RESEARCH ARTICLE

A Comparative Evaluation of Remineralizing Potential of Commonly Used Fluoridated Toothpaste, Herbal Toothpaste, Toothpaste with Zinc Hydroxyapatite, and Toothpaste with Calcium Sucrose Phosphate in Children: A Scanning Electronic Microscopic Study

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Abstract

Aim: The aim of this in vitro study is to evaluate the remineralizing potential of commonly used fluoridated toothpaste, toothpaste with calcium sucrose phosphate, toothpaste with zinc hydroxyapatite, herbal toothpaste, and to compare them.

Background: The process of demineralization and remineralization is balanced and occurs concurrently in the oral cavity, but even a mild disruption in this mechanism could lead to dental caries. Dental caries is a threat in the Third World countries and more common entity in childhood. The caries prevalence is reported to be 41% for children within the age of 2–11 years.

Materials and method: According to previous studies and standard sample size calculating formula the sample size of 48 was calculated, the teeth were subjected to demineralization – remineralization process and were observed under scanning electron microscope.

Results: The outcomes of this study suggest that the remineralizing potential of toothpaste with zinc hydroxyapatite ($p = 0.0001$*) was highest as compared to toothpaste with fluoride ($p = 0.0087$*), herbal toothpaste ($p = 0.9034$), and toothpaste with calcium sucrose phosphate ($p = 0.0002$*).

Conclusion: All the four toothpastes showed an adequate amount of remineralization but the highest amount of remineralization potential was seen with toothpaste containing zinc hydroxyapatite.

Clinical significance: The clinical significance of our study is to give clear and evidence-based idea to the dentist and the parents about the remineralizing toothpaste available in the market for children. Treating demineralization at an initial stage would help to remineralize the tooth structure and thereby limit further destruction of the tooth structure which will preserve the beautiful smile of the child.

Keywords: Children, Demineralization, Dental caries, Remineralization.

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Introduction

Dental caries is a major oral health threat in the Third World countries. The caries prevalence is reported to be 41% for children within the age of 2–11 years. The process of demineralization and remineralization is balanced and occurs concurrently in the oral cavity, but even a mild disturbance in the balance can lead to dental caries.[1]

The amalgamation of specific bacterial population that establishes in the oral cavity and the caries susceptible oral environment leads to demineralization of the enamel. This demineralization lesion is clinically demonstrated clearly when air dried as a white, opaque spot.[2]

A more biologically focused approach is used on demineralized lesion, diligent application of remineralization agents to early carious lesions has shown a good success rate in the literature. This creates a supersaturated environment around the early demineralized lesion, which further prevents the mineral loss and forces calcium and phosphate ions in the lesion leading to net mineral gain. Traditionally, these demineralizing agents consists of calcium phosphate along with or without fluoride [3]

A newer generation of toothpaste containing Zinc hydroxyapatite nanoparticles has been developed. This paste has shown effective result in reducing caries, plaque, and gingivitis. Along with its anticaries, antiplaque, and anti-gingival action, it has markedly shown remineralizing effects on initial carious lesions.[4]

Fluoridated toothpaste are commonly available in the market but its use is still debatable even after ample research on the subject. Dentists as well as the parents are in a dilemma for its use in children

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because of its deleterious effects. There has been an ever increasing quest to search for a newer and better alternative.[5] There are herbal toothpaste and toothpaste with calcium sucrose phosphate, which have gained popularity in the recent times and their usage has increased tremendously.

Hence, an in vitro Scanning electron microscope study was conducted with a purpose to evaluate and compare the remineralizing potential of fluoridated toothpaste, herbal toothpaste, toothpaste with zinc hydroxyapatite, and toothpaste with calcium sucrose phosphate.

Materials and Method

The sample size of 48 was calculated using standard sample size calculating formula and previous research articles,[5,12] the required data for the study was derived from noncarious extracted or exfoliated maxillary or mandibular premolar obtained from the Department of Pediatric and Preventive Dentistry. The inclusion criteria considered in our study were extracted caries-free premolar of maxillary or mandibular arch. The exclusion criteria were the teeth with white spot lesions, carious teeth, teeth with cracked areas, and hypoplastic discolored teeth.

Storage

Extracted caries-free premolar of maxillary or mandibular arch were stored in 10% formalin until further use.

