



Ana Paula Miranda Vieira

**Adição de extrato da casca de romã em um
enxaguatório bucal com polifosfato-fluoreto reduz a
desmineralização do esmalte dentário**

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desmineralização do esmalte dentário**

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Coorientadora: Prof^a. Dr^a. Marcelle Danelon

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*“Cada descoberta nova da ciência é uma porta nova pela qual
encontro mais uma vez Deus, o autor dela”*

Albert Einstein

Vieira, A. P. M. **Adição de extrato da casca de romã em um enxaguatório bucal com polifosfato-fluoreto reduz a desmineralização do esmalte dentário.** 2022 89f. Tese (Doutorado em Ciência Odontológica, área de Biomateriais) - Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2022.

Objetivos: Avaliar o efeito anti-desmineralizante de um enxaguatório bucal com extrato de casca de romã (3% PPE), trimetafosfato de sódio (0,3% TMP) e flúor (225 ppm F) em um estudo *in situ* e determinar seu potencial de irritação em um estudo *ex vivo*.

Métodos: Este estudo foi duplo-cego cruzado e realizado em quatro fases com 7 dias cada. Doze voluntários utilizaram dispositivos orais contendo blocos de esmalte bovino que foram submetidos a desafios cariogênicos com sacarose (30%) seis vezes ao dia. A formulação ETF (PPE+TMP+F), formulação TF (TMP+F), água deionizada e um enxaguatório comercial (CM) foram gotejados nos blocos de esmalte duas vezes ao dia. Em seguida, determinou-se a porcentagem de perda de dureza superficial (%SH), perda integrada de dureza subsuperficial (Δ KHN), bem como cálcio (Ca), fósforo (P) e fluoreto (F) no esmalte e nos biofilmes. Além disso, as concentrações de polissacarídeos extracelulares solúveis (EPS) foram analisadas a partir do biofilme coletado dos blocos. O potencial de irritação dos enxaguatórios foi avaliado pelo teste da membrana corioalantóica do ovo de galinha através do efeito vascular produzido durante 300 s de exposição.

Resultados: A formulação ETF promoveu os melhores resultados para %SH e Δ KHN entre todos os grupos ($p < 0,001$), apresentou a maior concentração de Ca e P no esmalte e no biofilme, e a menor quantidade de EPS no biofilme ($p < 0,001$). ETF produziu leve rubor, enquanto CM levou a hiperemia e hemorragia nos ovos.

Significado: A adição de PEE na formulação para enxaguatório sem álcool contendo TMP e F aumentou sua propriedade anti-desmineralizante, e esta formulação apresentou menor potencial de irritação quando comparada ao CM testado.

Palavras-chave: Romã; Fosfatos; Fluoretos; Cárie dentária; Desmineralização do dente; Embrião de galinha; Antissépticos bucais.

Abstract

Vieira, A. P. M. **Addition of pomegranate peel extract in a mouthwash with polyphosphate-fluoride reduces the tooth enamel demineralization.** 2022 89f. Tese (Doutorado em Ciência Odontológica, área de Biomateriais) - Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2022.

Objectives: This study evaluated the anti-demineralizing effect of a mouthwash comprising pomegranate peel extract (PPE 3%), sodium trimetaphosphate (TMP 0.3%), and fluoride (F 225 ppm) in an *in situ* study and assessed its irritation potential in an *ex vivo* study.

Methods: This double-blind crossover study was conducted in four phases with 7 days each. Twelve volunteers used palatal appliances containing bovine enamel blocks that were subjected to cariogenic challenges with sucrose (30%) six times daily. The ETF formulation (PPE+TMP+F), TF formulation (TMP+F), deionized water, and commercial mouthwash (CM) were dropped onto the enamel blocks twice daily. The percentage of surface hardness loss (%SH), integrated loss of subsurface hardness (Δ KHN), calcium (Ca), phosphorus (P), and F in enamel and biofilms were determined. In addition, alkali-soluble extracellular polysaccharide (EPS) concentrations were analyzed in the biofilms collected from the blocks. The irritation potential of the mouthwashes was evaluated using the hen's egg chorioallantoic membrane test through the vascular effect produced during 300 s of exposure.

Results: The ETF formulation presented the best results for %SH and Δ KHN among all groups ($p < 0.001$), showed the highest concentrations of Ca and P in the enamel and biofilm, and the lowest amount of EPS in the biofilm ($p < 0.001$). The ETF produced light reddening, whereas CM led to hyperemia and hemorrhage in the eggs.

Significance: The addition of PPE to alcohol-free mouthwash formulations containing TMP and F increased its anti-demineralizing property, and this formulation presented a lower irritation potential than the CM tested.

Keywords: Pomegranate; Phosphates; Fluorides; Dental Caries; Tooth Demineralization; Chick Embryo; Mouthwashes.

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Addition of pomegranate peel extract in a mouthwash with polyphosphate-fluoride reduces the tooth enamel demineralization

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1. Introduction

Oral health is a fundamental part of overall health and well-being, and talking, eating, smiling, and socializing without pain, discomfort, or embarrassment is directly related to good oral health [1, 2]. Nevertheless, oral diseases remain a global public health concern. Dental caries is the most prevalent dental condition among the 50 most common oral diseases evaluated between 1990 and 2017 by the Global Burden of Disease Study [3, 4]. Globally, an estimated 2.3 billion people suffer from untreated caries in permanent teeth [5]. Dynamic development and multifactorial etiology of caries involve complex interactions between host factors, oral microbes, and fermentable carbohydrates [4].

Presently, fluoride (F) is the main component for the prevention and noninvasive control of dental caries. However, changes in the diet of the population, including the increasing sugary and processed foods, have necessitated the inclusion of compounds that can improve the protective and therapeutic effects of F [6, 7]. Numerous studies have shown that adding phosphate salts, including sodium trimetaphosphate (TMP), to F products produces a synergistic effect by decreasing the dissolution of hydroxyapatite and increasing mineral apposition in dental enamel [8-12]. TMP adsorbs onto the enamel, forming a barrier that inhibits acid diffusion. However, this barrier prevents the precipitation of CaF_2 and subsequently reduces the obstruction of enamel pores, furthering ion diffusion into the enamel [9, 12-14]. The adsorbed TMP may retain both Ca^{2+} and CaF^+ in its negatively charged cyclic structure. When the pH drops due to the cariogenic challenge, calcium species are released, leading to the formation of neutral species ($\text{CaHPO}_4^0 + \text{HF}^0$) that have a higher diffusion coefficient, improving the ionic flux. Notably, it decreases enamel demineralization and increases the remineralization process [9, 12-14].

In the last decade, there has been an exponential exploration of herbal medicine [15, 16]. The plurality of therapeutic effects found in plant extracts makes them promising raw materials for the search for novel remineralizing agents [7, 17-19]. In this regard, the pomegranate (*Punica granatum*), a fruit-bearing tree native to Iran and cultivated worldwide,

stands out. Since ancient times, it has been known for its anti-inflammatory, antioxidant, antimicrobial, wound healing, anti-hyperglycemic, hepatoprotective, and anticancer properties [20]. Pomegranate fruit has various biocompounds with high biological value in all its parts, including the peel, carpelar membranes, arils, and seeds [21]. The peel represents 30–40% of the pomegranate and is a non-valued by-product that is usually discarded as residues by the agro-industry after processing the fruit [22, 23]. Notably, pomegranate peel has the highest amount of phenolic compounds compared with the juice and seeds of different pomegranate cultures, which is responsible for its beneficial properties [24].

This study aimed to evaluate the anti-demineralizing effect of mouthwash with pomegranate peel extract (PPE), TMP, and F in an *in situ* study and to assess the irritation potential of that mouthwash in an *ex vivo* study to achieve a sustainable approach with the use of natural compounds and the need for new remineralizing products for the prevention/treatment of dental caries. The null hypothesis was that mouthwash with PPE, TMP, and F would provide similar anticaries effects when compared with mouthwash containing only TMP and F, and it would present an irritation potential similar to a commercial mouthwash (CM).

2. Materials and Methods

2.1. *Punica granatum* peel extraction

Punica granatum (pomegranate) peel (dehydrated, ground, and sterilized) was obtained from a single allotment of the Company Santos Flora (Santosflora Com. De Ervas, Mairiporã, SP - Brazil). The peel powder was dissolved in ethanol 70% GL, and the maceration process used for extraction followed the method described by Fernandes et al. [25]. After exhaustive extraction, the resulting mixture was filtered and concentrated using a vacuum evaporator to obtain a soft extract. Subsequently, it was diluted in propylene glycol, and the

yield percentages were calculated at 32% w/v of pomegranate dry matter (according to solvent evaporation in an oven at 100– 105°C until a constant weight).

2.2. Mouthwash formulations

The mouthwash formulations were produced using gum arabic, potassium sorbate, disodium EDTA, xylitol, sorbitol, mint spicata essential oil, sodium hydroxide, purified water, and active compounds (PPE, TMP and F). For the formulations, the concentrations of TMP (0.3%) and F (225 ppm) followed the report by Favretto et al. [8] and the PPE concentration (3%) was based on the one by Fernandes et al. [25]. Previously, phytochemical analyzes revealed that PPE is rich in phenolic compounds ($114.98 \text{ mg} \pm 3.55 \text{ g}^{-1}$), punicalagin ($\alpha = 2.14 \pm 0.006 \text{ mg g}^{-1}$, $\beta = 2.286 \pm 0.03 \text{ mg g}^{-1}$) and ellagic acid ($0.458 \pm 0.006 \text{ mg g}^{-1}$) [25]. The formulations produced were as follows: E (formulation with 3% PEE), TF (formulation with 0.3% TMP + 225 ppm F), ETF (formulation with 3% PPE + 0.3% TMP + 225 ppm F), and N (formulation without PPE, TMP, and F). The pH of the formulations was adjusted using a pH electrode (2A09E, Analyzer, São Paulo, Brazil), and the amount of F was verified using an F-specific electrode (Orion 9609-BN; Orion Research Inc., Beverly, USA) connected to an ion analyzer (Orion 720 A+; Orion Research Inc.) (Sigma Aldrich, St. Louis, USA) [25]. Standards containing 0.5 to 8.00 ppm F were used to calibrate the electrodes.

2.3. *In situ* study design and Experimental protocol

This study was approved by the Human Ethics Committee of São Paulo State University (UNESP), School of Dentistry, Araçatuba, Brazil (Protocol: 10320719.9.0000.5420), and all participants provided informed consent statements before the study onset. This study was an *in situ* double-blind crossover study conducted in four phases of 7 days each [26]. The sample size of 12 volunteers (8 female, 3 males, aged 24 – 31 years) was used based on the study by do Amaral et al. [27], considering as the primary outcome the surface and cross-sectional hardness analysis, the mean difference between groups (30 and 1300, respectively),

standard deviation (20 and 900, respectively), an α -error of 5%, and a β -error of 20%. Volunteers (age $\pm$ 20–30 years) followed the inclusion and exclusion criteria based on their good general and oral health status (Appendix A).

Enamel blocks (4 x 4 mm, n = 192) were prepared from bovine incisor teeth, polished, and selected by initial surface hardness (approximately 365.9 Knoop hardness number (KHN), $p < 0.05$). These enamel blocks were fixed in acrylic resin palatal appliances and covered with a piece of plastic mesh to enable biofilm accumulation (leaving a 1 mm space from the block surface). Additionally, different palatal appliances were manufactured during all the experiment phases. Notably, to simulate a cariogenic diet, the volunteers were instructed to remove the appliance and drip 30% sucrose solution (Synth[®], Diadema, Brazil) onto each enamel block six times daily (8:00 am, 11:00 am, 2:00 pm, 5:00 pm, 7:00 pm, and 9:00 pm) (Figure 1) and wait five minutes for the sucrose solution to diffuse before reinserting the appliance into the mouth [14]. Moreover, the volunteers were instructed to use F-free toothpaste (Appendix B) to brush their natural teeth during the 7 days of pre-experimental, experimental, and wash-out periods. During the experimental period, the volunteers brushed thrice daily after the main meals while having the palatal appliance inside their mouths, being careful not to brush the enamel blocks. After brushing, the appliance was removed, and two drops of formulation were dropped onto each enamel block twice daily (2:30 pm and 9:30 pm), waiting for 1 min for formulation diffusion (Figure 1). All volunteers underwent four phases (n = 48) of treatment, lasting seven days each one and using the following treatments: TF, ETF, W (deionized water), and CM (essential commercial anticaries alcohol-free mouthwash, Johnson & Johnson[®], São Paulo, Brazil, Appendix C) (Figure 1). The appliance was used throughout the day and night, except while eating and drinking.

After each experimental period, biofilms formed on the blocks were collected for analysis of F, calcium (Ca), phosphorus (P), and insoluble extracellular polysaccharides (EPS). In enamel, the percentage of surface hardness loss (%SH), integrated loss of subsurface area (Δ KHN), F, Ca, and P (Figure 1).

2.3.1. Hardness analysis

SH was measured by five indentations on the surface of the enamel before (SHi) and after (SHf) each treatment phase using a Shimadzu HMV-2000 microhardness tester (Shimadzu Corp., Kyoto, Japan) under a 25 g load for 10 s [28]. The %SH was calculated as $\%SH = 100(SHf-SHi)/SHi$. Subsequently, the enamel blocks were longitudinally sectioned through their center and embedded in acrylic resin, with the cut face exposed and polished for determining cross-sectional hardness measurements. A Micromet 5114 hardness tester (Buehler® Ltd., Lake Bluff, USA) and the software Buehler OmniMet (Buehler) with a Knoop diamond indenter under a 5 g load for 10s were used to perform one sequence of 14 indentations spaced 100 μm apart (5, 10, 15, 20, 25, 30, 40, 50, 70, 90, 110, 130, 220, and 330 μm). The integrated hardness area (KHN x μm) from the lesion to the hard enamel was assessed using the trapezoidal rule (Graph Pad Prism, version 3.02) and subtracted from the hard enamel hardness integrated area to obtain the ΔKHN [28, 29].

