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Comparison between fluoride varnish and nanohydroxyapatite in the remineralisation of active white spot lesions: a prospective clinical study

Comparativo entre o verniz fluoretado e a nanohidroxiapatita na remineralização de lesões de manchas brancas ativas: estudo clínico prospectivo

Comparación entre barnices de flúor y tinción de nanohidroxiapatita para la remineralización de manchas blancas activas: un estudio clínico prospectivo

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Abstract
Objective: The initial expression of dental caries, white spot, is still frequent and requires professional care that may or may not be preventive. It is important to carry out research that evaluates different methods of remineralisation of these lesions, both in primary and permanent dentition. Thus, the aim of this study was to compare the remineralisation potential using fluoride varnish and nano-hydroxyapatite toothpaste in split-mouth design. Materials and Methods: For the research, 21 children between 6 and 12 years of age, with active non-cavitated white spots on primary and permanent teeth were selected. As a methodology, the remineralisation procedures were performed in different hemiarchs and each tooth received a different product: fluoride varnish and nano-hydroxyapatite toothpaste, both in 4 sessions, once a week. Results: Were analyzed by a calibrated examiner with no prior knowledge of the applied product; through visual criteria and measurement of white spots in their largest diameter with a digital caliper. Changes in gloss, color, texture and surface integrity were observed. The collected data received statistical treatment by means of the t-Student test, which identified the effectiveness in the reduction and remineralisation of white spots ($p<0.05$), by the use of fluoride varnish. Conclusion: The fluoride varnish presented statistically significant results in the treatment of active white spots. In the visual clinical evaluation, no differences were observed between fluoride varnish and nano-hydroxyapatite toothpaste. Therefore, more research is needed to offer patients viable and safe alternatives in carie prevention.
Keywords: Dental caries; Tooth remineralization; Hydroxyapatite; Nanoparticles.

Resumo
Objetivo: A expressão inicial da cárie dentária, mancha branca, ainda é frequente e requer cuidados profissionais que podem ou não serem preventivos. É importante realizar pesquisas que avaliem diferentes métodos de remineralização dessas lesões, tanto na dentição decídua quanto na permanente. Assim, este estudo comparou o potencial de remineralização, utilizando verniz fluoretado e creme dental nanoparticulado em boca dividida. Materiais e Métodos: Para a pesquisa, foram selecionadas 21 crianças entre 6 e 12 anos de idade, com manchas brancas ativas não cavitadas em dentes decíduos e permanentes. Como metodologia, os procedimentos de remineralização foram realizados em diferentes hemiarcos e cada dente recebeu um produto diferente: verniz fluoretado e creme dental nanoparticulado, ambos em 4 sessões, uma vez por semana. Resultados: Foram analisados por um examinador calibrado sem conhecimento prévio do produto aplicado; através de critérios visuais e medição de manchas brancas em seu maior diâmetro, com paquímetro digital. Foram observadas alterações no brilho, cor, textura e integridade da superfície. Os dados coletados receberam tratamento estatístico por meio do teste t-Student, que identificou a eficácia na redução e remineralização de manchas brancas ($p<0,05$), pelo uso de verniz fluoretado. Conclusão: O verniz fluoretado apresentou resultados estatisticamente significantes no tratamento de manchas brancas ativas. Na avaliação clínica

visual, não foram observadas diferenças entre o verniz fluoretado e o creme dental nanoparticulado. Portanto, mais pesquisas são necessárias para oferecer aos pacientes alternativas viáveis e seguras na prevenção da cárie.
Palavras-chave: Cárie dental; Remineralização dentária; Hidroxiapatita; Nanopartículas.

