A PRELIMINARY STUDY OF ENAMEL REMINERALIZATION BY DENTIFRICES BASED ON RECALDENT™ (CPP-ACP) AND NOVAMIN® (CALCIUM-SODIUM-PHOSPHOSILICATE)

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ABSTRACT
The purpose of this study was to investigate the enamel remineralization potential of two toothpastes, one of which was based on Recaldent™ (CPP- ACP) and the other on NovaMin® (Calcium-sodium-phosphosilicate).
Human permanent molar teeth were subjected to three consecutive demineralization cycles. These cycles were followed by remineralization of the experimental groups by toothpastes containing Recaldent™ and NovaMin® respectively. The samples were analyzed by Scanning Electron Microscope, (SEM) and energy-dispersive X-ray spectroscopy analysis (EDX). Extensive demineralization was noted in the control group (without remineralization) while the groups treated with the dentifrices demonstrated various degrees of remineralization, as shown by formation of different types of deposits on the enamel surface. The EDX analysis showed increased amounts of Ca, P, Si and Zn in the enamel of the experimental groups, compared to the control one.
Toothpastes containing Recaldent™ and especially NovaMin® have the potential to remineralize enamel, a property which might be important in finding a substitute to pit and fissure sealing.

Key words: toothpastes, tooth demineralization, tooth remineralization, dental enamel.

ESTUDIO PRELIMINAR DE LA REMINERALIZACIÓN DE ESMALTE PRODUCIDA POR DENTÍFRICOS EN BASE A RECALDENTTM (CPP-ACP) Y NOVAMIN® (PHOSPHOSILICATO DE CALCIO Y SODIO)

RESUMEN
El objetivo del trabajo fue investigar el potencial de remineralización del esmalte de dos pastas dentífricos, una de ella con formulación basada en Recaldent™ (CPP- ACP) y la otra en NovaMin® (phosphosilicato de calcio y sodio).
Se realizaron tres ciclos cosecutivos de desmineralización en molares permanentes humanos, seguidos de remineralización, en los grupos experimentales con los dentí fricos que contenían Recaldent™ y NovaMin® respectivamente. Se analizaron las muestras con microscopía electrónica de barrido (SEM) y análisis espectroscópico por dispersión de rayos X (EDX). En el grupo control (sin remineralización) se observó una extensa demineralización mientras que los grupos tratados con los dentífricos mostraron varios grados de remineralización, evidenciados por la formación de diferentes tipos de depósitos sobre la superficie del esmalte. El análisis EDX mostró cantidades aumentadas de Ca, P, Si y Zn en los grupos tratados en comparación con el grupo control.
Los dentífricos conteniendo Recaldent™ y especialmente NovaMin®, tienen potencial de reminalización del esmalte, una propiedad que puede resultar importante como substituto del sellado de fosas y fisuras.

Palabras clave: dentífricos, desmineralización dentaria, remineralización dentaria, esmalte dental.

INTRODUCTION

The potential for remineralization of damaged tooth surfaces, especially in children, is appreciable. Immature permanent teeth immediately after eruption undergo maturation, resulting in minerals being precipitated in the enamel rods. Standard procedures for protection of these teeth are fissure sealing and topical fluoride application[1]. So far, none of these procedures is completely efficient, therefore attempts have been made to find effective anticariogenic and remineralizing agents. The recently reported Recaldent™ - casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP) are an example[2], and they have been shown to have anticariogenic potential in various experimental studies[2-5].

Another group of mineralizing materials are bioactive glasses, though to date they have been mainly used in bone mineralization. These materials are capable of bonding chemically to bone and their components are oxides of calcium, sodium, phosphorus and silica in ratios that impart bioactivity. *In vivo*, these glasses are able to form a layer of hydroxyapatite on their surface as a first step in becoming fully incorporated into the human body[6,7].