2.3.2. Biofilm dental processing and analysis of EPS

The biofilm formed on each enamel block was collected, weighed, stored in microcentrifuge tubes (Axygen, Corning, Nova York, USA), and dried in a vacuum over P_2O_5 (Vetec Química Fina Ltd., Duque de Caxias, Brazil) for 12 h at room temperature [30]. For inorganic and carbohydrate analysis, hydrochloric acid (HCl, 0.5 mol L^{-1} , Sigma Aldrich) and sodium hydroxide (NaOH, 0.5 mol L^{-1} , Sigma Aldrich) were sequentially added to the biofilm under constant agitation [31, 32]. Afterward, the samples were vortexed and centrifuged ($11,000 \times g$) for 1 min, and the supernatant was separated for F, Ca, and P determination. The number of EPS was determined using the phenol-sulfuric acid method [33].

2.3.3. F, Ca, and P in enamel and dental biofilm

F present in the enamel was determined according to Weatherell, Robinson [34] and modified by Alves et al. [35] using a specific electrode 9409BN (Thermo Scientific, Beverly,

USA) and microelectrode reference (Analyzer[®], Sao Paulo, Brazil) coupled to an ion analyzer (SevenCompact S220-uMix, Mettler Toledo[®], Greifensee, Switzerland). The F present in the dental biofilm was analyzed using an ion-selective electrode (Orion 9409 BN) and a potentiometer (Orion 900200, Thermo Scientific[™], Beverly, USA) [32]. For the analysis of Ca present in enamel and biofilms, the Arsenazo III colorimetric method [36] was used, and P analysis followed the protocol established by Fiske and Subbarow [37]. The absorbance readings were recorded at 650 and 660 nm for Ca and P, respectively, using a plate reader (PowerWave 340, Biotek, Agilent Technologies[®], Santa Clara, USA). The results for enamel were expressed in $\mu\text{g}\cdot\text{mm}^{-3}$, and those for biofilms were expressed in moles per kilogram.

2.4. The hen's egg chorioallantoic membrane (HET-CAM) test

The HET-CAM assay followed the guidelines of the *Journal Officiel de la République Française* [38] according to the method proposed by Luepke with minor changes [39]. White Leghorn fertile eggs (Mario Salviato, Porto Ferreira, Brazil) were incubated in an egg incubator (model GP100, GP Chocadeiras[®], Salto, Brazil) at 37°C and 60–70% relative humidity for 10 days. The eggs were rotated daily for the first 8 days. On the tenth day, the shell above the airspace of the eggs was removed using a diamond disc (KG Sorensen[®], São Paulo, Brazil) coupled to a micromotor (Marathon 3 Champion, Talmax[®], Curitiba, Brazil). Afterward, the inner egg membrane was removed, and the CAM was exposed to the formulations E, TF, ETF, N, or CM (300 μL) for 20 s. The CAM was then washed with isotonic sodium chloride (NaCl 0.9%, w/v), and vascular reactions (hyperemia, hemorrhage, and coagulation) were observed for 300 s. Reactions were graded according to their time of appearance (Table 1). Subsequently, the grading of vascular reactions was combined to obtain an irritant score (IS) based on the following scale: $\text{IS} \leq 0.9$, no irritant; $0.9 < \text{IS} \leq 4.9$, slight irritant; $4.9 < \text{IS} \leq 8.9$, moderate irritant; $8.9 < \text{IS} \leq 21$, severe irritant. NaCl 0.9%, w/v, sodium dodecyl sulfate (DDS 1% w/v), and NaOH 0.1 N were used as test system controls as references for the irritation

index. Three eggs were used for each group, and the test was conducted thrice on different occasions.

2.5. Statistical analysis

SigmaPlot software (version 12.0; Systat Software Inc., San Jose, CA, USA) was used for statistical analyses with a confidence level of 95%. Data (Ca, P, F, EPS, %SH, and Δ KHN) were analyzed for normality and homogeneity of sample distributions using the Shapiro–Wilk and Bartlett test, respectively. Additionally, data were subjected to a one-way analysis of variance (ANOVA) and repeated measures, followed by the Student–Newman–Keuls test.

3. Results

3.1. Effect anti-demineralization of the mouthwash formulations

The mean (SD) concentrations of total F ($n = 3$) in the formulations containing F were as follows: TF formulation = 215.9 (8.7) and ETF formulation = 232.9 (6.3). The pH of all formulations was adjusted to 7.0 by adding NaOH.

The combination of PPE, TMP, and F in the formulation (ETF) resulted in the highest reduction in %SH compared with all the groups tested. ETF promoted ~46.6% and ~40.4% decreases in %SH compared to TF and CM, respectively ($p < 0.001$). No significant difference was observed between the TF and CM groups regarding %SH ($p = 0.170$). In addition, the capacity to reduce Δ KHN was ~27.5% and ~29.5% lower in TF and CM ($p < 0.001$), respectively, than in ETF (Table 2).

Similar F concentrations were observed in TF, ETF, and CM ($p > 0.01$), which were significantly higher than those in W ($p < 0.01$). ETF promoted the highest retention of Ca and P in the enamel and biofilm when compared with TF, CM, and W ($p < 0.01$). The lowest EPS in biofilms was observed when the ETF formulation was used ($p < 0.01$), followed by the TF, CM, and W (Table 2).

3.2. The irritation potential by the HET-CAM assay

Applying NaCl (negative control) to healthy membranes produced no visual responses. The positive controls (DDS and NaOH) were graded as severely irritating. Upon applying DDS, hyperemia, and hemorrhage were observed (cumulative score = 12 points), and NaOH produced all three signs of vascular response (cumulative score = 19 points), which are in accordance with the acceptance criteria for the “*Interagency Coordinating Committee on The Validation of Alternative Methods*” [40]. In the E and ETF formulations, no vascular response was noticeable within 30 s, whereas light reddening was visible after 30 s (cumulative score = 3 points, slightly irritant). The N and TF formulations showed no signs of vascular response (non-irritant), and CM produced hyperemia and hemorrhage and was classified as a moderate irritant (cumulative score = 10 points). Figure 1 shows the representative images for each group.

4. Discussion

Mouthwashes have become increasingly popular [41]. During the COVID-19 pandemic, their use was encouraged due to the effects of reducing the viral load of saliva, leading to part of the population using it daily [42, 43]. However, indiscriminate applicability is problematic, primarily because most mouthwashes contain alcohol or synthetic chemical agents that are unsuitable for everyday use [44]. Thus, developing herbal and biocompatible alternatives is a niche area to be explored in the dental market. Therefore, we produced an alcohol-free mouthwash formulation comprising PPE, polyphosphate, and F at lower concentrations. This formulation reduced demineralization on bovine enamel in an *in vitro* pH cycling test and prevented biofilms produced by *Streptococcus mutans* and *Candida albicans* [25]. In this study, we provide new insights into enhancing the anti-demineralizing activity of sodium TMP and F by adding PPE. This combination in a mouthwash formulation (ETF) was used in an *in situ* model that has already been well studied and successfully used to prove the anticaries

effectiveness of F compounds [14, 26, 32]. Furthermore, we demonstrated the biocompatibility of the ETF in an *ex vivo* model using a hen's egg chorioallantoic membrane. The null hypothesis of this study was rejected as ETF promoted a higher reduction in dental enamel demineralization than the TF mouthwash formulation and presented a lower irritation potential than the CM.

Our results showed that side effects on enamel produced by simulated cariogenic challenges were reversed by ETF, significantly reducing the mineral loss in tooth enamel. In addition, higher amounts of Ca and P were detected in the enamel and biofilm than in the other mouthwash treatments proposed in this study (TF, W, and CM mouthwashes). This finding demonstrated that the enamel treated with ETF was significantly more mineralized, which is considered important in cases of subsequent demineralization cycles [10, 12, 13]. Particularly, the Δ KHN results suggested that ETF increased the mobility of Ca and P ions in deeper regions of the enamel, which is crucial for the remineralization process occurring in progressive carious lesions [12, 13].

Regarding the concentration of alkali-soluble EPS, there was a significant reduction in biofilms treated with the ETF. The effectiveness of reducing EPS has been demonstrated in the literature when the active compounds present in ETF were tested separately against biofilms. PPE effectively inhibited the production of insoluble EPS in *S. mutans* biofilms [45]. F interferes with EPS synthesis by controlling gene expression via glycosyltransferase-related and glycolysis-related genes [46]. TMP associated with F decreased EPS in several *in situ* studies [12, 14]. This association also induced structural changes and reduced the protein, carbohydrate, and DNA content in the extracellular matrix in dual-species biofilms of *S. mutans* and *C. albicans* [47]. For the inorganic composition of the biofilm, our study showed an increase in the adsorption of Ca and P after ETF treatment, suggesting a biofilm with a higher degree of saturation regarding hydroxyapatite [14]. In previous studies [12], TMP alone could not increase P levels of *in vitro* biofilms, as it is not a source of free P [13, 48]. Comparatively, with our results, we hypothesize that PPE could play a significant role as a P source in biofilms.

The outcome showed that the association of PPE with remineralizing agents (TMP and F) was significant in our study. However, the mechanism of action of the ETF formulation on de- and remineralization enamel processes still needs to be elucidated. Fernandes et al. [25] demonstrated that a mouthwash formulation containing PPE alone does not decrease enamel demineralization in an *in vitro* cariogenic challenge study. Considering this, we demonstrated the additive effect of PPE in association with TMP+F on the reduction of enamel demineralization. The additive effect of herbal-containing toothpaste (*Prunus amygdalus Dulcis* and *Camellia sinensis*) on the remineralizing efficacy of F has already been reported by Forcin et al. [49]. *Prunus amygdalus dulcis* is an almond that contains proanthocyanidins and hydrolyzable tannins (including ellagic and gallic acids) [49, 50], and these components are also found in pomegranate peel extract [51]. It has been suggested that PPE forms an organic pellicle on the enamel surface, comprising monomeric and polymeric polyphenols. This supposedly provides the main contact of pellicle-containing gallotannins with the enamel surface, thereby increasing the deposition effectiveness of remineralizing ions [49].

Additionally, dentinal tubules are physically occluded, impeding acid diffusion [49]. PPE contains Ca and P in its composition, and there is also the possibility of PPE acting as an external source of these ions [22]. TMP also forms a layer on the surface enamel and increases the adsorption capacity of Ca and P [8, 14, 52]. Therefore, the bioavailability of Ca and P is increased because of the presence of PPE, and a state of supersaturation occurs and; consequently, larger retention of them in the enamel would ensue. This may explain the higher uptake of Ca and P observed in the enamel after ETF treatment. It is believed that the polyphenolic compounds present in PPE, more specifically gallic acid, facilitates mineral deposition on the surface layer by combining with Ca^{2+} from the surrounding medium and forming insoluble compounds with Ca [53-56]. Furthermore, gallic acid might act as a carrier of these Ca^{2+} ions to the lesion body [57], and this theory is supported by our findings on subsurface lesion reduction. Another issue to be emphasized is the ability of polyphenols to bind to oral surfaces, and even with salivary flow, they can remain bound for long periods,

acting as “slow-release” devices [58-61]. After rinsing with the ETF formulation, its astringent organoleptic feature was noted, resulting from tannins interaction with salivary proteins. As part of the calcium present in saliva is bound to proteins [62], precipitation of salivary protein could be another factor responsible for the increase in calcium deposition in enamel [63].

Furthermore, the promising results of ETF can be accentuated by its significant anti-demineralizing efficacy when compared with the CM evaluated. This mouthwash was chosen as a control because of its similar characteristics to the ETF formulation, such as F concentration, absence of alcohol, and presence of natural compounds. Mouthwashes based on essential oils (menthol, thymol, eucalyptol, and methyl salicylate) are popular commercial products, and the addition of F in its formulation proved effective in the remineralization of artificial caries lesions [64, 65]. In 2004, this mouthwash type was classified as effective and safe by the American Authority “*Food and Drug Administration*” [66], and it was selected as a comparative group in our study in the HET-CAM test.

The HET-CAM is an alternative *ex vivo* assay that evaluates the potential for irritation and local toxicity of compounds on mucosal surfaces [65, 66] and can be used in investigations of dental products [69-71]. Our results from the HET-CAM assay showed that ETF did not induce a highly irritating reaction (classification rating: slightly irritant), indicating that it is a substance without toxic effects. In comparison, the CM presented a higher irritant potential (classification rating: moderate irritant) owing to the induction of adverse reactions in the vascular plexus of the CAM. The toxicity of CM was proven in our previous study in which murine bone marrow-derived macrophages were used [25]. In that study, after 2 h of treatment with ETF, more than 75% of murine bone marrow-derived macrophages remained viable, whereas CM was cytotoxic, retaining only 20% of viable cells [25].

The a-toxic characteristics of the ETF formulation might be because of the concentrations of the active compounds (PPE, TMP, and F) selected to be in it [72-74]. In a recent study, monosodium glutamate (a flavor-enhancing agent added to various foods) produced congenital malformations in chicken embryos. The injection of PPE (0.3 mL)

ameliorated these harmful actions in embryos owing to its antioxidant properties [75]. Although *in vivo* tests are necessary to prove the safe clinical application of ETF, our preliminary data strongly suggest its biocompatibility.

