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Effect of Three Different Dentifrices on Enamel by Automated Brushing Simulator- *In vitro* Profilometric Study

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Authors' contributions

This work was carried out in collaboration between both authors. Author TK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SR managed the analyses of the study, managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Bacterial plaque control is critical in maintenance of oral health because dental plaque is the primary etiological factor for both caries and periodontal disease. Toothbrush and dentifrices play an integral part in accomplishing plaque removal. The aim of the study was to assess the enamel surface abrasion caused by three different dentifrices using automated brushing simulator and profilometer. A total of 24 samples (N=24) which are extracted for orthodontic purposes were divided into three groups based on the dentifrices used. They are Group 1-Colgate Swarnavedsakthi (n=8), Group 2-Dabur Herbal(n=8),Group 3-Ayush(n=8). Samples were subjected to take pre profilometric readings and brushing was done by an automated brushing simulator. A Laser 3D profilometer was used to detect the wear in the enamel surface. Pre and Post profilometric readings were compared. Statistically significant differences ($p<0.05$) were observed in the values of enamel abrasion among the Group 1(Colgate

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Swarnavedsakthi) and Group 3(Ayush). After analysing the profilometric values, significant differences were found among the Ayush group while comparing with other groups such as Colgate Swarnavedsakthi and Dabur herbal. This indicates the higher enamel surface abrasion in the ayush group.

Keywords: Enamel abrasion; dentifrices; brushing simulator; profilometer; abrasives.

1. INTRODUCTION

As dental plaque is the primary etiological factor in the initiation and development of both dental caries and periodontal disease, effective plaque control is critical in the maintenance of oral health [1]. The combination of the toothbrush and dentifrices has been a greater cleansing tool in plaque removal as necessary part of disease control [2]. And they aid in mechanical plaque control because of its positive chemical effects and delivery of various therapeutic agents. The ideal dentifrice should provide the greatest possible cleaning action on tooth surfaces with the lowest possible abrasion rates on the tooth surfaces [3,4].

Dentifrices (toothpaste and tooth powders) are complex formulations, and it is necessary to achieve a fine balance to provide cosmetic and oral health benefits, while limiting chemical and/or physical damage to teeth [5,6]. Toothpowder is the most common form of oral hygiene practice in semi urban and rural areas of India for economic reasons as well as due to misconception that these indigenous herbal products may be beneficial for dental and gingival health [7,8]. Abrasives are the insoluble components added to dentifrices to aid the physical removal of stains, plaque, and food debris. The most commonly used abrasives are silica and calcium carbonate. A high quality dentifrice contains silica, but its use increases the cost and hence low quality calcium carbonate, iron oxide, etc., are used to bring down the cost [9,10].

Toothpowders, in general, are known to be 5 times more abrasive than toothpastes due to the quantity of abrasives used (95%) and their particle size. Hence, concern has been expressed about its detrimental effects on tooth substance which pose an important oral health problem. The chemical composition of most of the tooth powders is not known, but they may contain chemicals of low pH, which could cause softening of the dental hard tissues [11,12].

Tooth wear is a complex process which is dependent on the interaction between the wearing agent and the sinuous surface of teeth. Tooth wear is classified as erosion (due to acids), abrasion (due to external mechanical factors, such as toothbrushing) and attrition (due to tooth to tooth contact) but tooth wear is rarely attributed to a single cause as the concomitant effects of erosion from dietary acids and tooth brush abrasion results in worse wear lesions [13,14]. Erosion is not only a surface but also a sub-surface process, penetrating up to 5μm into the sub-surface of the enamel and this sub-surface layer can be removed easily by further tooth brush abrasion. This effect can be made worse depending on the duration of the erosive challenge as the depth of the sub-surface effect is greater with longer acidic exposures [15,16]. Toothbrush abrasion is classified in two types: two-body abrasion, between the bristles of the toothbrush and the teeth, and three-body abrasion where the toothpaste slurry containing abrasive particles and loose enamel or dentine chips, acts as the third body of wear. The increasing prevalence of tooth wear at all age groups highlights the importance of prevention to avoid the long detrimental effects of wear and difficulties in restoring worn dentitions.

