Evaluation of the Occluding Characteristics of Nanosized Eggshell/Titanium Dioxide with or without Saliva

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Abstract

Objectives The study reports on the effectiveness of a ball-milled nanosized titanium dioxide composite (EB@TiO_2) for DH management in comparison with commercial desensitizing paste with and without saliva.

Materials and Methods Forty-nine dentine specimens were prepared from extracted bovine anterior teeth. Twenty-one of the specimens were brushed with three desensitizing toothpaste for 7 days, namely: Group 1; EB@TiO_2, Group 2; Colgate Pro-relief; and Group 3; Sensodyne repair (n = 7). Twenty-four specimens were brushed with the toothpaste for 7 days and stored in artificial saliva (control) after brushing. Each specimen was subsequently posttreated in citric acid solution to test its stability in acidic condition. Field scanning electron microscope was used to evaluate the effectiveness of the dentine tubules occlusion. The biocompatibility of the composite was tested using BHK21 cell line.

Statistical Analysis One-way analysis of variance was used to analyze the percentage occluded area ratio values for all specimens ($\alpha = 0.05$). Independent t-test was further used to evaluate the occlusion differences with saliva and without saliva.

Results and Conclusions The number of dentine tubules decreased significantly after 7 days of brushing. Overall, the occlusion observe for EB@TiO_2 were significantly better than for Colgate Pro-relief and Sensodyne repair ($p < 0.05$). BHK21 assay suggested that composite had no significant effect on the BHK21 cell line. This study demonstrated that the composite effectively occluded open dentine tubules within 7 days of brushing.

Keywords
- dentine hypersensitivity
- desensitizing paste
- remineralization

Introduction

Globalization has created a rapid change in the diets and lifestyles of millions of people worldwide. In South Africa for example, the reintroduction of the country to the global economy postapartheid in 1994 has witnessed the proliferation of foreign goods and a rapidly changing food environment.[1] Concerning, and in the context of oral health care,

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this change has brought about the increase in consumption of energy drinks, and acidic beverages; which is reportedly linked to the incidence of dental diseases, such as dental caries and erosion of the enamel surface.[2,3] More worrisome is that the excessive demineralization of the tooth surface due to erosion has been reported to initiate the onset of dentine hypersensitivity (DH).[4-6]

According to the Canadian Advisory Board on DH,[7] DH is characterized by distinctive short, sharp pain arising from exposed dentinal tubules particularly in response to external stimuli that are typically thermal, evaporative, tactile, electrical, osmotic, or chemical changes which cannot be ascribed to any other form of dental defects or pathology. As reported in the literature, DH is one of the most clinically encountered problems in dentistry affecting between 10 to 30% of people worldwide.[8] Although there have been conflicting reports on the exact prevalence of DH, nevertheless, the most common age range in which DH is frequently experienced is given as 20 to 50 years, with female patients predominantly affected.[9,10] Moreover, and as Schiff et al[11] points out, DH has a negative consequence on the quality of life for dental patient as they are less complaint with oral hygiene recommendation; thus posing a challenge for oral health care providers to manage.

Many theories have been reportedly proposed to explain the mechanism of DH. However, the hydrodynamic theory expanded upon by Brannstrom is now accepted by the dental community as the most likely mechanism for DH occurrence.[12] In an attempt to control this process, products or agents that typically aimed to reduce fluid flow, and or interfere with the nerve impulses have been reported in the literature.[11,13] At the first line of at-home therapy for DH management, the use of occluding agents is often recommended for effective treatment.[14]

Several different occluding agents such as potassium oxalates,[15] sodium fluoride and sodium monofluorophosphate,[12] strontium salt,[16] amorphous calcium phosphate containing casein phosphopeptide,[17] and calcium glycerophosphate[18] have been widely utilized in desensitizing paste for their dentine tubule occluding capabilities. Still, the effectiveness of the aforementioned occluding agents will depend on the flow of saliva. Moreover, due to the chemical composition of saliva, it can play a critical role in naturally reducing DH.[13,19] Kleinberg[19] revealed that saliva could reduce DH by depositing phosphate and calcium ions in the exposed tubules which ultimately result into the ions forming a protective layer on the surface of the tubules. In some patients; however, particularly those with conditions of hyposalivation and xerostomia, the flow of saliva is limited; which could further increase the risks of caries and tooth demineralization, thereby exacerbating DH.[20]

