UNIVERSIDADE DE UBERABA

MESTRADO ACADÊMICO EM ODONTOLOGIA

GIOVANNA SOUSA OLIVEIRA CHAGAS

Comparação dos níveis salivares de flúor após o uso de compostos fluoretados de aplicação profissional

UBERABA - MG
2023

GIOVANNA SOUSA OLIVEIRA CHAGAS

Comparação dos níveis salivares de flúor após o uso de compostos fluoretados de aplicação profissional

Dissertação apresentada como requisito para obtenção do título de Mestre em odontologia no Programa de Pós-Graduação em Odontologia da Universidade de Uberaba.

Área de concentração: Clínica Odontológica Integrada

Orientador: Prof. Dr. Vinícius Rangel Geraldo Martins

UBERABA – MG

2023

Catalogação elaborada pelo Setor de Referência da Biblioteca Central UNIUBE

C346c

Chagas, Giovanna Sousa Oliveira.
Comparação dos níveis salivares de flúor após o uso de compostos fluoretados de aplicação profissional / Giovana Sousa Oliveira Chagas. – Uberaba, 2023
47 f.

Dissertação (Mestrado) – Universidade de Uberaba. Programa de Pós-graduação em Odontologia. Área de Concentração em Clínica Odontológica Integrada.
Orientador: Prof. Dr. Vinícius Rangel Geraldo Martins.

1. Cáries dentárias. 2. Flúor. 3. Saliva. 4. Odontologia. I. Martins, Vinícius Rangel Geraldo. II. Universidade de Uberaba. Programa de Mestrado em Odontologia. Área de Concentração em Clínica Odontológica Integrada. III. Título.

CDD 617.67

GIOVANNA SOUSA OLIVEIRA CHAGAS

COMPARAÇÃO DOS NÍVEIS SALIVARES DE FLÚOR APÓS O USO DE COMPOSTOS FLUORETADOS DE APLICAÇÃO PROFISSIONAL

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Odontologia do Programa de Pós- Graduação em Odontologia - Mestrado da Universidade de Uberaba.

Área de concentração: Clínica Odontológica Integrada

Aprovado (a) em: 27/02/2023

BANCA EXAMINADORA:

Prof. Dr. Vinícius Rangel Geraldo Martins
Orientador
Universidade de Uberaba

Prof. Dr. Marcelo Rodrigues Pinto
Universidade de Uberaba

Profª. Drª. Carla Regina Costa
Universidade Federal do Triângulo Mineiro

Dedicatória

Dedico este trabalho a meus pais que sempre me apoiaram e acima de tudo acreditaram e embarcaram neste meu sonho. A vocês dois, Sérgio e Jackeline, o meu profundo e eterno agradecimento. Dedico esta pesquisa ao meu namorado Pedro Henrique cuja ajuda foi essencial para a conclusão deste trabalho. Grata pela sua compreensão com as minhas horas de ausência. Amo vocês.

Agradecimentos

Meu Deus, obrigada pelos teus planos para minha vida, pois são sempre maiores que meus próprios sonhos.

Aos meu pais, por todo incentivo e apoio nos caminhos difíceis que percorri.

Mãe, seu amor e carinho me ajudaram em todos os momentos, obrigada por ser tão incrível.

Pai, seus conselhos me ajudaram a tomar as melhores decisões da minha vida, obrigada por todo amor incondicional.

Ao meu namorado por sempre me incentivar a ser uma pessoa melhor. Você é um presente em minha vida, serei eternamente grata a Deus por dividir a vida com você.

As minhas avós, Edith e Conceição, obrigada por todo amor e carinho. Aos meus avôs, Vicente e Geraldo, obrigada por toda proteção, saudades eternas.

Aos meus familiares, tios e primos, pela ajuda e incentivo sempre.

Aos meus amigos, por me alegrar em momentos difíceis.

Ao meu orientador Prof. Dr. Vinícius Rangel, por todas as horas de dedicação e ajuda durante a realização deste trabalho.

A Profa. Dra. Carla Regina, por todo apoio e suporte prestado na pesquisa.

Aos funcionários e alunos, Cidney e Carlos, do departamento de Química da Universidade Federal do Triângulo Mineiro, a colaboração de vocês foi essencial para a realização desta pesquisa.

A aluna de graduação Nádia, pela ajuda na etapa de coleta das amostras.

Aos funcionários da Universidade de Uberaba, minha eterna gratidão por todos os momentos que passamos juntos.

A Universidade Federal do Triângulo Mineiro pelo empréstimo dos equipamentos.

Ao programa PAPE da Universidade de Uberaba pela compra de materiais utilizados durante a pesquisa.

Resumo

O objetivo da presente pesquisa foi avaliar a concentração de flúor na saliva após a aplicação de compostos profissionais de flúor, imediatamente e até uma hora após o tratamento. Este foi um estudo clínico, intervencional e randomizado. Nesta pesquisa, 40 participantes foram selecionados para receber tratamento tópico com flúor. As coletas de saliva foram realizadas no mesmo dia da aplicação dos compostos citados acima, nos seguintes horários: imediatamente antes da aplicação do produto, imediatamente após a aplicação do produto e 20, 40 e 60 minutos após o tratamento. A disponibilidade de flúor solúvel na cavidade oral após a aplicação de compostos fluoretados foi avaliada. O flúor foi quantificado em todas as amostras com o auxílio de um eletrodo de íon seletivo para fluoreto (F^-). As médias foram comparadas em todos os momentos por meio do teste ANOVA de 2 vias, seguido do teste de Tukey (α= 5%), $P>0.05$. Os resultados mostraram que nenhum composto conseguiu manter uma alta concentração salivar de flúor após 60 minutos de aplicação. Apenas o gel e a espuma elevaram os níveis de flúor imediatamente após a aplicação do produto, porém, após 20 minutos, os níveis de flúor retornaram aos valores basais. O dentifrício e o verniz não alteraram a concentração do íon F^- em nenhum momento, provavelmente devido à salivação, deglutição ou pela forma como o excesso de flúor foi removido da cavidade oral. Concluiu-se que a concentração de flúor na saliva aumentou imediatamente após a aplicação do gel e da espuma fluoretada, mas a concentração voltou aos níveis basais em 20 minutos após a aplicação dos produtos.

Palavras-chave: Cárie. Flúor. Gel. Pasta Profilática. Saliva. Verniz.

Abstract

The objective of the present research was to evaluate the fluoride concentration on saliva after the application of professional fluoride compounds, immediately and up to one hour after the treatment. This was a clinical, observational and randomized study. In this research, 40 participants were selected to receive topical fluoride treatment. Saliva collections were performed on the same day of application of the compounds mentioned above, at the following times: immediately before product application, immediately after product application, and 20, 40 and 60 minutes after treatment. The availability of soluble fluoride in the oral cavity after the application of fluoride compounds was evaluated. Fluorine was quantified in all samples using a fluoride ion-selective (F^-) electrode. Means were compared at all times using the 2 way ANOVA test, followed by the Tukey´s test (α= 5%), $P>0.05$. Results showed that no compound could maintain a high salivary fluoride concentration after 60 minutes of application. Only gel and foam raised fluoride levels immediately after product application, however, after 20 minutes, fluoride levels returned to their baseline. The dentifrice and varnish did not change the F^- ion concentration at any time, probably due to salivation, swallowing, or the way excess fluoride was removed from the oral cavity. It was concluded that fluoride concentration in saliva was increased only immediately after the application of the gel and the fluoridated foam, but the concentration returns to its baseline levels within 20 minutes after the application of the products.

Keyword: Caries. Fluorine. Gel. Prophylactic Paste. Spittle. Varnish.

Sumário

1. Introdução

A cárie dental é uma doença biofilme-sacarose dependente que afeta grande parte da população, promovendo a destruição localizada dos tecidos dentários. Sua origem é multifatorial e depende da correlação entre fatores determinantes e modificadores. Os fatores determinantes atuam diretamente no processo de desmineralização e remineralização que ocorre constantemente no esmalte dental. São considerados fatores primários o biofilme dental, a microbiota cariogênica, o hospedeiro (dieta) e o tempo, enquanto a saliva representa um fator determinante secundário, onde o pH e a presença dos íons atuam diretamente nos processos de obtenção e de perda mineral nos dentes. Já os fatores modificadores influenciam indiretamente na probabilidade de o indivíduo desenvolver lesões cariosas. Dentre esses fatores, destacam-se a renda, o conhecimento sobre higiene oral, o comportamento, a escolaridade, atitudes e classe social (TINANOFF *et al*., 2019).

