UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

BEATRIZ MARTINES DE SOUZA

Effect of TiF_4 varnish in the prevention and remineralization of caries lesions in permanent teeth of children living in a fluoridated region: randomized clinical trial of 18 months

BAURU
2019

BEATRIZ MARTINES DE SOUZA

Effect of TiF_4 varnish in the prevention and remineralization of caries lesions in permanent teeth of children living in a fluoridated region: randomized clinical trial of 18 months

Efeito de um verniz de TiF_4 na prevenção e remineralização de lesões cariosas na dentição permanente de crianças residentes em área fluoretada: estudo clínico randomizado de 18 meses

Thesis presented to the Bauru School of Dentistry of the University of São Paulo to obtain the degree of PhD in Science in the Applied Dental Science Program, Oral Biology, Stomatology, Radiology and Imaging concentration area.

Supervisor: Prof. Dr. Ana Carolina Magalhães

Tese apresentada à Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Doutor em Ciências no Programa de Ciências Odontológicas Aplicadas, área de concentração Biologia Oral, Estomatologia, Radiologia e Imaginologia.

Orientadora: Profa. Dra. Ana Carolina Magalhães

Versão corrigida

BAURU
2019

Souza, Beatriz Martines de
Effect of TiF_4 varnish in the prevention and remineralization of caries lesions in permanent teeth of children living in a fluoridated region: randomized clinical trial/ Beatriz Martines de Souza. – Bauru, 2019.
97p. : il. ; 31cm.

Tese (Doutorado) – Faculdade de Odontologia de Bauru. Universidade de São Paulo

Orientadora: Prof. Dr[a] Ana Carolina Magalhães

Nota: A versão original desta tese encontra-se disponível no Serviço de Biblioteca e Documentação da Faculdade de Odontologia de Bauru – FOB/USP.

Comitê de Ética da FOB-USP
Protocolo nº: 59787116.2.0000.5417
Data: 22/11/2016

(Cole a cópia de sua folha de aprovação aqui)

DEDICATÓRIA

Dedico essa tese de Doutorado aos meus pais que sempre me apoiaram em todas as decisões da minha vida, e que me ensinaram pelo exemplo, que tudo na vida se conquista com dedicação, trabalho, bondade e amor.

A minha irmã Juliana, que é minha companheira desde que meus olhos abriram pela primeira vez, e que me apoia e acalanta em minhas decisões.

A minha sobrinha Eduarda, que me ensinou, ainda que apenas com 2 anos (e 7 meses) de vida, que nenhum título ou especialidade é maior que o amor e a presença que uma criança pode gerar em nossas vidas.

Ao meu companheiro de vida, e noivo Thiago, que tão sutil e delicadamente entrou em minha vida, e a transformou em calmaria. Que trouxe a mansidão para suportar todos os percalços.

O amor sempre será o caminho.

AGRADECIMENTOS

Aos meus pais, **Milton e Ana Marisa,** que me aceitaram ter como filha nessa vida. Que me amaram desde a concepção até diante de todas as minhas falhas, tristezas e estranhezas. Vocês são a minha inspiração: de pessoas íntegras, honestas e boas; de casal, de cumplicidade e amor. Minha inspiração como pais, onde quero poder ensinar o que aprendi com vocês aos meus futuros filhos. Vocês são minha fortaleza, meu chão, o lugar para onde eu sei que posso sempre voltar. Vocês são a voz da consciência, o afago no dia difícil, o sorriso nas conquistas. Meu amor, respeito e gratidão por tudo que vocês são, e por tudo que vocês viveram e fizeram por mim. Amo vocês!

À minha querida irmã, **Juliana,** aquela que me puxa de volta para o planeta terra, com quem tenho conversas sobre a vida, sobre os medos e inseguranças. Que me acompanha desde sempre e para sempre. Não consigo imaginar minha vida sem você, e nem como poderia passar por tantas etapas na minha vida sem sua presença. Você simplesmente tem que estar! Na defesa, no casamento, na vida. Você é meu complemento, minha primeira amiga, minha sis querida. Te amo!

À minha sobrinha, **Eduarda**, que me mostrou que a vida tem muito mais cores que eu podia imaginar, que consegue me tirar um sorriso em qualquer situação. Que me fez entender que amor nasce antes mesmo de conhecer, ainda na barriga (assim como já amo bebê que a Ju gera). Que mudou o meu jeito de enxergar a vida e inclusive o jeito de atender as crianças na clínica. Amor da titia mais coruja.

Ao **Thiago Melancieri,** meu companheiro de vida, meu noivo e meu amigo. Obrigada pela paciência, por entender meus horários loucos, pelos finais de semana algumas vezes perdidos (e algumas madrugadas) nos quais tive que ficar no laboratório, e você sempre calmo e compreensivo. Obrigada por me dar o suporte e o apoio, por me ajudar seja arrumando meu computador, carregando o QLF, imprimindo desenhos, levando doce, chá ou café para mim. Saber que tinha alguém ao meu lado, torcendo e me amparando quando precisava, me deu forças para continuar. Você é minha calmaria, se tornou meu porto seguro e meu lar. Te amo!

Aos meus **familiares**, avós, tios, tias, primos e cunhado, pelo apoio e incentivo que me deram e dão até hoje. O amor e carinho de vocês é o que me motiva a ser melhor.

A minha amiga **Leticia Prestes,** pela amizade, pelo apoio e incentivo. Por me dar força nos meus momentos de pânico, por me ajudar em alguns atendimentos pelo simples fato de ser minha amiga e sem esperar nada em troca. Por ter me ajudado a superar meu pânico em voltar a fazer atendimento clínicos. Você me deu força, me amparou e me ajudou. Sou muito grata por isso. Sou muito feliz em ser sua amiga, poder participar da sua vida, por você ter me escolhido como madrinha do Lex, que é uma responsabilidade enorme, mas linda. Obrigada por tudo! Obrigada ao **Tiago Tadashi**, ao **Lex**, ao **Beto** e a **Deise**, minha família do coração. Porque família não são só aqueles com laços sanguíneos, mas também aqueles com laços afetivos. Amo vocês!

À **Thamyris Carvalho,** que com seu jeito manso foi entrando na minha vida, que me ajudou a atender as crianças que necessitavam de tratamento ao longo da pesquisa, que me ensinou a ter mais confiança nos meus procedimentos (porque como ela mesmo disse, conhecimento eu tinha). Obrigada por não me deixar surtar na clínica! Você em pouco tempo conquistou meu carinho e confiança. Que você seja luz por onde passa. Obrigada pela ajuda, pela amizade e pelo carinho.

À **Aline Dionísio** e **Isabela Sabino**, as amigas que a bioquímica me deu e que levo para vida. Obrigada pelo apoio, carinho, pelos muitos cafés ao longo desses anos. Uma relação que começou lá em 2009 com a iniciação científica, e que foi crescendo, amadurecendo e se tornando amizade. Nossas pesquisas não são nada parecidas, uma não pode de maneira direta ajudar a outra, mas mesmo assim, a ajuda sempre foi mútua seja pela conversa, pelo olhar ou pelo cuidado. Obrigada pela amizade e carinho ao longo desses anos, e tenho a certeza que é uma amizade além Bioquímica.

Ao **Victor Mosquim** e a **Mariele Vertuan**, que ao longo da minha vida de pós-graduação já foram meus alunos de iniciação científica e agora se tornaram colaboradores nas muitas pesquisas da bioquímica. Pessoas que tenho imenso carinho de poder chamar de amigos. Trabalhar com vocês é sempre muito bom e com muitas risadas que até esquecemos que ciclagem de pH e escovação é cansativo pra caramba. Obrigada por todo carinho e toda a disposição em ajudar (e por atenderam o Thiago como paciente de vocês na Dentística!).

À **Aline Braga**, **Daiana** e **Natalia Santos,** obrigada por terem participado da pesquisa, por terem ido nas escolas, ajudado com as crianças, e terem disponibilizado o tempo de vocês (conciliado suas próprias pesquisas com a minha). Um trabalho clinico não se faz sozinho, e vocês foram fundamentais. Obrigada pela convivência, pelo carinho, pelas risadas e pela amizade.

A todos os **colegas do laboratório de Bioquímica**, que para não ser injusta e não correr o risco de esquecer ninguém, prefiro não citar os nomes, pois são muitos. Cada um, seja com um bom dia, uma parada no café do laboratório, ou na cozinha da bioquímica, tem uma memória guardada comigo. Obrigada pelo convívio diário, pelas conversas, pelas risadas. Por toram o laboratório quase uma família.

À **Mayara Souza,** minha aluna de iniciação cientifica, que participou ativamente desse projeto, que ia em todas as escolas, ajudava a montar o QLF (trambolhão), que passou por todos os perrengues junto comigo (chuva, não ter nenhuma criança na escola, entre outros), que ajudava na escovação supervisionada e no manejo com as crianças. Você foi incrível, e muitas vezes fez além do que era necessário, sempre disposta, pró ativa e educada. Torço muito pelo seu sucesso. Obrigada!

À **Larissa Santi**, minha aluna de iniciação cientifica desde o começo do doutorado e ao longo de todo o período. Uma pessoa incrível, linda, educadíssima, esforçada e inteligente. Poder participar do seu crescimento e amadurecimento durante esses 3 anos foi um processo maravilhoso, te co-orientar foi leve e divertido, e sei que ganhei uma amiga no processo. Tenho certeza que seu futuro será brilhante.

À **Paula Fontana** e **Isabela Vieira**, alunas de iniciação cientifica que orientei ao longo do doutorado, obrigada pela oportunidade de convívio, de ensinamento e troca de aprendizado. Vocês foram importantes no meu processo de amadurecimento como pessoa e profissional.

Ao **Prof. Dr. Carlos Ferreira do Santos,** que me auxiliou nos momentos de dificuldades que surgiram durante o caminho. Suas palavras fortes de apoio estão gravadas em minha memória, e me deram suporte e equilíbrio para não desistir. Sua colaboração foi fundamental para a conclusão desse projeto. Sou imensamente grata.

As professoras **Prof.ª Dr.ª Marília Afonso Rabelo Buzalaf** e **Prof.ª Dr.ª Daniela Rios Honório,** por serem colaboradoras nesse projeto, por terem contribuído enriquecendo esse trabalho, pelas sugestões durante a etapa de qualificação, por estarem sempre disponíveis para esclarecem duvidas, e por cederem suas alunas para me auxiliar no desenvolvimento desse projeto.

Ao **Prof. Dr. Rodrigo Cardoso de Oliveira**, obrigada pelo convívio de trabalho no departamento de Bioquímica, por sempre ser tão atencioso e educados, e estar sempre disposto a ensinar e ajudar no que fosse possível.

Aos funcionários do departamento de Ciências Biológicas: **Thelma**, **Larissa e Dalva.** Vocês sempre foram muito prestativas, principalmente quando precisei da ajuda de vocês. Obrigada pela atenção e pelo esforço em ajudar.

À **Secretária Municipal de Educação,** na pessoa do diretor do **Departamento de Planejamento, Projetos e Pesquisas Educacionais, Prof. Me. Wagner Antônio Junior** e da senhora diretora da **Divisão de Projetos e Pesquisas Educacionais, Prof.ª Me. Kelli Cristina do Prado Corrêa,** por aprovarem o desenvolvimento dessa pesquisa nas escolas municipais de ensino fundamental.

Aos diretores, vice-diretores, coordenadoras pedagógicas, secretários, professores e funcionários das escolas **EMEF "Geraldo Arone", EMEF "Ivan Engler de Almeida", EMEF "Santa Maria", EMEF "Thereza Tarzia- Irmã Rosamaria Tarzia", EMEF "José Romão",** minha gratidão por terem concordado e permitido meu acesso à escola, por responderam meus e-mails (muitas vezes desesperados), por estarem disponíveis em ajudar (dando soluções quando as agendas não correspondiam) por abrirem muitas e muitas vezes as portas das escolas quando eu precisava repetir as análises. A ajuda e colaboração de vocês foram fundamentais.

Aos **pais e responsáveis** pelas crianças que participaram desse projeto. Por acreditarem no desenvolvimento e na credibilidade da pesquisa. Por permitirem que eu realizasse as intervenções necessárias em seus filhos. Obrigada pela confiança.

A todas **as crianças,** participantes da pesquisa, por terem aceitado participar da pesquisa, pelo carinho, sorrisos e abraços que ganhei ao longo da pesquisa, toda vez que chegava a escola. As cartinhas de carinho, aos desenhos pintados, ao "Tia, tava com saudade", tudo isso recarregava minha energia e me motivou a seguir, apesar de todas as dificuldades. Cada criança cativou um espaço em meu coração, e me deu a certeza de estar no caminho certo.

À **Faculdade de Odontologia de Bauru-USP**, na pessoa do senhor diretor **Prof. Dr. Carlos Ferreira do Santos** e da senhora presidente da **Comissão de Pós-Graduação, Profa. Dra. Izabel Regina Fischer Rubira de Bullen,** pela oportunidade em realizar pós-graduação em nível de Doutorado nesta instituição, ao qual tenho orgulho e profundo respeito, desde março de 2009, data em que iniciei meu curso de graduação em Odontologia. Muito obrigada, tenho muito orgulho de ser FOB-USP.

À **Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)**, pelo apoio financeiro concedido durante a realização deste estudo de Doutorado (Processo nº 2015/14149-1) e também ao estudo de Mestrado (Processo nº 2013/03942-7) e Iniciação Científica (Processo nº 2010/07001-4), realizados anteriormente.

Ao **CNPq,** pelo apoio financeiro com o auxílio universal para pesquisa (Processo nº 401313/2016) que foi fundamental para o desenvolvimento desse trabalho.

AGRADECIMENTOS ESPECIAIS

Agradeço em especial minha orientadora **Prof.ª Dr.ª Ana Carolina Magalhães,** no qual ao longo desses 10 anos, tenho o carinho de chamar de amiga. Não é possível escrever esse agradecimento sem relembrar de toda a trajetória, e de tudo que passamos juntas, e foram muitas coisas. Minha iniciação científica conturbada com tantos discos diamantados quebrados, que sei o desespero que dava receber minhas ligações, já que naquela época não tinha WhatsApp. E nesse período pude ver você se tornando Professora Livre-Docente, e depois sua primeira homenagem como professora homenageada da minha turma (muito merecidamente). Enfim, você me ajudou a tomar a decisão profissional, o que fazer depois de formada, e não era tão obvio assim minha escolha pela área acadêmica, porque ainda existiam muitas dúvidas, mas você me escutou, me apoiou e me aceitou no mestrado, mesmo com a minha ideia fixa de fazer especialização junto, mas você acreditou no meu potencial. E deu certo, perdi uns parafusos no caminho e ganhei alguns quilos durante esse processo de espe e mestrado. Foi com você que fiz minha primeira viagem internacional, você me treinou e me incentivou a apresentar no IADR. E foi durante o mestrado, já no fim, que pude participar de outro momento de sua vida, para lá de emocionante. Jamais vou me esquecer a primeira vez que vi a Ana Luísa, a posição no ultrassom igual a você na maca, a sua emoção. Então defesa de mestrado, Carol grávida, aquele turbilhão de coisas acontecendo, e você sendo forte, guerreira e eu ali, observando como você conseguia equilibrar tudo, e a admiração por você só aumentava. Se antes era meu exemplo como professora, ali se tornava meu exemplo de mulher batalhadora e mãe. Doutorado chegou, e junto com a entrada do doutorado veio o nascimento da AnaLu (e a primeira licença maternidade do meu doutorado). E assim tinha uma Carol orientadora, mãe, amiga, vivendo tantas coisas, equilibrando tantos papéis, e mesmo com tudo, não negligenciou nada. Como é possível? Por acaso era super-heroína? Mas só você sabe, só quem estava perto sabe, fácil não foi, mas você conseguiu. Agora minha orientadora era mãe, mas ora que enganou meu, mãe ela sempre foi, cuidava de nós alunos com carinho de mãe, já havia feito o test drive com a gente. Ana Luísa veio pra mexer com a sua vida, e mexeu comigo também. Emocionante poder participar do batizado dela, você ter me escolhido como madrinha de consagração, de poder ver ela crescendo. Opa,

mas aluna que é madrinha e amiga tem que participar ativamente, e lá fomos nós fazer um tour Atenas/Roma, como uma Ana Luísa bebê, participar de congresso, primeira apresentação oral, dar papinha, andar 8 km empurrando o carrinho, descobrir que acessibilidade na Europa (e no mundo) é péssima, e de novo aquele pensamento “Que mãe incrível!”. Doutorado seguiu, não foi um projeto fácil, mas você continuava acreditando no meu potencial, e tudo que podia acontecer, aconteceu. Pesquisa atrasada, escola que cancelava visita, criança que faltava, relatório que voltava e de repente renovação negada. Nunca é fácil, e você ali batalhando por mim, não abaixando a cabeça, não desistindo. E de repente, outro baby, e lá vamos nós para segunda licença maternidade. A vida tem que ser com emoção! Carol com duas filhas, alunos de pós, alunos de IC, família, casa...ufa, cansei. Você cansou, quantas noites mal dormidas, e mesmo assim, não deixava de responder e corrigir. Sério, um dia quero ter esse poder, e essa determinação. Você é meu exemplo: de pessoa, profissional e mãe. E em que momento dessa história todas, desses 10 anos, onde a chave virou, onde a amizade começou? Não sei se existe esse momento, mas sei que tenho uma amiga, que era minha orientadora, que será minha supervisora, e minha madrinha de casamento. Quero passar por muito mais histórias ao seu lado. Obrigada por ser assim, tão Carol!

“O que vale na vida não é o ponto de partida e sim a caminhada.

Caminhando e semeando, no fim terás o que colher. ”

Cora Coralina

ABSTRACT

Effect of TiF_4 varnish in the prevention and remineralization of caries lesions in permanent teeth of children living in a fluoridated region: randomized clinical trial of 18 months

This randomized-controlled clinical trial compared the effect of TiF_4 and NaF varnishes in the treatment of non-cavitated caries lesions and in the prevention of new lesions in permanent teeth. Sixty children (6-7 y/o) from 5 municipal schools of Bauru-SP (Brazil), according to the caries activity (at least 1 active white spot lesion), were selected and randomly divided into the following treatments (varnishes): 4% TiF_4 (2.45% F^-, FGM); 5% NaF (2.26% F^-, Duraphat®-Colgate) or placebo (without F^-, FGM). Varnishes were applied on permanent teeth, once a week (4 weeks); after the 6th and 12th month. Clinical examination (Nyvad/ICDAS) and quantitative fluorescence changes analysis (QLF) were performed after 1 month of the treatment and after 6, 12 and 18 months after the beginning of the study. Patient's satisfaction degree was reported after each varnish application by visual scale. Visual plaque index (VPI) was calculated and measured after the 3rd, 9th and 15th months. The 1st paper of this thesis was published in the *JMIR Research Protocols* showing the experimental design model. Two-way RM-ANOVA, ANOVA/Tukey and χ^2 were performed. No differences were found between treatments with respect to carious lesions regression/progression using Nyvad/ICDAS ($p>0.05$). Only TiF_4 varnish was able to reduce the mean fluorescence loss significantly after 18 months (-14.6 ± 4.0%) compared to the baseline value (-17.5 ± 3.9%, $p=0.003$). Regarding the prevention of new lesions, only TiF4 (99.2 ± 1.7%) presented a significantly higher percentage of sound surfaces at the end of the 18 months compared to placebo (94.9 ± 7.9%), while the NaF did not differ from the two groups (98.5 ± 2.1, p=0.014%). Around 97% of the participants reported being satisfied with treatments, regardless of varnish. The VPI percentage decreased significantly after 9 and 15 months compared to 3 months ($p<0.001$). TiF_4 varnish was the only treatment able to show beneficial effect under this study model. The 2nd article of this thesis showed the results and it will be sent to the *International Journal of Paediatric Dentistry.*

Key-words: Dental Caries. Topical Fluorides. Clinical Trial. Titanium.

