

Effect of Proarginine Technology Plus 1450 PPM Fluoride on Enamel Remineralization: An in Vitro Study

Abstract

AIM: The objective of this in vitro study was to evaluate the effects of new dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride for their ability to promote remineralization of demineralized enamel. **Methods:** Twenty human premolar extracted for orthodontic reasons were used. The specimens were immersed in demineralization solution followed by application of proarginine paste with fluoride. All the samples were assessed using DIAGNOdent at the baseline and after demineralization and remineralization and was statistical analyzed by using one way anova test. **Results:** There was statistically significant increase in the DIAGNOdent[®] values after remineralization of the tooth with a p value <0.001. The results of this in vitro study support the conclusion that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provide superior protection against caries lesion and have remineralization potential to reverse early enamel lesion. However more, randomized clinical trials are needed to be designed in order to test the efficacy of this product.

Key Words

Apex DSP; full strength NaOCl; apex locator; working length

Sharan S Sargod¹, Sham Bhat S²,
Sundeep Hegde K³, Sajid Ahamed K⁴

¹Professor, Department of Paedodontics & Preventive Dentistry, Yenepoya Dental Collage, Mangalore, Karnataka, India

²Professor & Head, Department of Paedodontics & Preventive Dentistry, Yenepoya Dental Collage, Mangalore, Karnataka, India

³Professor, Department of Paedodontics & Preventive Dentistry, Yenepoya Dental Collage, Mangalore, Karnataka, India

⁴Post Graduation Student, Department of Paedodontics & Preventive Dentistry, Yenepoya Dental Collage, Mangalore, Karnataka, India

INTRODUCTION

Dental professionals recognize that new approaches to intervene earlier in the caries process are an important step in taking conservative dentistry and minimal invasive therapy to the next stage. Infact, the profession are actively looking for new technologies that are proven to be effective in “high risk” children and adults.^[1,2] Fluoride does not act upon the plaque biofilm, an important modifiable factor in dental caries, to reduce cariogenic challenge. As fluoride’s benefits are focused on the host tissue as a means of damage control, once the caries process has been initiated and is in progress, combining fluoride with an agent that prevents the caries process at an earlier stage, by targeting the residual plaque biofilm, would have potential to deliver a step change improvement in caries prevention.^[3] From a biological perspective, saliva is a key source of nitrogen-based metabolites, such

as arginine and urea, which are derived from the breakdown of peptides and proteins by salivary enzymes. Arginine is metabolized by arginolytic bacteria using the arginine deiminase system to produce energy in the form of adenosine triphosphate, and ammonia and carbon dioxide. The important feature of this pathway is the production of ammonia which neutralizes acids and promotes a more alkaline pH that is unfavorable to cariogenic bacteria. Experiments with mixed bacteria showed that a higher ratio of arginolytic to non-arginolytic bacteria resulted in a less cariogenic Stephan pH curve, and vice versa.^{4,5}

Based upon its proposed mechanism of action, which is complimentary to the well-known mechanism of action of fluoride, arginine with an insoluble calcium compound has the potential to significantly enhance the caries preventive benefits of traditional fluoride dentifrices. For this



Fig. A: Collection of samples

Fig. B: pH adjusted to 4.5 using ph meter

Fig. C: Teeth evaluating using DIAGNOdent ®

Table 1

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		ANOVA ...F value	p value
				Lower Bound	Upper Bound		
Baseline	20	7.90	2.972	6.51	9.29	141.262	.000
Demineralization	20	21.80	3.679	20.08	23.52		
Remineralization	20	14.55	3.154	13.07	16.03		

Table 2

		Mean Difference	Std. Error	change (%)		
					P value	
Baseline	Demineralization	-13.900	.934	175.95	.000	HS
	Remineralization	-6.650	.748	84.18	.000	HS
Demineralization	Remineralization	7.250	.788	33.26	.000	HS

reason, a new and innovative dentifrice based upon 1.5% arginine and 1450 ppm fluoride, as sodiummonofluorophosphate, in an insoluble calcium base has been developed and clinically validated. Traditional caries clinical methods do not allow investigators to evaluate the effects of an intervention on the disease process. For this reason, a number of advanced and more discerning techniques, such as Quantitative Light-induced Fluorescence (QLF) and the Electrical Caries Monitor (ECM) have been developed, and their use in clinical trials has been refined and optimized to enable the detection and monitoring of pre-cavitated lesions over time. These advanced techniques are gaining wide acceptance as state-of-the-art caries detection methods.^[6,7] So the aim of the current study was to confirm the hypothesis that the new dentifrice containing 1.5% arginine, an insoluble calcium compound with 1450 ppm fluoride, have remineralization potential to reverse early enamel lesion using DIAGNOdent® (kavo).