In an attempt to address the above concern, Kleinberg in 2012 at the State University of New York-Stony Brook, patented novel occluding agents based on the understanding of the role that saliva plays in naturally reducing DH. This new technology comprises arginine (an amino acid with a pH 6.5–7.5), bicarbonate, pH buffer, and calcium carbonate.[19] The said technology is marketing under the brand name Colgate Pro-Argin.[11] It is reported that Pro-Argin technology function by occluding dentinal tubules using arginine to bind to the negatively charged dentin surface, which subsequently attracts a calcium-rich layer from the saliva to infiltrate and block the dentinal tubules.[20] However, its effectiveness in a highly acidic environment has been reported to be ineffective,[21] thus leading to the reopening of the dentine tubules. Given the above drawbacks, a new occluding material consisting of an eggshell modified titanium dioxide composite recently proposed in DH management.[22]

Importantly, studies[23,24] have projected that the future of tooth remineralization will be the use of eggshell owing to its high bioavailability of calcium. Likewise, the use of titanium dioxide, particularly in nano form, and their combination with other abrasive agents have been proposed in the literature to occlude open dentine tubules.[25] In a recent report, the authors demonstrated that eggshell modified with titanium dioxide ($EB@TiO_2$) significantly improved the composite acidic resistant to erosive acids.[26] This present study, therefore, aimed to evaluate $EB@TiO_2$ occluding characteristic against commercially available toothpaste containing Pro-Argin (Colgate Pro-relief) and NovaMin (Sensodyne repair) with and without saliva in reducing DH. The formulated hypothesis tested was: $EB@TiO_2$ significantly occlude the open dentine tubules with or without saliva.

Materials and Methods

Two commercially available toothpastes namely: Sensodyne repair (GlaxoSmithKline, United Kingdom) and Colgate Pro-relief (Colgate-Palmolive, Poland) were used as the test desensitizing paste. Titanium dioxide (Anatase form) and citric acid were purchased from Sigma-Aldrich (Germany), and Merck (South Africa), respectively.

Eggshell-Titanium Dioxide Composite Preparation

Eggshell and titanium dioxide composite was prepared in accordance with the method reported in literature.[22] An extensive details of the surface morphology, particle sizes, and phase of the prepared $EB@TiO_2$ can be found in other reported papers.[22,27,28]

Preparation of Artificial Saliva

Artificial saliva was prepared following the method reported by Saporeti et al[29] with a slight modification. As specified in ►**Table 1**, the listed chemicals were prepared in 1L of volumetric flask using deionized water. The pH of the prepared saliva was given as 6.5.

Forty-nine anterior teeth extracted from bovine were collected from an abattoir, South Africa. Disinfecting and cleaning of the teeth followed by immersing in 10% chloroxylenol solution. With the aid of a diamond saw operating at a minimal speed, and cooled with water, the teeth were sectioned below the enamel-dentinal to prepare a dentine specimen having a dimension of 5 mm × 5 mm × 1 mm. A silicon carbide paper with particle size of 600 grits were further used to wet ground the specimens for 60 seconds. Thereafter, the specimens were embedded in a resin (AMT composite,

Table 1 Composition of the prepared artificial saliva (mg/L)

Chemicals	Concentration (mg/L)	Mass (g)
$NaH_2PO_3H_2O$	780	0.078
NaCl	500	0.05
KCl	500	0.05
$CaCl_2H_2O$	795	0.0795
NaS_9H_2O	5	0.0005
$(NH_4)_2SO_4$	300	0.03
Citric Acid	5	0.0005
$NaHCO_3$	100	0.01
Urea	1000	0.1

Note: Preparation of dentine tooth specimens.

South Africa). The specimens were then soaked in a solution containing 4% wt. citric acid for 2 minutes to open up the tubules. As described in ►**Table 2**, the specimens were randomly assigned in different experimental groups.

Each specimen from the respective groups were brushed twice daily (morning and evening) with a toothbrush powered with 1.5v alkaline battery (Oralwise, China) for 1 minute and allowed to dry for 30 seconds before rinsing with deionized water. Brushing was performed at room temperature using 100 mg of respective toothpaste. The slurry of EB@TiO_2 was prepared by mixing 100 mg of the powder/200 µL of deionized water. After each brushing protocol, the specimens were immersed in saliva or without saliva as described in ►**Table 2**. At the end of the 7-day brushing, the treated specimens were exposed to 4% wt. citric acid solution for 2 minutes to determine the resistance to acidic challenge, and subsequently rinsed in deionized before blot drying.

