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## RESEARCH ARTICLE

### PREVENTIVE EFFECT OF TWO TOOTHPASTES ON ENAMEL EROSION PRODUCED BY A SOFT DRINK: A CONFOCAL MICROSCOPY INVITRO STUDY

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#### ABSTRACT

**Background:** The purpose of this study was to evaluate two new formulation tooth pastes (Colgate Sensitive Pro Relief and Clinprotooth Crème) on repairing enamel erosion produced by a soft drink (Coca Cola), using Confocal Microscopy.

**Methodology:** This in vitro study was conducted on 30 extracted premolar teeth which were free of caries and defects. The samples were assigned to 6 groups, each containing 5 teeth. Group 1: intact enamel, Group 2: enamel + soft drink, Group 3: enamel + Colgate Sensitive Pro Relief, Group 4: enamel + soft drink + Colgate Sensitive Pro Relief, Group 5: enamel + Clinprotooth Crème and Group 6: enamel + soft drink + Clinprotooth Crème. Groups 2, 4 and 6 were immersed in 6mL of the soft drink for 2 min at room temperature before rinsing with deionized water. Four consecutive intervals of the immersion procedure were carried out at 0, 8, 24 and 36 h for a total of 8 minutes. The toothpastes were then applied onto the surface of the specimens of groups 3, 4, 5 and 6 without brushing for 3 min at 0, 8, 24 and 36 h and then washed with distilled water. In groups 4 and 6 the toothpastes were applied after demineralization with Coca Cola. The Root Mean square roughness will be obtained from Confocal Microscopy images and statistical analysis was done by ANOVA test.

**Results:** Comparing Groups 4 (Enamel + Soft Drink + Colgate Sensitive Pro Relief) and Group 6 (Enamel + Soft Drink + Clinprotooth Crème) with Group 2 (Enamel + Soft Drink) a statistical difference ( $P < 0.05$ ) was registered, suggesting effectiveness in protecting enamel against erosion of the products investigated.

**Conclusion:** The application of the tested toothpastes can be considered effective on preventing enamel erosion produced by a soft drink, however Colgate Sensitive Pro-relief appears to be more effective.

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## INTRODUCTION

Dental erosion is thought to be increasing, due to the wide availability, and frequent consumption of acidic drinks such as soft drinks, sports drinks, and fruit juices (Lussi et al., 2006). Erosion is a chemical process in which the inorganic phase of the tooth is demineralized, thereby reducing the hardness of tooth substrates (Lussi et al., 2011). To prevent dental erosion, many strategies have been developed (Ceci et al., 2015). We also know that enamel has no spontaneous capacity to repair; Therefore, when enamel is exposed to oral environment the only possibility for reconstruction is the application of alloplastic materials. Hence, to improve enamel and dentin resistance, toothpastes were considered effective and affordable vehicles (Kato et al., 2010). Recently, tooth pates have been marketed which claim to prevent erosion.

Two such toothpastes are Colgate Sensitive Pro Relief (Colgate-Palmolive, New York, NY, USA) which is based on Arginine 8% (Pro-Argin™) (Poggio et al., 2014). This technology contains arginine, an amino acid naturally found in saliva, and a compound of insoluble calcium in the form of calcium carbonate and Clinprotooth Crème (3M ESPE, Saint Paul, MN, USA) which is functionalized tricalcium phosphate which prevents calcium from prematurely interacting with ionic fluoride and forming calcium fluoride thus delivering more calcium and fluoride ions to the surface. The aim of this study is to evaluate these two new formulation tooth pastes (Colgate Sensitive Pro Relief and Clinprotooth Crème) on repairing enamel erosion produced by a soft drink (Coca Cola), using Confocal Microscopy.

