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The Abrasive and Remineralising Efficacy of *Coturnix* Eggshell

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ABSTRACT

Aim: The objective of this research was to determine the abrasive and remineralising efficacy of quail eggshell (*Coturnix coturnix*) incorporated into a new experimental toothpaste.
Methods: An experimental study was designed to compare the surface microhardness of the dental enamel of bovine lateral incisors exposed to different toothpastes. In relation to microroughness (abrasive efficiency), the unit of analysis was each specimen with 16 polymethylmethacrylate (PMMA) blocks. For the remineralisation variable, the unit of analysis was each bovine lateral incisor tooth. A sample size of 16 was determined for each group.
Results: The unpolished PMMA specimens presented the highest mean surface microroughness with a mean of 4.60 ± 1.47 μm, whilst the quail paste, universal polishing paste, control paste, and Diamond Excel Paste showed a mean of 1.16 ± 0.40 μm, 0.63 ± 024 μm, 0.45 ± 0.22 μm, and 0.43 ± 0.17 μm, respectively, presenting significant differences ($P < .001$). When comparing the surface microhardness of bovine lateral incisor dental enamel exposed to different toothpastes, the universal polishing paste presented the lowest microhardness, with 192.43 ± 56.21 kg/mm^2, whilst the quail paste presented the greatest remineralising effect, with the highest average of 272 ± 21.18 kg/mm^2, followed by the control paste with 244 ± 41.43 kg/mm^2 and the Diamond Excel Paste with 228.72 ± 43.72 kg/mm^2. These differences were statistically significant ($P < .001$).
Conclusions: Significant differences were found in the surface abrasive efficiency (microroughness) of PMMA subjected to different toothpastes. The quail toothpaste presented statistically significant results compared to the control pastes, the Diamond Excel Paste, and the universal polishing paste.

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Introduction

In recent years, there has been a growing interest in finding new sources of calcium carbonate ($CaCO_3$). Early studies on the composition of eggshell showed that it is made up by approximately 97% of $CaCO_3$. Moreover, eggshell can also provide a great protective barrier against the penetration of microorganisms, and at the same time it is constituted by numerous porous layers permeable to water and gases, which allow the embryo to breathe. In addition, eggshell is composed of a bioceramic composite material to guarantee the calcium necessary for the skeletal formation of the chick.[1,2]

Fluoride has an effective remineralising effect on caries prevention. However, chronic low-level fluoride exposure can generate urogenital, respiratory, and gastrointestinal conditions. In addition, the application of fluoride ions alone cannot

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Table 1 – Characteristics of the toothpaste studied.

	Company	Formulation	Country
Quail paste	Own creation	Powdered quail eggshell 25.0 g; tetrasodium pyrophosphate 3.75 g; aerosil (hydrophilic fumed silica) 0.20 g; nipagin (sodium methylparaben) 0.15 g; crystallised sodium saccharin 0.20 g; menthol crystals 0.80 g; titanium dioxide (dye) 0.53 g; xanthan gum 1.05 g; sodium lauryl ether sulfate 28% 2.00 g; sodium fluoride (fluoride) 0.32 g; liquid glycerine 24.00 mL; distilled water 42.00 mL	Peru
Diamond Excel Paste	FGM Dental group	Micronised diamond-based polishing paste with extra-fine grit (2 to 4 microns)	Brazil
Universal Polishing Paste	Ivoclar Vivadent Inc.	Mixture of the substances listed below with nonhazardous additions. Naphtha (petroleum), hydrotreated heavy (Nota P, <0.1% benzene), Ammoniumoleat	Germany

completely remineralise carious lesions.[3,4] Therefore, it is necessary to find an efficient alternative to fluoride for caries prevention and incipient enamel lesion remineralisation.

The loss of minerals due to enamel demineralisation, caries, and erosion of the enamel can lead to multiple health problems. The carious process is a dynamic de- and remineralising process resulting from microbial metabolism, which eventually leads to cavitation. In contrast, the term dental erosion is used when exposure to acid, such as hydrochloric acid from chronic acid reflux, or extrinsic acids, such as fruit juice, affect the enamel of the tooth.[5] Tooth surface roughness is important to prevent bacterial plaque retention, staining, and subsequent adverse impacts on oral health. Smooth and well-polished denture surfaces are more prone to intraoral success.[6]

Thus, the aim of this study was to determine the abrasive and remineralising efficacy of quail eggshell (*Coturnix coturnix*) incorporated into a new experimental toothpaste.

