Anti-discoloration system: a new chlorhexidine mouthwash

G. Tetè[1], F. Cattoni[1] and E. Polizzi[2]

[1]Dental School, Vita-Salute University and Department of Dentistry, IRCCS San Raffaele Hospital, Milan, Italy; [2]Center for Oral Hygiene and Prevention, Department of Dentistry, IRCCS San Raffaele Hospital, Milan, Italy

Chlorhexidine is defined as biocompatible, which is why it is used as a mouthrinse for the patient before starting dental procedures (2). It has the ability to bind well to teeth and mucous membranes and is released for twelve hours, which is why it is used as a treatment for gingivitis and also in post-operative wound healing. The long-term side effects of chlorhexidine are pigmentations. To remedy this, various types of anti-discoloration have been tried out over time. Nowadays there are other types of anti-discoloration systems such as, for example, in our study we used a test group containing an anti-discoloration system called SPPD. A single-center, prospective, double-blind randomized clinical trial on 84 patients. The investigated treatments consisted of 4 mouthwashes (CHX 0.12% SPDD alcohol free; CHX 0.20% SPDD alcohol free; CHX 0.12% alcohol free with ADS; CHX 0.20% alcohol free with ADS). Despite the limitations of the study, all the mouthwashes tested showed good efficacy in reducing the amount of plaque. Comparing the two experimental concentrations (0.12% and 0.20%) tested here demonstrates that the 0.20% chlorhexidine concentration slightly surpasses its 0.12% equivalent with regard to the PI and BI parameters. The SPDD is an innovative anti-discoloration system and gives the mouthwash a great taste.

Chlorhexidine is a microbial agent introduced for clinical use in the 1950s. It has the ability to destroy the bacterial membrane and is therefore also used for inanimate surfaces (1). Chlorhexidine is defined as biocompatible, which is why it is used as a mouthrinse for the patient before starting dental procedures (2). It can bind well to teeth and mucous membranes and is released for twelve hours, which is why it is used as a treatment for gingivitis and also in post-operative wound healing (3). However, according to the new scientific guidelines, Chlorhexidine alone is no longer sufficient for Covid-19, especially in the case of patients with previous chronic diseases of various origins (4-8). Indeed, chlorhexidine is a powerful antimicrobial but has a less effective effect on viruses; therefore, it is recommended to use another rinse with hydrogen peroxide or povidone iodine together with chlorhexidine as their actions become complementary and fully effective (4-9). However, further studies are needed to confirm this, and chlorhexidine even when combined with other mouth rinses should be used. However, the diatonic structure of chlorhexidine also has some side effects such as altered taste, and, above in particular, discoloration of the teeth and mucous membranes; the latter effect is the most commonly reported by long-term users of the chlorhexidine products (10-11). Therefore, in recent developments, some have added ADS, an anti-discoloration system that reduces side effects with a chemical formulation.

Key words: Chlorhexidine, mouthwash, discoloration, plaque, hydrogen peroxide, povidone iodine together

corresponding author:
Prof. Francesca Cattoni,
Dental School,
Vita-Salute University and Department of Dentistry,
IRCCS San Raffaele Hospital, Milan, Italy
e-mail: cattonif@tiscalinet.it

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Nowadays there are other types of anti-discoloration systems such as, for example, in our study we used a test group containing an anti-discoloration system called SPPD.

The aim of our study is to compare 4 types of mouthwash in two different chlorhexidine concentrations (0.12% and 0.20%) without alcohol and with ADS and SPPD. Firstly, the inflammatory indices were evaluated and then the level of discoloration.

Study design

A single-center, prospective, double-blind randomized clinical trial on 84 patients. The investigated treatments consisted of 4 mouthwashes (CHX 0.12% SPDD alcohol free; CHX 0.20% SPDD alcohol free; CHX 0.12% alcohol free with ADS; CHX 0.20% alcohol free with ADS). Each patient provided a written informed consent before participation. The protocol was approved by the Local Ethical Committee. Participants were chosen among patients seeking care at the Center for Dental Hygiene and Prevention at the Department of Dentistry, IRCCS San Raffaele Hospital, Milan, Italy. Patient admissibility for the study was determined based on the inclusion/exclusion criteria.