Resumen

Objetivo: La manifestación inicial de la caries dental, mancha blanca, aún es frecuente y requiere de cuidados profesionales que pueden o no ser preventivos. Es importante investigaciones que evalúen diferentes métodos de remineralización de estas lesiones, tanto en dentición temporal como permanente. Así, este estudio comparó el potencial de remineralización utilizando barniz de flúor y pasta de dientes con nanopartículas em estúdio de boca dividida. Materiales y Métodos: Para la investigación se seleccionaron 21 niños entre 6 y 12 años de edad, con manchas blancas activas no cavitadas en dientes primarios y permanentes. Los procedimientos de remineralización se realizaron en diferentes hemiarcadas y cada diente recibió un producto diferente: barniz de flúor y dentífrico nanoparticulado, ambos en 4 sesiones, una vez por semana. Resultados: Fueron analizados por un examinador calibrado sin conocimiento previo del producto aplicado; mediante criterio visual y medición de manchas blancas en su mayor diámetro, con un pie de rey digital. Se observaron cambios en el brillo, el color, la textura y la integridad de la superficie. Los datos recolectados recibieron tratamiento estadístico por medio de la prueba t-Student, que identificó la efectividad en la reducción y remineralización de las manchas blancas ($p<0,05$), por el uso del barniz de flúor. Conclusión: El barniz de flúor mostró resultados estadísticamente significativos en el tratamiento de las manchas blancas activas. En la evaluación visual no se observaron diferencias entre el barniz de flúor y el dentífrico nanoparticulado. Se necesita más investigación para ofrecer a los pacientes alternativas viables y seguras en la prevención de caries.
Palabras clave: Caries dental; Remineralización dental; Hidroxiapatita; Nanopartículas.

1. Introduction

Early Childhood Caries, like other forms of caries, is considered to be a biofilm-mediated, sugar-driven, multifactorial, dynamic disease that results in the imbalance of demineralisation and remineralisation of dental hard tissues. Dental caries is determined by biological, behavioural, and psychosocial factors linked to an individual's environment. Early childhood caries shares common risk factors with other non-communicable diseases (NCDs) associated with excessive sugar consumption, such as cardiovascular disease, diabetes, and obesity. Excessive intake of sugars leads to prolonged acid production from tooth adherent bacteria and to a shift in the composition of the oral microbiota and biofilm pH. If sustained, tooth structures are demineralized (Pitts et al., 2019). Undoubtedly, dental caries is the oral disease drawing more attention of the dental professionals, being the main concern among parents. Although the aetiology of dental caries is long known, its prevalence is still high even today. The inclusion of early caries lesions in epidemiological surveys can be relevant for mapping the population's needs as many studies exclude white-spot lesions, which are not their priority (De Carvalho et al., 2020). Such changes occur due to an increase in the porosity of the enamel subsurface, and subsequently, a decrease in light reflection in the demineralized area. These are the first detectable clinical signs of mineral loss in the enamel, called "non-cavitated caries lesions". These lesions may be reversible as long as the biofilm removal and fluoride application are successful (Machiulskiene et al., 2013). Control of biofilm and balance between the demineralisation and remineralisation processes are important preventive approaches to avoid the occurrence of dental caries (Cury et al., 2009). Thus, the use of substances to control and/or reverse early enamel lesions has been investigated by several studies, such as those on fluoride varnishes, casein and nano-hydroxyapatite toothpaste (Hellwig et al., 2010; Patil et al., 2013; Oliveira et al., 2014; Savas et al., 2016; Kamath et al., 2017; Grewal et al., 2018; Oliveira et al., 2019).

The first fluoride varnish was developed in the 1960s to prolong the contact between fluoride and enamel. Adhesion to enamel and formation of calcium fluoride after application act as a long-term fluoride reservoir (Seppä et al., 2004). More recently, the research on nano-technology in dentistry could be useful for the release of molecules or medications in the repair of damaged tissues, including investigation and implementation of nano-technological methods and techniques aimed at finding therapeutic alternatives and developing nano-designed structures (Freitas & Nanodentistry, 2000; Patil et al., 2013; Martínez et al., 2011 Huang et al., 2009; Huang et al., 2010; Niwa et al., 2001). Niwa et al. (2001) demonstrated the presence

of perfectly grown hydroxyapatite nanocrystals at the interface between toothpaste and enamel. The clinical significance of this material consists in the fact that nanoparticulate toothpaste can be recommended as an alternative in the remineralisation process, as it has a lower fluoride concentration than fluoride varnish which can be beneficial for children, pregnant women and for those who have a high risk of dental fluorosis (Daas et al., 2018).

