Morphological and Elemental Evaluation of Investigative Mouthwashes to Repair Acid-Eroded Tooth Surface

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OBJECTIVE

- We investigated the effectiveness of mouthwashes containing hydrolyzed wheat protein (HWP) in repairing Erosive Tooth Wear through promotion of organized crystal growth on the eroded tooth surface.

INTRODUCTION

- Erosive tooth wear is the progressive loss of tooth structure due to exposure to acids of non-bacteria origin and its prevalence in deciduous teeth varies from 0-82%.
- Many interventions; including highly concentrated fluoride, hydroxyapatite, Casein Phosphopeptide Amorphous Calcium Phosphate or self-assembling peptides, applied either as toothpaste, mouthwash, brush-on liquid, varnish, or gel have been used to remineralize and/or arrest the eroded tooth surface.
- In the present era of tissue engineering and biomimetics, reconstructing enamel-like structures on the tooth surface is increasingly being studied in the material sciences and dentistry.
- One of these proteins being studied is hydrolyzed wheat protein, Enameguard™ (BASF Corporation, Florham Park, USA), a chelating agent that works by guiding and catalyzing the regeneration of lost enamel in an eroded tooth surface.

MATERIALS and METHODS

Eroded lesions were created on 210 bovine tooth blocks with 1% citric acid. Blocks were randomly assigned the following 7 mouthwashes (30/group).

- (A) 0.2% HWP
- (B) 1% HWP
- (C) 2% HWP
- (D) 1% HWP + 0.05% NaF
- (E) non-fluoridated Listerine™ mouthwash
- (F) 0.02% NaF Crest Pro-health Restore Enamel Mouthwash
- (G) Artificial saliva only.

Samples underwent the following treatment regimen for 28 days:

Daily event	Treatment
Day 1: All-day storage in Artificial Saliva. Then, subsequent days' treatment was as follows:	
1 minute	Mouthwash treatment
4 hours	Storage in Artificial Saliva
5 minutes (12 noon)	Erosive challenge
1 minute	Mouthwash treatment
4 hours	Storage in Artificial Saliva
1 minute	Mouthwash treatment
Till 8:00 am next day	Storage in Artificial Saliva

Table 1. pH cycling treatment sequence

Structural and elemental composition analysis: SEM coupled with EDX was used to investigate the structural and quantitative chemical composition of the eroded enamel surface and the mineral deposits laid by the different mouthwash formulations.

RESULTS

There was complete crystal growth on the eroded enamel surfaces in the groups that were exposed to the HWP-containing mouthwashes (Fig. 1B, C, D & E).

The crystals had fiber-like structures and the deposit was porous but increased in packing density in a dose-dependent manner (0.2, 1, 2% HWP).

The SEM images (Fig. 1F, G & H) from the groups exposed to Listerine™ mouthwash (F), NaF mouthwash (G), and Artificial saliva only (H), showed that the eroded enamel surfaces were covered by a structureless deposit layer that was firmly attached to the enamel surface (did not come off after sonication (Fig. 2C)).

These deposits appear to seal the exposed enamel prisms/interprismatic spaces that were visible on the eroded enamel surface (Fig. 1A).

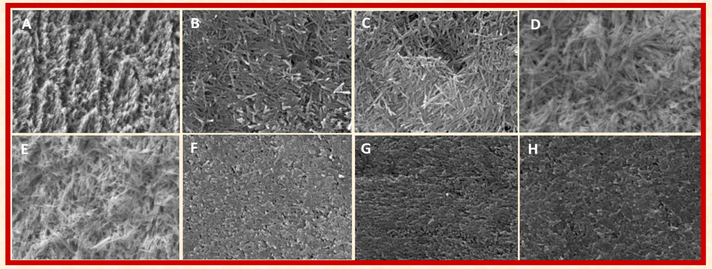

Figure 1. SEM images of enamel surface after acid erosion (A), and biomimetic mineralization of the eroded surface by deposition of fiber-like crystals with different concentrations of Enameguard in mouthwash; 0.2% (B), 1% (C), 2% (D), 1% + 225 ppm fluoride (E), and with Listerine™ mouthwash (F), 0.02% sodium fluoride mouthwash (G), and Artificial saliva only (H). All the images were taken under the same magnification x6,000

After sonication, the fiber-like crystals in the group treated with 0.2% HWP concentration were able to come off completely,(Fig. 2A), while groups treated with 1% and 2% HWP revealed evidence of deeper layer of crystals growth (Fig. 2B), with the crystals growing out in bundles perpendicular to the enamel surface.