Field Scanning Electron Microscope (FESEM; Carl Zeiss) was used to examine the treated specimens after each day of brushing from each respective group. The instrument was operated in controlled environment and scan at 20 kV. Prior to FESEM observation, the specimens were dehydrated, sputter coated with electric conductive gold film. Using the captured image of 1500 magnification, a software (ImageJ; National Institute of Health, United States, http://imagej.nih.gov./ij) was used to compute the occluded tubules ratios by dividing the area of the occluded tubules by the total tubules area ($n = 7$). The % occluded area ratio were counted and used for statistical evaluation.

Biocompatibility Test

A cytotoxicity assay was performed on the prepared EB@TiO_2 to evaluate its biocompatibility. Before culturing, the sample was dispersed in a solvent (Dimethyl Sulfoxide). The BHK21 hamster kidney cells were grown in the laboratory following the process of culturing normal tissues.[22] The cell viability were then evaluated using MTS assay. Auranofin was used as a negative control. All analyses were tested in duplicate and performed across two plates ($n = 6$).

Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyze the mean occluded area ratio within the different groups, followed by a Bonferroni test ($\alpha = 0.05$). In addition, the independent *t*-test was used to compare the mean occluded area ratio observe for the specimens treated with saliva and without saliva ($\alpha = 0.05$). All analysis was performed using statistical software (IBM SPSS Statistics v24; IBM Corp.).

Results

Dentine Specimens Treated in 7 Days (without Saliva)

►**Table 3** depicts the results of the dentine specimens measured in 7 days without saliva immersion. The total mean % ratio of the tubules occluded area for the dentine specimens

Table 2 The distribution of specimens according to the experimental group

Sample groups	Treatment condition		Brushing days	Total
	Without saliva	With saliva		
Artificial saliva	–	7	Twice daily (for 7 days)	7
EB@TiO_2	7	7		14
Colgate Pro-relief	7	7		14
Sensodyne repair	7	7		14
Total	21	28		49

Note: Surface examination of the treated specimens. EB@TiO_2, eggshell-titanium dioxide.

Table 3 ANOVA test comparison of the occluded area (without saliva)

Treatment group	*N*	Mean ± SD	Standard error	95% confidence interval		*p*-Value	Posthoc Bonferroni's test
				Lower bound	Upper bound		*p*-Value
EB@TiO_2	7	64.7 ± 1.3	0.655	63.318	66.070	0.000	$0.028^{1,2}$
Colgate Pro-relief	7	62.0 ± 3.2	0.655	60.624	63.376		$0.000^{1,3}$
Sensodyne repair	7	22.7 ± 4.8	0.655	21.358	24.111		$0.000^{2,3}$

Abbreviation: SD, standard deviation.
Note: Superscript numbers indicate significant differences between the sample groups (ANOVA, $p < 0.05$). EB@TiO_2, eggshell-titanium dioxide.

treated with EB@TiO_2, Colgate Pro-relief, and Sensodyne repair was statistically different ($p < 0.001$).

Notably, and after 7 days of brushing, the EB@TiO_2 group had the highest % mean occluded area (64.7 ± 1.3%), while the Sensodyne repair treated group had the lowest % mean occluded area (22.7 ± 4.8%). The Bonferroni's correction results are given in ►**Table 3**. The % tubules occluded for the test group (EB@TiO_2) were statistically higher when compared against the Colgate Pro-relief group ($p < 0.05$), and the Sensodyne repair group ($p < 0.001$). Equally, the % occluded area measured for the Colgate Pro-relief was significantly higher than that observed for Sensodyne repair ($p < 0.001$). ►**Fig. 1** illustrates the differences in the % tubules occluded per day with the three desensitizing paste materials (EB@TiO_2, Colgate Pro-relief, and Sensodyne).

The Paired sample test, mean, and standard deviation results for the dentine specimen's pre- and postacidic challenge are given in ►**Table 4**. There was no significant different found in the EB@TiO_2 group pre- and postacidic treatment ($p > 0.05$). In contrast, both the Colgate Pro-relief and Sensodyne repair treated group showed differences ($p < 0.001$).

