Original Article

Qualitative and Quantitative Assessment of Remineralizing Effect of Prophylactic Toothpaste Promoting Brushite Formation: A Randomized Clinical Trial

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Abstract

Background: Currently various studies are conducted to improve the effect of existing and developing new remineralizing agents. One of the trends in remineralizing therapy is the development of toothpaste allowing brushite crystals formation in the demineralized lesions of hard tooth tissues. **Aim:** The aim of this study was to assess the effect of toothpaste, forming a brushite, on the functional acid resistance of enamel and the speed of its remineralization. **Materials and Methods:** This was a randomized controlled double-blind clinical study. Sixty consent patients aged 20–25 years were enrolled in the three groups: test group (n = 20), positive control group (n = 20), and negative control group (n = 20), which used brushite-forming toothpaste, toothpaste with hydroxyapatite (HAP), and toothpaste without remineralizing agents, respectively. The hygiene indices, the rate of enamel remineralization, the dynamics of acid resistance of enamel, and the level of enamel sensitivity were determined at baseline, after 2 and 4 weeks to assess the effectiveness of toothpastes. Friedman rank sum test (for related variables) and the Kruskal–Wallis rank sum test (for independent variables) with Nemenyi *post hoc* test were used for statistical comparisons. **Results:** The study test and positive control groups showed significantly greater acid resistance of enamel ($P > 0.05$) and rate of its remineralization at the study endpoints as compared with negative control group.

In the test and positive control groups, Schiff index values significantly decreased after 4 weeks, whereas in the negative control group no significant differences were observed at the study time points. The oral hygiene level improved significantly after 2 and 4 weeks in all groups. **Conclusion:** The 30-day use of paste that promotes brushite formation and paste with hydroxyapatite resulted in faster enamel remineralization and higher enamel resistance. Brushite-containing toothpaste may be used as an alternative to HAP containing for remineralizing and desensitizing treatment.

Keywords: *Brushite, enamel acid resistance, hypersensitivity, remineralization, toothpastes*

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Introduction

The susceptibility of tooth enamel to cariogenic factors is evaluated not only by the remineralizing properties of the oral fluid but also by its resistance

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to demineralization.[1] Enamel demineralization may occur due to the exposure to organic acids produced by biofilm microorganisms during anaerobic glycolysis of fermentable carbohydrates. Organic acids increase the solubility of hydroxyapatite crystals in hard tooth tissues, leading to the exposure of dentine.[2,3] Thus, one of the clinical manifestations of enamel demineralization is hypersensitivity.

Different remineralizing agents are used for the prevention of further demineralization and the decrease of hypersensitivity.[4] The main components of these products are Ca, P, and F ions, which aid the restoration of enamel structure, as the deficiency of these ions weakens the enamel. The most common forms of remineralizing products include gels, solutions, mouthrinses, and pastes.[5] Toothpaste is the most popular dental disease prevention tool. The use of a manual toothbrush in combination with toothpaste has become most widespread among hygienic habits due to their effectiveness, ease of use, and low cost.[6,7] Traditionally, the most widely used anticariogenic pastes are those containing fluoride ions. But the modern dental market also offers anticariogenic toothpastes without fluoride, for example, containing hydroxyapatite and other remineralizing substances.[8]

Hydroxyapatite is characterized by high biocompatibility and bioactivity.[9] Its particles are similar to apatite crystals of natural enamel in morphology and crystal structure. Various *in vitro* studies have reported superior remineralizing efficacy of hydroxyapatite over fluorides.[10,11]

Currently various studies are still underway to improve the remineralizing effect of hydroxyapatite or to develop new effective remineralizing agents.[12,13] One of the trends in remineralizing therapy is the development of toothpaste allowing brushite crystals formation in the demineralized lesions of hard tooth tissues. Brushite ($CaHPO_4 \cdot 2H_2O$) is a precursor to hydroxyapatite [HAP, $Ca_5(PO4)_3OH$]. Materials, based on calcium phosphate, are used for a long time for filling defects in mineralized tissues.[14,15]

Brushite crystals form during the interaction of two substances: $Ca\ (NO3)_2 + (NH4)_2\ HPO_4 > CaHPO_4 + 2NH_4NO_3$.

