

Mechanical Brushing Effects *in vitro* of a Dentifrice Containing *Punica granatum* Linné

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ABSTRACT

Objectives: This study aimed to evaluate *in vitro* the effects of mechanical brushing with *Punica granatum* Linné (*P. granatum* L.) or pomegranate on the mass, roughness and color of artificial teeth in acrylic resin. **Methods:** The artificial teeth ($n=30$) were randomly distributed into 3 groups, according to the tested solution ($n = 10$ each group): Distilled Water (DW), Commercial Dentifrice (CD) and Dentifrice based on *P. granatum* L. (PD). All the samples were brushed for a simulated 5-year period. The effects of mechanical brushing were evaluated before brushing and after 12, 24, 36 and 60 months. Data were submitted to the Kolmogorov-Smirnov test and a significance level of 5% was adopted and compared by ANOVA with Bonferroni. **Results:** In the PD group, the mass changed was statistically significant at T4 ($0.3942 \pm 0.003g$). The roughness was altered only in T4 in the PD group (1.059 ± 0.053), while in the CD group was altered in T1 (0.982 ± 0.026) and T2 (0.965 ± 0.02) and finally the color changes

were only detected in the PD group in T4 ($2,899 \pm 0.992$), while in the DW group ($2,050 \pm 0,287 *$) and common dentifrice ($2,710 \pm 0,228*$) in T3.

Conclusion: The pomegranate dentifrice did not cause deleterious effects on the surface of artificial acrylic resin teeth.

Key words: Dental Prosthesis, Denture Stomatitis, Natural products, Tooth brushing, *Punica granatum*.

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INTRODUCTION

Dental prostheses are commonly used to replace missing teeth in people.¹ However, poor hygiene of the oral cavity associated with accumulation of biofilm, promote inflammatory processes, such as Prosthetic Stomatitis (PS), which develops in the proliferation of the species *Candida albicans*.^{2,3} This way, proper cleaning of these surfaces is critical to avoid the entrance of this infection.^{4,5}

The method most commonly used by patients is the association between brushing and the use of dentifrices; however, the major disadvantage of using this technique is the damage caused to the prostheses by abrasion of dentifrices on the surface of acrylic resin, besides being hard to be performed for patients with motor limitations.^{4,6} The result of this damage is the excessive use of acrylic with the coming of channels that allow an accumulation of micro-organisms and staining,⁷⁻⁹ plus the loss of material, polishing and shine.^{5,8}

Although there is an antifungal agent and for ideal cleaning of prostheses, the interest in natural products as antimicrobial and antifungal agents has grown, as a promising alternative to synthetic chemicals,^{10,11} in the case of studies with *Punica granatum* Linné (*P. granatum* L.) or pomegranate.

The pharmacological properties of *P. granatum* L. have been described and suggest that it presents powerful oral antifungal and antibacterial activity thanks to its rich bioactive composition, being the predominant class of its phytochemicals, the polyphenols, among which stand out the tannins, such as punicalagin.¹²⁻¹⁵ The effect of tannins on the fungi may be assessed by its action on the membranes.^{12,14}

On the other hand, there are few *in vivo* and *in vitro* studies, around the effects of the use of these substances on the acrylic resin of the DP.

Along these lines and the importance to show scientific evidence of new patented products, this study aimed to find out the possible effects of mechanical brushing with the use of a toothpaste of low abrasiveness containing *P. granatum* L. (Brazilian Patent Number BR1020170212203) on mass, roughness surface and color change of teeth in acrylic resin used for manufacture of prostheses, for a period of 5 years, simulated time considered ideal for replacement of prostheses.¹⁶

MATERIALS AND METHODS

Extract and preparation of the samples

Punica granatum were obtained from from Juruena Valle in the Brazilian state of Mato Grosso (Region: Midwest, Latitude: 10° 19' 05 "S, Longitude: 58° 21' 32" W, Height: 300 m). This samples are deposited at Federal University of Mato Grosso herbarium-voucher BI 31659. Chemical constituents (punicalagin, galic acid, quercetin and *p*-Coumaric acid) were identified by specialists at the Department of Chemistry, in the Federal University of Ceara (GC-MS, Shimadzu, model QP 5050, Japan).