The FESEM image of the occluded dentine tubules for dentine specimens treated without storing in artificial saliva after 7 days brushing test is reflected in ►**Fig. 2**. In day 1 (►**Fig. 2B1**), day 2 (►**Fig. 2B2**), the group treated with Colgate Pro-relief showed more evidence of tubule occlusion when compared against the group treated with EB@TiO_2 and Sensodyne repair. However, the EB@TiO_2 group showed a better evidence of tubule remineralization in day 3 (►**Fig. 2A3**) and day 4 (►**Fig. 2A4**). Similarly, there was a complete remineralization or sealing of the tubules in the EB@TiO_2 group in day 5 (►**Fig. 2A5**), day 6 (►**Fig. 2A6**), and day 7 (►**Fig. 2A7**).

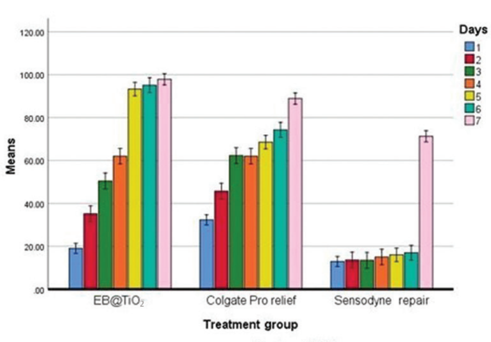

Fig. 1 Differences in mean tubules occluded of dentine specimens treated with EB@TiO_2 Colgate Pro-relief, and Sensodyne repair desensitizing paste materials after 2 minutes of brushing without saliva immersion (7-day brushing test [$n = 7$]). EB@TiO_2, eggshell-titanium dioxide.

The posttreatment in citric acid solution (4 wt.%) of the specimens are shown in ►**Fig. 2** (**A–C8**). Nonetheless, the specimen treated EB@TiO_2 (►**Fig. 2A8**) showed superior acid resistance with no visible differences pre- and postacidic challenge when compared against the specimens treated with Colgate Pro-relief (►**Fig. 2B8**) and Sensodyne repair (►**Fig. 2C8**), respectively.

Dentine Specimens Treated in 7 Days (with Saliva)

The mean, standard error, standard deviation, and ANOVA results for the dentine specimens stored in artificial saliva after brushing treatment are shown in ►**Table 5**. The total mean % ratio of the tubules occluded area for the dentine specimens stored in artificial saliva alone, treated with EB@TiO_2, Colgate Pro-relief, and Sensodyne repair was statistically different ($p < 0.001$).

It was found that the % occluded mean measured for the EB@TiO_2 group was the highest (72.0 ± 1.0%), while the specimens stored in artificial saliva alone without treatment had the lowest % mean occluded area (7.3 ± 2.3%). The Bonferroni's correction results are shown in ►**Table 5**. The EB@TiO_2 group mean % occluded area was statistically better when compared against the Colgate Pro-relief, and the Sensodyne repair ($p < 0.001$). More so, the % occluded area measured for the Sensodyne repair was significantly higher than that observed for Colgate Pro-relief ($p < 0.001$). All the treatment groups showed a significant improvement in occluding the tubules when compared against the samples stored in artificial saliva alone (< 0.001). ►**Fig. 3** illustrates the differences in the % tubules occluded per day with the three desensitizing paste materials (EB@TiO_2, Colgate Pro-relief, and Sensodyne) and artificial saliva.

►**Table 6** provides the paired sample test, mean, and standard deviation results for the dentine specimen's (stored in artificial saliva) pre- and postacidic challenge. No difference was found in the Sensodyne repair group pre- and postacidic treatment ($p > 0.05$). By contrast, there was a significant difference observed for the EB@TiO_2, Colgate Pro-relief, and specimens stored in artificial saliva alone ($p < 0.001$).

The FESEM images of the dentine specimens treated with EB-TiO_2, Colgate Pro-relief, and Sensodyne repair for 7 days and storing in artificial saliva are shown in ►**Fig. 4**. The observed images indicate the occlusion of EB-TiO_2 groups (A1–A7) were different from other test groups (Artificial saliva, Colgate Pro-relief, and Sensodyne repair). ►**Fig. 4** (**A–D8**)

Table 4 Paired sample test comparison of occluded area ratio pre- and postacidic treatment

Treatment group	Occluded area (%)		p-Value
	Preacidic challenge (mean ± SD)	Postacidic challenge (mean ± SD)	
EB@TiO_2	97.9 ± 1.3	97.1 ± 1.2	0.318
Colgate Pro-relief	88.9 ± 3.2	33.9 ± 4.1	0.000
Sensodyne repair	71.3 ± 4.9	9.3 ± 2.4	0.000

Abbreviation: SD, standard deviation.