Successive exposure of hard tooth tissues to these solutions may result in formation of amorphous calcium phosphate and dicalcium phosphate dihydrate (brushite) on the tooth surface. The possible mechanism of brushite action is the blockage of the fluid movement in the dentinal tubules as a result of the formation of a crystalline precipitate. The purpose of this study was to compare the effect of brushite and hydroxyapatite on hypersensitivity, enamel acid resistance, and its speed of remineralization.

Material and Methods

Study design

This study was a randomized double-blind controlled clinical study. The study was conducted from March 2019 to September 2019 at the Department of Therapeutic Dentistry, Sechenov University, Moscow, Russia. Ninety-eight volunteers aged 20–25 were surveyed and 60 consent patients with enamel hypersensitivity were selected for the study. Informed written consent was obtained for participation in the study and publication of the data for research and educational purposes. Participants were given freedom to withdraw from the trial at any point. Regular care was ensured to the participant in the case of withdrawal.

Ethical approval

The study was approved by the Ethics Committee of Sechenov University (Protocol no. 11–18). The study was registered in the ISRCTN registry no. ISRCTN29509071 (https://doi.org/10.1186/ISRCTN29509071).

Sampling criteria

The volunteers attending Dental Institute were invited to participate in the survey with the possibility to further participate in the study. The inclusion criteria were otherwise healthy individuals aged 20–25, with no gingival recession.

The exclusion criteria were the presence of orthodontic appliance, malocclusion allergic reactions to the components of toothpastes, systemic diseases, and pregnancy.

The sample size was determined according to the previous trial by the same authors, where Shiff index was assessed. The power was set at 80%, alfa-level was set as 0.05, and the means from the previous study were 2.6 ± 0.75 and 1.9 in toothpaste and placebo groups, respectively. The allocation ratio was equal to 1. The target sample size comprised 18 participants in each group, 54 patients total. The sample size was increased to 20 participants in each group to compensate for the attrition bias.

Material preparation

The composition of the toothpaste used by the test group was follows:

Bottle number 1 composition

Calcium nitrate, sorbitol, glycerin, drinking water, silica, aromatic composition, xanthan gum, xylitol, nettle extract, chamomile extract, yarrow extract, calendula extract, sage extract, echinacea extract,

stevioside, D-panthenol, sodium benzoate, potassium sorbate, papain, and natural indigo dye.

Bottle number 2 composition

Ammonium hydrogen phosphate, sorbitol, glycerin, drinking water, silica, aromatic composition, xanthan gum, xylitol, green tea extract, aloe vera extract, calamus extract, menthol, stevioside, D-panthenol, allantoin, sodium benzoate, potassium sorbate, natural dye, and beta carotene.

The composition of the toothpaste used by the positive control group

Aqua, sorbitol, hydrated silica, glycerin, hydroxyapatite 6% (nano), cellulose gum, sodium myristoyl sarcosinate, sodium methyl cocoyl taurate, aroma, xanthan gum, stevia rebaudiana extract, anethole, tetrasodium glutamate diacetate, tocopheryl acetate, eucalyptol, o-Cymen-5-ol, citric acid, *Vitis vinifera* (grape) seed extract, tannase, thymol, limonene, and fluoride free.

The composition of the toothpaste used by the negative control group

Hydrogenated starch hydrolysate, hydrated silica, aqua, glycerin, cellulose gum, dipotassium glycyrrhizate, polyglyceryl-4 laurate/sebacate, polyglyceryl-6 caprylate/caprate, xanthan gum, menthyl lactate, *Perilla frutescens* seed extract, and colloidal silver.

Patients received instructions on oral hygiene, including the use of medium toothbrush and dental floss.

Grouping method

Sixty people aged 20–25 years old were examined and randomly assigned to one of the study groups:

1. Group 1 used toothpaste promoting the formation of brushite (test group).
2. Group 2 used toothpaste with hydroxyapatite (positive control group).
3. Group 3 used toothpaste without fluoride and without hydroxyapatite (negative control group).

