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Remineralizing potential of synthetic saliva: an *in vitro* study

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ABSTRACT

Objective: To evaluate the remineralizing effect of synthetic saliva on eroded bovine dental enamel. **Materials and methods:** 50 enamel blocks were prepared from bovine teeth. The specimens were divided into 5 study groups according to treatment: group A (Colgate Total®, toothpaste containing fluoride), group B (MI Paste Plus®, toothpaste containing casein phosphopeptide, amorphous calcium phosphate [CPP-ACP] with fluoride), group C (Salival® Solution, synthetic saliva), group D (distilled water) and group E (no treatment). All specimens in groups A, B, C and D received 0.5% citric acid attacks for 2 minutes at 0, 8, 24 and 32 hours. After each acid attack, toothpastes corresponding to each treatment group were applied. The degree of mineralization was then evaluated by Raman microscopy and surface microhardness by Vickers microhardness. **Results:** Regarding the degree of mineralization and surface hardness, no significant differences ($p > 0.05$) were found in the eroded bovine tooth enamel treated with Colgate Total®, Mi Paste Plus® and Salival® Solution in comparison with the healthy enamel samples. But all presented a significantly higher degree of mineralization and surface hardness than the eroded bovine tooth enamel samples preserved in distilled water ($p < 0.05$). **Conclusion:** This *in vitro* study shows that the synthetic saliva Salival® Solution has a remineralizing potential on eroded bovine enamel.

Keywords: artificial saliva, dental erosion, dental remineralization.

INTRODUCTION

Demineralization and remineralization of tooth enamel are cyclic and dynamic processes that occur in the mouth (1-3). Demineralization is the process by which minerals, such as calcium and phosphate, are dissolved from the enamel surface due to the action of acids, thus weakening the tooth structure. On the other hand, remineralization is the process of replacing the minerals lost during demineralization. In this process, the action of saliva plays a fundamental role, since it takes the lost calcium and phosphates back to the dental tissue (4, 5).

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These processes are especially important in two highly prevalent oral diseases, which are caries and dental erosion. Dental erosion is a process in which dental tissues are worn away due to the action of acids, either of extrinsic or intrinsic origin. Extrinsic origin comes from dietary acids, excessive consumption of carbonated or acidic beverages or certain habits. Intrinsic origin comes from the acid reflux of the stomach and frequent vomiting. Dental caries is mainly caused by bacteria that produce acids from sugars and starches in the mouth (6, 7). Saliva plays an important role in the remineralization of tooth enamel as it helps regulate the pH of the mouth and stimulates salivary flow. An alkaline pH and increased salivary flow favor remineralization by shifting the chemical equilibrium toward mineral precipitation, and by providing the necessary minerals for hydroxyapatite crystal formation (8).

There are patients who may have problems with dry mouth, mainly due to a decrease in salivary secretion, which is frequent in the elderly and in patients under pharmacological treatment. These patients present an increased risk of developing caries, periodontal disease, candidiasis, among others. In this context, the use of artificial saliva is indicated as a palliative measure until the etiological causes of xerostomia are resolved (9, 10).

Artificial saliva can have remineralizing properties due to its mineral content, such as calcium and phosphate, which are essential in the remineralization process of dental enamel, thus promoting the formation of hydroxyapatite crystals and improving the resistance of enamel to acid decomposition caused by bacteria in dental plaque or acid attack of various agents (7, 11).

Saliva substitutes have been shown to be useful for the relief of dry mouth. They are made with a neutral pH and contain electrolytes in a concentration that is similar to normal saliva. Their matrix corresponds to mucin or methylcellulose. Mucin-based products are better tolerated and last longer. They are available as *sprays*, rinses and gel (12).