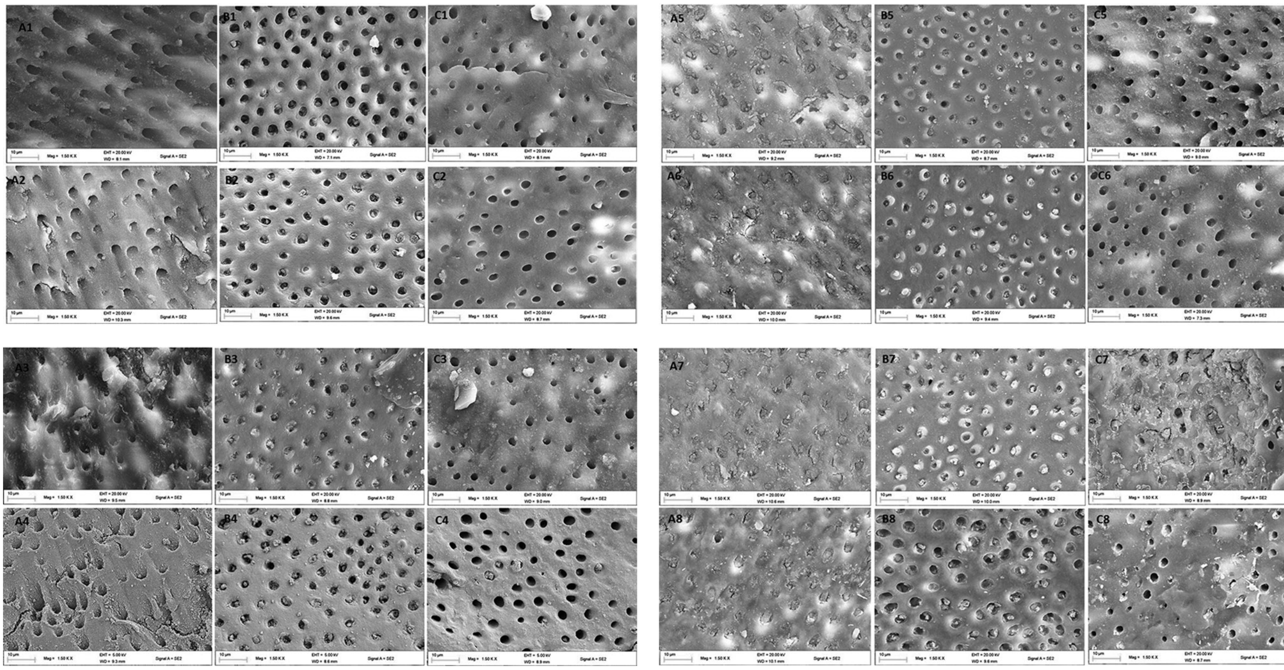

Fig. 2 Representative FESEM micrograph for the dentine surface after brushing for 7 days without saliva immersion using (**A**) $EB@TiO_2$; (**B**) Colgate Pro-relief; (**C**) Sensodyne repair (1–7 represents number of each day of brushing with the respective desensitizing paste, and 8 represent post acidic exposure). FESEM, field scanning electron microscope. $EB@TiO_2$, eggshell-titanium dioxide.

Table 5 ANOVA test Comparison of the occluded area (samples stored in artificial saliva)

Treatment group	*N*	Mean ± SD	Standard error	95% confidence interval		*p*-Value	Posthoc Bonferroni test
				Lower bound	Upper bound		*p*-Value
Artificial saliva		7.3 ± 2.3	0.636	5.953	8.578	0.000	0.000^{1-5}
$EB@TiO_2$		72.0 ± 1.0	0.636	70.708	73.333		$0.000^{2,3}$
Colgate Pro-relief		34.3 ± 8.6	0.636	33.034	35.659		$0.000^{3,4}$
Sensodyne repair		50.3 ± 3.0	0.636	49.014	51.639		$0.000^{2,4}$

Abbreviation: SD, standard deviation.
Note: Superscript numbers indicate significant differences between the sample groups (ANOVA, $p < 0.001$). $EB@TiO_2$, eggshell-titanium dioxide.