A cárie dentária é a doença crônica mais comum da infância. A literatura relata que 59% dos indivíduos de 12 a 19 anos apresentam pelo menos uma lesão de cárie nos dentes permanentes (TOMAR e REEVES, 2009). A cárie representa uma "epidemia silenciosa" que afeta desproporcionalmente as populações pobres, jovens, minorias e crianças que vivem abaixo do nível de pobreza (FRENCKEN *et al*., 2020). Nos Estados Unidos, 25% das crianças de 2 a 5 anos de idade de grupos socioeconômicos baixos apresentam 80% dos dentes comprometidos pela doença. Entre crianças de 3 a 5 anos, a cárie dentária não tratada foi significativamente maior para crianças negras e hispânicas (19,3% e 19,8% respectivamente) do que para crianças brancas não hispânicas (11,3%). Essa disparidade persistiu entre crianças de 6 a 9 anos e de 13 a 15 anos de idade (DYE *et al*., 2012).

O fluoreto, forma iônica do elemento químico flúor, é o principal responsável pelo declínio da cárie dentária em países desenvolvidos e no Brasil. Além da redução da prevalência da cárie, o flúor age reduzindo a velocidade de progressão de novas lesões (CLARCK *et al*., 2020). O efeito preventivo do flúor tem sido investigado extensivamente nos últimos anos. Várias modalidades e métodos de aplicação dos compostos fluoretados foram estudados, cada um com sua própria concentração recomendada, frequência de uso e esquema de dosagem. Numerosas

revisões baseadas em evidências confirmaram que o flúor é seguro e altamente eficaz para a prevenção e controle da cárie. O uso de flúor através de dentifrícios, vernizes, géis e enxaguatórios bucais é comum em programas de prevenção de cárie (CHI *et al*., 2013; FRANK *et al*., 2019).

A aplicação profissional de flúor é realizada nos consultórios odontológicos e é frequentemente recomendada para indivíduos com alto risco/atividade de cárie (CLARK *et al*., 2020). Essa aplicação envolve uso de géis fluoretados, vernizes, espumas e pastas profiláticas que podem, em alguns casos, serem implementados em escolas ou outras instituições como parte de programas de prevenção de cárie (SOARES *et al*., 2021). Os benefícios da aplicação tópica profissional de flúor na redução dos índices de cárie dentária estão estabelecidos com base em evidência de ensaios clínicos. Esse efeito anticárie está associado à reatividade do fluoreto com o esmalte e a dentina, formando produtos tipo fluoreto de cálcio, que funcionam como reservatório de liberação lenta de F^- para a cavidade bucal. Os meios profissionais são indicados para indivíduos com alta/moderada atividade de cárie ou risco de desenvolver a doença. Dentre os meios profissionais de uso de F^-, destacam-se o flúor fosfato acidulado em gel e o verniz fluoretado, que possuem concentração de F solúvel, pH e veículo distintos (NOWAK *et al*.,2019). Apesar da *American Dental Association* sugerir que indivíduos que apresentam alto risco à cárie deveriam receber verniz fluoretado duas vezes por ano, a literatura não é conclusiva sobre esse assunto, principalmente devido ao risco da fluorose dentária, que é o resultado da ingestão crônica de flúor durante o desenvolvimento dental que se manifesta como mudanças visíveis de opacidade do esmalte devido a alterações no processo de mineralização (BERG *et al*., 2011).

Dentifrício fluoretado é considerado um dos métodos mais racionais de prevenção das lesões de cárie, pois alia a remoção do biofilme dental à exposição constante ao flúor. Eles apresentam como vantagens a facilidade de aplicação e o baixo custo. A concentração típica de um creme dental regular é de aproximadamente 1.000 a 1.500 partes por milhão (ppm) de flúor. Revisões sistemáticas têm mostrado que apenas os dentifrícios que apresentam a partir de 1.000 ppm de flúor podem prevenir as lesões cariosas de forma eficaz. Contudo, ainda não existe o consenso sobre o uso desses dentifrícios em crianças que apresentam os dentes permanentes não erupcionados (VALKENBURG *et al*., 2019; SOARES *et al*., 2021). Dos tipos de compostos fluoretados, os dois mais utilizados nos dentifrícios são o fluoreto de sódio

(NaF) ou monofluorfosfato de sódio (MFP, Na_2PO_3F). Independentemente do composto utilizado, a ação na cavidade bucal será a mesma, pois ambos liberam o íon fluoreto na cavidade bucal, o primeiro por ionização quando em contato com água e o MFP pela ação de enzimas chamadas fosfatases, que estão presentes na cavidade bucal (POLLICK, 2018). Existem no mercado dentifrícios indicados não apenas para indivíduos que desejam prevenir o aparecimento das lesões de cárie, mas também os cremes dentais recomendados para o tratamento da gengivite, para a diminuição da hipersensibilidade dentinária e para o clareamento dos dentes. Nestes casos, a concentração e as fontes de flúor incorporados às pastas podem ser diferentes, pois eles precisam ser compatíveis com os compostos utilizados para cada tratamento específico (CVIKL & LUSSI, 2021). Embora a fonte de flúor não interfira na eficácia dos compostos fluoretados de uso caseiro ou profissional, os demais componentes da formulação de cada veículo devem ser compatíveis para evitar que o fluoreto reaja com espécie catiônicas formando compostos insolúveis e perdendo sua ação.

O objetivo final de todos os meios de utilização de F, sejam direcionados a populações ou indivíduos, é a manutenção do íon flúor na cavidade bucal, para interferir no desenvolvimento da cárie dentária (YU *et al*., 2021). Dentro deste contexto, torna-se importante avaliar a concentração salivar do flúor após a aplicação de cada um destes componentes, a fim de nortear o Cirurgião-Dentista e os serviços de saúde pública a utilizarem esses compostos de modo racional e eficiente. A hipótese nula era que os níveis de flúor na saliva não se alterariam ao longo de 1 hora após sua aplicação na cavidade oral.

2. Objetivo

O objetivo da presente pesquisa foi avaliar a concentração salivar de flúor após a aplicação de compostos fluoretados de uso profissional imediatamente e até uma hora após o tratamento.

3. Artigo

Comparison of salivary fluoride levels of professional fluoride compounds

Giovanna Sousa Oliveira Chagas[1]
Carla Regina Costa[2]
Carlos Eduardo Ferreira Cruz[2]
Cidney Luiz Junior[2]
Ruchele Dias Nogueira[1]
Cesar Penazzo Lepri[1]
Marcelo Rodrigues Pinto[1]
Vinicius Rangel Geraldo-Martins[1]

1- Mestrado em Odontologia – Universidade de Uberaba – Uberaba – MG, Brazil
2- Departamento de Química – Universidade Federal do Triângulo Mineiro – Uberaba – MG, Brazil

Corresponding Author:
Prof. Dr. Vinicius Rangel Geraldo Martins
School of Dentistry – Uberaba University
Av. Nenê Sabino, 1801 Sala 2D04 Zip Code: 38.055-500 – Uberaba-MG, Brazil,
Phone/Fax +55(34)3319-8913 e-mail: vinicius.martins@uniube.br

3.1 Abstract

The objective of the present research was to evaluate the fluoride concentration on saliva after the application of professional fluoride compounds, immediately and up to one hour after the treatment. This was a clinical, observational and randomized study. In this research, 40 participants were selected to receive topical fluoride treatment. Saliva collections were performed on the same day of application of the compounds mentioned above, at the following times: immediately before product application, immediately after product application, and 20, 40 and 60 minutes after treatment. The availability of soluble fluoride in the oral cavity after the application of fluoride compounds was evaluated. All samples were analyzed for fluoride using an ion-selective electrode adapted for microanalysis. Means were compared at all times using the 2 way ANOVA test, followed by the Tukey´s test (α= 5%). P>0.05. Results showed that no compound could maintain a high salivary fluoride concentration after 60 minutes of application. Only gel and foam raised fluoride levels immediately after product application, however, after 20', fluoride levels returned to their baseline. The dentifrice and varnish did not change the F ion concentration at any time, probably due to salivation, swallowing, or the way excess fluoride was removed from the oral cavity. It was concluded that fluoride concentration in saliva was increased only immediately after the application of the gel and the fluoridated foam, but the concentration returns to its baseline levels within 20 minutes after the application of the products.