Buccal surfaces of teeth are more prone to abrasion due to adverse brushing. Abrasion is most commonly associated with toothbrushing on the cervical margins of teeth. An upper limit of 250 for relative dentin abrasivity (RDA) or 40 for relative enamel abrasivity for a toothpaste is considered safe for everyday use in adults' International Organization for Standardization (ISO) [17,18]. To evaluate toothpaste abrasivity, many different techniques have been used, for example, the RDA method, weight, and volume loss techniques which are quantitative techniques, measuring the amount of abraded material removed, as well as profilometer and light reflection techniques, which are qualitative techniques measuring the roughness of the abraded material. The aim of this study was to evaluate the enamel surface abrasion using four different dentifrices and a customized automated brushing machine under a profilometer.

2. MATERIALS AND METHODS

2.1 Infection Control Protocol

Immediately after extraction, the soft tissue attached to the tooth surface was carefully removed with wet cotton. Occupational safety and health administration (OSHA) and the Centers for Disease Control and Prevention (CDC) recommendations and guidelines were followed. After collection, the samples were transferred to 100 ml of 5.25% sodium hypochlorite solution (Prime Dental Products Pvt. Ltd, Thane, India) stored in an amber-colored bottle. The solution was discarded after 30 min, and the teeth were transferred into separate jars containing artificial saliva (Wet Mouth, ICPA Health Products Ltd) to simulate the oral environment. The samples were removed with cotton pliers and rinsed in tap water. The samples were dried by placing them over paper towels and blotted for a few minutes before using them for study.

2.2 Study Criteria

Natural teeth which were extracted for orthodontic purposes are included in this study. Teeth extracted due to caries, periodontal problems were excluded from this study.

Groups

A total of 24 samples were selected for this study (N=24). The specimens were allotted to three groups based on the dentifrices used and they are,

Group 1- Colgate Swarnavedsakthi (n=8)
Group 2- Dabur Herbal (n=8)
Group 3- Ayush (n=8)

2.3 Specimen Collection and Preparation

A total of 24 samples (N=24) which are extracted for orthodontic purposes and were divided into three groups based on the dentifrices used. After infection control of natural teeth, each one was poured in rubber mould with dental stone. The mould is round in shape and it is checked to fit in both brushing simulator and profilometer. Dental stone is preferred for pouring the mould as it sustains prolonged duration of forces by brushing simulator. The specimens were subjected to take pre profilometric readings. They were noted in two and three dimensional views and noted.

2.4 Brushing Simulator

The toothbrushing station (DentTest, Germany) was developed for the simulation of the tooth cleaning process using both power and manual toothbrushes. The tooth brushing machine included eight holders for toothbrushes. Each toothbrush worked on up to three specimens. The specimens were mounted with standardized key lock fixations. The tooth brushes with soft bristles were used in brushing simulator. The bristles of the toothbrush were aligned without pressure contacting the specimen surface in perpendicular fashion [19].

Fig. 1. Specimen Mounted in Dental Stone

Table 1. Ingredients of Dentifrices

Dentifrices	Ingredients list
Dabur Herbal	Calcium carbonate, Water, Sorbitol, Glycerine, Silica, Sodium lauryl sulphate, Extracts of Babool, Lodhra, Jamun, flavor(tulsi oil),Xanthan gum, Sodium saccharin, Sodium benzoate, Formaldehyde
Colgate Swarnavedsakti	Calcium carbonate, Sodium lauryl sulphate, Silica, Zinc oxide, Carrageenan, Sodium monofluorophosphate, Zinc citrate trihydrate, Benzyl alcohol, Clove oil, Neem oil, Mint oil, Cinnamon leaf oil, Amla extract oil, Basil oil, Fennel oil, Honey extract, Eugenol
Ayush	Lavang, Choornam, Kadhira, Pippali, Daruharida, Jatipatri, Cinnamon, Roseoil, Maricha, Kankola, Jatiphal, Benzyl alcohol, silica, zinc oxide