Specimens were obtained from acrylic resin artificial teeth (upper central incisors) Vipi Dent Plus[®] brand (VIPI dental products, Pirassununga, SP, Brazil) on the color 60 and model 38. With the purpose of adapting the specimens to the brushing machine, the same were embedded in acrylic plates (30 mm diameter and 5 mm thick) and fixed through an array of heavy condensation silicone (Reflexdenso[®], Yller Biomaterials, Pelotas, RS, Brazil) and each disk was then identified by a number from 1 to 10. The samples were divided, at random, into three groups ($n = 10$ each

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group), according to the substance tested: distilled water (DW); *Punica granatum* Dentifrice (PD) and control dentifrice (CD).

The following toothpastes were tested:

- Punica Dentifrice (PD)

The dentifrice was handled by the Pharmacy School of the University Center of Catholic College located in the city of Quixada in the Brazilian state of Ceara, Brazil. For this purpose we have used micronized Calcium carbonate for reduction of abrasiveness of the final product, as well as Pharmaceutical Excipients as glycerin, hydroxyethylcellulose, sodium lauryl sulfate, sodium saccharin and menthol. In addition the *Punica granatum* glycolic extract was incorporated to a final concentration of 6.25%.

- Control Dentifrice (CD)

As control, there was used commercial dentifrice Even* (Industries Assembled Raymundo da Fonte S/A, Vila Torres Galvão Paulista, PE, Brazil), whose composition is: fluorine, glycerin, sodium saccharin, carboxymethylcellulose, sorbitol, sodium silicate, tetrasodium pyrophosphate, methylparaben, propylparaben, calcium carbonate, sodium lauryl sulfate, flavor, water, sodium monofluorophosphate.

Groups

The samples ($n=30$) were composed of acrylic artificial teeth were randomly distributed into 3 groups ($n = 10$), each one being numbered from 1 to 10.

DW = Compost by teeth that have been brushed with distilled water.

PD = Composed by the group of teeth that have been brushed with *P. granatum* L dentifrice.

CD = Composed by the group of teeth that have been brushed with commercial dentifrice Even*.

Brushing test

Soft brushes were used (Medfio*, Medfio Dental Articles Industry and Trade Ltd., Pinhais, PR, Brazil) with soft nylon bristles, containing 34 separate tufts. The cables were cut for these brushes to be engaged in shoes brushing machine.

Before and after the brushing test, acrylic teeth were taken to an ultrasonic tub (Unique-, Ultracleaner 1400*, Indaiatuba, São Paulo, Brazil), during 5 min, immersed in distilled water and then dry with absorbent paper and resealed in the matrix, with the purpose of remove any residues.

For the test, the samples were placed on simulation machine of brushing (Elquip-MSEI*, São Carlos, São Paulo, Brazil). There were simulated five years of brushing, when 12 months of brushing matched 17,800, with 200g load on the surface of the samples with excursion amplitude of movements in 20mm with a speed of 4.5 movements per second.¹⁷

Preparation of solutions

It was prepared a suspension with 100 mL of distilled water and 100 mL of each toothpaste (ratio 1:1), under temperature of 23°C, in order to simulate the dilution that occurs in the mouth by the saliva and, as a result, reduce the friction action. As soon as diluted, the toothpaste was placed in injection needles adapted to the brushing machine (Elquip-MSEI*).

Preparations of the solution were included in 20mL needles and taken to brushing machine (Elquip-MSEI*). The machine has been regulated to be injected the solution used in each group during 4 sec; every 30 sec and the temperature of brushing will be kept at 37°C.

Analyses

The analyses of the samples were done before the test of brushing (T0) and after intervals of 12 (T1), 24 (T2), 36 (T3) and 60 months (T4),

months of simulated brushing by performing five tests. The surface roughness and color stability were measured on vestibular surfaces.