RESUMO

Efeito de um verniz de TiF_4 na prevenção e remineralização de lesões cariosas na dentição permanente de crianças residentes em área fluoretada: estudo clínico randomizado de 18 meses

Este ensaio clínico randomizado, controlado, paralelo e duplo-cego de 18 meses comparou o efeito do verniz de TiF_4 ao do verniz de NaF no tratamento de lesões cariosas não cavitadas em esmalte e na prevenção de novas lesões em dentes permanentes. Foram selecionadas 60 crianças (6-7 anos de idade), de 5 escolas municipais de Bauru-SP, de acordo com a atividade de cárie (pelo menos 1 lesão de mancha branca ativa) e divididas aleatoriamente nos seguintes tratamentos: verniz de TiF_4 (2,45% F^-, FGM); verniz de NaF 5% (2,26% F^-, Duraphat® Colgate) e verniz placebo (sem F^-, FGM). Os vernizes foram aplicados em todos os dentes permanentes, uma vez por semana, por 4 semanas consecutivas e após 6 e 12 meses do estudo. O exame clínico (Índice de Nyvad/ICDAS) e a análise das mudanças quantitativas de fluorescência (QLF) foram realizados após 1 mês do tratamento e após 6, 12 e 18 meses do início do estudo. O grau de satisfação do paciente foi relatado após cada aplicação de verniz por escala visual. O índice placa visível (IPV) foi mensurado após 3, 9 e 15 meses. O 1º artigo desta tese foi publicado na *JMIR Research Protocols* e abordou o delineamento experimental do trabalho. ANOVA a 2 critérios com medidas repetidas, ANOVA seguida pelo teste de Tukey e teste Q^2 foram realizados. Não foram encontradas diferenças significativas em relação à regressão/progressão das lesões cariosas pelo Índice de Nyvad ($p>0,05$). Apenas o TiF_4 foi capaz de reduzir significativamente a perda média de fluorescência após 18 meses (-14,6 ± 4,0%) em comparação aos valores iniciais (-17,5 ± 3,9%, $p=0,003$). Em relação à prevenção de novas lesões, somente o TiF_4 (99,2 ± 1,7%) apresentou uma porcentagem significativamente maior de superfícies hígidas ao final dos 18 meses comparado ao placebo (94,9 ± 7,9%), enquanto o NaF não diferiu dos dois grupos (98,5 ± 2,1%, $p=0,014$). Uma média de 97% dos participantes relataram estar satisfeitos com os tratamentos, independentemente do grupo. A % de IPV reduziu significativamente após 9 e 15 meses em comparação aos 3 meses do estudo para todos os grupos ($p<0,001$). O verniz de TiF_4 foi o único tratamento capaz de mostrar efeito benéfico neste modelo de estudo. O 2º artigo

desta tese aborda os resultados obtidos e será enviado à revista *International Journal of Paediatric Dentistry*.

Palavras-chave: Cárie dentária. Fluoretos Tópicos. Ensaio Clínico. Titânio.

SUMÁRIO

1 INTRODUCTION

1 INTRODUCTION

Fluoride varnishes are a feasible approach for preventing and treating carious lesions at the individual level and in public health programs, due to its good cost-benefit compared to restorations of cavitated lesions, which in turn have a significant negative impact in quality of life (MARINHO et al., 2013; GAO et al., 2016).

Due to the polarization of dental caries (DOUGLAS et al., 2016) and inequality in health services access, treatment is available to restrict part of the population only (DOUGLAS et al, 2016; TELLEZ, WOLFF, 2016). This fact requires the attention of authorities and appropriate public health interventions reducing inequities (ARDENGHI et al., 2013; TELLEZ, WOLFF, 2016). Based on this new panorama of the disease, researchers have sought to improve the effect of conventional fluorides or alternatively to test nonconventional fluorides (eg, fluorides [F] containing polyvalent metals, such as stannous fluoride [SnF_2] and titanium tetrafluoride [TiF_4]) (WIEGAND; MAGALHÃES; ATTIN, 2010; COMAR et al., 2017) to achieve better results with population at high-risk for caries.

The protective effect of titanium tetrafluoride on dental caries has been intensively investigated under *in vitro* and *in situ* models (MAGALHÃES et al., 2008; COMAR et al., 2012; COMAR et al., 2017; SOUZA et al., 2018a; ALEXANDRIA et al., 2019; DOS SANTOS et al., 2019). Comar et al. (2017) recently demonstrated a better effect of TiF_4 varnish in remineralizing initial enamel caries lesions *in situ*, regardless of the caries activity (ranging from low to high), compared to NaF varnish, which was only able to remineralize enamel lesions under low cariogenic challenges.

The mechanism of action of NaF varnish is based on the calcium fluoride (CaF_2) deposition on the dental surface, which acts as a fluoride reservoir and a mechanical barrier (GAO et al., 2016; COMAR et al., 2018). Titanium tetrafluoride, on the other hand, has an additional effect due to titanium that reacts with apatite forming compounds such as hydrated titanium phosphate and titanium dioxide, responsible for the high acid-resistant layer formed on teeth (COMAR et al., 2018). Furthermore, TiF_4 varnish increases the deposition of CaF_2 on the enamel, due to its low pH, compared to NaF varnish (COMAR et al., 2018). Despite its low pH, TiF_4

varnish has similar levels of toxicity on murine fibroblast lineage (NIH/3T3) and gingival fibroblasts compared to NaF varnish (SALOMÃO et al., 2017; ARANDA SALOMÃO et al., 2019).

The mechanical barrier created by fluorides protects enamel against demineralization induced by bacteria acids. Furthermore, the fluoride reservoir can speed remineralization, inducing the growth of fluor-hydroxyapatite like-crystals. In case of TiF_4, the incorporation of titanium into the surface layer of demineralized enamel may improve its mechanical resistance (MAGALHÃES et al., 2016; COMAR et al., 2018). Due to this latter property, TiF_4 varnish can be considered a good option for the treatment of non-cavitated enamel caries lesions, avoiding cavitation and, consequently, the need of invasive approaches (SKÖLD et al., 2008).

There are few clinical studies testing the anti-cariogenic or remineralizing effect of TiF_4 (REED, BIBBY, 1976; POMARICO; VILARDI; MAIA, 2012) but no one them evaluated TiF_4 as varnish. All of them have shown benefit of TiF_4 solution application in comparison to other conventional fluorides on the prevention and treatment of enamel caries lesions (REED, BIBBY, 1976; POMARICO; VILARDI; MAIA, 2012). On the other hand, previous work of our group showed better effect of TiF_4 varnish compared to TiF_4 solution on enamel caries lesion *in situ* (COMAR et al. 2012), justifying the idea and the novelty of the thesis.

Therefore, the aim of this randomized, controlled, longitudinal and double-blind clinical trial was to compare the effect of 4% TiF_4 varnish with a commercial 5% NaF varnish (gold standard) on the treatment and prevention of non-cavitated enamel caries lesions in the permanent dentition of children 6 to 7 years old, residing in an optimally fluoridated area. The tested null hypothesis was there is no significant difference between the fluoride varnishes in the prevention (a) and/or regression/progression (b) of non-cavitated enamel caries lesions in permanent teeth. For the analysis of each effect we applied: a) ICDAS b) Nyvad index and quantitative light-induced fluorescence.

2 ARTICLES

2 ARTICLES

2.1 Article 1

Article formatted according to JMIR Research Protocols Guidelines.

Published on 26.01.2018 (Souza BM, Santos DMS, Braga AS, Santos NMD, Rios D, Buzalaf MAR, Magalhães AC. Effect of a Titanium Tetrafluoride Varnish in the Prevention and Treatment of Carious Lesions in the Permanent Teeth of Children Living in a Fluoridated Region: Protocol for a Randomized Controlled Trial. JMIR Res Protoc 2018;7(1):e26)

Effect of a TiF_4 varnish in the prevention and treatment of caries lesions in permanent teeth of children living in a fluoridated region: protocol of randomized controlled clinical trial

Beatriz Martines de Souza[1], DDS, MSc; Daiana Moreli Soares dos Santos[1], BSc; Aline Silva Braga[1] BSc, MSc; Natália Mello dos Santos[2], DDS, MSc; Daniela Rios[2], DDS, MSc, PhD; Marília Afonso Rabelo Buzalaf[1], DDS, MSc, PhD; Ana Carolina Magalhães[1*], DDS, MSc, PhD.

[1]Department of Biological Sciences. Bauru School of Dentistry, University of São Paulo. Bauru-SP, Brazil

[2] Department of Pediatric Dentistry, Orthodontics and Public Health. Bauru School of Dentistry, University of São Paulo, Bauru, Brazil.

The authors declare no conflict of interest.

Corresponding author:
Ana Carolina Magalhães
Department of Biological Sciences
Bauru School of Dentistry - University of São Paulo
Al. Octávio Pinheiro Brisolla, 9-75. Bauru-SP
Zip code: 17012-901 (Brazil)
Phone/Fax. + 55 14 32358497
E-mail: acm@usp.br

Abstract

Background: Titanium tetrafluoride (TiF_4) has became of great interest again due to new formulations that have shown to be more effective against tooth demineralization than NaF formulations *in vitro* and *in situ*.

Objective: To evaluate the effect of 4% TiF_4 varnish compared to a commercial 5% NaF varnish on the prevention of caries lesions and the treatment of non-cavitated enamel caries lesions in permanent teeth of children living in fluoridated area.

Methods: This randomized, controlled, parallel and single blind clinical trial involves 63 children, 6-7 years old, living in Bauru-SP/Brazil. Children were selected according to their caries activity (presence of at least 1 tooth with score Nyvad 1) and randomly divided into the following treatments: 4% TiF_4 varnish (2.45 % F^-, pH 1, FGM); 5% NaF varnish (2.26% F, pH 5, Duraphat®, Colgate) and control (placebo varnish, pH 5, FGM). The varnishes were applied on all permanent teeth, once a week for 4 weeks and they will be reapplied only once after 6 and 12 months of the beginning of the study. Two calibrated examiners carry out the clinical examination (ICDAS and Nyvad indexes, kappa> 0.8) at baseline (before the first application), after the 1st, 6th, 12th and 18th month. Furthermore, quantitative fluorescence changes are measured using QLF. The degree of patient satisfaction with the treatment is also computed. The data will be submitted to statistical analysis ($p<0.05$).

Results: This ongoing study is funded by the Brazilian funding agencies (FAPESP-2015/14149-1 and CNPq- 401313/2016-6). We expect to confirm the efficacy of TiF_4 compared to NaF varnish on the prevention and treatment of caries lesions. The subjects are under 1 month evaluation and the dropout was about 8.3%. No differences between the treatments were detected at the 1st month.

Conclusions: If our hypothesis is confirmed, TiF_4 varnish can be marketed and applied at individual level and in community programs to control dental caries.

Key-words: Clinical Trial; Dental Caries; Sodium Fluoride; Titanium; Topical Fluorides.

Introduction

Fluoride varnishes are a good approach for prevention and treatment of caries lesions at individual level and in public health programs, due to its good cost-benefit compared to restorations of initial caries lesions that eventually progress to cavitation and have a significant negative impact in quality of life [1,2].

Considering the polarization of caries disease [3], the treatment needs have been concentrated on a small part of the population, characterizing significant inequities in health [3,4]. This fact requires the attention of authorities and appropriate interventions in public health [4,5]. Based on this new panorama of the disease, researchers have sought to improve the effect of conventional fluorides or alternatively to test non-conventional fluorides (fluorides containing polyvalent metals, such as stannous fluoride -SnF_2 and titanium tetrafluoride - TiF_4) on the prevention and treatment of caries lesions [6,7].

Several *in vitro* and *in situ* studies have shown that an experimental 4% TiF_4 is more effective than NaF on the reduction of demineralization and improvement of remineralization [7-9]. The mechanism of action of TiF_4 is based on the effect of titanium ions that react with dental apatite, forming a "glaze"-like layer acid resistant, rich in hydrated titanium phosphate and titanium dioxide [10]. Furthermore, TiF_4 varnish induces a higher deposition of CaF_2 than NaF varnish on sound and demineralized enamel surface [10].

Recent *in situ* study demonstrated that 4% TiF_4 varnish was the only treatment able to improve enamel remineralization regardless of the cariogenic activity, while NaF varnish failed in preventing further demineralization under high cariogenic activity [7]. This result supports the hypothesis of the present study that TiF_4 varnish could be more effective than NaF varnish in preventing and treating caries lesions in permanent teeth of children living in fluoridated area.

Objective

The aim of this clinical protocol is to evaluate the effect of 4% TiF_4 varnish compared to a commercial 5% NaF varnish on the prevention of caries lesions and the treatment of non-cavitated enamel carious lesions in permanent teeth of children living in fluoridated area.

Methods

Ethical Aspects

The protocol of this study was submitted and approved by the local Ethics Committee (Number: 59787116.2.0000.5417, Ethics Committee of the Bauru School of Dentistry, University of São Paulo, Brazil) and by the registration of clinical research in the database - The Brazilian Clinical Trials Registry – ReBEC (Number: RBR-5VWJ4Y). The research protocol was also approved by the Municipal Secretariat for Education of Bauru (São Paulo, Brazil) and by 5 municipal schools (Table 1) enrolled in the study. Thereafter, the responsible for 6-7 years old children received and signed an informed consent prior to their involvement in the research. The children also received a consent form, with age-appropriate language, explaining how the research would be conducted. The children were free to agree or disagree with their participation in the research. Only after all approvals the research started.

Study Design

This is a randomized, controlled, parallel, single blind, and three-arms (4% TiF_4 varnish, 5% NaF varnish and placebo varnish) clinical trial of 18 months. It involves 63 children (37 males and 26 females) between 6-7 years old, coming from public schools of Bauru city (Brazil), an area optimally fluoridated. A number of 20 subjects per group was previously calculated considering α error of 5%, β error of 20%, a dropout of 30% and a caries incidence after a period of 2 years of 15% for fluoride group and 42% for control group [11].

Children were selected according to their caries activity (at least 1 active white spot lesion present on the smooth surface of the permanent dentition, score 1 by Nyvad index[12]) and randomly allocated in one of the 3 treatments options ensuring stratified block randomization into each school: 4% TiF_4 varnish (2.45% F, pH 1, FGM); 5% NaF varnish (2.26% F, pH 5, Duraphat®, Colgate) and control (placebo varnish, pH 5, FGM).

The treatment was conducted as further described. The teeth were submitted to clinical examination (ICDAS and Nyvad indexes) and quantitative fluorescence changes analysis by QLF device. The analyses were conducted after the 1^{st} month and they will be carried out at 6^{th}, 12^{th} and 18^{th} month. Figure 1 summarizes the study protocol.

Baseline analysis

Two trained examiners (inter- and intra-examiner agreement, kappa> 0.8), not involved in the treatment application, are responsible for examining the children (NMS and BMS). The selection of the children was based on the analysis of smooth surfaces using Nyvad index [12]. Only children 6-7 years old, presenting at least 1 smooth surface with active caries lesions and the signed consents, were selected. The exclusion criteria were children: under orthodontic treatment; who participated in another clinical study 3 months prior the present study; who underwent professional fluoride application 6 months prior the present study; under treatment with antibiotics or some other type of medicine (patients with chronic diseases); or with periodontal disease.

The distinction between active and inactive caries lesions were done through visual and tactile inspection. The active white spot lesions were defined as rough and opaque white surface [13]. All white spot lesions were further analyzed using QLF [14]. Furthermore, all permanent teeth surfaces were analyzed using ICDAS (International Caries Detection and Assessment System) index [15] and dmft was applied for the primary teeth (this data will be included in the regression analysis to check the influence of other variable on the results).

Treatment

All children were instructed regarding cariogenic diet and oral hygiene and were submitted to supervised toothbrushing. The researchers provided new toothbrushes (Colgate® Classic, Colgate-Palmolive, Brazil), dental floss (Colgate®, Colgate-Palmolive, Brazil) and fluoride toothpastes (Colgate®, 1450 ppm F as MFP, Colgate-Palmolive, Brazil). The oral hygiene kit will be replaced each 3 months of the research.

The varnishes were applied on all permanent teeth once a week for the first 4 consecutive weeks [16] and they will be reapplied once at 6^{th} and 12^{th} month of the research [17] by ASB. The application was done using a microbrush, under natural light, following the clinical steps: 1. Supervised toothbrushing by DMSS; 2. Relative isolation of teeth area with cotton rolls; 2. Drying of the teeth surfaces using sterile gauze; 3. Varnish application according to the manufacture's instruction; 4. Waiting for 5 minutes for solvent evaporation; and 5. Removal of the cotton rolls. The treatments were done during afternoon. The children were instructed to not ingest liquid for 30 min, to have soft meals and to perform oral hygiene only 4 h after the application.

After each application, a visual scale (Figure 2) (Wong-Baker Pain Scale - WBPS) was applied to assess the degree of patient satisfaction with the treatment. The scale is known to be one of the most effective tools for self-rated child pain [18]. The mean result of the four varnish applications is described in the Results section.

Clinical examination

Clinical examinations were performed after dental hygiene, based on the clinical criteria of caries activity diagnosis from Nyvad et. al. [19], which were re-coded [12] (Table 2). Only the smooth surfaces of all permanent teeth were considered and the clinical examination was carried out under illuminated environment, using clinical probe, clinical mirror and sterile gauzes. In addition, the International Caries Detection and Assessment System (ICDAS) index was applied on all surfaces at baseline and it will be re-applied at 18th month. The ICDAS is an international system of caries detection and evaluation that classifies the stages of the caries process [15] (Table 3).