This present study demonstrated that the addition of PPE (3%) to an alcohol-free mouthwash formulation containing TMP (0.3%) and F (225 ppm) increased its anti-demineralizing property, with a slight irritant potential. In commercial dental products, the ETF formulation could be used in the future for the prevention of dental caries. Therefore, controlled clinical trials are recommended to confirm our outcomes.

5. Conclusion

We concluded that, with the limitations inherent to an *in situ* study, ETF was able to reduce %SH and Δ KHN and increase the concentration of Ca and P in the enamel and biofilm. In addition, it acts on the dental biofilm, reducing the number of EPS. In the HET-CAM test, ETF had a lower irritation potential than the CM, proving to be a promising alternative for long-term use in patients at high risk of caries.

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[75] Al-Ghamdi FAM. Pomegranate peel extracts effects to reduce mono sodium glutamate toxic effects on chicken embryos: Morphological studies. *Saudi J Biol Sci.* 2022;29:975-83.

Supplementary Material

Inclusion criteria for volunteers

The volunteers who were included in this study were all residents of Araçatuba (São Paulo, Brazil) with a fluoridated water supply, good general health, clinical absence of active caries lesions, and periodontal disease, and they provided written informed consent. The exclusion criteria were as follows: use of medications interfering with the salivary flow rate, use of antibiotics, use of removable dentures, fixed or removable orthodontic appliances, smoking, pregnancy or breastfeeding, systemic diseases, and previous allergy to oral products or plant extracts. Notably, the participants were prohibited from using any antimicrobial or F product during the experiment.

Toothpaste formulation

Toothpastes were produced with the following components: titanium dioxide, carboxymethyl cellulose, methyl p-hydroxybenzoate sodium, saccharin, mint oil, glycerin, abrasive silica, sodium lauryl sulphate and deionized water.

Ingredients of essentials oil anticaries alcohol-free commercial mouthwash

Purified water, sorbitol, propylene glycol, poloxamer 407, sodium lauryl sulfate, benzoic acid, aroma (benzyl alcohol, d-Limonene), eucalyptol, methyl salicylate, thymol, sodium saccharina, sodium fluoride (220 ppm), sodium benzoate, sucralose, menthol, CI 47005/quinoline yellow, CI 42053/fast green.

Figure and tables captions

Figure 1. Experimental design of the study.

Figure 2. Representative images: **(Egg)** Fertilized chicken egg showing the chorioallantoic membrane. Sequence of photographs illustrating the macroscopic changes on the chorioallantoic membrane vasculature after administration of solutions for 360 s: **(NaOH)** sodium hydroxide 0.1N; **(DDS)** sodium dodecyl sulfate 1%; **(NaCl)** sodium chloride 0.9%; **(N)** formulation without pomegranate peel extract (PPE), sodium trimetaphosphate (TMP) and fluoride (F); **(E)** formulation with 0.3% PPE; **(TF)** formulation with 0.3% TMP and 225 ppm F; **(ETF)** formulation with 0.3% PPE, 0.3% TMP and 225 ppm F; **(CM)** commercial mouthwash of essential oil.

Table 1. Grading of vascular reactions (hyperemia, hemorrhage, and coagulation) in the chorioallantoic membrane of the embryonated egg chicken in the HET-CAM test.

Table 2. Mean (SD) initial surface hardness loss (SHi), percentage of surface hardness loss (%SH), integrated loss of subsurface hardness (Δ KHN), fluoride, calcium, and phosphorus concentration in enamel and biofilm according to the different treatments.

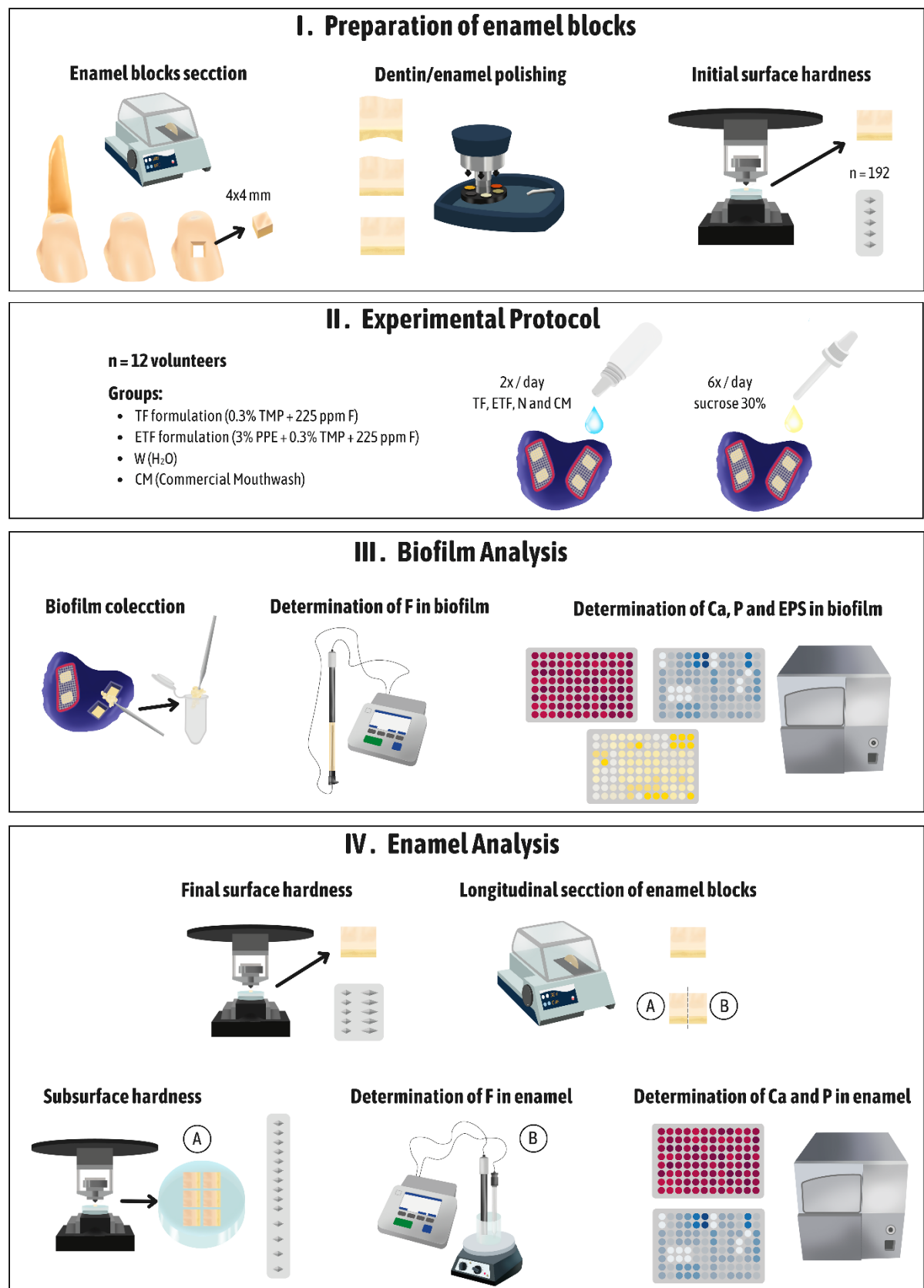


Figure 1. Experimental design of the study.

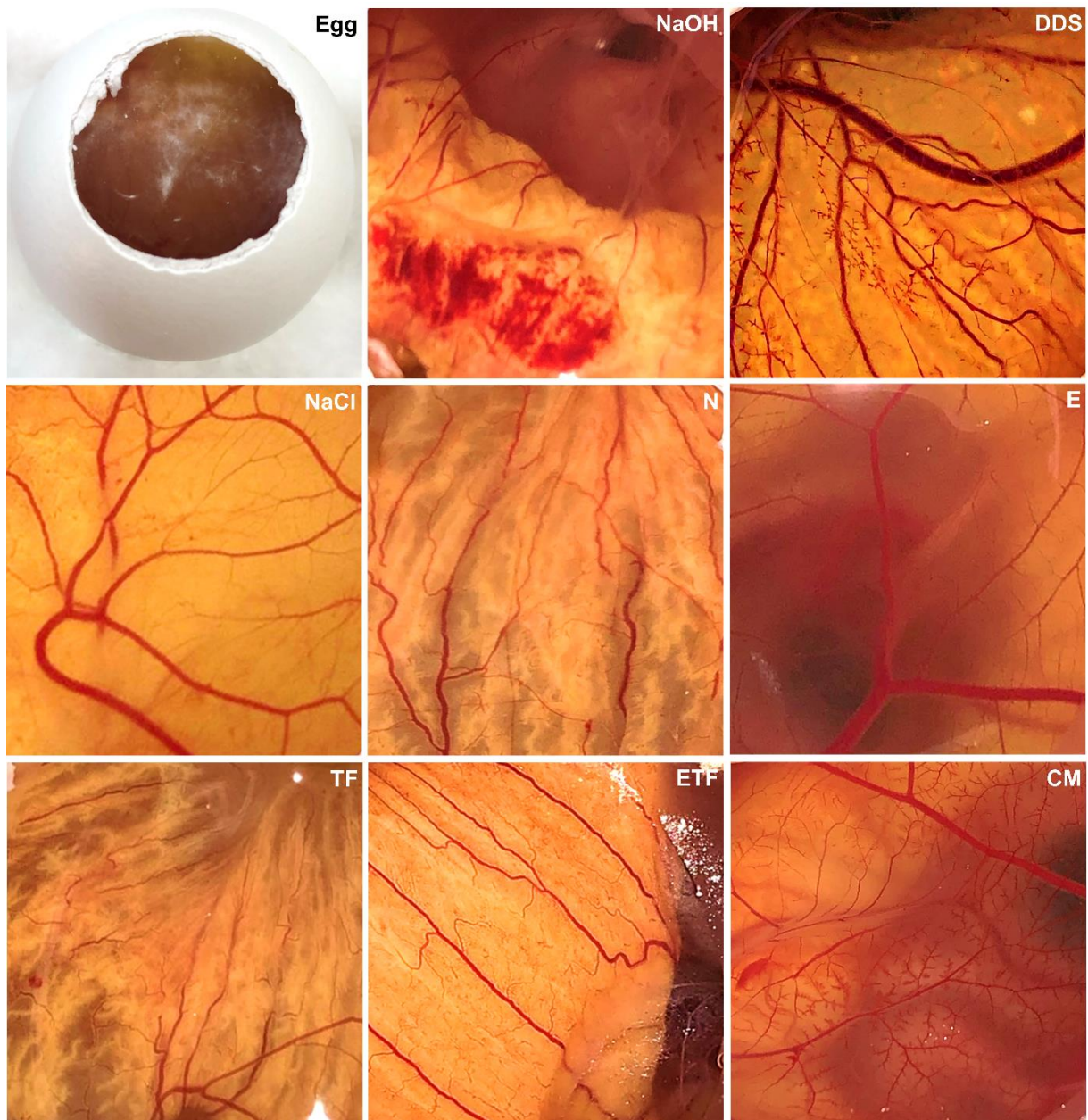


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Table 1. Grading of vascular reactions (hyperemia, hemorrhage, and coagulation) in the chorioallantoic membrane of the embryonated egg chicken in the HET-CAM test.

Effect	Score		
	< 30 s	30–60 s	60–300 s
Hyperaemia	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5

Table 2. Mean (SD) initial surface hardness loss (SHi), percentage of surface hardness loss (%SH), integrated loss of subsurface hardness (Δ KHN), fluoride, calcium, and phosphorus concentration in enamel and biofilm according to the different treatments.

Analysis	Mouthwash formulations			
	W	TF	ETF	CM
SHi (KHN)	366.2 ^a (0.2)	366.2 ^a (0.7)	366.2 ^a (0.7)	365.9 ^a (0.7)
%SH (KHN)	-76.8 ^a (5.9)	-27.3 ^b (4.5)	-14.6 ^c (2.9)	-24.5 ^b (5.3)
Δ KHN (KHN x μ m)	7607.3 ^a (503.6)	3926.6 ^b (380.2)	2845.4 ^c (365.7)	4032.8 ^b (298.2)
Enamel (μg mm⁻³)				
Fluoride	0.2 ^a (0.1)	0.5 ^b (0.2)	0.4 ^b (0.1)	0.4 ^b (0.2)
Calcium	378.0 ^a (83.3)	337.0 ^a (99.1)	668.0 ^b (197.1)	340.3 ^a (138.2)
Phosphorus	245.0 ^a (92.8)	219.0 ^a (43.1)	312.7 ^b (100.1)	219.4 ^a (88.2)
Biofilm (mol kg⁻¹)				
Fluoride	5.48E-04 ^a (9.46E-05)	1.26E-03 ^b (7.34E-04)	2.66E-03 ^c (9.30E-04)	2.74E-03 ^c (1.10E-03)
Calcium	2.62E-01 ^a (7.28E-02)	2.71E-01 ^a (3.81E-02)	3.54E-01 ^b (3.60E-02)	2.64E-01 ^a (7.27E-02)
Phosphorus	7.88E-02 ^a (2.54E-02)	9.81E-02 ^a (3.88E-02)	1.34E-01 ^b (5.54E-02)	9.55E-02 ^a (2.26E-02)
EPS	4.5 ^a (1.9)	1.7 ^b (0.8)	1.0 ^c (0.4)	1.9 ^b (0.5)

KHN (Knoop hardness); W: deionized water (n=12); E: formulation with 0.3% pomegranate peel extract (n=12); TF: formulation with 0.3% sodium trimetaphosphate and 225 ppm fluoride (n=12); ETF: formulation with 0.3% pomegranate peel extract, 0.3% sodium trimetaphosphate, and 225 ppm fluoride (n=12); CM: essential oil commercial mouthwash (n=12). Different superscript letters indicate significant differences among the treatments for each variable. Data were analyzed using repeated measures of one-way ANOVA, followed by the Student-Newman-Keuls test ($p < 0.05$).

