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Efficacy of dental floss impregnated with chlorhexidine on reduction of supragingival biofilm: a randomized controlled trial

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Muniz FWMG, Sena KS, de Oliveira CC, Veríssimo DM, Carvalho RS, Martins RS. Efficacy of dental floss impregnated with chlorhexidine on reduction of supragingival biofilm: a randomized controlled trial.

Abstract: *Background:* The use of a toothbrush has a limited ability to control the dental biofilm in interproximal areas. Therefore, specialized devices, such as dental floss, may be useful for these specific areas. *Objective:* This study aimed to investigate the efficacy of dental floss impregnated with 5% chlorhexidine gluconate on the reduction of the supragingival biofilm. *Methods:* This research was parallel, single-blind, controlled and randomized, and contained a sample of thirty dental students from the Faculty of Pharmacy, Dentistry and Nursing of the Federal University of Ceará, Brazil, who were divided equally into three groups. The negative control group (NC) did not utilize any kind of interproximal cleaning; the positive control group (PC) used waxed floss without impregnation twice a day; and the test group (T) used the same dental floss, which was impregnated with 5% chlorhexidine gluconate, twice a day. For all groups, this study lasted for 15 days. The presence of a biofilm was evaluated on four surfaces (mesiobuccal, distobuccal, mesiolingual and distolingual) by the Quigley–Hein Index, resulting in four scores for each tooth. *Results:* Group T had the lowest plaque scores, showing a significant difference compared to group NC ($P < 0.001$) and group PC ($P < 0.001$). Group PC also displayed a significant difference compared to NC ($P < 0.001$). *Conclusion:* It was concluded that the use of dental floss impregnated with 5% chlorhexidine gluconate resulted in additional reductions in the supragingival biofilm relative to the results achieved with conventional waxed floss on the anterior teeth of a well-motivated and well-instructed population.

Key words: biofilms; chlorhexidine; dental devices; dental plaque; dental plaque index; home care; oral hygiene; therapeutic use; treatment efficacy

Introduction

Dental biofilms are the main aetiological factor for periodontal disease (PD) (1). The best way to prevent this disease is through the mechanical control of the biofilm with the use of a toothbrush (2). However, toothbrushes should be supplemented with the use of other cleaning devices, as their efficacy in removing the dental biofilm that accumulates in interproximal surfaces is limited (3). In addition, PD does not necessarily affect all tooth areas with equal severity (4). Larger concentrations of dental biofilm accumulation occur in interproximal surfaces than in

non-proximal sites (5), suggesting that interdental cleaning is often not executed in an adequate manner.

To remedy this problem, several interproximal cleaning devices are regularly available, including single tufted brushes, toothpicks and dental floss. In choosing the most appropriate interdental device for each patient, the size and morphology of the interdental spaces should be considered (6). Additionally, the skill and ability of the patient to use these devices should be respected (7). Subjects without attachment loss who brush and floss regularly have less gum bleeding compared to those who solely use a toothbrush (8), indicating that dental floss could be beneficial for these patients.

According to Zimmer *et al.* (9), only a small portion of the population uses dental floss on a daily basis, and its consumption is greater in individuals with higher socioeconomic levels. Many people avoid flossing because they consider it to be time-consuming, awkward, difficult to use, and it shreds easily between teeth (3). It is also known that the frequency of flossing increases when its use is constantly encouraged (10), but all dental professionals do not routinely recommend this behaviour. All of these difficulties make the process of flossing less universal in its applicability.

Despite these evidences, one systematic review established that toothbrushing and dental flossing provide no benefit, when compared to toothbrushing alone, on removing plaque and reducing gingivitis (6). In addition, another systematic review found that there is weak and very unreliable evidence which suggests that flossing plus toothbrushing may be associated with a small reduction in plaque (11). However, it is still possible to found, in the literature, that flossing is effective, but it depends on the patient's situation whether it should be carefully recommended by a dental professional (6, 12).