One commercial bioactive glass that has been used in the treatment of the dentinal hypersensitivity is NovaMin®, a material which was originally developed as a bone regeneration material. NovaMin® is a ceramic material consisting of amorphous sodium-calcium-phosphosilicate which is highly reactive in water, and as a fine particle size powder can physically occlude dentinal tubules[8]. In the aqueous environment of the tooth, sodium ions from the NovaMin® particles rapidly exchange with hydrogen cations (in the form of H_3O^+). This leads to release of calcium and phosphate (PO_4^{3-}) ions from the material[9,10]. A localized, transient increase in pH occurs during the initial exposure of the material due to the release of sodium. This increase in pH helps to precipitate the extra calcium and phosphate ions provided by the NovaMin® particles to form a precipitated calcium phosphate layer. As these reactions continue, this layer crystallizes into hydroxycarbonate apatite which is chemically and structurally equivalent to naturally occurring biological apatite[11]. The combination of the residual NovaMin® particles and the newly formed hydroxycarbonate apatite layer physically occludes the dentinal tubules.

The purpose of the present study was to investigate the enamel remineralization potential of two agents containing calcium and phosphate ions, one of which was based on Recaldent™ (CPP- ACP) and the other on NovaMin® (Calcium-sodium-phosphosilicate). The null hypothesis was that there was no difference between the specimens treated with the potential remineralizing agents and the control group.

MATERIALS AND METHODS

Fifteen young immature permanent human molars, extracted for orthodontic reasons, were used in the study. The experiments were conducted in accordance with the Declaration of Helsinki and were approved by the Ethical Committee of the Faculty of Dentistry. All procedures were carried out with the adequate understanding and written consent of the subjects.

The roots were cut with a diamond bur with high speed dental handpiece at the level of the cemento-enamel junction, and the remnants of the pulp tissue discarded. The coronal segments were thoroughly ultra-sonicated and polished with pumice and polishing toothpaste. The excess toothpaste was cleaned by water-spray for 3 minutes. The teeth were divided randomly into 3 groups, consisting of 5 teeth each. The first group served as control, the second was treated with Recaldent™ containing dentifrice and the third with NovaMin® containing dentifrice.

All of the groups were submitted to three consecutive cycles of demineralization with 24 hours' duration. The demineralization was carried out by an acidic artificial caries gel, prepared according to the method described by Arends et al[12]. It consisted of: 6% by weight hydroxyl-ethyl cellulose; 0.1 mol/l lactic acid and 1.0 mol/l NaOH, adjusted to pH= 4.5. After each cycle of demineralization, the second and the third group were treated by GC Tooth Mousse (GC International, Itabashi-ku, Tokyo, Japan) and Nanosensitive® hca (Hager & Werken, GmbH & Co KG) (Table 1) respectively for 15 minutes. Then the dentifrices were cleaned with toothbrush for 5 minutes under copious water-spray to eliminate the possible leftovers.

The teeth were cut by half along the longitudinal axis. The first half of each sample was gold-sputtered and analyzed under Scanning Electron Microscope, (SEM) in secondary electron mode (Cambridge Stereoscan 360 High-Resolution Scanning Electron Microscope, Cambridge Instruments, Co., UK); while the other half was cast in Epo-Thin resin (Buehler®, USA, Batch No.20-8140-032), cured in a vacuum desiccator for 24 hours, polished with different sizes of carborundum grits up to 1μm diamond, carbon coated and analyzed under SEM in backscattered electron mode JEOL JSM 5310LV Scanning Electron Microscope. Also, quantitative energy-dispersive X-ray spectroscopy analysis point analysis (EDX) ISIS 300 Systems, (Oxford Instruments Co., UK) was performed on the enamel surface to determine the levels of Na, Mg, Al, Si, P, Cl, K, Ca and Zn. For each sample, five points were selected and the mean values calculated. The statistical analysis was performed by one-way ANOVA. When statistically significant differences appeared (at the level of significance $p<0.05$), the post hoc – Tukey Honest significant difference test was applied.

Table 1: Toothpastes used and their composition.

