journal of dentistry 38 (2010) 671–677

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden

Effect of fluoride and of calcium sodium phosphosilicate toothpastes on pre-softened dentin demineralization and remineralization *in vitro*

I. Diamanti [a], *H. Koletsi-Kounari* [a,*], *E. Mamai-Homata* [a], *G. Vougiouklakis* [b]

[a] *Department of Preventive and Community Dentistry, University of Athens, Dental School, 2 Thivon St. 115 27, Athens, Greece*
[b] *Department of Operative Dentistry, University of Athens, Dental School, 2 Thivon St. 115 27 Athens, Greece*

ARTICLE INFO

Article history:
Received 18 February 2010
Received in revised form
10 May 2010
Accepted 13 May 2010

Keywords:
Bovine root dentin
Fluoride
Calcium sodium phosphosilicate
Demineralization
Remineralization
Toothpaste
pH cycling
Acid resistance test

ABSTRACT

Objectives: The purpose of this *in vitro* study was to evaluate the effect of toothpastes containing sodium fluoride in different concentrations or a calcium sodium phosphosilicate system on pre-softened dentin demineralization and remineralization.
Methods: During a ten day pH-cycling protocol, pre-softened bovine root dentin slabs were immersed twice daily, after the demineralization periods, for 2 min, in the following toothpaste slurries: (a) non-fluoridated (control), (b) 7.5% calcium sodium phosphosilicate, (c) 1450 ppm F, (d) 2800 ppm F and (e) 5000 ppm F. Subsequently, the specimens were subjected to a 15-h acid resistance test. Surface microhardness was assessed initially and during the pH-cycling and the acid resistance test period. Repeated measurements in each group were analyzed through appropriate regression models for longitudinal data.
Results: All fluoride groups, during pH cycling, showed significantly less microhardness loss ($p \leq 0.010$, $p \leq 0.002$, $p \leq 0.002$) and subsequently exhibited increased acid resistance ($p \leq 0.010$, $p \leq 0.001$, $p \leq 0.001$) compared to the control. The 5000 ppm and 2800 ppm F toothpastes, inhibited demineralization significantly more effectively than the 1450 ppm F ($p \leq 0.001$, $p \leq 0.030$) and the calcium sodium phosphosilicate toothpaste ($p \leq 0.001$), while no significant differences were found between the two high fluoride groups ($p = 0.130$). The calcium sodium phosphosilicate toothpaste, during pH cycling showed a difference that approached statistical significance compared to control ($p = 0.079$), but its acid resistance behavior was similar to control ($p = 0.610$).
Conclusions: Under these experimental conditions, the high fluoride toothpastes promoted remineralization and inhibited demineralization more effectively, than the 1450 ppm F, the non-fluoridated (control) and the calcium sodium phosphosilicate toothpastes.

1. Introduction

Although root caries can be present in young individuals, the prevalence,[1] incidence[2] and severity[3,4] of the disease tends to increase with age and it is likely to become an important public health issue in the near future, as a result of coexistence of factors such as the population ageing and the growing tendency for retaining natural teeth at older age.[5]

Root caries initiation involves mineral loss due to surface attack by weak organic acids of bacterial origin and this process seems to be a prerequisite for enzymatic degradation of exposed denatured collagen matrix and cavity formation.[6] In root caries lesions, dentin is directly involved, due to the

* *Corresponding author at*: Tel.: +30 210 746 1291; fax: +30 210 746 1132.
E-mail address: hkounari@dent.uoa.gr (H. Koletsi-Kounari).
0300-5712/$ – see front matter
doi:10.1016/j.jdent.2010.05.010

fact that the thin cementum layer can be easily abraded with brushing or repeated scaling.[7] Although the average pH of natural dentin caries lesions is estimated to be approximately 5.0,[8] *in vitro* studies have shown demineralization of human tooth roots to occur at pH as high as 6.7.[9] Demineralization proceeds 2.0–2.5 times faster in dentin than in enamel at corresponding *in vitro*[10] and *in situ*[11] conditions and also, dentin shows less tendency to remineralize.[10] These observations are mainly attributed to dentin's higher solubility compared to enamel in the oral micro-environment,[12] although caries progression rate in the tissue also depends on diffusion phenomena on the crystal surfaces.[13] More specifically it has been suggested that, the speed of lesion formation is primarily influenced by acid diffusion rate through peritubular dentin[13] and that collagen matrix contributes at a later stage, by expansion of partially demineralized fibers and increase of their electropositive charge, to retardation or inhibition of hydrogen cation penetration inside the tissue.[14]