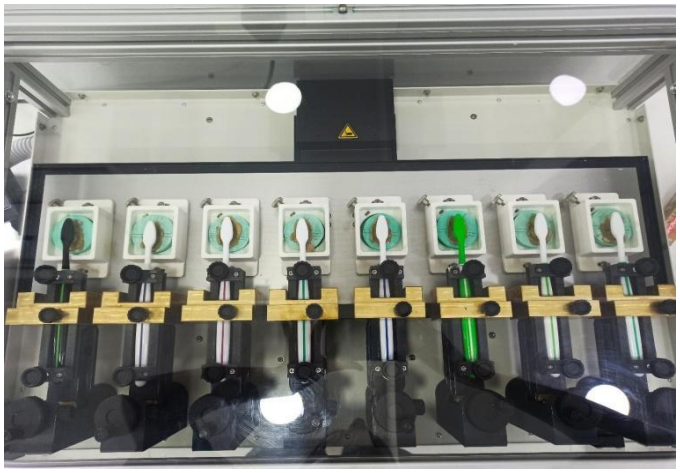


Fig. 2. Specimens in Brushing Simulator

A linear cleaning movement of 3 cm length and zig zag movement was selected for the experiments with power and manual toothbrushes. The movement length was sufficient to cover the specimens surfaces. A force of 2 N was chosen for brushing.

Specimens were randomly allocated to three groups. 8 specimens were assigned to each toothbrush. The total brushing strokes were calculated to be equivalent to 10 years of brushing, based on a brushing time of 160 seconds twice-daily of all teeth. Based on this estimation, the maximum contact time for one tooth surface per day is 8 seconds. The total brushing time was calculated to be 320 min. The brush head should be replaced after 45 days (a typical time period to replace the brush). This represents 270 minutes of cumulative use for 24 teeth with 8 s brushing per day. The movement of the power toothbrushes differs from brushing with a manual toothbrush. With oscillating-rotating technology, the brush head oscillates from a center point but does not rotate in a full circle. Considering these differences in brushing movement, each sample was submitted to 42,200 brushing strokes at a rate of 150 strokes per minute for manual toothbrushes. Brushing movements were executed with the slurry applied to the surface of the specimens. The flow rate of the slurry was set at 10 ml/minute. Specimens were rinsed with tap water for 30 seconds and received new slurry automatically every 2 minutes. After the final cleaning run all samples were stored in saline to avoid sample disintegration due to dehydration.

2.5 Profilometric Analysis

A noncontact type optical three-dimensional (3D) profilometer (R Tech Universal 3D profilometer, Nipkow confocal technologies, Japan) was used for taking profilometric readout for each group subjected to brushing and also for the control group. Before each measurement, the sample's surface was covered with distilled water for 30 s. Excess of water was blotted with absorbent tissue without touching the specimen surface and checked for any remnants macroscopically.