Supplementary Material

Ingredients of essentials oil anticaries alcohol-free commercial mouthwash

Purified water, sorbitol, propylene glycol, poloxamer 407, sodium lauryl sulfate, benzoic acid, aroma (benzyl alcohol, d-Limonene), eucalyptol, methyl salicylate, thymol, sodium saccharina, sodium fluoride (220 ppm), sodium benzoate, sucralose, menthol, CI 47005/quinoline yellow, CI 42053/fast green.

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Toothpaste formulation

Toothpastes were produced with the following components: titanium dioxide, carboxymethyl cellulose, methyl p-hydroxybenzoate sodium, saccharin, mint oil, glycerin, abrasive silica, sodium lauryl sulphate and deionized water.

ANEXO A: Resumo do estudo

OBTENÇÃO DO EXTRATO DA CASCA DA ROMÃ



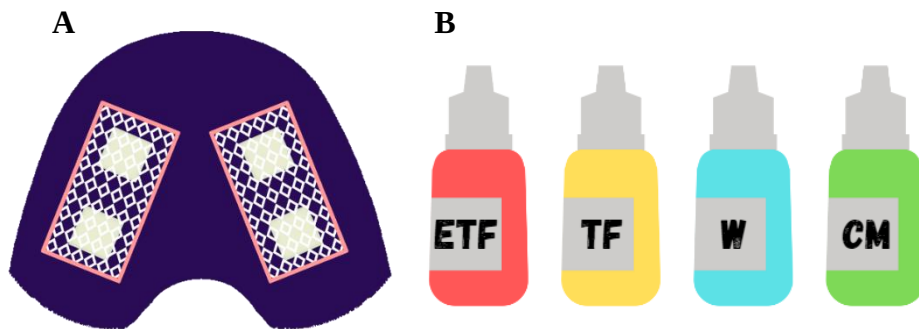
(A) Pó do extrato da casca da romã; (B) Maceração; (C) Retroevaporação; (D) Extrato mole; (E) Extrato da casca da romã diluído em propilenoglicol.

DESENVOLVIMENTO DAS FORMULAÇÕES LÍQUIDAS



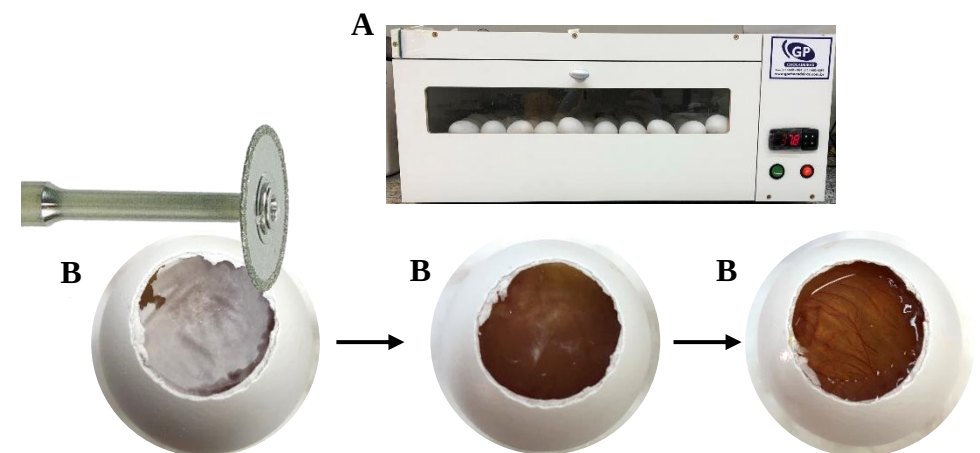
Formulação com extrato da casca da romã, trimetafosfato de sódio, fluoreto de sódio, estabilizante, conservante, quelante, edulcorante e água purificada q.s.p.

ESTUDO *IN SITU*



(A) Esquema representativo do dispositivo palatino em resina acrílica utilizado pelos voluntários, contendo quatro blocos de esmalte fixados no interior de lojas do dispositivo e cobertos com malha de plástico para permitir o acúmulo de biofilme oral. (B) Desenho dos frascos contendo os quatro grupos das formulações para enxaguatório bucal testados pelos voluntários: formulação ETF (contendo 3% de extrato da casca da romã + 0,3% de trimetafosfato de sódio + 225 ppm de flúor), formulação TF (contendo 0,3% de trimetafosfato de sódio + 225 ppm de flúor), W (água deionizada) e enxaguatório bucal comercial de óleos essenciais sem álcool (CM).

ENSAIO DE MEMBRANA CÓRIO-ALANTÓIDE DO OVO DE GALINHA



(A) Chocadeira automática com os ovos de galinha fertilizados; (B1) Abertura do ovo fertilizado com exposição da membrana da casca; (B2) ovo fertilizado após retirada da membrana da casca e exposição de membrana cório-alantóide; (C) ovo fertilizado após umectação da membrana cório-alantóide.

ANEXO B

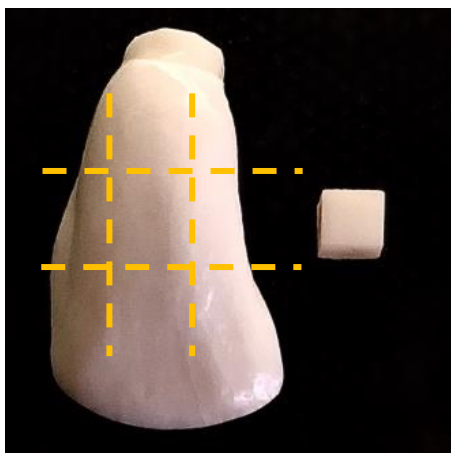
Padronização do extrato da casca da romã nas formulações em % de sólidos

Teor de sólidos de romã (mg mL^{-1}) no extrato glicólico da casca da romã e nas formulações com (E e ETF) e sem o extrato da casca da romã (P e TF) em sua composição.

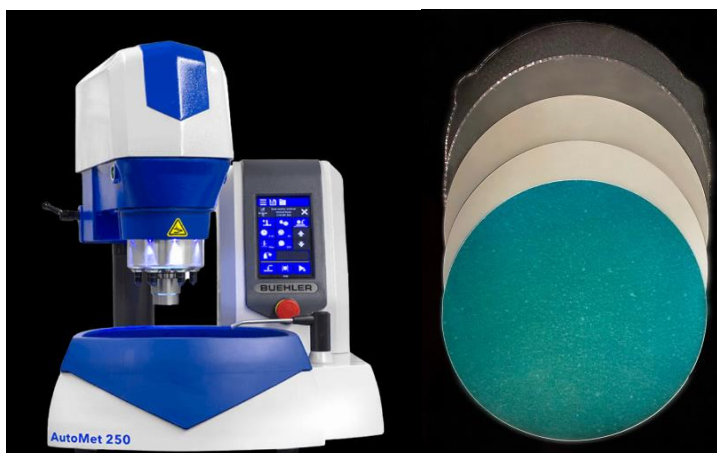
Triplicata	Absorbância	Massa (mg)	Teor (mg g ⁻¹)	Média (mg g ⁻¹)	Densidade	Teor (mg mL ⁻¹)	Média (mg mL ⁻¹)	Desvio padrão (mg mL ⁻¹)	CV (%)	Sólidos de romã (mg mL ⁻¹)
Extrato glicólico da casca da romã										
1	0,560	129,46	103,78	101,13	1,137	118,00	114,98	3,55	3,51	328,02
2	0,641	156,96	97,69			111,07				
3	0,552	129,99	101,91			115,87				
Formulação N (sem os ativos)										
1	0,01	622,5	0,44	0,46	1,056	0,463	0,49	0,06	12,50	-
2	0,009	623,48	0,42			0,443				
3	0,014	613,33	0,52			0,551				
Formulação E (3% PPE)										
1	0,557	619,87	10,78	10,78	1,072	11,556	11,56	0,01	0,07	32,16
2	0,569	633,35	10,77			11,548				
3	0,562	624,90	10,79			11,564				
Formulação TF (0,3% TMP + 225 ppm F)										
1	0,01	624,2	0,44	0,51	1,057	0,462	0,54	0,07	14,29	-
2	0,015	632,32	0,52			0,554				
3	0,017	618,43	0,57			0,607				
Formulação ETF (3% PPE + 0,3% TMP + 225 ppm F)										
1	0,57	634,56	10,77	10,79	1,074	11,568	11,59	0,06	0,55	32,22
2	0,57	635,99	10,75			11,542				
3	0,566	625,52	10,85			11,654				

ANEXO C

Preparo dos blocos de esmalte e análise de dureza de superfície inicial



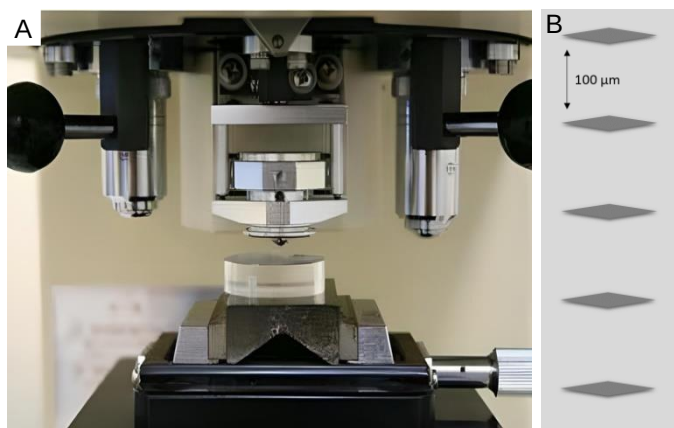
Seleção da região a ser seccionada da cora de incisivo central bovino e bloco de esmalte já seccionado (4 x 4 mm).



Politriz automática (AutoMet 250 Buehler, Lake Bluff, Illinois, USA) e lixas de diferentes granulações (CARBIMET Paper Discs, 30-5108-320, Buehler) utilizadas para planificação da dentina e polimento do esmalte bovino.



Bloco de esmalte fixado com cera pegajosa em disco de resina acrílica após o polimento.



(A) Bloco de esmalte posicionado para leitura de dureza de superfície inicial no esmalte utilizando-se microdurômetro Shimadzu HMV-2000 (Shimadzu Corp., Kyoto, Japan; Software de imagem Buehler OminMet), com penetrador tipo Knoop e carga estática de 25 gramas por 10 segundos. (B) Esquema das cinco endentações realizadas na superfície de esmalte com distanciamento padronizado entre si.

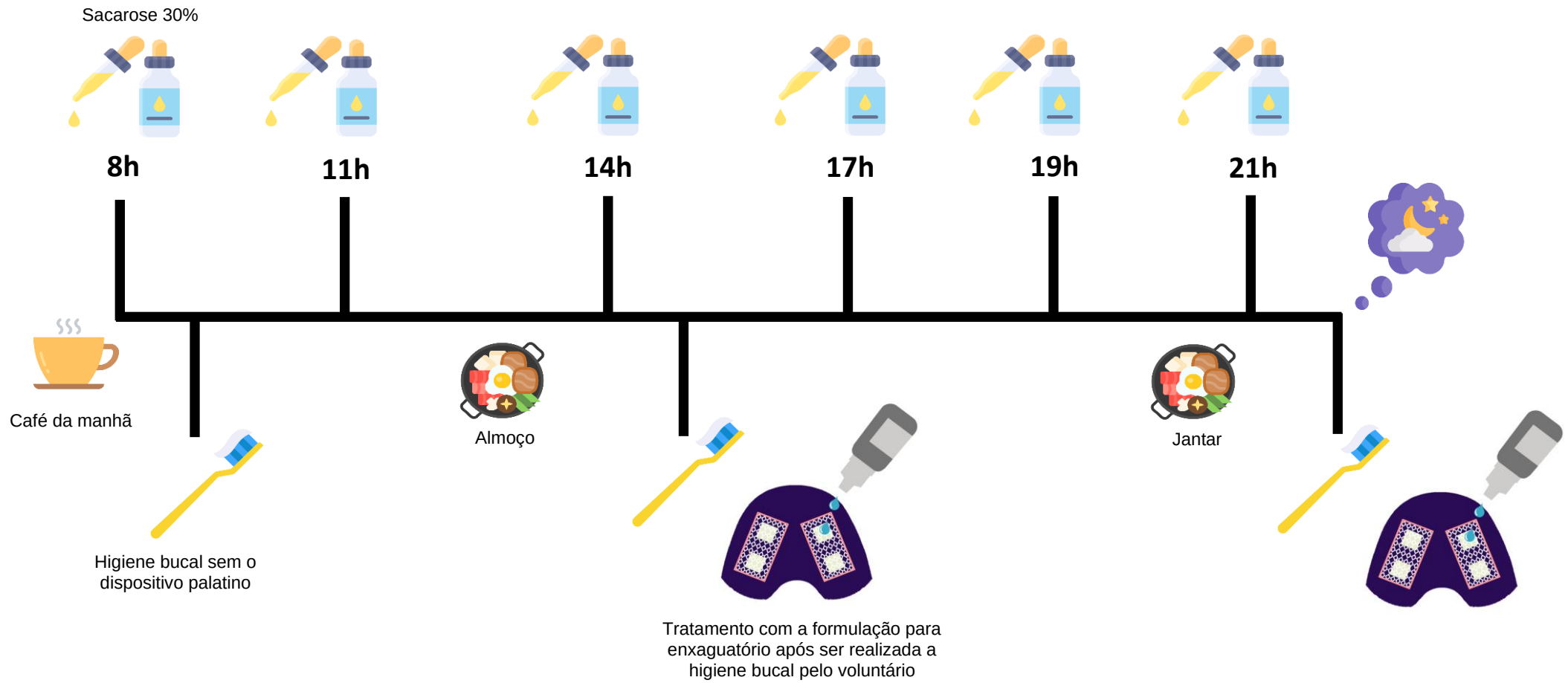
ANEXO D

Fase experimental *in situ*

Kit recebido pelos voluntários contendo: (A) dispositivo palatino, (B) escova de dentes, (C) dentífrico não fluoretado, (D) caixa para armazenamento, (E) sacarose e (F) enxaguatório bucal experimental.