Keywords: Caries. Fluorine. Gel. Prophylactic Paste. Spittle. Varnish.

3.2 Introduction

Dental caries is one of the most prevalent chronic diseases that affect all population worldwide. Caries incidence increased dramatically in post-industrial societies with increasing affluence, and in particular with the availability of processed sugar. It is an infectious disease that leads to localized destruction of dental tissues [11]. The disease is the result of a complex interaction between acid producing tooth-adherent bacteria and fermentable carbohydrates. Its occurrence is multifactorial and depends on the correlation between determining and modifying factors. The determining factors act directly on demineralization and remineralization process that constantly occurs in dental enamel. Dental biofilm, specific microbiota, diet and time are considered primary factors, while saliva represents a secondary determining fator[3]. On the other hand, the modifying factors, such as low-income families, knowledge about oral hygiene, behavior, education and social class indirectly influence the probability of the individual to develop carious lesions[20] .

Dental caries is the most common chronic disease of childhood[11]. Literature reports that 59% of individuals aged 12 to 19 years have at least one caries lesion on permanent teeth[21]. Caries represents a "silent epidemic" that disproportionately affects poor populations, youth, minorities and children living below 100% of the poverty level[8]. In the United States, 25% of children aged 2 to 5 years in low socioeconomic groups have 80% of their teeth compromised by the disease. Among children aged 3 to 5 years, untreated tooth decay was significantly higher for black and Hispanic children (19.3% and 19.8% respectively) than for non-Hispanic white children (11.3%). This disparity persisted between 6- to 9-year-olds and 13- to 15-year-olds[6].

Fluoride is the main responsible for the decline in dental caries in both high and low-income countries. In addition to reduce the prevalence of caries, F acts by reducing the progression of new lesions[4]. The preventive effect of fluoride has been investigated extensively in recent years. Different modalities and methods of application of fluoride compounds have been studied, each with its own recommended concentration, frequency of use, and dosing schedule. Evidence-based reviews have confirmed that fluoride is safe and highly effective for caries prevention and control [7,4,3].

Professional application of fluoride is performed in dental offices and is often recommended for individuals with high caries risk/activity[4]. This application involves the use of fluoride gels, varnishes, foams and dentifrices that can, in some cases, be

implemented in schools or other institutions as part of caries prevention programs[15]. The benefits of professional topical application of fluoride in reducing dental caries rates are established based on evidences from clinical trials[17]. This anticaries effect is associated with the reactivity of fluoride with enamel and dentin, forming calcium fluoride-like products, which function as a reservoir of slow release of F into the oral cavity[3]. Professional application is indicated for individuals with high/moderate caries activity or risk of developing the disease. Among the professional means of using F, acidulated fluor phosphate in gel and fluoride varnish stand out, which have different soluble F concentrations, pH and vehicle[13]. Although the American Dental Association suggests that individuals at high risk for caries should receive fluoride varnish twice a year, the literature is inconclusive on this issue, mainly due to the risk of dental fluorosis, which is the result of chronic fluoride ingestion during tooth development that manifests as visible changes in enamel opacity due to changes in the mineralization process[23].

The goal of all means of F utilization, whether directed to populations or individuals, is to maintain the fluoride ion in the oral cavity, to interfere with the development of dental caries[24]. Within this context, it is important to evaluate the salivary fluoride concentration after the application of each of these components, to guide the Dental Surgeon and the public health services to use these compounds in a rational and efficient way. Therefore, the objective of the present research was to evaluate the salivary concentration of fluoride after the professional application of fluoride compounds methods immediately and up to one hour after use. The null hypothesis is that fluoride levels in saliva do not change over 1 hour after its application in the oral cavity.

3.3 Materials and Methods

Experimental Design

This study was approved by the Ethics Committee in Research with Human Beings of the University of Uberaba (#47724521.8.0000.5145). This was a randomized interventional clinical study. In this research, fluoride compounds for professional application (gel, varnish, prophylactic toothpaste, and fluoride foam) were used. Forty participants received professional fluoride application. The number of participants was defined after consulting the literature, evaluating previous studies that used a methodology similar that used here[19]. Saliva collections were performed immediately before the application of the product, immediately after the application of the product, and 20, 40 and 60 minutes after the treatment. The availability of soluble fluor in the oral cavity was evaluated after the application of fluoride compounds.

Selection of Participants

Participants were selected at the Getúlio Vargas Dental Clinic, University of Uberaba. This research included individuals with good general health, non-smokers, aged between 18 and 45 years old, of both genders, who have active white spots lesions on enamel smooth surfaces, requiring fluoride therapy. Individuals who have caries lesions with cavitation, restorations with glass ionomer cement, who use any medication that can alter salivary flow, smokers or who make use of fluoride supplements were excluded. The selected participants signed the Terms of Free and Informed Consent, according to resolution 466/2012 of the Brazilian National Health Council.

Assessment of salivary fluoride concentration of compounds for professional use

The treatment was carried out in the morning, and participants (n=40) were instructed not to brush their teeth after waking up, so that the last fluoride exposure occurred the night before (at least 12 hours). First, to obtain a fluoride estimate prior to professional application, saliva samples (baseline) were collected. Then, participants were randomly divided in 4 groups, according to the fluoride compound

applied (Table 1). The products were applied according to the manufacturers' recommendations. The fluoride gel (Fluor in Gel Flugel - Nova DFL, Taquara-RJ) and the fluoride foam (Fluor Care, FGM Dental Group, Joinvile-SC) were applied with trays, for 1 minute (5g). After, excess gel were removed with gauze and patients were instructed to spit out the product for one minute. The varnish (Duraphat, Colgate-Palmolive, São Bernardo do Campo-SP) application was done with a brush using 0.50 g of the product on the teeth affected with the white spot (2 tooth per patient). After relative isolation with cotton rolls, the varnish was applied with disposable brushes. After 1 minute, the isolation was removed and saliva collections were performed. For the pophylactic paste (ClinProTM 5000, 3M ESPE, Sumaré-SP) 1.0 g of the product was used. The patient's soft tissues were protected and the teeth were brushed with a Robinson brush at low rotation for 1 minute. Then, the patient rinsed your mouth with 30 mL of deionized and deionized water for 30 seconds to remove excess paste and then discard the solution in the spittoon. Saliva from all participants was collected immediately after treatment, in the same way mentioned above. New collections were carried out after 20, 40 and 60 minutes of treatments. During this period, participants must remain seated and not ingest any food or drink. All samples were stored on ice until the analysis of salivary fluoride concentration. Each product was only once used in each participant.

Table 1. Professional fluoride compounds (NaF – Sodium Fluoride)

Group (n=10)	Product	Fluoride
1	Flugel (Nova DFL, Taquara-RJ, Brazil)	Neutral Gel NaF 2% (9,050 ppm)
2	Duraphat (Colgate-Palmolive (São Bernardo do Campo-SP, Brazil)	Varnish - NaF 5% (22,600 ppm)
3	Clinpro^{tm} 5000 (3M ESPE (Sumaré-SP, Brazil)	Prophilatic Toothpaste NaF (5,000 ppm)
4	Fluor Care (FGM Dental Group, Joinvile-SC, Brazil)	Foam- NaF 2% (9,050 ppm)

Fluoride Concentration Analysis

The ionic and ionizable fluorine present in the standards and samples were determined using a fluoride ion-selective eletrode (ISE 4010-C00). Potential measures were performer versus a Ag/AgCl reference electrode with a potentiometro. Calibration curve was determined from the linear regression of the E(mV) versus log [F^-] curve, obtained by standards with fluoride concentrations ranging from 0.4; 2.0; 4.0; 10.0; 20.0; 72.0; 100.0; 152.0 and 200.0 ppm F^- in deionized water, made from standard 1000 ppm F (ORION). In total, 200 saliva samples were analyzed. The accuracy of the readings were evaluated by tests with solutions of known fluoride concentrations. The volume of 1.25 mL of saliva were pipetted to which 250 µl of TISAB III (Total Ionic Strength Adjustment Buffer) were added and readings were taken in millivolts (mV), in triplicate for each standard and samples, which was recorded. The millivoltage potentials were converted into ppm F^- using a standard curve with regression coefficient $r^2 \geq 0.99$. The averages of the readings of the standards were entered in an Excel® worksheet and then the percentage of variation between the measured and the expected amount in the known standards were calculated.

