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# Clinical Evaluation of Residual Tetrasodium Pyrophosphate Released from Two Different Anticalculus Flosses

Vinícius Pedrazzi[1], Leandro Pereira Corsi[1], Hamilton Pedrazzi[1], Emilson I. Netto[3], Cássio do Nascimento[1], João Paulo Mardegan Issa[2]

[1]Department of Dental Materials and Prosthesis, School of Dentistry of Ribeirão Preto, USP - University of São Paulo, Ribeirão Preto, SP, Brazil
[2]Department of Morphology, Physiology and Basic Pathology, School of Dentistry of Ribeirão Preto, USP - University of São Paulo, Ribeirão Preto, SP, Brazil
[3]Chemical Engineer, Independent Researcher, São Paulo, SP, Brazil

Correspondence: Vinícius Pedrazzi, Avenida do Café, s/n, 14040-904 Ribeirão Preto, SP, Brasil. Tel: +55-16-3315-4008. e-mail: pedrazzi@forp.usp.br

The aim of this study was to compare the residual content of tetrasodium pyrophosphate released by two different anticalculus dental flosses (Reach PP® - entangled polypropylene floss and Reach NT® - texturized nylon) in the oral cavity. Ten healthy individuals (aged between 18 and 30 years) were enrolled in this randomized crossover clinical investigation. Participants received instructions on daily dental flossing and the interventions were randomly performed in 2 different groups (NT or PP) of five individuals each according to the dental flosses. Individuals were instructed to use each dental floss with a total of six slides on the two interproximal aspects of target teeth (3 slides on each interproximal aspect). A washout period of one week was used before start flossing interventions and after each type of dental floss to prevent any bias related to the exposure to any product that contained the active ingredient. Samples were collected by #35 sterilized absorbent paper points from interdental fluid after flossing and assessed by ion chromatography. The levels of residual tetrasodium pyrophosphate were evaluated by means of binomial generalized linear model proportions and canonical link function. Both dental flosses were effective in tetrasodium pyrophosphate release at therapeutic levels in the interdental gingival crevicular fluid for a period of up to 2 h after use. No significant differences were found between both groups ($p>0.05$). It may be concluded that both material composition and physical structure of the new dental floss did not affect the release or the maintenance of anticalculus agent at therapeutic levels for a period of up to 2 h after single use.

Key Words: dental floss, tetrasodium pyrophosphate, drug residue, entangled polypropylene dental floss, texturized nylon dental floss.

## Introduction

The human oral cavity comprehends a very complex ecosystem which may harbor several species of microorganisms in both soft and hard tissues (1,2). Bacteria, fungi, viruses and unclassified microbial species have been frequently reported colonizing oral tissues (3). The disruption of oral microbiome equilibrium by the increase of pathogenic species, associated to microbial proteases and metabolic products of their lysis may induce host responses such as inflammation and immunoreactions leading to inflammatory diseases. Several species of pathogenic and non-pathogenic bacteria, *Candida* and viruses, as well as opportunistic microorganisms have been related to caries (4), periodontitis/periimplantitis (5) and stomatitis (6).

Mechanical removal of oral biofilm, which includes tooth brushing, tongue scraping and flossing, are considered the most effective method of oral hygiene, significantly reducing microbial charge in the oral cavity (7,8). In recent years, the increase in demand for oral health and dental aesthetics have become more prominent, leading to the creation and development of dental materials that provide the desired effect. New preventive dental materials have arisen in the market worldwide and following this trend the industry has launched innovative products with traditional agents (9). Regarding flosses containing anti-caries, gingivitis and antiplaque agents, there is an increasing range of products with highly efficient physical and chemical components (10,11).