Clinical observations suggest that, fluoride provision in various vehicles is effective at preventing root caries occurrence[15] and at arresting[16] or possibly reversing[17] the process of lesion development. Among them, toothpaste formulations have been widely accepted as the principle means of delivering topical fluoride.[18] However, although dentin presents higher capacity for fluoride uptake compared to enamel at corresponding *in vitro*,[19] *in situ*[20] and *in vivo*[21] conditions, it has been indicated that, dentin, compared to enamel, requires significantly higher doses of fluoride for protection against cariogenic attack.[10,22,23]

It seems likely that, the higher the fluoride concentration, the greater the driving force for fluoride diffusion through cariogenic biofilm towards the tooth surface.[24] Moreover, in the light of the high 'critical pH' at which root dentin begins to dissolve in the oral environment, exposed root surfaces are considered to be at high caries risk and they may benefit from the use of a high fluoride toothpaste.[25]

Although fluoride is found to be clinically effective in reducing caries levels, it seems to be insufficient to overcome high caries challenge in many individuals and there is a need to find new approaches to enhance the remineralization process.[26,27] These new approaches may focus either on enhancement of calcium and phosphate delivery and effective localization, especially in persons with reduced salivary function, or at more effective antibacterial treatment in conjunction with improved remineralization means.[27] Recently developed remineralization strategies, involve the use of various commercialized calcium-phosphate based systems, where the manufacturers claim that, the specific form of calcium phosphate helps to overcome possible limited bioavailability of corresponding ions in the vicinity of hard tissue and so, it promotes mineral regain process in apatitic forms.[28]

Among them, a system based on calcium, sodium phosphosilicate glass particles which are reactive when exposed in aqueous environments such as saliva, results in the formation of a crystalline hydroxyl-carbonate apatite layer with time, which is claimed to be structurally and chemically similar to biological apatite.[29] One laboratory report from the company suggested that, the use of a toothpaste containing 5% calcium sodium phosphosilicate with or without the addition of 5000 ppm F (NaF) in a pH-cycling model, was significantly more effective at enhancing remineralization of bovine root dentin lesions, compared to the use of a 5000 ppm F (NaF) or a 1000 ppm F (NaF) dentifrice.[29]

The aim of this study was, to evaluate the effect of commercially available toothpastes containing 1.1% NaF (5000 ppm F), 0.616% NaF (2800 ppm F), 0.319% NaF (1450 ppm F), or a 7.5% calcium sodium phosphosilicate system, on demineralization and remineralization of pre-softened bovine root dentin in a pH-cycling model, followed by testing of "acquired acid resistance" of treated tissue, *in vitro*.

2. Materials and methods

A total of 50 bovine root dentin slabs were used. The following toothpaste slurries (1:3 in distilled water) were applied: (a) non-fluoridated (control), (b) 7.5% calcium sodium phosphosilicate, (c) 1450 ppm F, (d) 2800 ppm F and (e) 5000 ppm F. The slurries were applied on pre-softened bovine root dentin slabs that were exposed to ten daily demineralization/remineralization cycles, according to the methodology developed by Koulourides.[30]

2.1. De/remineralizing solutions

The composition of the demineralizing (DM) solution was 0.1 M lactic acid containing 3 mM Ca, 1.8 mM P (derived from 17 mM tricalcium phosphate) in 1% CMC adjusted to pH 4.5. The remineralizing (RM) solution contained 3 mM Ca, 1.8 mM (derived from 17 mM tricalcium phosphate) in 1% CMC and was adjusted to pH 7.0.

2.2. Preparation of dentin slabs

Sections, approximately 3 mm × 5 mm × 3 mm, were cut from previously unexposed to the oral environment root surfaces of permanent bovine incisors and mounted with sticky wax on Plexiglas blocks. The specimens were progressively polished with wetted silicon carbide papers, up to 4000-grit.

2.3. Preparation of artificial lesions

The slabs were immersed in DM solution, pH 4.5 (20 ml/slab) for 8 h at 37 °C.

2.4. Experimental procedure

Fifty pre-softened dentin slabs were divided to five groups of ten slabs each. Initially, dentin slabs were subjected to ten daily DM/RM cycles. Each daily cycle consisted of 30 min DM period followed by a 4-h RM period, a second 30 min DM period and overnight RM (16 h). The slabs of each group were placed in either DM or RM solution (20 ml/slab renewed at every change) at 37 °C. The slabs were immersed in the previously described toothpaste slurries twice daily, after the demineralization periods and the exposure time was 2 min. All slabs were rinsed with distilled water for 15 s before and after any DM/RM solution change or toothpaste slurry application and were wiped dry with soft tissue paper.