MATERIALS AND METHODS

Twenty humans sound premolar extracted for orthodontic reasons in patient aged 12-15 years were collected and cleaned with sterile gauze soaked in distilled water and was stored in sterile container with 5% formalin solution. The teeth were thoroughly cleaned of its debris, calculus, and soft

tissues. All the teeth were sliced mesio-distally into buccal and lingual halves using a diamond disk bur (Fig. A). The samples that showed no evidence of white spot lesion, enamel cracks, or caries on visual inspection was taken for evaluation. Each extracted tooth was coated with nail varnish, leaving an enamel window of 3mm × 3mm on the buccal surface in the middle one-third of the crown. The buccal halves of the teeth were used for the study, taking into consideration the technique sensitivity of DIAGNOdent® (KaVo). As recommended by the manufacturer, prior to every measurement session the instrument was calibrated against its own ceramic standards. The baseline value of each tooth was recorded. Samples showing a moment value between 3 and 10 on the digital display were selected. Followed by placement of teeth in demineralizing solution [2.2mMKH₂PO₄, 50mM acetic acid, and 2.2 mMCaCl₂] and pH was adjusted each day using ph meter to 4.5 (Fig. B), and teeth were kept in demineralizing solution for three days after which teeth was taken out, washed with deionized water, dried, and placed back in their respective clean container. The teeth were evaluated with DIAGNOdent® and the samples showing a moment value above 10 on the digital display was taken for further evaluation (Fig. C). The samples were then treated with the pro-arginine paste,

continuously 14 days applying twice daily using an applicator and were kept in incubator followed after which rinsed with deionised water, dried, and cleaned and DIAGNodent[®] reading was again taken to evaluate the remineralization and results were statistically analyzed.

Statistical Analysis

Statistical analysis was carried out by one-way ANOVA using SPSS version 11.5 with post-hoc tests to compare the statistical difference of between the DIAGNodent[®] values at baseline, demineralization and after remineralisation.

RESULTS

The present study evaluated the remineralization potential of proargininepaste with 1450ppm fluoride on artificial enamel subsurface lesions using DIAGNodent[®] (KAVO). The results of demineralization and remineralization value are tabulated below respectively. The mean baseline value answer was 7.90 ± 2.972 and mean demineralization values were 21.80 ± 3.679 . After application of the proarginine paste the mean remineralisationvalue were 14.55 ± 3.15 (p value < 0.001). The result in the study showed there was statistically significant increase in the DIAGNodent[®] values after remineralization of the tooth.

DISCUSSION

Early lesions can be arrested and reversed by remineralization. Re-mineralization occurs following de-mineralization, when the acid challenge is removed, saliva becomes saturated in calcium and phosphate, once again, and these calcium and phosphate ions are driven back into the demineralized tooth tissue resulting in net mineral gain and "repair" of the enamel's hydroxyapatite structure.^[8,9] Specifically, attempts to reduce caries risk have focused on reducing the tooth's susceptibility to acid attack by rendering the tooth's surface less vulnerable to de-mineralization. In addition, by enhancing the uptake of calcium and phosphate ions into de-mineralized tissue, remineralization can be increased.^[10] Kleinberg has developed a highly effective fluoride-free anticaries technology based on the protective benefits provided by saliva. This technology is based upon a combination of arginine, calcium carbonate, and a cariostatic anion, such as bicarbonate, to deliver anticaries benefits. The basic research that led to this new technology was followed by clinical research demonstrating that products containing it can actually deliver clinically meaningful benefits

in cavity prevention.^[11] Based upon its proposed mechanism of action, which is complimentary to the well-known mechanism of action of fluoride, arginine with an insoluble calcium compound has the potential to significantly enhance the caries preventive benefits of traditional fluoride dentifrices.^[12] For this reason, a new and innovative dentifrice based upon 1.5% arginine, 1450 ppm fluoride, as sodium monofluorophosphate, in an insoluble calcium base has been evaluated in this study. The results of study showed there was statistically significant increase in the DIAGNodent[®] reading after application of pro-arginine paste on the demineralized tooth surface with a p value < 0.001 which indicates effect of pro-arginine plus 1450 ppm fluoride on enamel remineralization. Previously two root caries studies in adults have each shown that the new dentifrice containing 1.5% arginine and 1450 ppm fluoride in a calcium base is significantly more effective in arresting and reversing root caries lesions than a dentifrice containing 1450 ppm fluoride alone.^[13,14]