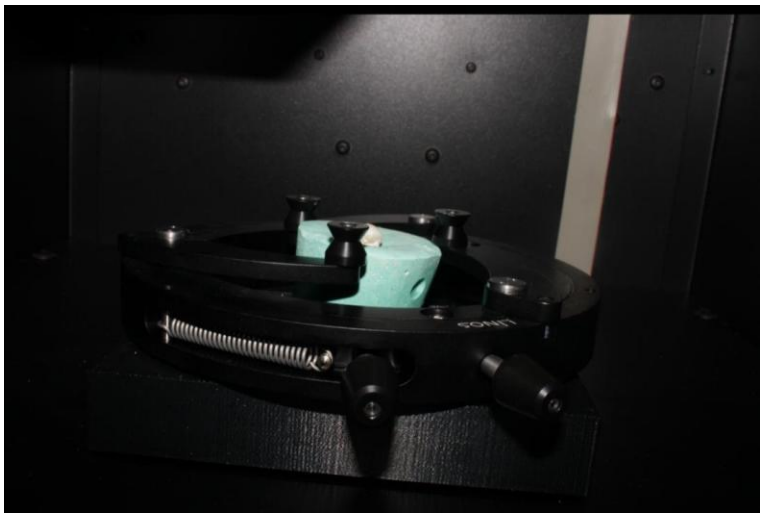


Fig. 3. Specimen in Profilometer

3. RESULTS AND DISCUSSION

3.1 Statistical Analysis

Statistical analysis was performed with the help of Epi Info (TM) 3.5.3. (Centre for Disease Control and Prevention, Kolkata, West Bengal, India) Epi Info is a trademark of the CDC. Descriptive statistical analyses were performed to calculate the means with corresponding standard deviations.

One-way analysis of variance (ANOVA) was done to compare the mean values across the five groups for numerical data (using the F distribution) followed by post hoc Tukey's test which was performed with the help of critical difference or least significant difference at 5% and 1% levels of significance to compare the mean values.

Abrasivity should be sufficient to remove surface deposits including dental plaque, but it should not damage enamel. Typically, this requires that particle size and shape of abrasive agents should be in a desirable range (i.e., 1–20 μm or 5–15 μm) and should not be sharp or angular [19,20]. Crude red ochre, which typically contains clay minerals and/or other impurities in addition to the red iron oxide, may not be suitable for the purpose [21,22].

The samples were also tested by adding them in artificial saliva to simulate the oral conditions during brushing, as saliva contains a buffer to resist changes in pH and also provides a constant supply of ions to the tooth surface. It also favors lubrication, hence, it may also control the abrasive wear to some extent. Similarly, many factors favours caries progression and the endodontic and conservative procedures in treating carious lesions [23–25,26–41].

Table 2. Showing profilometric analysis of surface roughness of Colgate swarnavedsakthi group

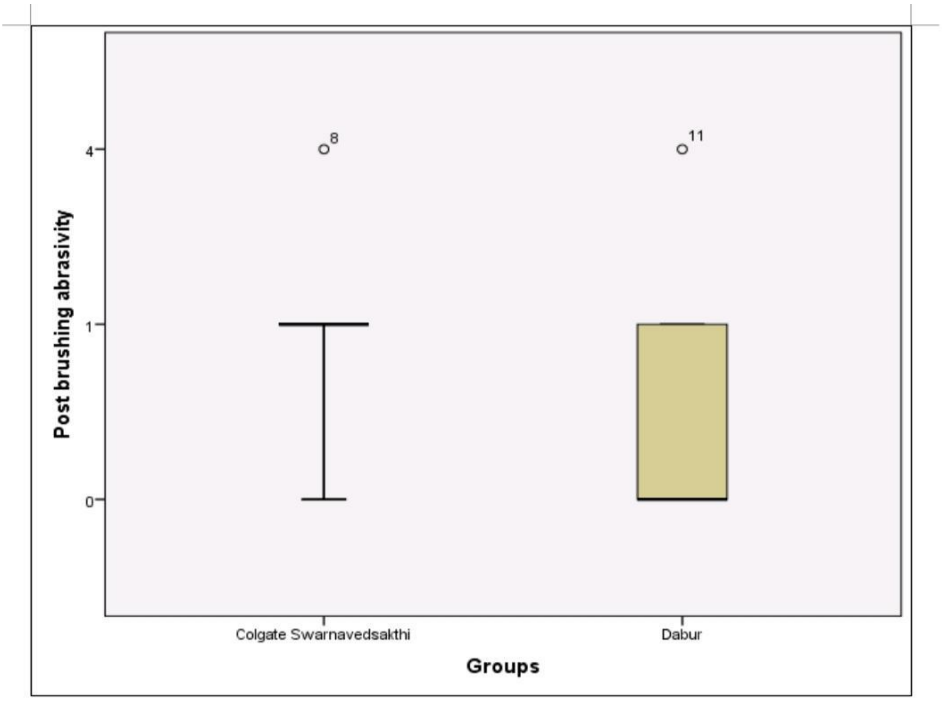
Group A(Colgate Swarnavedsakthi)	Pre brushing values (mm)	Post brushing values(mm)	Range
Group A-1	0.78	1.41	0.83
Group A-2	1.52	2.85	1.33
Group A-3	1.90	2.35	0.45
Group A-4	1.71	2.03	0.37
Group A-5	1.40	2.75	1.35
Group A-6	0.97	2.19	1.22
Group A-7	1.58	2.36	0.78
Group A-8	1.30	5.07	3.77