ANEXO E

Protocolo experimental



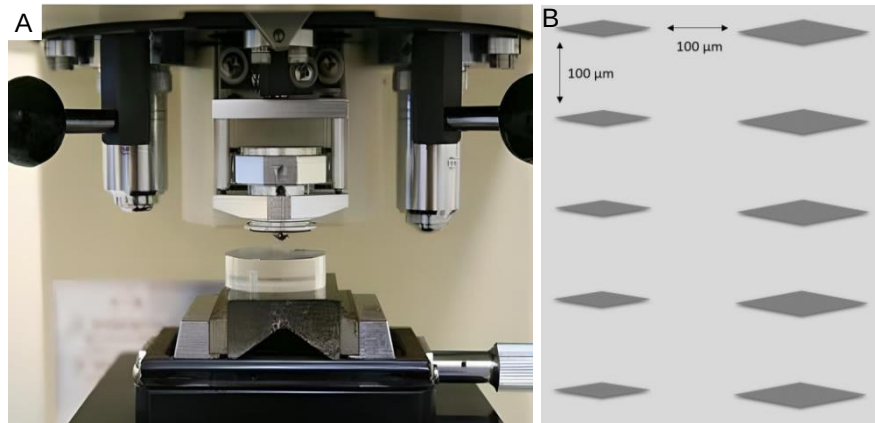
ANEXO F

Instruções aos voluntários:

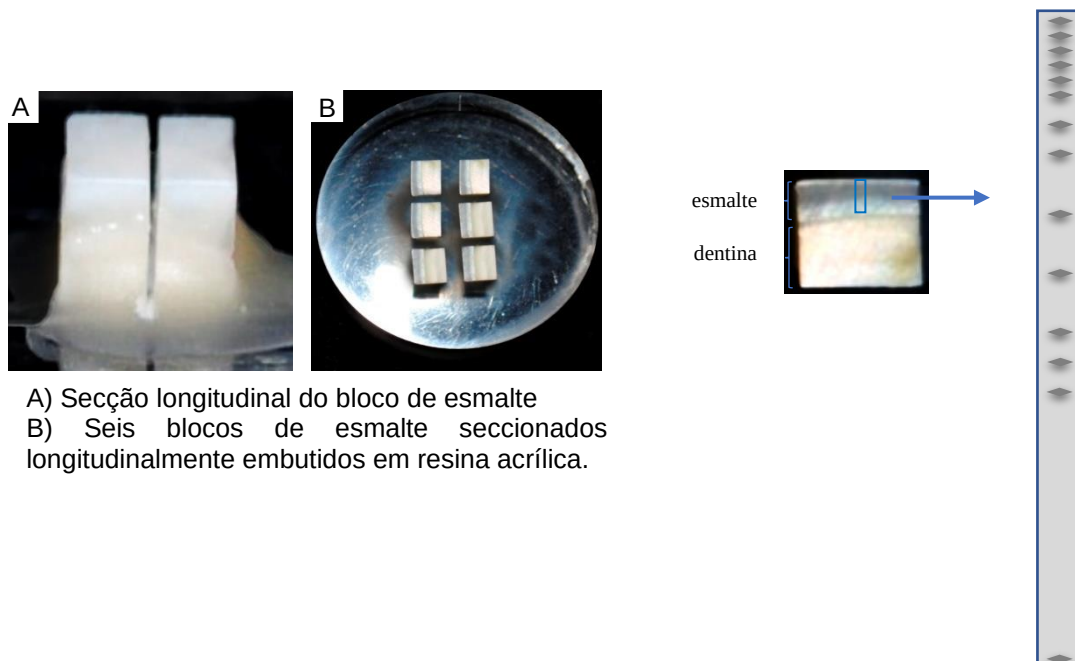
- O voluntário deverá fazer uso do dentifrício não fluoretado fornecido pela pesquisadora 7 dias antes e durante todo o período do estudo;
- A pesquisa será composta por 4 regimes experimentais (enxaguatórios bucais), com duração de 7 dias e intervalo de uma semana entre eles;
- Durante cada fase experimental e entre elas, não será permitido o uso de enxaguatórios bucais (exceto o oferecido pela pesquisadora responsável), dentifrício e fio dental fluoretados;
- O dispositivo intraoral palatino deverá ser utilizado durante todo o período experimental, inclusive para dormir e deverá ser removido apenas para ingestão de líquidos e sólidos, ou para o gotejamento da sacarose e do enxaguatório bucal;
- A sacarose deverá ser gotejada seis vezes ao dia, após gotejá-la o dispositivo intraoral palatino deverá permanecer 5 min fora da boca;
- A escovação deverá ser realizada somente 3 vezes ao dia com o dentifrício que foi fornecido e com o dispositivo intraoral palatino na cavidade bucal, nos seguintes períodos: após o café da manhã, após o almoço e após o jantar;
- Caso deseje escovar os dentes mais vezes é necessário retirar o dispositivo intraoral palatino;
- O enxaguatório bucal fornecido pela pesquisadora deverá ser gotejado duas vezes ao dia depois da escovação que foi realizada após o almoço e após o jantar. Gotejar duas gotas do enxaguatório bucal e aguardar 1 min para retornar o dispositivo intraoral palatino à cavidade bucal;
- Obrigada pela colaboração, qualquer dúvida entrar em contato com a pesquisadora Ana Paula Miranda Vieira.

ANEXO G

Análise de dureza de superfície final, secção longitudinal dos blocos e análise de dureza em secção longitudinal



(A) Bloco de esmalte posicionado para leitura de dureza de superfície final no esmalte utilizando-se microdurômetro Shimadzu HMV-2000 (Shimadzu Corp., Kyoto, Japan; Software de imagem Buehler OminMet), com penetrador tipo Knoop e carga estática de 25 gramas por 10 segundos. (B) Esquema das cinco endentações realizadas a 100 μm de distância entre si (coluna da esquerda endentações iniciais e coluna da direita endentações iniciais com a distância entre elas de 100 μm) na superfície de esmalte.



A) Secção longitudinal do bloco de esmalte
B) Seis blocos de esmalte seccionados longitudinalmente embutidos em resina acrílica.

A) Corte longitudinal do dente bovino indicando no esmalte região onde foram realizadas as leituras de dureza.
B) Esquema com quatorze endentações para leitura da dureza em sessão longitudinal (dureza de subsuperfície): sequência de impressões nas distâncias de 5, 10, 15, 20, 25, 30, 40, 50, 70, 90, 110, 130, 220 e 330 μm na superfície externa do esmalte.

ANEXO H

Microabrasão do esmalte para análise da concentração de fluoreto, cálcio e fósforo



Bloco de esmalte seccionado no sentido longitudinal e fixado com cola adesiva em mandril para peça reta para realização da microabrasão do esmalte.



Mensuração com micrômetro eletrônico digital após a microabrasão do esmalte (50 μm).



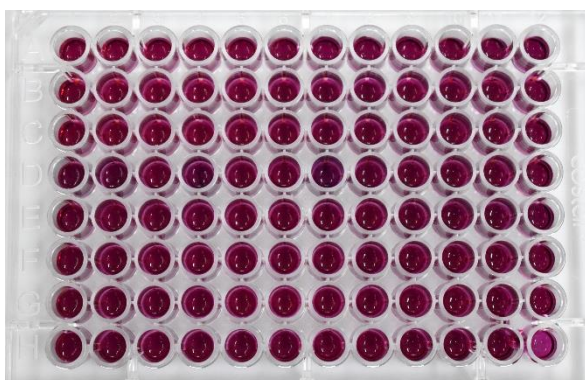
Pó (→) de esmalte derivado da microabrasão em lixa adaptada em frasco de polietileno.

ANEXO 1

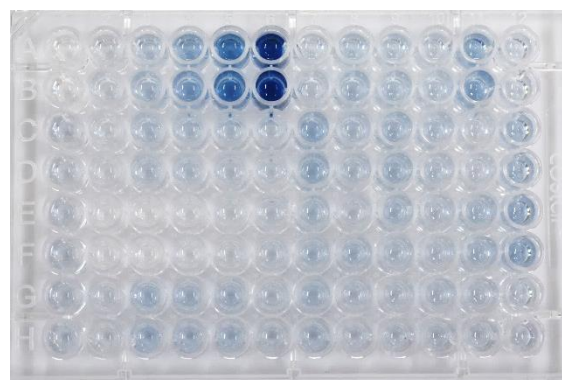
Análise de fluoreto, cálcio e fósforo no esmalte



Análise de fluoreto no esmalte com eletrodo específico 9409BN e microeletrodo de referência acoplados a um analisador de íons.



Determinação da concentração de cálcio no esmalte pelo método colorimétrico descrito por Vogel et al. 2009 utilizando-se Arsenazo III.



Determinação da concentração de fósforo no esmalte de acordo com o método colorimétrico descrito por Fiske & Subbarow 1925.

ANEXO J

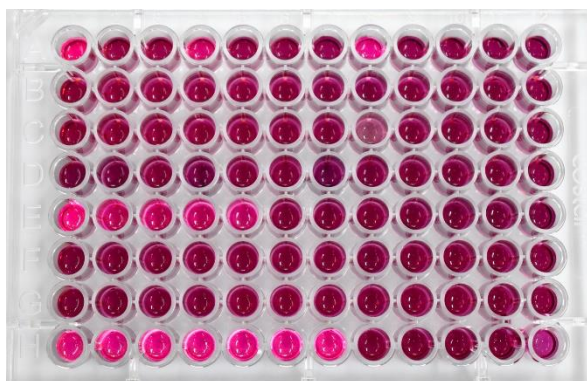
Análise de fluoreto, cálcio, fósforo e carboidratos álcali-solúveis no biofilme



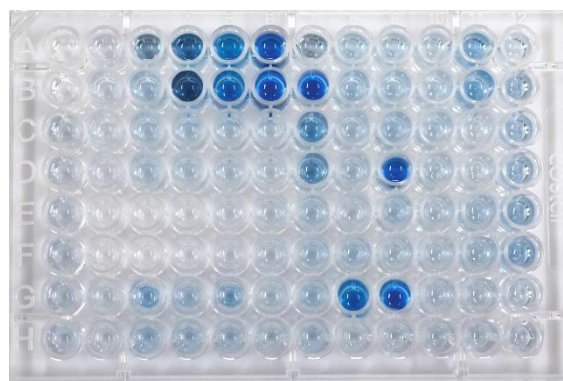
Análise de fluoreto no biofilme com eletrodo específico, potenciômetro e um analisador de íons.



Dosagem de carboidratos álcali-solúveis no biofilme de acordo com o método fenol-ácido sulfúrico descrito por Dubois et al. 1956.



Determinação da concentração de cálcio no biofilme pelo método colorimétrico descrito por Vogel et al. 2009 utilizando-se Arsenazo III.



Determinação da concentração de fósforo no biofilme de acordo com o método colorimétrico descrito por Fiske & Subbarow 1925.

ANEXO K

UNESP - FACULDADE DE
ODONTOLOGIA-CAMPUS DE
ARAÇATUBA/ UNIVERSIDADE



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do potencial anticariogênico de uma formulação para enxaguatório bucal sem álcool contendo extrato da casca de romã (*Punica granatum*), trimetafosfato de sódio e flúor

Pesquisador: ANA PAULA MIRANDA VIEIRA

Área Temática:

Versão: 2

CAAE: 10320719.9.0000.5420

Instituição Proponente: Faculdade de Odontologia do Campus de Araçatuba - UNESP

Patrocinador Principal: Universidade Estadual Paulista Júlio de Mesquita Filho

DADOS DO PARECER

Número do Parecer: 3.326.501

Apresentação do Projeto:

A apresentação do projeto está adequada as normas do CEP.

Objetivo da Pesquisa:

Objetivo Primário:

A proposta geral desse projeto será avaliar in situ a ação de uma formulação líquida para enxaguatório bucal (ETF) contendo extrato da casca da romã, TMP e F no processo de desmineralização do esmalte dentário.

Objetivo Secundário:

Avaliar in situ a atividade anticariogênica sobre a dureza de blocos de esmalte bovino, e quantificar no biofilme formado sobre eles: carboidratos álcali-solúveis, Ca, P e F, além do Ca, P e F presentes no esmalte após ser submetido a ação da ETF.