In addition to the mechanical control of dental biofilms, it is possible to use chemical control, and chlorhexidine is the gold standard for this purpose. Chlorhexidine is a dicationic compound that is able to join anionic compounds, such as phosphate and carboxyl radicals from the tooth surface and salivary glycoproteins. Its action damages the cytoplasmic membrane, leading to bacterial cell lysis (13). Additionally, chlorhexidine has a retention capacity and remains in oral tissues for a prolonged time, exhibiting a high substantivity (14).

Despite its potential benefits, chlorhexidine displays some disadvantages after extensive use, such as taste alterations and teeth and oral tissue pigmentation (15). However, although it has been suggested that chlorhexidine (CHX) and sodium lauryl sulphate (SLS) may counteract in the oral cavity (16), the SLS/CHX interaction studies showed that the antiplaque activity of 0.2% CHX mouthrinse is not reduced when immediately preceded or followed by toothbrushing with an SLS-containing dentifrice (17). In case of rinsing with CHX before or after toothbrushing during 30 s, an additional reduction of 33% for plaque and 26% for gingivitis has been determined (18).

This study aimed to verify the efficacy of the use of dental floss impregnated with 5% concentrated chlorhexidine gluconate on the reduction of the supragingival dental biofilm.

Materials and methods

Ethical aspects

The study was approved by the Ethical Committee of the Federal University Ceará under protocol 215/03, and all volunteers signed an informed consent form.

Study type

This study followed the CONSORT statement, and it was designed as a single-centre, parallel, single-blind, controlled and randomized clinical trial.

Sample selection

Thirty dental students of both genders from the Faculty of Pharmacy, Dentistry, and Nursing of Federal University Ceará were selected for this study. Recruitment consisted of a convenience sample, carried out from November 2011 to April 2012. Those volunteers who fulfilled the following criteria were included.

As inclusion criteria, all volunteers had to be in a good general health, they had to display interproximal spaces without any diastema or gingival recession. Only volunteers with gingival index equal to zero were included in this study. Moreover, they were right-handed and had not taken classes in periodontics.

Smokers and patients with any kind of dental prosthesis, caries or any other biofilm retentive factor, with exception of dental calculus, were excluded from this study. Those who used systemic antibiotics within 3 months prior to the start of the study and those who possessed systemic conditions that exerted an effect on periodontal health, such as diabetes, were also excluded.

Experimental design

Thirty dental students were equally divided into three groups. The negative control group (NC) ($n = 10$) did not use any kind of interproximal cleaning device and only used a toothbrush to eliminate the supragingival plaque; the positive control group (PC) ($n = 10$) used the same toothbrush and a waxed dental floss without any impregnation twice a day; and the test group (T) ($n = 10$) also used a toothbrush and the same dental floss impregnated with 5% chlorhexidine gluconate twice a day.

At baseline, all volunteers were examined by the same calibrated investigator (FWMGM), who also performed prophylaxis and supragingival scaling and planing, with the purpose of making the dentition 100% free of plaque, calculus and extrinsic pigmentation. Prophylaxis was performed with Robson brush, rubber cup and prophylactic paste. Disclosing tablets, composed of fuchsine basic 2% (Eviplac®; Biodinâmica, Ibiporã, Brazil), were used to confirm the absence of dental biofilm. Supragingival scaling and planing were performed, when necessary, with a Gracey curettes and a sickle Point Morse 0–00.

Techniques and personalized instructions for toothbrushing and flossing were individually and verbally provided by

another researcher (KSS). For this study, only the anterior teeth were selected for evaluation, totalling twelve interproximal spaces on the upper arch and twelve on the lower arch. The choice to use only anterior teeth only was a tentative to facilitate flossing by the volunteers and also to make the dental biofilm visualization easier for the examiner.