## MATERIALS AND METHODS

**Specimens Preparation:** Specimens were prepared from 30 Premolar teeth which were extracted for orthodontic reasons.

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The criteria for tooth selection included that the labial enamel surface had to be unaffected by any pre-treatment chemical agents, no cracks, no white spot lesions or caries and no hypoplastic enamel. The teeth were cleansed of soft tissue remnants, they were inspected and they were disinfected in 5.25% sodium hypochlorite solution for 60 minutes. All teeth were stored in artificial saliva during the whole experimentation. The specimens were cut at the cemento - enamel junction, with a high-speed diamond rotary bur with a water-air spray. The labial surfaces near the enamel dentin junction were ground using silicon carbide papers (grades 600 to 1200) under water irrigation to produce flat enamel surfaces. For ease of evaluation under confocal microscopy, the samples were then dried and placed horizontally in a block of silicone. The baseline root mean-square roughness,  $R_{rms}$ , was measured for all the specimens before starting experimentation. No statistical difference in  $R_{rms}$  values was recorded, suggesting that the specimens may be comparable

### Demineralization and Remineralization

A soft drink (Coca Cola, Coca Cola Company, Milano, Italy) was chosen for the demineralization process. The pH at 20°C, buffering capacity and concentration of calcium and phosphate of the beverage were measured. The pH of coke was measured with a pH meter. Two remineralizing agents were used: Colgate Sensitive Pro Relief and Clinprotooth Crème.

The samples were then randomly assigned to 6 groups, each made of 5 teeth

- Group 1: intact enamel (negative control )
- Group 2: enamel + soft drink (positive control )
- Group 3: enamel + Colgate Sensitive Pro Relief
- Group 4: enamel + soft drink + Colgate Sensitive Pro Relief
- Group 5: enamel + Clinprotooth Crème
- Group 6: enamel + soft drink + Clinprotooth Crème

The control specimens (group 1) were taken on storage for the whole experimentation and they did not receive any treatment. The specimens of groups 2, 4, and 6 were immersed in 6mL of the soft drink for 2 minutes at room temperature before rinsing with deionized water. Four consecutive intervals of the immersion procedure were carried out at 0, 8, 24, and 36 hour for a total of 8 minutes. The toothpastes were applied on the surface of the specimens of groups 3, 4, 5 and 6 without brushing for 3 minutes at 0, 8, 24 and 36 hour and then wiped off with distilled water washing. In groups 4 and 6 the toothpastes were applied after demineralization with Coca Cola.

### Confocal Microscopic Observations

Confocal microscopy enables 3-D view of the surface and offers quantitative roughness values. The labial surface of each tooth was observed at a magnification of 20X. An Area of 640 \* 640 micro meter was observed.

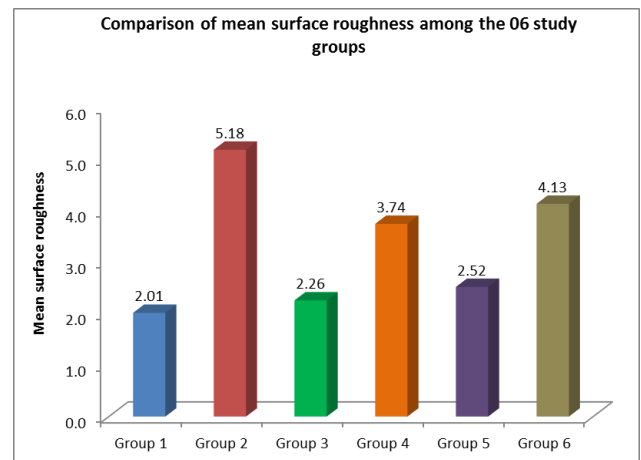
### Statistical Analysis

Differences in the averaged values among the groups were analyzed by ANOVA test. The Statistical difference was set at  $P < 0.05$ . Post hoc Bonferroni test was performed to assess the differences between the different groups.