The progression of white spot lesion will be considered when the initial lesion becomes a cavity (untreated cavity or restored tooth) or when a healthy surface is transformed into an active lesion (cavitated or not). The regression will be considered when initial active white spot lesion is transformed into inactive carious lesion or healthy surface. The data (df= baseline Nyvad's score - 1 month Nyvad's score) was submitted to Kruskal-Wallis test.

Quantitative light-induced fluorescence (QLF)

QLF is applied to measure the changes in the enamel fluorescence of white spot lesions and to quantify the lesions reversal or progression. The equipment has an xenon arc lamp as light source and an optical filter system, producing blue light with a maximum wavelength of 370 nm, led by a guide filled with liquid (Inspektor Research Systems BV, Amsterdam, The Netherlands). The fluorescence emitted by the tooth is collected with a CCD-video microcamera (Panasonic WV-KS 152, Matsushita Electric Industrial Co, Ltd, Osaka, Japan) equipped with high pass yellow filter (γ >520 nm) to exclude any excitation or ambient light that may reach the detector and with a special dental mirror to reflect the image of the lesion connected to the camera [14].

After drying the tooth surface (5 s), images of clinically detected white spot lesions are obtained by QLF, under a completely dark environment. A computer program (Software Inspektor QLF 2.00f; Inspektor Research System BV, Amsterdam, The Netherlands) is applied to display, store, browse and analyze the images. The QLF parameters are: 1 -The area of the lesion (WS, mm^2) that is the sum of all points within the lesion with fluorescence loss > 5%, 2- The mean fluorescence loss (ΔF, %, detection threshold of 5%), and 3 – The integrated fluorescence loss multiplied by the lesion area (ΔQ, mm^2%) [20].

The QLF analysis was performed at baseline and after 1 month. The differences between 1 month and baseline values were calculated as following: ΔWS area = WS area baseline – WS area after 1 month (the same for ΔΔF and ΔΔQ). The data were submitted to Kruskal-Wallis test. This analysis will be repeated after 6, 12 and 18 months of the treatment by BMS.

Statistical Analysis

The data will be submitted to statistical analysis using the Graph Pad Instat and Prism version 5.0 software for Windows (GraphPad Software - San Diego, CA, USA). Firstly, the data will be checked in respect to the normality and homogeneity. Parametric or similar non-parametric test will be applied to compare the treatments in respect to: 1. Prevention of new caries lesions (ICDAS and Nyvad indexes); 2. Regression or progression of previous active white spot lesions using the Nyvad index; 3. Regression or progression (gain or loss of fluorescence, respectively) of previous active white spot lesions using QLF (0, 1, 6, 12 and 18 months).

Results

This protocol refers to an ongoing clinical study funded by São Paulo Research Foundation (FAPESP- 2015/14149-1) and the National Council for Scientific and Technological Development (CNPq- 401313/2016-6).

Figure 1 shows the number of children by school enrolled in the research until the moment. All enrolled children (n=63) were submitted to 4 weeks of treatment and almost all of them (n=58) were analyzed after 1 month. The dropout was of 5 children (8.3%) at 1st month of analysis. No significant differences in caries prevention, regression or progression was found among the treatments at the 1st

month (Tables 4 and 5). The degree of patient satisfaction with the treatment after the varnish applications is displayed in Table 6.

We expect to confirm the efficacy of TiF_4 compared to NaF varnish on the prevention and treatment of caries lesions at the end of the present study (18 months) as we have previously found under *in vitro* and *in situ* protocols.

Discussion

Previous systematic reviews have shown no significant differences between the anti-caries performances of fluoride (mainly NaF) included in different products such as gel, varnish and toothpaste [1,2]. However, varnish has some advantages over the other products, since it adheres to the tooth surface allowing a long time of contact between fluoride and tooth. Besides, it presents low systemic toxicity and is well tolerated and accepted by the patient especially children [1,2,21].

Therefore, the inclusion of TiF_4 into a varnish allows longer time of contact with enamel, improving the reaction of titanium with the tooth apatite and allowing the formation of a "glaze"-like layer on the tooth surface rich in titanium dioxide, hydrated titanium phosphate and calcium fluoride [6,10]. Due to its low pH, TiF_4 varnish is able to enhance the enamel fluoride uptake compared to NaF varnish [6]. The varnish may also reduce the contact of TiF_4 with soft tissues compared to a rinse solution, reducing the possibility of cytotoxicity, due to its low pH [22]. A recent study from our group has shown that TiF_4 varnish presents similar toxicity on NIH3T3 cells compared to NaF varnish [22]. To check any possible side effect of TiF_4 varnish, the degree of patient satisfaction was evaluated by using a simple, but effective tool for self-rated child pain [18].

Previous studies have shown that the application of fluoride varnish once a week for 4 consecutive weeks (4 applications in a one-month interval) has been effective in accelerating the remineralization of white spot lesions [16]. On the other hand, bi-annual applications are effective for the prevention of new caries lesions [17,21]. The focus of the research is the prevention and the treatment of caries lesions on smooth surface, where fluoride varnish is predominantly indicated [1]. For occlusal surfaces, other treatments are often indicated, such as fissure sealants, despite recent systematic review has shown good results with the use of NaF varnish on occlusal surfaces either [23].

The most common method for caries detection is the visual-tactile by using ICDAS, Nyvad and DMFT indexes; however, other non-invasive techniques for detection of early caries have been developed such as QLF and DIAGNOdent, which are especially applied for research proposal [24]. The traditional DMFT index is based on the detection of caries lesions at the cavitated level only, but it fails in detecting caries lesions in very early stage [25]. On the other hand, ICDAS is an accurate and reproducible method for detecting early lesions on enamel and also for detecting changes over time [24]. Braga et al [26] compared two methods of visual inspection (Nyvad and ICDAS), and both presented good reproducibility and validity to detect and estimate the depth of the caries lesions, justifying their inclusion in the present study.

The QLF is a sensible quantitative clinical method with good repeatability and reproducibility, requiring a smaller number of participants (that may decrease the impact of dropout for longitudinal studies) compared to the visual analysis [14]. The QLF is able to quantify small mineral changes that might not be detectable in the visual inspection. However, the method fails in detecting the portion of the subsurface lesion that gains or losses minerals [14, 20]. Therefore, we combined the visual inspection with a complementary method (QLF) to better detect and quantify very early caries lesions [24].

Despite some tendency for better effect of TiF_4 varnish was seen at the 1st month of this study ($\Delta\Delta Q$ and $\Delta\Delta F$ values), the differences did not reach significance, since only slight lesion changes were detected at this stage. If TiF_4 varnish presents a better performance in caries control compared to NaF at the end of the present study, it shall be marketed and alternatively applied at individual level and in community programs to control dental caries in children in the future.

Acknowledgments

This study is funded by the São Paulo Research Foundation (FAPESP-2015/14149-1) and the National Council for Scientific and Technological Development (CNPq-401313/2016-6).

References

1. Gao SS, Zhang S, Mei ML, Lo EC, Chu CH. Caries remineralisation and arresting effect in children by professionally applied fluoride treatment - a systematic review. BMC Oral Health 2016;16:12. PMID: 26831727
2. Marinho VC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2013;(7):CD002279. PMID: 23846772
3. Douglas GV, Ramsdale MP, Vinall-Collier K, Csikar JI. Using High Fluoride Concentration Products in Public Policy: A Rapid Review of Current Guidelines for High Fluoride Concentration Products. Caries Res 2016;50 Suppl 1:50-60. PMID: 27100106
4. Tellez M, Wolff MS. The Public Health Reach of High Fluoride Vehicles: Examples of Innovative Approaches. Caries Res 2016;50 Suppl 1:61-67. PMID: 27099929
5. Ardenghi TM, Piovesan C, Antunes JL. [Inequalities in untreated dental caries prevalence in preschool children in Brazil]. Rev Saude Publica 2013;47(Suppl 3):129-137. PMID: 24626589
6. Wiegand A, Magalhães AC, Attin T. Is titanium tetrafluoride (TiF_4) effective to prevent carious and erosive lesions? A review of the literature. Oral Health Prev Dent 2010;8(2):159-164. PMID: 20589250
7. Comar LP, Souza BM, Martins J, Santos MG, Buzalaf MAR, Magalhães AC. Response of carious enamel to TiF_4 varnish treatment under diverse cariogenic activities in situ. J Dent 2017;63:81-84. PMID: 28579385
8. Magalhães AC, Comar LP, Rios D, Delbem AC, Buzalaf MA. Effect of a 4% titanium tetrafluoride (TiF4) varnish on demineralisation and remineralisation of bovine enamel in vitro. J Dent 2008;36(2):158-162. PMID: 18187248
9. Comar LP, Wiegand A, Moron BM, Rios D, Buzalaf MAR, Buchalla W, Magalhães AC. Effect of TiF_4 and NaF varnish and solution on enamel demineralisation *in situ*. Eur J Oral Sci 2012;120(4):342-348. PMID: 22813225
10. Comar LP, Souza BM, Al-Ahj LP, Martins J, Grizzo LT, Piasentim I, Rios D, Buzalaf MAR, Magalhães AC. Mechanism of action of TiF_4 on dental enamel surface: SEM/EDX, KOH-soluble F and X-ray diffraction analysis. Caries Res 2017;51(6):554-567. PMID: 29020673

11. Weintraub JA, Ramos-Gomez F, Jue B, Shain S, Hoover CI, Featherstone JD, Gansky SA. Fluoride varnish efficacy in preventing early childhood caries. J Dent Res 2006;85(2):172-176. PMID: 16434737
12. Nyvad B, Machiulskiene V, Baelum V. Construct and predictive validity of clinical caries diagnostic criteria assessing lesion activity. J Dent Res 2003;82(2):117-122. PMID: 12562884
13. Zero DT. Dental caries process. Dent Clin North Am 1999;43(4):635-664. PMID: 8615944
14. Tranaeus S, Al-Khateeb S, Björkman S, Twetman S, Angmar-Månsson B. Application of quantitative light-induced fluorescence to monitor incipient lesions in caries-active children. A comparative study of remineralisation by fluoride varnish and professional cleaning. Eur J Oral Sci 2001;109(2):71-75. PMID: 11347658
15. Shivakumar K, Prasad S, Chandu G. International Caries Detection and Assessment System: A new paradigm in detection of dental caries. J Conserv Dent 2009;12(1):10-16. PMID: 20379434
16. Almeida MQ, Costa OX, Ferreira JM, Menezes VA, Leal RB, Sampaio FC. Therapeutic potential of Brazilian fluoride varnishes: an in vivo study. Braz Dent J 2011;22(3):193-197. PMID: 21915515
17. Hedman E, Gabre P, Birkhed D. Dental hygienists working in schools – a two-year oral health intervention programme in swedish secondary schools. Oral Health Prev Dent 2015;13(2):177-188. PMID: 24914429
18. El Batawi HY. Effect of intraoperative analgesia on children's pain perception during recovery after painful dental procedures performed under general anaesthesia. Eur Arch Paediatr Dent 2015;16(1):35-41. PMID: 25260983
19. Nyvad B, Machiulskiene V, Baelum V. Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions. Caries Res 1999;33:252-260. PMID: 10343087
20. Sitthisettapong T, Doi T, Nishida Y, Kambara M, Phantumvanit P. Effect of CPP-ACP Paste on Enamel Carious Lesion of Primary Upper Anterior Teeth Assessed by Quantitative Light-Induced Fluorescence: A One-Year Clinical Trial. Caries Res 2015;49(4):434-441. PMID: 26228178

21. Divaris K, Preisser JS, Slade GD. Surface-specific efficacy of fluoride varnish in caries prevention in the primary dentition: results of a community randomized clinical trial. Caries Res 2013;47(1):78-87. PMID: 23207237
22. Salomão PMA, Oliveira FA, Rodrigues PD, Al-Ahj LP, Gasque KCDS, Jeggle P, Buzalaf MAR, Oliveira RC, Edwardson JM, Magalhães AC. The cytotoxic effect of TiF_4 and NaF on fibroblasts is influenced by the experimental model, fluoride concentration and exposure time. PLoS One 2017;12(6):e0179471. PMID: 28614381
23. Ahovuo-Saloranta A, Forss H, Hiiri A, Nordblad A, Mäkelä M. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in the permanent teeth of children and adolescents. Cochrane Database Syst Rev 2016;(1):CD003067. PMID: 26780162
24. Gomez J. Detection and diagnosis of the early caries lesion. BMC Oral Health. 2015;15(Suppl 1):S3. PMID: 26392124
25. Melgar RA, Pereira JT, Luz PB, Hugo FN, Araujo FB. Differential Impacts of Caries Classification in Children and Adults: A Comparison of ICDAS and DMF-T. Braz Dent J 2016;27(6):761-766. PMID: 27982192
26. Braga MM, Ekstrand KR, Martignon S, Imparato JC, Ricketts DN, Mendes FM. Clinical performance of two visual scoring systems in detecting and assessing activity status of occlusal caries in primary teeth. Caries Res 2010;44(3):300-308. PMID: 20530964

Tables

Table 1. Distribution of selected schools according to the region of Bauru city (São Paulo, Brazil)

Region	**Name of School**
North	EMEF “José Romão”
	EMEF “Geraldo Arone”
South	EMEF “Santa Maria”
East	EMEF ‘Thereza Tarzia- - Irmã Rosamaria Tarzia”
West	EMEF "Ivan Engler de Almeida"

Table 2. Modified Nyvad's scores [12]

Score	Description
Score 0	Sound enamel
Score 1	Active White spot lesion - not cavitated
Score 2	Inactive White spot lesion – not cavitated
Score 3	Cavitated enamel (tooth with cavity, restored, or extracted)

Table 3. ICDAS scores [15]

Score	Classification criteria
0	No or subtle change in enamel translucency after prolonged drying (5s) in area of biofilm accumulation
1	Visible white spot after drying (no loss of surface continuity) or pigmentation restricted to confines of a pit or fissure
2	White spot visible on wet surface (no loss of surface continuity) or pigmentation that extrapolates confines of a pit or fissure
3	Localized cavitation (or loss of continuity) in opaque or pigmented enamel
4	Underlying dark shadow from dentin, with or without cavitation on enamel
5	Cavitated enamel with exposure of the underlying dentin, involving up to half of the analyzed surface
6	Cavitated enamel with exposure of the underlying dentin, involving more than half of the analyzed surface

Table 4. Nyvad's scores [12] at the baseline and after 1 month of treatment

	TiF_4		NaF		Placebo	
	Baseline	**Final**	**Baseline**	**Final**	**Baseline**	**Final**
Nyvad Index (mean ± s.d.)	1.0±0.00	1.0±0.00	0.97±0.15	1.03±0.15*	0.99±0.06	1.08±0.24**
Df Median (min: max)	0.0 (0.0: 0.0)		0.0 (-0.7: 0.0)		0.0 (-1.0: 0.0)	

Df = Baseline – final value, where positive values indicate regression and negative values indicate lesions progression. Kruskal-Wallis Test (p=0.39).

*One patient presented 2 teeth that progressed from score 0 to 1 and 1 patient had 1 lesion that progressed from score 1 to 3.

** One patient presented 1 tooth that progressed from score 0 to 3 and 1 patient had 1 lesion that progressed from score 1 to 3.

Table 5. Median (minimum: maximum values) obtained in QLF analysis at the 1st month compared to the baseline

	ΔWS area (mm^2)	**ΔΔF (%)**	**ΔΔQ (mm^2%)**
TiF_4	0.01 (-9.15: 1.19)	-1.29 (-16.30: 4.74)	-6.40 (-27.20: 143.90)
NaF	0.17 (-2.38: 1.47)	-0.55 (-5.80: 6.10)	-6.83 (-42.13: 41.80)
Placebo	0.19 (-1.14: 4.36)	-0.23 (-5.17: 5.10)	-3.55 (-76.77: 19.70)

Kruskal-Wallis Test (p=0.59, p=0.45 and p=0.83, respectively).

For ΔWS and ΔΔQ, negative values mean progression (demineralization), and positive values, regression (remineralization). The opposite is valid for ΔΔF.

Table 6. Mean percentage of the degree of patient satisfaction after 4 applications of the varnishes using Wong-Baker Pain scale (%)

	TiF_4	**NaF**	**Placebo**
0	75.00±8.13	85.71±5.50	76.25±11.09
2	20.24±4.56	11.90±6.15	17.50±8.66
4	4.76±3.89	2.38±2.75	2.50±2.89
6	0.00±0.00	0.00±0.00	2.50±2.89
8	0.00±0.00	0.00±0.00	0.00±0.00
10	0.00±0.00	0.00±0.00	1.25±2.50

.

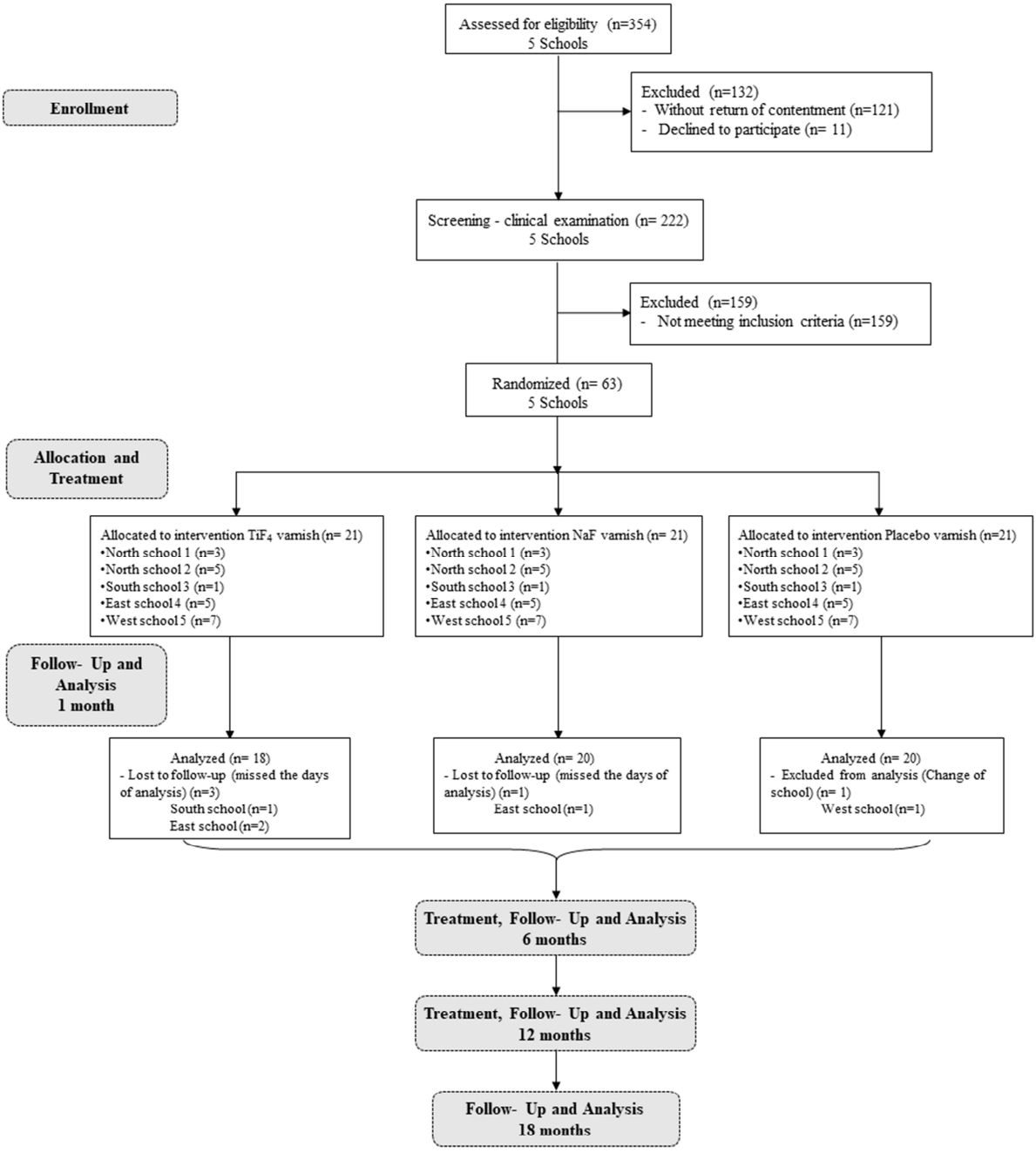


Figure 1. Flowchart of the study.

