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# Fluoride/zinc/essential oil-containing mouthrinse promotes fluoride uptake and microhardness in enamel *in vitro*

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## Abstract

***Objective*** To evaluate the effect of fluoride/zinc/essential oil-containing mouthrinse on fluoride uptake and microhardness in enamel.

***Materials and methods*** A double-blind randomized trial was performed. Thirty polished bovine enamel specimens (6 x 6 mm) were subjected to caries-likes lesion formation in the demineralizing solution. Then specimens were analyzed for microhardness (no difference in microhardness; $p > 0.05$) and divided into 3 groups (n=10/group). The fluoride and calcium contents in enamel were analyzed by fluoride electrode and atomic absorption spectrometer, respectively. The specimens in three groups were then treated with either fluoride/zinc/essential oil-containing mouthrinse (Listerine Total Care-LTC), 100 ppm NaF (positive control), or 5% ethanol (negative control) and subjected to 30 cycles of demineralization/remineralization ie. 10 min in demineralizing solution, 5 min in test solution, and 60 min in remineralizing solution. After the cycling, the fluoride, calcium and microhardness in enamel were determined. The changes of fluoride content (Δ F) and microhardness (Δ H) after treatment were analyzed between groups using a one way ANOVA and Tukey test.

***Results*** The Δ F obtained from the test mouthrinse (71.10 ± 45.50 ppm) was not statistically different ($p > 0.05$) from that of 100 ppm NaF (41.41 ± 26.75 ppm). However, both solutions showed significantly more fluoride uptake than 5% ethanol group (0.20 ± 2.30 ppm) ($p < 0.05$). The Δ H of LTC treated enamel (6.7 ± 2.8 Vickers) was not different ($p > 0.05$) from that of 100 ppm NaF treated samples (10.9 ± 6.1 Vickers). However, Δ Hs of both treatments were significantly more than in ethanol treated teeth (1.1 ± 1.7 Vickers) ($p < 0.05$).

***Conclusion*** Fluoride/zinc/essential oil-containing mouthrinse can promote enamel fluoride uptake and microhardness.

(CU Dent J. 2013;36:31-36)

***Key words:*** *essential oil; fluoride; fluoride uptake; microhardness; mouthrinse; zinc*

## Introduction

Mouthrinse is an oral health product growing in popularity in the dental health product market. The oral health benefits of mouthrinse mostly derive from their anti-microbial activities of their ingredients eg. triclosan, cetylpyridium chloride, essential oil, etc. Essential oil-containing mouthrinse has been established to improve oral health by reducing plaque, gingivitis and oral bacteria.[1,2] In order to broaden the range of mouthrinse action, new formulations of mouthrinse contain combinations of other active ingredients such as fluoride and zinc. Fluoride is accepted as an anti-caries agent by its anti-microbial activity, promotion of tooth remineralization and inhibition of demineralization.[3-6] Zinc can prevent calculus formation by inhibiting hydroxyapatite formation and promoting the formation of more soluble form of calcium phosphate.[7]

Essential oil mouthrinse containing either zinc or fluoride has been demonstrated to be similar or more effective than the original formula.[8-11] Zinc chloride-containing essential oil mouthrinse was clinically proven to reduce calculus formation by up to 21%.[12] Fluoride-containing essential oil mouthrinse was found to increase enamel fluoride uptake and microhardness.[9-11] Recently, a new essential oil mouthrinse containing both zinc chloride and fluoride in its formula has been developed. The objective of this study was to investigate the effect of this formula mouthrinse on enamel fluoride uptake and microhardness.

## Materials and methods

The study was performed as a double-blind randomized trial.

### Test reagents

Listerine Total Care (LTC) mouthrinse (0.022% NaF, 0.09% zinc chloride) was the test reagent using 100 ppm (0.022%) NaF as a positive control. Five percent ethanol, which is a major solvent of LTC, was used as a negative control.