Therefore, the studies in the literature support positively the use of different agents in the remineralisation process of white-spot lesions in the dental enamel. Nevertheless, it becomes clear the need to clinically compare the performance of fluoride varnish and nano-hydroxyapatite in both dentition.

2. Methodology

The present study was carried out with 21 patients aged between 6 and 12 years old from the Pediatric Dentistry Clinic of the São José dos Campos Institute of Science and Technology of the State University of São Paulo (ICT-UNESP) after approval by the human ethics research committee location according to CAAE protocol number: 03513818.4.0000.0077.

Children aged 6 to 12 years of both sexes with active lesions of white spots (i.e. opaque and rough) in the mixed dentition were selected, being selected 14 boys and 7 girls.

All patients received treatment with fluoride varnish and toothpaste with nanohydroxyapatite simultaneously, split-mouth design.

2.1 Eligibility criteria

The inclusion criteria were: Only children aged between 6 and 12 years old of both genders with active white-spot lesions (i.e. opaque and rugous) in the mixed dentition were selected. Patients with agreement to voluntarily participate in the research, being aware of the risks and benefits and before signing the Informed Consent Term (ICT).

The exclusion criteria were: Children with smooth and glossy white-spot lesions, hypoplastic spots on the enamel, fluorosis, molar-incisor hypomineralization (MIH) or cavitated caries lesions were excluded.

2.2 Sample

Power of test for the data on white-spot lesions was performed by using statistical software (MedCalc, version 18.11, 2018). For a sample size of 21, Student-t test (5%) detected a difference with clinical significance of 0.5 mm with 80-percent power for dependent samples and standard deviation (i.e. variability) of 0.75 mm.

2.3 Clinical procedures and evaluation

The remineralization procedures were performed on different hemi-arcs by the same calibrated and blinded operator. The operator was calibrated through previous training in a pilot study. He had experience in the area, both in clinical practice and in theory.

Procedures with Nano-Hydroxyapatite Toothpaste:

The Nano-Hydroxyapatite Toothpaste contains in its formula, 1,450 ppm of sodium fluoride, nanohydroxyapatite, hydroxyapatite and xylitol.

Prior to the application of nano-hydroxyapatite toothpaste, the selected tooth was submitted to prophylaxis with pumice and rubber cup, washed and dried for 15 seconds. Next, the lesion's size was measured (more than once) by using a digital calliper (i.e. the greatest diameter) and photographed for control purposes. Prophylaxis was continued by using a Robson toothbrush and a standard amount of the toothpaste for 30 seconds in the surface affected with white-spot lesions and

removal of excessive saliva. The patients were instructed to await a minimum of 30 minutes before consuming foods and beverages, according to the manufacturer's recommendations.

Procedures with Fluoride Varnish:

The Fluoride Varnish contains in your formula, 22.600 ppm of sodium fluoride, ethanol, colophonium, matrix, shellac, wax, saccharine and flavor.

Application of fluoride varnish was performed after prophylaxis of the selected tooth, which was cleaned with pumice and rubber cup, washed and dried for 15 seconds. Next, the lesion's size was measured (more than once) by using a digital calliper (i.e. the greatest diameter) and photographed for control purposes. The operative field was isolated with cotton rolls before applying the fluoride varnish with a small brush on the surfaces affected with active white-spot lesions. After removal of the cotton rolls, the patients were instructed to not consume any food or beverage before two hours and to not brush the treated tooth before 12 hours, according to the manufacturer's recommendations. The dental suction device remained within the oral cavity throughout the procedure. It is important to emphasise that the application of fluoride varnish is safe and does not interfere with the other quadrants of the tooth, since there is no systemic action and its sticky aspect when in contact with saliva is limited to the tooth being treated.

