LETTER TO THE EDITOR

Airborne contamination during a full-mouth disinfection session: Pilot study before COVID-19 pandemic

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To the Editor,

The World Health Organization (WHO) released guidelines for the use of special Personal Protective Equipment (PPE) and avoiding and minimising use of aerosols during the COVID-19 pandemic (1). Professional dental hygiene is the most aerosol-producing chair-side activity in dentistry. A small fraction of contaminated droplets usually goes back into the air during almost all dental procedures, and virus, bacteria, and fungi could contaminate each surface in the dental office for days (2).

The One-Stage Full-Mouth Disinfection (FMD) introduced by Quirynen and co-workers in 1995 is the most used approach in professional oral hygiene (3). It consists of treating the patient's mouth all in one session, thus reducing the overall treatment time and site-to-site cross-infection risk. The standard procedure includes the use of chlorhexidine mouthwashes before the debridement (4). Different studies have reported the benefits of combining full-mouth debridement with the use of mouth rinses with antiseptic agents before or during the session (5). A recent review by James P. et al. confirmed the benefits of pre-operative Chlorhexidine oral rinses in terms of better clinical outcome and reduced bacterial contamination (6). Different alternatives to Chlorhexidine have been suggested to overcome Chlorhexidine side effects and explore the potential new adjunctive effect of other products such as ozonised water that is effective against different micro-organisms such as bacteria, virus, and fungi (7).

The aim of the present pilot study was to evaluate the antimicrobial efficacy of pre-operative rinses using ozonised water and Chlorhexidine before FMD in terms of airborne contamination prevention.

MATERIALS AND METHODS

To assess the bacterial load on the surfaces near dental chairs in a dental clinic (due to the contamination of aerosol during these treatment procedures), a slide was positioned 35 cm in front of the patient's oral cavity. The present clinical study was conducted in accordance with the 2008 Declaration of Helsinki. Each patient had to sign an informed written consent and answer a specific anamnestic questionnaire about their general and oral health. According to the inclusion and exclusion criteria, all consecutive patients needing a professional oral hygiene section were enrolled in the study (after the screening) between January and December, 2019.

The inclusion criteria were: patients aged between 25 and 60 years and with moderate gingivitis; patients with good compliance with their oral hygienist (both

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for home and professional treatments); and patients with good general health.

The exclusion criteria were: pregnancy; systemic conditions impairing oral health; professional hygiene or antibiotic therapy within the previous four months; mental disorders or physical disabilities affecting patient compliance; abscess, or other relevant inflammatory phenomena in the oral cavity; smoking more than ten cigarettes per day.

Study protocol

At least 40 patients were required for the study. The treatment procedure consisted of a periodontal examination (probing with a standard periodontal North-Caroline probe), after which the patients were randomly allocated to one of the two groups of treatment. At baseline (T0) patients were simply motivated to carry out a better home oral hygiene by using a chlorhexidine mouthwash and a sonic or electric toothbrush to reduce oral inflammation and plaque, according to the principle of modified full-mouth disinfection (MFMD) introduced by Genovesi and co-workers (7).

At T1 (after ten days), a full-mouth disinfection session was performed to patients according to the treatment group. Control (C) group (Control-group, n=30): All patients rinsed their mouths for 1.5 min with physiological water, then ultrasonic debridement, scaling and deplaquing were performed. The session concluded with air-polishing and again a 1.5-min rinse with physiological water. In the test (T) group (Ozone-group, n=30), all patients rinsed their mouths for 1 min and 30 s with ozoned water (1:3) and then 1 min with digluconate chlorhexidine 0.20% (Plakout Active - Polifarma Benessere Srl), for decontamination purposes. Then, ultrasonic debridement, scaling and deplaquing were performed. The session concluded with air-polishing and, again, 1-min rinse with digluconate chlorhexidine 0.20. Three patients (of the same group) were treated consecutively with the same microbiological test plate positioned at a 35-cm distance from them.

Microbiological analysis

The Mikrocount® (supplied by De Marco S.r.l. Via F. Tajani, 9 - 20133 Milan, produced by Schülke, Germany) is a simple microbiological test that uses small dip slides of 12.5cm². Mikrocount® dip slides are a tool to systematically monitor environmental hygiene in a dental or medical clinic. These types of slides are used for sterilisation and disinfection control of surfaces and airborne disinfection, and their protocol has been validated in different studies. As reported in the literature, this test is very rapid, convenient, safe, and uncomplicated (8). One dip slide is sufficient to determine the total plate count within the scope of microbiological monitoring. It contains nutrients for the growth of the most common bacteria, enterobacteria, yeasts and fungi; specific characteristics are as follow:

- Presentation: 4.5 ml medium each side
- Agar surface: 12.5 cm²
- Shelf-life: 9 months
- Storage: 10–25°C.

One slide of Mikrocount® TPC test was unscrewed and extracted from the cylindrical container without touching the culture media surfaces. The surface was positioned at a 90° angle from the patient's head. After

