Poster # 2321

A Novel Enzyme Whitening Toothpaste is Safe for Enamel

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Introduction

A novel enzyme whitening toothpaste was developed that utilizes a perhydrolase enzyme to catalyze the reaction of hydrogen peroxide and triacetin to generate peracetic acid (PAA) during tooth brushing. PAA can then bleach a broad spectrum of both extrinsic and intrinsic stains including those tough stains from coffee, tea, wine, soda and tobacco. The PAA generated during tooth brushing provides tooth whitening effects that have been demonstrated *in-vitro* as well as clinically.

Rapid PAA generation by the enzyme whitening toothpaste is essential to providing the consumer with tooth whitening efficacy. Kinetic studies were conducted to quantify the generation of PAA by the enzyme whitening toothpaste over a time-period relevant to consumer tooth brushing.

The International Organization for Standardization (ISO) 28399 has established criteria for external tooth bleaching products used to whiten natural teeth via chemical means. *In-vitro* studies were conducted to demonstrate that the enzyme whitening toothpaste is safe for the enamel by evaluating the change in surface microhardness after *in-vitro* brushing with the enzyme whitening toothpaste. In addition, the surface morphology of the enamel pre- and post-brushing was examined via confocal microscopy.

Conclusions

This novel enzyme whitening toothpaste generates PAA at the point of use through the action of a perhydrolase enzyme catalyst. Over 2 minutes, between 580-720 ppm of PAA was generated by the toothpaste. Based on *in-vitro* and clinical studies, this quantity of PAA is sufficient to deliver whitening efficacy.

According to the ISO standard for an external tooth-bleaching product, the enzyme meets the established criteria for enamel surface microhardness. Confocal images revealed that the enamel surface morphology remains consistent before and after brushing with the enzyme whitening toothpaste. These *in-vitro* study results support that the enzyme whitening toothpaste is safe for enamel.

Results

The enzyme toothpaste steadily generates between 578-720 ppm PAA over the course of 2 minutes (Table 1). The average PAA values over 2 minutes are plotted in Figure 1.

Table 1. PAA values measured at specified time points

Time (seconds)	Run 1 (%PAA)	Run 2 (%PAA)	Average (%PAA)
0	0.0000	0.0000	0.0000
15	0.0627	0.0720	0.0674
30	0.0597	0.0640	0.0619
60	0.0578	0.0605	0.0592
90	0.0587	0.0626	0.0607
120	0.0601	0.0635	0.0618

Figure 1. The average amount of PAA generated by the enzyme whitening toothpaste over 2 minutes

