JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

The investigation of antibacterial activity of hyperlight fluid fusion subcellular essential complex

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Abstract

Introduction: This study aimed to investigate the possibility of applying the hyperlight fluid fusion essential complex in dental plaque control, and to evaluate the effectiveness of new and modern agents used for the prevention and early treatment of gingivitis.

Methodology: The study included 60 subjects randomly divided into two groups. The control group was assigned to 0.12% chlorhexidine (CHX) mouth rinse, whereas the test group used a solution based on hyper-harmonized hydroxylated fullerene water complex (3HFWC), twice daily for 2 weeks. The plaque, gingivitis and bleeding scores were evaluated and recorded. Collected plaque samples were seeded on blood agar and incubated aerobically at a temperature of 37 °C for 24-48 hours. In order to isolate anaerobic bacteria, samples were seeded on Schaedler Agar and incubated anaerobically at 37 °C for seven days. Serial dilutions in saline from 10^1 - 10^6 were made, and grown colonies were counted and identified using the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) system.

Results: The reduction in the number of bacteria was significant in both control and test groups. The reduction was greater in the control group compared to the experimental group, but without statistically significant difference.

Conclusions: 3HFWC treatment causes significant reduction in the number of dental plaque microorganisms. Since 3HFWC solution exhibited a bacteriostatic effect similar to chlorhexidine it could be an adequate addition to solution of a growing problem in prevention and early treatment of gingivitis and periodontitis.

Key words: Chlorhexidine; dental plaque; gingivitis; 3HFWC.

J Infect Dev Ctries 2023; 17(5):643-648. doi:10.3855/jidc.17740

(Received 04 December 2022 – Accepted 07 January 2023)

Introduction

Gingivitis is an inflammatory disease of the gingiva present in a large number of people whose manifestation is of variable intensity and most often reversible. The main etiological factor that leads to the appearance and development of gingivitis is dental plaque that can be detected on the tooth surface, in the gingival sulcus, on conservative or prosthetic restorations, and inside the gingival or periodontal pocket.

According to some data, about 350 species of microorganisms that inhabit the oral cavity and participate in the complex interaction with the host tissue have been identified [1]. According to the latest data, the number of species of microorganisms is over 700, of which only 350 can be cultivated [2]. Most of these microorganisms are commensals, which live in a unique form of symbiosis and tolerance with the host tissue. However, the disease appears and develops when there is a change in the microbiome, i.e., a change in the composition and concentration of bacteria that inhabit one region in a certain period [3].

Despite the polymicrobial theory of periodontal infection, the opinion that only certain microorganisms of dental plaque exhibit periodontopathogenic potential was adopted in 1996.

It has been noticed that during the disease there is a change in the percentage of certain microorganisms concerning healthy periodontium, which is a characteristic of each individual [4]. Unlike healthy periodontium, whose dental plaque contains about 75% of Gram-positive microorganisms, inflammatory conditions of tooth-supporting structures are characterized by approximately 65% of Gram-negative bacteria. Dental plaque of the gingival sulcus is mostly inhabited by bacteria from the genera *Streptococcus*

and *Actinomyces* (*Streptococcus sanguinis*, *Streptococcus mitis*, *Actinomyces viscosus* and *Actinomyces naeslundii*) followed by *Prevotella intermedia*, *Fusobacterium nucleatum*, *Capnocytophaga*, *Neisseria* spp., *Veillonella* spp., *Campylobacter* spp., (about 13%), while spirochetes and motile bacilli are present in the lowest percentage [2,5,6].

With the development of inflammation and clinical signs of gingivitis, the number of Gram-positive aerobic microbes decreases, and with the deepening of gingival and periodontal pockets, the number of Gram-negative anaerobic microorganisms increases.

In the active phase of the disease, an increase in the number of bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythya*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* has been observed, the elimination of which leads to a significant improvement in the condition and to a decrease in the number of *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum* and *Treponema denticola*, characteristic of the remission phase of the disease [2,7].

Although the presence of dental plaque microorganisms plays a key role in the development of gingivitis and then periodontitis, the degree of periodontal damage depends on the intensity of the host's immunoinflammatory reaction to the presence of bacterial noxa.