A 2-year conventional caries clinical study has proven that two dentifrices containing 1.5% arginine and 1450 ppm fluoride in a calcium base, one with di-calcium phosphate and the other with calcium carbonate, are significantly more effective in preventing the formation of cavitated caries lesions than a dentifrice containing 1450 ppm fluoride alone.^[15] Drawbacks of the present study include period of remineralization used in the study was 14 days, which could not remineralize artificial caries completely, thus the period of application for complete remineralization cannot be described for remineralising agents used in the study. Although surface remineralization was confirmed, enamel subsurface remineralization was not evaluated in the study.

CONCLUSION

The following conclusion can be drawn from the study that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride can provide protection against caries lesion and have remineralization potential to reverse early enamel lesion. More, randomized clinical trials are needed to be designed in order to test the efficacy of this product.

REFERENCES

1. Cummins D. The impact of research and development on the prevention of oral diseases in children and adolescents: an industry

- perspective. *Pediatric Dentistry*. 2006;28:118-27.
2. Fisher J, Johnston S, Hewson N, van Dijk W, Reich E, Eisele JL, *et al*. FDI Global Caries Initiative; implementing a paradigm shift in dental practice and the global policy content. *International Dental Journal*. 2012;62:169-74.
 3. Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. *Caries Research*. 2004;38:182-92.
 4. Traudt M, Kleinberg I. Stoichiometry of oxygen consumption and sugar, organic acid and amino acid utilization in salivary sediment and pure cultures of oral bacteria. *Archives of Oral Biology*. 1996;41:965-78.
 5. Kanapka J, Kleinberg I. Catabolism of arginine by the mixed bacteria in human salivary sediment under conditions of low and high glucose concentrations. *Archives of Oral Biology*. 1983;28:1007-15.
 6. Cortes DF, Ellwood RF, Ekstrand KR. An in vitro comparison of a combined Foti/visual examination of occlusal caries with other diagnostic methods and the effect of stain on their diagnostic performance. *Caries Research*. 2003;37:8-16.
 7. Stookey GK. Quantitative light fluorescence: a technique for early monitoring of the caries process. *Dental Clinics of North America*. 2005;49:753-70.
 8. Kleinberg I. The other side of confectionary use and dental caries. *Journal of the Canadian Dental Association*. 1989;55:837-8.
 9. Featherstone JD. The caries balance: contributing factors and early detection. *Journal of the Californian Dental Association*. 2003;31:129-33.
 10. Cummins D. Dental caries: a disease which remains a public health concern in the 21st century. The exploration of a breakthrough technology for caries prevention. *Journal of Clinical Dentistry*. 2013;24:1-14.
 11. Acevedo AM, Machado C, Rivera LE, Wolff M, Kleinberg I. The inhibitory effect of an arginine bicarbonate/calcium carbonate Cavistat-containing dentifrice on the development of dental caries in Venezuelan school children. *Journal of Clinical Dentistry*. 2005;16:63-70.
 12. Cummins D. Dental caries: a disease which remains a public health concern in the 21st century. The exploration of a breakthrough technology for caries prevention. *Journal of Clinical Dentistry*. 2013;24:1-14.
 13. Hu DY, Yin W, Li X, Feng Y, Zhang YP, Cummins D, *et al*. A clinical investigation of the efficacy of a dentifrice containing 1.5% arginine and 1450 ppm fluoride as sodium monofluorophosphate on primary root caries. *Journal of Clinical Dentistry*. 2013;24:23-31.
 14. Souza MLR, Cury JA, Tenuta LMA, Zhang YP, Mateo LR, Cummins D, *et al*. Comparing the efficacy of a dentifrice containing 1.5% arginine and 1450 ppm fluoride to a dentifrice containing fluoride alone in the management of primary root caries. *Journal of Dentistry*. 2013;41:35-41.
 15. Kraivaphan P, Amornchat C, Triratana T, Mateo LR, Ellwood R, Cummins D, *et al*. Two-year caries clinical study of the efficacy of novel dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450ppm fluoride. *Caries Research*. 2013. 10,1159/000353183.