The ability to perform effective flossing depends on the human skills and dental floss constitution, which includes physical structure, shape, flexibility and presence of an antimicrobial agent (12). Dental flosses that slide easily between interproximal contacts without shredding are more effective and comfortable in the cleaning of oral tissues. Texturized dental floss made from nylon and entangled floss made from polypropylene could be commonly found for oral hygiene; both may have antimicrobial agents (such as triclosan, chlorhexidine and tetrasodium pyrophosphate) added to reduce the microbial colonization in the subgingival sulcus and in the interproximal spaces. Texturized nylon-based flosses may shred during dental flossing and fail in the mechanical removal of biofilm. In addition, they may fail to release the antimicrobial agent during flossing (13,14).

The current literature is scarce or still lacking information on the antimicrobial effectiveness of dental flosses to clean the interdental space. Recent progress in the fabrication

of dental flosses and their material composition has raised interest in relation to their effectiveness in the release of active ingredients for calculus control and the duration of residual effect. Some questions concerning the actual efficacy of these agents for oral health promotion have dictated the need of investigating if the new constructions of dental floss containing anticalculus agent can provide a durable effect of the active ingredient applied directly to the site. The aim of this clinical trial was to evaluate whether the most appropriate structure of entangled propylene floss is as effective as the traditional texturized nylon dental floss in releasing anticalculus agent locally and lasting for the same time in the gingival sulcus.

## Material and Methods

The study design was approved by the institutional Ethics Committee (Protocol #2005.1.657.58.8) and all participants signed an informed consent form before the clinical phase.

This work applied a double-blind methodology so that neither the participants or the investigators were aware of the distribution of the products being tested. There were 10 volunteers aged between 18 and 30 years. Other criteria for inclusion in the study of these volunteers were good oral health and have the same studied teeth in the oral cavity. Exclusion criteria were: pregnant or lactating patients; periodontal treatment or antibiotics in the previous 3 months; smokers; systemic disease that might disturb the periodontium, or patients who required pre-medication for dental treatment.

These volunteers were subjected to a structured crossover experiment through a Latin square 2x2 (two sequences of two products replicated four times) according to a crossover study with two types of dental floss (15,16). Two sequences of flosses were randomly assigned by computer to the individuals included in the study, forming two groups of five subjects each, so that all individuals used both flosses in different periods.

Each individual used the assigned floss, totalizing six slides in the target area defined as both interproximal surfaces of selected teeth, or three slides on each side in sequence, for each floss. The chosen sample collection areas were: the interproximal surfaces of the central incisors, lateral incisors and canines, and finally, between the second premolar and first molar of the individual, all maxillary teeth. This choice was made in order to simulate what occurs in the oral cavity (17).

The tested dental flosses had different physical structures (mesh structure - physical platform) and different ways of introducing tetrasodium pyrophosphate, the active principle. In the texturized nylon dental floss, introduction of the active ingredient was made by simple immersion of the floss in a solution containing the active principle, in contrast to floss made from entangled polypropylene, where the insertion of the active ingredient was obtained with a crystal micro-wax containing in addition to the studied active ingredient, long lasting flavoring agents.

Before starting to use the first floss, as well as between control and different tested flosses, a one-week washout period was performed without using any product that contained the active ingredient (tetrasodium pyrophosphate). The following items were delivered to each individual: one Johnson & Johnson toothbrush (São Paulo, SP, Brazil) with a small soft head and flat trim bristles; one conventional toothpaste without tetrasodium pyrophosphate or any therapeutic agent, and one pack containing the same floss (PP or NT) to be tested, but without the active ingredient to be used during the washout period. After flossing, samples from interproximal aspects of target teeth were collected for further processing. After that, participants were instructed to follow the same procedures using anticalculus dental flosses (on the test day).

The responses of the experiment were the mean values of tetrasodium pyrophosphate percentage detected in crevicular fluid collected from each patient before and after application of tested dental floss at the following times: Before flossing - control group; 0 - immediately after flossing; 1 h after flossing; 2 h after flossing; and 4 h after flossing.

For this study, was used the relative variation of the mean index, before and after use. It is important to clarify that the flosses with active ingredients were used only once, on the day of crevicular fluid collection. In the remaining days (washout time) the subjects used the same type of the dental floss, but without tetrasodium pyrophosphate, the active ingredient.

