# Comparative Evaluation of the Remineralizing Potential of Commercially Available Agents on Artificially Demineralized Human Enamel: An *In vitro* Study

## Abstract

**Background:** Caries is highly prevalent multifactorial disease, but its progression can be prevented in the initial stage of demineralization through remineralization (RML). Various materials have been proposed for the same, successful outcome can prove to be a boon in the prevention of caries. **Aim:** The aim of the study is to assess the RML potential of four commercially available agents so as to restore the enamel closest to its previous microhardness levels. **Materials and Methods:** Sixty permanent intact premolars were randomly divided into six groups: Four test groups – (1) bioactive glass (BAG) Novamin (SHY-NM), (2) nano-hydroxyapatite (nHAp) (Acclaim), (3) functionalized tricalcium phosphate (f-TCP) (Clinpro Tooth Crème), and (4) grape seed extract (GSE); one positive control – (5) fluoride (1000 ppm) containing dentifrice (Colgate Calci-Lock); and one negative control – (6) distilled water. The samples were initially evaluated for baseline surface microhardness (SMH); later on, these samples were placed in the demineralizing solution for 48 h in an incubator at 37°C, and postdemineralization again SMH was measured. Thereafter, the samples were subjected to the pH cycling for consecutive 21 days, and SMH was recorded. The SMH was evaluated using a Vickers microhardness tester. Statistical analysis was done using a *post hoc* Tukey test for each group based on the stage of treatment and one-way ANOVA for comparison among different groups. **Results:** BAG Novamin showed SMH recovery at 96.75% followed by f-TCP at 95.83%, nHAp at 90.88%, and GSE at 48.71%. Statistically significant differences were observed between the first three groups and the rest of the groups after RML stage. **Conclusion:** BAG Novamin, f-TCP, and nHAp showed considerable RML followed to a lesser extent by GSE.

**Keywords:** *Bioactive glass, calcium phosphate, fluoride, microhardness, nano-hydroxyapatite, pH-cycling, remineralization*

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## Introduction

Enamel white spot lesions are the earliest macroscopic evidence of enamel caries in which the enamel surface layer stays intact during subsurface demineralization, but without any intervention, cavitation will take place.[1,2] In the first stage of enamel demineralization, removal of interprismatic mineral content takes place subsequently followed by a well-defined surface layer formation that constitutes an early carious lesion.[3] The progression of the early enamel lesion is a slow process, and this early lesion is reversible through the process of remineralization (RML).[4] Voids and surface roughness on demineralized surfaces of enamel and partially dissolved crystallites serve as nucleating sites for the formation of new crystallites or regrowth of existing crystallite structures.[5]

Till date, the researches in the literature support that fluoride treatment remains the best remineralizing method for early enamel caries.[6-9] However, it is difficult for fluoride to result in oriented and ordered mineral crystals on the surface of enamel under physiological conditions due to the lack of ability to guide the formation of mineral crystals. The ordered orientation is essential for the mechanical properties of enamel. Thus, the aim of an ideal mineralizing agent should be to achieve the organization and microarchitecture of mineral crystal as close the natural ones as possible.[10]

Novamin is a trade name that has been given to bioactive glass (BAG) (e.g., Bioglass) that has been ground into a fine

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particulate with a median size of fewer than 20 μ.[11,12] BAG which has the ability to act as a biomimetic mineralizer matching the body's own mineralizing traits, while also affecting cell signals in a way that benefits the restoration of tissue structure and function. When it comes in contact with an aqueous environment, it releases bioavailable calcium, sodium, and phosphate ions contributing to the RML process.[13] Another material is tricalcium phosphate, which is a new hybrid material created with a milling technique that fuses beta-tricalcium phosphate (β-TCP) and sodium lauryl sulfate (SLS) or fumaric acid. This blending results in "functionalized" calcium and a "free" phosphate, increasing the efficacy of fluoride RML.[14] The functionalized tricalcium phosphate (f-TCP) is produced by altering 98% w/w β-TCP with 2% w/w SLS using a ball milling technique.[15] Nano-hydroxyapatite (nHAp) has also been advocated for RML of teeth. It is hydrophilic and has more surface area than the conventional hydroxyapatite crystals. Hence, they have enhanced wettability which helps it to form a thin but strong layer on enamel surface that bonds to the tooth structure.[16,17] Finally, grape seed extract (GSE) has recently been advocated for its positive effects on the RML process of artificial carious lesions of the enamel in human primary teeth due to the GSE being rich in proanthocyanidin (PA), which is mainly composed of monomeric catechin and epicatechin, gallic acid, and polymeric and oligomeric procyanidins. PA has been reported to strengthen collagen-based tissues by increasing collagen cross-links.[18]