An artificial saliva present in the Peruvian market is Salival® Solution, which is a preparation of synthetic saliva similar to the mixture of the secretion from the three pairs of salivary glands (parotid, submaxillary and sublingual). It is characterized by being a colorless, somewhat opalescent and viscous liquid. Its composition is very similar to natural saliva, especially in its inorganic ions Ca^{++}, Mg^{++}, K^{++} and $C1^{-}$ and in the percentage ratio of these ions with respect to the high purified aqueous volume. It also has a viscosity and pH equivalent to natural saliva. The objective of this *in vitro* study was to evaluate the remineralizing effect of a synthetic saliva on eroded bovine dental enamel.

MATERIALS AND METHODS

Type of study

It is an experimental *in vitro* study.

Samples

Heads of cattle were purchased for commercial reasons, i.e., for consumption. Permanent mandibular incisors were then extracted. Selected teeth were free of cracks, fractures, hypoplasia, and white spot lesions. They were subsequently stored in 0.1% thymol solution (pH 7.0). Based on previous studies, it was decided to use 10 bovine enamel samples per study group (13, 14). 50 bovine tooth enamel blocks (4 mm × 4 mm × 1 mm) were prepared from the vestibular surface of the bovine crown. Specimens were cut using a low-speed diamond blade. Surfaces were polished using granulation water sandpaper 600, 800, 1000, 1200, 1500, 1800 and 2000 to obtain a homogeneous surface on all samples. Finally, these were encased in acrylic resin to facilitate handling, and were kept at 100% humidity with distilled water until the start of the experiment.

Study Groups

Samples were randomly divided into five study groups according to treatment:

1. Group A: Colgate Total® (toothpaste containing fluoride)
2. Group B: MI Paste Plus® (toothpaste containing casein phosphopeptide-amorphous calcium phosphate [CPP-ACP] with fluoride)
3. Group C: Salival® Solution (synthetic saliva)
4. Group D: Control distilled water
5. Group E: Control enamel samples without treatment or acid cycle

Procedures

All specimens in groups A, B, C and D received acid attacks by immersing the bovine enamel of all samples (ten per group) in a container with 6 mL of 0.5% citric acid for 2 min at room temperature at 0, 8, 24 and 32 hours. It was washed with distilled water after each acid attack. Group E samples did not receive acid attack.

After each acid attack, toothpastes corresponding to each treatment group were applied for 3 minutes with an electric toothbrush and then rinsed with distilled water.

During the whole process, the samples of groups A, B and C were preserved in artificial saliva, which was changed every day. On the other hand, the samples corresponding to group D were preserved in distilled water until the next acid attack. Group E samples were preserved in distilled water. The degree of mineralization was then evaluated by Raman microscopy and the surface microhardness by Vickers microhardness.

Degree of mineralization

The degree of mineralization was evaluated with Raman microscopy, using a confocal Raman microscope alpha 350RA (WITec GmbH, Ulm, Germany) of the Laboratory of Mycology and Biotechnology "Marcel Gutiérrez-Correa" from Universidad Nacional Agraria La Molina, equipped with a laser of 785 nm wavelength (50 mW power) and a UHTS spectrometer (300 lines/mm) with a CCD camera as detector.

At each sample, twenty equidistant points along an eighty-five µm line were measured, and at each point 20 Raman spectra of 0.1 s each were taken and calculated. The location of the study area on each specimen was determined using a Zeiss EC 50x objective and Köhler white light LED illumination provided by the Raman microscope.

Characteristic spectra of tooth enamel were obtained based on the concentration of molecular compounds. The value given to the peak area corresponding to the vibrational mode of the phosphate ion v1 was counted to calculate the degree of mineralization by normalizing all values with respect to that found in group E.

Surface microhardness

Surface hardness was evaluated using an LG Vickers microhardness tester (HV 1000). Microhardness was measured after the acid cycling with 50 g pressure for 5 seconds. 5 microhardness measurements were recorded for each specimen and values in kg/mm2 were obtained. In addition, the hardness loss percentage (HLP) with respect to group E was analyzed.