Table 3. Showing profilometric analysis of surface roughness of Dabur herbal group

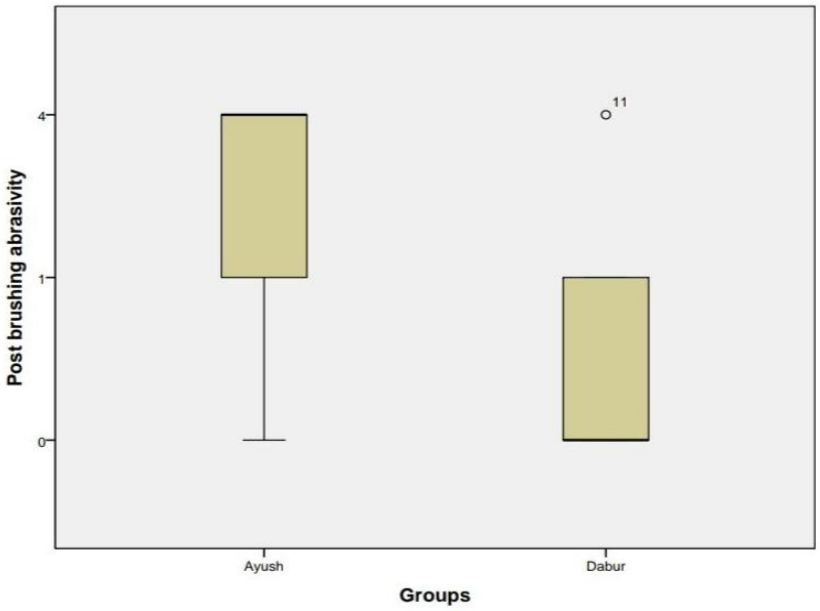
Group B (Dabur Herbal)	Pre brushing Values	Postbrushing Values	Range
Group B -1	1.37	1.79	0.42
Group B -2	1.46	1.65	0.19
Group B -3	1.33	5.01	3.68
Group B -4	1.58	2.14	0.56
Group B -5	1.99	1.67	-0.32
Group B -6	5.02	5.11	0.09
Group B -7	1.18	1.52	0.34
Group B -8	1.78	2.05	0.27

Table 4. Showing profilometric analysis of surface roughness of Ayush group

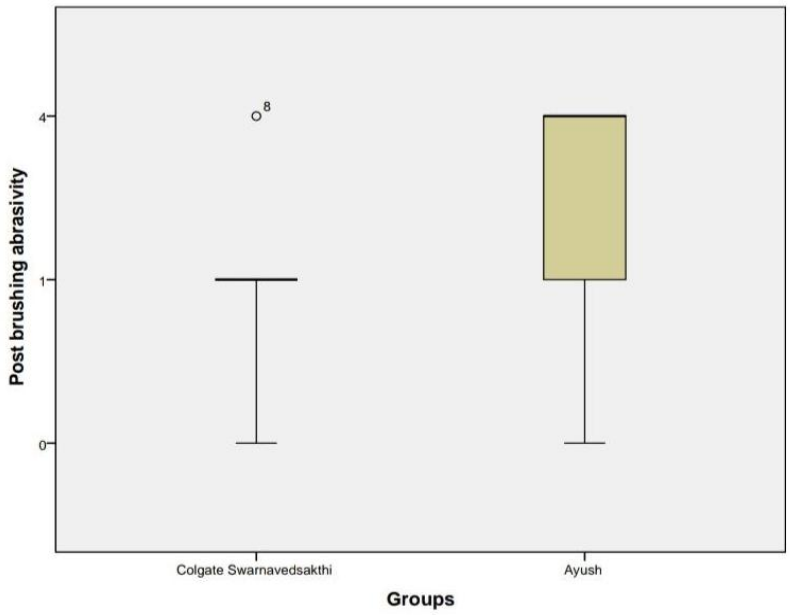
Group C(Ayush)	Pre Brushing Values	Post Brushing Values	Range
Group C-1	1.47	5.17	3.71
Group C-2	1.52	2.23	0.71
Group C-3	1.58	2.06	0.48
Group C-4	1.26	5.05	3.79
Group C-5	1.41	5.19	3.78
Group C-6	1.54	5.42	3.88
Group C-7	0.90	1.69	0.81
Group C-8	1.78	2.85	1.31