In this research, microhardness tests were used to evaluate the degree of RML of the tooth samples. Surface microhardness (SMH) indentations provide a relatively simple, nondestructive and rapid method in demineralization and RML studies.[19]

An *in vitro* pH-cycling technique to mimic the intraoral scenario of demineralization and RML was used in this study. Our literature is enriched with several studies comparing the remineralizing potential of different agents, but there are few studies in the literature comparing the remineralizing potential of the four agents together. Hence, this study was performed with an aim of comparing RML potential of all the available novel agents: SHY-NM containing BAG Novamin, Acclaim containing nHAp, Clinpro Tooth Crème containing f-TCP, and GSE on artificially demineralized human enamel.

## Materials and Methods

A total of 60 teeth were divided into six groups. Selection criteria for the teeth as samples were as follows:

### Inclusion criteria

Permanent premolars extracted for the orthodontic purpose were selected for the study. The teeth selected were noncarious, with an intact surface and no visible cracks, and were unrestored.

### Exclusion criteria

Any tooth with visible cracks, hypoplasia, enamel white spot lesion or caries on any surface, and restored teeth was excluded from the study.

### Sample distribution

After the collection of teeth, the samples were divided into six groups. The first four groups were test groups, which had 12 samples each, and the two were control groups, Group 5 – positive control and Group 6 – negative control had 6 samples each [Figure 1].
- Group 1 – BAG Novamin (SHY-NM, Group Pharmaceuticals Limited, Mumbai, India) – 12
- Group 2 – nHAp (Acclaim, Group Pharmaceuticals Limited, Mumbai, India) – 12
- Group 3 – f-TCP (Clinpro Tooth Creme, 3M ESPE Dental Products, Ontario, Canada) – 12
- Group 4 – GSE (Inlife Group, Hyderabad, India) – 12
- Group 5 – Fluoride (1000 ppm) (Colgate Calci-Lock, Colgate-Palmolive Limited, Mumbai, India) – 6
- Group 6 – Distilled water (Aquarch, Ahmedabad, India) – 6.

### Preparation of different agents used in the study

The solutions that were used in the entire study were as follows:

#### Artificial saliva

- Artificial saliva was prepared using analytical grade chemicals and distilled water. It was prepared using the following agents in definite proportions – calcium chloride ($CaCl_2$) 1.5 mmol/l, potassium chloride (KCl) 50 mmol/l, potassium dihydrogen phosphate ($KH_2PO_4$) 0.9 mmol/l, and Tris buffer 20 mmol/l [Figure 1].

#### Demineralizing solution

An artificial demineralizing solution prepared by mixing analytical grade chemicals. The composition of the demineralizing solution was as follows: calcium chloride ($CaCl_2.2H_2O$) 2.2 mmol/l, potassium dihydrogen phosphate ($KH_2PO_4.7H_2O$) 2.2 mmol/l, and lactic acid 0.05 mmol/l [Figure 1].

The final pH was adjusted to 4.5 with 50% sodium hydroxide (NaOH).

### Remineralizing agent slurry

The remineralizing agent slurry was prepared by manually mixing peanut-sized toothpaste (equals to the volume of a standardized lid of toothpaste) to the distilled water (three times volume of the toothpaste) with a plastic spatula at a speed of 30 rotations in 30 s. The following agents were used as follows: SHY-NM, Acclaim, Clinpro Tooth Crème, GSE, and Colgate Calci-Lock. Each remineralizing agent was used in its respective group.

## Sample storage

The samples were placed in 10% formalin for 1 week and then were stored in artificial saliva till further use at 37°C in an incubator and at pH 7.4 [Figure 2]. A digital pH meter (Systronics Company, Ahmedabad, India) [Figure 2] was used to check pH during and after preparation of the solution. The solution of artificial saliva was replaced every 24 h.