Since an individual's response to gingival irritation with dental plaque is determined by local, systemic and genetic factors, it is known that gingivitis will progress to periodontitis only in a certain number of people. It is not possible to predict with certainty the moment of disease progression, and control of dental biofilm is the best method of prevention of periodontal diseases. Personal oral hygiene and professionally performed control of dental plaque are the main and basic ways of preventing periodontal diseases caused by oral biofilm microorganisms. However, mechanical plaque control is sometimes not sufficient to prevent the recurrence or progression of inflammatory periodontal disease, usually due to poorly acquired oral hygiene skills by the patient. To overcome such conditions, and to achieve the desired goal in terms of calming inflammation, the use of a complementary therapeutic method in the form of various antiseptic agents for the control of the oral biofilm is indicated. Regardless of the fact that plaque control can be two-fold, priority is given to the means and methods of mechanical control. Auxiliary chemical agents will only work if the oral biofilm is mechanically disintegrated beforehand.

The mechanism of action of chemical agents is manifested through the interference of the phases of dental plaque formation. This refers to preventing the adhesion of microorganisms to the tooth surface or inhibiting coaggregation and plaque maturation, which prevents further colonization of oral tissues [8]. The most commonly used means of controlling the dental biofilm is with chlorhexidine due to its high anti-plaque effect, which has been proven to slow down bacterial recolonization. Chlorhexidine is still an antiseptic of choice and a kind of gold standard for chemical plaque control due to its broad action on Gram-positive and Gram-negative bacteria. In addition, the effect on certain fungi and viruses is noticeable, as well as on bacteria localized in other oral niches on which the means of mechanical control do not have a direct impact (tongue, tonsils, oral mucosa) [9].

The application of this solution at a concentration of 0.12% exhibits a bacteriostatic effect in such a way that it damages the bacterial membrane, while a concentration of 0.2% has a bactericidal effect thanks to the coagulation of the bacterial cytoplasm [10]. However, chlorhexidine has side effects such as discolouration of teeth, tongue, and prosthetic restorations and changes in taste perception. This has led scientists to look for other antiseptics of similar effectiveness.

Modern trends in the development of new oral hygiene products include the use of cosmetic products with nanomaterials [11]. Fullerene C60 is an allotropic modification of carbon and a nanomaterial with great potential for use in biomedical purposes [12]. To improve the effects of fullerene, derivatization of the basic molecule is applied, which improves the physical and chemical properties. Hydroxylated fullerene has increased hydrosolubility, antioxidative and antimicrobial properties [13,14]. The hyper-harmonized hydroxylated fullerene water complex (3HFWC) is a patented hydroxylated fullerene derivative surrounded by arranged layers of water that are stabilized by hydrogen bonding [15]. Arranged layers of water prevent direct contact of the 3HFWC nucleus with the external environment and thus protects biomolecules from possible side effects of fullerol, as well as the 3HFWC nucleus itself from external influences [16,17].

As already mentioned, dental plaque control is the key solution in the prevention of both gingivitis and periodontitis. The current, causal approach of eliminating one of the etiological factors for the occurrence to these diseases is to remove or reduce the

number of dental plaque microorganisms from the tooth surface.

This study aimed to investigate the possibility of applying of Hyperlight Fluid Fusion Essential Complex (composition: 3HFWC, Bioptron, Beograd, Srbija) in dental plaque control, as well as to evaluate the effectiveness of new, modern agents used for the prevention and early treatment of the most common periodontal diseases.

Methodology

The study included 60 subjects, aged 20 to 50 years, with visible clinical signs of gingivitis and periodontitis.

Criteria for inclusion in the study were:

a. presence of gingivitis or the initial stage of periodontitis,
b. existence of not less than 10% and not more than 30% of places that bleed on probing
c. values of clinical attachment level ≤ 2 and periodontal pocket depth ≤ 3.

Criteria for exclusion from the study

a. allergy to any of the components of the solution,
b. presence of an orthodontic appliance or prosthetic restorations,
c. carious lesions in the cervical third of the tooth crown,
d. use of antibiotics in the last two months,
e. pregnant and lactating women.

All patients were provided with written informed consent and all procedures were carried out with adequate understanding. The study protocol and consent forms were in accordance with the Declaration of Helsinki of the World Medical Association and were approved by The Medicines and Medical Devices Agency of Serbia.

The respondents were divided into two groups using the method of random selection. The control group was given a mouthwash solution based on 0.12% chlorhexidine (Hibidex DAP, Galenika, Beograd, Srbija) while the experimental group was given a solution based on 3HFWC (Hyperlight Fluid Fusion, Bioptron, Beograd, Srbija). As part of the first visit, soft and hard deposits had been removed from all subjects using an ultrasound machine at the Clinic of Periodontology, Faculty of Dentistry in Pancevo. The subjects were then instructed on how to properly perform the modified Bass tooth brushing technique and flossing.