Avaliação dos Riscos e Benefícios:

Riscos:

A participação nesta pesquisa não infringe as normas legais e éticas: o voluntário deverá fazer uso de um dispositivo palatino, o qual irá fixar-se no palato por um período de 7 dias em cada fase experimental 1) Formulação com extrato da casca da romã a 3%, 0,3% TMP e 225 ppm F, 2)

Endereço: JOSE BONIFACIO 1193

Bairro: VILA MENDONÇA

CEP: 16.015-050

UF: SP

Município: ARACATUBA

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E-mail: andrebertoz@foa.unesp.br

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ARAÇATUBA/ UNIVERSIDADE



Continuação do Parecer: 3.326.501

Formulação com 0,3% TMP e 225 ppm F, 3) LISTERINE® anticáries zero álcool, 4) Formulação placebo (sem o extrato da casca da romã, sem

TMP e sem F) , o que poderá talvez ocorrer, um certo desconforto. Os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em

Pesquisa com Seres Humanos conforme Resolução nº. 466/12 do Conselho Nacional de Saúde. Nenhum dos procedimentos usados oferece riscos

à sua dignidade. O risco será Mínimo.

Benefícios:

Ao participar desta pesquisa o(a) sr.(a) não terá nenhum benefício direto. Entretanto, esperamos que este estudo resulte em informações

importantes sobre uma nova formulação de um enxaguatório bucal sobre o processo de remineralização de lesões iniciais de cárie. Além disso, seria

interessante aprofundar os conhecimentos sobre o sinergismo entre o extrato da casca da romã, TMP e F, de forma que o conhecimento que será

construído a partir desta pesquisa possa contribuir para a Odontologia, onde pesquisador se compromete a divulgar os resultados obtidos,

respeitando-se o sigilo das informações coletadas, conforme previsto no item anterior.

Comentários e Considerações sobre a Pesquisa:

Nada a declarar.

Considerações sobre os Termos de apresentação obrigatória:

Adequados

Recomendações:

Nada a declarar

Conclusões ou Pendências e Lista de Inadequações:

Não há.

Considerações Finais a critério do CEP:

Não havendo pendências, o CEP propõe a aprovação do projeto de pesquisa salientando que, de acordo com a Resolução 466 CNS de 12/12/2012 (título X, seção X.1., art. 3, item b, e, título XI, seção XI.2., item d), há necessidade de apresentação de relatórios semestrais, devendo o primeiro relatório ser enviado até 01/11/2019. O CEP reitera a necessidade de entrega de uma via (não cópia) do TCLE ao sujeito participante da pesquisa e solicita ao pesquisador responsável leitura da carta circular 003/2011 CONEP/CNS antes do início do projeto.

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Continuação do Parecer: 3.326.501

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1314323.pdf	18/04/2019 11:05:08		Aceito
Projeto Detalhado / Brochura Investigador	PP.pdf	18/04/2019 10:57:14	ANA PAULA MIRANDA VIEIRA	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	17/04/2019 16:46:14	ANA PAULA MIRANDA VIEIRA	Aceito
Folha de Rosto	Folha_de_rosto.pdf	17/04/2019 16:45:31	ANA PAULA MIRANDA VIEIRA	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

ARACATUBA, 15 de Maio de 2019

**Assinado por:
Aldiéris Alves Pesqueira
(Coordenador(a))**

Endereço: JOSE BONIFACIO 1193

Bairro: VILA MENDONÇA

CEP: 16.015-050

UF: SP

Município: ARACATUBA

Telefone: (18)3636-3200

Fax: (18)3636-3332

E-mail: andrebertoz@foa.unesp.br

ANEXO L



19/12/2019 870190136418
12:52

29409161913227919

**Pedido nacional de Invenção, Modelo de Utilidade, Certificado de
Adição de Invenção e entrada na fase nacional do PCT**

Número do Processo: BR 10 2019 027251 1

Dados do Depositante (71)

Depositante 1 de 2

Nome ou Razão Social: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA
FILHO

Tipo de Pessoa: Pessoa Jurídica

CPF/CNPJ: 48031918000124

Nacionalidade: Brasileira

Qualificação Jurídica: Instituição de Ensino e Pesquisa

Endereço: Rua Quirino de Andrade, 215

Cidade: São Paulo

Estado: SP

CEP: 01049-010

País: Brasil

Telefone: 11 56270217

Fax: 11 56270103

Email: auin@unesp.br

**PETICIONAMENTO
ELETRÔNICO**

Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 19/12/2019 às
12:52, Petição 870190136418

Depositante 2 de 2

Nome ou Razão Social: APIS FLORA INDL. COML. LTDA

Tipo de Pessoa: Pessoa Jurídica

CPF/CNPJ: 49345358000307

Nacionalidade: Brasileira

Qualificação Jurídica: Pessoa Jurídica

Endereço: Av Barao Do Bananal, 2305, Jd Anhanguera

Cidade: Ribeirão Preto

Estado: SP

CEP: 14092-000

País: BRASIL

Telefone:

Fax:

Email:

Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): Composição, obtenção e uso de formulações de cuidado bucal

Resumo: A presente invenção, pertencente ao campo da odontologia, fornece formulações de cuidado bucal, constituídas por: fluoreto, fosfato e extrato da Punica granatum. Além disso, há a inclusão de veículos oralmente aceitos para administração da composição. O termo bucal aqui utilizado abrange qualquer superfície mole ou sólida dentro da cavidade bucal, incluindo língua, palato duro e mole, gengiva, mucosas bucais e superfícies dentárias naturais ou artificiais. São reivindicadas as formulações de cuidado bucal, método de obtenção e seus usos.

**PETICIONAMENTO
ELETRÔNICO**

Esta solicitação foi enviada pelo sistema Petição Eletrônica em 19/12/2019 às 12:52, Petição 870190136418

Dados do Procurador

Procurador:

Nome ou Razão Social: Renan Padron Almeida

Numero OAB:

Numero API:

CPF/CNPJ: 33778301896

Endereço: Rua Joaquim Antunes 819

Cidade: São Paulo

Estado: SP

CEP: 05415012

Telefone: 1156270570

Fax:

Email: renan.padron@unesp.br

**PETICIONAMENTO
ELETRÔNICO**

Esta solicitação foi enviada pelo sistema Petição Eletrônica em 19/12/2019 às 12:52, Petição 870190136418

Dados do Inventor (72)

Inventor 1 de 5**Nome:** DEBORA DE BARROS BARBOSA**CPF:** 25228297855**Nacionalidade:** Brasileira**Qualificação Física:** Professor do ensino superior**Endereço:** Rua José Bonifácio, 1193**Cidade:** Araçatuba**Estado:** SP**CEP:** 16015-050**País:** BRASIL**Telefone:****Fax:****Email:****Inventor 2 de 5****Nome:** ANDRESA APARECIDA BERRETTA E SILVA**CPF:** 25897916829**Nacionalidade:** Brasileira**Qualificação Física:** Enfermeiro de nível superior, nutricionista, farmacêutico e afins**Endereço:** Rua Triunfo, 945**Cidade:** Ribeirão Preto**Estado:** SP**CEP:** 14020-670**País:** BRASIL**Telefone:****Fax:****Email:****Inventor 3 de 5****PETICIONAMENTO
ELETRÔNICO**

Esta solicitação foi enviada pelo sistema Petição Eletrônica em 19/12/2019 às 12:52, Petição 870190136418

Nome: ALBERTO CARLOS BOTTAZZO DELBEM

CPF: 09546471810

Nacionalidade: Brasileira

Qualificação Física: Professor do ensino superior

Endereço: Rua José Bonifácio, 1193

Cidade: Araçatuba

Estado: SP

CEP: 16015-050

País: BRASIL

Telefone:

Fax:

Email:

Inventor 4 de 5

Nome: GABRIELA LOPES FERNANDES

CPF: 37059940855

Nacionalidade: Brasileira

Qualificação Física: Odontólogo

Endereço: Rua Compadre João Bertoni, nº 882

Cidade: Araçatuba

Estado: SP

CEP: 16020-367

País: BRASIL

Telefone:

Fax:

Email:

Inventor 5 de 5

**PETICIONAMENTO
ELETRÔNICO**

Esta solicitação foi enviada pelo sistema Petição Eletrônica em 19/12/2019 às 12:52, Petição 870190136418

Nome: ANA PAULA MIRANDA VIEIRA

CPF: 40768181801

Nacionalidade: Brasileira

Qualificação Física: Odontólogo

Endereço: Rua Passeio Canindé, nº 413

Cidade: Ilha Solteira

Estado: SP

CEP: 15385-000

País: BRASIL

Telefone:

Fax:

Email:

Documentos anexados

Tipo Anexo	Nome
Procuração	Proc e Posse 07-2018.pdf
Procuração	Procuração Apis Flora.pdf
Comprovante de pagamento de GRU 200	GRU PI 5 29409161913227919 comprov.pdf
Relatório Descritivo	Relatório Descritivo.pdf
Reivindicação	Reivindicações.pdf
Resumo	Resumo.pdf

Acesso ao Patrimônio Genético

- ☒ Declaração Negativa de Acesso - Declaro que o objeto do presente pedido de patente de invenção não foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, o acesso foi realizado antes de 30 de junho de 2000, ou não se aplica.

Declaração de veracidade

- ☒ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

**PETICIONAMENTO
ELETRÔNICO**

Esta solicitação foi enviada pelo sistema Petição Eletrônica em 19/12/2019 às 12:52, Petição 870190136418



UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

AGÊNCIA UNESP DE INOVAÇÃO



PROCURAÇÃO

Pelo presente instrumento,

a **UNIVERSIDADE ESTADUAL PAULISTA "JULIO DE MESQUITA FILHO" - UNESP**, autarquia estadual de regime especial, criada pela Lei nº 952 de 30.01.1976, com sede na Rua Quirino de Andrade, 215, Centro, CEP 01.049-010, São Paulo/SP, inscrita no CNPJ/MF sob nº 48.031.918/0001-24, doravante designada simplesmente UNESP, neste ato, representada por seu Magnífico Reitor, Prof. Dr. **SANDRO ROBERTO VALENTINI**, de acordo com o Art. 34, I de seu Estatuto, ou quem legalmente o substitua,

nomeia e constitui seu procurador, **RENAN PADRON ALMEIDA**, brasileiro, portador do RG nº 43.746.608-5, SSP/SP, inscrito no CPF/MF sob o nº 337.783.018/96,

outorgando-lhe poderes para representá-la perante o Instituto Nacional da Propriedade Intelectual – INPI e outras instituições competentes, para o fim de requerer e processar direitos de propriedade intelectual, tais como patentes de

Agência UNESP de Inovação
Rua Quirino de Andrade, 215 – 9º andar - Centro
CEP: 01049-010, São Paulo/SP - Brasil
Fone: +55 11 5627 0696 - e-mail: auin@unesp.br



UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"



AGÊNCIA UNESP DE INOVAÇÃO

invenção, de modelos de utilidade, desenhos industriais, registros de marcas de produto, de serviço, coletivas ou de certificação, de indicações geográficas, cultivares, direitos de autor, de programas de computador e mantê-los em vigor com amplos e ilimitados poderes para assinar petições, autorizações para cópias, termos de cessão de direitos, termos de gestão e compartilhamento de propriedade intelectual, documentos diversos relacionados ao processo administrativo de proteção de direitos de propriedade intelectual, incluindo, mas não se limitando, aos documentos já utilizados pelo INPI, bem como àqueles que vierem a ser adotados e utilizados para instrução processual de patentes, modelos de utilidades, marcas, desenhos industriais e programas de computador, pagar taxas, retribuições, impostos, fazer prova de uso das invenções patenteadas ou das marcas registradas, efetuar pagamentos e receber restituições, dando as respectivas quitações, apresentar oposições, recursos, réplicas, desistir, renunciar, anotar, averbar contratos de licença e transferências de tecnologia, elaborar notificações extrajudiciais, requerer prorrogação dos prazos de proteção, fazer declarações, opor, protestar, impugnar, recorrer, pedir reconsideração, manifestar-se sobre oposições e recursos, obter vista de processos, cumprir exigências, apresentar defesas escritas ou orais, desistir, replicar, transigir, receber, juntar e retirar documentos, requerer caducidade e contestar pedido de caducidade, requerer e contestar nulidade administrativa e licença compulsória, preencher qualquer tipo de formalidade, requerer anotação e averbação de cessão, alterações de nome e sede, proceder à publicação de editais de chamamento para instruir, elaborar, firmar e acompanhar contratos de transferência de tecnologia e/ou de licenciamento com exclusividade ou não, e praticar para o fim mencionado

Agência UNESP de Inovação
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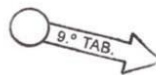
UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"



AGÊNCIA UNESP DE INOVAÇÃO

todos os atos necessários perante as autoridades administrativas competentes no Brasil em benefício da Outorgante.

São Paulo, 16 de julho de 2018.



UNESP

pl Prof. Dr. Sandro Roberto Valentini

Reitor

SERGIO ROBERTO NOBRE
VICE-REITOR NO EXERCÍCIO DA REITORIA

9.º TABELÃO DE NOTAS

Rua Mesquita, 127 - 9º andar - CEP 01047-000 - São Paulo
Telefone: (11) 2556-2000 - Fax: (11) 2174-6858
www.notaartorio.com.br

Reconheço a 1 firma com valor econômico por semelhança de SERGIO ROBERTO NOBRE, do que dou fé.

Em tesº da verdade.