## Sample preparation

After thorough scaling with an ultrasonic scaler and polishing with pumice paste and a rubber cup, the teeth were examined for visible cracks under the surgical microscope (Prima DNT, Fremont, USA) at ×10 magnification. The teeth having cracks or hypoplastic teeth were discarded. The radicular part of each of the 60 teeth was horizontally sectioned at a level – 1 mm apical to the cementoenamel junction with the help of a diamond disc at 15,000 rpm, attached to a slow-speed straight handpiece in micromotor with constant water coolant. Thereafter, impenetrable stickers of 4-mm × 4-mm dimension were applied on the buccal surface of the crown portion of the samples, and then the samples were coated with different colored nail varnish (according to the group) on the rest of the surfaces to keep tooth surface exposed for testing purpose. Group 1: SHY-NM – Yellow; Group 2: Acclaim – Orange; Group 3: Clinpro Tooth Crème – Green; Group 4: GSE – Blue; Group 5: Colgate Calci-Lock – Magenta; Group 6: Distilled water – Pink [Figure 3].

Now, the specimens were mounted in acrylic resin mold by pouring self-cure acrylic resin in the preformed heavy body mold of (dimension 2 by 2 cm). The mounted blocks were also stored in artificial saliva at 37°C and pH 7.4 until further use. Following this, all the samples were tested for SMH at different stages.



**Figure 1: Materials used as – remineralizing agent, artificial saliva, demineralizing agent**



**Figure 3: Acrylic blocks with different nail varnish application for each group**

## Baseline surface microhardness measurement

Baseline microhardness of the samples was taken using digital Vickers microhardness tester and Vickers Microhardness Software (Fuel Instruments and Engineers Pvt. Ltd., Kolhapur, India). A load of 100 g was applied to the surface for 10 s [Figure 4]. Three such indentations were placed on the surface, and the average value of the three readings was taken into consideration. A built-in scaled microscope measured the diagonal length of the indentation [Figure 5]. After the measurement of baseline microhardness, the samples were subjected to demineralization process for the induction of early enamel carious lesion.

## Induction of demineralizing lesion

All the prepared specimens of six groups were immersed in individual glass containers containing 10 ml of demineralization solution for 48 h in an incubator at a temperature of 37°C. The solution was replenished every 24 h. A digital pH meter was used to check pH during and after preparation of the solution. Then, the samples were thoroughly washed with distilled water and placed in artificial saliva until further use.

After induction of enamel lesions, the SMH test of all the samples was again measured using the same
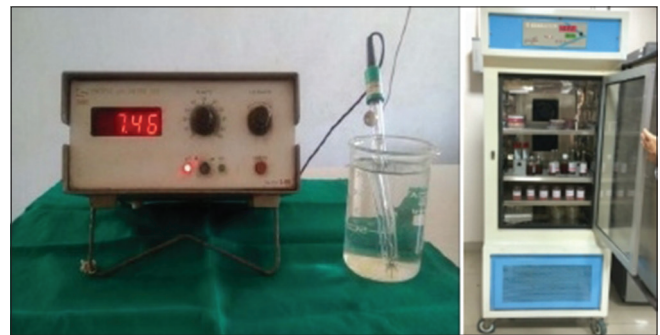


**Figure 2: pH meter and incubator used in the study**



**Figure 4: Surface microhardness measured using digital Vickers microhardness tester**

above-mentioned protocol. After measuring post demineralization microhardness, the samples were exposed to pH cycling regime to mimic the intraoral pH environment.

## The pH-cycling model (demineralization–remineralization model)

The experimental process attempted to imitate the changes in pH of the oral environment. The specimens were subjected to a pH cycling regimen of alternative demineralization and RML for 21 consecutive days. In a 24-h cycle, the samples were subjected to demineralization for 3 h twice a day and received 2 min treatment with the respective remineralizing agent slurry twice daily with a soft toothbrush. The solutions (artificial saliva and demineralizing solution) were replenished every 24 h. The 24-h pH cycling regime was designed in such a way that it mimics a routine intraoral scenario of an individual [Figure 6].

After the completion of pH cycling regime for 21 days, SMH measurement was done again for each sample, and all the readings were recorded and evaluated by application of the suitable statistical test.