Statistical analysis

Comparisons of fluoride concentration in saliva were performed at each of the collection times and among fluoride compounds. The data were entered and processed in the SPSS v statistical package. 13.0. The means obtained were evaluated for normality using the Kolmogorov-Smirnov test in the Graphpad Instat v 3.01 program. Once confirmed the normal distribution of the data, a parametric SPSS test for statistical inferences (ANOVA) was used. The significance level was 5%.

3.4 Results

Table 2 represents the mean fluoride concentration in saliva before, immediately after application (0') and 20, 40 and 60 minutes after using each fluoride compound (Gel, dentifrice, varnish and foam). Data from pre-treatment stage indicated that there was similarity in fluoride concentration in all analyzed groups. The analysis of saliva data collected immediately after application (0') of the fluoride compound revealed that patients treated with fluoride gel (270 ± 60 ppm) or foam (230 ± 30 ppm) had higher ppm when compared to those treated with varnish (170 ± 20 ppm) or dentifrice (180 ± 20 ppm). After 20 minutes, the saliva samples of the foam-treated patients had higher concentration of fluoride than the other patients. After 40 minutes, the fluoride concentration in saliva decreased in all groups and, after 60 minutes, they returned to pre-brushing levels.

Table 2 and figure 1 show the trend in fluoride concentration over time. For gel, it was observed that there was a significant increase in ppm at 0 minute, but it was reduced at 20 minutes and 40 minutes, reaching the same ppm before the application of the compound at 60 minutes. Table 3 shows the percentage increase in ppm over time. In this table, it is possible to observe that the salivary concentration of fluoride after using the gel increased by 124% when compared to pre-treatment levels. The fluoride concentration after the application of dentifrice and fluoride varnish remained similar at all analyzed times. In both cases, salivary concentration showed higher values immediately after treatment, however, it did not show statistically significant values when compared to other periods. The fluoridated foam showed the same behavior observed for the gel, where there was a significant increase in ppm immediately after product application (36.12%), followed by a decline in the salivary fluoride concentration just after 20 minutes of treatment.

Table 2. Mean (± standard deveation) of fluoride concentration (ppm) in saliva before and after application of fluoride compounds. Lowercase letters compare rows and uppercase letters compare columns (α= 0.05).

	Pre-treatment	0’	20'	40'	60'
GEL	120 (30) Aa	270 (60) Ab	140 (20) Aa	130 (20) Aa	130 (20) Aa
Dentifrice	160 (10) Aa	180 (20) Ba	170 (10) Aa	170 (10) ABa	160 (10) Aa
Varnish	150 (20) Aa	170 (20) Ba	160 (20) Aa	160 (10) ABa	150 (20) Aa
Foam	170 (10) Aa	230 (30) Ab	190 (10) Bab	180 (10) Ba	170 (10) Aa

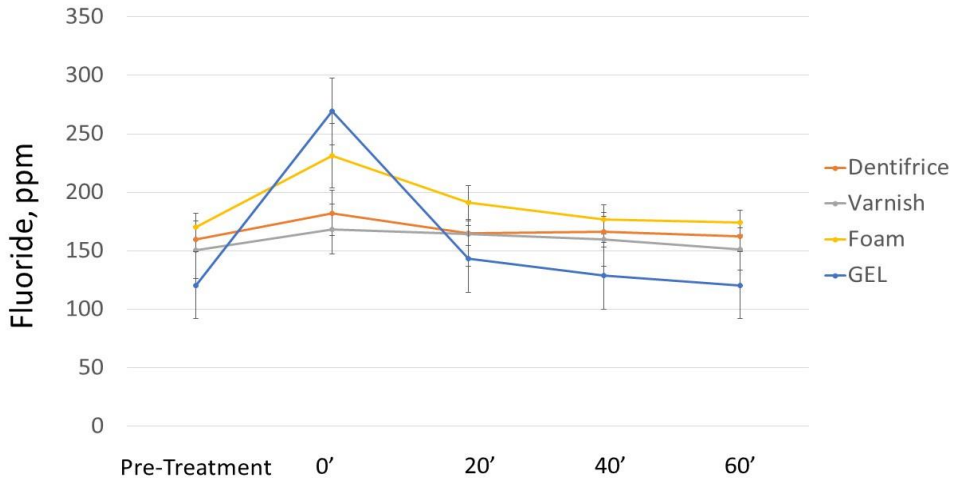

Figure 1. Trend graphs to examine ppm progress over time for all fluoride compounds. Error bars represent the 95% confidence intervals.

Table 3. Percentage (± standard error) of increase in fluoride concentration in saliva when compared to pre-treatment data.

	0'	20'	40'	60'
GEL	124,3 (20,8)	19,3 (7,2)	6,6 (3,9)	0,5 (1,1)
Dentifrice	14,7 (9.1)	3,8 (6.1)	4,6 (3.3)	2,64 (2.4)
Varnish	12,3 (4,7)	9,7 (2,3)	6,4 (3,3)	0,8 (0,9)
Foam	36,12 (7,4)	12,4 (5,6)	3,9 (1,8)	2,6 (1,4)

3.5 Discussion

The objective of this study was to evaluate the fluoride levels in saliva after professional application of fluoride compounds, immediately and up to one hour after use. The results showed that the fluoridated gel and foam increase fluoride levels immediately after the application of the compounds, but these levels regress to the baseline level after 20 minutes. So, the null hypothesis that fluoride levels in saliva do not change over 1 hour after its application in the oral cavity was rejected.

The presence of free fluoride in oral fluids is critical in caries prevention. During bacteria-produced acid challenges, fluoride exerts its caries-controlling effect on dental hard tissues, while intra-oral fluoride reservoirs serve to maintain elevated salivary fluoride levels. Salivary fluoride levels tend to rise after brushing teeth or applying fluoride compounds. Fluoride fixation also occurs in the buccal mucosa and tongue of the individual, and fluoride levels in the mucosa are higher than in saliva[19]. Other fluoride reservoirs in the oral cavity incudes calculus, dental biofilm, and caries lesions[18]. Because of this, and to standardize the study, the pre-treatment analysis was carried out 12 hours after each participant's last toothbrushing. In addition, each participant performed a 2-minute mouthwash with distilled water to renew saliva in the oral cavity, as recommended in previous studies[19,12].

Fluoride levels in saliva before brushing may vary depending on the patient's salivary flow. The pre-treatment salivary fluoride levels ranged from 120 to 170 ppm, which was considered statistically similar among participants (Table 2). This can be explained by the release of fluoride from the patients' buccal mucosa or tongue, which reached the saliva during the non-stimulated collection of each sample.

Dentists have been routinely using fluoride therapy for preventing and managing dental caries, mainly in preventive programs carried out in schools and in underprivileged communities. The common professionally applied fluoride agents are 1.23% acidulated phosphate fluoride gel, 2 - 5% sodium fluoride (NaF) varnish (22,600 ppm), and 38% silver diamine fluoride (SDF) solution (44,800 ppm)[4]. In the present study, a neutral 2% NaF (9,050 ppm) gel was used. This compound has advantages such as chemical stability, acceptable taste, low cost, simplicity in the application technique, in addition to not causing staining on teeth and restorations. In general, prefabricated trays are used to apply the gel during 1 minute, which allows better contact between gel and tooth. After application, patient should spit out the excess gel

until there are no significant amounts left in the mouth. The protocol recommends to not remove gel is with water jets or mouthwash[4]. After that, saliva collection was performed. This explains the higher amount of fluorine in saliva at 0' (Table 2). The concentration of fluorine in saliva was decreased over time, returning to pre-treatment levels after 20' (Figure 1). This is caused by an increase in the volume of saliva produced and swallowing by the patient over time. Subsequently, the fluoride clearance phase passes into a slower phase (40 to 60', which is thought to reflect a combination of continued clearance of fluoride from saliva as well as release of fluoride into saliva from various oral reservoirs Therefore, it makes sense to advise the patient to refrain from drinking or eating for at least 30 minutes[3,9].

Fluoride foams are especially indicated for children. Its composition is similar to that of the gel, but due to its lower density, a smaller amount of fluoride is used per application, making it safer in terms of toxicity. Furthermore, elderly patients often have an impaired saliva function due to aging and polypharmacy and perceive tooth brushing as physically difficult. In these cases, fluoride mouth rinses and self-applied fluoride foams could be an option that merits further clinical research[22]. The method of application is the same as described for the gel. As with the gel, the salivary fluoride concentration of patients treated with the foam increases significantly immediately after application of the product. However, the ion concentration remains higher at 20' when compared to the gel and the other compounds. Upon fluoride exposure an immediate increase occurs in the fluoride concentration in saliva, which is followed by an exponential decrease, the latter resulting from swallowing and secretion of saliva. Table 3 shows that there is an increase of more than 30% in F^- ppm concentration compared to pre-treatment, which agrees with other studies[16,2].