Statistical analysis

For the statistical analysis of microhardness and relative mineral amount data obtained by Vickers hardness and Raman microscopy assays, respectively, STATA 16 software (StataCorp LLC, Texas, USA) was used. For descriptive statistics, mean values and standard deviations were considered for each study group, as well as box plots for a better data observation. For inferential statistics, Bartlett test was first used to assess homoscedasticity. Once the homogeneity of variances was verified, a one-factor ANOVA test was performed to find differences between groups with a significance of 5% ($p = 0.05$). The Bonferroni post-test was then used to compare the mean between groups.

RESULTS

Figure 1 shows the box plot regarding the degree of mineralization by Raman microscopy, in which it can be observed that the remineralizing activity in bovine enamel, produced by Salival® Solution artificial saliva, is similar to Colgate Total® and MI Paste Plus® pastes.

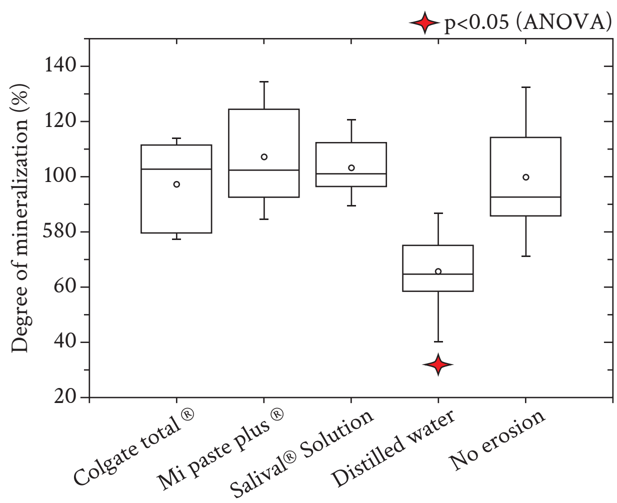

Figure 1. Box plot from the degree of mineralization in the groups studied. The star indicates the group with significant differences with respect to the others at the 0.05 level.

Table 1. Descriptive statistics of the results obtained for the degree of mineralization in the groups studied.

	Degree of mineralization (%)		
	Mean	**SD**	*
Group A: Colgate Total®	97.51	15.10	a
Group B: MI Paste Plus®	107.49	17.27	a
Group C: Salival® Solution	103.10	9.44	a
Group D: Distilled water	65.33	12.55	b
Group E: No erosion	100.00	17.87	a

* Comparison between groups using ANOVA and Bonferroni post-test. Different letters represent groups with significant differences ($p < 0.05$).

Table 1 shows the mean and standard deviation values of the mineralization degrees obtained. ANOVA test determined that there were significant differences between groups, and Bonferroni post-test showed that the group of specimens subjected to erosive cycle and stored in distilled water presented significant differences with respect to the control group with no erosion ($p < 0.05$), while the other groups (Colgate Total®, MI Paste Plus and Salival® Solution) did not show significant differences with respect to the control group.

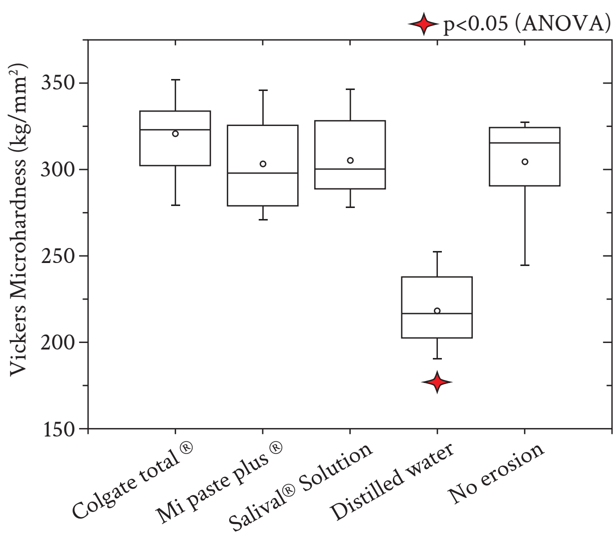

Figure 2. Box plot of Vickers microhardness in the groups studied. The star indicates the group with significant differences with respect to the others at 0.05 level.