The allocation concealment was performed by the use of containers numbered by a "third party" (person, who did not participate in the study). The toothpastes in white bottles without any titles were placed in the containers. The weight of the pastes and bottles in different groups was the same. The patient on enrolment received a sealed container with a toothpaste. Neither patients nor researchers were aware of the type of a toothpaste received by each patient.

Observational parameters

At the baseline visit, the following procedures were performed:

- written informed consent was reviewed and obtained;
- a unique screening/enrollment number was assigned to each patient;
- medical history, and medication history to determine eligibility based on inclusion/exclusion criteria, all current medications, including medications over-the-counter and herbal medications, and demographics (age, race, ethnicity, gender) were reviewed and recorded;
- physical examination was performed and simplified oral hygiene index (OHI-S), enamel acid resistance test, enamel remineralization rate, and Shiff test were administered.

All patients received oral hygiene instructions (including the use of the assigned toothpastes twice daily, brushing technique, the use of interdental flosses, and/or brushes) at the baseline visit.

The patients used the prescribed pastes for a month. Control examinations were carried out in the following periods: 2 weeks and 4 weeks. After each study time point (baseline, 2 weeks, and 4 weeks) the patients were to visit the dental office daily to assess the rate of enamel mineralization (the procedure is described below).

The examination included the following:

1. OHI-S,
2. enamel acid resistance test,[16]
3. rate of enamel remineralization,[17]
4. Schiff test for the assessment of teeth sensitivity.

For the assessment of enamel acid resistance, a drop of hydrochloric acid buffer solution (pH: 0.3–0.6) was applied on the buccal surface of maxillary central incisor (middle third) for a minute. After that the acid was rinsed off with water, and 2% methylene blue aqueous solution was applied for 5 min. The assessment of staining intensity was performed using 10-point printed scale.

To evaluate the rate of enamel remineralization, the staining was repeated daily until the loss of staining ability. The number of days was fixed and served as a quantitative parameter of enamel remineralization rate.

Schiff test was performed to assess enamel sensitivity in all groups as follows: air from air/water syringe was applied perpendicular to the cervical areas of all teeth from a distance of 1 cm for a second. Enamel sensitivity was estimated in accordance with the following criteria: 0—no reaction; 1—discomfort but the patient does not insist on stopping the test; 2—discomfort, accompanied by a request to discontinue the test; and 3—severe pain

reaction with pronounced motor reactions aimed at the immediate termination of the test.

Statistical analysis

Data were analyzed by using Microsoft Excel (2007, Redmond, WA, USA) and Statistical Package for the Social Sciences (SPSS) software program, version 23.0 (Armonk, NY, USA). To describe the quantitative characteristics, the mean values (*M*) and standard deviation (*m*) were calculated. As the distributions of variables were not normal, nonparametric methods were used: Friedman rank sum test for related variables and the Kruskal–Wallis rank sum test for independent variables. Nemenyi test adjusted for multiple comparisons was used for *post hoc* comparisons. Cohen's *d* was used to estimate the effect size. The level of statistical significance was set as $\alpha = 0.05$.

The primary outcome was a hypersensitivity relief as measured with the Shiff index, and the secondary outcomes were the increase in the enamel resistance to acid and enamel remineralization rate.

Results

Ninety-eight volunteers aged 20–25 were surveyed and 65 patients reported tooth hypersensitivity, but two of these patients did not satisfy the inclusion criteria (one woman was pregnant, and the other woman had diabetes mellitus). Sixty-three consent patients with enamel hypersensitivity were enrolled and randomly assigned in the three groups: test group ($n = 21$), positive control group ($n = 20$), and negative control group ($n = 22$) [Table 1]. However, three of the enrolled patients did not present to one of the follow-up visits and were withdrawn from the study [Figure 1]. All the three persons explained the reason why they did not come as the "lack of time." They denied any adverse reactions related to the use of the assigned toothpastes. Finally, 20 patients in each group were included in the analysis of the primary outcome (the decrease in tooth hypersensitivity according to Shiff index). During the study, no harms or unintended effects were registered.