Toothpastes	Manufacturer	Active substance	Ingredients
GC Tooth Mousse (TM)	GC International, Itabashi-ku, Tokyo, Japan	Recaldent CPP-ACP (Casein phosphopeptide - amorphous calcium phosphate) 10.0%	Glycerol, Propylene glycol, D-glucitol, Colloidal Silica, Sodium carboxyl methyl cellulose (CMC-Na), Titanium dioxide, Xylitol, Guar Gum, Phosphoric acid, Sodium saccharin, Zinc oxide, Magnesium oxide, Ethyl 4-hydroxybenzoate, Propyl 4-hydroxybenzoate
Nanosensitive® hca (NS)	Hager & Werken, GmbH & Co KG	Calcium Sodium Phosphosilicate (NovaMin) 7.0%	Glycerine, PEG-8 distearate, Silica, Sodium Lauryl Sulfate, Titanium Dioxide, Aroma, Carbomer, Potassium Acesulfame

Table 2: Elemental analysis (EDX) of the enamel.

Elem%	Na	Mg	Al	Si	P	Cl	K	Ca	Zn
Control	0.58 (0.27)	0.19 (0.12)	0.07 (0.03)	0.29^{a} (0.12)	20.55^{ab} (2.87)	0.57 (0.27)	0.04 (0.03)	36.89^{ab} (3.25)	0.12^{a} (0.08)
GC Tooth Mousse	0.55 (0.24)	0.15 (0.10)	0.07 (0.04)	0.36^{b} (0.15)	23.22^{a} (2.44)	0.66 (0.32)	0.02^{a} (0.04)	39.66^{a} (3.40)	0.11^{b} (0.08)
Nanosensitive® hca	0.68 (0.18)	0.18 (0.13)	0.11 (0.11)	0.42^{ab} (0.21)	23.26^{b} (4.02)	0.60 (0.36)	0.06^{a} (0.06)	39.93^{b} (4.51)	0.20^{ab} (0.15)
p (ANOVA)	0.08	0.45	0.13	0.03	0.01	0.58	0.02	0.01	0.01

Values are expressed as means and standard devistions in parenthesis
* identical superscripts indicate statistically significant difference at p< 0,05

RESULTS

The EDX analysis (Table 2) represents the element distribution in the enamel of the tested groups. Unlike the control, in general higher quantities of Si, P, Ca and Zn appeared in the enamel of the experimental groups. GC Tooth Mousse significantly increased the level of Ca and P only; while the treatment with Nanosensitive® hca significantly increased all four elements.

The SEM images of the control samples (Fig. 1) show decomposed enamel surface with lost integrity. On the higher magnification images (Fig. 1c, d) it is noticeable that the enamel is comprised of indistinct and completely destructed enamel rods with wide inter-rod spaces and leftovers of the fractured enamel prisms' bases.

The results from the two experimental groups (Fig. 2 and 3) were completely different. Teeth samples from both groups had plugs that practically sealed

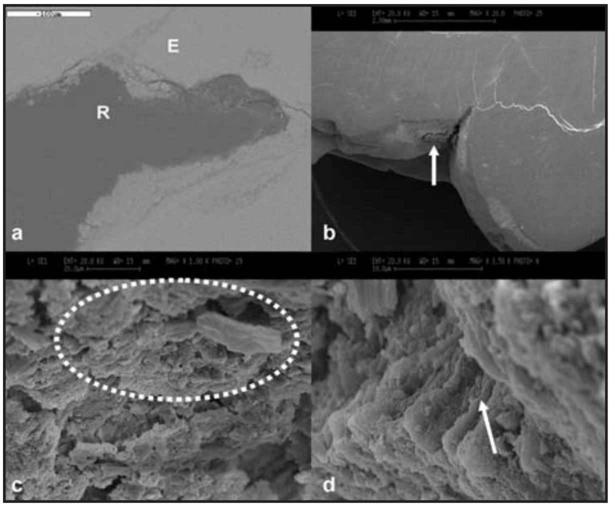

Fig. 1: SEM images of the tooth samples from the control group: a. backscattered image; b., c., d. secondary electron image of the bottom of the fissure representing demineralized enamel surface (a., b. lost enamel integrity; c. decomposition of the enamel, indistinguishable enamel rods and fractured enamel segments; d. bases of fractured enamel rods; E- Enamel, R- Resin used for casting.