### Statistical analysis

The values obtained were then subjected to statistical analysis using SPSS Software (Statistical Package for the Social Sciences version 20; SPSS Chicago, USA). Comparison of the various treatment groups was done using one-way ANOVA. This was followed by *post hoc* Tukey analysis as each group contained three subgroups based on the stage of treatment. ANOVA is a technique by which the total variation is split into two parts, one between groups and the other within the groups. If "*F*" value is significant, there is a significant difference between groups. To find out which mean between the two groups is significantly different *post hoc* analysis, Tukey's test is perfo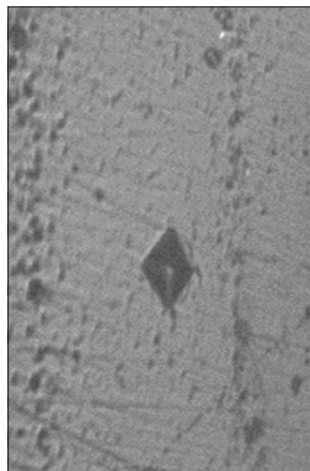rmed. In case *F* value is not significant, it indicates that there is no significant difference between the groups, and it stops the analysis at this stage and the Tukey test is not used. For the test, a value $P < 0.05$ was considered to be statistically significant.

## Results

Percentage SMH recovery (SMHR) for each of the treatment groups was calculated using the following formula:

% SMH Recovery

$$= \frac{(\text{VHN Remineralization} - \text{VHN Demineralization}) \times 100}{\text{VHN Baseline} - \text{VHN Demineralization}}$$

(VHN: Vickers hardness number)

Percentage SMHR of the samples of Group 1 was 96.75%, Group 2 was 90.88%, Group 3 was 95.83%, Group 4 is 48.71%, Group 5 is 40.52%, and Group 6 was 27.82% [Figure 7].

There was no statistical difference between various groups at baseline SMH and SMH after demineralization, but all the groups showed a significant difference in SMH after RML [Tables 1 and 2]. Meanwhile, on analysis for multiple comparisons between various groups, statistically

**Figure 5: Digital image of indentation obtained through the software**
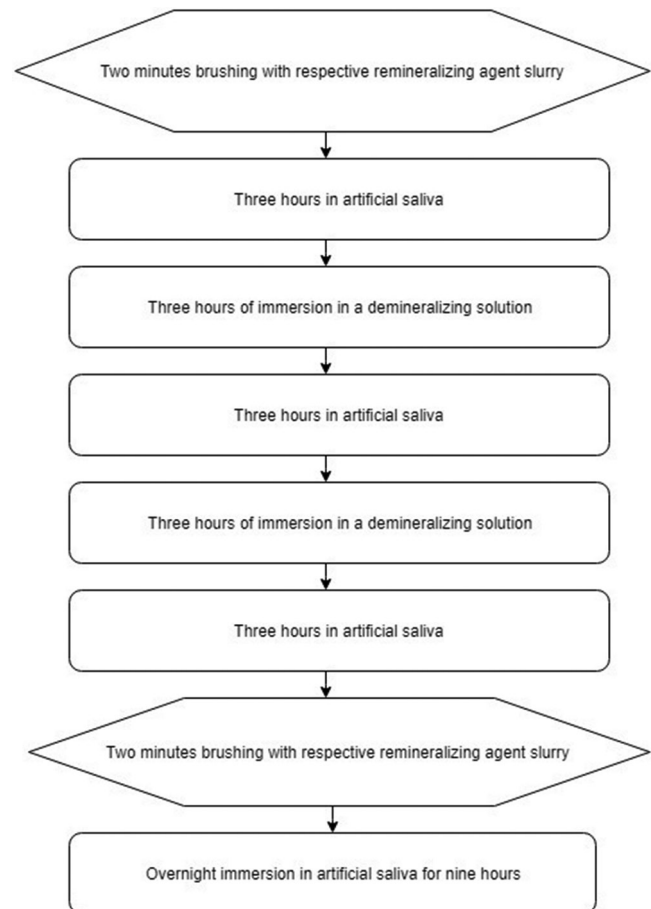
**Figure 6: pH cycling regimen**

Flowchart content:
- Two minutes brushing with respective remineralizing agent slurry
- Three hours in artificial saliva
- Three hours of immersion in a demineralizing solution
- Three hours in artificial saliva
- Three hours of immersion in a demineralizing solution
- Three hours in artificial saliva
- Two minutes brushing with respective remineralizing agent slurry
- Overnight immersion in artificial saliva for nine hours