In the test and positive control groups, Schiff index values significantly decreased after 2 weeks ($P = 0.02$ and $P = 0.0034$, respectively), whereas in the negative control group no significant differences were observed at the study time points **[**Figure 2**]**. The effect size of the hypersensitivity relief was large (–1.047, CI: –1.73; –0.36) in the test group compared to the negative control group, and negligible (0.05 CI: –0.59; 0.69) in the test group compared to the positive control group [Table 2].

The differences in oral hygiene level between groups were not statistically significant at baseline, after 2 and 4 weeks ($P > 0.05$). The OHI-S values were significantly lower after 2 and 4 weeks compared with the previous measurements in all groups ($P < 0.001$) [Table 3].

Table 1: Age and gender of the study participants

Group	Age	Male		Female		Total	
		Abs.	%	Abs.	%	Abs.	%
Study group	20	1	5	5	25	6	30
	21	2	10	3	15	5	25
	22	1	5	0	0	1	5
	23	1	5	1	5	2	10
	24	2	10	3	15	5	25
	25	1	5	0	0	1	5
	Total	8	40	12	60	20	100
Negative control group	20	2	10	5	25	7	35
	21	3	15	2	10	5	25
	22	1	5	2	10	3	15
	23	1	5	1	5	2	10
	24	0	0	0	0	0	0
	25	2	10	1	5	3	15
	Total	9	45	11	55	20	100
Positive control group	20	2	10	7	35	9	45
	21	2	10	1	5	3	15
	22	0	0	2	10	2	10
	23	2	10	1	5	3	15
	24	1	5	0	0	1	5
	25	1	5	1	5	2	10
	Total	8	40	12	60	20	100