Materials and methods

Sample size and study design

An in vitro, longitudinal, comparative, experimental design was developed for this study. The unit of analysis was each surface of the polymethylmethacrylate (PMMA) block. Finally, for the remineralization variable, the unit of analysis consisted of each enamel of the bovine lateral incisor tooth. In relation to the variable microroughness, the unit was PMMA blocks. For the remineralization variable, the unit was formed by the bovine lateral incisors for each group. The sample was calculated in relation to the means and standard deviations previously obtained in a pilot test using the formula for comparison of means with the statistical software Stata®15, determining an n=16 for each group evaluated.

Group A: Dental bovine enamel exposed to the experimental toothpaste including $CaCO_3$ from *Coturnix* eggshell vs commercial pastes (universal polishing paste, Diamond Excel Paste, and control paste without quail eggshell).

Group B: Polymethyl-methacrylic blocks exposed to the experimental paste based on $CaCO_3$ from *Coturnix* eggshell vs commercial pastes (universal polishing paste, Diamond Excel Paste, and control paste without quail eggshell).

Preparation of the toothpaste

Fresh quail eggs were selected and crushed. The broken shells were emptied, and 125 g of eggshell were collected. After selecting a mortar, the other components were added and weighed based on the proportions established in the formulation (tetrasodium, aerosil, pyrophosphate, menthol nipagin, titanium dioxide, and saccharin). First, all the components collected in the mortar were mixed until a homogeneous compound was achieved to combine gum, sodium lauryl ether sulfate, and fluoride solution. When the final compound obtained good viscosity, glycerin was added followed by distilled water. Finally, when the paste obtained good viscosity, the procedure was completed by labeling the product in a container (Table 1).

Sample preparation and demineralisation

Bovine incisors were collected for this study. The enamel blocks were 4 mm long, 4 mm wide, and 2 mm thick and were embedded in acrylic blocks. Moreover, the samples were immersed in a solution of potassium dihydrogen phosphate (KH_2PO_4), calcium chloride dihydrate ($CaCl_2$ $2H_2O$), methylhydroxydiphosphonate, and acetic acid (CH_3COOH) (Merck) at pH 4.95 and placed in an incubator (37.8 °C; BR 6000; Heraeus Kulzer) for 14 days. During the demineralisation period, the solutions were not shaken or changed. The pH values of the demineralising solutions were monitored daily (pHelectrode GE 100 BNC by a pH meter GMH 3510; Greisinger). In addition, small amounts of hydrochloric acid were added to maintain a constant pH value of between 4.94 and 4.96 for the enamel during the demineralisation period.

Remineralisation process

The samples were then stored separately in an eggshell-based experimental paste for 2 and 5 weeks (37 °C). The toothpastes were diluted in a remineralising solution based on the EN ISO 11609 (European standards for preparing artificial saliva/toothpastes) to obtain a homogeneous suspension. These pastes were commercially available (all without fluorides) as active ingredients. After preparation, the suspensions pH values were measured directly. Subsequently, the samples were brushed manually with minimal pressure using a soft toothbrush. Brushing procedures were performed on each subgroup twice a day for 5 seconds each (an additional contact time of 115 seconds with the suspension resulted in 120 seconds of total contact time). After each brushing procedure, the samples were cleaned with deionised water (10 seconds). The remineralising solutions were replenished every 2 days (250 mL each time per group), and the pH values were verified. Three weeks later, half of the

exposed surfaces were varnished with enamel to determine the effect after 2 weeks (intermediate effect).

Microhardness testing

The surface microhardness of all the specimens was analysed using Vickers microhardness testing equipment. A load of 25 g was applied for 5 seconds and 5 notches were applied to each specimen with a spacing of 100 microns.

Preparation of acrylic PMMA specimens

The acrylic blocks (1 × 1 × 1 × 1 cm) used to elaborate the PMMA specimens were prepared. According to the manufacturer's recommendations, all specimens were polymerised (Vitacryl). Using a milling cutter (tungsten carbide) at 18,000 rpm, the samples were trimmed after the polymerisation procedure. Before achieving the final brilliance, the samples were polished with abrasive paper (silicon carbide CC768) and were finally polished with each of the evaluated pastes.

Surface roughness analysis

Using a roughness tester (Mitutoyo), the microroughness surfaces of the acrylic specimens were analysed. The measuring instrument was calibrated with a 0.8-mm cutoff filter, evaluating a length of 4.00 mm with a range of 5.1 mm. Four surface roughness measurements were performed in each specimen, and the mean Ra values were used for evaluation.