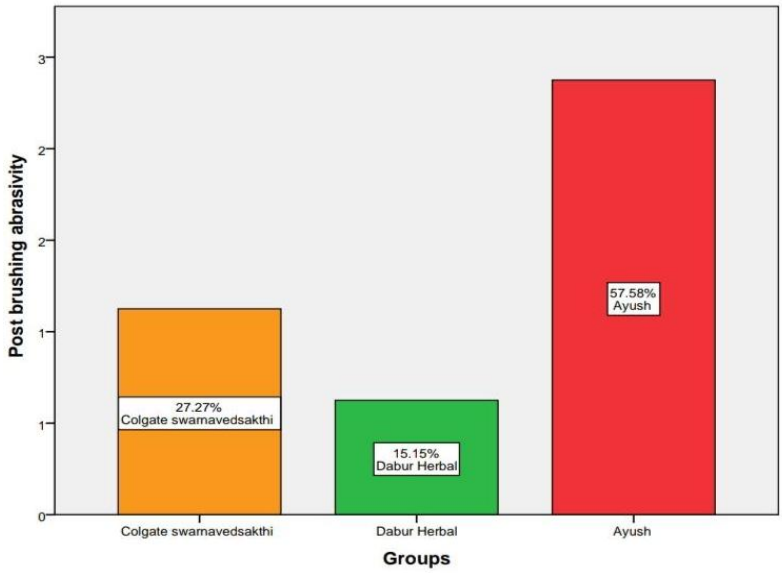
Graph 1. Box plot showing the association between Colgate swarnavedsakthi and Dabur dentifrices and Post brushing abrasivity values, the box plot of Colgate swarnavedsakthi shows the mean values are at different ranges(0.02-2.1) whereas the box plot of Dabur shows the mean values are at near ranges.(0.01-1.01)



Graph 2. Box plot showing the association between Ayush and Dabur dentifrices and Post brushing abrasivity values, the box plot of Ayush(0.03-3.92) shows the mean values are at different ranges whereas the box plot of Dabur shows the mean values are at near ranges.(0.01-1.01)



Graph 3. Box plot showing the association between Colgate swarnavedsakthi and Ayush dentifrices and Post brushing abrasivity values, the box plot of Ayush shows the mean values are at different ranges(0.03-3.92) whereas the box plot of Colgate swarnavedsakthi shows the mean values are at near ranges.(0.04-1.00)



Graph 4. Bar chart showing comparison of abrasivity between the three groups, X axis represents the experimental groups and Y axis represents the abrasivity of groups post brushing. After analysing the pre and post readings of the profilometer, significant differences in values were found among the Ayush group (red colour) while comparing with other groups such as Colgate swarnavedsakthi and Dabur herbal. There is a significant difference among the groups in Pearson chi square statistical test.(p value-0.02<0.05)

The accuracy of the scanner was measured by repeated scanning of a calibrated gauge of known dimensions and was found to be accurate to 3.1µm. Therefore, samples were scanned by oversampling with a step-over distance of 50µm in order to increase the accuracy of the scan. The chosen stylus would have affected the accuracy of horizontal measurements to a greater degree than vertical measurements so this effect would not affect our results greatly [42,43]. The brushing protocols simulated accumulated wear over 1 year and a force of 2N was chosen as it simulates normal toothbrushing habits and has previously been used by other authors [44].