The fluoride content of the prophylactic toothpaste used here is 5,000 ppm. The dentifrice was applied for one minute with a Robinson brush, and the excess of dentifrice was removed with water jets and, at the end, the participant spitted out the remained dentifrice. Although table 3 shows a 14% increase in ppm at moment 0' compared to pre-treatment, saliva analyses did not show statistically significant differences when compared to pre-treatment samples at all periods evaluates here. Nevertheless, literature showed that frequent exposures to high-fluoride toothpaste result in significantly higher fluoride concentrations in saliva compared to exposures to 1,000 and 1,450 ppm fluoride toothpaste, respectively[14,19]. According to previous studies, the salivary concentration of fluoride tends to decrease over time, but fluoride

remains in higher concentrations in the patient's mucosa, which works as a fluoride reservoir throughout the day[19]. It is believed that the main factor responsible for this decline in fluoride levels would be the fact that there is a culture of removing excess of toothpaste from the oral cavity with water jets (in the professional technique) or with mouthwashes with water (home use)[10]. Perhaps if the remaining dentifrice in adult patients was eliminated without rinsing, fluoride levels in saliva would remain higher for a longer time.

Fluoride varnish is a vehicle for topical application with a high concentration of fluoride (22,600 ppm), based on a natural resin, and was developed with the main objective of increasing the preventive and therapeutic effects of topical fluoride formulations[3]. It presents characteristics such as a higher concentration of fluoride, retention on the applied dental surfaces, providing an increase in the time of exposure to fluorides and consequent increase in the formation of calcium fluoride on the dental surface[1]. In the present research, varnish was applied in a lower volume when compared to the other compounds, since the varnish is indicated for topical application only on teeth with white spot lesions. The results showed that, although the varnish increased the initial ppm of fluoride by 12% when compared to the pre-treatment level, the varnish did not show significantly higher concentrations in saliva in the long term (Figure 1). When applied to the tooth surface, fluoride varnish forms an adherent layer that can remain for approximately 24 hours. During this period, the patient is advised not to brush over the varnish. This situation promotes the formation of calcium fluoride (CaF_2) on the applied surface, releasing, slowly and in high concentrations, fluoride ions that actively participate in the process of remineralization of dental enamel [9]. However, as noted here, salivary concentration is not significantly altered when varnish is applied. Therefore, the action of this compound seems to be exclusive to the area where it was applied[5].

Under the conditions of the present study, it was observed that no compound could maintain a high salivary fluoride concentration after 60 minutes of application. Only gel and foam raised fluoride levels immediately after product application, however, after 20', fluoride levels returned to their baseline. This was most likely due to salivation, swallowing, or the way excess fluoride was removed from the oral cavity. According to the literature, the benefits of the fluoride reservoir appear to be more important than the salivary fluoride concentration. In this way, new clinical studies should be conducted to determine whether the fluoride concentration in the patient's

soft tissues remains high in the long term following the application of the compounds used in the present study. Evaluation of fluoride in soft tissues will be performed in future studies

3.6 Conclusions

Considering the results obtained here, it was concluded that:

1- The fluoridated gel and foam significantly increased salivary fluoride concentration immediately after application. However, this salivary fluoride concentration returned to pretreatment levels before 60 minutes after application.

2- The dentifrice did not significantly increase the salivary fluoride concentration probably due to the method of removing the dentifrice from the cavity.

3- The varnish also did not increase the salivary concentration of fluoride, probably indicating that its action is restricted to the site where it was applied.

3.7 References

1. Agarwal D, Purohit B, Ravi P, Priya H, Kumar V. Effectiveness of topical fluorides in prevention of radiation caries in adults: A systematic review and meta- analysis. Oral Oncol. 2022 Jun;129:105869. doi: 10.1016/j.oraloncology.2022.105869.
2. Braxton A, Garrett L, Versluis-Tantbirojn D, Versluis A. Does fluoride gel/foam application time affect enamel demineralization? J Tenn Dent Assoc. 2014 Spring-Summer;94(1):28-31
3. Chan AKY, Tamrakar M, Jiang CM, Tsang YC, Leung KCM, Chu CH. Clinical evidence for professionally applied fluoride therapy to prevent and arrest dental caries in older adults: A systematic review. J Dent. 2022 Oct;125:104273. doi: 10.1016/j.jdent.2022.104273.
4. Clark MB, Keels MA, Slayton RL; SECTION ON ORAL HEALTH. Fluoride Use in Caries Prevention in the Primary Care Setting. Pediatrics. 2020 Dec;146(6):e2020034637. doi: 10.1542/peds.2020-034637.
5. Dehailan LA, Martinez-Mier EA, Eckert GJ, Lippert F. An In Vitro Investigation of Anticaries Efficacy of Fluoride Varnishes. Oper Dent. 2019 Sep/Oct;44(5):E234-E243. doi: 10.2341/18-040-L
6. Dye BA, Li X, Thorton-Evans G. Oral health disparities as determined by selected healthy people 2020 oral health objectives for the United States, 2009-2010. NCHS Data Brief. 2012 Aug;(104):1-8.
7. Frank M, Keels MA, Quiñonez R, Roberts M, Divaris K. Dental Caries Risk Varies Among Subgroups of Children with Special Health Care Needs. Pediatr Dent. 2019 Sep 15;41(5):378-384.
8. Frencken JE, Giacaman RA, Leal SC. An assessment of three contemporary dental caries epidemiological instruments: a critical review. Br Dent J. 2020 Jan;228(1):25-31. doi: 10.1038/s41415-019-1081-1
9. Jurasic MM, Gibson G, Orner MB, Wehler CJ, Jones JA, Cabral HJ. Topical Fluoride Effectiveness in High Caries Risk Adults. J Dent Res. 2022 Jul;101(8):898-904. doi: 10.1177/00220345221081524.
10. Machiulskiene V, Richards A, Nyvad B, Baelum V. Prospective study of the effect of post-brushing rinsing behaviour on dental caries. Caries Res. 2002 Sep-Oct;36(5):301-7. doi: 10.1159/000065955.
11. Mascarenhas AK. Who Needs More than 1,000 ppm? The Epidemiology of High-Risk Populations. Caries Res. 2016;50 Suppl 1:1-8. doi: 10.1159/000442673.
12. Naumova EA, Dickten C, Jung R, Krauss F, Rübesamen H, Schmütsch K, Sandulescu T, Zimmer S, Arnold WH. Dynamics of Fluoride Bioavailability in the Biofilms of Different Oral Surfaces after Amine Fluoride and Sodium Fluoride Application. Sci Rep. 2016 Jan 5;6:18729. doi: 10.1038/srep18729.
13. Nowak B, Sicilio L, Kizior C, Tedder G, Zimmerman N, Bobo N. Advocating for Oral Health Through Fluoridation. NASN Sch Nurse. 2019 Sep;34(5):288-294. doi: 10.1177/1942602X19831655.