After the volunteers used the tested dental flosses, the fluids were collected with sterile absorbent paper cones gently placed at 1 mm depth inside the gingival sulcus, taken at different time and stored in individual microtubes for chromatographic analysis. The cones containing the crevicular fluid from each individual were analyzed by the ion chromatography method. The analysis was performed in the ion chromatograph (IC 2000; Sunnyvale, CA, USA) using an EG40 KOH eluent generator which suppresses the ASRS ultra 4 mm to 17 mA conductivity, AS-11 analytical column and pre-column AG-11.

For the preparation of samples, the cones were transferred and increased to 10 mL volumes with 50 mM KOH. After the samples rested for 30 min, decantation and filtration in ultrasound were performed. Finally,

the samples were transferred to 5 mL vials and injected into the ion chromatograph apparatus. Both tested strands contained 11 mg of tetrasodium pyrophosphate per 18" of floss that corresponds to 11 mg of the active principle every 45.72 cm. The composition of tetrasodium pyrophosphate-based dental flosses used in the study was as follows: Reach® PP: entangled polypropylene and Reach® NT: texturized nylon.

Occurrence of tetrasodium pyrophosphate was analyzed by binomial generalized linear model proportions and canonical link function. The statistical analysis used the maximum likelihood method for the detection of parameters. Calculations were performed with SAS 9.1 (SAS Institute, Cary, NC, USA) system running in Linux operating system (Mandriva. 2006).

## Results

All volunteers who started the study completed the study, i.e., there was no abandonment during the two study phases. Concerning the safety of the study, there were no adverse reactions from the use of the flosses reported or observed in any phase of the study.

For detection of tetrasodium pyrophosphate, a statistical test compared the distribution of events by treatment and time. The postulated distribution was binomial. The level of significance referred to the hypothesis: distributions in the populations are equal (the ratios of corresponding categories are the same).

No differences in relation to tetrasodium pyrophosphate release was found among the tested dental flosses (floss A or B) (p=1.00), i.e., both tested flosses were effective in releasing the active principle at therapeutic levels in the same period of time. Time effect was the only factor to present significant difference between groups (p<0.0001). Table 1 presents the estimated percentages of pyrophosphate detection for each sample and time.

## Discussion

Two types of dental flosses were tested in this study, texturized nylon and entangled polypropylene, regarding tetrasodium pyrophosphate release over time. The results showed no differences in the antimicrobial agent release comparing the different dental flosses. The results of this investigation showed that both tested flosses were able to release tetrasodium pyrophosphate over time (since baseline – time 0).

Previous studies emphasized that floss is the most effective means for cleaning the interproximal dental spaces and can lead to interdental papillae therapeutic agents acting as a vehicle (17-19). According to previous studies, some toothpastes which contain in their compositions anticalculus agents such as pyrophosphate, may provide reduction in biofilm formation by approximately 15% when compared to toothpastes without this active principle (20).

Previous studies have also demonstrated effectiveness for inhibiting formation of supra-gingival dental calculus with toothpaste containing soluble pyrophosphate, achieving a reduction of 31% (21). This reduction in the formation of dental plaque and calculus and the possibility of the tested product to deliver the active principle to the site shows their importance in the fight against periodontal disease. According to other studies, the amount of tetrasodium pyrophosphate, which was added to the structure of the tested dental floss (11 mg of active principle every 46 cm of dental floss) is effective in reducing dental calculus formation by 37% when compared with flosses without the active ingredient, proving the efficiency of the studied material (22).