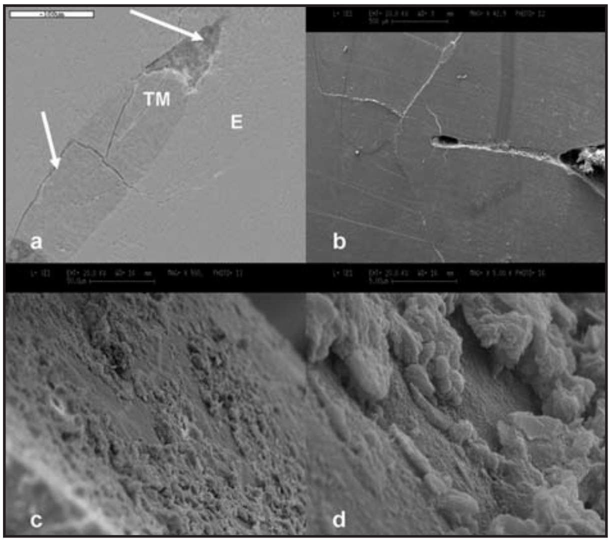

Fig. 2: SEM images of the tooth samples treated with GC Tooth Mousse: a. backscattered image; b., c., d. secondary electron image of the occlusal surface with formation of plugs that seal the fissure system (a. plugs that do not occlude the fissure intimately, empty space at the bottom; b. presence of some loose material above the plug; c., d. amorphous deposits on the surface); TM- GC Tooth Mousse, E- Enamel.

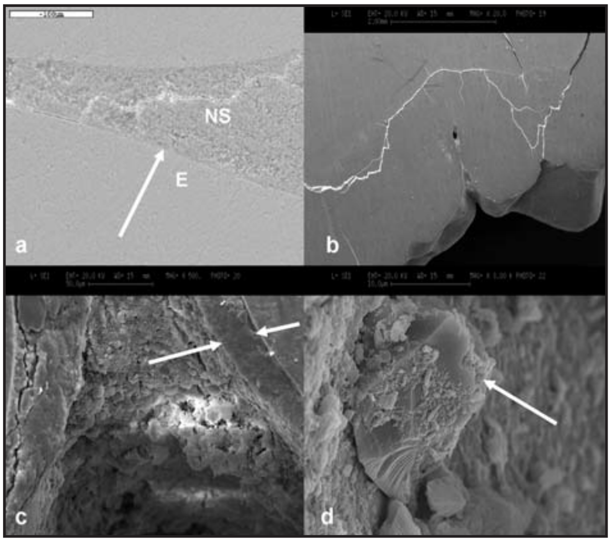

Fig. 3: SEM images of the tooth samples treated with Nanosensitive® hca: a. backscattered image; b., c., d. secondary electron image of the occlusal surface with formation of plugs that seal the fissure system (a. excellent sealing of the fissure system; b. absence of loose segments on the surface of the sealed fissure; c. intimate contact between the enamel and the bioactive glass particles, formation of a layer that resembles the ion-exchange layer in glass-ionomer cements (arrows); d. numerous angular deposits on the surface); NS- Nanosensitive® hca, E- Enamel.

the fissures, although the plugs created by Nanosensitive® hca appeared to be more compact and intimately attached to the enamel surface. In addition, both dentifrices left deposits which were firmly fixed to the enamel surface. The deposits formed by GC Tooth Mousse were smaller and amorphous (Fig. 2c,d), while Nanosensitive hca created larger, more angular deposits (Fig. 3c, d).