Figure 2 shows the box plot for surface microhardness, in which, similar to the previous case, ANOVA test determined that there were significant differences between the groups; and by means of Bonferroni post-test it could be seen that the group of specimens subjected to erosive cycle and stored in distilled water presented microhardness values with significant differences with respect to the control group with no erosion ($p < 0.05$). On the other hand, the other groups (Colgate Total®, MI Paste Plus® and Salival® Solution) showed no significant differences in comparison with the control group.

Table 2. Descriptive statistics of the Vickers microhardness results obtained in the groups studied.

	Vickers microhardness (kg/mm^2)		
	Mean	**SD**	*
Group A: Colgate Total®	319.60	21.63	a
Group B: MI Paste Plus®	302.99	25.16	a
Group C: Salival® Solution	305.51	21.67	a
Group D: Distilled water	218.51	20.81	b
Group E: No erosion	303.71	27.52	a

* Comparison between groups using ANOVA and Bonferroni post-test. Different letters represent groups with significant differences ($p < 0.05$).

Mean and standard deviation values are presented in Table 2. In addition, Table 3 shows the HLP. It can be observed that group D subjected to erosive cycle and stored in distilled water loses 28.48% of surface microhardness significantly compared to the control group without erosive cycle.

Table 3. Values of hardness loss percentage (HLP) with respect to samples with no erosion.

	HLP **(%)**
Group A: Colgate Total®	4.61
Group B: MI Paste Plus®	–0.82
Group C: Salival® Solution	7.21
Group D: Distilled water	–28.48
Group E: No erosion	0.00

DISCUSSION

The objective of this study was to evaluate *in vitro* the remineralizing effect of synthetic saliva by assessing the degree of enamel mineralization and surface microhardness, using bovine enamel previously subjected to an erosive cycle. We found that the effect produced by Salival® Solution synthetic saliva is similar to Colgate Total® and MI Paste Plus® commercial toothpastes.

A remineralizing agent can be defined as a substance capable of promoting remineralization of dental tissue (15). Saliva is the natural remineralizing agent par excellence (7) and, at the same time, a key vehicle for promoting enamel remineralization through other remineralizing agents. Under physiological conditions, it efficiently maintains the equilibrium between the dental substance and the biofilm. In adverse conditions, when pH tends to decrease due to a deficient removal of biofilm or ingestion of sugars, it seeks to reverse the demineralization process. Demineralization is a reversible process. Therefore, partially demineralized hydroxyapatite (HA) crystals in teeth can grow up to their original size if exposed to oral environments that favor remineralization (16).

Saliva contains minerals such as calcium and phosphate in adequate concentrations to remineralize tooth enamel. These minerals are deposited on the surface of the enamel and help to strengthen it, compensating for the loss of minerals during demineralization (17-19). Several studies have shown that supersaturation of saliva substitute with calcium and phosphates significantly increases its ability to remineralize dental hard substances (17, 18). Also, fluoride supplementation appears to play an important role in the maintenance of the crystal structure (16-19). After an acid attack, the salivary fluid buffers the H+ produced by bacteria. When pH is higher than 5.5, remineralization naturally occurs, since saliva is supersaturated with Ca^{+2}, $PO4^{-}3$ and F^{-} with respect to the dental mineral (20).

Saliva has a basic pH that can neutralize the acids produced by bacteria in dental plaque. This helps to reduce acidity in the mouth and prevent demineralization of tooth enamel (7, 9-11).