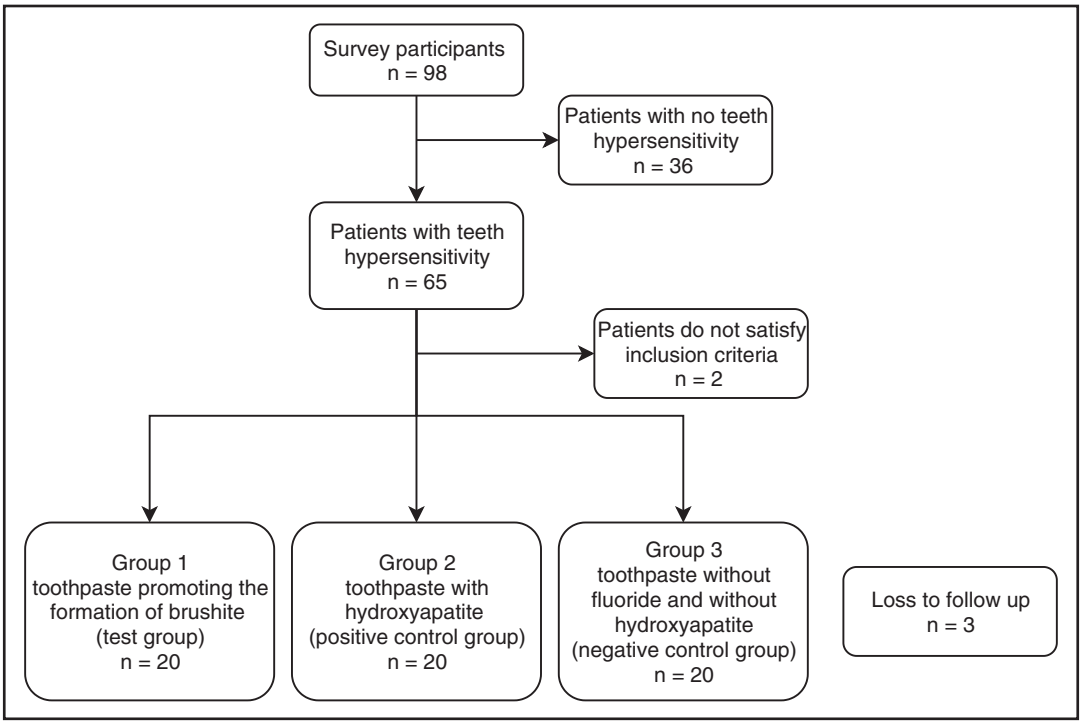

Figure 1: Participant flow diagram

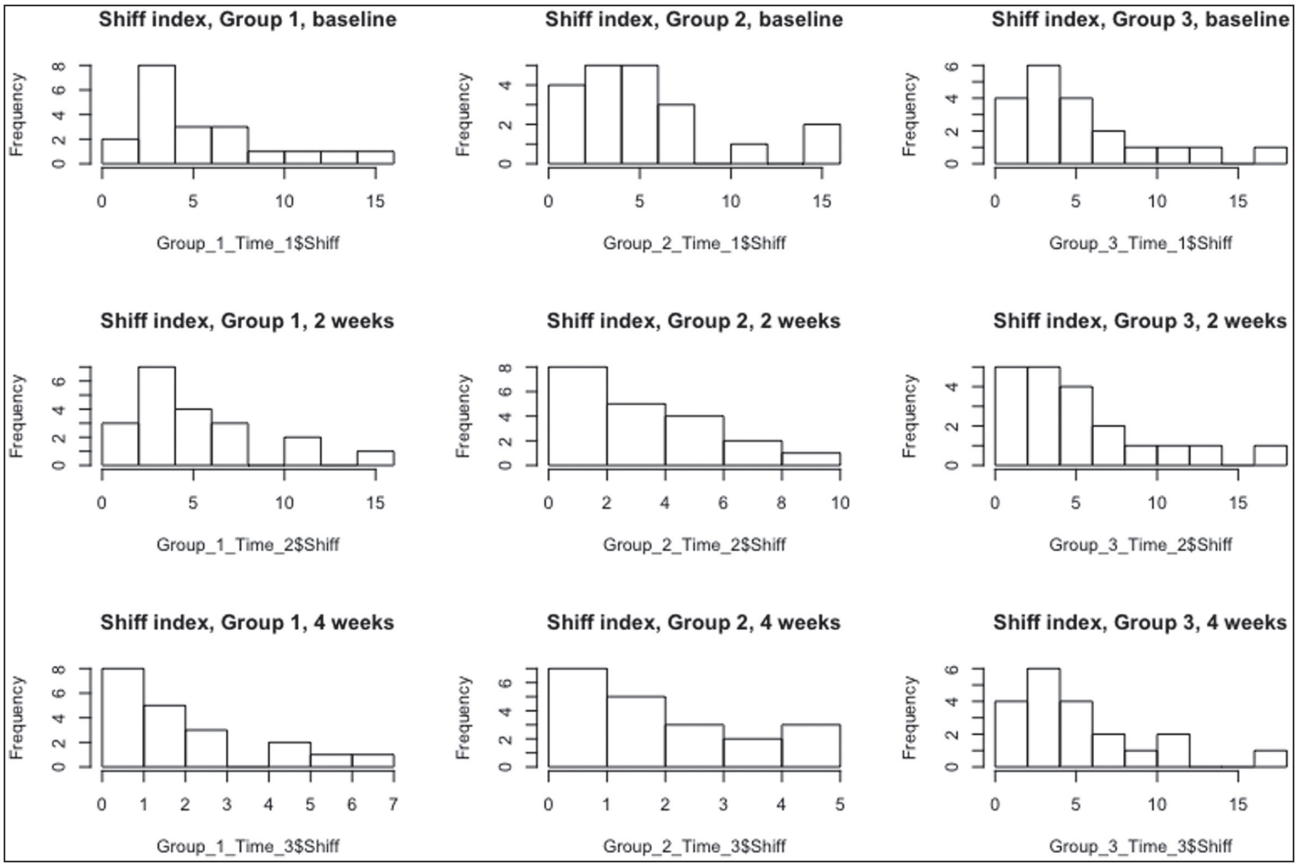

Figure 2: Dynamics of Shiff test

The acid resistance of enamel at baseline did not differ between groups (P = 0.583). Positive control group showed significantly higher level of acid resistance compared with other groups after 2 and 4 weeks. Positive control group (HAP) showed a significant rise in enamel acid resistance after 2 and 4 weeks compared with the previous measurements (P < 0.001). Test group showed a significant increase in acid resistance after 4 weeks compared with the previous measurements (P < 0.001). Negative control group showed nonsignificant changes in enamel acid resistance at different time points [Table 4]. Enamel remineralization rate showed similar dynamics [Figure 3] and [Table 5]. The effect sizes of the influence of the toothpaste promoting brushite formation compared with negative control on enamel acid resistance and remineralization rate were large (–1.73, CI: –2.48; –0.98 and –1.47, CI: –2.19; –0.75, respectively). The effect sizes of the toothpastes used in the test group compared with positive control group on enamel acid resistance and remineralization rate were small and medium (0.24, CI: –0.40; 0.88 and 0.65, CI: –0.005; 1.31, respectively) [Table 2].