Demineralization

The selected teeth were dried and coated with nail varnish (acid resistant), leaving a window of 4 cm × 3 mm for demineralization process, on the buccal or lingual surface. The rectangular window was then coated with 37% phosphoric acid for 3 mins to produce artificial carious lesions.[5]

Sectioning and Division into Groups

The teeth samples were vertically sectioned using a diamond disk in buccolingual direction, such that each section consists of a demineralized area and normal enamel. Total number of sound sections obtained were 48, which were then allocated into four groups, with 12 sections in each group.

The four groups were as follows:

Group 1: Toothpaste containing fluoride
Group 2: Herbal toothpaste
Group 3: Toothpaste containing zinc hydroxyapatite
Group 4: Toothpaste containing calcium sucrose phosphate

Recording Demineralization Rates

These 48 demineralized sections were then studied under the stereomicroscope to evaluate the lesion depth (Fig. 1). The evaluated values were noted and tabulated. The values noted by two examiners were taken and the average value of all two was noted. The cut inner part of the section was then painted with nail varnish except the artificial carious lesion surface for further exposure to remineralization toothpaste.

Recording the Remineralization Rates

After the application of remineralizing toothpaste, the sections were studied under stereomicroscope to evaluate the remineralizing potential at 2nd, 4th , and 6th day (Fig. 2). The evaluated values will be noted and tabulated. The values recorded by two examiners were taken and the average value of all two was noted. The evaluated values for the demineralized (control group) sections and remineralized sections were recorded in units by using "micrometer eyepiece." The units value was then converted into micrometer using the following formulas:

$$\text{Micrometer} = \frac{\text{No. of units} \times 1{,}000}{\text{Eyepiece magnification} \times \text{zoom}}$$

Scanning Electronic Microscope Examination

For the SEM examination, a sample specimen from baseline, demineralized tooth structure as well as from each group was randomly selected to observe surface change. To compare the morphological variations between the different treated samples scanning electron microscope was used. For better observation of the results, both sound and demineralized enamel surfaces were examined. The resultant images obtained were of ×500 magnification.

Results

The obtained results were recorded and the data was entered into MS excel sheet (Microsoft Corp.) and the data was analyzed using

Fig. 1: Demineralized sections: Viewed under the stereomicroscope

Fig. 2: Remineralized sections: Viewed under the stereomicroscope

IBM SPSS software (version 20.0 Chicago IL, USA).The remineralized values were compared with the evaluated demineralized (control group) values of the same specimen. This was done for all the four groups. The results of comparison of the four test groups were done using one-way ANOVA test, Dependent "t" test, and Tukey's post hoc test.

The current study was designed to test the effects of four toothpaste on demineralized enamel lesion depth in vitro condition. Tooth sections obtained from extracted human premolar teeth were subjected to experimental application. The depths of the artificial carious produced in enamel were measured in millimeter using stereomicroscope and an intergroup comparison was done at the end of 2nd, 4th , and 6th day.

The difference in mean lesion depths was not statistically significant when comparison was done between four groups at baseline after demineralization (Table 1). When a pair-wise comparison was done between all the four groups at different time intervals, the results of the comparison showed a statistically significant difference in the remineralizing potential with all of the groups apart from when Herbal toothpaste (Group 2) was compared to Toothpaste containing calcium sucrose phosphate (Group 4), the results were not statistically significant (Table 1).

Figure 3 showed the remineralizing potential of all the four toothpastes at different time points, i.e., 2nd, 4th, and 6th day. Toothpastes containing zinc hydroxyapatite (Group 3) showed the maximum amount of remineralization at day 2 (1491.57) itself, which gradually continued up till day 6 (1508.33) where complete remineralization of the tooth structure was seen. Toothpaste containing fluoride (Group 1) also showed a complete remineralization of the tooth structure, which was gradual from day 1 to 6 (1491.67). Herbal toothpaste (Group 2) and toothpaste containing calcium sucrose phosphate (Group 4) showed appreciable amount of remineralization at day 6, which was 1241.67 and 1033.33, respectively but no complete remineralization of the tooth structure was seen.

Figure 4 compared different time points with remineralization scores in four groups and it was pleasant to see that toothpaste containing zinc hydroxyapatite (Group 3) showed a linear graph from the point of demineralization at baseline to day 2 which stayed steady till day 6. It also showed a highest amount of remineralization than compared to all the other three groups.

Discussion

To survive, all higher forms of life are reliant on consumption of food that fuels one's life. Teeth are the tool used to ensure survival of life. Nature has fashioned the hard enamel covering to serve this function. It is a paradox that though enamel is the hardest biological substance, its acellular and avascular nature make it incompetent of any natural defense.[6]

Dental caries is a multifactorial disease affected not only by oral cavity environment but also influenced by community, family, and individual but most essential of all is by time.[7] Demineralization of teeth is mostly caused by exposure of tooth surface to the acids.