DISCUSSION

In the current study, two types of remineralizing agents were used and incorporated into toothpastes, namely CPP-ACP and bioactive glass. Although their mechanisms of action are different, the results obtained indicate that both of the toothpastes enhanced enamel remineralization. Therefore, the null hypothesis (that there was no difference between the control group and the experimental groups) was rejected.

CPP-ACP is known to be a source of calcium and phosphate close to the sites of possible demineralization, and this is likely to inhibit demineralization, enhance remineralization or possibly both[13]. The rate of calcium loss from plaque during cariogenic attack in the presence of CPP-ACP will thus decrease, and permit a rapid return to resting calcium concentrations[14]. This will allow immediate remineralization. Another possible mechanism to prevent demineralization is that casein is able to buffer plaque acid either directly or indirectly through bacterial catabolism. This agent is able to release amino acids, and these accept protons and act as buffers[15].

The group treated with CPP-ACP was remineralized by calcium and phosphorus, and the resulting calcium-phosphate layer was found to be amorphous. Previous studies have demonstrated that CPP-ACP enhances the remineralization of artificially formed dentinal lesions and the suggested mechanism for this is the stabilization of calcium phosphates on the tooth surface by the casein phosphopeptides, which leads to high concentration gradients of calcium and phosphate ions, thus promoting the remineralization of hard tissues[16].

Enamel remineralization by the bioglass-containing toothpaste occurred by a different mechanism, namely the incorporation of different elements into the enamel structure. Microscopic observations in the present study demonstrate the existence of an ion-enriched layer, firmly attached to the enamel surface. The basis of their bonding is the chemical reactivity of the glass in the presence of body fluids. The surface chemical reaction results in the formation of a hydroxycarbonate apatite (HCA) layer[9]. Glasses of this type incorporated into glass-ionomer cements have been shown previously to assist dentine remineralization and promote ion-exchange bonding at the tooth surface[6,7].

Previous studies have used bioactive glass to treat dentine hypersensitivity by obturation of the exposed dentinal tubules. Gillam et al[17] used a conventional bioglass and an experimental dentifrice in this manner. They found that bioactive glass particles can partially occlude the dentinal tubules, but they can be displaced by washing, so they thought that the inclusion of bioactive glass particles in a suitably formulated medium (a toothpaste, for example), would be the possible solution to this problem. The findings of the present study indicate that the deposits formed on the surface are firmly attached, since they were not removed by thorough washing and brushing. Unlike the deposits from the group treated with CPP-ACP, these deposits had silica and zinc as components, as well as calcium and phosphorus.

Bioactive glasses interact with human dentine after exposure to whole saliva and can adhere to dentine under these conditions[18]. A study which used nanoparticulate bioactive glass tried to remineralize dentine, but the newly precipitated apatite mineral was not mechanically stable[19] and little or no remineralization occurred. By contrast, the results in the present study suggest that the enamel is easily remineralized.

Another possible anticariogenic mechanism of the bioactive glasses is the antibacterial effect of high rates of ion release with their associated local changes in pH[20,21]. A previous study, using a toothpaste containing Novamin®, concluded that this was the first report of toothpaste with a therapeutic effect on gingival tissue that did not contain antibiotics and/or fluoride[22].

In the present study, a thorough toothbrushing and water spray were used to eliminate the leftovers of the toothpastes, in order to simulate (as much as possible) the conditions in the oral cavity. The deposits appear to be firmly attached to the surface, since they were not affected even by the desiccation of the samples during their preparation for the SEM and the EDX. Additionally, charging does not appear on the SEM micrographs, which suggests that no loose segments are present at the enamel surface.

The combined favorable properties of toothpastes containing Recaldent™ and Novamin® reported in this study show its potential as a remineralizing agent in permanent immature teeth.

CONCLUSIONS

Within the limitations of an *in vitro* study, the results lead to conclusion that toothpastes containing bioactive glass particles and CPP-ACP enhance the remineralization potential of the enamel in teeth. Further development of this concept may lead to alternative or supplement to fluoride application and fissure sealing in providing better enamel protection against demineralization.

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