### Table 1: Comparison of various groups at each treatment stage by one-way ANOVA

| SMH | Groups | Mean | SD | P | SMH | Mean | SD | P | SMH | Mean | SD | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| At baseline | 1 | 378.00 | 18.30 | 0.632 | After DMZ | 308.17 | 17.77 | 0.539 | After RML | 375.75 | 18.93 | <0.001* |
| | 2 | 373.83 | 21.29 | | | 304.25 | 19.23 | | | 367.33 | 20.30 | |
| | 3 | 372.42 | 20.93 | | | 302.58 | 18.89 | | | 369.50 | 20.61 | |
| | 4 | 383.92 | 17.88 | | | 315.75 | 17.34 | | | 348.83 | 17.72 | |
| | 5 | 370.67 | 13.78 | | | 305.67 | 11.06 | | | 331.33 | 14.65 | |
| | 6 | 372.50 | 18.10 | | | 305.00 | 17.79 | | | 323.67 | 22.02 | |
| | Total | 375.95 | 18.80 | | | 307.22 | 17.59 | | | 357.78 | 25.66 | |

*Significant difference $P<0.05$. SD: Standard deviation; SMH: Surface microhardness; DMZ: Demineralization; RML: Remineralization

### Table 2: Mean microhardness values of all the groups after demineralization and remineralization (in Vickers hardness number)

| Groups | Stage | Mean | SD | SE | Mean difference | P |
|---|---|---|---|---|---|---|
| 1 | After DMZ | 308.17 | 17.77 | 5.13 | 67.58 | <0.001* |
| | After RML | 375.75 | 18.93 | 5.46 | | |
| 2 | After DMZ | 304.25 | 19.23 | 5.55 | 63.08 | <0.001* |
| | After RML | 367.33 | 20.30 | 5.86 | | |
| 3 | After DMZ | 302.58 | 18.89 | 5.45 | 66.92 | <0.001* |
| | After RML | 369.50 | 20.61 | 5.95 | | |
| 4 | After DMZ | 315.75 | 17.34 | 5.00 | 33.08 | <0.001* |
| | After RML | 348.83 | 17.72 | 5.12 | | |
| 5 | After DMZ | 305.67 | 11.06 | 4.51 | 25.67 | <0.001* |
| | After RML | 331.33 | 14.65 | 5.98 | | |
| 6 | After DMZ | 305.00 | 17.79 | 7.26 | 18.67 | <0.001* |
| | After RML | 323.67 | 22.02 | 8.99 | | |

*Significant difference $P<0.05$. SD: Standard deviation; DMZ: Demineralization; RML: Remineralization; SE: Standard error



Figure 7: Percentage surface microhardness recovery of each group after remineralization

significant difference was seen in values of SMH at the RML stage between Group 1 with Group 4, 5, and 6; Group 2 with Group 5 and 6; and Group 3 also with Group 5 and 6 [Table 3]. From the results, it could be inferred that the first three groups showed statistically significant difference with control groups, but among them, there was no statistically significant difference.

## Discussion

Permanent maxillary and mandibular premolars extracted for the orthodontic purpose were selected for the purpose of the study owing to their easy availability, the adequate thickness of enamel on buccal surfaces of the tooth, fewer chances of microcracks in young tooth, and to avoid age-related changes of enamel. No variations among them in terms of demineralization and RML strategies were noticed as confirmed by the $P$ value ($P > 0.05$).