Both application procedures were performed in four consecutive sessions once a week, according to protocols established in the literature (Oliveira et al., 2019; De Amorim et al., 2008). Considering that both materials present only topical actions.

The children and their guardians followed the hygiene and diet guidelines, according to the teaching philosophy Pediatric Dentistry Clinic of the São José dos Campos Institute of Science and Technology of the State University of São Paulo (ICT-UNESP). Household hygiene was performed with standardized children's fluoride toothpaste (1,100ppm), from the time of initial screening until the end of the survey. They received guidance and previous training for oral brushing and appropriate material. Being, the guidelines conferred and reinforced at each session with patients and guardians. Treated teeth were carefully dried and evaluated under adequate lighting by a calibrated examiner blinded to the applications. Based on visual criteria, filling out a specific questionnaire, the results were recorded in previously prepared medical records. The evaluation showed changes in gloss, colour, texture and surface, even disappearance of the lesions. In case of doubt, the examiner used a blunt-tipped probe for further investigation.

The examiner used a digital calliper (in millimeters) to measure the most extremal parts of the white-spot lesion in its greatest mesio-distal or cervico-incisal/occlusal diameters in order to assess the size reduction (more than once).

The data were submitted to statistical analysis with software (MedCalc, version 18.11, 2018), using the Student t test with significance of 5% ($\alpha = 0.05$) for the comparison between the datas.

3. Results

3.1 Recruitment and Demographic Data

Patient screening was initiated during the discipline of paediatric dentistry, in which 31 patients were initially selected. After a careful anamnesis, some patients were excluded and thus 25 ones were found to be eligible for study. However, three patients dropped out of the study due to personal problems and one patient had the selected teeth extracted. Therefore, the final sample had 21. All patients received treatments with fluoride varnish and nano-hydroxyapatite toothpaste on a simultaneous basis. Briefly, children aged between 6 and 12 years old participated in the study in which 14 were males and 7 were females. The teeth most affected by active white-spot lesions were deciduous canines (31%), followed by permanent first molars (24%).

3.2 Clinical Analysis:

Clinical evaluation was performed by a blinded evaluator and previously calibrated, made through an individual analysis before and after 4 weeks of application of the material. After clinical assessment, it was found that 21 spots were present, five remained active, 16 became inactive, 20 were white and one was brownish after application of fluoride varnish for four weeks. With regard to the nano-hydroxyapatite toothpaste, it was found that seven spots remained active, 14 became inactive, 18 were white and three brownish. No cavitations were observed in any of the white-spot lesions treated with both materials.

3.3 Statistical Analysis

Analysis of data collected from 21 patients showed that fluoride varnish was effective in reducing active white spot lesions ($P < 0.05$). The results listed in Table 1 illustrate the difference between the initial and final sizes of white spot lesions in each patient, as well as the relationship between the initial size and the difference, including percentages of reduction or increase.

On the other hand, the results obtained with the use of nano-hydroxyapatite toothpaste did not show a statistically significant reduction in white spot lesions. Table 2 shows the difference between the initial and final sizes of white spot lesions in each patient, as well as the relationship between the initial size and the difference, obtaining its reduction or possible percentage increase.

The negatives results reflect an increase in the size of the white spot lesions in the Tables 1 and 2. Table 3 lists the values of the initial and final sizes of the white-spot lesions regarding the materials used in each patient, including colour and spot, namely: ab (active-brownish), aw (active-white) and iw (inactive-white). According to the data obtained, it is possible to observe that both materials had a similar performance regarding colour and caries activity as the spots were inactive and white (i.e. iw) in the majority of the patients. No inactive brownish spot was found. Table 4 shows the percentage values for caries activity (i.e. active or inactive) and colour (white or brownish) of the white-spot lesions after application of fluoride varnish and nano-hydroxyapatite toothpaste.