The systematic use of the dental floss was established according to the American Dental Association (ADA) recommendations (19). The volunteers were instructed to introduce the dental floss in each interproximal space and perform three buccal–lingual movements against the mesial surfaces and three more against the distal surfaces. With the exception of the initial visit, daily oral hygiene procedures were not supervised. The researcher motivated the volunteers to perform this procedure twice a day in 12-h intervals. Subjects used only one piece of dental floss on the lower arch and another on the upper arch. On the posterior teeth, regardless of group distribution, the use of any of dental floss was allowed.

At baseline appointment, the volunteers received a printed frame that had 30 cells, each cell represented a period of the day, being two cells for each day. They were asked to mark one cell immediately after flossing. These printed frames were received in the last appointment (day 15) and assessed how often the floss was used.

To achieve standard conditions, each volunteer received a kit that contained a new toothbrush with soft bristles (Reach Eco; Johnson & Johnson®, New Brunswick, NJ, USA) and a manipulated dentifrice composed of carboxymethyl cellulose 5% gel and 8% mint essence. Furthermore, the volunteers were requested to not use any kind of antiseptic mouthwash during the 15 study days. Dental floss was distributed to the volunteers at the baseline appointment and at the 7th day of the experimental period. On day 15, subjects attended another appointment to complete the study, when the clinical evaluation was performed.

Laboratory phase

The laboratory phase used is an adaptation of the protocol developed by Oppermann *et al.* (20) in toothpicks impregnated with chlorhexidine. Initially, 30-cm lengths of waxed dental floss (Reach Dental Floss – Total Care; Johnson & Johnson®) were cut with the help of a ruler. In an autoclave, these floss segments were sterilized and then placed in previously sterilized falcon tubes with a solution of 5% chlorhexidine gluconate.

During 24 h, these falcon tubes remained in a microbiological oven at a constant temperature of 37°C as a tentative to impregnate the solution on the floss. After that time, the floss passed through a drying stage in the same microbiological oven for the same period of time and was then subsequently placed in appropriate packages. The dental floss for the PC group passed only through the autoclave process. The period between dental floss preparation and its distribution to volunteers did not exceed 1 week. As this is the first work performed in this field, and the dental floss' expiry date was not assessed so far, it was assumed that this amount of time was reasonable to achieve a positive clinical result.

Visually, no change was observed in any sterile floss. It is worth noting that the researchers were extremely careful in all laboratory stages, such as temperature control, as a tentative to avoid any changes in the dental floss properties. After this process, the chlorhexidine antimicrobial activity was not assessed.

Clinical evaluation

Fifteen days after the baseline appointment, the presence of the dental biofilm was evaluated only on four surfaces, resulting in four scores for each tooth (mesiobuccal, distobuccal, mesiolingual and distolingual). This amount of time was chosen for this study because 15 days after the biofilm control, carried out by a dental professional, the biofilm accumulation assessment may appear significantly. The four surfaces were assessed according to the Quigley–Hein Index (21), modified by Turesky *et al.* (22).

One calibrated examiner (FWMGM), who was unaware of the kind of dental floss used by the volunteers, performed these clinical analyses. At first, the biofilm was disclosed with tablets composed of 2% basic fuchsine. The volunteers were asked to masticate the tablet and smoothly spread the disclosing with tongue help. Before to assess any score, the teeth were washed and dried with gentle air jets to remove the tablet excess.

In that same appointment, before teeth disclosing, the same examiner (FWMGM) performed a visual search for any tooth and tongue extrinsic pigmentation. Another researcher (KSS) asked the volunteers whether they had experienced any side effect since the beginning of the study, such as palate alterations and any kind of hypersensibility.

Sample size estimate

The sample size calculation was based on results obtained in a clinical trial that also used mechanical and chemical biofilm control (23). This study found a mean ± SD of plaque scores in the control group of 1.81 ± 0.21 and 1.44 ± 0.28 in the test group after 6 months of treatment. Through Student's *t*-test, it was estimated a sample size of eight volunteers per group taking into consideration a power of 80% and an alpha of 5%. However, a dropout rate of approximately 20% was added to the sample size totalling 30 volunteers.