Profilometry has offered an opportunity to clinical academics to characterize dental disease and its progression to attempt correlation with social, pathological environmental and ultimately, genetic factors. Accurate quantification of changes in dental tissues may guide diagnosis and aid in treatment planning to dental phenomics will have a larger role in clinical practice [45,46].

Techniques such as quantitative light fluorescence are playing a larger role in accurate quantification of the carious process whereas two-dimensional and three-dimensional imaging can shed more information in craniofacial development or pathological processes such as tooth wear or phenotyping of genetic disorders such as hypodontia. Contacting and non-contacting profilometry has been widely used to measure tooth wear *in vitro*, in situ and *in vivo* with the use of surface metrology or surface matching software [47,48].

Conventionally, enamel samples are polished flat and a protected section of the sample serves as a reference area to measure wear. This two-dimensional method gives rise to bias as profile selection to measure wear is reliant on the operator. Further to this, interpretations of wear from a whole sample are made from a finite number of profiles, which disregard wear from the rest of the sample. Finally, the polishing procedure removes surface irregularities and the aprismatic layer of enamel which has been shown to offer resistance to the wear episodes [49,50], so wear measurements may be overestimated as the protective effect of this layer cannot be assessed. Measurements of wear using native enamel may be desirable for a better understanding of the complex interactions between the wearing agents and the tooth surface. To this effect, surface metrology

software allows automatic superimposition and measurements of wear of samples, thus removing operator bias and giving better indication of the complex wear process over a sample with an intact surface [51].

The three-dimensional method described on this article using surface matching software with external datum (ball bearings) allowed measurements of wear over the whole surface and this technique can be used to measure wear from areas of interest such as cervical wear or can be used to measure the effect of the aprismatic layer on resistance to wear. Furthermore, our method removes operator bias as wear is measured from whole samples rather than individual profiles.

The difference in wear between enamel and dentine may be attributed to the difference in abrasivity in the dentifrices and the structural differences of enamel versus dentine. Although this was not investigated in this study, it is hypothesised that the silica particles in Ayush may have caused dislodgement of eroded dentine and these particles could have acted as pumice, thus worsening wear. Enamel is a harder substrate compared to dentine so it is more resistant to dislodgement of particles by the abrasive agents in CT. Previous research has shown that dentifrices with medium and high relative enamel abrasion values wear enamel to similar levels. Dentine is a softer substrate which is more susceptible than enamel to erosive/abrasive wear and dentine loss appears to correlate with increased toothpaste abrasivity [52].

The particle size and the type of abrasive in each of the dentifrices might have caused the difference in the results among the experimental groups. All dentifrices selected were aqueous based to keep the vehicle constant to avoid any discrepancy in the result obtained. Among the constituents mentioned by the manufacturer, silica particles present in Ayush might have resulted in the highest enamel abrasion which is statistically significan t($P < 0.05$), followed by Colgate Vedshakti and Dabur.

4. CONCLUSION

After analysing the pre and post readings of the profilometer, significant differences in values were found among the Ayush toothpaste while comparing with other groups such as Colgate Swarnavedsakthi and Dabur herbal as abrasive content is higher in Ayush toothpaste. This indicates the higher enamel surface abrasion in the ayush toothpaste.

5. CLINICAL SIGNIFICANCE

Abrasivity in dentifrices should be sufficient to remove the surface deposits including dental plaque, but it should not damage the enamel. Thus, the choice of dentifrices should be made with utmost care as it influences oral health.

6. LIMITATIONS

Natural teeth obtained from different age groups might give differences in abrasion results while using different dentifrices and this study involved smaller sample sizes.

7. FUTURE SCOPE

A larger sample size could be taken into consideration in future studies and preferably natural teeth of the same age group can be considered for experimentation.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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