São Paulo/Capital, 24 de julho de 2018. ANDREI BARRETO DA SILVA - Valor recebido R\$ 9,25
Válido somente com selo de autenticidade. Selo pago por verba



Agência UNESP de Inovação

Rua Quirino de Andrade, 215 – 9º andar - Centro

CEP: 01049-010, São Paulo/SP - Brasil

Fone: +55 11 5627 0696 - e-mail: auin@unesp.br

terça-feira, 29 de novembro de 2016

Diário Oficial Poder Executivo - Seção I

São Paulo, 126 (222) - 3

Artigo 1º - É declarada de utilidade pública a Associação Mestre Custódio Prossidônio Martins, com sede em Apiaí.

Artigo 2º - Esta lei entra em vigor na data de sua publicação.

Palácio dos Bandeirantes, 28 de novembro de 2016.

GERALDO ALCKMIN

Marcos Fernando Elias Rosa

Secretário de Justiça e da Defesa da Cidadania

Samuel Moreira da Silva Junior

Secretário-Chefe da Casa Civil

Publicada na Assessoria Técnica de Casa Civil, aos 28 de novembro de 2016.

Atos do Governador

DECRETOS

DECRETOS DE 28-11-2016

Diagmando, a pedido e a partir de 25-11-2016, João Beltrame Moraes de Andrade, RG 3.704.467.4, da função de Diretor Presidente da Fundação Memorial da América Latina.

Designando, Inês Feres Carvalho, RG 6.951.115.4, Chefe de Gabinete, da Fundação Memorial da América Latina, para responder pelo expediente da Presidência da Fundação.

Nomeando, com função de Assessoria Técnica de Casa Civil, nos termos do art. 3º do Estatuto da Universidade Estadual Paulista "Júlio de Mesquita Filho" - Unesp, aprovado pelo Dec. 29.720/81, e alterações:

Sendo Roberto Valente para exercer a função de Rector da aludida Universidade, com mandato de 4 anos, a partir de 16-1-2017.

Sendo Roberto Nêze para exercer a função de Vice-Rector da aludida Universidade, com mandato de 4 anos, a partir de 16-1-2017.

DESPACHOS DO GOVERNADOR

DESPACHOS DO GOVERNADOR, DE 28-11-2016

No processo SE-542-2016 (SG-118.809.16), sobre ressarcimento de débito: "Dante dos elementos de instrução constantes dos autos, em especial da representação do Secretário da Educação e da Casa 255-2016, do Assessoria Jurídica do Gabinete do Procurador Geral do Estado, autoriza que o ressarcimento do débito do Município de Niterói para com o Estado, decorrente da não aprovação de conta dos adiantamentos feitos ao Conselho celebrado em 2-7-2011, exercícios 2012, 2013 e 2015, seja-se em 24 parcelas mensais e consecutivas, observadas as normas legais e regulamentares pertinentes à espécie e às recomendações assinaladas no pronunciamento do órgão jurídico-consultivo."

No processo SE-1048-2016 (SG-118.810.16), sobre ressarcimento de débito: "Dante dos elementos de instrução constantes dos autos, em especial da representação do Secretário da Educação e da Casa 255-2016, do Assessoria Jurídica do Gabinete do Procurador Geral do Estado, autoriza que o ressarcimento do débito do Município de Garça para com o Estado, decorrente da não aprovação de conta referente ao exercício de 2015 do adiantamento do Conselho celebrado em 5-7-2011, seja-se em 24 parcelas mensais e consecutivas, observadas as normas legais e regulamentares pertinentes à espécie e às recomendações assinaladas no pronunciamento do órgão jurídico-consultivo."

No processo GBA-16-1075-16 (SG-1107.997.16), sobre contratação de guarda-vídeo: "A vista dos elementos de instrução do processo, com fundamento no art. 146, par. 1º da LC 1.093/2009, regulamentada pelo Dec. 54.682-2009, bem como das manifestações das Secretarias de Planejamento e Gestão e da Fazenda, autoriza, com base na necessidade temporária de excepcional interesse público, a Polícia Militar do Estado de São Paulo a retribuir as providências necessárias para a realização de processo seletivo simplificado, visando à contratação de 600 Guardas-Vídeo, por tempo determinado e pelo prazo máximo de 5 meses, correspondente ao período de novembro/2016 a março/2017, tendo por limite o valor disponível no período relativo à contratação anterior (Inov/015 a mar/2016), de modo que não haja expansão das despesas a serem cobradas pelo erário, obedecidos os demais preceitos legais e regulamentares pertinentes à espécie."

Casa Civil

GABINETE DO SECRETÁRIO

Despacho do Secretário, de 23-11-2016

No processo 34.860-2016, em que é interessada Casa Civil, sobre pagamento pelo indenização à Empresa Amarelo Turismo e Eventos ME, devido a fornecimento de refeições não constantes em contrato inicialmente celebrado: "A vista dos elementos que instruem os autos, notadamente o contrato no Relatório Final apresentado pela Comissão de Avaliação Preliminar, de fls. 315/320, complementados à fls. 334/335, no qual verifica-se que não houve má-fé por parte das envolvidas, bem como incorreram de eventual irregularidade, o Rector do Conselho Indicial da Secretaria de Governo 478-2016, de fls. 338/343, que se manifestou pela Viabilidade do pagamento, uma vez preenchidos todos os requisitos indicados nos incs I e II do art. 1º do Dec. 40.177-95, bem como o despacho da Chefe de Gabinete, de fls. 344/346, no qual foi lido no art. 265, parágrafo 3, da Lei 10.261-68, com redação dada pela LC 942-2003, impõe o arquivamento da Ação Preliminar, tendo em vista que não ficou caracterizado ilícito administrativo, em face do uso do princípio geral do direito que prescreve o empacotamento sem causa, autoriza o pagamento à empresa Amarelo Turismo e Eventos - Ltda - ME, a título indenizatório, da importância de R\$ 11.300,00, decorrente da prestação de serviços de fornecimento de 300 refeições, sem cobertura contratual, no dia 19 de março do corrente ano, no Hotel Fazenda Horta Abaeté/SP, aos participantes da III Conferência Estadual LGST. Consegue-se a Concordância Geral da Administração, conforme disposto no art. 1º, V alínea III, do Dec. 53.334-2008."

Governo

FUNDO SOCIAL DE SOLIDARIEDADE DO ESTADO DE SÃO PAULO

CHEFIA DE GABINETE

Extrato de 2º Termo de Adiantamento ao Convênio Consórcio FUSFESP 216/2014 - Processo FUSFESP 37326/2014

Parer C2 198/2016

Participa Fundo Social de Solidariedade do Estado de São Paulo e o Município de Burtama, por meio de seu Fundo Social de Solidariedade.

Clausula Primeira: O 1º termo de adiantamento ao convênio supracitado, celebrado em 23-12-2014 e o Plano de Trabalho que o integra, jantares, respectivamente, às fls. 85 a 88 e 71 a 75 dos autos do Processo FUSFESP 37326/2014, foram verificadas para constar que serão capacitados 1 e não 8 turnos por mês da

amem ora aditada, ficando restabelecido, assim, o número de turnos previsto no instrumento original do ajuste.

Parágrafo Primeiro - A vista do contrato no "caput" desta cláusula fica reafirmada a cláusula primeira do aludido 1º termo de adiantamento para constar que será transferido ao CONVEN-TE, no total, a quantia de R\$ 7.320,00.

Parágrafo Segundo - Os recursos financeiros remanescentes, sob a responsabilidade do FUSFESP, serão transferidos ao presente termo de adiantamento, para constar que o valor correto do convênio é de R\$ 56.992,63, dos quais R\$ 30.382,63 a cargo do FUSFESP e R\$ 26.610,00 a cargo do CONVEN-TE.

Clausula Terceira - A carga horária inerente ao Cargo de Assistente de Cabineiros, ministrado no âmbito do Projeto "Escola de Beleza" fica reduzida a partir da 3ª turma, em conformidade com o plano de trabalho a que se refere o 2º da cláusula primeira deste termo.

Clausula Quarta - A cláusula sexta do convênio original, alterado pelo 1º termo de adiantamento, sobre nova modificação e passa a vigorar com a seguinte redação:

"Cláusula Sexta (O prazo de vigência do presente convênio é de 42 meses, contados da data de assinatura do presente instrumento."

Data de assinatura: 28-11-2016.

CASA MILITAR

Resolução CM 17-610 - Ceder, de 28-11-2016

Edita o Plano Preventivo de Defesa Civil para erosão costeira, inundações costeiras e enchentes/alagamentos causados por eventos meteorológicos-oceanoográficos, extremos (ressacas do mar e marés altas).

Considerando as atribuições legais consubstanciadas nos Decs. 10.231-16, de 16-06-25 e nº 48.526, de 04-03-04, desta Secretário Chefe da Casa Militar e Coordenador Estadual de Defesa Civil;

Considerando que a Coordenadoria Estadual de Defesa Civil (CEDEC) desenvolve, de acordo com as peculiaridades de cada região, planos preventivos e de contingência visando à minimização de desastres;

Considerando o aumento do número de inundações e da magnitude de eventos meteorológicos-oceanoográficos extremos, como as ressacas do mar e marés altas anormais no litoral do Estado de São Paulo, em especial desde o final da década de 1990;

Considerando que 52% das zonas do Estado de São Paulo se encontram em risco alto e muito alto de erosão costeira;

Considerando os efeitos desses perigos costeiros, traduzidos em elevados prejuízos socioeconômicos e diversos tipos de transtornos à população, ao patrimônio público e privado, aos serviços e ao meio ambiente;

Considerando a necessidade da articulação do Sistema Estadual de Defesa Civil, para que, em conjunto com os municípios localizados nessas áreas, possam enfrentar as situações adversas em razão desses eventos meteorológicos-oceanoográficos extremos, resolve:

Artigo 1º - Editar o Plano Preventivo de Defesa Civil (PPDC) para erosão costeira, inundações costeiras e enchentes/alagamentos causados por eventos meteorológicos-oceanoográficos extremos (ressacas do mar e marés altas), que passa a vigorar nos termos desta resolução e seus anexos.

Parágrafo único - O PPDC, a que se refere o "caput" deste artigo, abrange os pontos sensíveis costeiros do Estado de São Paulo, embasados em Coordenadorias Estaduais de Defesa Civil de Registro (REDEC), de fls. 11, Baixada Santista (REDEC-3) e São José dos Campos e Litoral Norte (REDEC-3).

Artigo 2º - O Plano Preventivo de Defesa Civil (PPDC) para erosão costeira, inundações costeiras e enchentes/alagamentos causados por eventos meteorológicos-oceanoográficos extremos (ressacas do mar e marés altas) tem a seguinte composição:

I - Orgão Central: a Casa Militar, representada pela Coordenadoria Estadual de Defesa Civil (CEDEC).

II - Órgãos Regionais: as Coordenadorias Regionais de Defesa Civil de Registro (REDEC-1), Baixada Santista (REDEC-3) e São José dos Campos e Litoral Norte (REDEC-3).

III - Órgãos Setoriais: a Marinha do Brasil, o Instituto Nacional de Meteorologia (INMET), o Instituto Oceanográfico (IO) da Universidade de São Paulo, o Instituto Geológico (IG), Centro de Pesquisas de Tempo e Estudos Costeiros (CPTC), o Instituto de Pesquisas Tecnológicas (IPT), o Departamento de Ciências do Mar da Universidade Federal de São Paulo, o Centro de Estudo e Pesquisas sobre Desastres (CEPED-SP), o Corpo de Bombeiros e a Polícia Ambiental do Estado de São Paulo.

IV - Órgãos Municipais: as Prefeituras Municipais envolvidas no Plano de Contingência, representadas pelas respectivas Coordenadorias Municipais de Defesa Civil (COMDEC).

V - Entidades privadas com reconhecimento atuação na área.

Artigo 3º - Cabe às Coordenadorias Municipais de Defesa Civil envolvidas neste Plano, apoiadas pelas respectivas Coordenadorias Regionais de Defesa Civil, a edição de planos preventivos e de contingência específicos para cada município, em consonância com os pressupostos presentes nos anexos desta resolução.

Artigo 4º - O período de vigência deste plano será intermitente, devendo suas ações serem deflagradas conforme aviso e boletins emitidos pelos órgãos setoriais.

ANEXO I

Normas e procedimentos do Plano Preventivo de Defesa Civil (PPDC) para erosão costeira, inundações costeiras e enchentes/alagamentos causados por eventos meteorológicos-oceanoográficos extremos (ressacas do mar e marés altas).

TÍTULO I

Disposições Preliminares

Artigo 1º - O Plano Preventivo de Defesa Civil (PPDC) para erosão costeira, inundações costeiras e enchentes/alagamentos causados por eventos meteorológicos-oceanoográficos extremos (ressacas do mar e marés altas), tem como objetivo principal prevenir as ações das Coordenadorias Regionais e Municipais de Defesa Civil na minimização dos efeitos desses eventos no âmbito dos quatro setores costeiros do Estado de São Paulo.

Artigo 2º - O Plano se baseia na adoção de medidas para conhecimento antecipado das ocorrências de eventos extremos especificadas no artigo anterior, nas ações dos órgãos de defesa civil e nas ações de Planos de Contingência para os municípios sujeitos a esses eventos.

Artigo 3º - Para efeito desta resolução, seguem as seguintes considerações e conceitos:

I - Eventos Meteorológicos-Oceanoográficos Extremos: Marem Meteorológico Proximo e Anomalo de Mar.