After two weeks, as part of the second visit, soft deposits were removed from all subjects with a rotating brush and gingival index according to Loe Silness (GI), the index of bleeding gingiva (IKG), the periodontal index with an assessment of treatment needs (CPITN) and the plaque index according to Silness Lou (PI) were determined. The periodontal probe of the University of North Carolina (UNC-15; Hu-Friedy, Chicago, IL, USA) was used for this purpose and the measurement was performed on six tooth sides (mesio-buccal, medio-buccal, disto-buccal, disto-lingual, medio-lingual and mesio-lingual tooth surface). Dental plaque was then sampled using a paper point (Diadent - Group International Inc, Cheongju, Korea). A paper point was applied to the deepest gingival/periodontal pocket for 20 seconds and stored in sterile Eppendorf tubes (volume 1.5 mL, Ismaning, Germany) with the addition of 100 μL of physiological solution.

The samples were seeded on blood agar (Columbia 5% Sheep Blood Agar, ProMedia, Kikinda, Srbija) in the laboratory of the Faculty of Dentistry in Pancevo, and the plates were incubated aerobically at a temperature of 37 °C for 24-48 hours. In order to isolate anaerobic bacteria, samples were seeded on Schaedler Agar (ProMedia, Kikinda, Srbija) which was incubated anaerobically at 37 °C for seven days. Serial dilutions in saline from 10^1 - 10^6 were made, and grown colonies were counted and identified using the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) system (Bruker Daltonic GmbH, Bremen, Germany) [2,18-20].

Data from the experiment were prepared for statistical analysis by entering them into a computer database (Excel software package). The data obtained by statistical analyses are presented in tables and graphs including the statistical parameters necessary for deriving conclusions regarding these surveys. Descriptive statistics of the results obtained in this experiment were calculated using the SPSS statistical package (IBM SPSS Statistics for Windows, Version 24.0.). The mean value is shown as a measure of central tendency for the corresponding variables, while the standard deviation is shown as a measure of variance. The sample sizes for the respective sample groups (3HFWC and chlorhexidine treatment) are also shown. The Kolmogorov-Smirnov Goodness of Fit Test was used to test for normality of our data. The null hypothesis for the test states that data are taken from normal distributed population. The Kolmogorov-Smirnov Test had a p value of 0.0037 leading to rejection of the null hypothesis that our data follows a normal distribution. Nonparametric tests were used in further analysis. Wilcoxon's test was used to test the association between applied treatments (3HFWC and chlorhexidine) and the presence of bacteria. A p value

Table 1. Mean values and standard deviations of differences in the number of dental plaque bacteria formed after the action of hyperlight fluid fusion subcellular essential complex (3HFWC) and chlorhexidine (H).

Treatment	n	Mean value	Standard deviation
3HFWC	22	46034	21305
H	19	112136772	31387500

Table 2. Wilcoxon test of differences in the reduction of the number of dental plaque bacteria as a result of the action of hyperlight fluid fusion subcellular essential complex and the reduction of the number of bacteria as a result of the action of chlorhexidine.

	Z- statistics	Pr > |Z|
Wilcoxon Two-Sample test	1.8463	0.0648

of 0.05 was taken as the threshold value for accepting/rejecting the null hypothesis. The values of the Wilcoxon statistic and the corresponding *p* values are shown.

Results

The results of the pilot/feasibility experiment are shown in Figures 1 and 2 and Tables 1 and 2. The number of dental plaque bacteria both after chlorhexidine and after 3HFWC treatment were significantly reduced in patients included in this pilot experiment (Figures 1 and 2).

The most frequently isolated strains were: *Streptococcus mitis*, *Rothia dentocariosa*, *Actinomyces viscosus*, *Streptococcus salivarius*, *Sterptococcus sanguinis*, *Streptococcus anginosus*, *Streptococcus cristatus*, *Neisseria flava*, *Lactobacillus casei*, *Candida albicans*, *Candida dubliniens*, *Klebsiella oxytoca*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*.

A decrease in the number of bacteria was present in all patients included in the experiment. The average reduction was several orders of magnitude in both groups of patients. The reduction in the number of bacteria varied significantly in different patients both in the experimental group treated with 3HFWC and in the control group treated with chlorhexidine.

