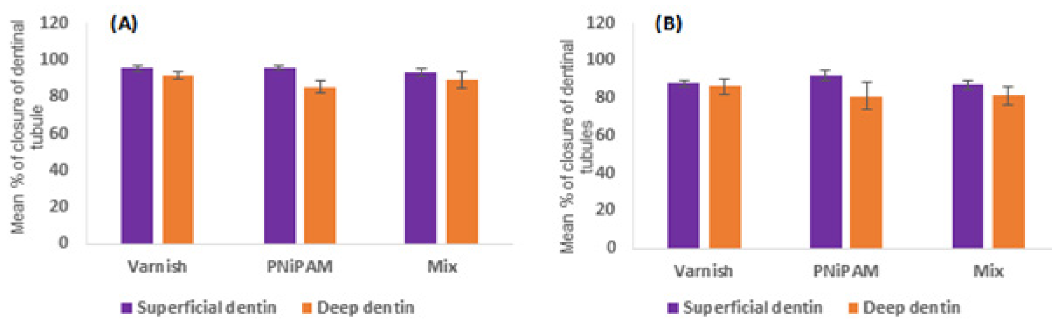

Figure-9 Representing effect of dentin type on degree of occlusion for different materials (Varnish, p(NIPAM) and Mix) on a) immediate testing, b) after thermocycling.

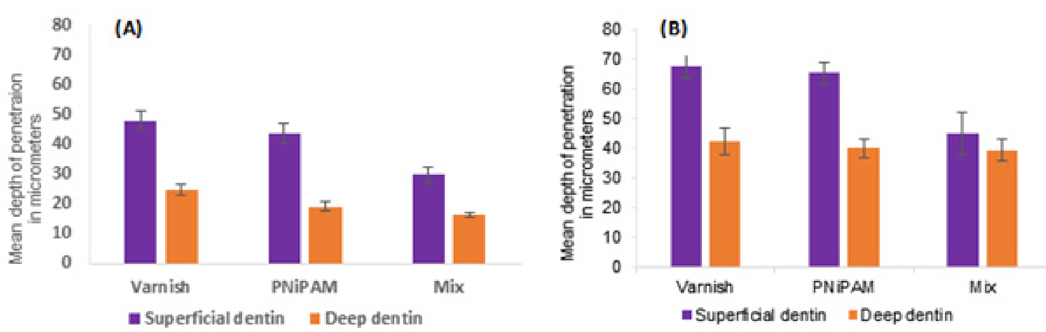

Figure-10 Representing effect of dentin type on depth of penetration for different materials (Varnish, p(NIPAM) and Mix) on a) immediate testing, b) after thermocycling.

Discussion

Dentin hypersensitivity is a painful disorder influencing oral comfort and function. It is characterized by brief, sharp torment, emerging from the uncovered dentin in reaction to stimuli; regularly; warm, evaporative, osmotic or chemical [9,1]. The most accepted theory so far used to explain mechanism of dentin hypersensitivity is the hydrodynamic theory presented by Bransstrom in 1963 [4,1]. According to this theory, the pulp nerves are indirectly activated by movement of dentinal fluid inside exposed dentinal tubules [1] and consequently, any decrease in the movement (conductance) of dentinal fluid, should show reduction in the dentin hypersensitivity. Individuals with dentin hypersensitivity show a greater number of open dentinal tubules (8×) that are wider (2×) than non-sensitive surfaces [2,5,6,16,17] specially at the buccal cervical area. In addition, fluid movement inside the dentinal tubules increase as their width increase and consequently the pain/sensitivity response is increased. Thus, the width of the tubule was of great importance to be measured. Along with the hydrodynamic theory, the permeability of opened dentinal tubules decrease as their practical radius decrease, thus decreasing dentin hypersensitivity [18]. The depth to which the desensitizing agents penetrate into the dentinal tubule, is an important factor in the treatment's long term effectiveness, because the deeper they reach into the dentinal tubules, the more the effectiveness [18,19] while the less the penetration level is, the more chance of removal of deposits by brushing or dietary acids.

Thermocycling was used to show how temperature variations influenced the adaptation of desensitizing agents to surface of tooth in different dentin depths, it was carried out at 5°C-55°C with total number of 500 cycles which is simulating 18 days in the oral environment [20]. These variables seem to be tolerated by the oral tissues and are suitable for clinical conditions [21]. The current study contained three variables; desensitizing agents, dentin depth and aging, they were all tested using environmental scanning electron microscope because it was able to display deviations in the morphology of the dentinal surfaces caused by the different desensitizing agents used without needing any sample preparation [6]. 600 grit silicon carbide paper was used in a circular motion to create standardized smear layer [6], then all dentin specimens were treated with 50 % citric acid for 1 minute, to remove the smear layer and smear plugs for simulating the hypersensitive dentin [9,22].

Therefore, the aim of the present study was to compare the effect of a new desensitizing agent microgel P(NIPAM) with Clinpro white varnish 5 % NaF + TCP and the combination of the two desensitizing agents for the treatment of dentin hypersensitivity on different dentin depth with aging and it was evaluated using environmental electron microscope.