Eventos associados à influência de furios meteorológicos (ondas extratropicais, frentes frias), oceanográficos (ondas de ressaca do mar e ondas energéticas), atmosféricos (inundações de chuva e de equinócios) e astronômicos (efeito astronômico devido ao aquecimento do oceano durante o verão). Quanto maior o número de fatores associados em conjugação, maiores serão os impactos, os efeitos danosos e os prejuízos na zona costeira.

II - Marem altas anormais: Principais perigos gerados por esses eventos na zona são: erosão costeira, inundações costeiras, enchentes e alagamentos.

III - Marem altas anormais: Trata-se de um tempo quando se verifica a sobreposição de uma ou mais causas de uma maré alta a uma maré meteorológica positiva, em especial se conjugada a uma maré de sizígia. Podem ocorrer sem a atuação de forte agitação marítima, podendo-se associar com uma ressaca.

III - Erosão costeira

O resultado do conjunto de processos sedimentares que atuam na praia pode ser medido por meio do seu balanço sedimentar que é, em outras palavras, a relação entre as perdas e ganhos de sedimentos nessa praia. Quando o balanço sedimentar da praia for negativo, ou seja, quando a saída de sedimentos for maior do que a entrada/ganho de sedimentos, haverá déficit sedimentar, predominando assim o processo erosivo.

IV - Inundação costeira

Sobressa temporária de terrenos marginais à linha de costa costeira e estuarina/lagunas, causada pela combinação de marés altas anormais e ressacas.

V - Enchentes associadas a marés altas anormais e ressacas: Submersão temporária de áreas marginais a cursos de água doce ou salobra na planície costeira, associada ao transbordamento do canal fluvial/devidado a ocorrência de precipitação intensa e à incapacidade de escoamento das águas para o estuário/laguna, ou o canal de maré ou a praia, pelo efeito do empilhamento de água na costeira alta anormal.

VI - Alagamentos associados a marés altas anormais e ressacas: Acúmulo de água em ruas, calçadas ou outras infraestruturas urbanas devido à entupimento da capacidade de escoamento de sistemas de drenagem urbana, em decorrência de precipitação intensa, maré alta anormal e resaca (que prejudicam sobre estruturas urbanas em áreas com erosão costeira acelerada).

VII - Vento previsto do quadrante sul: Durante os eventos meteorológicos-oceanoográficos extremos os ventos sopram do quadrante sul, apresentando direções SW, SSW, S e SE.

VIII - Altura e Direção de Ondas Significativas: A altura de uma onda marítima é definida como a diferença de nível entre a sua crista e o seu cavado. Como as alturas das ondas podem variar bastante, para se medir o estado do mar é utilizada a altura significativa das ondas, que corresponde à média do tempo superior das ondas com maior altura registada durante um período de tempo.

TÍTULO II

Do Funcionamento

CAPÍTULO I

Das Diretrizes Técnicas

Artigo 4º - O Plano Preventivo tem como base fundamental para erosão costeira:

1. Prevenção de condições meteorológicas associadas à elevação do nível do mar junto à costa;

2. Evacuação do nível do mar prevista (altura das ondas, elevação do mar e mare astronômica);

3. Mapa de risco à erosão costeira;

Parágrafo único: Para inundações costeiras e enchentes/alagamentos causados por eventos meteorológicos-oceanoográficos extremos (ressacas do mar e marés altas), o Plano tem como base:

1. Prevenção de condições meteorológicas associadas à elevação do nível do mar junto à costa;

2. Evacuação do nível do mar prevista (altura das ondas, elevação do mar e mare astronômica);

3. Mapa de risco a inundações costeiras e enchentes/alagamentos causados por marés altas e ressacas.

Artigo 5º - O Plano Preventivo para os perigos costeiros tratados nesta resolução está estruturado em 3 (três) níveis, indicando progressivamente a possibilidade de ocorrência de eventos meteorológicos-oceanoográficos extremos (ressacas do mar e marés altas), a saber:

I - Observação: Vento previsto do quadrante sul (SSW a SSE) até 40 km/h, ondas de quadrante sul (SSW a SSE) com altura significativa inferior a 2,0 metros e elevação do nível (astronômica) prevista até 1,8 metros;

II - Alerta: Vento previsto do quadrante sul (SSW a SSE) entre 60 e 80 km/h, ondas de quadrante sul (SSW a SSE) com altura significativa de 2,0 a 3,0 metros ou elevação de maré (astronômica) prevista entre 1,8 a 2,0 metros;

III - Alerta: Vento previsto do quadrante sul (SSW a SSE) acima de 80 km/h, ondas de quadrante sul (SSW a SSE) com altura significativa acima de 3,0 metros ou elevação de maré (astronômica) prevista acima de 2,0 metros.

S.T. - Para cada nível estão previstos procedimentos operacionais que visam à minimização das consequências desses eventos.

CAPÍTULO II

Do Procedimento Operacional

Artigo 6º - Os procedimentos operacionais de contingência previstos para os diferentes níveis, segundo o artigo 5º, são os seguintes:

I - Nível de Observação

1) Coordenadoria Estadual de Defesa Civil (CEDEC):

a) monitorar os critérios de vento e ondas do quadrante sul e elevação do mar;

b) acompanhar, através das REDECs, as Coordenadorias Municipais de Defesa Civil (COMDEC) na operação dos Planos de Contingência;

c) convocar, quando necessário, os órgãos envolvidos para avaliação da operação do Plano;

d) emitir as recomendações meteorológico-oceanoográficas às REDECs e COMDECs;

2) Coordenadoria Regional de Defesa Civil (REDEC):

a) atender à comunicação da CEDEC, para reunião dos órgãos envolvidos;

b) acompanhar as Coordenadorias Municipais de Defesa Civil (COMDEC) na operação dos Planos de Contingência;

c) acompanhar as previsões, avisos e alertas emitidos pela CEDEC;

3) Coordenadorias Municipais de Defesa Civil (COMDEC):

a) acompanhar as previsões, avisos e alertas emitidos pela CEDEC;

b) elaborar e desenvolver o Plano de Contingência Municipal para os perigos costeiros associados a eventos meteorológicos-oceanoográficos extremos;

I - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e boletim meteorológico;

e) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

f) emitir alertas para a população e veículos de comunicação;

g) adaptar as medidas previstas nos respectivos planos de contingência municipal;

II - Nível de Alerta

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

e) emitir alertas para a população e veículos de comunicação;

f) adaptar as medidas previstas nos respectivos planos de contingência municipal;

III - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

e) emitir alertas para a população e veículos de comunicação;

f) adaptar as medidas previstas nos respectivos planos de contingência municipal;

IV - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

e) emitir alertas para a população e veículos de comunicação;

f) adaptar as medidas previstas nos respectivos planos de contingência municipal;

IV - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

e) emitir alertas para a população e veículos de comunicação;

f) adaptar as medidas previstas nos respectivos planos de contingência municipal;

V - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

e) emitir alertas para a população e veículos de comunicação;

f) adaptar as medidas previstas nos respectivos planos de contingência municipal;

VI - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

e) emitir alertas para a população e veículos de comunicação;

f) adaptar as medidas previstas nos respectivos planos de contingência municipal;

VII - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

e) emitir alertas para a população e veículos de comunicação;

f) adaptar as medidas previstas nos respectivos planos de contingência municipal;

VIII - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

e) emitir alertas para a população e veículos de comunicação;

f) adaptar as medidas previstas nos respectivos planos de contingência municipal;

IX - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

e) emitir alertas para a população e veículos de comunicação;

f) adaptar as medidas previstas nos respectivos planos de contingência municipal;

X - Nível de Atenção

1) Coordenadoria Estadual de Defesa Civil (CEDEC)

a) proceder a totalidade dos itens definidos para o nível de observação;

b) convocar reunião dos órgãos envolvidos, quando da mudança do nível, se for o caso;

c) registrar as informações acerca das vitórias de campo efetuadas pelas Coordenadorias Municipais de Defesa Civil (COMDEC);

d) comunicar o evento ao REDEC, COMDEC e órgãos de apoio, por meio de SMS e mudança de nível do Plano;

do Norte: Rua Rui Barbosa, 1.213,18 m² de recapamento, no trecho entre as Ruas Luiz Gonzaga e R. Lacerda; Rua Luiz Gonzaga, 868,50 m² de recapamento, no trecho entre as Ruas R. Barbosa e Bernardino Pinto.

PARÁGRAFO ÚNICO: Inalterado.

CLÁUSULA SEGUNDA: A Cláusula Terceira, que trata das Obrigações dos Participantes, passa a ter a seguinte redação: Para a execução do presente Convênio o ESTADO e o MUNICÍPIO terão as seguintes obrigações:

I - COMPETE AO ESTADO:

a) Inalterado;

b) Inalterado;

c) Inalterado;

d) Inalterado;

e) Inalterado;

f) Inalterado;

g) Inalterado;

h) Inalterado;

i) Inalterado;

j) Inalterado;

k) Inalterado;

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m) Inalterado;

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p) Inalterado;

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er) Inalterado;

es) Inalterado;

PROCURAÇÃO

Pelo presente instrumento, a **APIS FLORA INDL. COML. LTDA**, confere poderes especiais à **UNIVERSIDADE ESTADUAL PAULISTA "JULIO DE MESQUITA FILHO" - UNESP**, autarquia estadual de regime especial, criada pela Lei nº 952 de 30.01.1976, com sede na Rua Quirino de Andrade, 215, Centro, CEP 01.049-010, São Paulo/SP, inscrita no CNPJ/MF sob nº 48.031.918/0001-24, doravante designada simplesmente **UNESP**, neste ato, representada por seu Magnífico Reitor, Prof. Dr. **SANDRO ROBERTO VALENTINI**, de acordo com o Art. 34, I de seu Estatuto, ou quem legalmente o substitua, para o fim de requerer e processar direitos de propriedade intelectual face ao pedido de patente intitulado "**Composição, obtenção e uso de formulações de cuidado bucal**", a ser depositado junto ao INPI, para mantê-lo em vigor com amplos poderes para assinar petições e documentos, pagar taxas, anotar transferências, fazer prova de uso da invenção patenteada, apresentar oposições, recursos, réplicas, anotar, elaborar notificações extrajudiciais, e praticar para os fins mencionados todos os atos necessários perante as autoridades administrativas competentes no Brasil e no exterior, em benefício da Outorgante, ratificando os atos já praticados.

São Paulo, 16 de dezembro de 2019.



Apis Flora Indl. Coml. Ltda.

Raul de Barros Ferreira

CEO

ANEXO M



DENTAL MATERIALS



ISSN: 0109-5641

DESCRIPTION

Online submission and editorial system now available:
["https://www.editorialmanager.com/dentma/default.aspx"](https://www.editorialmanager.com/dentma/default.aspx)

Dental Materials publishes original research and review articles.

Academy of Dental Materials members click ["https://sciencedirect.com/science/activate/adm"](https://sciencedirect.com/science/activate/adm) to register for free access to Dental Materials online.

The principal aim of *Dental Materials* is to promote rapid communication of scientific information between academia, industry, and the dental practitioner. Original Manuscripts on clinical and laboratory research of basic and applied character which focus on the **properties** or **performance** of **dental materials** or the **reaction** of host tissues to materials are given priority publication. Other acceptable topics include application technology in **clinical dentistry** and dental laboratory technology.

Comprehensive reviews and editorial commentaries on pertinent subjects will be considered.

AUDIENCE

Dental research scientists, materials scientists, clinicians, students of dentistry, dental materials and equipment manufacturers.

IMPACT FACTOR

2021: 5.687 © Clarivate Analytics Journal Citation Reports 2022

GUIDE FOR AUTHORS

INTRODUCTION

Authors are requested to submit their original manuscript and figures via the online submission and editorial system for *Dental Materials*. Using this online system, authors may submit manuscripts and track their progress through the system to publication. Reviewers can download manuscripts and submit their opinions to the editor. Editors can manage the whole submission/review/revise/publish process. Please register at: <https://www.editorialmanager.com/dentma/default.aspx>.

Dental Materials now only accepts online submissions.

The Artwork Quality Control Tool is now available to users of the online submission system. To help authors submit high-quality artwork early in the process, this tool checks the submitted artwork and other file types against the artwork requirements outlined in the Artwork Instructions to Authors on <https://www.elsevier.com/artworkinstructions>. The Artwork Quality Control Tool automatically checks all artwork files when they are first uploaded. Each figure/file is checked only once, so further along in the process only new uploaded files will be checked.

Manuscripts

Submitted manuscripts must relate directly to both Materials Science and Dentistry.

The journal is principally for publication of **Original Research Reports**, which should preferably investigate a defined hypothesis. Maximum length 6 journal pages (approximately 20 double-spaced typescript pages) including illustrations and tables.

Systematic Reviews will however be considered. **Prior approval must be sought from the Editor before submission of Review Manuscripts.** Authors should send the Editor a structured abstract of the proposed review topic. Intending authors should communicate with the Editor beforehand, by email, outlining the proposed scope of the review. Maximum length 10 journal pages (approximately 33 double-spaced typescript pages) including figures and tables.

Three copies of the manuscript should be submitted: each accompanied by a set of illustrations. The requirements for submission are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", *Annals of Internal Medicine*, 1997, 126, 36-47. All manuscripts must be written in American English. Authors are urged to write as concisely as possible.

The Editor and Publisher reserve the right to make minimal literary corrections for the sake of clarity. Authors for whom English is not the first language should have their manuscripts read by colleagues fluent in English. If extensive English corrections are needed, authors may be charged for the cost of editing. For additional reference, consult issues of *Dental Materials* published after January 1999 or the Council of Biology Editors Style Manual (1995 ed.).

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