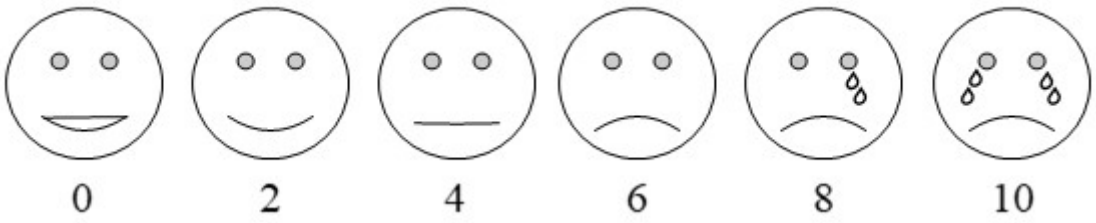


Figure 2. Wong-Baker Visual Scale (WBPS), where 0 is very good (no pain/discomfort) and 10 is very dissatisfied (worst possible pain/discomfort).

2.2 Article 2

Article formatted according to *International Journal of Paediatric Dentistry* guidelines.

Titanium tetrafluoride varnish for treatment of initial dental caries in children permanent dentition: a randomized controlled trial

Running title: Effect of TiF_4 on initial dental caries

Beatriz Martines de Souza[a], Mayara de Souza Silva[a], Aline Silva Braga[a], Daiana Moreli Soares dos Santos[a], Thamyris de Souza Carvalho[a], Natália Mello dos Santos[b], Daniela Rios[b], Marilia Afonso Rabelo Buzalaf[a], Ana Carolina Magalhães[a].

[a]Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo. Bauru-SP, Brazil
[b]Department of Pediatric Dentistry, Orthodontic and Public Health, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil

Author's contributions: B.M.S., M.S.S., A.S.B, D.M.S.S, N.M.S and T.S.C. carried out the research; B.M.S. and T.S.C performed the clinical care of the lesions that progressed; A.C.M., M.A.R.B. and D.R. conceived the ideas; A.C.M. mentored and assisted the clinical investigators; B.M.S. and A.C.M. wrote the manuscript; all authors revised the paper.

Corresponding author:
Ana Carolina Magalhães
Department of Biological Sciences
Bauru School of Dentistry - University of São Paulo
Al. Octávio Pinheiro Brisolla, 9-75. Bauru-SP
Zip code: 17012-901 (Brazil)
Phone/Fax. + 55 14 32358497
E-mail: acm@usp.br

Acknowledgments
We thank FAPESP for the concession of scholarship to the first author (number 2015/14149-1). We thank CNPq for the financial support (number- 401313/2016-6). We thank FGM-DentsCare (Joinville-SC, Brazil) for manufacturing the experimental varnishes (TiF_4 and placebo). We thank Municipal Secretariat for Education of Bauru (São Paulo, Brazil) that approved and supported the development of this study in the municipal schools.

Word Count (excluding tables): 4,991

Effect of Titanium tetrafluoride varnish on dental caries lesions control in children permanent dentition: a randomized controlled trial

Abstract

Background: TiF_4 varnish has shown superior anticaries effect compared to NaF varnish.

Aim: This randomized-controlled clinical trial compared the effect of TiF_4 and NaF varnishes in the prevention and treatment of initial caries lesions in permanent teeth.

Design: Sixty children (6-7 y/o; Bauru-SP/Brazil) were selected and randomly divided into treatments: 4% TiF_4 (2.45% F^-, FGM); 5% NaF (2.26% F^-, Duraphat®-Colgate) or placebo (FGM). Varnishes were applied on permanent teeth, once a week (4 weeks); after the 6^{th} and 12^{th} month. Clinical examination (Nyvad/ICDAS) and quantitative fluorescence changes analysis were performed. Patient's satisfaction degree was reported. Visual plaque index (VPI) was calculated. Two-way RM-ANOVA/ANOVA and Tukey were performed.

Results: After 18 months, no differences were found between treatments with respect to carious lesions regression/progression using Nyvad ($p>0.05$). Only TiF_4 was able to reduce the mean fluorescence loss significantly compared to the baseline value ($p=0.003$). With respect to prevention, TiF_4 showed higher percentage of sound surfaces compared to placebo by ICDAS ($p<0.014$). Regardless of the treatment, around 97% of the participants reported being satisfied. The VPI percentage decreased significantly after 9 and 15 months compared to 3 months ($p<0.001$).

Conclusion: Only TiF_4 varnish was able to show slight remineralizing and prevention effect.

Key words: Clinical Trial, Dental Caries, Sodium Fluoride, Titanium, Topical Fluorides.

Introduction

The protective effect of titanium tetrafluoride on dental caries has been intensively investigated under *in vitro* and *in situ* models[1-6]. Comar et al.[3] recently demonstrated a better effect of TiF_4 varnish in remineralizing initial enamel caries lesions *in situ*, regardless of the caries activity (ranging from low to high), compared to NaF varnish, which was only able to remineralize enamel lesions under low cariogenic challenges.

The mechanism of action of NaF varnish is based on the calcium fluoride (CaF_2) deposition on the dental surface, which acts as a fluoride reservoir and a mechanical barrier[7,8]. Titanium tetrafluoride, on the other hand, has an additional effect due to titanium that reacts with apatite forming compounds such as hydrated titanium phosphate and titanium dioxide, responsible for the high acid-resistant layer formed on teeth[8]. Furthermore, TiF_4 varnish increases the deposition of CaF_2 on the enamel, due to its low pH, compared to NaF varnish[8]. Despite its low pH, TiF_4 varnish has similar levels of toxicity on murine fibroblast lineage (NIH/3T3) and gingival fibroblasts compared to NaF varnish[9,10].

The mechanical barrier created by fluorides protects enamel against demineralization induced by bacteria acids. Furthermore, the fluoride reservoir can speed up remineralization, inducing the growth of fluor-hydroxyapatite like-crystals and, in case of TiF_4, the incorporation of titanium into the surface layer of demineralized enamel may improve its mechanical resistance[8,11]. Due to this latter property, TiF_4 varnish can be considered a good option for the treatment of non-cavitated enamel caries lesions, avoiding cavitation and, consequently, the need of invasive approaches[12].

There are few clinical studies testing the anti-cariogenic or remineralizing effect of TiF_4[13,14], but no one them evaluated TiF_4 as varnish. A previous work of our group showed better effect of TiF_4 varnish compared to TiF_4 solution on enamel caries lesion *in situ*[2].

Therefore, the aim of this randomized, controlled, longitudinal and double-blind clinical trial was to compare the effect of 4% TiF_4 varnish with a commercial 5% NaF varnish (gold standard) on the treatment and prevention of non-cavitated enamel caries lesions in the permanent dentition of children 6 to 7 years old, residing in an optimally fluoridated area. The tested null hypothesis was that there is no significant difference between the fluoride varnishes in the prevention (a) and/or

regression/progression (b) of non-cavitated enamel caries lesions in permanent teeth. For the analysis of each effect, we applied: a) ICDAS b) Nyvad index[15] and quantitative light-induced fluorescence.

Material and Methods

Ethical aspects and subjects selection

This randomized-controlled, parallel, double blind, and three-arm clinical trial was firstly registered in the Brazilian Clinical Trials Registry (ensaiosclinicos.gov.br, identifier RBR-5VWJ4Y), then approved by the local Ethics Committee (Number: 59787116.2.0000.5417, Ethics Committee of the Bauru School of Dentistry, University of São Paulo, Brazil) and by the Municipal Secretariat for Education of Bauru (São Paulo, Brazil). Five municipal schools were selected contemplating the 4 most populous regions of the city (2 schools in the northern region [A and B]; 1 in the southern region [C]; 1 in the western region [D] and 1 in the eastern region [E] (Figure 1). The central region was not contemplated due to the absence of municipal school. Written consent was obtained from the parents/guardian of each child, who also assigned a receive consent form, with age-appropriate language, explaining how her/his participation in the research would be. The recruitment period was divided into 2 blocks: Block 1 from March to May 2017 and Block 2 from June to August 2017. The last follow up were done in November 2018 for block 1 and in March 2019 for block 2.

Sixty healthy children (34 boys and 26 girls, 6-8 years old), coming from 5 public schools from Bauru-SP (Brazil), an area optimally fluoridated, were selected according to the study inclusion and exclusion criteria. The inclusion criteria consisted of: 1) the approved and signed consent from the parent/guardian and the child; 2) child with at least one active non-cavitated enamel caries lesion (white spot lesion)[15] in the smooth surface of the permanent dentition at the baseline examination. Exclusion criteria included: 1) users of orthodontics appliances; 2) child who participated in other clinical trial 3 months prior to the selection day; or 3) who was submitted to professional topical fluoride application 6 months prior to the selection day; 4) under treatment with antibiotics; 5) presenting periodontal disease; or 6) any type of systemic chronic disease.

Study Design and treatment

The sample calculation was performed based on previous clinical trial (caries incidence after a period of 2 years: 15% for fluoride varnish and 42% for control - without fluoride treatment)[16], considering a dropout of 30%, a statistical power of 80% and an alpha-error of 5%.

Children were selected and randomly distributed to the treatments according to the caries activity (Nyvad index[15]), in two blocks, block 1 (n=11/ per treatment) and block 2 (n=9/ per treatment) (A.C.M.): 4% TiF_4 varnish (2.45% F, pH 1, FGM-DentsCare); 5% NaF varnish (2.26% F, pH 5, Duraphat®, Colgate) or placebo varnish (without F, pH 5, FGM-DentsCare). The treatment was double blind to children and their parents/guardians and to the researchers who made the clinical examination and QLF analysis.

At the baseline, dmfs and DMFS of all selected children were computed. The treatment was performed after the initial clinical examination and consisted of 4 consecutive weekly application of varnish on all smooth surface of permanent teeth[17] (with more than ½ of the crown exposed), and a single application at the 6th and 12th month of study[18]. After each day of treatment, a visual pain scale was applied to evaluate the satisfaction degree of patients (A.S.B. and D.M.S.S.)[19]. Children should note the face that better represents them after treatment. For each face was attributed a number for the statistical analysis where zero was very pleased (absence of pain, discomfort) and ten was extremely upset (worst possible pain/discomfort) (Figure 2). Since the degree of satisfaction kept constant along to the six varnishes application, a mean was calculated.

During the visits, the children were instructed with respect to cariogenic diet and oral hygiene. All participants received an oral hygiene kit, instruction and motivation to brush their teeth twice a day, at each 3 months. The kit consisted of toothbrush (Colgate® Classic, Colgate-Palmolive, Brazil), toothpaste (Colgate®, 1450 ppm F^- as monofluoride phosphate [MFP], Colgate-Palmolive, Brazil) and dental floss (Colgate-Palmolive, Brazil).

Before the oral hygiene instruction, the visual plaque index (VPI)[20] was calculated (M.S.S.), at the 3th, 9th and 15th months. Two trained examiners (inter and intra-examiner agreement, kappa>0.8), not involved in the treatment, were responsible for the children's examination (B.M.S. and N.M.S) with respect to the caries lesions analyses.

Clinical procedures

The varnish was applied on all smooth surface of permanent teeth, under natural light, following the clinical steps: 1) supervised toothbrushing (A.S.B. and D.M.S.S.); 2) relative isolation of teeth area with cotton rolls; 3) drying of dental surfaces using sterile gauze; 4) varnish application using microbrush (approximately 30 mg of varnish for each arche[21]); 5) 5 minutes of wait for solvent evaporation; 6) removal of the cotton rolls. The application was done after the school break. All the collaborators (teachers and parents) received a written recommendation advising for children do not ingest liquid for 30 minutes after the treatment, to have soft meals and just perform brushing 4 h after the treatment. The treatment was performed once a week for four consecutive weeks without interruption, at the 6th and 12th months of the study (T.S.C).

Clinical Examination

The clinical examination was performed under natural illumination, after dental hygiene, using WHO probe and mirror (B.M.S. and N.M.S). We applied the clinical criteria of Nyvad et al.[15] modified by the researchers to facilitate the data analysis of the surfaces that presented caries lesions at the baseline, as following: Score 0- sound enamel; Score 1 – non-cavitated and inactive white spot lesion; Score 2- non-cavitated and active white spot lesion and Score 3 – cavitated lesion (tooth with cavity, restored or extracted). Only the smooth surfaces of permanent teeth (with at least ½ of crown exposed at the baseline) were considered in the analysis. The Nyvad's index was applied at baseline, after 1 month of the last application of the 4 consecutive ones, and after 6, 12 and 18 months of the baseline analysis. In addition, the International Caries Detection and Assessment System (ICDAS) was performed at the baseline and after 18 months of the study[22] on all permanent teeth smooth surfaces, which were sound at the baseline. The dmfs and DMFS were measured at the baseline for random distribution proposal.

Complementary exam: Quantitative light-induced fluorescence (QLF)

QLF system (Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to measure the fluorescence loss of non-cavitated enamel carious lesion allowing the analysis of their regression or progression over time[22]. The QLF

analyses were performed at baseline, after 1 month of the last application of the 4 consecutive ones, and after 6, 12 and 18 months of the baseline analysis (B.M.S.).

The QLF images were taken only from the teeth with non-cavitated enamel carious lesions, which were previously cleaned and dried, under completely dark environment. The software QLF 2.00f (Inspektor Research System BV) was used for image storage and analysis. The area of the lesion (mm^2) and the mean fluorescence loss (ΔF, %, detection threshold is 5%) were determined.

Visual plaque index (VPI)

The VPI[20] was applied on all teeth surfaces (including primary and permanent teeth) at the 3rd, 9th and 15th month of the study. The VPI was applied before hygiene instruction and supervised brushing. Score 0 was equivalent to absence of dental plaque and Score 1 to the presence of dental plaque. The VPI was converted into percentage value of surfaces with visible dental plaque.

Statistical Analysis

Abandonment in clinical trial is expected, however, losses may compromise statistical analysis, decreasing study strength and increasing bias. Therefore, for all periods, amputation of the missing values was used since it corresponded to less than 20% of the sample, as suggested by Spineli et al.[23]. In this case, we applied the average of the other two treatments as a conservative estimate of missing values.

The means Nyvad, ICDAS and QLF parameters per child were calculated. The data were submitted to statistical analysis using GraphPad Prism version 7.0 software for Windows (GraphPad Software - San Diego, CA, USA). Two-way repeated measures ANOVA was performed for Nyvad index, QLF parameters and VPI. For ICDAS and dmfs/DMFS, ANOVA/Tukey was applied. For the visual satisfaction scale, $\chi 2$ test was done. The level of significance was set at 5% (n=20 children).

Results

The number of children selected and followed during the entire study is described in the flowchart indicated by CONSORT (http://www.consort-statement.org) (Figure 1). During the follow up and at the end of the study, we lost

the maximum of 4 subjects per group (within of 20%). The most common causes of lost were miss the day of analysis or change of school.

At the baseline, all groups presented children with similar dmfs (TiF_4 1.65 ± 2.0; NaF 1.45 ±2.4 and Placebo 1.55 ±2.4; ANOVA p=0.96) and DMFS (TiF_4 0.00 ± 0.0; NaF 0.05 ± 0.2 and Placebo 0.10 ± 0.3; ANOVA p=0.36) values.

From 60 children, 128 smooth surfaces (5.1% of the total surfaces) presented Score 2 of modified Nyvad Index at the baseline (TiF_4 n=45; NaF n=41; Placebo n=42 surfaces). The most affected smooth surfaces were from mandibular posterior teeth (48.4%), followed by maxillary posterior teeth (28.9%), maxillary anterior teeth (14.1%) and mandibular anterior teeth (8.6%).

The number of children who presented caries lesion progression (cavitation) at the end of the study (13% of the children) was: TiF_4 (n=2), NaF (n=4) and placebo (n=2). With respect to regression, the following numbers were 1, 3 and 3 for TiF_4, NaF and placebo, respectively. In both cases, no differences were found among the groups by using Nyvad Index (Table 1). For most of children, the lesions did not present clinical changes along the period of study regardless of the treatments.

With respect to the prevention of new lesions, only TiF_4 (99.2% ± 1.7 surfaces prevention fraction) presented a significant higher percentage of sound surfaces compared to placebo (94.9% ± 7.9), while NaF did not differ from both groups (98.5% ± 2.1) after 18 months of follow up (Figure 3, ANOVA p=0.014)

With respect to QLF analysis, no significant differences were found among the treatments with respect to lesion area - WS (Table 2) and mean fluorescence loss – ΔF (Table 2). When the periods of analysis were compared within of each treatment group, only TiF_4 reduced ΔF means after 18 months compared to baseline values (2-way ANOVA, p= 0.0003).

The VPI means were similar among the treatment groups (2-way ANOVA, p>0.05, Table 3). For all treatments groups, there was a significant reduction in the percentage of dental surfaces with visible plaque at 9th and 15th months compared to 3rd month.

Regardless of treatment group, around 97% of children were very pleased or pleased with treatment. No association was found between the degree of satisfaction and the type of treatment ($\chi 2$, Table 4).

Discussion

The tested null hypothesis was partially rejected. TiF_4 varnish was not able to change the clinical appearance of the white spot lesions, however, this treatment improved the amount of mineral gain resulting in significant reduction of enamel fluorescence loss after 18 months of study, in agreement with previously *in vitro* and *in situ* studies[1-6]. With respect to prevention of new caries lesions, TiF_4 varnish also presented a significant higher percentage value of sound surfaces at the end of study compared to placebo. The positive results are explained by the reaction of Ti with apatite, producing an acid-resistant layer (able to prevent demineralization) and by its incorporation into the lesions porous, improving mineral gain (enhancing remineralization)[8].