Evaluation of Mass

There was used a precision weighing-machine (Bel Engineering*, Piracicaba, SP, Brazil) to gauge the initial mass of each sample and assess if the frequent brushing would lead to change of the mass. The weighing-machine (Bel Engineering*) was calibrated before each reading. The mass variation (pm) was calculated in grams (g), by $pm=m1-m2$, where $m1$ is the initial weight and $m2$ the final weight presented after brushing the tested substances.

Readings were performed before and after each brushing with the different substances, being before dried with absorbent sheets of paper for elimination of moisture present on the surface; and, from the obtained values there were calculated the variations for each situation.

Surface roughness

To assess there were conducted three readings far between in 3 mm on the surface of each more flat sample. Since, there was recovered from an arithmetic average of the standard deviations of profile roughness with the aid of the roughness tester (Hommel Tester T1000*, Jenoptik, Jena, Thuringia, Germany). The roughness tester (Hommel Tester T1000*) was programmed to move a diamond tip (5 μ m of radius), following a rectilinear path of 4.8 mm long, during 10 sec.

Color stability

It was used for reading the portable spectrophotometer (Vita Easyshade*, Vita Zahnfabrik h. Rauter GmbH and Co, Germany). It was applied to quantify the magnitude of difference ΔE relationship colorimetric ($\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, in which L^* represents lightness, a^* means the chromaticity red-green and b^* chromaticity blue-yellow) of each specimen, using the default Note recommended by the C.I.E. (Commission Internationale de l'Éclairage), after periods of brushing, compared to its initial parameters. We used a white apron, a jig-assisted condensation silicone (Reflexdenso*) to ensure the setting of reading in the center of each sample before and after the sinking and keep out ambient light. ΔE values less than 1 are considered as no appreciable by the human eye; between 1 and 3.3, noticeable by specialized observers, larger than 3.3, noticeable for lay observers. Variations above 3.3 were pointed to as clinically unacceptable.^{18,19}

Statistical analysis

Data was tabulated in Microsoft Excel* (Microsoft, Redmond, Washington, USA) and exported to the software GraphPad Prism 5.0* (GraphPad Software, Inc., La Jolla, California, USA) at which the analyses were conducted adopting a confidence of 95%.

The data were expressed in the form of average and standard error of the mean (Kolmogorov-Smirnov test, $p<0.05$) and compared by means of ANOVA tests 1-way and 2-way ANOVA for repeated measures, both followed by Bonferroni post-test.

RESULTS

Evaluation of Mass

The isolated effect of brushing on mass of specimens can be observed in the groups brushed with distilled water, with maintenance of the mass. The Group CD, maintenance of initial mass until T2. There was a significant increase in the mass of specimens in the CD group, from T3. Brushing with dentifrice the pomegranate base promoted significant changes from T1 to the end of the period evaluated (Table 1).

Surface roughness

According to Table 2, the DW group suffered a discreet significant increase of roughness only in T2. The CD Group showed a significant increase after brushing on T1 and T2. In the PD group, brushing with pomegranate extract caused an increase of roughness on T4 rating, significantly higher compared to the same evaluation in the DW group in the same period. Significant difference was observed between the groups throughout the ages.

Color stability

According to Table 3, the DW group experienced a significantly higher color change in T3 (Table 3). A similar pattern of darkening of color could be observed in the CD group in the period T3 (Table 3). The subjects brushed with pomegranate extract keeps staining of specimens for longer; however it was observed a significant increase in the value of ΔE in the evaluation of five years (Table 3).