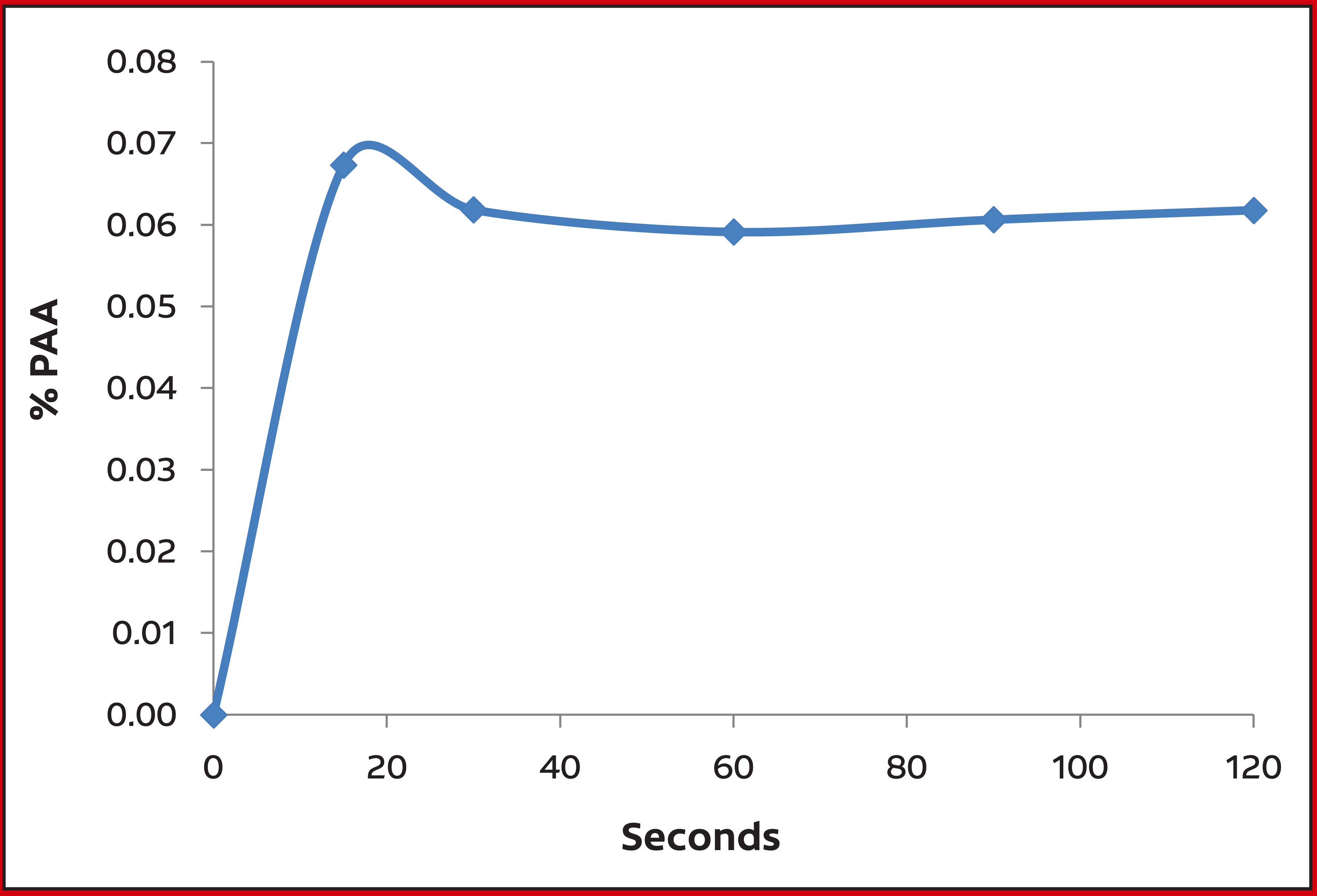
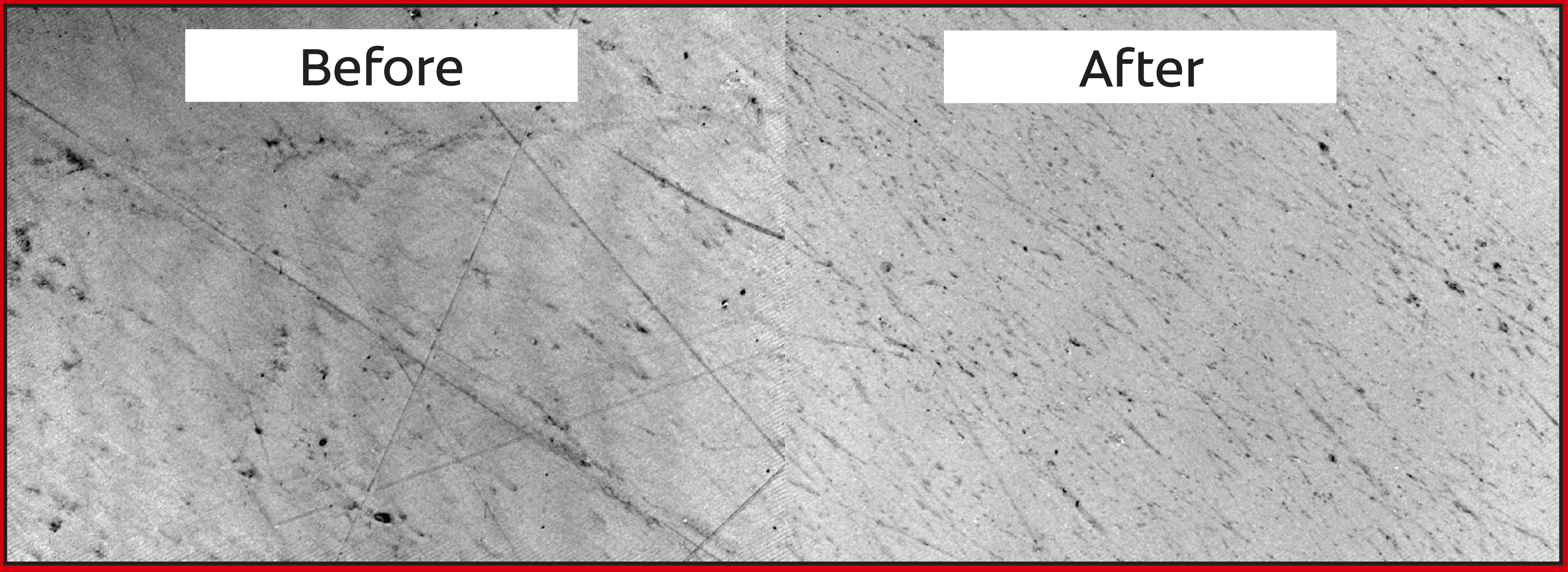