The tooth remineralization is a natural repair process in the oral cavity, supersaturation of ions such as calcium, phosphate, and fluoride around the tooth is important to restoring the strength and function of the tooth. These ions are deposited in the crystal voids of enamel making it more resistant to the carious process.[8–10]

Our study incorporated the use of human permanent premolar teeth. The primary reason for the same was that they are the most

Table 1: Pair-wise comparison of four groups with remineralization at different time points by Tukey's multiple post hoc procedures

Time points	*Group 1 vs Group 2*	*Group 1 vs Group 3*	*Group 1 vs Group 4*	*Group 2 vs Group 3*	*Group 2 vs Group 4*	*Group 3 vs Group 4*
Baseline	$p = 0.9873$	$p = 0.9984$	$p = 0.7984$	$p = 0.9592$	$p = 0.6026$	$p = 0.8777$
Day 2	$p = 0.0013$*	$p = 0.0018$*	$p = 0.0087$*	$p = 0.0002$*	$p = 0.9034$	$p = 0.0002$*
Day 4	$p = 0.0002$*	$p = 0.9954$	$p = 0.0002$*	$p = 0.0002$*	$p = 0.5729$	$p = 0.0002$*
Day 6	$p = 0.2773$	$p = 0.9994$	$p = 0.0091$*	$p = 0.2256$	$p = 0.4359$	$p = 0.0065$*

*statistically significant

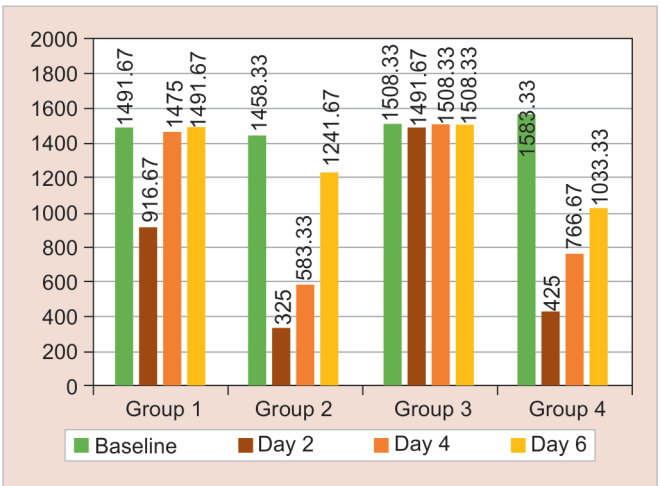

Fig. 3: Comparison of different time points with remineralization score in four groups

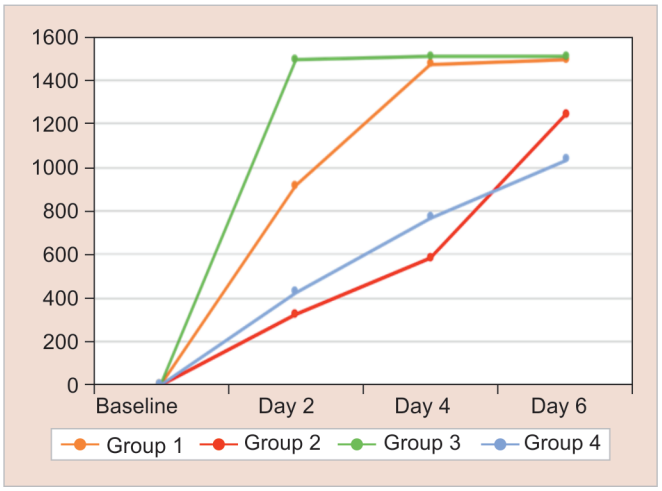

Fig. 4: Graph showing comparison of different time points with remineralization scores in four groups

common teeth to be extracted for orthodontic purpose. The physical and chemical composition of the teeth will vary between deciduous and permanent dentition and also between the types of teeth (incisors, canine, premolar, and molar). Hence, in order to avoid the microscopic variation, which may occur, in the present study, only permanent premolars have been selected.[11]

Cessation of dental caries has always been a forte of a dentist. In this study, 37% phosphoric acid was used for demineralization to mimic a white spot lesion. This study showed that toothpaste containing zinc hydroxyapatite had the highest amount of remineralization potential; this can be attributed to the fact that it contained zinc hydroxyapatite crystals in it. These crystals are thought to be deposited in the demineralized voids which on repeated deposition detailed in a sound tooth structure.[12]