## RESULTS

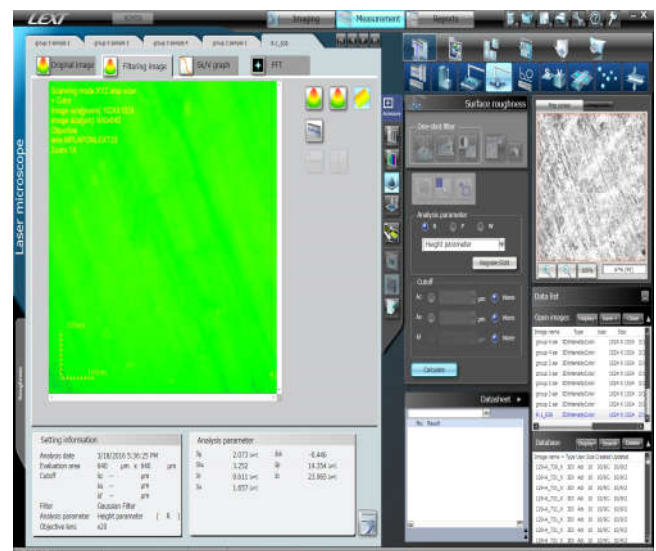
Multiple comparisons using Bonferroni post hoc analysis revealed that among all the groups,

### Graph and Table

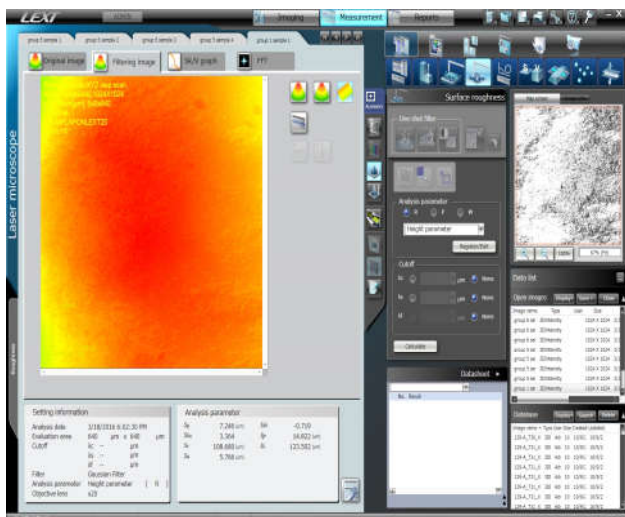


Groups	Mean
Group 1 - Intact Enamel	2.01
Group 2 - Enamel + Soft Drink	5.18
Group 3 - Enamel + Colgate Sensitive Pro Relief	2.26
Group 4 - Enamel + Soft Drink + Colgate Sensitive Pro Relief	3.74
Group 5 - Enamel + Clinprotooth Crème	2.52
Group 6 - Enamel + Soft Drink + Clinprotooth Crème	4.13

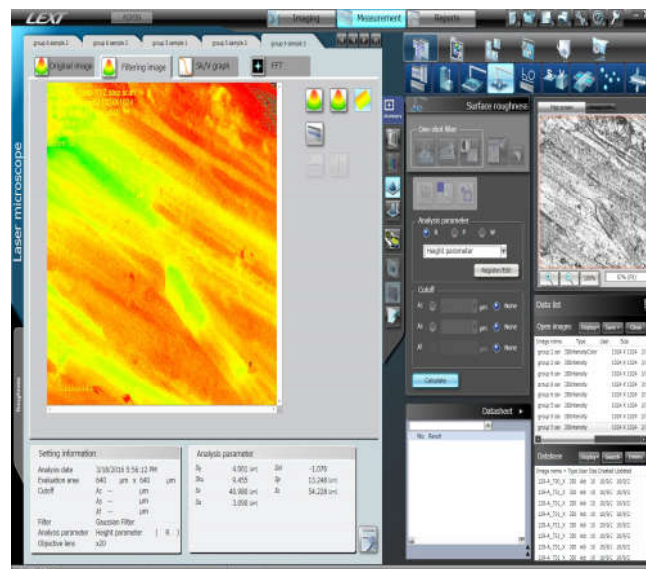
Group 1 (intact enamel) showed lesser mean surface roughness and it showed statistically significant difference with Group 2 (enamel + soft drink) at  $P < 0.001$  & with Groups 4 (enamel + soft drink + Colgate Sensitive Pro Relief) & 6 (enamel + soft drink + Clinprotooth Crème) at 0.01 & 0.001 respectively. Group 2 (enamel + soft drink) showed higher mean surface roughness than Groups 3 (enamel + Colgate Sensitive Pro Relief) & 5 (enamel + Clinprotooth Crème) at  $P < 0.001$  & 0.001, whereas Group 3 (enamel + Colgate Sensitive Pro Relief) showed statistically significant lesser mean scores compared to Group 4 (enamel + soft drink + Colgate Sensitive Pro Relief) & 6 (enamel + soft drink + Clinprotooth Crème) at 0.04 & 0.004 respectively. Similarly, Group 5 (enamel + Clinprotooth Crème) also showed statistically significant difference with Group 6 at  $P = 0.02$ .