Results showed regarding degree of occlusion; the effect of materials on superficial dentin on immediate testing (before thermocycling); the highest mean value was for the p(NIPAM) group **(Figure-1), (Table-1)**, this could be explained by the hydrodynamic theory, as the diameter of opened dentinal tubules is decreased, the permeability is reduced and thus dentin sensitivity is reduced [11], as well as Yang et al., 2017 [23] stated that the further decrease in dentinal tubule diameter can be ascribed to the remineralization process. However, this was contradicted with Mohsen et al., [24] who stated that when the temperature was underneath the 34^0C the microgel was not able to seal the membrane, while above this temperature the polymer phase separates. Moreover, this could be also explained on basis of the p(NIPAM) properties, that can significantly alter their adaptation and thus modifying their particle size and surface charge density in response to the environmental changes which are vastly sensitive to it. Additionally, one favorable characteristic of microgel is their adaptability, it can be made with explicit properties to outfit a necessary application. In addition, in the presence of aqueous media and temperature beneath 34^0C, the P(NIPAM) promptly isolates and swells, this is due to its hydrophilic nature [24,25,26]. This swelling may be the reason behind its occlusion to the dentinal tubules on immediate testing before being subjected to thermocycling.

While in deep dentin; the effect of material on the degree of occlusion on immediate testing, the highest mean value for the varnish group in **(Table-1), (Figure-2)**, these results were compatible with Tosun et al., 2016 [9] who discovered that the presence of a slight layer of Clinpro WV (5 % NaF + TCP) varnish results in a layer that seals the opened dentinal tubules by forming salts quickly due to reactive fluoride ions. As well as Machado et al., 2019 [6] who concluded that Clinpro WV was one of the groups in his study with the lowest dentin permeability after treatment, nearly no dentinal tubules were calculated, or were visible enough to be counted. This can be ascribed also to the mechanism of action of NaF, were calcium fluoride crystal (CaF_2) is formed and deposited in the dentinal tubule through the interactions that occur between NaF and calcium ions in dentinal fluid. However, Ipcy et al., 2009 [14] stated that NaF when used alone will not occlude the dentinal tubule effectively and requires multiple applications as when combined with other chemicals as TCP (tri calcium phosphate) that caused a more homogenous modification in the dentin surface. Therefore, NaF varnish containing TCP (Clinpro varnish) was used in this study. Additionally, this could be explained as well by the way the Clinpro white varnish works; after application, the modified rosins quickly cover and flow into dentinal tubules, then slowly hardens to lacquer like state forming immediate occlusion. Polish like film gradually wears away over time, NaF and $CaPO_4$ in the coating breakdown into ions, allowing fluoride ions to react with free calcium in the mouth or as a result of Clinpro white varnish in order to form insoluble calcium fluoride [27]. Those insoluble calcium fluoride globules seal exposed dentin tubules for continued tooth hypersensitivity relief. This blocks the transmission of ions in solution and decreases dentinal hypersensitivity. Another study by Ritter et al., 2006 [28] stated that a defensive layer of calcium fluoride that is formed by fluoride varnish protects the dentin surface.

Regarding aging, and the effect of materials used on the degree of occlusion in the superficial dentin; the difference between the p(NIPAM) and Varnish groups was not statistically significant, however, the p(NIPAM) group showed the highest mean value **(Table-2), (Figure-3)** this was in agreement with Mohsen et al., 2013 [24], who found that the p(NIPAM can flocculate and block a 5 micrometers pore size membrane, when suitable conditions as temperature, PH and electrolyte concentration are available. They concluded that the microgel p(NIPAM) could be used to treat dentin hypersensitivity. This was accepted as well with another study by P.Tempesti et al., 2017 [15] who stated that p(NIPAM) microgel represents a faultless case study for blocking of dentinal tubules because of its volume transition temperature (34^0C) that is near enough the average temperature of the oral cavity, this is considered one of the characteristics of the p(NIPAM) as below this temperature its considered in the cold state and it swells, while above this temperature its considered in the warm state and it tends to shrink (collapse). He stated as well that the importance of being close enough to the oral cavity's average temperature so that the thermal transition of the p(NIPAM) is impulsively triggered when it is presented in the mouth. In both studies it was concluded that flocculation was the reason behind the blockage. Mohsen et al., 2013 [24] described flocculation as the histrionic increase in hydrodynamic diameter that occurs around the volume transition temperature of 34^0C, they claimed that as the temperature increases, the microgel to flocculated blocking the areas with the higher permeability. Flocculation is assumed to be happened in the current study after thermocycling where the temperature used in the current study was between 5^0C to 55^0C, for 500 cycles. Meanwhile, in deep dentin, the varnish group showed the highest mean value followed by Mix group, p(NIPAM) group and the least was the control group **(Table-2), (Figure-4)**, this could be explained according to the particle size that made the varnish penetrated easily in deep dentin. And the presence of modified rosins that quickly cover and flow into dentinal tubules, then gradually toughens to lacquer like state forming immediate occlusion. Lacquer like film gradually wears away over time, NaF and $CaPO_4$ in the coating liquify and release as ions, a short time later interactions occur between fluoride ions and free calcium that is either present in the mouth naturally or discharged from Clinpro white varnish forming insoluble calcium fluoride, which aim to provide continued relief of tooth hypersensitivity through dentinal tubules occlusion. Additionally, to the rosins present in the Clinpro varnish acts as a barrier, preventing the removal of sodium fluoride.