Table 1 - Initial and final measurements of the white-spot lesions treated with fluoride varnish and the relationship between difference and initial size (in percentage).

Patient	Varnish-i	Varnish-f	Varnish-Dif	Dif/i(%
1	3.5	3.4	0.1	2.9
2	3.7	3.4	0.3	8.1
3	2.0	1.4	0.6	30.0
4	3.6	3.3	0.3	8.3
5	5.7	5.8	-0.1	-1.8
6	4.0	3.8	0.2	5.0
7	3.6	3.5	0.1	2.8
8	2.4	2.1	0.3	12.5
9	4.0	3.9	0.1	2.5
10	4.9	4.6	0.3	6.1
11	4.1	3.9	0.2	4.9
12	4.4	4.1	0.3	6.8
13	5.6	5.0	0.6	10.7
14	5.8	5.5	0.3	5.2
15	3.6	3.4	0.2	5.6
16	4.0	3.7	0.3	7.5
17	3.2	2.8	0.4	12.5
18	2.6	2.7	-0.1	-3.8
19	4.7	4.7	0.0	0.0
20	4.2	4.0	0.2	4.8
21	2.5	2.7	-0.2	-8.0

Source: Authors (2022).

Note: Varnish-I, initial sizes; Varnish-f, final sizes; Varnish-Dif, relationship between the differences; Dif/i(%), percentages of reduction or increase.

Table 1 illustrate the difference between initial and final sizes of white-spot lesions in each patient as well as the relationship between the difference and initial size, including the percentages of reduction or increase, with fluoride varnish.

Table 2 - Initial and final measurements of the white-spot lesions treated with nano-hydroxyapatite toothpaste and the relationship between difference and initial size (in percentage).

Patient	Toothpaste-i	Toothpaste-f	Toothpaste-Dif	Dif/i(%)
1	4.2	4.1	0.1	2.4
2	3.9	4.0	-0.1	-2.6
3	1.8	1.9	-0.1	-5.6
4	3.0	3.1	-0.1	-3.3
5	5.3	5.5	-0.2	-3.8
6	3.5	3.1	0.4	11.4
7	6.1	6.1	0.0	0.0
8	4.0	3.8	0.2	5.0
9	4.6	4.7	-0.1	-2.2
10	1.0	0.9	0.1	10.0
11	3.9	3.7	0.2	5.1
12	4.1	4.0	0.1	2.4
13	5.0	4.8	0.2	4.0
14	3.6	3.4	0.2	5.6
15	2.9	2.6	0.3	10.3
16	4.9	3.5	0.4	8.2
17	6.1	5.7	0.4	6.6
18	3.5	3.6	-0.1	-2.9
19	2.5	2.6	-0.1	-4.0
20	6.9	7.0	-0.1	-1.4
21	4.2	4.0	0.2	4.8

Source: Authors (2022).

Note: Toothpaste-I, initial sizes; Toothpaste-f, final sizes; Toothpaste-Dif, relationship between the difference; Dif/i(%), percentages of reduction or increase.

Table 2 shows the difference between initial and final sizes of white-spot lesions in each patient as well as the relationship between the difference and initial size, including the percentages of reduction or increase, with nano-hydroxyapatite toothpaste.

Table 3 - Initial and final values for the white-spot lesions treated with fluoride varnish and nano-hydroxyapatite toothpaste and the combination colour-spot for both materials.

