Effect of Novel Amelotin-Coated Hydroxyapatite Nanoparticles on the Remineralization of Artificial Carious Lesions *in vitro*

O'Hagan-Wong K.[1], Enax, J.[2], Meyer F.[2], & Ganss B[1]

Faculty of Dentistry, University of Toronto[1] Department Oral Care Dr. Kurt Wolff GmbH & Co. KG, Germany[2]

Introduction

Hydroxyapatite Toothpaste

- Hydroxyapatite toothpaste (HAP-TP) was approved for use in Canada in 2015 [1]
- The main ingredient in HAP-TP is calcium and phosphate in the form of HAP
- HAP –TP are currently being explored as a fluoride free anti-caries agent
- Clinical trials have demonstrated the non-inferiority of HAP toothpaste compared to fluoride toothpaste as an anti caries agent [2]

Comparison of fluoride and HAP-TP [3]

	Hydroxyapatite Toothpaste	Fluoride Toothpaste
Mechanism of Action	Deposition of HAP particles on demineralized enamel	Improvement of the natural tooth surface remineralization process
Source of Ca^{2+} and PO_4^{2-}	Does not rely on calcium and phosphate in saliva	Relies on salivary flow for calcium and phosphate ions
Adverse Effects	No adverse heath events reported	Dental fluorosis, systemic toxicity when used in excess
Whitening	HAP gel found to produce increased brightening effects *in vitro* and i*n vivo*	No significant difference in tooth shade score from baseline
Sensitivity	Decreased dentinal hypersensitivity and increased tubule occlusion	No significant difference between fluoridated toothpaste and placebo

Amelotin

- Amelotin (AMTN) is a recently discovered protein that is specifically expressed during the maturation stage of dental enamel formation [4]
- *In vivo* studies demonstrate AMTN knockout mice display hypomineralized enamel [5]
- *In vitro* studies have demonstrated that AMTN coated hydroxyapatite nanoparticles (AMTN-HAP) promote significantly more mineralization on demineralized dentin disks and collagen gels [6]
- AMTN may be a novel strategy to promote enamel mineralization

Objectives

1. To develop a protocol to create artificial white spot lesions (WSL) *in vitro*
2. To investigate the effect of AMTN-HAP on the remineralization of artificial WSL *in vitro*

Materials & Methods

- Extracted human third molars were painted with 2 coats of nail varnish except for a 2x3mm window on each surface (n=6)
- Teeth were demineralized in a lactic acid buffer for 7 days (pH=4.5) at 37C to create WSL on each surface of the tooth [7]
- Human recombinant AMTN was expressed in *E.Coli* and purified as described [6]
- AMTN was mixed with a 1% solution HAP nanoparticles [6]
- WSL were incubated with 1- recombinant AMTN, 2- HAP nanoparticles, or AMTN-HAP or artificial saliva for 2 hours at 37C
- Mineral density and lesion depth were measured using micro computer tomography (µCT)
- Unpaired non-parametric t test

Results

Demineralization at 7 days and 37C produced a non-cavitated WSL

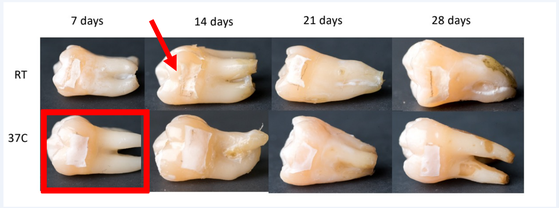

Figure 1 Photo of WSL produced after demineralization at different timepoints and temperatures. Demineralization of 7 days at 37 C was chosen for all subsequent experiments as it produced a non-cavitated enamel lesion (red box). Surface cavitations can be seen at 14 days (red arrow)

Results

The artificial WSL produced had an average mineral density of 1.57 ± 0.21g/cm3 and lesion depth of 167 ±0.03um as measured by µct

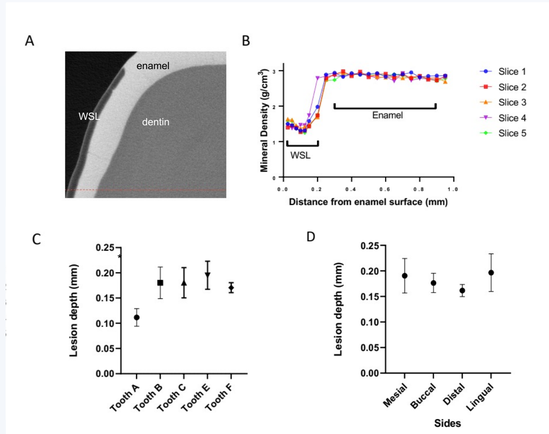

Figure 2 Characterization of artificial WSL by µCT **A.** µCT slice **B**. Plot of mineral density of WSL and distance from enamel surface **C**. Variability in lesion depth in different teeth **D.** Demineralization of different surfaces on one tooth produced a uniform WSL

Naturally occurring WSL have a thicker surface layer and appear more diffuse compared to artificial WSL

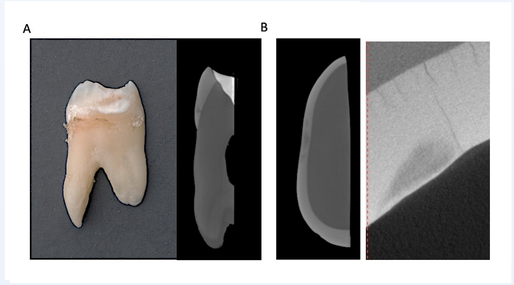

Figure 3 Characterization of naturally occurring WSL **A.** Clinical photo and **B.** µct slice of interproximal WSL

Results

Treatment of artificial WSL with AMTN-HAP resulted in a significant increase in mineral density and decrease in lesion depth by µct compared to control

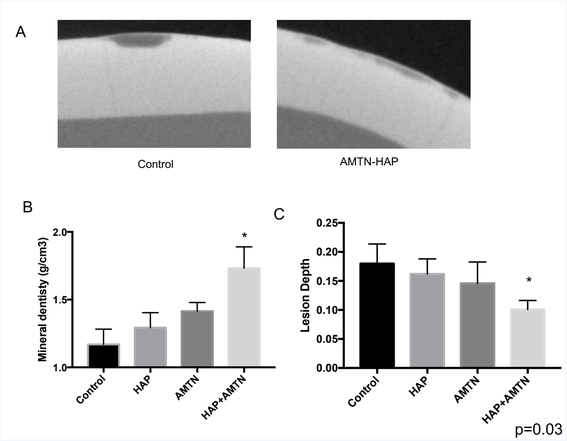

Figure 4 Treatment of artificial WSL with AMTN-HAP **A.** µCT slice of control and AMTN-HAP treated artificial WSL **B.** Increased mineral density and **C.** Decreased lesion depth by µCT of AMTN-HAP group compared to untreated group

Conclusion

Our early research suggests that AMTN-HAP are effective at remineralizing artificial WSL

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