Chitosan MS boosts fluoride uptake and limits bacterial adhesion on human teeth

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Poster 48

Daniel Bacqueville[1], Mélanie Souyris[1], Andreas Kiesow[2], Maria Morawietz[2], Catherine Feuillolay[3], Alberto Ogalla[4], Perrine Coumes[4], Nathalie Castex-Rizzi[1]

[1] Pierre Fabre Dermo-Cosmétique & Personal Care, Toulouse, France
[2] Fraunhofer Institute for Microstructure of Materials and Systems, Halle (Saale), Germany
[3] ACM Pharma Fonderephar, Toulouse, France
[4] Pierre Fabre Oral Care, Lavaur, France

Introduction

The aim of this *in vitro* study was to evaluate the benefit of a chitosan associated with mandelic acid (CMS) for improving enamel fluoride uptake (EFU) from Nicomethanol hydrofluoride (NHF 1400 ppm, Fluorinol®) and preventing the adhesion of *Streptococcus mutans* (*Sm*), a cariogenic bacterial strain.

Methods

Enamel Fluoride Uptake analysis by ToF-SIMS

EFU was analyzed by *Time-of-Flight Secondary Ion Mass Spectrometry* (ToF-SIMS, depth profiling and cross-section mapping, M6, Iontof) on enamel from human molars (N=3). Embedded and polished enamel samples were slightly demineralized (1% citric acid, pH 3.8) for 15 min to mimic an "early erosion" and treated twice a day for 10 days with a solution (1min) or toothpaste slurry (2 min; 1:2 w/w toothpaste:deionized water) containing NHF 1400 ppm ± CMS. Samples were stored in artificial saliva between the treatments. For references, non-treated controls were tested for both not demineralized enamel (sound enamel) and demineralized enamel. As negative control, teeth were demineralized and treated in water for 1 min without a subsequent cyclic procedure.

***Depth profiling* was performed to quantify EFU up to about 4500 nm with a measurement area of 75x75 µm² on the enamel surfaces and sputtering in an alternating mode. Mean values of the respective intensities per sample were calculated in Sputter time (sec) over the whole spectra from 150 to 7000 sec (about 10 nm to 4500 nm depth) as well as between the regions of interest 1500-2500 sec and 5000-7000 sec. Statistical evaluation was conducted by ANOVA and post-hoc Bonferroni (p<0.05).**

***Cross-section mapping* was performed to visualize the EFU up to 10 µM. Image acquisition was done from the previous samples that were cross-sectioned and embedded in epoxy resin before ToF-SIMS analysis.**

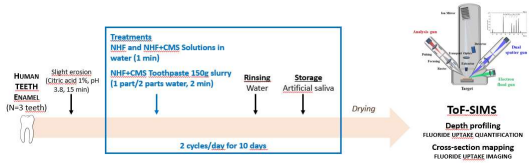

Figure 1: Experimental setup of Enamel Fluoride Uptake analysis

Bacterial adhesion by CFU counting

Adhesion of *Streptococcus mutans* was performed on hydroxyapatite disc preincubated with human saliva to form a biofilm. Bacteria were cultured 24h and treated thrice with toothpaste or water as control (2 min). Adhesion was evaluated by counting of colony forming units (Log CFU/carrier). Results are mean ± SEM and statistics were conducted by unpaired t test (N=3).

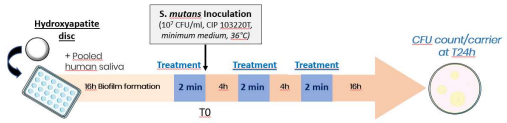

Figure 2 Experimental setup of Streptococcus mutans adhesion

Results

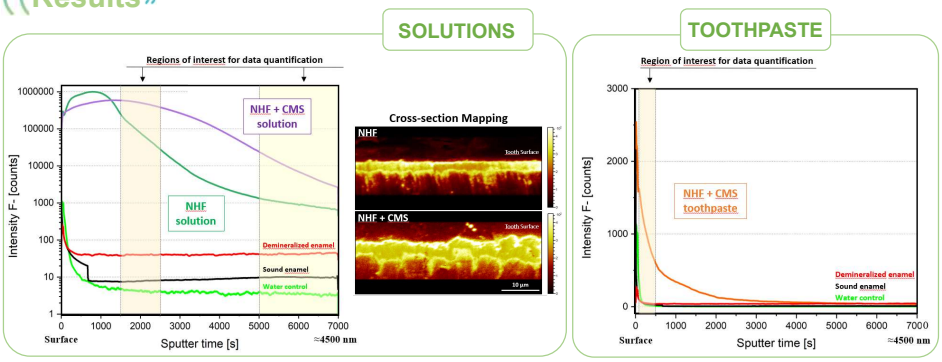

Figure 3: Enamel Fluoride Uptake analysis

- ToF-SIMS depth profiling showed that **NHF and NHF+CMS solutions presented a higher EFU than the references** (sound enamel, demineralized and water-treated enamel).
- **CMS increased EFU in comparison to NHF without CMS with 5-fold induction in the region 1500-2500** (497656±165061 vs 94496±140325, p<0.01) **and 10-fold induction in the interval 5000-7000 sec** (8888±1255 vs 933±1145 counts, p<0.0001).
- EFU visualization by cross-section mapping confirmed the depth profiling and **CMS boosted Fluoride uptake from NHF on teeth** up to about 10 µm.
- **NHF+CMS toothpaste also significantly increased EFU** (910±147 vs 47 counts for demineralized enamel sputter-time 150-500 sec, p<0.0001).

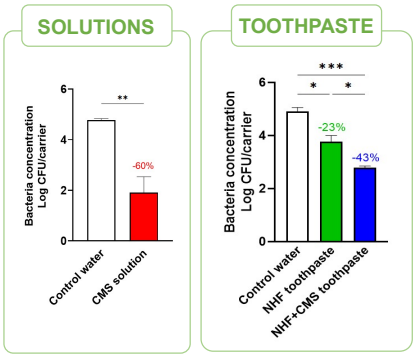

Figure 4: Bacterial adhesion analysis

- **CMS solution inhibited Sm adhesion** (-60%, Log CFU/disc 1.92±0.62 *versus* 4.77±0.06, p<0.001).
- **NHF toothpaste inhibited Sm adhesion** (-23%, Log CFU/disc 3.777±0.225 *versus* 4.907±0.150, p<0.05).
- **NHF+CMS toothpaste was more efficient than NHF toothpaste** to decrease Sm adhesion (-43%, LogCFU/disc 2.787±0.064, p<0.001). Difference between toothpastes was significant (p<0.05).

Conclusions

Nicomethanol hydrofluoride (Fluorinol®) + Chitosan MS combination favors Fluor uptake and inhibits bacterial adhesion. Thus, toothpaste containing both active ingredients could help remineralize enamel and protect teeth against caries.