Discussion

In this study, we assessed the effect of HAP-containing and brushite-forming toothpastes on the functional acid resistance of enamel, speed of its remineralization, and hypersensitivity.

Both HAP-containing and brushite-forming toothpastes significantly increased enamel acid resistance and speed of its remineralization. However, in the HAP-toothpaste group the effect developed earlier (after 2 weeks).

Table 2: Effect sizes of the study outcomes (Cohen's *d*) with 95% confidence intervals

Groups	Shiff index		Enamel resistance to acid		Enamel remineralization rate	
TG vs. PC	0.05 (–0.59; 0.69)	Negligible	0.24 (–0.40; 0.88)	Small	0.65 (–0.005; 1.31)	Medium
TG vs. NC	–1.047 (–1.73; –0.36)	Large	–1.73 (–2.48; –0.98)	Large	–1.47 (–2.19; –0.75)	Large

TG = test group, PC = positive control, NC = negative control

Table 3: Simplified oral hygiene index values

Group/time	Baseline	After 2 weeks*	After 4 weeks**
Test group	1.06 ± 0.32	0.95 ± 0.30 (P = 0.015)	0.80 ± 0.28 (P = 0.007)
P value (TG vs. PC)	–	–	–
Positive control group	1.05 ± 0.29	0.90 ± 0.26 (0.004)	0.75 ± 0.26 (0.004)
P value (NC vs. PC)	–	–	–
Negative control group	1.07 ± 0.31	0.92 ± 0.28 (P = 0.004)	0.74 ± 0.25 (P = 0.004)
P value (NC vs. TG)	–	–	–
P value (Kruskal–Wallis rank sum test)	*0.9917*	*0.9171*	*0.8105*

P value indicates the level of significance of the differences between groups

*P value in brackets is given for the differences between baseline and 2 weeks; **P value is given for the differences between the 2 weeks and 4 weeks

TG = test group, PC = positive control, NC = negative control

Table 4: Enamel acid resistance (points)

Group/time	Baseline	After 2 weeks*	After 4 weeks**
Test group	4.35 ± 0.59	4.15 ± 0.49 (P = 0.0034)	3.15 ± 0.58 (P = 0.0307)
P value (TG vs. PC)	–	0.45	0.025
Positive control group	4.5 ± 0.69	3.7 ± 0.86 (0.012)	3.0 ± 0.65 (0.139)
P value (NC vs. PC)	–	0.00064	0.00074
Negative control group	4.35 ± 0.67	4.40 ± 0.59 (P = 1)	4.3 ± 0.73 (P = 1)
P value (NC vs. TG)	–	>0.001	>0.001
P value (Kruskal–Wallis rank sum test)	0.5835	0.01208	>0.001

P value indicates the level of significance of the differences between groups

*P value in brackets is given for the differences between baseline and 2 weeks; **P value is given for the differences between the 2 weeks and 4 weeks

TG = test group, PC = positive control, NC = negative control

In the study of Hanning and Hanning,[9] the nanocrystals have been used in tooth pastes and mouth rinses to promote the repair of demineralized enamel or dentine surfaces. Hydroxyapatite crystals have been shown to repair micrometer-sized tooth surface defects *in vitro*.

Hydroxyapatite is capable of remineralizing enamel by forming a surface apatite coating to cover the enamel structure. According to Roveri *et al.*,[18] HAP-containing products filled micro defects at the etched enamel surface after 10-min application.

