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Comparative Evaluation of The Remineralizing Potential of Three Commercially Available Dentifrices On Artificial Carious Lesions: In-Vitro Study

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ABSTRACT

The study aims to evaluate and compare the remineralizing potential of three commercially available dentifrices with varying fluoride concentrations on artificial carious lesions, utilizing Vicker's Microhardness Number (VHN) as a primary measurement tool. Inclusion criteria for the study required the use of extracted premolars with sound, intact crowns, while exclusion criteria eliminated teeth with developmental defects, existing caries, restorations, fractures, or those previously subjected to pulp therapy. A total of 21 extracted premolar teeth were sourced from the Department of Oral and Maxillofacial Surgery at Dr. D Y Patil Vidyapeeth's Dental College, Pune. The dentifrices tested included Dente91® Cool Mint Toothpaste containing Nano-Hydroxyapatite and xylitol, Jordan* Junior Toothpaste (6-12 years) with sodium fluoride, and Colgate® Total Advanced Health Anticavity Toothpaste with 0.24% sodium fluoride and triclosan. Microhardness of teeth samples treated with Dente91 Cool Mint, Jordan Junior TMMC (1450 ppm), and Colgate Total Advanced Anticavity Toothpaste (n=14 each) was measured using a Reichert tester. Baseline values were 324 HV (Group 1), 328 HV (Group 2), and 330 HV (Group 3). After 96 hours of demineralization, values dropped to 261 HV (Group 1), 277 HV (Group 2), and 258 HV (Group 3). Group 2 showed the highest resistance to demineralization, while Group 3 had the lowest hardness.

Keywords: *Dental Caries, Fluoride, Remineralization*

1. INTRODUCTION

Dental caries, though one of the most common chronic diseases of the mankind, is preventable. Prevention of caries has been given a great emphasis by introduction of several strategies. Yet, developing countries like India, have a dental caries prevalence rate of 31.5% to 89%[1-3]. Continuous oral microbial exposure leads to the early development of caries in children[4]. Toothbrushing daily is a gold standard for reversing early enamel caries, and toothpastes containing remineralizing and biofilm reducing materials are widely accepted and economical.

For 25 years, use of fluoride has led to a great decline in dental caries, in the developed countries[5].Yet, fluoride's remineralization impact is of utmost significance since it stops the early caries process and creates an enamel surface that is more resistant to decay. Rather than focusing on remineralization it is important to regain the lost ions for restoring the normal structure. Also, even though the effectiveness of fluoride is dose-dependent and rises with dose, there is a maximum

amount of fluoride that can be administered without increasing the risk of fluorosis in children[6].To avoid all this there is need of new-age strategies which are effective against caries. Nanohydroxyapatite (HAP; $Ca_5(PO_4)_3(OH)$) is a highly biomimetic material recently introduced in dental care, providing calcium and phosphate for enamel remineralization. Studies have shown that HAP combined with fluoride toothpaste (>1000 ppm) can effectively remineralize teeth and prevent caries. This study compares the remineralizing potential of three commercially available dentifrices with varying fluoride concentrations on artificial carious lesions using a Vickers microhardness tester. The null hypothesis states there is no difference in their remineralizing effects.

2. MATERIALS AND METHODS

This was a comparative in-vitro study conducted in the Department of Pediatric and Preventive Dentistry, Dr. D. Y. Patil Dental College and Hospital, Pimpri, Pune. Ethical approval and waiver of consent were obtained for using extracted premolars.

Inclusion & Exclusion Criteria

- **Inclusion:** Sound, intact extracted premolars
- **Exclusion:** Teeth with caries, restorations, fractures, developmental defects, or prior pulp therapy

Materials Used

- **Teeth:** 21 extracted premolars collected from the Oral Surgery Department.
- **Toothpastes:**
    - Group 1: Dente91® Cool Mint (Nano-Hydroxyapatite + Xylitol)
    - Group 2: Jordan® Junior Toothpaste (Sodium Fluoride)
    - Group 3: Colgate® Total (Sodium Fluoride 0.24%, Triclosan)

Other Materials: Artificial saliva, 10% formalin, diamond disc, demineralizing & remineralizing solutions, Vicker's Mic), thermocycling unit (Figure 2: Thermocycling machine), and cold-cure acrylic.