Randomization

A simple randomization process was carried out to distribute the subjects into three group, negative control, positive control and test groups, using a random command from Microsoft Excel 2007 (Microsoft Corporation®, São Paulo, São Paulo, Brazil) by one of the researchers (CCO). The volunteers were related in a column, according to its complete names, and then in another column, it was distributed three sequences of ten numbers (1, 2 and 3), which was related to each experimental group (1 – group negative control, 2 – group positive control and 3 – group

test). After that, the RAND function in Excel was executed to generate the distributions of subjects for each group. The results from this process were kept as a secret until the beginning of the baseline appointments. Only on that day, the researcher responsible for oral hygiene instructions (KSS) was able to know in what group each volunteer was designed to. The researcher responsible for clinical examinations (FWMGM) did not have any contact with this process, remaining blind during the whole study.

Intraexaminer calibration

Training on the plaque scores was provided with the help of a calibrated examiner. Each score was extensively discussed until similar criteria were established. After the training period, intraexaminer calibration was performed by examining five dental students twice within at least a 1-h interval.

To assess intraexaminer calibration, the intraclass correlation coefficient (ICC) (24) was calculated with SPSS 9.0 (SPSS, Chicago, IL, USA). The scores obtained by the Quigley–Hein Index for each tooth/surface were considered in the calculations. The ICC was 0.92, indicating an excellent level of intraexaminer calibration (25).

Statistical analysis

Plaque scores were considered as the primary outcome variable. The Kruskal–Wallis test, followed by the Dunn's test, was used to assess differences between groups regarding the plaque scores.

Kolmogorov–Smirnov was used to test the pattern distribution regarding the age in the samples. When the normality distribution was found, it was used one-way ANOVA to compare the mean ages between the three groups.

Only the positive control group and test group used dental floss in this study. To compare the average use of dental floss between those two groups, it was used Student's *t*-test for independent samples. In all those tests, a value of $P < 0.05$ was considered to represent a significant difference.

Results

From the 67 volunteers evaluated to participate in the study, 30 were randomized to the experimental groups (Fig. 1). All volunteers completed this clinical trial without any complications or side effects, such as dental or tongue staining and loss of taste. The subject characteristics are shown in Table 1. All the volunteers were adults with ages between 18 and 26 years. According to ANOVA test, there are no age differences between the three groups ($P = 0.994$) (Table 1).

The adherence to dental floss used was measured through a printed frame, and it was noticed a high compliance in both groups. Group PC had a mean use of 89.0 ± 8.61%, and group T had 93.45 ± 7.94%. Student's *t*-test showed no differences between the average use of dental floss between group PC and group T ($P = 0.245$) (Table 1).

At the end of baseline appointment, all the volunteers started the study with mean plaque score zero. After 15 days, the mean plaque score of all teeth examined was 2.96 ± 0.07,