The relief of hypersensitivity was significant after 2 weeks in both groups compared with negative control. Hydroxyapatite was shown to provide quick and sustained relief from symptoms when compared to potassium nitrate and sodium monofluorophosphate, although there was a decrease in hypersensitivity in all the treatment groups.[19,20]

According to Shetty *et al.*,[21] both 25% and 100% concentrations of hydroxyapatite provided significant desensitizing effect. However, the higher concentration probably enhanced better penetration of the hydroxyapatite particles into the dentinal tubules. Pain relief was obtained in most of the patients after one or two applications. In this study, we used toothpaste with

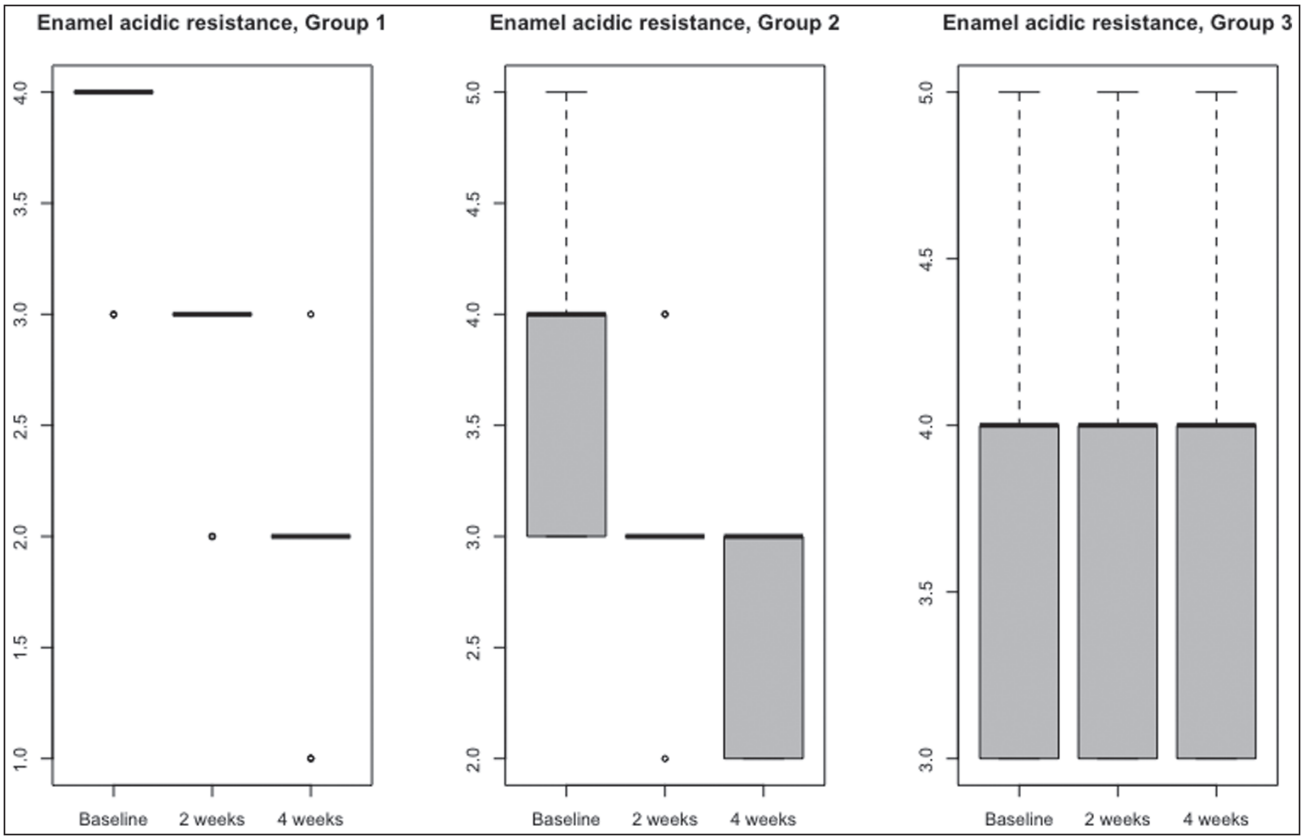

Figure 3: Enamel acidic resistance

Table 5: The rate of enamel remineralization (days)