Figure 1: Vicker's Microhardness Assessment Machine

Figure 2: Thermocycling Machine

Sample Preparation

- Teeth were cleaned, stored in formalin, and sectioned bucco-lingually to create 42 halves.
- Each section was embedded in acrylic resin and a 4×4 mm test window was created.
- Samples were thermocycled and baseline microhardness was measured using Vickers Hardness Number (VHN).

Demineralization Process

- Samples were immersed in demineralizing solution (10 ml) for 96 hours to simulate early caries lesions.
- Surface microhardness was measured post-demineralization.

Remineralization Protocol

- Samples were randomly assigned to 3 groups (n=14) and subjected to 10-day pH cycling using Tencate's method:
    - Alternating treatments with dentifrice slurries and de/re-mineralizing solutions.
    - Fresh solutions were prepared daily with pH maintained at 4.4 (demin) and 7.0 (remin).

Assessment

- Vickers microhardness was measured at three stages: baseline, post-demineralization, and post-remineralization. All assessments were performed by a blinded examiner.

Statistical Analysis: Statistical analysis included One-Way ANOVA, Post-hoc Tukey, and paired t-tests to compare mean microhardness values across three groups at baseline, post-demineralization, and post-remineralization, with significance set at $p<0.05$.

All groups showed a significant decrease in microhardness after demineralization and an increase after remineralization. No significant differences were found between groups at baseline. Post-remineralization, Group 2 (Jordan Junior TMMC Toothpaste, 1450 ppm) showed the highest microhardness, followed by Group 3 (Colgate Total Advanced Health, 1100 ppm), and Group 1 (Dente91 Cool Mint) showed the least.

Intergroup comparison revealed no significant difference between Groups 1 and 3 post-remineralization. Jordan and Colgate toothpastes had similar remineralizing potential, with Jordan being the most effective overall.

3. RESULTS

The microhardness of teeth samples of three groups treated with Dente91 Cool mint Toothpaste, Jordan Junior TMMC Toothpaste (1450 ppm) and Colgate Total advanced Health Anticavity Toothpaste (n=14 each group) were assessed using microhardness Tester, Reichert Austria Make, Sr.No.363798 with 100g load for baseline values. Collected baseline showed the baseline surface microhardness (in Vickers Hardness, HV) of untreated teeth samples across three groups, each with 14 samples. The mean microhardness values were **324 HV** for Group 1, **328 HV** for Group 2, and **330 HV** for Group 3. Overall, the microhardness readings were relatively consistent across all groups, with Group 3 showing the highest average value. After 96 hours of demineralization, the mean surface microhardness values decreased to 261 HV (Group 1), 277 HV (Group 2), and 258 HV (Group 3). Group 2 showed the highest resistance to demineralization, while Group 3 had the lowest post-demineralization hardness. Finally, all the samples were exposed to 10 days PH cycle involving application of dentifrice using toothbrush to simulate normal tooth brushing onto teeth. The samples were evaluated for remineralization (Table 1). All testing was carried out at Praj Metallurgical Laboratory, Pune (Maharashtra). The comparison between remineralizing values amongst 3 groups were carried out.

Table No. 1: Surface microhardness of teeth after remineralization

Group	Mean Microhardness (HV)
Group 1	289
Group 2	300
Group 3	297

After remineralization, Group 2 showed the highest recovery in microhardness (300 HV), followed by Group 3 (297 HV) and Group 1 (289 HV), indicating effective remineralization across all groups.

4. DISCUSSION

Dental caries is a multifactorial, irreversible disease leading to cavitation of the tooth. Though it is irreversible, it can be reversed in the initial stage when there is subsurface demineralization with intact hard tissue matrix.[1-3] Remineralization is a natural process that regains minerals in ionic forms in the hydroxyapatite (HAP) crystal lattice to repair the damage. As calcium and phosphate mineral ions from saliva and plaque fluid are redeposited within the caries lesion under favorable pH conditions, newly formed HAP crystals are produced that are larger and more resistant to acid breakdown.[7]