A study by Salem-Milani *et al*.[20] has shown that immersion of samples in 10% formalin for up to 2 weeks does not cause any change in the enamel microstructure, and subsequently, its SMH. Hence, for the purpose of effective disinfection, the extracted teeth were stored in 10% formalin for 1 week. In general, the enamel samples are abraded before microhardness measurement, but Xue
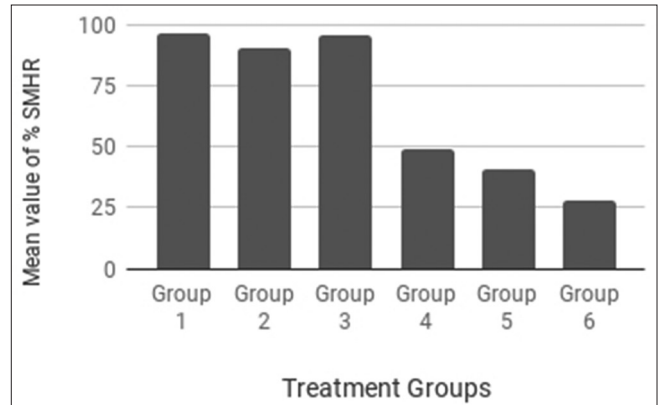
*et al*.[21] reported that the *in vitro* demineralization pattern of unabraded samples more closely resembles the pattern of a natural white spot lesion than that of the abraded samples. Hence, the samples were not abraded. The sectioned teeth were mounted in acrylic blocks for the purpose of stabilization and prevention of any kind of movement error of the samples during microhardness measurement. Hua *et al*.[22] proved that dehydration increases the SMH of swine enamel. To mimic the intraoral environment and to prevent dehydration of the samples which can affect the microhardness of the enamel, the tooth samples were embedded in the acrylic blocks and were stored in artificial saliva at 37°C in an incubator.

The composition of artificial saliva used was according to the one used by Wang *et al*.[23] Demineralizing solution used for the induction of incipient lesions was the one used by Patil *et al*.[24] The solutions used in the study were created to replicate supersaturation by apatite minerals found in saliva and were similar to those previously utilized by Ten Cate and Duijsters.[25] All the solutions were replenished every 24 h, and this regimen was followed for 21 days to mimic the intraoral real-life scenario. The duration of demineralization was kept 48 h based on the values obtained after the pilot study. Seventy-two and ninety-six hours of demineralization led to complete softening of enamel and 24 h of immersion in demineralizing solution did not yield adequate reduction of SMH. (The concentration of both calcium and phosphate, in the demineralizing solution, was

**Table 3: *Post hoc* Tukey honestly significant difference analysis for multiple comparisons between various groups at baseline, after demineralization, and after remineralization**

| SMH | Groups | Mean difference | SE | P | SMH | Mean difference | SE | P | SMH | Mean difference | SE | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| At baseline | 1-2 | 4.17 | 7.775 | 0.994 | After DMZ | 3.92 | 7.236 | 0.994 | After RML | 8.42 | 7.876 | 0.892 |
| | 1-3 | 5.58 | 7.775 | 0.979 | | 5.58 | 7.236 | 0.971 | | 6.25 | 7.876 | 0.967 |
| | 1-4 | −5.92 | 7.775 | 0.973 | | −7.58 | 7.236 | 0.899 | | 26.92 | 7.876 | 0.015* |
| | 1-5 | 7.33 | 9.523 | 0.971 | | 2.50 | 8.863 | 1.000 | | 44.42 | 9.646 | <0.001* |
| | 1-6 | 5.50 | 9.523 | 0.992 | | 3.17 | 8.863 | 0.999 | | 52.08 | 9.646 | <0.001* |
| | 2-3 | 1.42 | 7.775 | 1.000 | | 1.67 | 7.236 | 1.000 | | −2.17 | 7.876 | 1.000 |
| | 2-4 | −10.08 | 7.775 | 0.785 | | −11.50 | 7.236 | 0.609 | | 18.50 | 7.876 | 0.193 |
| | 2-5 | 3.17 | 9.523 | 0.999 | | −1.42 | 8.863 | 1.000 | | 36.00 | 9.646 | 0.006* |
| | 2-6 | 1.33 | 9.523 | 1.000 | | −0.75 | 8.863 | 1.000 | | 43.67 | 9.646 | <0.001* |
| | 3-4 | −11.50 | 7.775 | 0.679 | | −13.17 | 7.236 | 0.462 | | 20.67 | 7.876 | 0.109 |
| | 3-5 | 1.75 | 9.523 | 1.000 | | −3.08 | 8.863 | 0.999 | | 38.17 | 9.646 | 0.003* |
| | 3-6 | −0.08 | 9.523 | 1.000 | | −2.42 | 8.863 | 1.000 | | 45.83 | 9.646 | <0.001* |
| | 4-5 | 13.25 | 9.523 | 0.732 | | 10.08 | 8.863 | 0.863 | | 17.50 | 9.646 | 0.466 |
| | 4-6 | 11.42 | 9.523 | 0.835 | | 10.75 | 8.863 | 0.829 | | 25.17 | 9.646 | 0.113 |
| | 5-6 | −1.83 | 10.996 | 1.000 | | 0.67 | 10.234 | 1.000 | | 7.67 | 11.138 | 0.983 |

*Significant difference *P*<0.05. SMH: Surface microhardness; DMZ: Demineralization; RML: Remineralization; SE: Standard error; SD: Standard deviation

at 50% saturation level, causing dissolution of only enamel subsurface which simulated the naturally occurring early enamel lesions having intact surface layer.)