14. Pessan JP, Conceição JM, Grizzo LT, Székely M, Fazakas Z, Buzalaf MA. Intraoral fluoride levels after use of conventional and high-fluoride dentifrices. Clin Oral Investig. 2015 May;19(4):955-8. doi: 10.1007/s00784-015-1426-3.
15. Soares RC, da Rosa SV, Moysés ST, Rocha JS, Bettega PVC, Werneck RI, Moysés SJ. Methods for prevention of early childhood caries: Overview of systematic reviews. Int J Paediatr Dent. 2021 May;31(3):394-421. doi: 10.1111/ipd.12766.
16. Song W, Toda S, Komiyama E, Komiyama K, Arakawa Y, He D, Arakawa H. Fluoride Retention following the Professional Topical Application of 2% Neutral Sodium Fluoride Foam. Int J Dent. 2011;2011:209349. doi: 10.1155/2011/209349
17. Sousa GP, Lima CCB, Braga MM, Moura LFAD, Lima MDM, Moura MS. Early childhood caries management using fluoride varnish and neutral fluoride gel: a randomized clinical trial. Braz Oral Res. 2022 Jul 11;36:e099. doi: 10.1590/1807-3107bor-2022.vol36.0099.
18. Souza JGS, Del Bel Cury AA, Cury JA, Tenuta LMA. On the release of fluoride from biofilm reservoirs during a cariogenic challenge: an *in situ* study. Biofouling. 2020 Aug;36(7):870-876. doi: 10.1080/08927014.2020.1826455.
19. Staun Larsen L, Baelum V, Richards A, Nyvad B. Fluoride in Saliva and Oral Mucosa after Brushing with 1,450 or 5,000 ppm Fluoride Toothpaste. Caries Res. 2019;53(6):675-681. doi: 10.1159/000501264.
20. Tinanoff N, Baez RJ, Diaz Guillory C, Donly KJ, Feldens CA, McGrath C, Phantumvanit P, Pitts NB, Seow WK, Sharkov N, Songpaisan Y, Twetman S. Early childhood caries epidemiology, aetiology, risk assessment, societal burden, management, education, and policy: Global perspective. Int J Paediatr Dent. 2019 May;29(3):238-248. doi: 10.1111/ipd.12484
21. Tomar SL, Reeves AF. Changes in the oral health of US children and adolescents and dental public health infrastructure since the release of the Healthy People 2010 Objectives. Acad Pediatr. 2009 Nov-Dec;9(6):388-95. doi: 10.1016/j.acap.2009.09.018
22. Twetman S, Keller MK. Fluoride Rinses, Gels and Foams: An Update of Controlled Clinical Trials. Caries Res. 2016;50 Suppl 1:38-44. doi: 10.1159/000439180.
23. Yan IG, Zheng FM, Gao SS, Duangthip D, Lo ECM, Chu CH. A Review of the Protocol of SDF Therapy for Arresting Caries. Int Dent J. 2022 Oct;72(5):579-588. doi: 10.1016/j.identj.2022.06.006.
24. Yu L, Yu X, Li Y, Yang F, Hong J, Qin D, Song G, Hua F. The additional benefit of professional fluoride application for children as an adjunct to regular fluoride toothpaste: a systematic review and meta-analysis. Clin Oral Investig. 2021 Mar 29. doi: 10.1007/s00784-021-03909-5.

ACKNOWLEDGEMENTS

Authors are grateful to the financial support offered by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, an by the Programa de Apoio à Pesquisa da Universidade de Uberaba (PAPE-UNIUBE #2021/017).

4. Conclusão

De acordo com o trabalho apresentado e as condições do presente estudo, conclui-se que o gel e a espuma fluoretados aumentaram significativamente a concentração salivar de flúor imediatamente após a aplicação. No entanto, essa concentração salivar de flúor voltou aos níveis pré-tratamento antes de 60 minutos após a aplicação. O dentifrício não aumentou significativamente a concentração salivar de flúor, provavelmente devido ao método de remoção do dentifrício da cavidade. O verniz também não aumentou a concentração salivar de flúor, provavelmente indicando que sua ação é restrita ao local onde foi aplicado.

Referências

Berg, J. *et al*. **American Dental Association Council on Scientific Affairs Expert Panel on Fluoride Intake From Infant Formula and Fluorosis. Evidence-based clinical recommendations regarding fluoride intake from reconstituted infant formula and enamel fluorosis: a report of the American Dental Association Council on Scientific Affairs.** J Am Dent Assoc. 2011 Jan;142(1):79-87. doi: 10.14219/jada.archive.2011.0032.

Chi, D. L.; Rossitch K. C.; Beeles, E.M. **Developmental delays and dental caries in low-income preschoolers in the USA: a pilot cross-sectional study and preliminary explanatory model.** BMC Oral Health. 2013 Oct 12;13:53. doi: 10.1186/1472-6831-13-53.

Clark, M. B.; Keels, M. A.; Slayton, R. L.; SECTION ON ORAL HEALTH. **Fluoride Use in Caries Prevention in the Primary Care Setting.** Pediatrics. 2020 Dec;146(6):e2020034637. doi: 10.1542/peds.2020-034637.

Cvikl, B.; Lussi, A. **Supragingival Biofilm: Toothpaste and Toothbrushes.** Monogr Oral Sci. 2021;29:65-73. doi: 10.1159/000510201.

Dye, B. A.; Li, X.; Thorton-Evans, G. **Oral health disparities as determined by selected healthy people 2020 oral health objectives for the United States, 2009-2010.** NCHS Data Brief. 2012 Aug;(104):1-8.

Frank, M. *et al*. **Dental Caries Risk Varies Among Subgroups of Children with Special Health Care Needs.** Pediatr Dent. 2019 Sep 15;41(5):378-384.

Frencken, J. E.; Giacaman, R. A.; Leal S. C. **An assessment of three contemporary dental caries epidemiological instruments: a critical review.** Br Dent J. 2020 Jan;228(1):25-31. doi: 10.1038/s41415-019-1081-1

Nowak, B. *et al*. **Advocating for Oral Health Through Fluoridation.** NASN Sch Nurse. 2019 Sep;34(5):288-294. doi: 10.1177/1942602X19831655.

Pollick, H. **The Role of Fluoride in the Prevention of Tooth Decay.** Pediatr Clin North Am. 2018 Oct;65(5):923-940. doi: 10.1016/j.pcl.2018.05.014.

Soares, R. C. *et al*. **Methods for prevention of early childhood caries: Overview of systematic reviews.** Int J Paediatr Dent. 2021 May;31(3):394-421. doi: 10.1111/ipd.12766.

Tinanoff, N. *et al*. **Early childhood caries epidemiology, aetiology, risk assessment, societal burden, management, education, and policy: Global perspective.** Int J Paediatr Dent. 2019 May;29(3):238-248. doi: 10.1111/ipd.12484.

Tomar, S.L.; Reeves, A.F. **Changes in the oral health of US children and adolescents and dental public health infrastructure since the release of the Healthy People 2010 Objectives.** Acad Pediatr. 2009 Nov-Dec;9(6):388-95. doi: 10.1016/j.acap.2009.09.018

Valkenburg, C.; Van der Weijden, F. A.; Slot, D.E. **Plaque control and reduction of gingivitis: The evidence for dentifrices.** Periodontol 2000. 2019 Feb;79(1):221-232. doi: 10.1111/prd.12257.

Yu, L. *et al*. **The additional benefit of professional fluoride application for children as an adjunct to regular fluoride toothpaste: a systematic review and meta-analysis.** Clin Oral Investig. 2021 Mar 29. doi: 10.1007/s00784-021-03909-5.

Anexo

Scope and policy

1 SCOPE

The **Journal of Applied Oral Science** (JAOS) it is committed to publishing the scientific and technological advances achieved by the orofacial, dental and speech-language pathology and audiology communities, according to the quality indicators and peer-reviewed material, with the objective of assuring its acceptability within its knowledge fields. The primary goal of JAOS is to publish the outcomes of original research/clinical investigations in the field of Oral Sciences, with emphasis in dentistry, speech-language pathology and audiology, and related areas.

Submissions of case reports (including case series and clinical protocols) and short communication are no longer accepted by JAOS and review manuscripts (including systematic reviews) can only be submitted under the editor's invitation.

This Journal adopts Creative Commons license CC-BY:

"This license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit you for the original creation. This is the most accommodating of licenses offered. Recommended for maximum dissemination and use of licensed materials."

1.1 Publication fee

There is no fee to authors for submitting to JAOS nor Article Processing Charge (APC).

2 GENERAL GUIDELINES

2.1 Originality

The papers sent for publication **must be original** and the simultaneous submission to another journal, either natioEnal or international, is not allowed. JAOS shall retain the copyright of all papers published, including translations; future reproduction as a transcription are also allowed, provided the source is properly mentioned.

2.2 Acceptance of preprints

A *preprint* is defined as a manuscript ready for submission to a journal that is deposited on trusted *preprint* servers before or in parallel with submission to a journal. Submissions of papers previously deposited on these servers will be accepted, which will be duly evaluated for their recognition and quality by JAOS.

In case of acceptance and publication of the article in JAOS, the authors must update the registration status on the *preprint* server, informing the complete reference of the publication in the journal.

2.3 Data repository

JAOS **requires** the citation and referencing of data, program codes and any other underlying contents of the texts of the articles. Papers resulting from original research projects must be submitted with a footnote on the cover page about data availability, stating whether the dataset is publicly available or not, and how to access it.

To ensure full transparency of the data obtained, JAOS requires authors to declare that any piece of data that is not deposited in open access repositories **must** be made available to reviewers upon request.

To make these data available in accordance with SciELO's policies, authors **must** fill in the Open Science Compliance form.