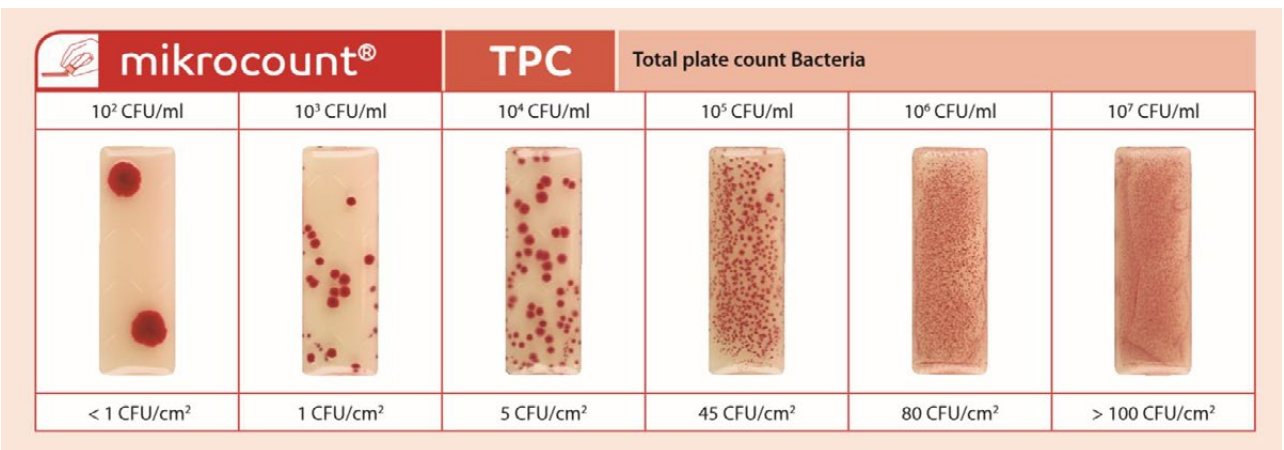

Fig. 1. *The estimated bacterial concentration using the mean distribution in the slide (1 CFU/Site= 1/1.25 x 104 CFU/10 M2).*

three consecutive patients, the slide was inserted in the container and incubated at 30±1°C for 24–48 h as indications of the producer. After incubation, the bacterial growth on media (plate count agar with triphenyl tetrazolium chloride and neutralising) was examined, and the bacterial concentration was estimated using the visual scheme shown in Fig. 1 (1 CFU/Site= 1/1.25 x 104 CFU/10 M2), classifying the slide in VI classes. One slide was used for three consecutive patients of the same groups, i.e. 10 times for each group of treatment.

Statistical analysis

Data were expressed as a mean ± standard deviation (SD) of values assigned for each patient; 1 point for class I, 2 points for class II, 3 points for class III, 4 points for class IV, 5 points for class V, 6 points for class VI. The *t*-test for independent samples was used to evaluate the statistical difference between the control group and test groups. A value of $P \leq 0.01$ was taken as being statistically significant. A Microsoft Excel spreadsheet (Microsoft Windows 2019) was used to display the statistics.

RESULTS

Sixty patients were considered eligible and enrolled in the study. The anamnestic data are resumed in Table I, and no significant differences were registered between the control group (C-group) and test group (T-group) ($p<0.05$).

In the C-group, no slides were associated with class I, class V or class VI while one slide was classified as class II, (3 patients); six slides were in class III and three slides in class IV. In the T-group, one slide was associated with class I, six slides with class II, three slides with class III and 0 slide with class VI. These data are shown in Table II.

A score was assigned to each patient corresponding to the slide class. The mean (± SD) score in the C group was 3.2±0.63, in the T group 2.2±0.63. A high score represents a high microbe contamination of the slides. The score in the T group was statistically significantly less than the C-group with p-value ≤ 0.01.

Table I. *Anamnestic data: sample size, age, gender, smoking. Only patients who smoked less than 10 cigarettes per day were admitted to the study.*

	C group	**T group**
Sample size	30	30
Age	39.38±5.08	41.0 ±7.14
Gender F/M	16/14	15/15
Smoking habits* (less Y/N)	7/23	9/21

Table II. *Class attribution for each slide. One slide corresponds to 3 patients.*

Classes	**I**	**II**	**III**	**IV**	**V**	**VI**
C-group (slide/patients)	0/0	1/3	6/18	3/9	0/0	0/0
T-group (slide/patients)	1/3	6/18	3/9	0/0	0/0	0/0

DISCUSSION

The aim of the present pilot trial was to evaluate the bacterial contamination of a surface 35 cm distant from the oral cavity of patients treated by non-surgical periodontal therapy. As reported in the results, in the T group, a water-ozone rinse was used immediately before the FMD, showing effectiveness in preventing airborne contamination. The Mikrocount® TPC test revealed significantly fewer bacteria, yeasts, and fungi in patients who received the pre-operative rinse by ozonised water than patients who received a physiological water rinse. In other fields of medicine, pre-operative oral decontamination by an antimicrobic rinse has also been recommended by recent systematic reviews (9). The classic cross-over study of Sekino and co-workers reported that a Chlorhexidine mouthwash used before and after dental treatments reduces plaque formation significantly (as well as decreasing the bacteria count) (10). Nevertheless, other studies have reported that prolonged use of Chlorhexidine has several side effects, and that the personal microbiota of patients will return in the same pattern as before the use of the antimicrobic substance (11). However, more worrying, is that even in healthy individuals, a major shift in their saliva will lead to more acidic conditions and a lower nitrite availability (12).

Ozone is a triatomic molecule with three atoms of oxygen that could be used as a carrier for airborne disinfection, using its antimicrobial property against bacteria, viruses, fungi, and protozoa, and its anti-inflammatory and analgesic properties for stimulating healing, blood circulation, and the modulation of the immune system.

A limitation of the present study may be related to the microbiological test used. It is an efficient and validated test, but it is limited only to bacteria and fungi, not viruses. Furthermore, it would be interesting to compare several antimicrobic pre-operative protocols, such as including a larger sample and a stratification of patients according to plaque index (considering that some patients may have more plaque and tartar, therefore contributing to more airborne contamination). The present pilot study reports that a rinse with watered ozone, immediately before a session of FMD, has positive effects in preventing airborne contamination after an oral hygiene session, which is significantly greater than a rinse with physiological water.

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