Previous clinical trials have tested the effect of TiF_4 as solution[13,14] showing promising effects on prevention of tooth demineralization and improving of remineralization. The annual application of 1% TiF_4 significantly reduced the appearance of new lesions in permanent teeth (33% reduction) compared to 1.25% APF (acidulated phosphate fluoride) in a follow-up of 3 years[13]. Pomarico et al.[14] demonstrated that permanent teeth treated with 4% TiF_4 solution (once) plus MFP toothpaste (daily) for 4 weeks significantly had lower lesion area (74.5%) compared to teeth treated with MFP toothpaste only (67% reduction). In our work, the TiF_4 varnish reduced the mean fluorescence loss in 16.3% after 18 months compared to the baseline values, which was not reflected in clinical appearance changes.

On the other hand, NaF varnish did not have protective or remineralizing effect on enamel compared to placebo. Following the recommendation for prevention of caries[18,24], the varnishes were applied each 6 months. Some clinical studies evaluating the potential of bi-annual application of fluoride varnish (NaF), to prevent dental caries in primary teeth, were unable to find significant differences between the fluoridated and non-fluoridated groups (or just brushing) after 24-month follow-up[25,26]. However, Arruda et al.[27] demonstrated in school children, who had their permanent teeth treated with 5% NaF varnish (bi-annual), a reduction of caries increment (new lesions) in 41% compared to placebo in a follow-up of 12 months. When we compare the results of Arruda et al. [27] with ours, it is obvious to infer that the protective effect of NaF was found by them due to the high-caries risk level of the tested population (DMFS 5.9) compared to the children from our study (DMFS 0.05).

In a longer follow-up period (26 months) with bi-annual application of NaF varnish, Hardman et al.[28] found no differences in caries incidence (NaF, 16% children) compared to placebo (19%) on the first permanent molar, similarly to our results. In agreement, Milson et al.[29] also demonstrated that DFS increment was 0.65 for patients treated with NaF varnish (annual application) and 0.67 for those belonged to placebo group, with no significant differences between them after 36 months of follow-up. As happened in our study, the above-cited works tested NaF varnish in children at low-risk level for caries.

Marinho et al.[30] suggest that fluoride varnishes have a good potential as caries inhibitor (permanent and primary teeth), regardless of the frequency of application (two or four times a year); however, the quality of evidence is still moderate due to the high risk of vies of the clinical studies included in the systematic review. This systematic review also showed that the side effects and information on acceptability was inconclusive, because they were often not reported in clinical trials[30]. It was not possible to demonstrate influence of external factors on the potential of fluoride varnishes in the treatment and prevention of carious lesions (such as initial lesion severity and exposure to other fluoride sources)[30]. However, these factors cannot be excluded in the interpretation of the results.

On the other hand, the beneficial effect of NaF varnish in remineralization is more evident for primary teeth than for permanent dentition[31,32]. Furthermore, there is still no consensus on the frequency of fluoride varnish application to stop or reverse non-cavitated enamel lesions on smooth surfaces. We found authors who applied 5% NaF varnish every 3 to 6 months[24,29] while others performed one application per week during 4 consecutive weeks[17,32]. Accordingly, we followed the protocol of Almeida et al.[17] to improve remineralization and we further reapplied the varnishes each 6 months to achieve the preventive effect[18].

Güçlü et al.[32] demonstrated no difference in the remineralization of white spot lesions in permanent teeth treated with NaF varnish (once week for 4 weeks) compared to control, after a follow-up of 3 months, by visual examination and fluorescence quantification (DIAGNOdent). Our study also did not show remineralizing effect of NaF. A slight remineralizing effect was found only for TiF_4 by using QLF. The main hypothesis to justify the low effect of fluorides varnishes, especially NaF one, on the prevention or regression of dental caries at the present study is the low caries rate incidence found in the population. Recently, a paper

revised the rate of caries progression, showing that the progression is proportional to the severity, with a mean of DMFS increment about 0.11[33]. The authors suggested longer follow-up periods for permanent teeth (> 36 months)[33]. Considering that our population had no severe caries lesions (DMFS 0.05 ± 0.22 at baseline), we advise a longer follow up periods for future studies with this population or to test the products in a high-caries risk level population.

A recent systematic review demonstrated that ICDAS is a visual diagnosis system with high accurate for carious lesions detection but not for assessing the caries lesion progression[34]. We applied ICDAS instead of DMFS index in the present study to detect new caries lesions, since the latter one does not allow evaluating initial caries lesions in enamel. Considering the limitation of ICDAS, to assess lesion progression/regression we combined two other methods (Nyvad index and QLF)[35]. QLF and DIAGNOdent systems are noninvasive techniques used for research purpose, by quantifying the mineral loss of early caries lesions by fluorescence[35]. A recent *in vitro* study showed that QLF was more efficient in detecting early and shallow caries lesions than a spectrophotometer (which evaluates the subtle color difference from sound to the demineralized enamel). However, QLF is limited for detecting very deep lesions since light scattering may not reflect fully deep mineral loss (> 200 μm depth)[36]. Therefore, QLF is a sensible quantitative complementary method with good repeatability and reproducibility, able to quantify small mineral changes that might not be detectable by visual inspection[37], as it was the case of the present study.

The biofilm control is an important factor that could have influenced the effect of the treatments[30]. In fact, we did not find differences among the groups with respect to percentage of visible plaque. Instead, we found a biofilm control improvement after 3 months of study, highlighting that the frequent presence of the researchers at the schools motivated the children to better brush their teeth. The improvement of biofilm control might have prevented dental caries development and progression, minimizing the effect of treatments.

With respect to degree of acceptance, the majority of the children reported to be pleasant, regardless of the treatment. This result is very interesting once we expected that TiF_4, due to its low pH, could have caused an unpleased taste change, as reported in an *in situ* study[38]. Furthermore, no subject reported tooth staining or any other side-effect due to the fluorides varnishes application. A recent *in situ* study

showed that 27% of the subjects complained about the taste change and 40% about a temporary tooth staining, due to the daily use of TiF_4 solution as mouthwash for 5 days[38]. Differently from mouthrinses, varnishes are applied in low quantity on tooth surfaces, with low direct contact with tongue, reducing eventual effect on the taste change. Furthermore, it is applied each 6 months; therefore, the risk of tooth staining is low compared to the daily application of a mouthrinse.

As conclusion, TiF_4 varnish is the only treatment able to show slight remineralizing and preventive effect under this study model. Considering the limitations of this RCT, the authors suggest that future studies shall be done during longer periods (> 36 months), despite of the high probability of dropout overtime, to allow seeing caries lesion progression/regression clinically. Furthermore, a high caries-risk population shall be included in the RCT to check the effect of TiF_4 varnish under a worst scenario.

Why this paper is important to pediatric dentists

This work shows some remineralizing and preventive potential of TiF_4 varnish applied on permanent teeth of young children.

In addition, it shows that, despite TiF_4 varnish has acid pH, most of subjects felt pleased after its application.

Finally, this study shows that, under very-well controlled conditions, the caries progression is low even after 18 months and, therefore, the effect of fluoride measures are limited.

References

1. Magalhães AC, Comar LP, Rios D, Delbem AC, Buzalaf MA. Effect of a 4% titanium tetrafluoride (TiF_4) varnish on demineralisation and remineralisation of bovine enamel in vitro. J Dent 2008;36(2):158-162.

2. Comar LP, Wiegand A, Moron BM, Rios D, Buzalaf MAR, Buchalla W et al**.** Effect of TiF_4 and NaF varnish and solution on enamel demineralisation *in situ***.** Eur J Oral Sci 2012;120(4):342-348.

3. Comar LP, Souza BM, Martins J, Santos MG, Buzalaf MAR, Magalhães AC. Response of carious enamel to TiF(4) varnish treatment under diverse cariogenic activities in situ. J Dent 2017;63:81-84.

4. Souza BM, Fernandes Neto C, Salomão PMA, Vasconcelos LRSM, Andrade FB, Magalhães AC. Analysis of the antimicrobial and anti-caries effects of TiF_4 varnish under microcosm biofilm formed on enamel. J Appl Oral Sci 2018;26:e20170304.

5. Alexandria AK, Nassur C, Nóbrega CBC, Branco-de-Almeida LS, Dos Santos KRN, Vieira AR et al. Effect of TiF(4) varnish on microbiological changes and caries prevention: in situ and in vivo models. Clin Oral Investig 2019;23(6)2583-2591.

6. Dos Santos DMS, Pires JG, Silva AB, Salomão PMA, Buzalaf MAR, Magalhães AC. Protective effect of 4% titanium tetrafluoride varnish on dentin demineralization using microcosm biofilm model. Caries Res 2019;14:1-8.

7. Gao SS, Zhang S, Lo EC, Chu C. Caries remineralization and arresting effect in children by professionally applied fluoride treatment – a systematic review. BMC Oral Health 2016;16:12

8. Comar LP, Souza BM, Al-Ahj LP, Martins J, Grizzo LT, Piasentim IS et al. Mechanism of Action of TiF_4 on Dental Enamel Surface: SEM/EDX, KOH-Soluble F, and X-Ray Diffraction Analysis. Caries Res 2018;51(6):554-567.

9. Salomão PMA, Oliveira FA, Rodrigues PD, Al-Ahj LP, Gasque KCDS, Jeggle P et al. The cytotoxic effect of TiF_4 and NaF on fibroblasts is influenced by the experimental model, fluoride concentration and exposure time. PLoS One 2017;12(6):e0179471.

10. Aranda Salomão PM, de Oliveira FA, Dos Santos DMS, Domezi JP, Dionísio TJ, de Oliveira RC et al. TiF(4) and NaF varnishes induce low levels of apoptosis in murine and human fibroblasts through mitochondrial Bcl-2 family and death receptor signalling. Arch Oral Biol 2019;97:245-252.

11. Magalhães AC, Dos Santos MG, Comar LP, Buzalaf MA, Ganss C, Schlueter N. Effect of a Single Application of TiF_4 Varnish versus Daily Use of a Low-Concentrated TiF_4/NaF Solution on Tooth Erosion Prevention in vitro. Caries Res 2016;50(5):462-470.

12. Sköld UM, Petersson LG, Birkhed D, Norlund A. Cost-analysis of school-based fluoride varnish and fluoride rinsing programs. Acta Odontol Scand 2008;66(5):286-292.

13. Reed AJ, Bibby BG. Preliminary report on effect of topical applications of titanium tetrafluoride on dental caries. J Dent Res 1976;55(3):357-358.

14. Pomarico L, Villardi M, Maia LC. In vivo effect of titanium tetrafluoride and sodium monofluorophosphate dentifrice on remineralization: a preliminary split-mouth clinical trial. Gen Dent 2012;60(4):e249-254.
15. Nyvad B, Machiulskiene V, Baelum V. Construct and predictive validity of clinical caries diagnostic criteria assessing lesion activity. J Dent Res 2003;82(2):117-122.
16. Weintraub JA, Ramos-Gomez F, Jue B, Shain S, Hoover CI, Featherstone JD et al. Fluoride varnish efficacy in preventing early childhood caries. J Dent Res 2006;85(2):172-176.
17. Almeida MQ, Costa OX, Ferreira JM, Menezes VA, Leal RB, Sampaio FC. Therapeutic potential of Brazilian fluoride varnishes: an in vivo study. Braz Dent J 2011;22(3):193-197.
18. Hedman E, Gabre P, Birkhed D. Dental hygienists working in schools – a two-year oral health intervention programme in swedish secondary schools. Oral Health Prev Dent 2015;13(2):177-188.
19. El Batawi HY. Effect of intraoperative analgesia on children's pain perception during recovery after painful dental procedures performed under general anaesthesia. Eur Arch Paediatr Dent 2015;16(1):35-41.
20. Alaluusua S, Malmivirta R. Early plaque accumulation--a sign for caries risk in young children. Community Dent Oral Epidemiol 1994;22(5 Pt 1):273-276.
21. Comar LP, Souza BM, Grizzo LT, Buzalaf MA, Magalhães AC. Evaluation of fluoride release from experimental TiF_4 and NaF varnishes in vitro. J Appl Oral Sci 2014;22(2):138-143.
22. Souza BM, Santos DMS, Braga AS, Santos NMD, Rios D, Buzalaf MAR, et al. Effect of a Titanium Tetrafluoride Varnish in the Prevention and Treatment of Carious Lesions in the Permanent Teeth of Children Living in a Fluoridated Region: Protocol for a Randomized Controlled Trial. JMIR Res Protoc 2018;7(1):e26.
23. Spineli LM, Fleming PS, Pandis N. Addressing missing participant outcome data in dental clinical trials. J Dent 2015;43(6):605-618.
24. Urquhart O, Tampi MP, Pilcher L, Slayton RL, Araujo MWB, Fontana M, et al. Nonrestorative Treatments for Caries: Systematic Review and Network Meta-analysis. J Dent Res 2019;98(1):14-26.
25. Agouropoulos A, Twetman S, Pandis N, Kavvadia K, Papagiannoulis L. Caries preventive effectiveness of fluoride varnish as adjunct to oral health promotion and

supervised tooth brushing in preschool children: a double-blind randomized controlled trial. J Dent 2014;42(10):1277-1283.

26. Jiang EM, Lo EC, Chu CH, Wong MC. Prevention of early childhood caries (ECC) through parental toothbrushing training and fluoride varnish application: a 24-month randomized controlled trial. J Dent 2014;42(12):1543-1550.

27. Arruda AO, Senthamarai Kannan R, Inglehart MR, Rezende CT, Sohn W. Effect of 5% fluoride varnish application on caries among school children in rural Brazil: a randomized controlled trial. Community Dent Oral Epidemiol 2012;40(3):267- 276.

28. Hardman MC, Davies GM, Duxbury JT, Davies RM. A cluster randomised controlled trial to evaluate the effectiveness of fluoride varnish as a public health measure to reduce caries in children. Caries Res 2007;41(5):371-376.

29. Milsom KM, Blinkhorn AS, Walsh T, Worthington HV, Kearney-Mitchell P, Whitehead H, Tickle M. A cluster-randomized controlled trial: fluoride varnish in school children. J Dent Res 2011;90(11):1306-1311.

30. Marinho VC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2013;7:CD002279.

31. Patil SK, Fatangare M, Jadhav RG, Shinde GR, Pawar SS, Kathariya MD. Caries preventive effect of sodium fluoride varnish on deciduous dentition: a clinical trial. J Contemp Dent Pract 2017;18(12)1190-1193.

32. Güçlü ZA, Alaçam A, Coleman NJ. A 12-Week Assessment of the Treatment of White Spot Lesions with CPP-ACP Paste and/or Fluoride Varnish. Biomed Res Int 2016;2016:8357621.

33. Hummel R, Akveld NAE, Bruers JJM, van der Sanden WJM, Su N, van der Heijden GJMG. Caries progression rates revised: a systematic review. J Dent Res 2019;9: 22034519847953.

34. Ekstrand KR, Gimenez T, Ferreira FR, Mendes FM, Braga MM. The International Caries Detection and Assessment System - ICDAS: A Systematic Review. Caries Res 2018;52(5):406-419.

35. Gomez J. Detection and diagnosis of the early caries lesion. BMC Oral Health 2015;15 Suppl 1:S3.

36. Kim HE, Kim BI. Early caries detection methods according to the depth of the lesion: An in vitro comparison. Photodiagnosis Photodyn Ther 2018;23:176-180.

37. Sitthisettapong T, Doi T, Nishida Y, Kambara M, Phantumvanit P. Effect of CPP-ACP Paste on Enamel Carious Lesion of Primary Upper Anterior Teeth Assessed by Quantitative Light-Induced Fluorescence: A One-Year Clinical Trial. Caries Res 2015;49(4):434-441.
38. de Souza BM, Santi LRP, de Souza Silva M, Buzalaf MAR, Magalhães AC. Effect of an experimental mouth rinse containing NaF and TiF(4) on tooth erosion and abrasion in situ. J Dent 2018;73:45-49.

Table 1. Mean ± S.D. of modified Nyvad's scores at baseline, after 1, 6, 12 and 18 months of treatment with TiF_4, NaF or placebo varnish (regression/progression analysis)

	Baseline	1 month	6 months	12 months	18 months
TiF_4	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.01 ± 0.06	1.99 ± 0.13
NaF	2.00 ± 0.00	2.00 ± 0.00	2.09 ± 0.25	2.09 ± 0.25	1.99 ±0.45
Placebo	2.00 ± 0.00	2.03 ± 0.11	2.03 ±0.11	2.04 ± 0.13	1.93 ±0.32

Two-way RM-ANOVA (time p=0.05 and treatment p=0.588). No statistical differences were found for both factors. * 2 means non-cavitated active caries lesion.

Table 2. Mean ± S.D. of the data obtained by QLF at baseline, after 1, 6, 12 e 18 months of treatment with TiF_4, NaF or placebo varnish

		Baseline	1 month	6 months	12 months	18 months
WS (mm^2)	**TiF_4**	3.4±1.5	3.8±2.6	3.8±1.8	3.8±1.8	3.6±2.4
	NaF	3.7±1.9	3.6±2.1	4.0±1.9	3.9±1.5	3.6±1.4
	Placebo	4.1±1.9	3.5 ±1.9	3.6±1.7	4.0±1.9	3.6±1.6
ΔF (%)	**TiF_4**	-17.5±3.9^{a}	-16.7±3.6^{a}	-16.1±3.0^{ab}	-16.3±3.2^{ab}	-14.6±4.0^{b}
	NaF	-15.7±3.2^{a}	-15.3±3.1^{a}	-16.4±2.7^{a}	-15.9±2.2^{a}	-14.9±2.2^{a}
	Placebo	-16.4±3.2^{a}	-16.2±3.6^{a}	-14.5±1.6^{a}	-15.5±1.6^{a}	-14.4±2.0^{a}

Two-way RM-ANOVA (WS: time p=0.555 and treatment p=0.971; ΔF: time p=0.0003 and treatment p=0.327). Different lowercase letters mean statistical difference among times within each treatment group.

Table 3. Mean ± S.D. of the percentage (%) of children with dental surfaces showing visible plaque after 3, 9 and 15 months of the study

	3 months	9 months	15 months
TiF_4	58.3 ± 34.1[a]	35.1 ± 29.2[b]	21.9 ± 17.5[b]
NaF	65.8 ± 34.7[a]	30.3 ± 30.9[b]	28.3 ± 27.0[b]
Placebo	64.4 ± 33.4[a]	22.8 ± 17.3[b]	22.3 ± 16.2[b]

Two-way RM-ANOVA (time $p<0.0001$ and treatment $p=0.169$). Different lowercase letters mean statistical difference among times within each treatment group.