### Enamel specimens

Bovine teeth were collected in 10% v/v phosphate buffered (pH 6.8) formalin solution and cleaned. The 6 x 6 mm enamel specimens were prepared by grinding and polishing with 800 and 1200 sand paper and 1 μm alumina, and mounted with acrylic resin. The specimens were immersed in the demineralizing solution (0.1 M lactic acid, 0.2% Carbopol C907 solution, 50% saturated with calcium hydroxyapatite at pH 5.0) at 37°C for 96 h to induce caries-like lesions.[13]

### Treatments

Thirty specimens were analyzed for initial surface microhardness using a Microhardness Tester (Future-Tech FM700E, Japan) with 300 g load and 5 indentations for each specimen. The average surface microhardness among the specimens was not significantly different ($p > 0.05$) and the specimens were divided into 3 groups (n = 10/group). A 1.6 mm diameter area was circumscribed on each enamel sample. Then the fluoride content was determined by acid etching with 0.5 M $HClO_4$ in the 1.6 mm diameter area, and analyzed using TISAB III and fluoride ion selective electrode (Selection Sensor, Select Biosciences Ltd, Sudbury, UK). The calcium content in the etching acid solution was also analyzed by an atomic absorption spectrometer (Varian AA 280FS, USA) to normalize the fluoride content to the calcium content to account for possible differences in the amount of etching which may have occurred.[14,15] The specimens were subjected through a demineralization/remineralization cycle model modified from Schemehorn *et al.*[16] The cycling was performed on a shaker (200 rpm) at room temperature for 30 cycles. Each cycle composed of 10 min in demineralizing solution, 5 min in either Listerine Total Care (LTC) mouthrinse, 100 ppm NaF (positive control), or 5% ethanol (negative control), and 60 min in remineralizing solution (22% gastric mucin, 6.5 mM NaCl, 1.5 mM $CaCl_2$, 5.5 mM $KH_2PO_4$, 15 mM KCl, pH = 7.0) (Table 1). After the cycling, fluoride and calcium content, and the surface microhardness of each

specimen were determined. During the preparation and between the treatments, specimens were kept in a humidified box at room temperature, and in the remineralizing solution at 4°C when stored overnight.

### Statistical analysis

The changes of fluoride content (Δ F) and microhardness (Δ H) after the cycling were analyzed between groups using a one way analysis of variance and where significant differences were indicated, the Tukey test was used to determine significant differences among the individual means.

## Results

After the 30 cycle treatments, the means of enamel fluoride uptake from the LTC, NaF and ethanol were 71.10 ± 45.50, 41.41 ± 26.75, and 0.20 ± 2.30 ppm, respectively (Table 2). The Δ F of the LTC treated group was not statistically different from the NaF treated group ($p > 0.05$). However, both values were statistically greater than the ethanol treated group ($p < 0.05$).

The changes in microhardness after treatment with LTC, NaF and ethanol were 6.7 ± 2.8, 10.9 ± 6.1 and 1.1 ± 1.7 Vickers, respectively (Table 3). The Δ H of the LTC treated group was not statistically different from the NaF treated group ($p > 0.05$). However, both values were statistically higher than the ethanol treated group ($p < 0.05$).

## Discussion

Enamel microhardness has been reported to be significantly increased after *in vitro* and *in situ* treatments with fluoride/essential oil containing mouthrinse when compared to the non-fluoride/essential oil control and as effective as the NaF rinse.[9-11]

**Table 1** pH cycling regimen

| Duration | Procedure |
|---|---|
| 10 minutes | Demineralizing solution |
| ↓ | ↓ |
| 5 seconds | Deionized water rinse |
| ↓ | ↓ |
| 30 seconds | Deionized water wash |
| ↓ | ↓ |
| 5 minutes | Test reagent |
| ↓ | ↓ |
| 5 seconds | Deionized water rinse |
| ↓ | ↓ |
| 30 seconds | Deionized water wash |
| ↓ | ↓ |
| 60 minutes | Remineralizing solution |
| ↓ | ↓ |
| 5 seconds | Deionized water rinse |
| ↓ | ↓ |
| 30 seconds | Deionized water wash |