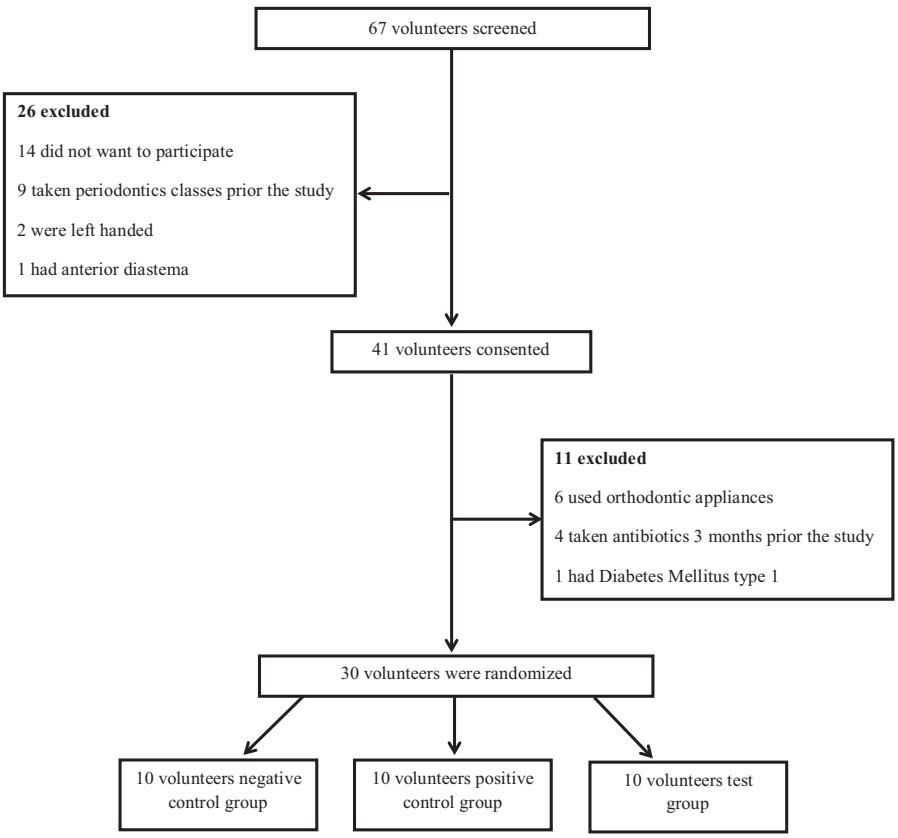

Fig. 1. Study flow chart.

Table 1. **Subject characteristics, dental floss use frequency and mean biofilm index after 15 days**

	Group NC	Group PC	Group T	*P*-value
Age	21.30 ± 2.87	21.40 ± 2.22	21.30 ± 1.49	–
Gender (female/male)	7/3	8/2	7/3	–
Dental floss use frequency	–	89.00 ± 8.61%	93.45 ± 7.94%	0.245*
Mean plaque scores	2.96 ± 0.07	2.57 ± 0.07	2.14 ± 0.08	<0.001†
				<0.001‡
				<0.001§
Mean plaque scores (mesiobuccal and distobuccal surfaces only)	2.90 ± 0.09	2.43 ± 0.10	2.00 ± 0.10	<0.001†
				<0.001‡
				0.015§
Mean plaque scores (mesiolingual and distolingual surfaces only)	3.01 ± 0.10	2.71 ± 0.10	2.28 ± 0.10	0.15†
				<0.001‡
				0.015§

*P-value obtained from Student's *t*-test for independent samples.
†Represents the *P*-value, obtained from Dunn's test, when group NC and PC were compared.
‡Represents the *P*-value, obtained from Dunn's test, when group NC and T were compared.
§Represents the *P*-value, obtained from Dunn's test, when group PC and T were compared.

2.57 ± 0.07 and 2.14 ± 0.08 in groups NC, PC and T, respectively. Group T had the lowest plaque scores, showing significant differences relative to groups NC ($P < 0.001$) and PC ($P < 0.001$). Group PC also showed a significant difference compared to NC ($P < 0.001$) (Table 1).

When only the plaque scores for the mesiobuccal and distobuccal surfaces was analysed, the group T had also the lowest plaque scores, showing significant differences between groups NC ($P < 0.001$) and PC ($P = 0.015$). Group PC also showed significant differences relative to group NC ($P < 0.001$) (Table 1). On the other hand, when only the mesiolingual and distolingual surfaces were analysed, only group T had significant differences from groups NC ($P < 0.001$) and PC ($P = 0.015$) (Table 1).

Discussion

This study evaluated the efficacy of dental floss impregnated with chlorhexidine gluconate 5% on the reduction of the supragingival biofilm *in vivo*. This is the first time that a dental floss impregnated with chlorhexidine has been described in the literature.