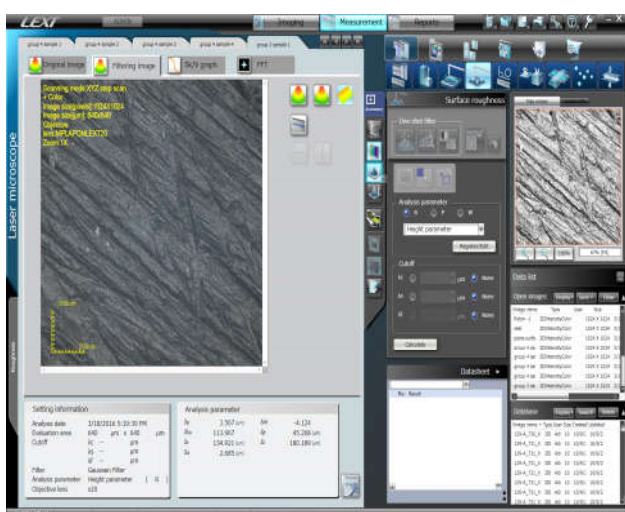
Group 1. Intact Enamel



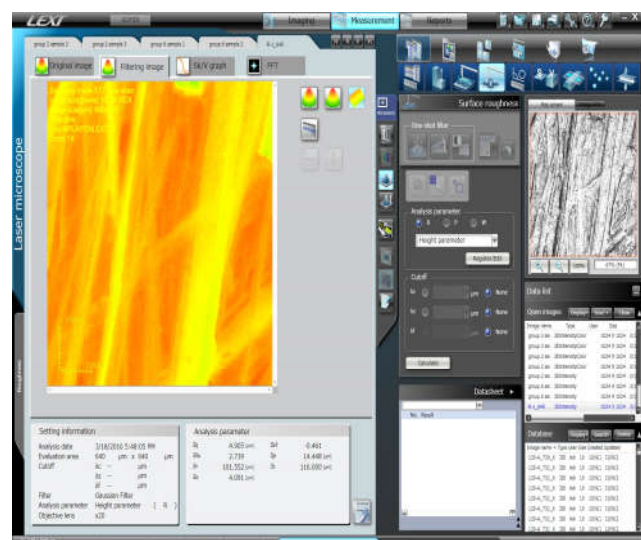
Group 2. Enamel + Soft Drink



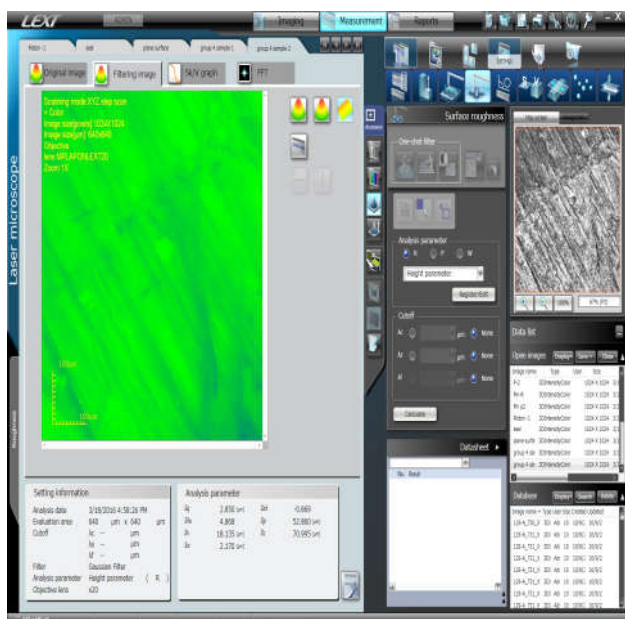
Group 5. Enamel + Clinprotooth Crème



Group 3. Enamel + Colgate Sensitive Pro Relief



Group 6. Enamel + Soft Drink +Clinprotooth Crème



Group 4. Enamel + Soft Drink + Colgate Sensitive Pro Relief

And groups 4 & 6 having higher mean scores compared to groups 3 & 5.

## DISCUSSION

In the present in vitro study, confocal microscopy was used to evaluate the Pro- Arginin Paste and Tricalcium Phosphate paste on repairing enamel erosion produced by a soft drink. Confocal Microscopy combines high resolution optical imaging with depth selectivity which allows for optical sectioning to be done and also helps to construct a 3-D view of the surface and a quantitative value of roughness to be obtained. Enamel surface is often aprismatic and more highly mineralized than enamel subsurface. In order to reduce individual enamel variations and standardize the samples, silicon carbide paper was used to grind teeth surfaces (Torres *et al.*, 2010). This procedure was also carried out to eliminate enamel Aprismatic outermost layer which is gradually worn off during mastication but is still retained in protected areas, this prism-free enamel layer with a high mineral content, is in fact less permeable to acids (Poggio *et al.*, 2010). Most consumed soft drinks are composed of calcium and phosphate with acetic acid or lactate.

In conclusion, there is a statistically significant difference in the mean surface roughness among all the study groups, with group 2 having highest mean score and group 1 having the least.

Most consumed soft drinks are composed of calcium and phosphate with acetic acid or lactate. Erosion is correlated to pH and temperature; moreover, there was a negative correlation between calcium concentration and erosion, but no clear relationship between phosphate concentration and erosion (Hemingway *et al.