Here, we show after 30 cycles of demineralization/remineralization treatment, the LTC mouthrinse significantly increased fluoride uptake and microhardness of the tested bovine enamel specimens when compared to the negative control, ethanol, which is the major solvent of the LTC. When compared to the positive control, NaF containing no other active ingredient of LTC, the fluoride uptake of LTC group tended to be greater than the NaF group, however no statistical difference was found between them. The higher fluoride uptake of the LTC group when compared to the NaF solution is similar to the *in situ* study of Zero *et al.*[9] This may be due to the lower pH (pH 4.2) of the LTC since acidity tends to promote the uptake of fluoride. No difference in microhardness changes was found between the LTC and NaF group which is similar to other reports studying in different fluoride-containing mouthrinses.[9-11] The increase in microhardness after demineralization/remineralization cycling of the LTC is most likely to be from the uptake of fluoride in the mouthrinse. To the best of our knowledge, the effect of zinc chloride on enamel microhardness has never been reported, however it has long been demonstrated to have a positive effect against enamel demineralization and recent study also revealed its positive benefit for fluoride remineralization.[17-19] This effect of zinc and the greater acidity of the LTC may help explain the tendency for higher fluoride uptake of the LTC than the NaF solution in our study. Normally, zinc chloride has been included in toothpaste and mouthrinse for its anti-microbial activity and its ability to displace calculus-forming ions, resulting in prevention of calculus build up.[12,20-21]

## Conclusion

Fluoride/zinc/essential oil-containing mouthrinse can promote the fluoride uptake and microhardness of the enamel.

## Acknowledgement

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**Table 2** Enamel fluoride uptake from different treatments

| Treatment | Fluoride (ppm) | | |
|---|---|---|---|
| | Before | After | Δ |
| Listerine Total Care | 66.00 ± 30.58 | 137.05 ± 30.21 | 71.10 ± 45.50* |
| 100 ppm NaF | 55.01 ± 23.45 | 96.42 ± 34.34 | 41.41 ± 26.75* |
| 5% Ethanol | 53.86 ± 14.02 | 54.08 ± 13.55 | 0.20 ± 2.30 |

*Statistical difference from the ethanol control at $p < 0.05$

**Table 3** Enamel microhardness from different treatments

| Treatment | Microhardness (Vickers) | | |
|---|---|---|---|
| | Before | After | Δ |
| Listerine Total Care | 17.2 ± 8.8 | 23.9 ± 11.3 | 6.7 ± 2.8* |
| 100 ppm NaF | 15.4 ± 8.5 | 26.3 ± 11.0 | 10.9 ± 6.1* |
| 5% Ethanol | 20.5 ± 18.3 | 21.6 ± 17.9 | 1.1 ± 1.7 |

*Statistical difference from the ethanol control at $p < 0.05$

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# น้ำยาบ้วนปากที่ผสมฟลูออไรด์/สังกะสี/น้ำมันหอมระเหยส่งเสริมการได้รับฟลูออไรด์และความแข็งของเคลือบฟันในห้องปฏิบัติการ

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## บทคัดย่อ

***วัตถุประสงค์*** เพื่อประเมินผลของน้ำยาบ้วนปากที่ผสมฟลูออไรด์/สังกะสี/น้ำมันหอมระเหยต่อการได้รับฟลูออไรด์และความแข็งของเคลือบฟัน