Dental students were selected as participants in this study because they present, according to Kleisner and Imfeld (26), sufficient levels of knowledge, similar patterns of biofilm control, good manual dexterity and similar ages. The Quigley–Hein Index (21), modified by Turesky *et al.* (22), was used to allow small changes in the evaluations of the biofilm amount and also for being one of the most frequently used indices in product testing (27, 28). All those conditions are very important to show the efficacy of the dental floss impregnated with chlorhexidine. On the other hand, the results of the present study cannot be extrapolated as if an effect study was performed, as assessment of gingival inflammation and tooth staining would be necessary, and therefore, further studies need to be conducted in these areas. Additionally, it is not possible to say that the results would be the same if this study was conducted in the general population with smaller cleaning ability. As a tentative to enlarge the external validity, dental students that did not taken classes in periodontics were used, as this is the first class that teaches brushing techniques.

In this study, disclosed biofilm was used to assess plaque scores. Although the use of disclosing solutions in the management of biofilm control can be somehow discouraging for some patients, the use of disclosing solutions combined with index scales enables comparisons between new and existing oral hygiene products (28). The choice for disclosing tablets was based on the fact that this technique is largely used and probably removes less plaque when compared to liquid disclosing applied with cotton, which may requires scratches on tooth surfaces.

Only anterior teeth were used in this study, mostly as a tentative to promote an easier visualization and usage to the volunteers. The manipulated dentifrice was made only by carboxymethyl cellulose gel and mint essence, which has no effect on dental biofilm. For that reason, the group test (dental floss impregnated with chlorhexidine) was the only group that received an additional benefit by the chemical control of biofilm during the study. However, these facts decrease the external validity and may be a limitation of the study.

After the laboratory phase, the dental flosses impregnated with chlorhexidine were not tested for any antimicrobial activity. This is another limitation of this study; however, the efficacy of this process was previously certified by Oppermann *et al.* (20) in toothpicks. Additionally, our research group intend to assess the antimicrobial activity in further study, estimating the expiry date as well.

This unsupervised study used print frames as a tentative to reduce any possible compliance matter. The volunteers were asked to fulfil the frame as soon as they used the dental floss, which assessed how often the floss was used. Despite all the difficulties related to flossing (3), the returned frames indicated that the volunteers followed the given instructions conscientiously, as the compliance rate was considered higher (a mean use larger than 89% in both groups). The choice of use only right-handed was a tentative to avoid variations in flossing

quality between right-handed, two-handed and left-handed (29). If left-handed were included in this study, it is not possible to say that the results would be the same because it seems that they clean their teeth in a different manner when compared to right-handed (29).

Even more, this study did not assess clinical attachment loss and probing depth, which represent a potential selection bias. If these clinical parameters have been evaluated, it is possible that the differences would be even greater. The gingival index was not assessed in the last appointment because this study aimed to investigate only the antiplaque effect of the dental floss impregnated with chlorhexidine.

This study found out that the dental floss groups, whether or not the floss was impregnated with chlorhexidine gluconate, exhibited lower levels of biofilms in than the group that did not use any device for interproximal cleaning. This result is supported by the classic study of Gjermo and Flötra (1970) (30), which established the use of dental floss for the mechanical control of dental biofilms. There was a 40% reduction in the amount of biofilm in the interproximal areas for the group that used dental floss for over 15 days (30). However, significant reductions were not observed between the group that used toothpicks and the group that did not use any kind of interproximal cleaning device. In a study by Terézhalmy *et al.* (31), various types of dental floss, such as unwaxed, woven, shred-resistant and powered flosser, in association with the use of a toothbrush, similarly reduced the level of dental biofilms to a significantly greater extent than the use of a toothbrush alone.