Table 4. Mean ± S.D of percentage (%) of subjects reporting different degrees of satisfaction at the end of 6 varnishes applications using a visual scale

	Very Pleased (0)	**Pleased (2)**	**No reaction (4)**
TiF_4	77.5 ± 9.4	18.3 ± 5.2	4.2 ± 4.9
NaF	85.8 ± 4.9	11.7 ± 6.1	2.5 ± 2.7
Placebo	76.7 ± 5.2	20.8 ± 7.4	2.5 ± 4.2

No child scored > 4. Means were calculated from each child from the values obtained after the six applications. χ^2 showed no association between the type of treatment and the degree of satisfaction (p=0.515).

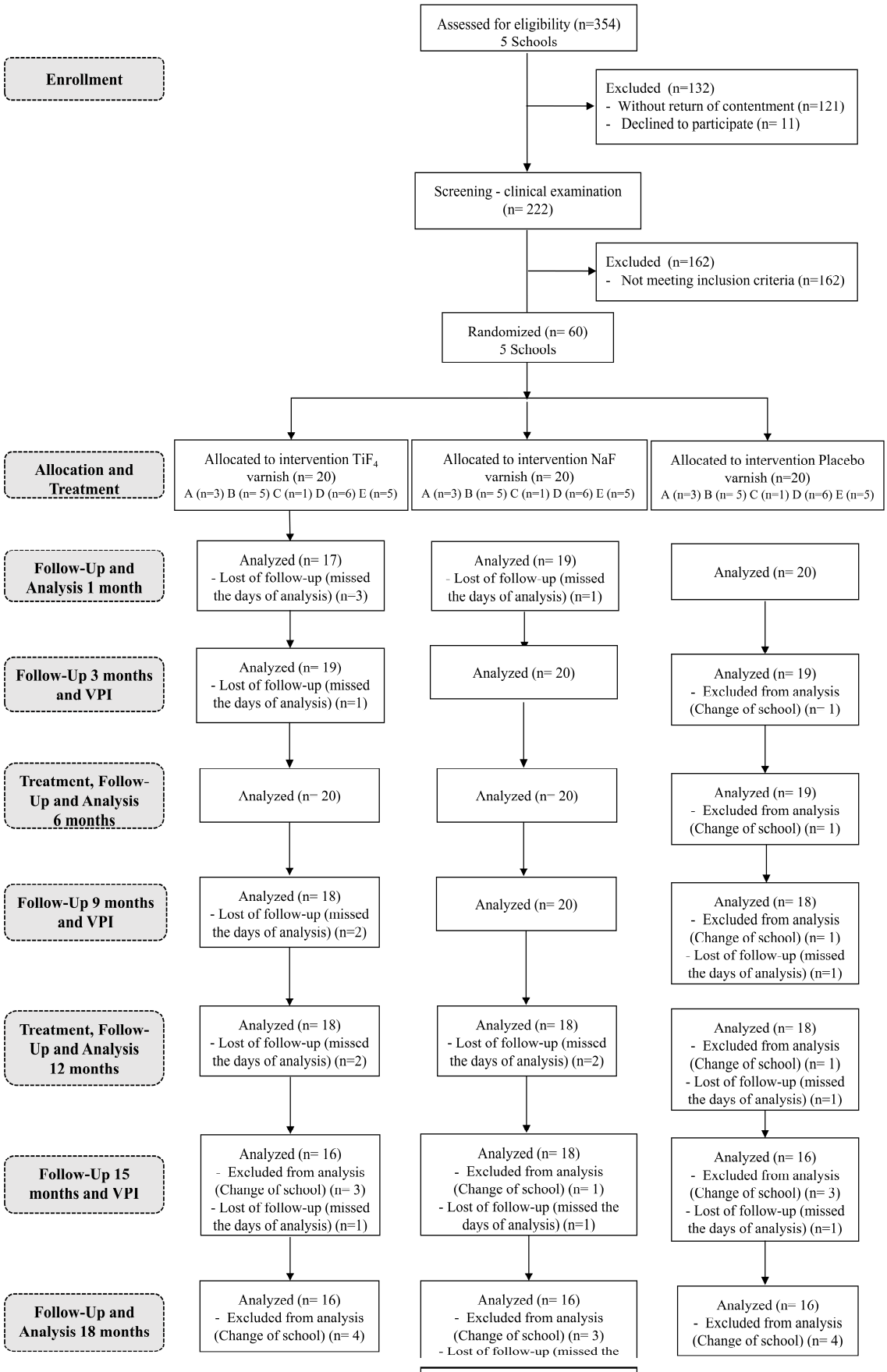


Figure 1: Consort flow diagram of the study. Schools' distribution: A- Northern region 1; B- Northern region 2; C- Southern region; D- Western region; E – Eastern region.

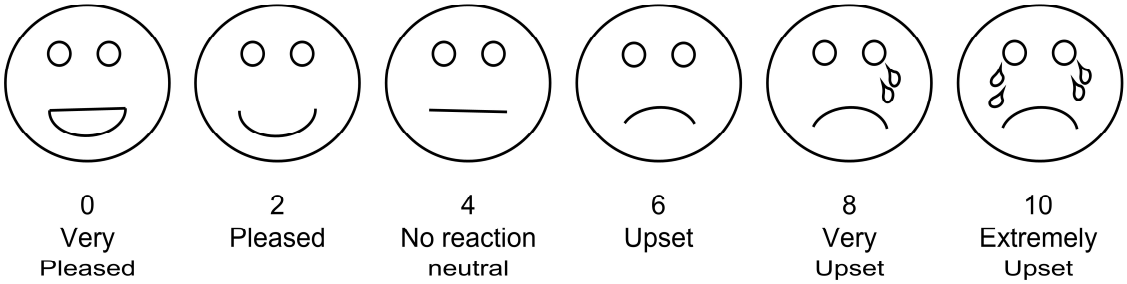


Figure 2. Visual scale to assess the degree of patient satisfaction.

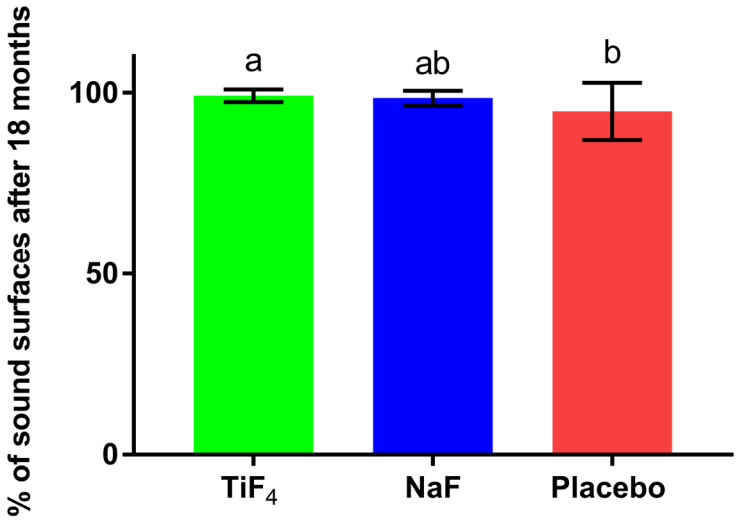


Figure 3. Mean and standard deviation of the percentage of children with dental surfaces scored ICDAS 0 at the end of study (18 months). ANOVA/Tukey (p=0.014)

3 DISCUSSION

3 DISCUSSION

The first paper reported the research protocol applied at the beginning of study. However, some changes are seen in the 2nd paper. After the first month, we realized the need of quantifying dental plaque, to better understand the risk of population for caries. We also changed the way to report the QLF data (from Δ values, we ended the study reporting absolute values). Furthermore, we modified the Nyvad index (index 1 by 2, and vice-versa) to facilitate the interpretation of the data.

Based on the results (2nd paper), the tested null hypothesis was partially rejected. TiF_4 varnish was not able to change the clinical appearance of the white spot lesions, however, it improved the amount of mineral gain resulting in significant reduction of enamel fluorescence loss after 18 months of study, in agreement with previously *in vitro* and *in situ* studies (MAGALHÃES et al., 2008; WIEGAND; MAGALHÃES; ATTIN, 2010; COMAR et al., 2012; SOUZA et al., 2018; ALEXANDRIA et al., 2019; DOS SANTOS et al., 2019). With respect to prevention of new caries lesions, TiF_4 varnish also presented a significant higher percentage value of sound surfaces at the end of study compared to placebo. The positive results are explained by the reaction of Ti with apatite, producing an acid-resistant layer (able to prevent demineralization) and by its incorporation into the lesions porous, improving mineral gain (enhancing remineralization) (COMAR et al., 2018).

Previous clinical trials have tested the effect of TiF_4 as solution (REED, BIBBY, 1976; POMARICO; VILARDI; MAIA, 2012) showing promising effects on prevention of tooth demineralization and improvement of remineralization. The annual application of 1% TiF_4 significantly reduced the appearance of new lesions in permanent teeth (33% reduction) compared to 1.25% APF (acidulated phosphate fluoride) in a follow-up of 3 years (REED; BIBBY, 1976). Pomarico, Vilardi e Maia (2012) demonstrated that permanent teeth treated with 4% TiF_4 solution (once) plus MFP toothpaste (daily) for 4 weeks significantly had lower lesion area (74.5%) compared to teeth treated with MFP toothpaste only (67% reduction). In our work, the TiF_4 varnish reduced the mean fluorescence loss in 16.3% after 18 months compared to the baseline values, which was not reflected in clinical appearance changes.

On the other hand, NaF varnish did not have protective or remineralizing effect on enamel compared to placebo. Following the recommendation for prevention of dental caries (HEDMAN; GABRE; BIRKHED, 2015; URQUHART et al., 2019), the varnishes were applied each 6 months. Some clinical studies evaluating the potential of bi-annual application of fluoride varnish (NaF), to prevent dental caries in primary teeth, were unable to find significant differences between the fluoridated and non-fluoridated groups (or just brushing) after 24-month of follow-up (AGOUROPOULOS et al., 2014; JIANG et al., 2014). However, Arruda et al. (2012) demonstrated in school children, who had their permanent teeth treated with 5% NaF varnish (bi-annual), a reduction of caries increment (new lesions) in 41% compared to placebo in a follow-up of 12 months. When we compare the results of Arruda et al. (2012) with ours, it is obvious to interfere that the protective effect of NaF was found by them due to the high-caries risk level of the tested population (DMFS 5.9) compared to the children from our study (DMFS 0.05).

In a longer follow-up period (26 months) with bi-annual application of NaF varnish, Hardman et al. (2007) found no differences in caries incidence (NaF, 16% children) compared to placebo (19%) on the first permanent molar, similarly to our results. In agreement, Milson et al. (2011) also demonstrated that DFS increment was 0.65 for patients treated with NaF varnish (annual application) and 0.67 for those belongs to placebo group, with no significant differences between them after 36 months of follow-up. As happened in our study, the above-cited works tested NaF varnish in children at low-risk level for caries.

Marinho et al. (2013) suggested that fluoride varnishes have a good potential as caries inhibitor (permanent and primary teeth), regardless of the frequency of application (two or four times a year); however, the quality of evidence is still moderate due to the high risk of vies of the clinical studies included in the systematic review. This systematic review also showed that the side effects and information on acceptability was inconclusive, because they were often not reported in clinical trials (MARINHO et al., 2013). It was not possible to demonstrate influence of external factors on the potential of fluoride varnishes in the treatment and prevention of carious lesions (such as initial lesion severity and exposure to other fluoride sources) (MARINHO et al., 2013). However, these factors cannot be excluded in the interpretation of the results.

On the other hand, the beneficial effect of NaF varnish in remineralization is more evident for primary teeth than for permanent dentition (GÜÇLU et al., 2016; PATIL et al., 2017). Furthermore, there is still no consensus on the frequency of fluoride varnish application to stop or reverse non-cavitated enamel lesions on smooth surfaces. We found authors who applied 5% NaF varnish every 3 to 6 months (MILSOM et al., 2011; URQUHART et al., 2019) while others performed one application per week during 4 consecutive weeks (ALMEIDA et al., 2011; GÜÇLU et al., 2016). Accordingly, we followed the protocol of Almeida et al. (2011) to improve remineralization and, we further reapplied the varnishes each 6 months to achieve the preventive effect (HEDMAN; GABRE; BIRKHED, 2015).

Güçlü et al. (2016) demonstrated no difference in the remineralization of white spot lesions in permanent teeth treated with NaF varnish (once week for 4 weeks) compared to control, after a follow-up of 3 months, by visual examination and fluorescence quantification (DIAGNOdent). Our study also did not show remineralizing effect of NaF. A slight remineralizing effect was found only for TiF_4 by using QLF. The main hypothesis to justify the low effect of fluorides varnishes, especially NaF one, on the prevention or regression of dental caries at the present study is the low caries rate incidence found in the population. Recently, a paper revised the rate of caries progression, showing that the progression is proportional to the severity, with a mean of DMFS increment about 0.11 a year (HUMMEL et al., 2019). The authors suggested longer follow-up periods for clinical studies on permanent teeth (> 36 months) (HUMMEL et al., 2019). Considering that our population had no severe caries lesions (DMFS 0.05 ± 0.22 at baseline), we advise a longer follow up periods for future studies with this population or to test the products in a high-caries risk population.

The most common method for caries detection is the visual-tactile by using ICDAS, Nyvad and DMFT indexes. The traditional DMFT index does not include enamel caries lesions (MELGAR et al., 2016). Therefore, we applied ICDAS, which is an accurate and reproducible method for detecting and monitoring early enamel lesions over time (GOMEZ, 2015). Braga et al (2010) compared two methods of visual inspection (Nyvad and ICDAS), and both presented good reproducibility and validity to detect and estimate the depth of the caries lesions, justifying their inclusion in the present study. A recent systematic review demonstrated that ICDAS is a visual

diagnosis system with high accurate for carious lesions detection but not for assessing the caries lesion progression (EKSTRAND et al., 2018). Considering the limitation of ICDAS to assess lesion progression/regression, we combined Nyvad index and QLF analysis (GOMEZ, 2015).

QLF and DIAGNOdent systems are noninvasive techniques used for research purpose, by quantifying the mineral loss of early caries lesions by fluorescence (GOMEZ, 2015). QLF is a sensible quantitative complementary method with good repeatability and reproducibility, requiring a smaller number of participants (that may decrease the impact of dropout for longitudinal studies) compared to the visual analysis (TRANAEUS et al., 2001). A recent *in vitro* study showed that QLF was more efficient in detecting early and shallow caries lesions than a spectrophotometer (which evaluates the subtle color difference from sound to the demineralized enamel). However, QLF is limited for detecting very deep lesions since light scattering may not reflect fully deep mineral loss (> 200 µm depth) (KIM, KIM, 2018). Therefore, QLF is an appropriate method, with good repeatability and reproducibility, to quantify small mineral changes that might not be detectable by visual inspection (SITTHISETTAPONG et al., 2015), as it was the case of the present study.

The biofilm control is an important factor that could have influenced the effect of the treatments (MARINHO et al., 2013). In fact, we did not find differences among the groups with respect to percentage of visible plaque. Instead, we found a biofilm control improvement after 3 months of study, highlighting that the frequent presence of the researchers at the schools motivated the children to better brush their teeth. The improvement of biofilm control might have prevented dental caries development and progression, minimizing the effect of treatments.

With respect to degree of acceptance, the majority of the children reported to be pleasant, regardless of the treatment. This result is very interesting, once we expected that TiF_4, due to its low pH, could have caused an unpleased taste change, as reported in an *in situ* study (DE SOUZA et al., 2018). Furthermore, no subject reported tooth staining or any other side-effect due to the fluorides varnishes application. A recent *in situ* study showed that 27% of the subjects complained about the taste change and 40% about a temporary tooth staining, due to the daily use of TiF_4 solution as mouthwash for 5 days (DE SOUZA et al., 2018). Differently from

mouthrinses, varnishes are applied in low quantity on tooth surfaces, with low direct contact with tongue, reducing eventual effect on the taste change. Furthermore, it is applied each 6 months; therefore, the risk of tooth staining is lower compared to the daily application of a mouthrinse.

As conclusion, TiF_4 varnish is the only treatment able to show slight remineralizing and preventive effect under this study model. Considering the limitations of this RCT, the authors suggest that future studies shall be done during longer periods (> 36 months), despite of the high probability of dropout overtime, to allow seeing caries lesion progression/regression clinically. Furthermore, a high caries-risk population shall be included in the RCT to check the effect of TiF_4 varnish under a worst scenario.

REFERENCES

REFERENCES

Agouropoulos A, Twetman S, Pandis N, Kavvadia K, Papagiannoulis L. Caries preventive effectiveness of fluoride varnish as adjunct to oral health promotion and supervised tooth brushing in preschool children: a double-blind randomized controlled trial. J Dent. 2014;42(10):1277-1283.

Ahovuo-Saloranta A, Forss H, Hiiri A, Nordblad A, Mäkelä M. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in the permanent teeth of children and adolescents. Cochrane Database Syst Rev. 2016;(1):CD003067.

Alaluusua S, Malmivirta R. Early plaque accumulation--a sign for caries risk in young children. Community Dent Oral Epidemiol. 1994;22(5 Pt 1):273-276.

Alexandria AK, Nassur C, Nóbrega CBC, Branco-de-Almeida LS, Dos Santos KRN, Vieira AR et al. Effect of TiF(4) varnish on microbiological changes and caries prevention: in situ and in vivo models. Clin Oral Investig. 2019;23(6):2583-2591.

Almeida MQ, Costa OX, Ferreira JM, Menezes VA, Leal RB, Sampaio FC. Therapeutic potential of Brazilian fluoride varnishes: an in vivo study. Braz Dent J. 2011;22(3):193-197.

Aranda Salomão PM, de Oliveira FA, Dos Santos DMS, Domezi JP, Dionísio TJ, de Oliveira RC et al. TiF(4) and NaF varnishes induce low levels of apoptosis in murine and human fibroblasts through mitochondrial Bcl-2 family and death receptor signalling. Arch Oral Biol. 2019;97:245-252.

Ardenghi TM, Piovesan C, Antunes JL. Inequalities in untreated dental caries prevalence in preschool children in Brazil. Rev Saude Publica. 2013;47(Suppl 3):129-137.

Arruda AO, Senthamarai Kannan R, Inglehart MR, Rezende CT, Sohn W. Effect of 5% fluoride varnish application on caries among school children in rural Brazil: a randomized controlled trial. Community Dent Oral Epidemiol. 2012;40(3):267- 276.

Braga MM, Ekstrand KR, Martignon S, Imparato JC, Ricketts DN, Mendes FM. Clinical performance of two visual scoring systems in detecting and assessing activity status of occlusal caries in primary teeth. Caries Res. 2010;44(3):300-308.