In recent years, a remineralizing effect has begun to be demanded from saliva substitutes, as some of these have a pH considerably lower than the limit value under which demineralization of enamel (approximately pH 5.5) or dentin (approximately pH 6-6.5) is known to occur. However, several studies have shown that there is no definite correlation between the pH of a saliva substitute and its effect on dental hard tissues (9, 10).

The wide range of products currently available and the different pharmaceutical forms facilitate the choice of the most suitable product for each patient, but those with a neutral pH, with fluoride supplements and the highest possible calcium and phosphate content should preferably be recommended (11). According to Laboratorios Unidos S. A. (LUSA) specifications, Salival® Solution has in its composition per 100 mL: sodium chloride (0.084 g), potassium chloride (0.120 g), calcium chloride dihydrate (0.015 g), magnesium chloride hexahydrate (0.005 g), sodium carboxymethylcellulose (0.375 g), propylene glycol (4.000 g), methylparaben (0.100 g), propylparaben (0.010 g) and purified water q.s. (100.00 mL).

Saliva contains enzymes and antimicrobial proteins that can help control the growth of bacteria in dental plaque, which helps to prevent caries formation and maintain a healthy oral environment (7). On the other hand, some saliva substitutes contain polymers as basic substances, which seem to influence both demineralization and remineralization of dental hard tissues due to their film-forming properties. Moreover, mucins and carboxymethylcellulose may bind calcium and thus limit the remineralizing power of a saliva substitute (12, 21-23).

In this study, the remineralizing effect achieved by Salival® Solution does not differ significantly from the initial situation prior to the onset of the acid cycle, achieving enamel remyelination. On the other hand, it contrasts significantly with the control sample that was preserved in distilled water, where the strong demineralization produced by citric acid is noticeable.

While fluoride-mediated remineralization is the cornerstone of current caries management philosophies, new strategies have been marketed or are being developed. These strategies claim to promote deeper remineralization of lesions, reduce the potential risks associated with high-fluoride oral care products, and facilitate lifelong caries control. These non-fluoride remineralizing systems can be classified into biomimetic enamel regenerative technologies and approaches that repair caries lesions by enhancing the efficacy of fluoride (24).

On the market, we find fluoride toothpastes, but there are also remineralizing dental products without fluoride. Currently, most commercially available non-fluoride remineralizing systems are intended to enhance the efficacy of fluoride, minimizing the potential risks associated with fluoride. These formulations are a promising alternative; however, it is necessary to investigate and characterize their remineralizing capacities. These studies are especially necessary for products that are already on the market, such as nanohydroxyapatite (nHA), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), tricalcium phosphate, calcium sodium phosphosilicate, amorphous calcium phosphates (ACP), polyphosphates, and sodium trimetaphosphate (24).

The results of this study comparatively show with commercial pastes that the effect of the use of artificial saliva used confers similar effect to that obtained by fluoride toothpastes and CPP-ACP, from the point of view of enamel remineralization.

The results show greater remineralization in the group exposed only to synthetic saliva. We should mention that the samples subjected to the toothpastes had a brushing process after each acid attack before being subjected to artificial saliva, and that the use of the electric toothbrush may have generated some additional loss of minerals, in relation to mechanical brushes (25, 26).

Carey (27) evaluated *ex vivo* remineralization in human teeth using SalivaMAX® artificial saliva supersaturated with calcium phosphate and obtained a marked increase in remineralization that provides relief from xerostomia and mucositis, allowing them to remineralize early enamel lesions without the requirement for acid pH cycling.

These findings show that the use of Salival® Solution artificial saliva is effective for enamel remineralization under *in vitro* conditions and could potentially complement the activity of toothpastes.

As limitations of this *in vitro* study, we can mention that, although the methodology standardizes the use of artificial saliva for all samples, it does not consider the variations that could occur in oral physiology and in the particular characteristics of the saliva present in individuals, so in light of the results, it is recommended to complement this study with *in vivo* and clinical studies.

CONCLUSION

Salival® Solution synthetic saliva has a remineralizing potential on eroded bovine enamel.

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