In contrast with these results, a systematic review concluded that routine instruction to use dental floss is not supported by the literature, and the dental professionals should analyse carefully what patient should benefit the most from this type of interproximal cleaning device (6). Additionally, another clinical trial did not report any additional benefits of the use of dental floss when it was compared to the use of a toothbrush alone (9). That study found that after 8 weeks, the group that used only a toothbrush and toothpaste showed a reduction of interproximal biofilms that was quite similar to the group that used toothbrush, toothpaste and dental floss. On the other hand, this study did not provide specific instructions on the use of dental floss to their volunteers, which indicates that the proper flossing technique is difficult to achieve. It also indicates the need for an accurate manual dexterity to obtain the proper efficacy of dental floss. In the present study, at baseline, all volunteers were instructed on the proper way to use this device.

This study results showed that dental floss impregnated with 5% chlorhexidine gluconate provided statistically significant lower biofilm indices than those of the other two groups. Chlorhexidine is a substance that exhibits bactericidal and bacteriostatic characteristics, and it damages the bacterial cytoplasmic membrane, leading to lysis (13). These characteristics may have influenced the superiority of group T parameters, especially on the mesiolingual and distolingual surfaces, as only this group showed significant differences, when only these surfaces were analysed, relative to the other two groups. Compared to buccal surfaces, lingual surfaces most likely required more accurate manual dexterity for proper interproximal hygiene, which explains the lack of significant differences between groups NC and PC for the mesiolingual and distolingual surfaces. When the mechanical action of flossing was combined with the chemical action of chlorhexidine, it was possible to achieve more satisfactory results in reducing the interproximal biofilms on these surfaces.

These results corroborate with the results of other studies that combined mechanical and chemical methods to control dental biofilms. In a study by Sharma *et al.* (23), essential oil mouthwash was used daily combined with dental floss. A reduction in the biofilm index of 13% was observed in the group that used only dental floss, and a 55% reduction was observed in the group that used dental floss plus essential oil mouthwash, demonstrating that, when mechanical and chemical methods are combined, biofilm control is more effective than that achieved with either individual method. In addition, a study by Gisselsson *et al.* (32) showed that after 3 years, the number of caries lesions was lower in children that used dental floss combined with chlorhexidine 1% gel than in children that used dental floss and a placebo gel.

The results of this study should be analysed carefully before dental floss impregnated with 5% chlorhexidine gluconate is used on a large scale. So far, this study does not have the intention to influence the clinicians to start recommend the dental floss impregnated with chlorhexidine. Mainly, because chemical control of biofilm should be prescribed only for patients that do not beneficiate of mechanical control alone, but also because this is the first clinical trial developed in this field and some few limitations, hereby presented, should be clarified in further studies. First of all, more investigations of the laboratory phase are needed, mainly, to assess how is the chlorhexidine action after the impregnation. Further clinical trials with longer experimental periods should be performed to confirm or reject the absence of side effects, especially in non-professional individuals with a larger sample. Furthermore, the ability to reduce gingivitis of this floss should also be evaluated in a new study, using specific bleeding index, such as Eastman Interdental Bleeding Index and Löe-Silness Gingival Index.

Conclusion

In conclusion, within the limitations of the present study design, the use of dental floss impregnated with 5% chlorhexidine gluconate produced additional reductions in the supragingival biofilm relative to the use of conventional waxed floss on the anterior teeth of well-motivated and well-instructed right-handed individuals.

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Clinical relevance

Scientific rationale for study

The proper flossing technique is difficult to achieve, resulting in a poor control of biofilm in the interproximal area. Chlorhexidine is considered as the gold standard to control supragingival biofilm. The control of biofilm can be enhanced with the combination of mechanical and chemical methods.

Principal findings

The dental floss impregnated with chlorhexidine gluconate 5% showed the lowest plaque scores when compared to the use of conventional waxed floss and the use of a conventional toothbrush.

Practical implications

The use of dental floss impregnated with gluconate chlorhexidine can be a useful product in the management of supragingival plaque on the anterior teeth of a well-motivated and well-instructed population.

Conflict of interest and sources of funding

The authors declare no conflict of interests between them and the company that gave the products to realize this study. Johnson & Johnson (Fortaleza, Ceará, Brazil) provided some of the toothbrushes and dental floss used in this study.

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