Early carious lesions can be reversed if treated before cavitation. Remineralizing agents, especially fluoride toothpastes, are popular, cost-effective, and commonly used by both adults and children. According to the 2019 Bangkok Declaration, children should brush twice daily with age-appropriate toothpaste containing 1000 ppm fluoride, without reducing its anti-cavity effect[8]. Fluoride's role in preventing caries by promoting remineralization of enamel was well documented in the 1980s. When sugar lowers oral pH, fluoride replaces lost calcium and phosphate ions, forming acid-resistant fluorapatite crystals (Fejerskov, 2015). Both children and adults benefit from topical fluoride, which helps protect teeth by strengthening enamel, as shown by Fazzi et al. (1997). [9]According to Walsh t et.al 2010[6] advocated the use of toothpaste in the higher concentration and increased frequency in containing the lesions in patient with higher caries index. This statement justifies our study about comparing and evaluating fluoride toothpastes with higher concentration. With the formulation in the use of fluoride, researches were conducted to introduce newer biomimetic materials which has capability to create apatite crystals. Nano-hydroxyapatite is a newly introduced ingredient in toothpaste that, in addition to fluoride, may be used to restore demineralized surfaces.

Nanoscale HAP resembles the apatite crystal found in tooth enamel which adheres appropriately in the vacancy created by HA crystals in demineralized enamel. Tencate studied that the demineralization can be reversed by deposition of nanohydroxyapatite particles. According to Narayana SS[7], these crystals are adsorbed on the superficial surface to form highly mineralized layer which reduces further demineralization by inhibiting the diffusion of acids into the deeper layers of enamel. Thus, application of nano Hain both subsurface and surface carious lesions. Unlike n-HAP which fills the last HA block in toto, fluoride ions replace the hydroxyl ion of the hydroxyapatite structure (Roveri N et.al 2009)[5].SH Huang et al (2009)[11] stated that nanohydroxyapatite of a proper concentration of about 10% or 15% can bring about remineralization if used daily. Vyavhare S (2016)[12] compared remineralizing potential of Nano-hydroxyapatite pastes with CPP-ACP and sodium fluoride (1000ppm) pastes using SEM analysis. Results conveyed that n-HA paste showed surface as well as subsurface remineralization and hence can replace fluoride. In our study we have compared toothpastes containing varying amount of fluoride with concentration above 1000 ppm vs Nano-Hydroxyapatite toothpastes which indicated for caries treatment for the same.

The artificial caries lesions were prepared by exposing the target site of tooth to differential pH for particular period tenth repetitive in a standardized protocol of pH cycling suggested by Ten Cate (1988)[13]. The samples were exposed to demineralization for 96 hours in accordance with Ten Cate (1988) and Duijsters. Similar protocol for exposure to demineralization and pH cycling model was used study conducted by Adwani S. et.al 2020[14].Vicker's microhardness tester were used in study as it is most commonly used in assessment of microhardness of different materials by various authors (Wang L 2003)[15]. The Vickers indenter is preferred in tooth hardness studies because it maintains a consistent square indent near the enamel-dentin junction (EDJ), minimizing measurement errors common with other testers. Its simpler, precise indent shape makes it ideal for assessing remineralization, as used in the study by C. Joshi (2019)[16]. Microhardness testing is important for materials with fine, nonhomogeneous, or crack-prone structures like enamel. After remineralization, all groups showed increased microhardness, with Group 2 highest (300 HV), followed by Groups 3 (297 HV) and 1 (289 HV). Group 1 (hydroxyapatite toothpaste) also showed positive results, indicating all tested pastes can effectively remineralize incipient caries. Further research on subsurface remineralization with n-HAP is needed. The growing use of dentifrices has created a competitive and profitable global market[17].

Most of the study has focused on dentifrices produced in affluent countries, but less attention has been paid to the content and effectiveness of dentifrices produced in developing nations. In contrast to the oral cavity, which is a dynamic, complex living eco-system, remineralization occurs in vitro. However, as mentioned by White[18], in vitro designs are mechanically constrained in three important aspects.

1. Poor depiction of intricate and varied intraoral circumstances
2. There was trouble mimicking saliva volume, content, and tooth surface area.
3. Limited sample size
4. Evaluation utilizing a qualitative test
5. Any placebo or control group

5. CONCLUSION

Remineralizing agents have been proven to be a blessing for early management of caries. Fluoride is the best material to provide remineralizing effects. We discovered that Jordan* Junior TMMC toothpaste is appropriate for children between the ages of 6 and 12. For the purpose of mineral gain, sodium fluoride toothpaste is superior. As determined by Vicker's microhardness tester, all the pastes demonstrated equivalent efficacy in reducing surface roughness. To evaluate the efficacy of nanohydroxyapatite toothpaste and to better understand how remineralization is dose- and time-dependent, more studies must be conducted over a longer period.

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