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In vitro assessment of artificial saliva formulations on initial enamel erosion remineralization

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ABSTRACT

Objectives: Various formulations of artificial saliva are present in the literature and little guidance is available on the standardization of type of saliva for use in in vitro protocols for erosive studies. The aim of this study was to evaluate the remineralizing capacity of different formulations of artificial saliva on initial enamel erosive lesion.

Methods: Bovine enamel blocks were subjected to short-term acidic exposure by immersion in citric acid 0.05 M (pH 2.5) for 15 s, resulting in surface softening without tissue loss. Then 90 selected eroded enamel blocks were randomly and equally divided into 6 groups according to saliva formulation ($n = 15$): Saliva 1 (contain mucin); Saliva 2 (Saliva 1 without mucin); Saliva 3; Saliva 4; Saliva 5 (contain sodium carboxymethyl cellulose) and control (C) (deionized water). After demineralization enamel blocks were subjected to remineralization by immersion in the saliva's formulations for 2 h. Enamel remineralization was measured by superficial hardness test (% superficial hardness change). The data were tested using ANOVA and Tukey's test ($p < 0.05$).

Results: All the tested formulations of artificial saliva resulted in significantly higher enamel remineralization compared to control ($p < 0.001$). Saliva 3 showed higher percentage of enamel remineralization than Saliva 5 ($p < 0.05$).

Conclusions: Besides the variety of artificial saliva for erosion in vitro protocols, all the formulations tested were able to partially remineralize initial erosive lesions.

1. Introduction

Dental erosion has been defined as a chemical process that involves gradual loss of dental hard tissue by intrinsic or extrinsic acids of non-bacterial origin.[1–3] The erosive lesion presents two distinguished aspects. In initial erosive lesions, termed as enamel softening, the acid promotes loss of structural integrity and mechanical strength, allowing remineralization.[4,5] The subsequent wear process induced by

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prolonged erosive challenge with repeated softening events corresponds to the erosive tooth wear and it is irreversible.[4,5]

Considered as a multifactorial condition, the knowledge of chemical, biological and behavioural etiological factors is essential for its prevention and treatment.[6] The protective potential of saliva has been described in the literature as the most important biological factor on the dental erosion pathogenesis.[7–11] Saliva contains calcium, phosphate and fluoride in a supersaturated state, enabling replacement of mineral lost in initial erosive lesion,[12] especially when saliva is stimulated.[13] The saliva also contains a variety of proteins responsible for the lubrication function and for the formation of acquired pellicle, which diminishes the direct contact of acids with the tooth surface.[14–16] Furthermore, the saliva can act as a diluting agent removing acids gradually by swallowing process.[17]

The increase in tooth erosion prevalence[18] has resulted in a rising number of studies searching for dental erosion preventive therapies. Various therapies are initially tested using in vitro protocols and saliva is usually used as a control to mimic oral conditions. However there is no standardization on saliva's formulation[19] and different types of saliva can result in higher or less remineralization. Thus the effect of preventive agents may be influenced by saliva, impairing comparison and application of the results. Taking these aspects into consideration the aim of this study was to evaluate the remineralizing potential of different saliva's formulations on initial softened erosive lesions.

2. Material and methods

2.1. Experimental design

This study compared saliva substitute formulations on enamel remineralization. The factor under investigation was formulation at 6 levels: Saliva 1[20]; Saliva 2 – Saliva 1 without mucin; Saliva 3[21]; Saliva 4[22]; Saliva 5[23] and control (C) (deionized water). After the development of enamel initial erosion, enamel blocks ($n = 90$) were randomly divided into the studied groups. In sequence blocks were immersed in the formulations for 2 h. The response variable was superficial hardness change. The null hypothesis tested was that there is no difference on the rehardening effect among artificial saliva formulations and deionized water.