Ethics approval

This study was reviewed and approved by the Institutional Ethics Committee of Universidad Cientifica del Sur with code (N° 174-CIEI-AB-CIENTÍFICA-2020).

Statistical analyses

Descriptive analysis was performed for the numeric variables (antimicrobial, remineralising, and abrasive activity). Normality was determined using the Shapiro–Wilk test. The analysis of variance and Kruskal–Wallis tests were used for bivariate analysis. Finally, the Bonferroni test was selected for post hoc analysis. All statistical analyses were performed with the Stata 15 programme, establishing a significance level of $P < .05$.

Results

It was observed that the unpolished PMMA specimens had the highest mean surface microroughness, with a mean of 4.60 ± 1.47 μm, whilst the quail paste, universal polishing paste, control paste, and Diamond Excel Paste presented means of 1.16 ± 0.40 μm, 0.63 ± 024 μm, 0.45 ± 0.22 μm, and 0.43 ± 0.17 μm, respectively. On the other hand, significant differences were observed between the groups when comparing abrasion (surface microroughness; $P < .001$; Table 2).

Table 2 – Comparison of the abrasive efficiency (surface microroughness) (μm) of acrylic subjected to different toothpastes during polishing.

Group	Mean	SD	Min	Max	P*	P
Polymethylmethacrylate, unpolished	4.60	1.47	2.74	7.65	.050	<.001†
Quail paste	1.16	0.40	0.66	1.88	.080	
Control paste	0.45	0.22	0.16	.82	.166	
Diamond Excel Paste	0.43	0.17	0.16	.78	.850	
Universal polishing paste	0.63	0.24	0.28	1.25	.286	

* Shapiro–Wilk test.
† Analysis of variance.
All values were expressed in μm and met the assumption of homogeneity of variances by Bartlett test with $P > .05$ in all the groups evaluated.

The post hoc analysis showed that there were only significant differences amongst the microroughness produced by the quail paste, control paste, and Diamond Excel Paste vs unpolished PMMA ($P < .001$; Table 3).

Comparison of the surface microhardness of the dental enamel of the bovine incisors exposed to the different toothpastes showed that the universal polishing paste presented the lowest microhardness, with 192.43 ± 56. 21 kg/mm^2, whilst the quail paste had the highest remineralising effect with the highest surface microhardness at 272 ± 21.18 kg/mm^2, followed by the control paste with 244 ± 41.43 kg/mm^2 and the Diamond Excel Paste at 228.72 ± 43.72 kg/mm^2 (Table 4).

Discussion

By the end of 2025, it is projected that the global market for $CaCO_3$ will be in the billions.[7] $CaCO_3$ from eggshells can be used as a substitute for food additives or supplements, for soil conditioning, or as a material for making pharmaceuticals, biomaterials, etc.[8,9]

Despite the efficacy of different toothpaste formulations with several antibacterial properties,[10,11] there has been an increase in the consumption of oral care products containing natural agents.[12] For instance, some natural herbal toothpastes are composed of sodium bicarbonate and other components with medicinal properties such as chamomilla extract with anti-inflammatory properties, echinacea extract which stimulates immune response, sage extract which reduces bleeding, myrrha extract with antiseptic properties, and *Mentha piperita* extract which has antiseptic and antimicrobial properties.[13,14] Therefore, there is a promising demand for the use of eggshell in stomatology and medical applications.

Although there seem to be different applications for the use of eggshells, there is limited evidence regarding the abrasive potential of the eggshell powder related to the smoothness of dental prostheses.[15,16] Moreover, $CaCO_3$ can be used in solid dosage forms as a diluent and is also used for dental and medicinal preparations, dissolutions, and buffering aids in the form of dispensable tablets. Likewise, it can be used in food additives and for calcium supplementation.[17-19]

This study showed that the use of eggshell in toothpaste maintained a surface microroughness of 1.16 μm, compared

Table 3 – Post hoc analysis of the abrasive efficiency (surface microroughness) (μm) of acrylic subjected to different toothpastes during polishing.

	Polymethylmethacrylate unpolished	Quail paste	Control paste	Diamond Excel Paste
Quail paste	0.001	—	—	—
Control paste	0.001	0.056	—	—
Diamond Excel Paste	0.001	0.045	1.000	—
Universal polishing paste	0.001	0.379	1.000	1.000

All post hoc analyses were performed using the Bonferroni test.

