Journal of Dentistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

The combined enamel remineralization potential of arginine and fluoride toothpaste

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ARTICLE INFO

Keywords:
Arginine
Enamel
Fluoride
Remineralization
Sodium fluoride

ABSTRACT

Objective(s): This study examined the remineralization potential of arginine (Arg) in NaF toothpaste.
Methods: Fifty enamel specimens allocated to five groups (n = 10) were subjected to artificial caries formation. A 10-day pH-cycling was performed to treat specimens as per group – [1]: 2% Arg – NaF, [2]: 4% Arg – NaF, [3]: 8% Arg – NaF, [4]: NaF and [5]: deionized water. The test solutions were subjected to pH measurement, fluoride estimation, Na-Cl element analysis using ICP-EOS and FTIR analyses. Mineral density of the specimens were assessed using micro-CT; while Ca/P ratio and surface fluorine concentration were determined using energy dispersive x-ray spectroscopy (EDS) and enamel fluoride uptake (EFU) by acid-etch method.
Results: pH, fluoride concentration and Na-Cl ratio exhibited significant difference amongst groups ($p < 0.001$). FTIR analysis showed presence of free amino acids in 2% and 4% Arg-NaF group. The mean mineral gain (0.40 ± 0.07 g/cm^3) and percent remineralization (27.91 ± 4.66%) of 2% Arg-NaF group were significantly higher than the other 4 groups ($p < 0.001$). Conversely, the median Ca/P ratio for 2% Arg-NaF (1.60) was significantly higher than deionized water (1.53) ($p = 0.029$). The mean surface fluorine concentration of specimens treated with 2% Arg-NaF (1.51 ± 0.14%) was significantly higher than treatment with NaF (1.02 ± 0.28%) ($p < 0.001$). The EFU of 2% Arg-NaF group (6.84 ± 1.59 μg/cm^2) was significantly higher than NaF group (5.22 ± 1.88 μg/cm^2) ($p < 0.001$).
Conclusion: Incorporation of 2% arginine in NaF toothpaste significantly increased the remineralization of enamel caries-like lesion when compared to NaF toothpaste; while 4% and 8% arginine in NaF toothpastes were ineffective in improving enamel remineralization.
Clinical Significance: In high-risk patients, daily use of 2% arginine in NaF toothpaste might provide a synergistic anti-caries effect given the proven prebiotic benefits of arginine in caries prevention and the demonstrated remineralization effect in the present study.

1. Introduction

Dental caries is one of the most prevalent conditions with a global prevalence of 35% for all ages combined [1,2]. The pathophysiology of dental caries is due to bacterial metabolism of fermentable carbohydrates, producing acid and demineralization of dental hard tissues [3]. The caries preventive agents mainly act by inhibiting bacterial acid production or by changing the de/remineralization equilibrium [4]. Fluoride has been identified as a potent caries preventive agent with significant benefits [5–9]. Daily brushing with fluoride toothpaste is the most common topical fluoride application method. However, there are problems associated with fluoride applications, such as toxicity at high doses. The availability of fluoride over the past few decades has now led to the evolution of fluoride-resistant *S. mutans* and other oral bacterial species [10]; hence, its actions on acid-producing microbes may be diminishing. Thus, it is imperative to supplement fluoride toothpaste with potent modifiers that enhances its remineralization potential and combats acidogenic microorganisms.

Arginine is a prebiotic-based organic compound that has recently been introduced as an additive to fluoride toothpaste and other oral care products with significant anti-caries benefits [11]. Arginine is a naturally occurring amino acid in dietary proteins. The high-protein diet metabolism leads to the presence of arginine in the oral cavity. Substantial salivary arginine (available in micro-concentrations) favors

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https://doi.org/10.1016/j.jdent.2018.06.009
Received 19 March 2018; Received in revised form 17 June 2018; Accepted 19 June 2018

the existence of less cariogenic biofilm by increasing oral biofilm pH owing to alkali production [12–14]. External arginine supplementation further strengthens the potential to produce oral alkali [15]. Therefore, arginine may complement the limitations of fluorides on oral biofilms.

The synergistic effects of arginine with fluorides and calcium in enamel remineralization have been reported previously [16,17]. Arginine has been added to toothpaste comprehending its potential as a biofilm modifier evident through clinical data [18]. Proteins (esp. serum albumin) enhances remineralization as a result of its affinity to adsorb fluorides [19,20]. Hence, it is quite possible that arginine (as a residual protein) might have a similar remineralization effect in incipient carious lesions. Moreover, the positively charged guanidinium group of arginine favors the attraction of highly electronegative fluorides [16].

Arginine in fluoride toothpaste is commercially available as 1.5% and 8% arginine with insoluble calcium base and 1450-ppm sodium monofluorophosphate (MFP). The 1.5% arginine-fluoride toothpaste was a priori introduced as a caries preventive agent [18]; while the 8% arginine-fluoride toothpaste with arginine bicarbonate variant, initially marketed for treatment of dentin hypersensitivity, was recently explored as a potent caries-preventive agent [21,22]. The effect of arginine with readily dissociable sodium fluoride (NaF) has been reported in several *in vitro* studies [16,23]. The arginine-NaF solution synergistically inhibits *S. mutans* and enhances *S. sanguis* in a multi-species biofilm [23]. An *in vitro* study found the remineralization effect of 2.5% arginine with 500-ppm NaF solution on artificial enamel carious lesion similar to that of control 500-ppm NaF solution; while the enamel fluoride uptake for the arginine-F solution was significantly higher [16]. Therefore, the interaction of arginine-NaF seems favorable in terms of enhancing the existing anti-caries effect of NaF.