This variation in the number reduction of bacteria was seen in the large values of standard deviations in both treatments (Table 1).

The Wilcoxon Two-Sample test (Table 2), compared the reduction in dental plaque bacteria in the two groups of patients. Although the reduction in the number of bacteria was greater in the control group of patients treated with chlorhexidine compared with patients treated with 3HFWC, no statistically significant differences were obtained in the application of these two treatments to dental plaque bacteria (p = 0.0648) (Table 2).

One patient reported side effects three days after starting hyperlight fluid fusion solution. The anamnesis did not reveal the existence of a systemic disease, nor the use of drugs. He was a non-smoker. The patient stated that he was prone to allergies. Clinical examination revealed oral mucosa diffuse enanthema localized on the mucosa of the fornix of the upper jaw on both sides and on the mucous membrane of the floor of the mouth. Minor erosion of the buccal mucosa was noticeable in the region of the upper left premolars. After discontinuing the hyperlight fluid fusion solution, the symptoms subsided after five days.

Figure 1. Average number of dental plaque bacteria in patients before and after chlorhexidine administration.

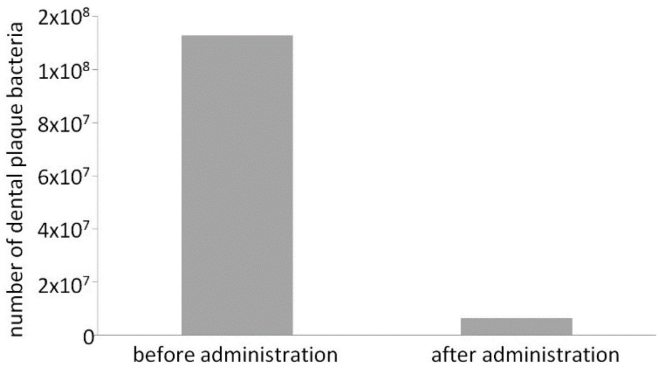
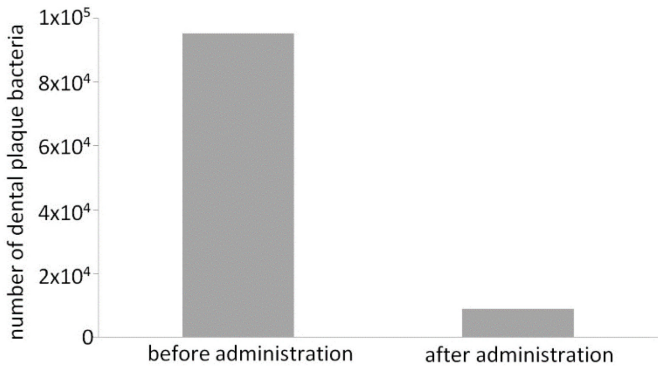


Figure 2. Average number of dental plaque bacteria in patients before and after hyperlight administration fluid fusion subcellular essential complex (3HFWC).



Discussion

Hyperlight fluid fusion subcellular essential complex has proven effects in removing microorganisms. This is supported by the results of this study, where it was shown that there is no statistically significant difference in reducing the number of dental plaque bacteria using these solutions. Although chlorhexidine solution is still the gold standard for chemical plaque control, this study showed that 3HFWC may be an adequate alternative because it does not lead to tooth or tongue discolouration, or dysgeusia, which are listed as side effects of chlorhexidine solution use. Taking into consideration that chlorhexidine solution is the only solution with a proven anti-plaque effect, we believe that the synergistic effect of these two solutions would give the best results in the chemical control of dental plaque in patients with gingivitis and periodontitis. We consider that it is necessary to plan a prospective study that would include *in vitro* testing on reference strains, so that the combination of these two solutions can become a future part of the protocol in the treatment of diseases of the tooth-supporting structures. Bacterial reductions were present in all patients included in the experiment and varied significantly in different patients in both the 3HFWC-treated experimental group and the chlorhexidine-treated control group. Although the reduction in the number of bacteria was greater in the control group of patients treated with chlorhexidine compared with patients treated with 3HFWC, no statistically significant differences were obtained in the application of these two treatments to dental plaque bacteria. Given that 3HFWC solution showed a bacteriostatic effect on oral bacteria, but without statistical significance, we believe that a prospective study should be planned that would include *in vitro* testing on American Type Culture Collection (ATCC) reference strains of the most important aerobic and anaerobic oral bacteria causing caries and periodontal disease. The study would compare the minimum inhibitory concentrations (MIC) values for the 3HFWC solution and 0.12% chlorhexidine. If statistically significant effects of the test solution were obtained, the *in vivo* testing in selected patients would be continued in the second phase of the study.