Table 4 – Comparison of remineralising efficacy (surface microhardness) of bovine tooth enamel subjected to different toothpastes.

Group	Mean	SD	Min	Max	P*	P†
Untreated tooth	290.02	11.01	259.10	302.66	.014	<.001
Quail paste	272.32	21.18	206.00	296.64	.001	
Control paste	244.89	41.43	133.62	285.12	.001	
Diamond Excel Paste	228.72	43.72	165.20	302.66	.634	
Universal polishing paste	192.43	56.21	1.51	249.58	.001	

* Shapiro–Wilk test.
† Kruskal–Wallis test.
All specimens were expressed in Vickers (kg/mm^2).

to other pastes indicated for polishing dental materials such as Diamond Excel Paste and universal polishing paste, with 0.43 and 0.63 μm, respectively. This is congruent with the study by Onwubu et al that showed that surface roughness is an important factor affecting biofilm retention and staining. Therefore, it is essential to note that good polishing of PMMA denture surfaces ensures successful clinical performance. This is supported by several studies that report an increase microbial adhesion on rough surfaces.[6,16,20]

According to Onwubu et al,[16] microroughness is generally evaluated in Ra (μm) values, and the values reported in their study were under the threshold value of 0.2 μm. Therefore, the eggshell powder was found to be effective in decreasing the microroughness of the polymethylmethacrylate surface. These differences are attributed to the diverse particle sizes included in the experimental paste and can be explained by the suggestion that abrasive materials of smaller particle size quickly wear away surface roughness and thereby have better clinical performance.[21]

When comparing our results with other abrasive materials (Colgate Great Regular Flavor; Colgate Palmolive Ltd.), it was found that wear occurred after toothbrushing. However, the values were low. On the other hand, the data of another study suggest that abrasion of tooth enamel caused by the diamond particles in the DD2 diamond dentifrice was directly associated with the diamond particle load.[22,23]

In relation to microhardness, some in vitro studies have mainly shown that different commercial toothpastes have remineralisation capacity on enamel and dentin lesions. In addition, toothpaste with fluoride aggregates showed the least hard tissue remineralisation as well as an increment in lesion depth. Similar to the results of the present study, the literature justifies the use of bovine enamel and dentin samples, as this source represents an accepted surrogate of human enamel. Remineralisation is a complex process involving different ions such as calcium and phosphate, which are returned to the internal structure of the tooth to ensure a strong tooth structure. However, when these minerals are lost the pores are larger, favouring demineralisation of the enamel.[24,25]

Generally, after the application of remineralising agents on caries lesions, it seems that mineral ions are diffused into the surface layer and thereby clog the surface porosities. The present study showed a significant difference in the remineralisation of the superficial enamel generated by the quail toothpaste (mean of 272.32 kg/mm^2), similar to the results of Roza et al,[3] who described that the microhardness significantly decreased in the samples after demineralisation and rose significantly after exposure to therapeutic solutions. Microhardness did not significantly differ between groups after pH immersions. Based on our results, the eggshell paste is effective as a remineralising agent in enamel caries-type lesions. Likewise, Kattimani et al[26] demonstrated eggshell biocompatibility, good in vitro material properties, and successful use of eggshell as graft material for bone defect grafting.

It is important to take into account that toothpastes based on pure $CaCO_3$ from eggshells are a novel and organic alternative. However, more studies are needed to establish the factors associated with antimicrobial efficacy of this product compared to commercial toothpastes using $CaCO_3$ of synthetic origin. The main limitation of our study was the limited literature on toothpaste elaboration[27-29] based on quail eggshell, which did not allow the comparison of the results in greater depth. Another limitation was the lack of specialised culture media to cultivate some facultative anaerobic bacteria. Nonetheless, the reproduction of microbiological assays was feasible. Finally, another limitation was the large quantity of quail eggshell required vs the small amount available of this natural resource.

Conclusions

Significant differences were found in the surface abrasive efficiency (microroughness) of polymethylmethacrylate subjected to the quail, control, Diamond Excel, and universal polishing pastes during polishing. The post hoc test showed that there were only significant differences in surface microroughness between unpolished polymethylmethacrylate and the other pastes. Finally, comparison of the remineralising efficacy (surface microhardness) of bovine dental enamel subjected to different toothpastes as remineralising agents showed significant differences between the quail vs the control, Diamond Excel, and universal polishing toothpastes.

Conflict of interest

None disclosed.

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