The packing density of the crystals increased with increase in the concentration of the HWP. This difference in density and bundle formation was more obvious with the group exposed to 1% HWP plus 0.05% NaF.

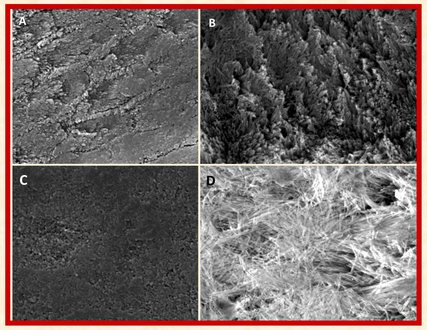

Figure 2. SEM images (x6,000), after sonication, of eroded enamel surface treated with (A) 0.2% and (B) 2% of Enameguard mouthwash, which were previously covered by deposits of fiber-like crystals, and (C) representative of groups not treated with Enameguard. (D) Higher magnification SEM image (x7,500) of group treated with 1% Enameguard + 225 ppm F (not sonicated).

RESULTS

Atomic element levels of Ca and phosphorous (P) obtained with EDX were expressed in percentage (%), and the Ca/P ratio was calculated for the sound enamel surface, eroded enamel surface, and remineralized eroded surface of each group (Table 2, Fig. 3A, B). However, it is only the groups exposed to 1% HWP + 0.05% NaF and Crest Pro-health Mouthwashes that showed fluoride in EDS.

Groups	Atomic % (Mean±SD)		
	Calcium	Phosphorus	Ca/P ratio
Sound Enamel	19.5	13.45	1.45±0.04
Eroded enamel	15.63	11.8	1.32±0.05
0.2% HWP mouthwash	19.35	13.2	1.47±0.01
1% HWP mouthwash	20.75	13.75	1.51±0.01
2% HWP mouthwash	20.45	13.6	1.52±0.02
1% HWP + 225 ppm Fluoride	20.3	13.5	1.51±0.01
Listerine mouthwash	20.25	13.7	1.48±0.01
Crest Pro-health mouthwash (0.02% NaF)	18.1	12.6	1.44±0.08
Artificial saliva	20.25	13.55	1.50±0.04

Table 2. Mean, standard deviation (SD) and Ratio of mineral content of sound enamel surface, the eroded enamel surface, and eroded surface with mineral deposits in eroded surface of each group. Ca – Calcium, P – Phosphorus, HWP - Hydrolyzed wheat protein.

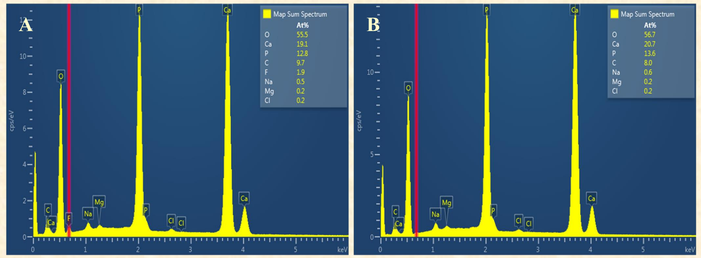

Figure 3. EDS spectrum of (A) eroded enamel surface and (B) remineralized eroded enamel surface with deposits of fiber-like crystals.

CONCLUSIONS

- Treatment of eroded enamel surface with a mouthwash containing Enameguard resulted in repair of the damaged tissue and remineralization of the enamel.
- The Enameguard-containing mouthwash formulations led to an increase in the Ca and P content of the enamel layer.
- The use of Enameguard in concentrations of 1% and 2%, is a promising approach. Not only did Enameguard remineralize the eroded tissue, it also replaced the missing tissue.
- Demineralized and eroded enamel tissue can be remineralized using mouthwashes containing Enameguard as a remineralizing agent.

Mackenzie Hatfield Cvelich is an officer of the US Navy, however, the views expressed in this article reflect the results of research conducted by the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the United States Government.

* Mackenzie H. Cvelich is the presenter, but not the first author.