So far, the published studies have only studied the effect of arginine in a low concentration NaF solution. No study has evaluated the interactive effect of arginine with high concentration (greater than 1000-ppm) NaF toothpaste, which is recommended for caries prevention in high caries-risk patients [6]. In addition, the optimum concentration of arginine to be incorporated in high concentration NaF toothpaste to impart effective anti-caries effect demands further evaluation. Hence, the aim of the present study was to examine the remineralization potential of arginine in NaF toothpaste. The null hypothesis tested was that the incorporation of arginine, regardless of its concentration, in NaF toothpaste has no additional remineralizing effect on artificial enamel caries-like lesions when compared to toothpaste with only NaF.

2. Materials and methods

2.1. Experimental study design

The experimental study design and ethical concerns were duly reviewed and approved by the Institutional Review Board of The University of Hong Kong – Hospital Authority Hong Kong West Cluster (Reference number: UW 17-058). A schematic representation of this study design is presented in Fig. 1. The outline represents significant steps in the experiment process. The study involved *in vitro* evaluation of enamel specimens, which underwent artificial enamel caries-like lesion formation, followed by investigations on the remineralization efficacy of three increasing arginine concentrations, supplemented to 1100-ppm F toothpaste, compared to respective positive and negative controls.

2.2. Sample power calculation

The prospective sample power calculation was done using G*Power 3.1 (Franz Faul, Germany) based on the results of a preliminary study. Considering the primary variable - mineral gain and based on the study protocol, the expected effect size was estimated to be 0.52 on standardized β/α: 4. Finally, the software computed the total sample size of 50 for 5 groups at significance of p-value < 0.05 with actual power quantified as 0.81 at critical F: 2.58, non-centrality parameter (λ): 13.52 using a priori protocol for F – tests sample power analysis.

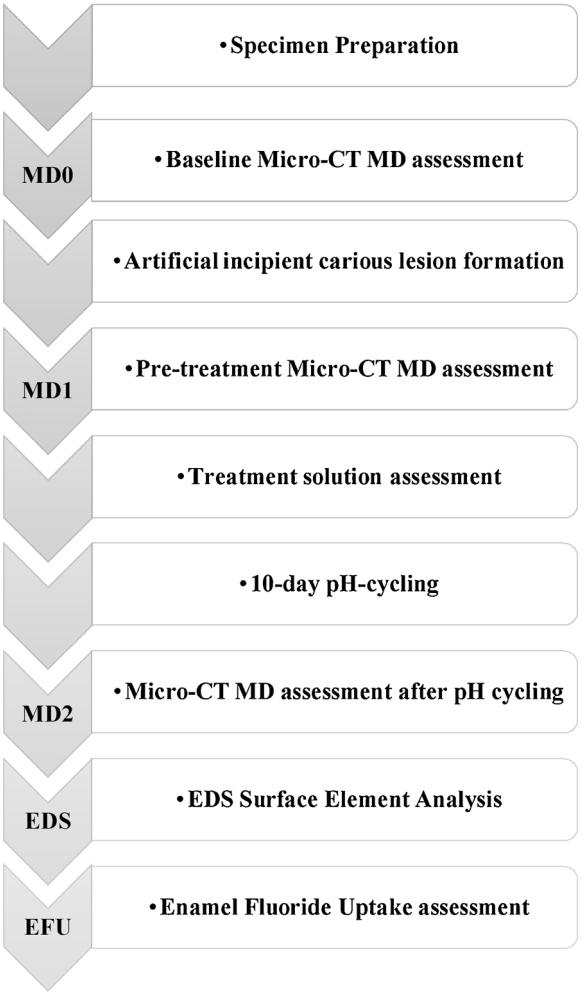

Fig. 1. Schematic representation of experimental study design.

2.3. Specimen preparation

Freshly extracted sound human third permanent molars collected after obtaining informed consent from patients were used in this study. The collected teeth were thoroughly debrided and disinfected before specimen preparation. The extracted teeth were primarily assessed using stereomicroscope (Carl Zeiss Stereo 475002, Germany) at 0.8x to rule out enamel defects like hypomineralisation, hypoplasia and fluorosis. Subsequently, the teeth were stored in 0.5% thymol solution at 4 °C. Fifty quadri-sectioned enamel specimens were prepared using 150-μm thick saw on a microtome (SYD Mikki Pulley, Japan). Initially, the selected teeth were decoronoted at the cemento-enamel junction. The obtained coronal structures were equally sectioned to acquire uniform sample outline. A minimum of 2.5 mm specimen height was maintained. A window of 3 × 3 mm^2 was covered with an equidimensional-masking strip; while other surfaces were covered with dual-applied acid-resistant nail varnish (Revlon®, New York, USA). Afterwards, the window was exposed to receive further treatment.

2.4. Demineralizing and remineralizing solutions

The demineralizing and remineralizing solutions were prepared as per the saturation concentration of hydroxyapatite minerals in saliva