An in vivo study was conducted where two pediatric dentifrices were compared to one regular dentifrice. This study hypothesized that the pediatric toothpaste containing sodium monofluorophosphate showed an appreciable amount to remineralization. Our study showed a similar results where toothpaste containing fluoride showed a meritorious result in terms of remineralization, which can be attributed to its content of sodium monofluorophosphate, which reacts with the hydroxyapatite crystals to form fluorapatite crystals which are more resistant to future demineralization.[13]

In our study, toothpaste containing calcium sucrose phosphate and herbal toothpaste did show a fair amount of remineralization but could not remineralize the tooth completely even at day 6 after repeated applications.

Stereomicroscopic analysis was carried out in our study, samples were randomly selected at baseline, after demineralization and from all the four groups. The sample at baseline showed the normal tooth structure without any depressions in enamel rods (Fig. 5). Once the demineralization process took place, the enamel rods showed concavities on the surface (Fig. 6).

Toothpaste was then applied on the demineralized tooth surface to check their remineralizing potential. In the first group, they were treated with fluoridated toothpaste which showed calcific deposits surrounding the enamel rods (Fig. 7). In the second group, the demineralized surface was treated with herbal toothpaste which showed very less amount of remineralization; moreover the enamel rods still had the depressions present (Fig. 8). In Group 3, the samples were treated with toothpaste containing zinc hydroxyapatite, the SEM analysis showed some astonishing results, complete remineralization of tooth surface was observed, and the enamel rods

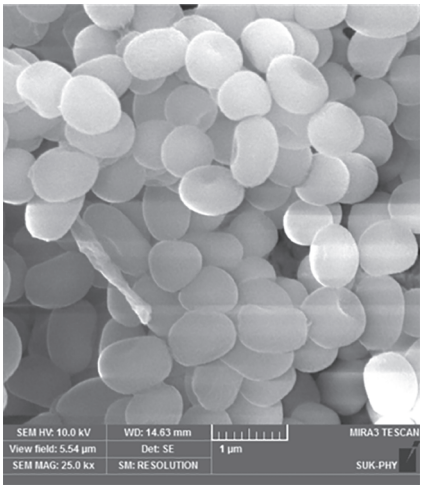

Fig. 5: Normal tooth anatomy as seen under SEM with 500 magnification

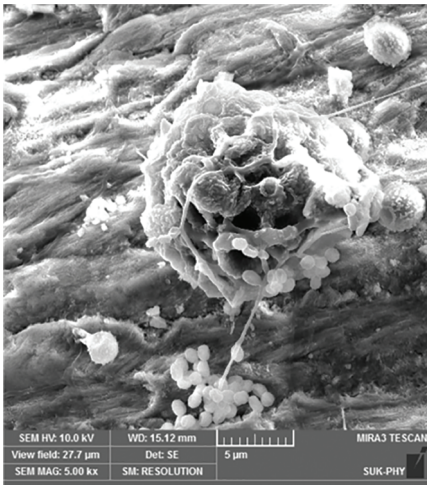

Fig. 7: Toothpaste containing fluoride showing remineralization of demineralized area under SEM with 500 magnification

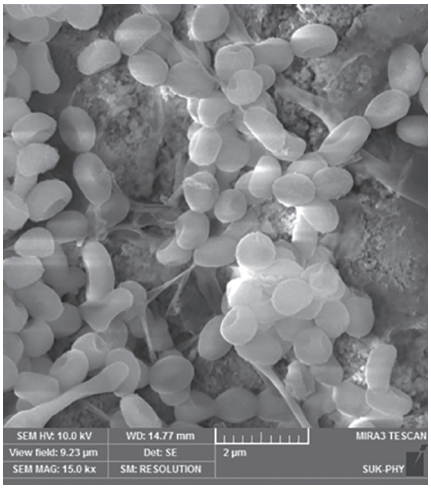

Fig. 6: Tooth anatomy after demineralization as seen under SEM with 500 magnification