DISCUSSION

The loss of mass (weight) and surface roughness are the most common methods of analysis of abrasion resistance.²⁰ The Group of distilled water there was no loss of mass. Corroborating this study, de Freitas and Paranhos *et al.* (2006), assessed the resistance to abrasion of teeth of acrylic resin front of dentifrices and not specific to dental hygiene and distilled water. The study demonstrated that all dentifrices have caused loss of mass, while distilled water caused minimal loss of mass.²¹ Policastro *et al.* (2016), observed in his survey about artificial teeth mass loss when subjected to different methods of brushing, using commercial dentifrices (Colgate) and coconut soap. After one year of follow-up, in

brushed with dentifrice and soap, there were found losses ranging from 0.1% to 0.9% and 0.09% to 0.2% of initial weight, respectively.²⁰In this study, the results obtained by analyses carried out concerning the mass indicated that brushing during five years of simulated specimens with herbal dentifrice hydroalcoholic extract of pomegranate to 6.25%, not promoted mass loss of artificial resin teeth Acrylic ($p>0.005$). With respect to specimens brushed with distilled water, both intra and inter-group analysis, it has not been possible to observe significant mass change corroborating with the data obtained in other studies.^{20,21} However, with respect to dentifrices tested, when on intra-group comparison, it was found that brushing artificial teeth of acrylic resin a discrete mass gain, in a maximum period of five years, simulated ranging from 0.1% 0.5% 0.6% CD group and the 1.1% in Group PD brushed with the dentifrices. Low abrasiveness of dentifrices tested in this study could be proven by the absence of mass loss of specimens of the CD groups and PD. In this sense, one can deduce that the low abrasiveness of dentifrices may have contributed, albeit indirectly, to the discrete mass gain observed. In this way, this mass gain could be interpreted as resulting from an accumulation on the surface of the artificial teeth of Glycerin, humectant substance present in dentifrices. However, it is important to note that the percentage gain observed in PD group, was just over 1% and clinically irrelevant.

Regarding roughness, Roselino *et al.* evaluated the effect of tooth brushing (Colgate Luminous White (CLW), Close Up White Now (CWN) and Trihydral (THD) and a dentifrice based on *Recinus communis* (castor bean) in artificial teeth. The study concluded that there were no significant alterations, intra or intergroup, of the roughness for any of the dentifrices tested.²² Tanaka *et al.* in 2013 examined the abrasive and cleaning effects of melamine foam and other cleaning agents on the surfaces of artificial composite dental specimens and observed a significant increase in roughness in the treated samples with conventional water and dentifrice.²³ In the present study, statistically significant alterations were observed in the roughness of the artificial teeth in the statistical analyzes performed in the 3 groups tested, however this result is expected by the natural wear of the prosthesis by the friction of the toothbrush itself. The data obtained from the intragroup analyzes indicated that the significant alterations in the roughness of the artificial acrylic resin teeth were more marked in the groups of teeth brushed with CD and DW, since they occurred in the first year and second year respectively. The teeth of the PD group, differently, only showed significant change in roughness after 5 years of simulated brushing, which generally corresponds to the useful life of prosthesis.²⁴ The results of the roughness analysis suggest that the dentifrice formulation tested presented a minimum degree of abrasiveness and did not promote any deleterious effect on the surface of the artificial tooth. This finding allows us to infer that this low abrasiveness of the dentifrice based on pomegranate extract

Table 1: Average results (± standard error) for mass, in grams, according to the treatment.

	Distilled Water	Commercial Dentifrice	Pomegranate Dentifrice	p-Value ^b
T0	0.3888±0.003	0.3865±0.003	0.3901±0.003	0.411
T1	0.3903±0.003	0.3875±0.003	0.3933±0.003*	
T2	0.3901±0.003	0.3876±0.003	0.3925±0.003*	
T3	0.3909±0.003	0.3888±0.003*	0.3944±0.003*	
T4	0.3899±0.003	0.3872±0.003*	0.3942±0.003*	
p-Value ^a	0.771	<0.001	<0.001	

* $p<0.05$ versus T0, ($n = 10$)

^a ANOVA-1-way for repeated measures/Bonferroni

^b ANOVA-2-way for repeated measures/Bonferroni

Table 2: Average results (± standard error) for roughness in µm, according to the treatment.