Patient selection

84 consecutive patients, 30 female and 54 males, aged between 20 and 45 years, were enrolled.
The inclusion criteria were:

- the absence of concomitant local or systemic pathologies.
- the absence of pregnancy.
- no medical history of allergy.
- no intake of substances (including hallucinogenic drugs) featured by a potential pharmacological interaction with the active ingredients to be tested.
- no intake of antibiotics and/or anti-inflammatory drugs in the 6 months prior to beginning of the study.
- no physical or mental disability such as might impair normal domestic oral hygiene practice.
- The exclusion criteria were:
- non-compliance with the inclusion criteria.
- smoking habit.
- the presence of fixed prostheses from upper right second premolar to upper left second premolar.
- the use of temporally prosthesis.
- the presence of orthodontic therapy.

The patients were randomly assigned to 4 groups, each of which consisted of 21 subjects. Assignment of the mouthwashes to the groups was randomized by a computer-generated sequence and double-blind. The concealment of the allocation was preserved by sequentially numbered sealed envelopes.

The Groups were as follows:

- Group 1: Chlorexhidine SPDD 0.12% without alcohol.
- Group 2: Chlorexhidine SPDD 0.20% without alcohol.
- Group 3: Chlorexhidine 0.12% without alcohol with ADS.
- Group 4: Chlorexhidine 0.20% without alcohol with ADS.

To provide standardization of home dental hygiene procedures, each patient was offered two 250 ml bottles of mouthwash, a medium-bristled toothbrush and a chlorhexidine-free toothpaste. Patients were instructed to wash their mouths with mouthwash for 1 minute twice a day half an hour after brushing their teeth. In order to avoid bleaching drinks and foods, they were also asked to abstain from consumption (3). At the first follow-up (T0), each patient filled out a medical history questionnaire and underwent a professional oral hygiene session, which revealed plaque and bleeding scores (O'Leary Plaque Index (PI), and Bleeding Index (BI) respectively) by means of a periodontal probe (DP-10, Hu Friedy, USA).

In order to follow the colour variations of the tooth surface during the test period, we verified the pre-treatment post-treatment colour of the maxillary right central incisor (the intrinsic technical characteristics of the spectrophotometer do not allow, due to its size and the alignment necessary to make the measurement, to operate on distal elements) by using a spectrophotometer (SpectroShadeTM MHT S.p.A. Medical High Technologies, Verona, Italy), which provides objective assessment of chromium, colour, value on the basis of the CIELAB system (Delta E). Successive follow-ups at 7 (T1), 14 (T2) and 21 (T3) days monitored plaque index (PI) and

bleeding index (BI) values, along with tooth colour.

During the entire duration of the study, all patients were supervised by a dental hygienist, who highly recommended proper oral hygiene at home and trained all patients on the appropriate use of their mouthwash. In addition, the conformity of the patients at home with the trial protocol was evaluated by questionnaires that the patients answered at each follow-up. Each one patient responded to questions on taste satisfaction.

Outcome measure

Outcome measure were assessed as primary outcomes PI and BI according to the Plaque Control Record and the Gingival Bleeding Index respectively.

Plaque Index (PI): it was assessed according to the Plaque Control Record. A dental hygienist, by means of a probe (PCP UNC 15, Hu Friedy, USA) detected the presence of plaque at six points on the tooth surface (disto-buccal, mesio-buccal, buccal and lingual, mesio-lingual, disto-lingual), revealed by plaque detector. The number of surfaces with plaque divided by the number of available tooth surfaces and multiplied by 100 expresses the percentage of plaque presence.

Bleeding Index (BI): index measuring the presence of gingival bleeding on gentle probing of six tooth surfaces (disto buccal, mesio-buccal, buccal and lingual, mesiolingual, disto-lingual). A dental hygienist, by means of a probe (PCP UNC 15, Hu Friedy, USA) assigned a positive score when bleeding happened within 10-15 seconds, running along the cervical surface of all teeth. The number of positive areas was divided by the number of areas tested, and the result was multiplied by 100 to express the index as a percentage. The absence/reduction of the gingival bleeding index was interpreted as an enhancement of the inflammatory status.

Delta E: the colour difference before and after treating the maxillary right central incisor, recorded according to the CIELAB system.

Randomization

A computer-generated list of random numbers was used to allocate participants to the four groups. The randomization sequence was generated by using specific statistical software (SPSS 17.0, SPSS Inc., Chicago, Illinois, USA). A dental hygienist (blinded and calibrated at baseline) carried out patient recruitment, professional oral hygiene procedures and outcome evaluation. Another dental specialist made the allocation to each group for mouthwash preparation; and each patient was given mouthwash in an unmarked bottle according to the randomization list.