Table 2 includes the microhardness measurements of the 10 enamel blocks. The average surface microhardness change after brushing with the enzyme toothpaste was -1.27% which is within ±10% of sound enamel.

Sample#	Sound	After Brushing	%Change
1	328.8	328.4	-0.12
2	320.4	330.8	3.25
3	341.8	332.0	-2.87
4	352.2	318.6	-9.54
5	353.2	374.6	6.06
6	323.4	320.4	-0.93
7	339.0	331.0	-2.36
8	333.2	353.4	6.06
9	340.8	315.0	-7.57
10	322.2	307.2	-4.66
Average	**335.5**	**331.1**	**-1.27**

Confocal images of the enamel blocks were taken after completion of the 14 brushing treatments. There is no significant difference in surface morphology after brushing with the enzyme whitening toothpaste.

Figure 2. Surface of enamel block before and after brushing with the enzyme whitening toothpaste at 150k magnification



Methods and Materials

To quantify PAA production, the enzyme toothpaste was mixed 1:1 with 100 mM sodium phosphate buffer, pH 7.2. Samples were taken at multiple time points up to 2 minutes, and the reactions were quenched with phosphoric acid. The amount of PAA generated was quantified by the Karst method, in which PAA oxidizes methyl *p*-tolyl sulfide in a 1:1 ratio and the resulting sulfoxide is quantified via HPLC.

To assess enamel safety, baseline surface microhardness of 10 polished sound bovine enamel blocks were measured using the Knoops Hardness test. All microhardness values were measured under 50g of force. The reported microhardness value for each block is an average of 5 measurements. The enamel blocks were then brushed with the enzyme toothpaste twice a day for 7 days. The blocks were submerged in artificial saliva in between brushings. After 14 brushings, the enamel blocks were dried overnight and the final microhardness was measured. The change in surface microhardness was compared to the ISO standard ISO28399 for safety of external tooth-bleaching products.

To visually assess the surface morphology after brushing, the enamel blocks were placed directly onto a Leica DCM8 microscope set to confocal mode and images were taken at a magnification of 150X.

References

1. U. Pinkernell, S. Effkemann, and U. Karst. 1997. Simultaneous HPLC Determination of Peroxyacetic Acid and Hydrogen Peroxide. *Anal.Chem.* 69: 3623-3627.
2. International Organization for Standardization. (2011). Dentistry – Products for external tooth bleaching (ISO Standard No. 28399).

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