	Distilled Water	Commercial Dentifrice	Pomegranate Dentifrice	p-Value ^b
T0	0 0.848±0.032	0 0.773±0.024	8 0.806±0.041	0 0,016
T1	0.950±0.025	0 0.982±0.026*	0.974±0.037	
T2	0 0.972±0.029*	0.965±0.024*	0 0.905±0.031	
T3	0. 0.933±0.027	0.897±0.032	0 0.938±0.030	
T4	0. 0.897±0.026	0. 0.932±0.035	1 0.059±0.053*†	
p-Value ^a	0 0,011	<0 0,001	<0 0,001	

* $p<0.05$ versus T0, † $p<0, 05$ versus water group in the same time; ($n = 10$)

^a ANOVA-1-way for repeated measures/Bonferroni

^b ANOVA-2-way for repeated measures/Bonferroni

Table 3: Results of average (± standard error) for ΔE, in different periods in relation to the baseline, according to the treatment.

	DiDistilled Water	Commercial Dentifrice	Pomegranate Dentifrice	p-Value ^b
T0	0 0.000±0.000	0 0.000±0.000	0 0.000±0.000	0.434
T1	1. 1.196±0.215	1.582±0.183	1 1.882±1.127	
T2	1 1.423±0.199	1. 1.599±0.235	2. 2.430±1.006	
T3	2 2.050±0.287*	2. 2.710±0.228*	2. 2.403±0.984	
T5	1 11.560±0.216	2. 2.330±0.206	2. 2.899±0.992*	
p-Value ^a	<0<0.001	< <0.001	0 0.001	

* $p<0.05$ versus T1, ($n = 10$)

^a ANOVA-1-way for repeated measures/Bonferroni

^b ANOVA-2-way for repeated measures/Bonferroni

may have contributed in some way to the accumulation of residues on the surface of the artificial teeth and to have caused the observed mass gain. This fact also explains the divergence of the results of the research with previous ones also carried out on artificial teeth.

Regarding the analyzes regarding a possible color change, Freire *et al.* evaluated the effect of different cleaning and artificial aging protocols on the color stability of denture teeth made of acrylic resin. A group of teeth were subjected to simulated brushing less than 200 g strength for 33247 cycles using distilled water / dentifrice solution of 1: 1. The results showed that the teeth had significant color changes and were clinically unacceptable ($\Delta E > 3.3$).⁷ Roselino *et al.* (DSW), Colgate Luminous White (CLW), Close Up White Now (CWN) and Trihydral (THD) and a dentifrice based on *Ricinus communis* (castor bean) The study simulated two years of brushing and concluded that only SDB and CLW significantly altered color. In the present study, using a higher number of cycles (89,000) and only one type of commercial dentifrice (Even) the occurrence of significant color changes in the 3 groups tested (CD, PD and DW), however, within a clinically acceptable range ($\Delta E < 3.3$) for all groups analyzed. significantly after 5 years of brushing, revealing the superiority of the pomegranate extract dentifrice in maintaining the color of the artificial teeth for a longer period of time considering the importance of the color stability of the artificial teeth as one of the factors essential to meet the aesthetic criteria, the effect caused by using pomegranate-based dentifrice is quite encouraging.²² Unfortunately, in the analysis of the literature, no studies with other phytotherapies were found that allowed the comparative analysis of the mechanical effects of brushing.

In addition, given the importance of intellectual property, where investments in patents are increasingly widespread, influencing the functioning of innovation and scientific and technological advancement, this dentifrice had the patent application deposited in national Office Industrial property INPI (patent number BR1020170212203).

As follows, based on the obtained results and, within the limitations of an *in vitro* study, the dentifrice to base of hydroalcoholic extract of pomegranate under concentration of 6.25% caused no deleterious effects to the surface of the acrylic resin artificial teeth. It can be suggested that brushing of the DP using dentifrice pomegranate extract-based would be an effective method for keeping and durability of artificial teeth.

CONCLUSION

Even though the promising results found in this research with the pomegranate-based dentifrice and unpublished experimental type of research on acrylic resin artificial teeth, it must be carried out research to assess the effects of dentifrice on the resin thermo-cured used at the base of the dentures and cobalt-chromium amalgams, in order to ensure their use in dentures and removable to cleats.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CD: Commercial Dentifrice; **DW:** Distilled Water; **PD:** *Punica granatum* L. Dentifrice.

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