Patient	Varnish-initial	Varnish-final	Toothpaste-initial	Toothpaste-final	Varnish colour-spot final	Toothpaste colour- spot final
1	3.5	3.4	4.2	4.1	iw	ab
2	3.7	3.4	3.9	4.0	iw	iw
3	2.0	1.4	1.8	1.9	iw	iw
4	3.6	3.3	3.0	3.1	iw	iw
5	5.7	5.8	5.3	5.5	aw	aw
6	4.0	3.8	3.5	3.1	iw	iw
7	3.6	3.5	6.1	6.1	iw	iw
8	2.4	2.1	4.0	3.8	iw	iw
9	4.0	3.9	4.6	4.7	iw	iw
10	4.9	4.6	1.0	0.9	aw	iw
11	4.1	3.9	3.9	3.7	iw	iw
12	4.4	4.1	4.1	4.0	iw	aw
13	5.6	5.0	5.0	4.8	iw	aw
14	5.8	5.5	3.6	3.4	ab	ab
15	3.6	3.4	2.9	2.6	aw	aw
16	4.0	3.7	4.9	4.5	aw	ab
17	3.2	2.8	6.1	5.7	iw	iw
18	2.6	2.7	3.5	3.6	iw	iw
19	4.7	4.7	2.5	2.6	iw	iw
20	4.2	4.0	6.9	7.0	iw	iw
21	2.5	2.7	4.2	4.0	iw	iw

Source: Authors (2022).

Note: ab (active-brownish); aw (active-white); iw (inactive-white).

Table 3 lists the values of the initial and final sizes of the white-spot lesions regarding the materials used in each patient, including colour and spot.

Table 4 - Distribution of the color changes and caries activity (in percentage) after application of the fluoride varnish and nano-hydroxyapatite toothpaste.

	Active	Inactive	White	Brownish
Fluoride Varnish	23,8%	76.2%	95.2%	4.8%
Toophpaste	33,3%	66.7%	85.7%	14.3%

Source: Authors (2022).

Table 4 lists the percentage values for caries activity (i.e. active or inactive) and colour (white or brownish) of the white-spot lesions after application of fluoride varnish and nano-hydroxyapatite toothpaste.

4. Discussion

The diagnosis of white-spot lesions should be performed as earlier as possible so that preventive measures can be taken. It is a general consensus among researchers that the increasing use of fermentable sugars in the diet of the individuals has considerably increased. This is the reason why new strategies should be developed in order to remineralise the enamel efficiently.

The progression patterns of caries lesions are relevant in the decision making process as they guide the choice of treatments, particularly for white-spot lesions, which can be treated by non-operative measures (De Carvalho et al., 2020). This corroborates the methodology of the present study. According to Oliveira et al., 2019, topical application of fluoride is a non-invasive treatment for the early stages of the disease, being shown to be effective in the prevention and control of dental caries. Fluoride varnish applied once a week during four weeks consecutively favours the remineralisation of white-spot lesions on the surface of and beneath the enamel, although some authors have suggested that the mineral deposition into the deeper parts of the enamel is not enough, leading to a partial remineralisation only. A 4-week protocol of application was used in this study, but a clinical study confirmed the effectiveness of fluoride varnish in reducing the white spots ($P < 0.05$) and remineralising them. In 76.2% of the patients treated with fluoride, the white-spot lesions became inactive and there was reversion of the dental caries process, thus being an important ally in the preventive methods used by the dental practitioner. With regard to colour and caries activity, only one white-spot lesion was brownish and remained active, whereas five were white and remained active. Nevertheless, for a successful therapy, it is important to use joint efforts to motivate the children to improve their oral hygiene habits by intervening in the diet, providing dental prophylaxis and oral hygiene guidance, and using fluoride toothpaste (De Carvalho et al., 2020; Almeida et al., 2011).

In this clinical study, it was possible to observe that fluoride varnish provided a significantly greater remineralisation. On the other hand, Daas et al., 2018, conducted an in vitro study and reported that there were no significant differences in the performance of fluoride varnish and nano-hydroxyapatite toothpaste. The authors suggest that fluoride materials can be used as an alternative in cases where one desires a lower concentration of fluoride, such as pregnant women, children and patients who are at high risk of developing fluorosis. In this sense, clinical alternatives for enamel remineralisation have been studied by several authors, particularly regarding the use of nano-particulate materials to provide the patients with individual prevention of dental caries on an at-home basis (Kamath et al., 2017; Huang et al., 2009; Huang et al., 2011). In view of this, this study used nano-hydroxyapatite toothpaste for remineralisation of white-spot lesions and obtained a success rate of 66.7%, which are positive results compared to the literature (Kamath et al., 2017; Grewal et al., 2018; Huang et al., 2009; Daas et al., 2018; Juntavee et al., 2021).