***วัสดุและวิธีการ*** ใช้การวิจัยเชิงทดลองแบบสุ่มชนิดปิดบังสองทาง โดยตัวอย่างเคลือบฟันวัวจำนวน 30 ชิ้น (6 x 6 มิลลิเมตร) ที่ขัดแล้ว และทำให้เกิดรอยผุจำลองในสารละลายสลายแร่ธาตุ จากนั้นนำมาวิเคราะห์ความแข็งของผิวเคลือบฟัน (ไม่มีความแตกต่างของความแข็ง; $p > 0.05$) และแบ่งออกเป็น 3 กลุ่ม กลุ่มละ 10 ชิ้น นำมาวิเคราะห์หาปริมาณฟลูออไรด์และแคลเซียมในเคลือบฟันโดยใช้ฟลูออไรด์อิเล็กโทรดและอะตอมมิกแอบซอร์พชันสเปกโตรมิเตอร์ ตามลำดับ จากนั้นนำตัวอย่างทั้งสามกลุ่มทดสอบด้วยสารอย่างใดอย่างหนึ่ง คือ น้ำยาบ้วนปากที่มีฟลูออไรด์/สังกะสี/น้ำมันหอมระเหย (ลิสเตอรีนโทเทิลแคร์-แอลทีซี) 100 พีพีเอ็ม โซเดียมฟลูออไรด์ (ตัวควบคุมบวก) หรือ เอธานอล ร้อยละ 5 (ตัวควบคุมลบ) โดยให้ผ่านกระบวนการสลายแร่ธาตุและกระบวนการสะสมกลับของแร่ธาตุเป็นจำนวน 30 รอบ กล่าวคือ กระบวนการสลายแร่ธาตุ 10 นาที ผ่านสารทดสอบ 5 นาที และกระบวนการสะสมกลับของแร่ธาตุ 60 นาที หลังจากครบจำนวนรอบทำการตรวจสอบปริมาณของฟลูออไรด์ แคลเซียม และความแข็งของเคลือบฟัน วิเคราะห์ค่าที่เปลี่ยนไปของฟลูออไรด์ ($\Delta F$) และความแข็ง ($\Delta H$) หลังการทดสอบระหว่างกลุ่มต่าง ๆ ด้วยการวิเคราะห์ความแปรปรวนทางเดียวและการทดสอบทูคีย์

***ผลการศึกษา*** ค่าที่เปลี่ยนไปของฟลูโอไรด์ที่ได้จากน้ำยาบ้วนปากที่ทดสอบ (71.10 ± 45.50 พีพีเอ็ม) ไม่มีความแตกต่างกันทางสถิติ ($p > 0.05$) จาก 100 พีพีเอ็ม โซเดียมฟลูออไรด์ (41.41 ± 26.75 พีพีเอ็ม) อย่างไรก็ตามน้ำยาทั้งสองทำให้มีการได้รับฟลูออไรด์มากกว่ากลุ่มเอธานอล ร้อยละ 5 อย่างมีนัยสำคัญ (0.20 ± 2.30 พีพีเอ็ม) ($p < 0.05$) ค่าที่เปลี่ยนไปของความแข็งของเคลือบฟันที่ทดสอบด้วยแอลทีซี (6.7 ± 2.8 วิคเคอร์ส) ไม่มีความแตกต่าง ($p > 0.05$) จากตัวอย่างที่ทดสอบด้วย 100 พีพีเอ็ม โซเดียมฟลูออไรด์ (10.9 ± 6.1 วิคเคอร์ส) อย่างไรก็ตามค่าที่เปลี่ยนไปของความแข็งของเคลือบฟัน จากการทดสอบทั้งสองนั้นมีค่ามากกว่าฟันที่ทดสอบด้วยเอธานอลอย่างมีนัยสำคัญ (1.1 ± 1.7 วิคเคอร์ส) ($p < 0.05$)

***สรุป*** น้ำยาบ้วนปากที่ผสมฟลูออไรด์/สังกะสี/น้ำมันหอมระเหยสามารถส่งเสริมการได้รับฟลูออไรด์และความแข็งของเคลือบฟันได้

(ว ทันต จุฬาฯ 2556;36:31-36)

***คำสำคัญ:*** *การได้รับฟลูออไรด์; ความแข็ง; น้ำมันหอมระเหย; น้ำยาบ้วนปาก; ฟลูออไรด์; สังกะสี*