Group/time	Baseline	After 2 weeks*	After 4 weeks**
Test group	2.9 ± 0.3	2.65 ± 0.49 (*P* = 0.559)	1.8 ± 0.61 (*P* = 0.01)
P value (TG vs. PC)	0.047	–	0.012
Positive control group	3.25 ± 0.44	2.75 ± 0.44 (0.09)	2.5 ± 0.5 (0.5)
P value (NC vs. PC)	0.109	–	0.113
Negative control group	2.95 ± 0.51	2.96 ± 0.59 (*P* = 1)	2.96 ± 0.53 (*P* = 1)
P value (NC vs. TG)	0.934	–	>0.001
P value (Kruskal–Wallis rank sum test)	0.0284	0.1706	>0.001

P value indicates the level of significance of the differences between groups

**P* value in brackets is given for the differences between baseline and 2 weeks; ***P* value is given for the differences between the 2 weeks and 4 weeks

TG = test group, PC = positive control, NC = negative control

6% concentration of hydroxyapatite; this may explain longer time (2 weeks) of desensitizing effect development. These results correspond to the study by Al Asmari and Khan,[22] in which the use of the desensitizing toothpaste containing zinc-carbonate hydroxyapatite provided significant pain relief after 4 weeks.

It has been shown that in addition to its remineralizing effect, hydroxyapatite powder was also an effective abrasive cleaning agent for oral hygiene.[22]

The rate of remineralization of enamel is determined by many factors, including the remineralizing effect of saliva, the amount of which, even in healthy people, may decrease under the stress.[23]

In this study, the oral hygiene level improved in all groups probably due to motivation, given instructions, and regular recalls. At the same time, enamel acid resistance and speed of its remineralization increased significantly in test and positive control group compared with negative control. This result may indicate that these parameters depend not only on the level of oral hygiene but also on the structural and functional features of its surface layer.

To the best of our knowledge, no studies assessed brushite effectiveness for enamel remineralization and hypersensitivity relief. In this study, the effect of brushite-containing toothpaste was almost equal to HAP-containing toothpaste. Brushite particles are similar to hydroxyapatite in morphology and crystal structure. The mechanism of action can be due to the penetration of soluble calcium and phosphate compounds into the foci of demineralization.

The limitations of the study include the limited age group that was assessed (20–25-year-old patients). It is possible that in the elder age groups the effect of brushite-containing toothpaste will be less prominent due to the differences in enamel mineralization and structure. In addition, longer follow-up could be useful to reveal the long-term effect of the use of the assessed toothpastes. Although no adverse reactions or harms were found in this study, it is possible that these reactions will become evident after a more prolonged use of the toothpastes. It is advisable that further studies include population of different areas, of different age, and include longer follow-up.

Within the limitations of this study, it can be concluded that brushite-containing toothpaste may be used as an alternative to HAP-containing for remineralizing and desensitizing treatment.

The study was registered in the ISRCTN registry no. ISRCTN29509071 (https://doi.org/10.1186/ISRCTN29509071).

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Conflicts of interest

Nil.

Author contributions

Conception or design of the work (Polyakova MA, Arakelyan MG, Novozhilova NE, Babina KS, Sokhova IA, Margaryan EG, Doroshina VYu); data acquisition (Polyakova MA, Arakelyan MG), analysis (Novozhilova NE, Babina KS), interpretation of data for the work (Polyakova MA, Arakelyan MG, Novozhilova NE, Babina KS); drafting the work or revising it critically for important intellectual content (Polyakova MA, Arakelyan MG, Novozhilova NE, Babina KS, Sokhova IA, Margaryan EG, Doroshina VYu); final approval of the version to be published (Polyakova MA, Arakelyan MG, Novozhilova NE, Babina KS, Sokhova IA, Margaryan EG, Doroshina VYu). Agreement was reached for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical policy and institutional review board statement

This study was approved by the Ethics Committee of Sechenov University (protocol number 11–18; December 5, 2018). All the procedures have been performed as per the ethical guidelines laid down by Declaration of Helsinki (2013).

Patient declaration of consent

Informed written consent was obtained from all the participants for participation in the study, publication of the data for research, and educational purposes.

Data availability statement

The data set used in this study is available from corresponding author (Dr. Marianna G. Arakelyan, e-mail: arakelyan.mg@1msmu.ru) on request.

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