*, 2006). For these reasons, the beverage with the lowest pH and highest concentration of calcium was chosen for the present *in vitro* study: Coca Cola. Coca Cola was kept at a constant temperature of 20°C. Although erosion proceeds more slowly *in vivo* than *in vitro* owing to the protective effect of saliva and acquired pellicle, the effect of temperature can be expected to be significant. The soft drink was replenished every 2 min to stress its demineralizing potential, to ensure that it was carbonated and to reduce the buffering effect from ions dissolved from the enamel surface (Poggio *et al.*, 2013). In order to simulate the application of any topically applied remineralizing agent, remineralization cycle was carried out at an interval of 0, 8, 24, and 36 hours from the beginning of the experimentation (Agrawal *et al.*, 2014). The most common topographical parameter was therefore determined, such as the ROOT MEAN SQUARE ROUGHNESS (Rrms), to quantitatively evaluate the surface aspect. After demineralization with an acidic substance such as Coca Cola, the surface appears rougher & enamel demonstrated a high degree of surface porosity. Leading to an increase of Rrms. Comparing the Rrms values of groups 4 and 6 (enamel + soft drink + toothpastes) with group 2 (eroded enamel) a statistical difference ( $P < 0.05$ ) was registered, suggesting a remineralizing power on eroded enamel of the two products. Group 3 (enamel + Colgate Sensitive Pro Relief) showed statistically significant lesser mean scores compared to Group 4 & 6. This could be due to the ability of Colgate Sensitive Pro Relief to regenerate a homogenous, very compact, thick and uniform surface layer.

### Conclusion

Under the limitations of the present *in vitro* study, the application of the tested toothpastes can be considered effective on preventing enamel erosion produced by a soft drink, however Colgate Sensitive Pro-relief appears to be more effective with a P value of 0.004.

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**Conflicts of Interest:** There are no conflicts of interest.

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