To ensure the quality and recognition of the data generated, JAOS recommends and follows the FAIR principles (Findable, Accessible, Interoperable and Reusable).

JAOS recommends the following data repositories:

SciELO Data - https://data.scielo.org/
Figshare - https://figshare.com/
Havard Dataverse - https://dataverse.harvard.edu/
Data Mendley - https://data.mendeley.com/
Zenodo - https://zenodo.org/
Repositório de dados científicos da USP
- https://uspdigital.usp.br/repositorio/

2.4 JAOS will only accept submissions of manuscripts written in English. The English language should also be adopted in all

documents during the procedures involving the articles. The content of the texts, quotations and references of the papers submitted and published are of the sole responsibility of the authors, not necessarily reflecting the opinion of JAOS's Editor-in-Chief or Editorial Board.

2.5 JAOS has the right to submit all manuscripts to the Editorial Board, which is fully authorized to determine the appropriateness of their content to peer review (otherwise, manuscripts will receive an Immediate Rejection decision), and the subsequent decision regarding acceptance/rejection, or return them to the authors with suggestions and modifications to the text and/or for them to adapt it to the editorial standards of the journal. In this case, the manuscript will be reevaluated by the Editor-in-Chief and the Editorial Board.

2.6 The dates of receipt of the original article and its acceptance will appear when published in the JAOS.

2.7 JAOS is published exclusively in electronic format.

3 REFEREEING CRITERIA

3.1 Pre-evaluation: manuscripts will be appreciated by Editors-in-Chief and Associate Editors for their adequacy to the Journal's scope, priority and potential publication and citation impact, degree of novelty and methodology. Manuscripts considered inadequate at this step will be rejected and returned to authors, while the manuscripts considered adequate will follow the regular peer review process.

3.2 Technical review: manuscripts approved in the pre-evaluation stage will be then evaluated for their compliance to the publication norms and presence of mandatory documents required for submission to JAOS. Manuscripts not in accordance with instructions will be returned to authors for adjustments before being reviewed by Associate Editors and referees.

3.3 Merit and content evaluation: papers approved by Associate Editors will be evaluated in their scientific merit and methods by at least two *ad hoc* referees from different institutions of that of the authors, besides the Editor-in-Chief.

3.3.1 As part of the evaluation process, all manuscripts will be submitted to analysis by a plagiarism software.

3.3.2 The Editor-in-Chief will decide on manuscript acceptance. When revision of the original is required, the manuscript will be returned to the corresponding author for modification. A revised version with modifications will be re-submitted by the authors, and that will be re-evaluated by the Editor-in-Chief, Editorial Board and *ad hoc* referees, if necessary.

3.4 After approval of the scientific merit, manuscripts will pass through a final review performed by a professional assigned by JAOS. If manuscripts are still considered inadequate, they will be returned to authors for revision.

3.5 Authors and referees will be kept anonymous during the review process.

4 GALLEY PROOFS

4.1 Galley proofs of each article will be sent to the corresponding author by electronic mail in pdf format for final approval.

4.2 If necessary, authors will have 48 hours to make corrections and return the revised article.

4.3 The only corrections accepted will be minor spelling corrections and the verification of figures. Major corrections will cause the manuscript to undergo a new revision by referees and delay in its publication.

4.4 If the galley proof is not returned in 48 hours, the Editor-in-Chief will consider it the unchanged final version.

4.5 Inclusion of new authors is **not allowed** at this stage of the publication process.

4.6 It is the sole responsibility of the authors to verify the proper use of their citation names and affiliation in the manuscript.

Form and preparation of manuscripts

1 PRESENTATION OF THE MANUSCRIPT

1.1 Structure of the manuscript

1.1.1 Cover page must be submitted as a supplementary file and should contain only:

- Title of the manuscript in English.
- Names of the authors in direct order with their respective affiliations in English. Affiliations must be written in Portuguese for Brazilian authors, in Spanish for Spanish authors, and in English for the other nationalities.
- Full address of the corresponding author, to whom all correspondence should be addressed, including phone number as well as e-mail address.
- Information about the deposit of the manuscript on a *preprints server*, when applicable, indicating the access address and DOI number, if required (attach the Open Science Compliance form). Citation and referencing of the research data specifying the repository and the DOI number (attach the Open Science Compliance form).
- Mandatory note stating whether the manuscript is derived from any dissertations or theses and their respective access address when available.

1.1.2 Text

- The paper must be previously translated into English language or proofread by a copyediting professional or company.
  Authors with English as native language must submit as supplementary file a signed letter taking responsibility for the quality of the English language and editing of the text.
- Title of the manuscript in English.
- Abstract structured in a sole paragraph: should comprise at most 300 words, highlighting a little introduction, objective, methodology, results and conclusions.
- Keywords: words or expressions that identify the contents of the manuscript. The authors must check
  both MeSH and DeCS. Authors must use periods to separate the keywords, which must have the first letter of the first word in capital letters. Ex: Dental implants. Fixed prosthesis. Photoelasticity. Passive fit.
- Introduction: summary of the rationale and proposal of the study including only proper references. It should clearly state the hypothesis of the study.
- Methodology: the material and the methods are presented with enough detail to allow confirmation of the findings. Include the city, state and country of all manufacturers right after the first appearance of the products, reagents or equipments. Published methods should be referred to and

briefly discussed, except if modifications were made. Indicate the statistical methods employed, if applicable. Please refer to item 3 for ethical principals and registration of clinical trials.

- Results: must be presented in a logical sequence in the text, tables and illustrations. Data contained in tables and illustrations should not be repeated in the text, and only important findings should be highlighted. Please, use the fewest number tables and figures possible.
- Discussion: this section should emphasize the new and important aspects of the study, discussing them with prior investigations. Any data or information mentioned in the introduction or results should not be repeated.The authors should stress the implications of their findings, as well as their limitations.
- Conclusion(s): Provide short conclusions that can be taken from the research. Go further than reaffirming the results, establishing relevant conclusions to the objectives based on the data. In most cases, the conclusions are only true for the study population.
- Acknowledgments (when appropriate): Acknowledge those who have contributed to the work. Specify sponsors, grants, scholarships and fellowships with respective names and identification numbers.
- References (please refer to item 2.3)

2 TECHNICAL NORMALIZATION

The text body of the manuscript should be typed as follows: 1.5 spacing in 11 pt Arial font, with 3-cm margins at each side, on an A4 page, adding up, to at most, 12 pages, thus excluding figures, tables, figure legends and references.

2.1 Illustrations and Tables

2.1.1 The illustrations (photographs, graphs, drawings, charts, etc.), regarded as figures, should be limited to the least amount possible and should be uploaded in separate files, consecutively numbered with Arabic numbers according to the order they appear in the text.

2.1.2 All illustrations must be inserted in the body of the Word file but sent separately as individual files during the submission of the article. Photographs should be sent in .jpg format with at least 10 cm width and at least 300 dpi.

2.1.3 The tables should be logically arranged, consecutively numbered with Arabic numbers. The legend shall be placed above

the tables. Tables should be open in the right and left laterals, removing any internal horizontal or vertical lines, as well as any colors or shades. Tables must be sent in .xls format.

2.1.4 The corresponding legends for figures and tables should be clear, concise and typed at the end of the manuscript as a separate list preceded by the corresponding number.

2.1.5 Footnotes should be indicated by asterisks and restricted to the least amount possible.

2.2 Citation of the Authors

Citation of the authors in the text may be performed in two manners:

1) Just numeric: ...and interfere with the bacterial system and tissue system.[3,4,7-10]

References must be cited in a numeric ascending order within the paragraph.

2) or alphanumeric

- one author - Gatewood[31] (2012)
- two authors - Cotti and Mercuro[19] (2016)
- three authors - Azar, Safi, Nikaein[27] (2012)
- more than three authors - Gealh, et al.[28] (2014)

2.3 References

The references must follow the "Uniform requirements for manuscripts submitted to Biomedical Journals - Vancouver" available at: http://www.nlm.nih.gov/bsd/uniform_requirements.html.

2.3.1 All references must be cited in the text. They should be numbered consecutively in the order in which they are first mentioned in the text. Abbreviations of the titles of the journals cited should follow MEDLINE.

2.3.2 Personal communications and unpublished data with no publication date must not be included in the reference list.

2.3.3 Abstracts, monographs, dissertations and theses will not be accepted as references.

2.3.4 Avoid referencing articles published in languages other than English (if included, limit them to a maximum of 3). The English translation of the title must appear between brackets and the original title by the end of the reference.