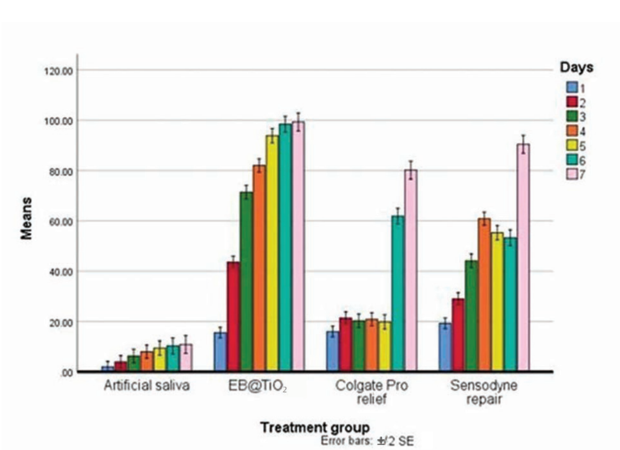

Fig. 3 Differences in mean tubules occluded of dentine specimens treated with $EB@TiO_2$ Colgate Pro-relief, and Sensodyne repair desensitizing paste materials after 2 minutes of brushing and immersed in saliva (7 day brushing test [$n = 7$]). $EB@TiO_2$, eggshell-titanium dioxide.

revealed dissimilarity posttreatment of the specimens in citric acid solution (4 wt.%). The occluded tubules remain intact after acidic challenge for both the $EB@TiO_2$ and Sensodyne treated group. On the contrary, the tubules in the Colgate Pro-relief (►**Fig. 4C8**) treated specimens were visibly reopened postacidic challenge.

Biocompatibility Testing

The biocompatibility of $EB@TiO_2$ with the BHK21 cell line is shown in ►**Fig. 5**. In comparison to the negative control, the $EB@TiO_2$ appear to show little effect on the BHK21 cell lines. However, there was 56% cell viability at 100 µg/mL.

Discussion

Over the last decade, DH has been extensively researched owing to its widespread prevalence and noticeable painful oral health problem affecting many individuals.[30] The main aim of the paper to evaluate the effectiveness of a modified

Table 6 Paired sample test comparison of occluded area ratio pre- and postacidic treatment (samples stored in artificial saliva)

Treatment Group	Occluded area (%)		*p*-Value
	Preacidic challenge (mean ± SD)	Postacidic challenge (mean ± SD)	
Artificial saliva	10.9 ± 2.3	5.7 ± 1.8	0.001
EB@TiO$_2$	99.3 ± 1.0	85.0 ± 3.8	0.000
Colgate Pro-relief	80.1 ± 8.6	9.0 ± 2.2	0.000
Sensodyne repair	90.4 ± 3.0	88.1 ± 3.9	0.245

Abbreviation: SD, standard deviation.

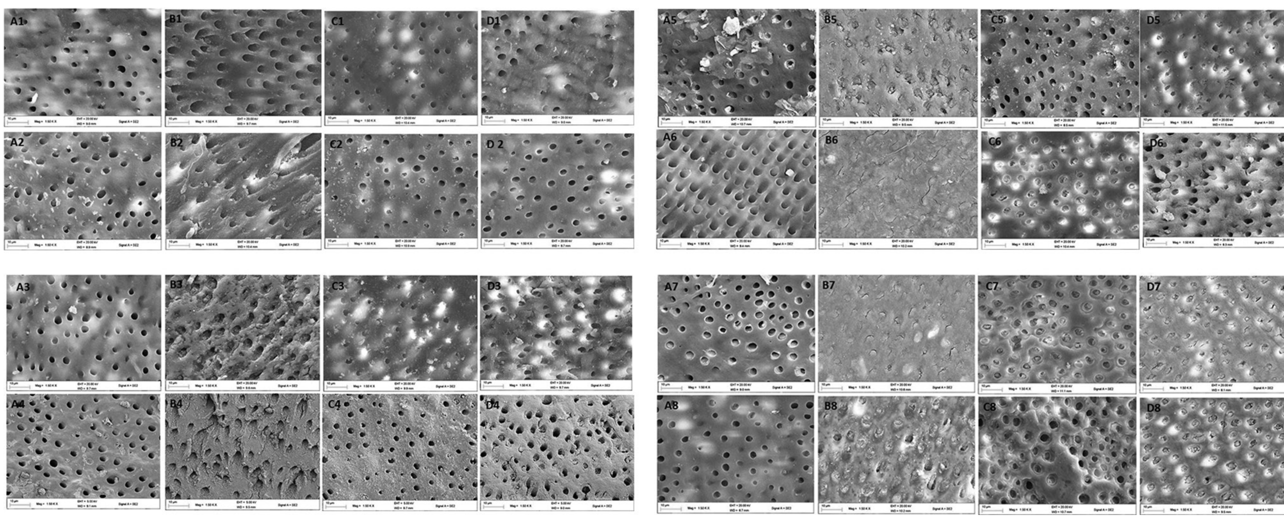

Fig. 4 Representative FESEM micrograph for the dentine surface after brushing for seven days with saliva immersion using (**A**) Artificial saliva; (**B**) EB@TiO$_2$; (**C**) Colgate Pro-relief; (**D**) Sensodyne repair (1–7 represents with the respective desensitizing paste, and 8 post acidic exposure). FESEM, field scanning electron microscope. EB@TiO$_2$, eggshell-titanium dioxide.