2.2. Enamel blocks preparation

Enamel blocks (4 mm × 4 mm × 3 mm, $n = 130$) were prepared from the labial surfaces of bovine incisors crowns. The blocks were cut using an ISOMET low speed saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4-mm thickness spacer. The blocks' surfaces were ground flat with water-cooled silicon carbide discs (320, 600, and 1200 grade papers; Buehler, Lake Bluff, IL, USA), and polished with felt paper wet by diamond spray (1 μm; Buehler, Ltd., Lake Bluff, IL, USA). The blocks were cleaned using an ultrasonic device for 2 min and checked regarding the presence of white spots and cracks using a microscope (40×).

2.3. Initial erosive lesion

Bovine enamel blocks were subjected to short-term acidic exposure by immersion in citric acid 0.05 M (pH 2.5) for 15Ys (17.6 ml per block), resulting in surface softening without tissue loss. A surface Knoop hardness (KHN) test was performed (5 indentations in the centre of the slab spaced 200 μm apart, 25 g, 5Ys, HMV-2000; Shimadzu Corporation, Tokyo, Japan) to select 90 bovine enamel blocks (SHi) with hardness values between 108 and 221 KHN (mean surface hardness of 181 ± 16 KHN).

2.4. Saliva's formulations testing effect

The volume of 17.6 ml of the testing solutions was used to immerse each enamel block for 2 h. The formulations are given: Saliva 1[20] – (0.33 g KH_2PO_4, 0.34 g Na_2HPO_4, 1.27 g KCl, 0.16 g NaSCN, 0.58 g NaCl, 0.17 g $CaCl_2$, 0.16 g NH_4Cl, 0.2 g urea, 0.03 g glucose, 0.002 g ascorbic acid, 2.7 g mucin in 1000 ml distilled water/pH 7); Saliva 2 – Saliva 1 without mucin; Saliva 3[21] – (0.1029 g $CaCl_2{\cdot}2H_2O$, 0.04066 g $MgCl_2$, 0.544 g KH_2PO_4, 4.766 g Hepes buffer acid form, 2.2365 g KCl in 1000 ml distilled water/pH 7); Saliva 4[22] – (0.381 g NaCl, 0.213 g $CaCl_2{\cdot}2H_2O$, 1.114 g KCl, 0.738 g KH_2PO_4, and 2.2 g mucin in 1000 ml distilled water/pH 7); Saliva 5[23] – (2 g methyl-p-hydroxybenzoate, 10 g sodium carboxymethyl cellulose, 0.625 g KCl, 0.059 g $MgCl_2{\cdot}6H_2O$, 0.166 g $CaCl_2{\cdot}2H_2O$, 0.804 g K_2HPO_4 and 0.326 g KH_2PO_4 in 1000 ml of water/pH 7) and control (C) (deionized water).

The degrees of saturation with respect to hydroxyapatite (HA), dicalcium phosphate dehydrate (DCPD) and octacalcium phosphate (OCP) were calculated based on added ions concentrations using a specifically program to evaluate the saturation of complex solutions with respect to biominerals.[24]

The surface hardness determination was performed again (SHf) with 5 measurements localized at 100 μm distance in relation to initial indentations. Percentage of superficial hardness change (%SMC) was calculated [(SHf − SHi)/SHi) × 100] for each block and averaged to represent the studied groups.

2.5. Statistical analysis

Statistical analysis was performed with SigmaPlot version 12.3 (2011 Systat Software, Germany), following the recommendations for dental research.[25] The assumptions of equality of variances and normal distribution of errors were checked using Shapiro–Wilk test. Since the assumptions were satisfied, one-way ANOVA and Tukey's post hoc test were applied. The significance level was set at 5%.

3. Results

Table 1 gives the degree of saturation with respect to calcium phosphates of the tested artificial salivas. The percentage of hardness gain of the evaluated formulations is displayed in Table 2. Deionized water (negative control) showed no remineralizing effect. All saliva formulations were able to promote enamel remineralization, showing statistically significant difference compared to control group ($p < 0.05$). Saliva