Colour changes were also observed in this study, which were more expressive in teeth treated with nano-hydroxyapatite toothpaste as 14.3% of the white-spot lesions were active and brownish, but without cavitation. Kamath et al., 2017 found potentially beneficial results in the re-establishment of uniformity and integrity of the enamel surface treated with nano-hydroxyapatite toothpaste, which was applied to white-spot lesions in extracted deciduous teeth during three minutes for 14 days. The fact that there was no statistically significant reduction in the size of the white-spot lesions in our sample does not mean that our findings are invalid, thus being necessary to corroborate them with clinical data of the patients. According to Tschoppe et al., 2011, treatment failure might be related to the individual characteristics of the patients such as diet, oral hygiene, salivary buffer capacity, individual predisposition, activity and depth of the lesion, presence of biofilm, and brushing with fluoride toothpaste, all factors which can modulate the natural process of repair or advance of the lesion. Children's poor diet and oral hygiene habits were the variables affecting the research results, as both materials had a low performance in remineralising the white-spot lesions. High frequency of fermentable sugar consumption, presence of visible biofilm and poor tooth brushing act decisively for the progress of dental caries lesions.

In order to minimize individual factors, control for independent variables and biases, and standardize procedures, Grewal et al., 2018 performed an in vitro study in which the therapeutic role of nanohydroxyapatite toothpaste could be observed in mineral loss situations. or damage to enamel. The authors showed that the adoption of biocompatible and bioactive remineralizing materials can be an alternative to fluoride toothpastes. Juntavee et al., 2021 evaluated the effects of two concentrations of nanohydroxyapatite gel on remineralization of an artificial carious lesion compared with nanohydroxyapatite toothpaste and fluoride varnish. The results obtained in this study also showed that nanohydroxyapatite in both toothpaste and gel form was able to remineralize better than fluoride varnish. Although this evaluation was carried out in artificial carious lesions, it does not corroborate our results. Huang et al., 2010 also support the importance of daily use of nano-hydroxyapatite toothpastes. And it reinforces the importance of prospective studies for results close to the reality of patients, since there is a lack of this type of studies as well as good scientific evidence, which is a constant challenge in dentistry, and this corroborates the results found in this clinical study. Therefore, further studies are needed to establish a standardized protocol as an alternative clinical treatment. Although a positive effect of nanohydroxyapatite has not yet been proven in in vivo studies under everyday conditions and with long-term follow-ups, the results of in situ studies may have some clinical relevance for the future. In daily clinical practice, dentists are often confronted with patients who, despite numerous attempts to elucidate compelling evidence about fluoride, totally resist the use of fluoride products. If the remineralizing effects of nanohydroxyapatite also proved to be valid in in vivo situations, then nanohydroxyapatite may be a viable option for those patients who also have active caries lesions, provided they are involved in optimized management and remotivated to perform adequate hygiene. (Wierichs et al., 2022).

The success in using nano-technology resides in the fact that it is possible to synthesise inorganic crystals of hydroxyapatite with nanometric dimensions. These hydroxyapatite crystals are characterised by particles with increased surface area and higher bioreactivity, thus allowing them to bind to the enamel biomemetically, which is an important step in the remineralisation process (Roveri & Iafisco, 2010). The development of nano-particles for dental purposes is in progress and will provide an improvement in the quality of oral health care of adults and children.

5. Conclusion

With the fluoride varnish and the toothpaste with nano-hydroxyapatite tested in the treatment of active white spots, it was possible to observe that the fluoride varnishes presented statistically better results, in relation to the toothpaste with nano-hydroxyapatite. However, through visual clinical analysis, no differences were observed between the materials. Therefore, we observe the importance of these evaluations and suggest new clinical studies to offer safe and practical alternatives to children's patients in the prevention of caries.

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