Comar LP, Souza BM, Al-Ahj LP, Martins J, Grizzo LT, Piasentim IS et al. Mechanism of Action of TiF_4 on Dental Enamel Surface: SEM/EDX, KOH-Soluble F, and X-Ray Diffraction Analysis. Caries Res. 2018;51(6):554-567.

Comar LP, Souza BM, Grizzo LT, Buzalaf MA, Magalhães AC. Evaluation of fluoride release from experimental TiF_4 and NaF varnishes in vitro. J Appl Oral Sci. 2014;22(2):138-143.

Comar LP, Souza BM, Martins J, Santos MG, Buzalaf MAR, Magalhães AC. Response of carious enamel to TiF(4) varnish treatment under diverse cariogenic activities in situ. J Dent. 2017;63:81-84.

Comar LP, Wiegand A, Moron BM, Rios D, Buzalaf MAR, Buchalla W et al**.** Effect of TiF_4 and NaF varnish and solution on enamel demineralisation *in situ***.** Eur J Oral Sci. 2012;120(4):342-348.

de Souza BM, Santi LRP, de Souza Silva M, Buzalaf MAR, Magalhães AC. Effect of an experimental mouth rinse containing NaF and TiF(4) on tooth erosion and abrasion in situ. J Dent. 2018;73:45-49.

Divaris K, Preisser JS, Slade GD. Surface-specific efficacy of fluoride varnish in caries prevention in the primary dentition: results of a community randomized clinical trial. Caries Res. 2013;47(1):78-87.

Dos Santos DMS, Pires JG, Silva AB, Salomão PMA, Buzalaf MAR, Magalhães AC. Protective effect of 4% titanium tetrafluoride varnish on dentin demineralization using microcosm biofilm model. Caries Res. 2019;14:1-8.

Douglas GV, Ramsdale MP, Vinall-Collier K, Csikar JI. Using High Fluoride Concentration Products in Public Policy: A Rapid Review of Current Guidelines for High Fluoride Concentration Products. Caries Res. 2016;50(Suppl 1):50-60.

Ekstrand KR, Gimenez T, Ferreira FR, Mendes FM, Braga MM. The International Caries Detection and Assessment System - ICDAS: A Systematic Review. Caries Res. 2018;52(5):406-419.

El Batawi HY. Effect of intraoperative analgesia on children's pain perception during recovery after painful dental procedures performed under general anaesthesia. Eur Arch Paediatr Dent. 2015;16(1):35-41.

Gao SS, Zhang S, Lo EC, Chu C. Caries remineralization and arresting effect in children by professionally applied fluoride treatment – a systematic review. BMC Oral Health. 2016;16:12.

Gomez J. Detection and diagnosis of the early caries lesion. BMC Oral Health. 2015;15(Suppl 1):S3.

Güçlü ZA, Alaçam A, Coleman NJ. A 12-Week Assessment of the Treatment of White Spot Lesions with CPP-ACP Paste and/or Fluoride Varnish. Biomed Res Int. 2016;2016:8357621.

Hardman MC, Davies GM, Duxbury JT, Davies RM. A cluster randomised controlled trial to evaluate the effectiveness of fluoride varnish as a public health measure to reduce caries in children. Caries Res. 2007;41(5):371-376.

Hedman E, Gabre P, Birkhed D. Dental hygienists working in schools – a two-year oral health intervention programme in swedish secondary schools. Oral Health Prev Dent. 2015;13(2):177-188.

Hummel R, Akveld NAE, Bruers JJM, van der Sanden WJM, Su N, van der Heijden GJMG. Caries progression rates revised: a systematic review. J Dent Res. 2019;9: 22034519847953.

Jiang EM, Lo EC, Chu CH, Wong MC. Prevention of early childhood caries (ECC) through parental toothbrushing training and fluoride varnish application: a 24-month randomized controlled trial. J Dent. 2014;42(12):1543-1550.

Kim HE, Kim BI. Early caries detection methods according to the depth of the lesion: An in vitro comparison. Photodiagnosis Photodyn Ther. 2018;23:176-180.

Magalhães AC, Comar LP, Rios D, Delbem AC, Buzalaf MA. Effect of a 4% titanium tetrafluoride (TiF_4) varnish on demineralisation and remineralisation of bovine enamel in vitro. J Dent. 2008;36(2):158-162.

Magalhães AC, Dos Santos MG, Comar LP, Buzalaf MA, Ganss C, Schlueter N. Effect of a Single Application of TiF_4 Varnish versus Daily Use of a Low-Concentrated TiF_4/NaF Solution on Tooth Erosion Prevention in vitro. Caries Res. 2016;50(5):462-470.

Marinho VC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2013;7:CD002279.

Melgar RA, Pereira JT, Luz PB, Hugo FN, Araujo FB. Differential Impacts of Caries Classification in Children and Adults: A Comparison of ICDAS and DMF-T. Braz Dent J. 2016;27(6):761-766.

Milsom KM, Blinkhorn AS, Walsh T, Worthington HV, Kearney-Mitchell P, Whitehead H, Tickle M. A cluster-randomized controlled trial: fluoride varnish in school children. J Dent Res. 2011;90(11):1306-1311.

Nyvad B, Machiulskiene V, Baelum V. Construct and predictive validity of clinical caries diagnostic criteria assessing lesion activity. J Dent Res. 2003;82(2):117-122.

Nyvad B, Machiulskiene V, Baelum V. Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions. Caries Res. 1999;33:252-260.

Patil SK, Fatangare M, Jadhav RG, Shinde GR, Pawar SS, Kathariya MD. Caries preventive effect of sodium fluoride varnish on deciduous dentition: a clinical trial. J Contemp Dent Pract. 2017;18(12)1190-1193.

Pomarico L, Villardi M, Maia LC. In vivo effect of titanium tetrafluoride and sodium monofluorophosphate dentifrice on remineralization: a preliminary split-mouth clinical trial. Gen Dent. 2012;60(4):e249-254.

Reed AJ, Bibby BG. Preliminary report on effect of topical applications of titanium tetrafluoride on dental caries. J Dent Res. 1976;55(3):357-358.

Salomão PMA, Oliveira FA, Rodrigues PD, Al-Ahj LP, Gasque KCDS, Jeggle P et al. The cytotoxic effect of TiF_4 and NaF on fibroblasts is influenced by the experimental model, fluoride concentration and exposure time. PLoS One. 2017;12(6):e0179471.

Shivakumar K, Prasad S, Chandu G. International Caries Detection and Assessment System: A new paradigm in detection of dental caries. J Conserv Dent. 2009;12(1):10-16.

Sitthisettapong T, Doi T, Nishida Y, Kambara M, Phantumvanit P. Effect of CPP-ACP Paste on Enamel Carious Lesion of Primary Upper Anterior Teeth Assessed by Quantitative Light-Induced Fluorescence: A One-Year Clinical Trial. Caries Res. 2015;49(4):434-441.

Sköld UM, Petersson LG, Birkhed D, Norlund A. Cost-analysis of school-based fluoride varnish and fluoride rinsing programs. Acta Odontol Scand. 2008;66(5):286-292.

Souza BM, Fernandes Neto C, Salomão PMA, Vasconcelos LRSM, Andrade FB, Magalhães AC. Analysis of the antimicrobial and anti-caries effects of TiF_4 varnish under microcosm biofilm formed on enamel. J Appl Oral Sci. 2018a;26:e20170304.

Souza BM, Santos DMS, Braga AS, Santos NMD, Rios D, Buzalaf MAR, et al. Effect of a Titanium Tetrafluoride Varnish in the Prevention and Treatment of Carious Lesions in the Permanent Teeth of Children Living in a Fluoridated Region: Protocol for a Randomized Controlled Trial. JMIR Res Protoc. 2018b;7(1):e26.

Spineli LM, Fleming PS, Pandis N. Addressing missing participant outcome data in dental clinical trials. J Dent. 2015;43(6):605-618.

Tellez M, Wolff MS. The Public Health Reach of High Fluoride Vehicles: Examples of Innovative Approaches. Caries Res. 2016;50 Suppl 1:61-67.

Tranaeus S, Al-Khateeb S, Björkman S, Twetman S, Angmar-Månsson B. Application of quantitative light-induced fluorescence to monitor incipient lesions in caries-active children. A comparative study of remineralisation by fluoride varnish and professional cleaning. Eur J Oral Sci. 2001;109(2):71-75.

Urquhart O, Tampi MP, Pilcher L, Slayton RL, Araujo MWB, Fontana M, et al. Nonrestorative Treatments for Caries: Systematic Review and Network Meta-analysis. J Dent Res. 2019;98(1):14-26.

Weintraub JA, Ramos-Gomez F, Jue B, Shain S, Hoover CI, Featherstone JD et al. Fluoride varnish efficacy in preventing early childhood caries. J Dent Res. 2006;85(2):172-176.

Wiegand A, Magalhães AC, Attin T. Is titanium tetrafluoride (TiF_4) effective to prevent carious and erosive lesions? A review of the literature. Oral Health Prev Dent. 2010;8(2):159-164.

Zero DT. Dental caries process. Dent Clin North Am. 1999;43(4):635-664.

APPENDIX

APÊNDICE A- **TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO**

Universidade de São Paulo
Faculdade de Odontologia de Bauru
Departamento de Ciências Biológicas
Disciplina de Bioquímica

Convite ao Participante

Nome do participante da pesquisa:_____

1 - Título do Trabalho Experimental

"**Efeito de um verniz de TiF_4 na prevenção e remineralização de lesões cariosas na dentição permanente de crianças residentes em área fluoretada: estudo clínico randomizado de 18 meses**".

2 – Objetivo

Avaliar o efeito de um novo verniz com flúor (material resinoso aplicado no dente de permanência temporária) para a prevenção e tratamento de manchas brancas (estágio inicial da cárie).

3 - Procedimentos da Fase Experimental

Estamos convidando o seu filho para participar deste estudo clínico, em que serão selecionadas crianças, entre 7-12 anos de idade, regularmente matriculadas na rede de EMEFs do município de Bauru-SP. As crianças selecionadas não podem ter ingerido antibióticos e nem terem sido submetidas à aplicação de flúor nos últimos meses. Ainda precisam apresentar pelo menos uma lesão de mancha branca visível (lesão de cárie). Os profissionais realizarão avaliação clínica e utilizarão um equipamento chamado QLF do qual tirarão fotos dos dentes com lesões de cárie para quantificar a gravidade da lesão. Estes procedimentos serão realizados na própria escola. Caso seja detectada alguma lesão de cárie com cavitação ("buraco"), a criança será encaminhada para tratamento sob responsabilidade da pesquisadora, para que o dente com "buraco" seja restaurado ("obturado") com material apropriado (resina composta ou CIV ou amálgama) conforme indicação. Neste caso, o exame radiográfico e tratamento serão realizados na Faculdade de Odontologia de Bauru, conforme agendamento, sendo que a pesquisadora responsável arcará com os custos do transporte. O tratamento restaurador, caso necessário, será realizado antes do início da aplicação do verniz (tratamento referente à pesquisa).

As crianças serão alocadas para um dos tratamentos, conforme sorteio aleatório: verniz TiF_4 (novo) ou verniz NaF (padrão) ou verniz placebo (sem flúor). Não haverá direito de escolha do grupo de tratamento e o participante só saberá que tratamento fez parte ao final do estudo. Os profissionais aplicarão o produto 1x/semana, durante 4 semanas, na própria escola. Ao término das aplicações, será recomendado ao participante que não ingira liquidos por 30 min e evite comer alimentos duros (bolachas, frutas, balas, chicletes...)ou escovar os dentes por pelo menos quatro horas após a aplicação, conforme orientação do fabricante (Colgate). Todos os participantes receberão as mesmas instruções de higiene bucal e aconselhamento sobre hábitos alimentares saudáveis. Além disso, os participantes receberão um Kit de higiene a cada 3 meses, contendo escova de dentes macia adequada ao tamanho da boca da criança, pastas de dente com flúor e fio dental, para a higienização diária. As crianças serão novamente avaliadas após 1 mês, 6 meses, 12 meses e 18 meses do término do tratamento.

4 - Benefícios do Experimento

O uso dos vernizes com flúor é mundialmente aceito para prevenção e tratamento da cárie dentária. Dessa forma, a aplicação dos vernizes, associada à educação em saúde bucal, ajudará no controle desta doença, reduzindo a progressão da lesão e a necessidade de tratamentos mais invasivos, e consequentemente, melhorando a qualidade de vida do seu filho.

As crianças participantes se beneficiarão diretamente do estudo, pois receberão avaliações clínicas periódicas, escovas de dente, pastas de dente e fio dental e orientações sobre higiene bucal

Rubrica do Participante da Pesquisa :

Rubrica do Pesquisador Responsável :

Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-901 – C.P. 73
e-mail: droliveir@fob.usp.br - Fone (0xx14) 3235-8497
http://www.fob.usp.br

Universidade de São Paulo
Faculdade de Odontologia de Bauru
Departamento de Ciências Biológicas
Disciplina de Bioquímica

e dieta (educação em saúde bucal). De acordo com o exame clínico, radiográficas e o tratamento restaurador poderão ser indicados, caso haja suspeita e confirmação da presença de lesões cavitadas ("buracos"), respectivamente. A consulta para a realização de radiografias e do tratamento restaurador ("obturação") ocorrerá na Faculdade de Odontologia de Bauru, no Laboratório de Fisiologia e Farmacologia Clinica (LAFFIC) alocado na Disciplina de Farmacologia e pertencente ao Departamento de Ciências Biológicas da FOB-USP, sob responsabilidade da pesquisadora, que tratará o seu filho gratuitamente e arcará com as despesas de transporte do participante e seu responsável até a FOB-USP.

Após a conclusão da pesquisa (18 meses após a finalização do tratamento), todos os participantes receberão a aplicação do verniz fluoretado (novo ou padrão) que apresentar o melhor resultado.

5 - Riscos do Experimento

O uso de vernizes fluoretados é um procedimento já consolidado para prevenção e tratamento da cárie dentária. A criança será submetida ao exame clínico sob luz natural e com espelho clínico. Nós também utilizaremos uma micro câmera de captura de imagem (QLF) de tamanho compatível com a cavidade bucal da criança, e sem nenhuma estrutura que possa machucar a mesma.

Portanto, o risco ao participante é mínimo, que acontecerá no caso de alergia a algum componente do verniz utilizado, o que pode gerar enjoos ou edema (inchaço), e neste caso será necessária a remoção do verniz. Caso isso aconteça, o participante/responsável deverá entrar em contato com a pesquisadora responsável, que irá ao local (escola ou residência) realizar a remoção do produto. Vale resaltar, que tais reações têm sido reportadas como raríssimos pela bula do fabricante. O participante pode apresentar também problemas comportamentais na hora do exame e da aplicação do tratamento. Em todos os casos, o participante será dispensado da participação na pesquisa, sem prejuízo ao mesmo.

A possibilidade do seu filho estar alocado no grupo verniz placebo também não causará prejuízo ao mesmo, pois todas as crianças receberão instrução de dieta e higiene bucal necessárias para a prevenção da cárie dentária. Caso lesões cariosas cavitadas ("buracos") sejam detectadas durante o estudo, tratamento restaurador ("obturação") gratuito será oferecido e, ao final do experimento, o verniz fluoretado que obtiver melhor resultado será aplicado nos dentes da criança.

Os gastos que forem gerados por este trabalho (deslocamento do participante à FOB-USP) ficará a cargo da responsável pelo projeto. Importante ressaltar que não está sendo considerado nenhum pagamento ou recompensa material pela participação neste estudo. O participante terá garantido o direito à indenização compensatória caso fique comprovado que a participação acarretou algum problema à criança.

Fica claro que o responsável poderá, a qualquer momento, retirar seu CONSENTIMENTO LIVRE E ESCLARECIDO e deixar de participar do estudo alvo da pesquisa e ciente que todo trabalho realizado torna-se informação confidencial guardada por força do sigilo profissional (Art. 9º do Código de Ética Odontológica).

O seu filho também receberá um termo de assentimento para assinatura, com linguagem própria para a idade dele, explicando como será a sua participação na pesquisa. Ele terá a liberdade em concordar ou discordar da participação na pesquisa, a qualquer momento, sem nenhum prejuízo ao mesmo.

Para esclarecimento de dúvidas sobre a participação na pesquisa, entrar em contato com qualquer um dos membros da equipe do projeto por meio do endereço Al. Dr. Octávio Pinheiro Brisolla, 9-75 (CEP: 17012-901), telefone (14) 3235-5846 ou com a pesquisadora responsável

Universidade de São Paulo
Faculdade de Odontologia de Bauru
Departamento de Ciências Biológicas
Disciplina de Bioquímica

Beatriz Martines de Souza (telefone 14 98154-8927, e-mail beatriz.martines.souza@usp.br), referindo-se à pesquisa intitulada **"Efeito de um verniz de TiF_4 na prevenção e remineralização de lesões cariosas na dentição permanente de crianças residentes em área fluoretada: estudo clínico randomizado de 18 meses"**. Em caso de denúncias e/ou reclamações, entrar em contato com Comitê de Ética em Pesquisa FOB/USP, à Alameda Dr. Octávio Pinheiro Brisolla, 9-75, Vila Universitária, ou pelo telefone (14)3235-8356, e-mail: cep@fob.usp.br.

Universidade de São Paulo
Faculdade de Odontologia de Bauru
Departamento de Ciências Biológicas
Disciplina de Bioquímica

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Pelo presente instrumento que atende às exigências legais, o Sr.(a) _____, portador da cédula de identidade _____, responsável pelo menor _____, após leitura minuciosa das informações constantes neste TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO, devidamente explicada pelos profissionais em seus mínimos detalhes, ciente dos serviços e procedimentos aos quais será submetido, não restando quaisquer dúvidas a respeito do lido e explicado, DECLARA e FIRMA seu CONSENTIMENTO LIVRE E ESCLARECIDO concordando em participar da pesquisa proposta. Fica claro que o participante da pesquisa, pode a qualquer momento retirar seu CONSENTIMENTO LIVRE E ESCLARECIDO e deixar de participar desta pesquisa e ciente de que todas as informações prestadas tornar-se-ão confidenciais e guardadas por força de sigilo profissional (Art. 9º do Código de Ética Odontológica)

Por fim, como pesquisador(a) responsável pela pesquisa, DECLARO o cumprimento do disposto na Resolução CNS nº 466 de 2012, contidos nos itens IV.3 e item IV.5.a e na íntegra com a resolução CNS nº 466 de dezembro de 2012.

Por estarmos de acordo com o presente termo o firmamos em duas vias igualmente válidas (uma via para o participante da pesquisa e outra para o pesquisador) que serão rubricadas em todas as suas páginas e assinadas ao seu término, conforme o disposto pela Resolução CNS nº 466 de 2012, itens IV.3.f e IV.5.d.