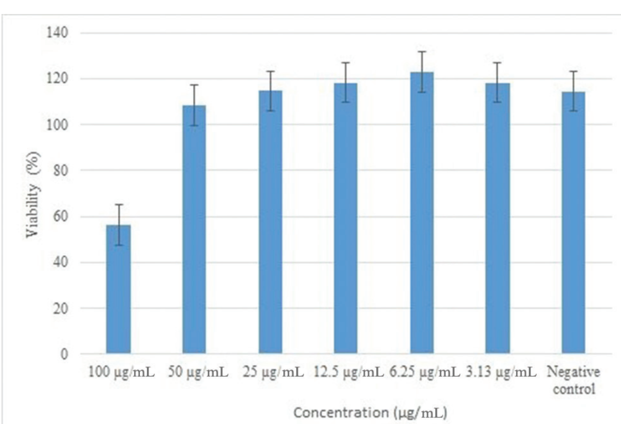

Fig. 5 Percentage cell viability.

nanosized eggshell powder titanium dioxide composite (EB@TiO$_2$) in reducing DH. The composite was prepared through the mechanochemical activation method. Importantly, this method utilizes a mechanical energy to create structural changes as well as stimulate chemical reactions.[31] Consequently, it becomes possible to cause structural changes and particle size reduction in the EB@TiO$_2$ composite.[27,28] Conversely, the effectiveness of the prepared EB@TIO$_2$ in reducing DH was compared against Colgate Pro-relief and Sensodyne with or without immersion saliva. As suggested in the literature,[32-37] their occluding capabilities were evaluated using the bovine model. The morphological changes pre- and postacidic treatment of the specimens were evaluated with FESEM. The EB@TiO$_2$ treated specimens showed good tubule occlusion that still remain effective in acidic condition for both samples treated with and without saliva. This leads to the acceptance of the study hypothesis.

With respect to time, in the specimens treated without saliva, Colgate Pro-relief showed instant occluding of the dentine tubules. This is consistent with clinical studies[38,39] that Pro-Argin technology provides instant relief of DH. According to the mechanism proposed by Kleinberg,[19] it may be assumed that dentine with a negative charge surface attracts the positive charge arginine constituent of the Colgate Pro-relief which subsequently causes the adherence of calcium carbonate to the dentin surface. This in turn promotes the occlusion of the tubules. Despite this, the overall dentine tubule occlusion observed in the samples treated with EB@TiO$_2$ were significantly better than Colgate Pro-relief ($p < 0.05$) and Sensodyne repair ($p < 0.001$), respectively. These differences could be attributed to the modification of the carbonate structure in eggshell with titanium dioxide.[22] According to Cutler,[25] nanosized titanium dioxide, together with abrasive materials facilitate the occluding of dentine tubules, thus contributing to reducing of DH. Added to this, the nanosized calcium carbonate materials have unique high surface energy—thus facilitating the attachment of calcium-rich ions on the oral tooth surface.[40]

Furthermore, the occlusion measured for Sensodyne repair was statistically lower compared against the Colgate Pro-relief ($p < 0.001$). At the end of 7 days brushing, $EB@TiO_2$ had the highest occluded area (97.8 ± 1.3%) followed by Colgate Pro-relief (88.9 ± 3.2%), and lastly Sensodyne repair (71.3 ± 4.9%). Overall, the highest (64.7 ± 1.3) occlusion measured was in the $EB@TiO_2$ group, while Sensodyne repair had the lowest (22.7 ± 4.8; ►**Table 3**). This difference may be related to the constituent of the various test materials. Although studies[41,42] have shown that calcium sodium phosphosilicate (NovaMin) constituent of the Sensodyne repair could obstruct dentine tubules to some extent, Yu et al[43] however, argued that the Ca^{2+} and PO_4 are protected by glass particles which need to be trapped for the Ca^{2+} and PO_4 to be localized. The consequence of this is that there might be a delay in the action of the NovaMin to effectively promote the closing of dentine tubules.[43] Since the brushing test was performed in 7 days, without saliva, the inferior occluding characteristics observe for Sensodyne repair could be attributed to the absence of saliva to trap the Ca^{2+} and PO_4^{3-}.