However, on comparing the results before and after thermocycling **(Figure-5)** it can be concluded that degree of occlusion was higher before thermocycling in either superficial or deep dentin. In a study done by Schmalz et al., 2016 [29] it was stated that thermocycling loading was done to simulate thermal stress, as thermal changes may cause defects on a dentin-adhesive surface. And accordingly, in the current study, it can be concluded that thermocycling affected the material applied/interface on the tooth surface, leading to higher results done before thermocycling.

Regarding the depth of penetration; on immediate testing in the superficial dentin there was no statistically significant difference among the varnish and the p(NIPAM) groups. However, the highest mean value was for the varnish group followed by p(NIPAM) and then Mix group **(Table-3), (Figure-6)**. Furthermore, in deep dentin on immediate evaluation, there was a statistically significant difference between the groups but yet the varnish showed the highest degree of penetration followed by p(NIPAM) and then mix group **(Table-3), (Figure-7)**. This was in agreement with Samet et al., 2016 [9], who confirmed by CLSM (confocal laser scanning microscope) that a layer of 5% NaF + TCP coated the surface of dentinal tubules in addition to its deposition on the internal walls. Moreover, in a study by Mitra et al 2017 [18], who evaluated the penetration of various types of desensitizing agents into dentinal tubules; he concluded that sodium fluoride varnish was able to mechanically seal the entrances and penetrate into tubules. It was also found in acceptance with Machado et al., 2019 [6] who previously proved that Clinpro WV capable of penetrating into dentinal tubule up to 13 micrometers.

The depth of penetration in the superficial dentin; after thermocycling there was no statistical difference between the varnish and the p(NIPAM) groups. However, the highest mean value was for the varnish group followed by the p(NIPAM) group then the mix group. As well as in the deep dentin there was no statistical difference between varnish, p(NIPAM) and mix groups with the highest mean value for the varnish group followed by the p(NIPAM) and then the mix group **(Table-4)**.

However, the depth of penetration of materials increased after thermocycling than before thermocycling either in superficial or deep dentin **(Figure-8)**. This could be explained according to Pazinatto et al., 2003 [21] and Sowbhagya et al., 2012 [30] who found that the reasons that helped in the increase of penetration of different materials where the dwell time that is 30 second, the increase in the number of cycles, increase in linear expansion and the shrinkage. In the current study the temperature used was between 5^0C and 55^0C with a dwell time 30 second according to Pazinatto et al.,2003 [21] he mentioned that the 30 seconds dwell time promoted great penetration of material. While p(NIPAM) showed higher penetration in dentinal tubules after thermocycling as Al-Moaleem et al., 2011 [20] stated that shrinkage promotes deeper penetration, p(NIPAM) microgel at temperature above 34^0C it shrinks resulting in water exclusion and breakdown of the hydrogen bond (hydrophobization) and successive sedimentation of the particles on the tooth surface and this cause better filling of the dentinal tubules [15]. While as for sodium fluoride, it was stated by Sowbhagya et al., 2012 [30] that its thermal expansion increases linearly with temperature, which explains the reason for its high penetration into dentinal tubule after thermocycling, that was found also in acceptance with Al- Moaleem et al., 2011 [20] and Pazinatto et al., in 2003 [21] who stated that penetration increases with increase in linear coefficient of thermal expansion. Furthermore, Al-Moaleem et al., 2011 [20] stated that the more the number of thermocycling the more is the penetration, in the current study specimens where subjected to 500 cycles of thermocycling that is simulating 18 days in the oral environment.

Moreover, all materials showed better penetration and occlusion of dentinal tubules in superficial dentin rather than deep dentin regardless aging **(Figures-9,10)** and this may be attributed to the significant correlation between dentinal tubule morphology and dentin hypersensitivity [17]. Superficial dentin has narrower diameter of dentinal tubule as well as fewer number than deep dentin. And thus, the materials were performing better in superficial dentin.

The complex of ecosystem is influenced by localized behaviors such as brushing, chewing and saliva. As a result, further in-vivo studies are required to better evaluate various desensitizing agents, as their efficacy is still debatable, and their capability to resist the challenges of the oral environment is still uncertain.

Conclusion

Within the limitations of the current study, we could conclude the following; P(NIPAM) showed the highest mean value with no statistically significant difference with the varnish group regarding the degree of occlusion weather on immediate evaluation or after thermocycling. NaF varnish showed the highest depth of penetration in deep dentin than any other group. All desensitizing materials showed better occlusion and depth of penetration in superficial dentin than in deep dentin. They as well showed better degree of occlusion of dentinal tubules on immediate testing than after thermocycling, however, they showed better depth of penetration after thermocycling than on immediate testing.

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