Rubrica do Participante da Pesquisa :

Rubrica do Pesquisador Responsável :

Bauru, SP, _____ de _____ de _____.

_____
Assinatura do Responsável pelo menor

_____
Beatriz Martines de Souza
Pesquisadora Responsável

Nome legível:_____

O **Comitê de Ética em Pesquisa – CEP**, organizado e criado pela **FOB-USP**, em 29/06/98 (**Portaria GD/0698/FOB**), previsto no item VII da Resolução CNS nº 466/12 do Conselho Nacional de Saúde do Ministério da Saúde (publicada no DOU de 13/06/2013), é um Colegiado interdisciplinar e independente, de relevância pública, de caráter consultivo, deliberativo e educativo, criado para defender os interesses dos participantes da pesquisa em sua integridade e dignidade e para contribuir no desenvolvimento da pesquisa dentro de padrões éticos.

Qualquer denúncia e/ou reclamação sobre sua participação na pesquisa poderá ser reportada a este CEP:

Horário e local de funcionamento:

Comitê de Ética em Pesquisa
Faculdade de Odontologia de Bauru-USP - Prédio da Pós-Graduação (bloco E - pavimento superior), de segunda à sexta-feira, no horário das **14hs às 17 horas**, em dias úteis.
Alameda Dr. Octávio Pinheiro Brisolla, 9-75
Vila Universitária – Bauru – SP – CEP 17012-901
Telefone/FAX(14)3235-8356
e-mail: cep@fob.usp.br

APÊNDICE B – **TERMO DE ASSENTIMENTO**

Universidade de São Paulo
Faculdade de Odontologia de Bauru

Departamento de Ciências Biológicas

Disciplina de Bioquímica

Convite ao Participante

Nome do participante da pesquisa:_____

Você está sendo convidado a participar da pesquisa **"Efeito de um verniz de TiF_4 na prevenção e remineralização de lesões cariosas na dentição permanente de crianças residentes em área fluoretada: estudo clínico randomizado de 18 meses".** Seus pais permitiram que você participasse deste estudo.

Neste estudo testaremos um novo produto com flúor para prevenir e tratar a cárie dentária antes que ela se torne um buraco no seu dente. Esperamos que este novo produto seja melhor do que o que está sendo usado atualmente. Para descobrir se ele é melhor, temos que testá-lo.
Você deverá ter entre 7 anos e 12 anos de idade e não ter sido submetido à aplicação de flúor por dentista ou ter tomado remédio nos últimos meses.
Você não precisa participar da pesquisa se não quiser, é um direito seu. Não haverá problema se desistir. Antes de começar esse estudo, caso seja encontrada alguma cárie (buraco no dente), trataremos seu dente (colocar massinha no buraco).
A pesquisa será feita na sua própria escola da seguinte forma:

1° dia

-Você vai receber um kit com escova, pasta de dente e fio dental. O primeiro passo é você deixar os dentes bem limpinhos.
- Após a escovação, vamos examinar a sua boca com espelhinho e tirar fotos dos seus dentes com uma micro câmera fotográfica.

2° dia

-Vamos aplicar o verniz com flúor ("uma capinha de proteção temporária") em todos os seus dentes. Você terá que ficar pelo menos 30 min sem ingerir líquidos (água, suco, leite), ficar sem comer alimentos duros (bolachas, chicletes e frutas) e escovar os dentes depois de 4 horas, para a "capinha" ficar mais tempo na sua boca. Não há problema em não escovar os dentes por algumas horas.
- Vamos repetir a aplicação por mais 3 semanas seguidas!!!! Tudo de novo: escovar os dente e abrir a boca para que possamos aplicar o produto.

Durante o período de 1 ano e meio (18 meses), faremos visitas periódicas na escola para ver se o tratamento funcionou. Nós faremos o exame clínico e as fotos dos seus dentes após 1, 6, 12 e 18 meses do término do tratamento. Durante este período você receberá novas escovas de dente, pastas de dente e fio dental.
O uso verniz é considerado seguro, mas talvez você sinta um sabor desagradável após a aplicação, mas é temporário. Se você engolir o produto, não haverá problema algum. O importante é você seguir as orientações. Caso você tenha algum incomodo, você pode nos ligar (14)98154-8927 (Beatriz Martines de Souza).
Ninguém saberá que você está participando da pesquisa, não falaremos a outras pessoas, nem daremos informações sobre você a estranhos. Os resultados da pesquisa serão publicados, mas sem identificar as crianças que participaram do estudo. Se você tiver alguma dúvida, você poderá sempre perguntar para a pesquisadora Beatriz.

Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-901 – C.P. 73
e-mail: droliveir@fob.usp.br - Fone (0xx14) 3235-8497
http://www.fob.usp.br

Universidade de São Paulo
Faculdade de Odontologia de Bauru
Departamento de Ciências Biológicas
Disciplina de Bioquímica

Termo de Assentimento

Eu _____
aceito participar da pesquisa **"Efeito de um verniz de TiF_4 no controle de lesões iniciais de cárie dentária na dentição permanente de crianças residentes em área fluoretada: estudo clínico randomizado de 18 meses"**. Entendi as coisas ruins e as coisas boas que podem acontecer. Entendi que posso dizer "sim" e participar, mas que, a qualquer momento, posso dizer "não" e desistir, que ninguém vai ficar bravo ou triste. Os pesquisadores tiraram minhas dúvidas e conversaram com os meus responsáveis. Recebi uma cópia deste termo de assentimento e li e concordo em participar da pesquisa.

Bauru, ____de _____de _____.

_____ _____
Assinatura do menor Beatriz Martines de Souza

O **Comitê de Ética em Pesquisa – CEP**, organizado e criado pela **FOB-USP**, em 29/06/98 (**Portaria GD/0698/FOB**), previsto no item VII da Resolução CNS nº 466/12 do Conselho Nacional de Saúde do Ministério da Saúde (publicada no DOU de 13/06/2013), é um Colegiado interdisciplinar e independente, de relevância pública, de caráter consultivo, deliberativo e educativo, criado para defender os interesses dos participantes da pesquisa em sua integridade e dignidade e para contribuir no desenvolvimento da pesquisa dentro de padrões éticos.
Qualquer denúncia e/ou reclamação sobre sua participação na pesquisa poderá ser reportada a este CEP:

Horário e local de funcionamento:
Comitê de Ética em Pesquisa
Faculdade de Odontologia de Bauru-USP - Prédio da Pós-Graduação (bloco E - pavimento superior), de segunda à sexta-feira, no horário das **14hs às 17 horas**, em dias úteis.
Alameda Dr. Octávio Pinheiro Brisolla, 9-75
Vila Universitária – Bauru – SP – CEP 17012-901
Telefone/FAX(14)3235-8356
e-mail: cep@fob.usp.br

APÊNDICE C – **FICHA CLÍNICA**

Região_____

Projeto de Pesquisa Doutorado
Bauru 2017-2019

EMEF_____ **Sala**_____

Nome _____

Sexo: (___) Feminino (___) Masculino **DN:**_____/_____/_____ - **Idade**_____

Exame: (___) Baseline (___) 1 mês (___) 6 meses (___)12 meses (__)18 meses

Data: _____/_____/_____

QLF_____

NYVAD/ICDAS

DENTE	V		M		D		L/P		OCLUSAL	
16										
12									X	X
11									X	X
21									X	X
22									X	X
26										
36										
32									X	X
31									X	X
41									X	X
42									X	X
46										

OBSERVAÇÕES_____

_____

_____

APÊNDICE D – **FICHA CLÍNICA DO ÍNDICE DE PLACA VISÍVEL (IPV)**

Projeto de Pesquisa Doutorado FOB/USP
Bauru 2016 - 2019

EMEF_____ Sala_____

Nome _____

Data _____/_____/_____

Verniz ( ) A ( ) B ( ) C

FICHA DE IPV

ANTES DA ESCOVAÇÃO

16	15	14	13	12	11

21	22	23	24	25	26

55	54	53	52	51

61	62	63	64	65

46	45	44	43	42	41

31	32	33	34	35	36

85	84	83	82	81

71	72	73	74	75

ANNEX

ANEXO A – **APROVAÇÃO PELO CEP**

FACULDADE DE ODONTOLOGIA DE BAURU-USP

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito de um verniz de TiF4 na prevenção e remineralização de lesões cariosas na dentição permanente de crianças residentes em área fluoretada: estudo clínico randomizado de 18 meses

Pesquisador: Beatriz Martines de Souza

Área Temática:

Versão: 2

CAAE: 59787116.2.0000.5417

Instituição Proponente: Universidade de Sao Paulo

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.829.117

Apresentação do Projeto:

idem ao Parecer 1.750.208

Objetivo da Pesquisa:

idem ao Parecer 1.750.208

Avaliação dos Riscos e Benefícios:

idem ao Parecer 1.750.208

Comentários e Considerações sobre a Pesquisa:

Todas as pendências foram esclarecidas

Considerações sobre os Termos de apresentação obrigatória:

Todos os temos foram apresentados

Recomendações:

Sem recomendações

Conclusões ou Pendências e Lista de Inadequações:

Sem recomendações

Endereço: DOUTOR OCTAVIO PINHEIRO BRISOLLA 75 QUADRA 9
Bairro: VILA NOVA CIDADE UNIVERSITARIA **CEP:** 17.012-901
UF: SP **Município:** BAURU
Telefone: (14)3235-8356 **Fax:** (14)3235-8356 **E-mail:** cep@fob.usp.br

FACULDADE DE ODONTOLOGIA DE BAURU-USP

Continuação do Parecer: 1.829.117

Considerações Finais a critério do CEP:

Esse projeto foi considerado APROVADO na reunião ordinária do CEP de 16.11.2016, com base nas normas éticas da Resolução CNS 466/12. Ao término da pesquisa o CEP-FOB/USP exige a apresentação de relatório final. Os relatórios parciais deverão estar de acordo com o cronograma e/ou parecer emitido pelo CEP. Alterações na metodologia, título, inclusão ou exclusão de autores, cronograma e quaisquer outras mudanças que sejam significativas deverão ser previamente comunicadas a este CEP sob risco de não aprovação do relatório final. Quando da apresentação deste, deverão ser incluídos todos os TCLEs e/ou termos de doação assinados e rubricados, se pertinentes.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_603544.pdf	27/10/2016 16:23:45		Aceito
Outros	Oficio_cep_Beatriz.pdf	27/10/2016 16:23:14	Beatriz Martines de Souza	Aceito
Folha de Rosto	folha_de_rosto_atualizada.pdf	26/10/2016 17:11:21	Beatriz Martines de Souza	Aceito
Projeto Detalhado / Brochura Investigador	projeto_detalhado_atualizado_beatriz.pdf	26/10/2016 17:09:50	Beatriz Martines de Souza	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	termo_assentimento_atualizado_beatriz.pdf	26/10/2016 17:09:25	Beatriz Martines de Souza	Aceito
Outros	termo_aquiescencia_secretariamunicipal_BeatrizSouza.pdf	01/09/2016 10:15:20	Beatriz Martines de Souza	Aceito
Outros	termo_de_aquiescencia_clinica_LAFFIC.pdf	31/08/2016 16:21:05	Beatriz Martines de Souza	Aceito
Outros	termo_de_compromisso_com_os_resultados.JPG	31/08/2016 14:56:59	Beatriz Martines de Souza	Aceito
Outros	termo_de_aquiescencia_departamento.JPG	31/08/2016 14:53:49	Beatriz Martines de Souza	Aceito
Outros	QuestionarioTecnicoPesquisador_Bia.pdf	31/08/2016 14:51:14	Beatriz Martines de Souza	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Beatriz_Souza.pdf	31/08/2016 12:13:50	Beatriz Martines de Souza	Aceito

Situação do Parecer:

Endereço: DOUTOR OCTAVIO PINHEIRO BRISOLLA 75 QUADRA 9
Bairro: VILA NOVA CIDADE UNIVERSITARIA **CEP:** 17.012-901
UF: SP **Município:** BAURU
Telefone: (14)3235-8356 **Fax:** (14)3235-8356 **E-mail:** cep@fob.usp.br

FACULDADE DE ODONTOLOGIA DE BAURU-USP

Continuação do Parecer: 1.829.117

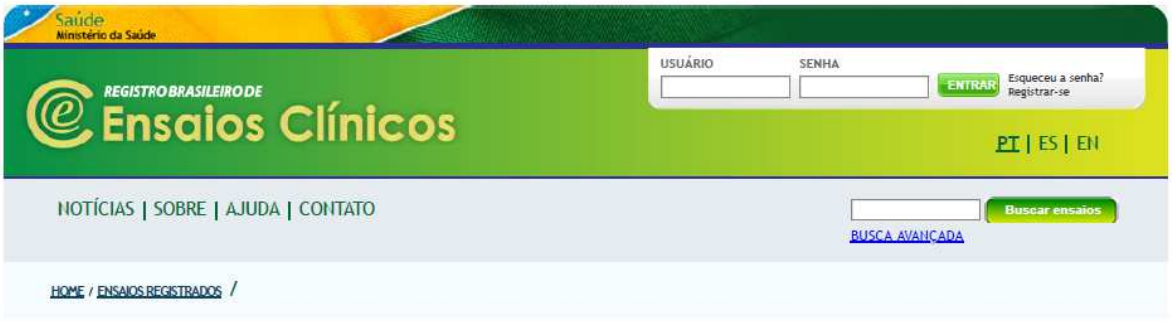
Aprovado

Necessita Apreciação da CONEP:
Não

BAURU, 22 de Novembro de 2016

Assinado por:
Ana Lúcia Pompéia Fraga de Almeida
(Coordenador)

ANEXO B - **CADASTRO NO REGISTRO BRASILEIRO DE ENSAIOS CLÍNICOS (REBEC)**



RBR-5vwj4y

Efeito de um Verniz de TiF4 na Prevenção e Remineralização de lesões cariosas na dentição permanente de crianças residentes em área fluoretada: estudo clínico randomizado de 18 meses

Data de registro: 5 de Out. de 2015 às 15:27

Last Update: 18 de Out. de 2017 às 17:04

Tipo do estudo:

Intervenções

Título científico:

PT-BR	EN
Efeito de um Verniz de TiF4 na Prevenção e Remineralização de lesões cariosas na dentição permanente de crianças residentes em área fluoretada: estudo clínico randomizado de 18 meses	Effect of a TiF4 Varnish in the Prevention and Remineralization of caries lesions in permanent teeth of children living in a fluoridated region: randomized clinical trial of 18 months

Identificação do ensaio

Número do UTN: U1111-1175-2380

Título público:

PT-BR	EN
Efeito de um Verniz com Flúor na Prevenção da Cárie Dentária em dentes permanentes: estudo clínico randomizado de 18 meses	Effect of a Fluoride Varnish on the Prevention of Dental Caries in permanent teeth: randomized clinical study of 18 months

Acrônimo científico:

Acrônimo público:

Identificadores secundários:

59787116.2.0000.5417

Órgão emissor: Plataforma Brasil

1.829.117

Órgão emissor: Comitê de Ética em Pesquisa em seres Humanos da Faculdade de Odontologia de Bauru -FOB/USP

Patrocinadores

Patrocinador primário: Universidade de São Paulo

Patrocinadores secundários:

Instituição: Faculdade de Odontologia de Bauru - FOB/USP

Instituição: Universidade de São Paulo

ANEXO C – **ARTIGO PUBLICADO JMIR RESEARCH PROTOCOLS (ARTIGO 1)**

JMIR RESEARCH PROTOCOLS Souza et al

Protocol

Effect of a Titanium Tetrafluoride Varnish in the Prevention and Treatment of Carious Lesions in the Permanent Teeth of Children Living in a Fluoridated Region: Protocol for a Randomized Controlled Trial

Beatriz Martines Souza[1], DDS, MSc; Daiana Moreli Soares Santos[1], BSc (Biology); Aline Silva Braga[1], BSc (Biology), MSc; Natália Mello Dos Santos[2], DDS, MSc; Daniela Rios[2], DDS, MSc, PhD; Marilia Afonso Rabelo Buzalaf[1], DDS, MSc, PhD; Ana Carolina Magalhães[1], DDS, MSc, PhD

[1]Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil

[2]Department of Pediatric Dentistry, Orthodontic and Public Health, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil

Corresponding Author:
Ana Carolina Magalhães, DDS, MSc, PhD
Department of Biological Sciences
Bauru School of Dentistry
University of São Paulo
Al Octávio Pinheiro Brisola, 9-75
Bauru, 17012-901
Brazil
Phone: 55 14 32358497
Email: acm@usp.br

Abstract

Background: Titanium tetrafluoride (TiF_4) has regained interest due to new formulations that have been shown to be more effective against tooth demineralization than sodium fluoride (NaF) formulations in vitro and in situ.

Objective: The aim of this study is to evaluate the effect of two types of varnishes (4% TiF_4 and a commercial 5% NaF) on the prevention of carious lesions and the treatment of noncavitated enamel carious lesions in the permanent teeth of children living in a fluoridated area.

Methods: This randomized, controlled, parallel and single-blind clinical trial involves 63 children, 6-7 years old, living in Bauru, São Paulo, Brazil. Children were selected according to their caries activity (ie, presence of at least 1 tooth with a Nyvad score of 1) and randomly divided into the following treatment categories: 4% TiF_4 varnish (2.45 % F^-, pH 1, FGM); 5% NaF varnish (2.26% F^-, pH 5, Duraphat, Colgate) and control (placebo varnish, pH 5, FGM). The varnishes will be applied on all permanent teeth, once a week for 4 weeks and they will be reapplied only once 6 and 12 months after the study begins. Two calibrated examiners will carry out the clinical examination (International Caries Detection and Assessment System [ICDAS] and Nyvad indexes, kappa>.8) at baseline, before the first application, after the 1st, 6th, 12th, and 18th month of the study begins. Furthermore, quantitative fluorescence changes will be measured using Quantitative Light-Induced Fluorescence (QLF). The degree of patient satisfaction with the treatment will also be computed. The data will undergo statistical analysis (P<.05).

Results: This ongoing study is funded by funding agencies from Brazil (São Paulo Research Foundation, FAPESP-015/14149-1, and National Council for Scientific and Technological Development, CNPq-401313/2016-6). We expect to confirm the efficacy of TiF_4 on the prevention and treatment of carious lesions by comparing it to NaF varnish. The subjects are under 1 month evaluation and the dropout was about 8%. No differences between the treatments have been detected at the first month so far (P>.05).

Conclusions: If our hypothesis is confirmed, TiF_4 varnish can be marketed and applied at the individual level and used in community programs to control dental caries.

Trial Registration: Brazilian Clinical Trials Registry: RBR-5VWJ4Y; http://www.ensaiosclinicos.gov.br/rg/?q=RBR-5VWJ4Y (Archived by WebCite at http://www.webcitation.org/6wUurEnm7)