The present study used the modified pH cycling regimen from the one used by Mehta *et al*.[26] rather than using the traditional pH-cycling method, in an attempt to simulate the real-life situation. In this study pH, cycling regime was structured in such a way that it reflects the dietary habits, brushing habits, and thereby intraoral environment of a normal human.

Several techniques introduced in the literature to assess the process of demineralization and RML.[16] Vickers SMH (VSMH) test had been selected to evaluate the RML potential of the test materials owing to the importance of the surface layer in caries progression and also microhardness measurement is appropriate for a material having a fine microstructure, nonhomogenous structure, or prone to cracking such as enamel. SMH test indentations provide a relatively simple, nondestructive, and rapid method in demineralization and RML studies.[27] In the present study, VSMH test was chosen over the Knoop microhardness test because the shape of indent obtained in VSMH was easy and accurate to measure.[26] Thus, Vickers hardness number values are indirect measurements of RML.

Group 1 having SHY-NM (BAG, calcium sodium phosphosilicate, Novamin technology),[28] gave the best results. It achieved the highest percentage SMHR 96.75%. Vahid Golpayegani *et al*.[29] also found increased post-RML microhardness values where Novamin dentifrice appeared to have a greater effect on RML of carious-like lesions. Mehta *et al*.[26] also found that the BAG had superior RML efficacy than that of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). On the contrary, in a study

by Balakrishnan *et al*.,[30] CPP-ACP showed the better remineralizing potential than the f-TCP and BAG Novamin using VSMH, but there was no statistically significant difference between f-TCP and BAG Novamin groups, this non difference in the values could be due to the number of days the RML was carried out and also the pH cycling regime was not followed.

nHAp (Group 2) has been reported to provide novel prevention strategies for the treatment of dental caries, specifically in the control and management of dental plaque biofilm and RML of initial dental caries.[31] Swarup and Rao[32] compared the RML potential of 10% biomimetic nHAp with 2% sodium fluoride (NaF) and found former to give more favorable results. Huang *et al*.[33] concluded that 10% of nHAp is an optimal concentration for RML. Also, both the studies, by Swarup and Rao and Huang *et al*. utilized separately procured 10% nHPa powder formed in a slurry to be used in the study but in the present study, the materials being tested are commercial dentifrices, the Acclaim, which contains nHPa 1%[34] achieved 90.88% SMHR, a higher percentage of nHPa in the commercial product can be expected to produce even better results. The results of nHAp in the current study are in accordance with a study performed by Tschoppe *et al*.[35] in which they evaluated nHAp on enamel and dentin RML and showed that nHAp toothpaste had greater efficacy for enamel and dentin RML than amine fluoride toothpaste. A study by Haghgoo *et al*.[36] found SMH to be higher in the teeth treated with Novamin (BAG) toothpaste (422.67 kgf/mm$^2$) than in the teeth treated with nHAp (384.2 kgf/mm$^2$). However, this difference was not statistically significant. These results are in accordance with our study.

Clinpro Tooth Crème (Group 3) is a 0.21% w/w NaF anticaries dentifrice that contains 950 ppm fluoride and

a f-TCP ingredient. The f-TCP contains 2% SLS, which prevents calcium phosphate reaction with fluoride. Organic coating of SLS on the TCP "functionalizes" the TCP and prevents undesirable interactions with fluoride. The organic component subsequently dissolves away when placed in saliva, to leave the particles active.[30] Karlinsey *et al.*[37] found significant differences among the four groups in which the placebo and 500 ppm F dentifrices providing significantly less RML relative to the 1150 ppm F and 500 ppm F plus f-TCP dentifrices.