Blinding

The treatment identity was blind to the practitioner who conducted patient recruitment and outcome evaluation, the data analysts, and the participants. Only the practitioner who performed the group allocation was conscious of the allocated group.

Statistical analysis

Dedicated software (SPSS 17.0, SPSS Inc., Chicago, Illinois, USA) was used to perform the statistical analysis. The data were subjected to the Kolmogorov-Smirnov test to assess the normality of the distribution. Subsequently, each of the study variables (PI, BI and Delta E) was subjected to multivariate analysis of variance and Tukey's post hoc test for multiple comparisons. For all statistical tests, statistical significance was set at α=0.05. The initial null hypotheses were "there is no association between the different mouthwash formulae used and the change in PI and BI; there is no associations between the different mouthwash product formulations used and the change in tooth colour".

RESULTS

Kolmorogov-Smirnov confirmed the normality of data distribution ($p>0.05$). There are no significant differences in modifications both of bleeding indices (in the statistical analysis); the are no significant differences in modifications of plaque indices; moreover, all patients report less long-term discoloration with the new SDPP system and a more pleasing taste

As show in Table I all the test formulations were efficient in enhancing the periodontal indices here analyzed. Mouthwashes that contain 0,20% chlorhexidine reduce BI more than 0,12 chlorhexidine,

with a medium reduction of 85% (table I); In fact, the group 1 and 2 achieve a better result in term of PI and BI, respectively 0.12% combination 49% and 0.20% combination 55% in PI and 0.12% combination 9% and 0.20% combination 87% in BI.

Furthermore, all mouthwashes can be defined as efficiency in terms of PI. The group 2 and 4 are more efficacy in term of BI. In general mouthwashes with SPDD have the better results but non significatively. Comparison of T1 with T0 data point out that all of the tested mouthwashes demonstrate the efficacy of the chlorhexidine molecule, in terms of reduction both in PI and BI. The group 2 mouthwash was the most effective in reducing PI and obviously its high ability in reducing BI.

The pre- and post-procedures data for pigmentation, which we take with a spectrophotometry, showed changes in Delta E, very similar between the 4 groups; moreover, the test groups (1 and 2) had better results in term of Delta E. Most pigmentations were found in the control groups (3, 4).

DISCUSSION

Despite the limitations of the study, all the mouthwashes tested showed good efficacy in reducing the amount of plaque. Comparing the two experimental concentrations (0.12% and 0.20%) tested here demonstrates that the 0.20% chlorhexidine concentration slightly surpasses its 0.12% equivalent regarding the PI and BI parameters. No statistically significant differences were found between the two types of mouthwash in terms of plaque and bleeding index, both alcohol-free.

There are differing views on alcohol-containing mouthwashes in the literature. Recent studies have shown that the high alcohol content in long-acting mouthwashes may potentially increase the risk of oral and oropharyngeal cancers (12-14). Indeed, chlorhexidine is considered the antibacterial "gold standard" and it is used in many fields of dentistry. Chlorhexidine is commonly used in non-causal therapy in the treatment of periodontitis and implant maintenance (15-17). Commonly used as a home oral hygiene aid and as a mouthwash of choice before starting oral hygiene procedures (18). However, considering that antiviral power of chlorhexidine is rather low, and that the literature stresses the involvement of the mouth in covid-19 pathology, it should be accompanied by another rinse (povidone iodine, hydrogen peroxide) before starting dental procedures for the safety of the patient and the operator (19-20).

However, in this study we analyzed two mouthwashes with chlorhexidine as an excipient with two different color protection systems; a control group (3-4) with ADS already described in the literature and the test group (1-2) with an innovative protection system and a very pleasant minty taste, as reported by the patients.

A meta-analysis shows that there is no significant effect of ADS on tooth stains in situations where mouthwash is used in addition to toothbrushing (21). While other authors point out that there are no statistically significant differences in terms of plaque and bleeding indices, but the test group with ADS had less staining than the control group during a 15-day period of use (22, 23).

This study lays the groundwork for further studies investigating the potential of the SPDD system, as it has been shown to be effective for pigmentations and to offer excellent patient-perceived taste.

Table I. *Test formulations for periodontal indices.*

Group	PI	BI
1 SPDD 0.12%	49%	9%
2 SPDD 0.20%	55%	87%
3 ADS 0.12%	48%	7%
4 ADS 0.20%	53%	82%

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