2.3.5 The names of all authors should be cited up to 6 authors; in case there are more authors, the 6 first authors should be cited, followed by the expression ", et al.", which must be followed by period and should not be written in italics. Ex: Cintra LT, Samuel RO, Azuma MM, Ribeiro CP, Narciso LG, Lima VM, et al.

2.3.6 At most 40 references may be cited.

Examples of references:

Book

Preedy VR, organizator. Fluorine: chemistry, analysis, function and effects. London: Royal Society of Chemistry; 2015.

Book chapter

Buzalaf CP, Leite AL, Buzalaf MA. Fluoride metabolism. In: Preedy VR, organizator. Fluorine: chemistry, analysis, function and effects. London: Royal Society of Chemistry; 2015. p. 54-72.

Papers published in journals

Gorduysus M, Nagas E, Torun OY, Gorduysus O. A comparison of three rotary systems and hand instrumentation technique for the elimination of Enterococcus faecalis from the root canal. Aust Endod J. 2011;37(3):128-33.

Papers published in journals in languages other than English

Merz WA. Die Streckenmessung an gerichteten Strukturen im Mikroskop und ihre Anwendung zur Bestimmung von Oberflächen-Volumen-Relationen im Knochengewebe [Distance measurement of directed structures in the microscope and its use in the determination of surface and volume relationship in bone tissue]. Mikroskopie. 1968;22(5):132-42. German

Online-only journal article (with electronic identifier)

Rudolph H, Ostertag S, Ostertag M, Walter MH, Luthardt RG, Kuhn K. Reliability of light microscopy and a computer-assisted replica measurement technique for evaluating the fit of dental copings. J

Appl Oral Sci [Internet]. 2018[cited 2017 Dec 12];26:e20160590. Available from: http://dx.doi.org/10.1590/1678-7757-2016-0590

Journal article with DOI

Wagner F, Strasz M, Traxler H, Schicho K, Seemann R. Evaluation of an experimental oblique plate for osteosynthesis of mandibular condyle fractures. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;124(6):537-41. doi: 10.1016/j.oooo.2017.09.004

Journal article Epub ahead of print/In press/Forthcoming

Cavalla F, Letra A, Silva RM, Garlet GP. Determinants of periodontal/periapical lesion stability and progression. J Dent Res. Forthcoming 2020. doi: 10.1177/0022034520952341

Preprint

Weissheimer T, Só MV, Alcalde MP, Cortez JB, Rosa RA, Vivan RR, et al. Evaluation of mechanical properties of coronal flaring nickel-titanium instruments. Research Square rs-49258/v1 [Preprint]. 2020 [cited 2020 Sept 2]. Available from: https://doi.org/10.21203/rs.3.rs-49258/v1

Research data

Mazzi-Chaves JF, Silva-Sousa YT, Leoni GB, Silva-Sousa AC, Estrela L, Estrela C, et al. Micro-computed tomographic assessment of the variability and morphological features of root canal system and their ramifications [dataset]. 2020 Feb 11 [cited 2020 Sept 2]. In: figshare [Internet]. London: Digital Science. 416.15 KB. Available from: https://doi.org/10.6084/m9.figshare.11839527.v1

Papers with more than 6 authors

The first 6 authors are cited, followed by the expression ", et al."

Grubbs V, Plantinga LC, Crews DC, Bibbins-Domingo K, Saran R, Heung M, et al. Vulnerable populations and the association between periodontal and chronic kidney disease. Clin J Am Soc Nephrol. 2011;6:711-7

Volume with supplement and/or Special Issue

Davisdson CL. Advances in glass-ionomer cements. J Appl Oral Sci. 2006;14(sp. Issue):3-9.

The authors are fully responsible for the correctness of the references.

3 ETHICAL PRINCIPLES AND REGISTRATION OF CLINICAL TRIALS

3.1 Experimental procedures in humans and animals

JAOS reassures the principles incorporated in the Helsinky Declaration and insists that all research involving human beings, in the event of publication in this journal, be conducted in conformity with such principles and others specified in the respective ethics committees of authors' institution. In the case of experiments with animals, the same ethical principles must also be followed. When surgical procedures in animals were used, the authors should present, in the Methodology section, evidence that the dose of a proper substance was adequate to produce anesthesia during the entire surgical procedure. All experiments conducted in human or animals must accompany a description, in the Methodology section, that the study was approved by the respective Ethics Committee of authors' affiliation and provide the number of the protocol approval.

3.1.1 Papers presenting **experimentalstudies in human volunteers or in animals** must contain the Ethical Committee approval of the reports **as mandatory supplementary file**.

3.1.2 Papers describing studies in animals must be submitted with the ARRIVE Checklist as mandatory supplementary file. The ARRIVE Checklist is available at

https://mc04.manuscriptcentral.com/societyimages/jaos-scielo/ARRIVEChecklist.docx

3.1.3 Ethics Committee certificate written in different languages from English, Spanish and Portuguese must be full translated into English.

3.2 Clinical Trial Registration

JAOS supports the policies of the World Health Organization (WHO) and the International Committee of Medical Journal Editors (ICMJE) for the registration of clinical trials. The journal recognizes the importance of such initiatives for the registration and international publication of clinical studies with an open access.

Therefore, JAOS will publish only those clinical trials that have previously received an identification number validated by the criteria established by the WHO and ICMJE.

The WHO defines clinical trials as "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes. Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioral treatments, process-of-care changes, preventive care, etc".

3.2.1 Manuscripts presenting clinical trials in human volunteers must be submitted with the following mandatory supplementary files:

- CONSORT 2010 checklist;
- registration number of the research in a database that meets the requirements of the WHO and the ICMJE
- Suggestions: for Brazilian authors
- Suggestions for Brazilian and non-Brazilian authors/ (ISRCTN).

3.2.2 Survey-based studies must be submitted following the same instructions of the item above.

3.3 Systematic Reviews

Systematic Reviews **SHOULD ONLY BE SUBMITTED AT THE INVITATION OF JAOS.** And even in these cases, JAOS will only receive Systematic Reviews of any kind (Traditional, Overviews, Umbrella Reviews and Scoping Reviews) if they meet 2 **mandatory** requirements:

- The prior publication of the research protocol of the Systematic Review in a scientific journal or the proof of the prior registration number of the Systematic Review protocol in a specific online platform for this purpose. **Submissions with a registration date from after the date of submission of the article to JAOS will not be accepted.** For registering the Systematic Review, the following platforms are suggested:
- PROSPERO International Prospective Register of Systematic Reviews)
- Open Science Framework
- Joanna Briggs Institute
- Cochrane Library

- Duly filled PRISMA Checklist, including all applicable extensions for particular cases.

3.4 The Editor-in-Chief and the Editorial Board reserve the right to refuse manuscripts that show no clear evidence that the methods used were not appropriate for experiments in humans or animals.

4 ANY FURTHER QUERIES SHALL BE SOLVED BY THE Editor-in-Chief AND EDITORIAL BOARD

Sending of manuscripts

1 MANUSCRIPT SUBMISSION

1.1 Articles must be submitted through the following address https://mc04.manuscriptcentral.com/jaos-scielo

1.2 The **original file** containing the main manuscript must be submitted **without the author's**' **identification** and affiliations.

1.3 The cover page must be submitted as a supplementary file containing the names of the authors, affiliations and correspondence address, citation and reference of the data repository and preprint server used (when appropriate) and the obligatory note for when the manuscript is derived from a dissertation/thesis.

1.4 Figures must be submitted as supplementary files according to the specifications of item 2.1 regarding the form and preparation of manuscripts.

1.5 Tables must be prepared in Excel format and must be submitted as a supplementary files.

1.6 Documents for proof – Ethics Committee protocol, Clinical trial registration, CONSORT Checklist, ARRIVE Checklist and PROSPERO registration – must be submitted as mandatory files.

1.7 The copyediting certificate for English language (signed by a professional or editing company) must be submitted as mandatory supplementary file.

1.8 The submission form, signed by ALL authors, must be submitted as a mandatory file.

1.9 The Open Science Compliance Form **MUST** be submitted as a supplementary file.

1.10 JAOS requires the inclusion of the ORCID registration number of the authors for manuscript submission. All authors must associate the ORCID registration number to their profile on ScholarOne.

Copyright Notice

All content of the journal, except where identified, is licensed under a Creative Commons attribution-type CC-BY.