Group 4 containing GSE showed slightly less RML than previous three groups but better than control groups as shown by the %SMHR and SMH values after RML. Mirkarimi *et al.*[18] conducted a study in which GSE enhanced the RML process of artificial enamel lesions of primary teeth. In this scanning electron microscopy (SEM) analysis, after treating the enamel surface with GSE, scaffolding deposits on the enamel surface with cluster-like structures resembling RML process initiation were observed. Spherical particles were also visible on sound enamel surface, and to a more extent, on the treated enamel surface. Cheng *et al.*[38] also supported a similar mechanism, where they found out that PAs and gallic acid present in the GSE are responsible for facilitating mineral deposition on enamel. It has been shown by Xie *et al.*[39] that GSE positively affects the RML process of root, since collagen can serve as a substrate for apatite formation. The present study was designed to assess whether GSE, mainly consisting of PA, can effectively influence the RML of artificially demineralized human enamel or not. Based on data obtained in this *in vitro* study, it may be proposed that GSE promotes the RML process of artificial demineralized lesions in the permanent tooth enamel.

Fluoride's anticariogenic effects take place through two mechanisms, inhibiting demineralization when fluoride is present at the crystal surface during an acid challenge and enhancing RML by forming a low solubility veneer similar to the acid-resistant mineral fluorapatite on the remineralized crystals.[40] Fluoride (Group 5) in this study was employed as a positive control, which was found to be effective in the process of demineralization and RML. Fluoride treatment inhibited further demineralization of existing artificial lesions and increased the microhardness value of lesions. Even Group 6 (distilled water) showed some amount of RML, this could be due to artificial saliva, which has been shown to have the potential to remineralize initial enamel lesions which goes in accordance with the study done by Huang *et al.*[33]

Results of the present study after the treatment with the dentifrices showed an increase in mean microhardness values in all the groups. This is in accordance with the previous studies done by Vahid Golpayegani *et al.*,[29] Karlinsey *et al.*,[37] Swarup and Rao,[32] and Kiranmayi *et al.*[9] for determining the RML potential of the agents that were used in the study either individually or in comparison with other agents.

Rirattanapong *et al.* effectively summarized in their study that although β-TCP had poor bioavailability than f-TCP, β-TCP also got promoted in lesioned enamel and dissolution profile showed that calcium ions released by f-TCP were double the amount of that released by β-TCP, and this confirmed the solubility of TCP. The role of SLS was also considered important for strong adherence to the enamel surface and for uptake of mineralizing ions to enamel surface.[41] The high remineralizing capacity of the BAG Novamin observed in the current study may be attributed to its high content of calcium and phosphate and at the micro level where BAG plug could be more compact and intimately attached to the enamel surface. SEM analysis done in one study showed that the deposits formed by BAG Novamin were larger, more angular as compared to other remineralizing agents which can be attributed to high microhardness.[26] The efficacy of the nHAp can be attributed to its particle size, which is fairly small, can enter into the enamel surface continuously and fill the vacant position of enamel crystal. Although it is very dense, partial penetration of certain ions and molecules through the enamel structure is possible because it contains small and intercrystalline spaces, rod sheaths, enamel cracks, and other defects.[42] Maybe due to all these abovementioned reasons BAG Novamin, f-TCP, and nHAp showed promising results as demonstrated by their high percentage SMHR after RML and also there was no statistically significant difference among them, but there was a statistically significant difference in the RML potential between Group 1 and Group 2-3; Group 2 and Group 3-4; Group 3 and 4.

Limitations of the study are that RML *in vitro* may be quite different when compared to the dynamic complex biological system which usually occurs in the oral cavity *in vivo*. Thus, direct extrapolations to clinical conditions must be exercised with caution because of obvious limitations of *in vitro* studies. Further, long-term clinical trials should be conducted to prove the superiority of these materials in the vital teeth.

## Conclusion

Within the limitations of the present study, it can be concluded that all the four remineralizing agents used in the study had the variable potential to remineralize the artificially demineralized human enamel. BAG Novamin (SHY-NM) showed the maximum RML potential in the current treatment protocol followed by f-TCP (Clinpro Tooth Creme), nHAp (Acclaim), and finally, GSE as demonstrated by percentage recovery of SMH after 21 days of pH cycling regime. However, no statistically significant difference was observed among the first three groups.

## Acknowledgments

## Conflicts of interest

There are no conflicts of interest.

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