On the other hand, for the specimens treated with saliva immersion, all the tested material demonstrated a significant occlusion difference when compared with the those found in saliva alone ($p < 0.001$). While saliva is reported to facilitate remineralization by the deposition of calcium and phosphate,[13,19] the finding from this study suggests that the occlusion of specimens in saliva alone without desensitizing paste treatment were highly inferior (►**Table 5**). This may; however, be attributed to the treatment duration (►**Table 2**). As reported in literature,[12] the occluding capabilities of saliva occur gradually within a long time. In support of the role saliva plays in reducing DH, the dentine tubules occlusion observed for Sensodyne repair showed an outstanding occlusion when compared against the samples treated without saliva. Similar significant occluding abilities were measured for $EB@TiO_2$ treated with saliva immersion ($p < 0.05$).

Contrary to the above, the dentine tubules occlusion observed for the samples treated with Colgate Pro-relief with saliva treatment were consistently inferior at each day of brushing to those measured for the samples treated without saliva ($p < 0.001$; ►**Table 6**). In contrast, other studies[11,38] claimed that the interaction of calcium carbonate and arginine encourages endogenous calcium and phosphate ions to deposit and occlude the dentin tubules. However, Yang et al[44] found that Colgate Pro-relief showed no significant changes after treatment and immersion in artificial saliva for 14 days. The above author findings corroborate with the same observation found in this study.

Moreover, the stability of occluding agents, particularly in a high acidic oral environment, is an important criterion for evaluating the efficiency of desensitizing paste in occluding dentine tubules.[45] This is more important as the oral cavity is often bombarded with citric acid that is highly common in the soft drinks and fruit juices found in our daily diets. In light of these, the effectiveness of the dentine occlusion observed with the different desensitizing paste was assessed posttreatment in a solution containing 4wt.% citric acid. The results observed for Colgate Pro-relief suggests that the product demonstrated an acid resistant to a certain extent. This can further be supported by the FESEM images that visibly showed that some of the closed dentine tubules were reopened after exposure to the citric acid solution (►**Fig. 2B8**, and **4C8**). This; however, could be attributed to the solubility of calcium phosphates in an acidic environment.[21]

In terms of the Sensodyne repair, the acid resistance effectiveness measured exhibit different behavior in the samples treated with and without saliva. In the group treated without saliva, nearly all the tubules were reopened after the exposure to citric acid (►**Fig. 2C8)**. Similar findings were observed by Yu et al[43] where the deposits created by NovaMin on the dentine surface were almost completely removed by the citric acid solution. In contrast, the samples treated with saliva (►**Fig. 4D8**), the Sensodyne repair demonstrated an outstanding acidic resistance characteristic ($p > 0.05$). The difference observed for both sample treatments may be associated with the role the saliva plays. It can, therefore, assume that the occlusion for the samples treated with Sensodyne and immersed in saliva had depth and penetration, thereby contributing to its acidic resistance.

As for the $EB@TiO_2$ group, the acidic resistant properties observed for the samples treated without saliva, pre- and postcitric acid exposure were comparable ($p > 0.05$). However, slight differences were observed for the samples treated and immersed in saliva. It was found that after citric exposure, some of the obstructing tubules were reopened (►**Fig. 4B8**). This notwithstanding, the FESEM images visibly validate that the acid resistant characteristics of $EB@TiO_2$ were superior to that of Colgate Pro-relief and to some extent Sensodyne repair. Consistent with Tao et al,[46] the stability of $EB@TiO_2$ in an acidic condition may have been influenced by the modification of eggshell with titanium dioxide. Further clinical research is; however, needed to substantiate the efficiency of $EB@TiO_2$ as biocomposite material for the management of DH.

Conclusion

In conclusion, and within the study limitation, the study established that the $EB@TiO_2$ composite successfully occludes open dentine tubules with and without saliva. It was also established that $EB@TiO_2$ achieved effectiveness after 3 days of brushing. The composites also provide outstanding acid resistant stability. Despite this, and given the size of the sample used for the study, larger and longer duration of treatment would be required to conclusively determine the efficiency of $EB@TiO_2$ in reducing DH.

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Conflict of Interest
None declared.

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