According to the obtained data, no differences in relation to tetrasodium pyrophosphate release were found among the tested dental flosses. Tetrasodium pyrophosphate release was reported at therapeutic levels for up to 2 h after the use of the specific dental floss. These data may not support that only anticalculus dental floss provides a striking reduction in dental biofilm and calculus formation, because nothing should replace the complete and well performed mechanical oral hygiene. However, the residual effect of tetrasodium pyrophosphate in the crevicular fluid may be relevant

Table 1. Estimated percentages of pyrophosphate detection for each sample and time

| Pyrophosphate detection treatment | Time | No | Yes |
|---|---|---|---|
| | | Estimated % | Estimated % |
| A | Control | 100 | 0 |
| | 0 | 5 | 95 |
| | 1 h | 55 | 45 |
| | 2 h | 90 | 10 |
| | 4 h | 100 | 0 |
| B | Control | 100 | 0 |
| | 0 | 5 | 95 |
| | 1 h | 55 | 45 |
| | 2 h | 90 | 10 |
| | 4 h | 100 | 0 |

Control: before flossing; 0: immediately after flossing; 1 h: 1 h after flossing; 2 h: 2 h after flossing; and 4 h: 4 h after flossing.

to prevent calculus formation and maturation. Several studies have demonstrated that the combination of tooth brushing with regular flossing provides a significant improvement of oral health as a whole (23-25), but the development of a high quality preventive dental material can and should contribute to improved levels of oral health of the population. New methodologies that bring improvements over time of the active principle in crevicular fluid are required, so the production of the anticalculus effect could be maximized.

Few studies were found in the literature regarding the potential activity of antimicrobial dental flosses on the oral microbiota. In the present investigation, it was proposed to evaluate over time the rate of antimicrobial agents release during flossing. This study proved that an active agent incorporated in two common physical structures of dental floss may remain in the floss and be released in significant amounts over time. Data provided by this study are preliminary and may help further studies assessing the impact of these products on the microbial colonization of oral surfaces.

These results should be interpreted with caution, because only sites from the anterior maxilla, which have lower salivary flow rates, were evaluated in the present study. These sites were used because they are easier to standardize and to reproduce the experimental design. However, the retention of an active substance in these areas could be enhanced by dry surface. Further studies including other areas of oral cavity are required to corroborate the study.

In conclusion, within the limitations of this investigation, the results of the study have confirmed the tested hypotheses: 1) both investigated dental flosses were equally effective in tetrasodium pyrophosphate release at therapeutic levels in the interdental gingival crevicular fluid for a period of up to 2 h after use; 2) the physical structure of the dental flosses did not influence the release of soluble pyrophosphate nor did the time of application.


## Resumo

O objetivo deste estudo foi comparar o teor residual de pirofosfato de tetrasodio liberado por dois tipos diferentes de fio dental anti-cálculo (Reach PP® - polipropileno entrelaçado e Reach NT® - nylon texturizado) na cavidade oral. Dez indivíduos saudáveis (com idade entre 18 e 30 anos) foram selecionados para este estudo clínico em modelo *crossover*. Os participantes receberam instruções sobre como utilizarem os fios dentais e as intervenções foram randomicamente realizadas em 2 grupos de 5 participantes de acordo com o tipo de fio avaliado. O participantes foram instruídos a passarem cada fio dental em um total de 6 passadas ao redor dos 2 espaços interproximais dos dentes selecionados (3 passadas em cada face interproximal). Um período de *washout* de 1 semana foi utilizado antes do início do estudo e após cada intervenção com o objetivo de prevenir a ocorrência de viés relacionado à exposição de algum agente terapêutico. Amostras do fluido interdental após as intervenções foram avaliadas por meio de cromatografia de íons. A porcentagem residual de pirofosfato tetrassódico foi verificada empregando-se o método da cromatografia de íons, pela colheita do fluido interdental com cones de papel absorvente esterilizados. Os teores de pirofosfato tetrassódico residual foram comparados para cada fio dental e para cada tempo de colheita do fluido interdental. Não foram observadas diferenças significantes entre os grupos avaliados (p>0,05). Conclui-se que a composição do material e a estrutura física dos fios dentais não exerceram influência na liberação e na permanência do agente anti-tártaro, que permaneceu em níveis terapêuticos por um período de até 2 h após o uso único.



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