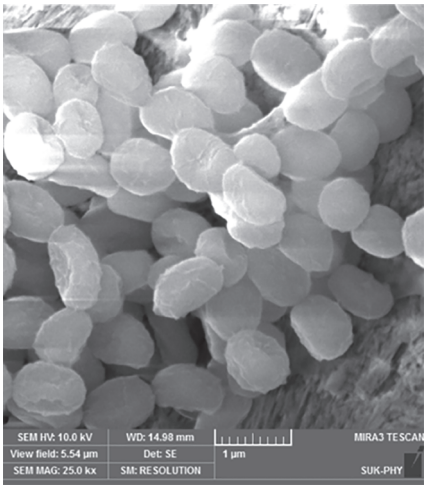

Fig. 8: Herbal toothpaste showing remineralization of demineralized area under SEM with 500 magnification

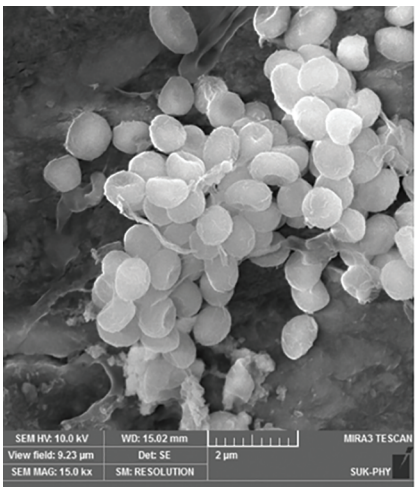

Fig. 9: Toothpaste containing zinc hydroxyapatite showing remineralization of demineralized area under SEM with 500 magnification

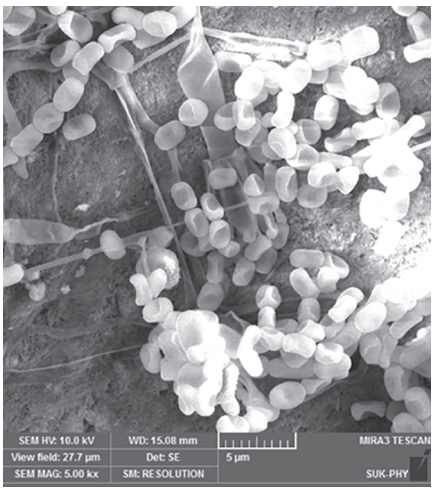

Fig. 10: Toothpaste containing calcium sucrose phosphate showing remineralization of demineralized area under SEM with 500

showed their normal anatomy (Fig. 9). The last group of samples was treated with toothpaste containing calcium sucrose phosphate, this group showed minimal amount of remineralization further more enamel rods appeared to be demineralized with significant number of concavity present in them (Fig. 10).

The remineralization is a steady process in the oral cavity. In the entire process of dental caries, the tooth is subjected to repeated cycles of demineralization and remineralization. These cycles are of uncertain duration and intensities. Clinically, remineralization is substantial when the enamel destruction can be stopped or reversed without the use of restorative techniques or material.[13]

The finding of our study has dental health implications. The patient and the parent should be educated toward effective preventive approaches such as maintenance of good oral hygiene via brushing twice a day and diet modifications. The lesions produced in the study are initial and in its first progression phase, which often is not detected in clinical examination. Some of the agents tested in this study did show ability to resist early lesion development. Prevention of such early lesions is considered as primary level of prevention because these lesions are not visible clinically or radiographically, hence are undiagnosed and can be effectively dealt with by just use of right remineralizing agent.[14,15]

This study can be a road map for the dentist to choose the toothpaste wisely for the betterment of the children and also to progress toward the goal of caries-free society. Our study showed that toothpaste containing zinc hydroxyapatite had better remineralization potential compared to the other toothpaste used in our study. The limitation of our study that it is an in vitro study so an in vivo study for longer duration shall be carried out. Various measures have been taken to conduct a clinical trial among children in this regard.

Conclusion

The conclusions drawn from the present study are as follows:

- All the four toothpaste showed a remineralizing potential
- The toothpaste containing zinc hydroxyapatite showed a superior remineralizing prospect compared to the toothpaste containing fluoride, toothpaste containing calcium sucrose phosphate, and herbal toothpaste; it could be attributed to the zinc hydroxyapatite content in it.

Clinical Significance

The clinical significance of our study is to give clear and evidence-based idea to the dentist and the parents about the remineralizing toothpaste available in the market for children. White spot lesion, which is an early sign of demineralization and an initial carious lesion, is very common among children. Treating them at the initial stage would help to remineralize the tooth structure and thereby limit further destruction of the tooth structure, which will preserve the beautiful smile of the child. It will also help the child and the parent to overcome physical, social, and economic barrier. A good oral health is a reflection of a overall good general health.

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