In this study the efficacy of a 3HFWC solution and 0.12% chlorhexidine was examined *in vivo*. A decreased number of bacteria which is higher in the control group of patients treated with chlorhexidine compared to patients treated with 3HFWC was registered, but without a statistically significant difference.

Conclusions

Hyperlight fluid fusion subcellular essential complex has proven effects in removing microorganisms of dental plaque. 3HFWC solution exhibited a bacteriostatic effect similar to chlorhexidine, which is a gold standard for chemical plaque control, but without any side effects. Thus, it could be used in the prevention and early treatment of gingivitis and periodontitis. We believe that a prospective study should be planned that would include in vitro testing on reference strains, from the ATCC of the most important aerobic and anaerobic oral bacteria that are the causative agent of caries and periodontal diseases.

Authors' contributions

Zoran Tambur designed the study. Ema Aleksić, Milana Čabrilo, Katarina Kalevski and Miljan Puletić took samples. Dolores Opačić performed the experiments. Stevan Avramov analysed the data. Zoran Tambur wrote the paper with input from all authors.

References

1. Socransky SS, Haffajee AD (2005) Periodontal microbial ecology. Periodontol 2000, 38: 135-187.
2. Abusleme L, Dupuy AK, Dutzan N, Silva N, Burleson JA, Strausbaugh LD, Gamonal J, Diaz PI (2013) The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J 7: 1016-1025.
3. Pflughoeft KJ, Versalović J (2012) Human microbiome in health and disease. Annu Rev Pathol 7: 99-122.
4. Rosan B, Lamont RJ (2000) Dental plaque formation. Microbes Infect 2: 1599-1607.
5. Noack B, Görgens H, Hoffmann T, Fanghänel J, Kocher T, Eickholz P, Schackert HK (2004) Novel mutations in the cathepsin C gene in patients with pre-pubertal aggressive periodontitis and Papillon-Lefèvre syndrome. J Dent Res 83: 368-370.
6. Marsh PD (2006) Dental plaque as a biofilm and a microbial community-implications for health and disease. BMC Oral Health 6 Suppl 1: S14.
7. Feng ZM, Weinberg A (2006) Role of bacteria in health and disease of periodontal tissues. Periodontol 2000 40: 50-76.
8. Brecx M (1997) Strategies and agents in supragingival chemical plaque control. Periodontol 2000 15: 100-108.
9. Graziani F, Karapetsa D, Alonso B, Herrera D (2017) Nonsurgical and surgical treatment of periodontitis: how many options for one disease? Periodontol 2000 75: 152-188.
10. Marui VC, Souto MLS, Rovai ES (2019) Efficacy of preprocedural mouth rinses in the reduction of microorganisms in aerosol: a systematic review. J Am Dent Assoc 150: 1015-1026.
11. Carrouel F, Viennot S, Ottolenghi L, Gaillard C, Bourgeois D (2020) Nanoparticles as anti-microbial, anti-inflammatory, and remineralizing agents in oral care cosmetics: a review of the current situation. Nanomaterials (Basel) 10: 140.

12. Castro E, Hernandez-Garcia A, Zavala G, Echegoyen L (2017) Fullerenes in Biology and Medicine. J Mater Chem B 5: 6523-6535.
13. Zhu X, Sollogoub M, Zhang Y (2016) Biological applications of hydrophilic C60 derivatives (hC60s)- a structural perspective. Eur J Med Chem 115: 438-452.
14. Aoshima H, Kokubo K, Shirakawa S, Ito M, Yamana S, Oshima T (2009) Antimicrobial activity of fullerenes and their hydroxylated derivatives. Biocontrol Sci 14: 69-72.
15. Koruga DJ (2021) Compositions comprising hyper harmonized hydroxyl modified fullerene substances. International Publication No: WO 2021/110234 A1, June 10.
16. Miljković S, Jeftić B, Sarac D, Matović V, Slavković M, Koruga D (2020) Influence of hyper harmonized fullerene water complex on collagen quality and skin function. J Cosmet Dermatol 19: 494-501.
17. Lazović J, Zopf LM, Hren J, Gajdoš M, Slavković M, Jović Z, Stanković I, Matović V, Koruga D (2021) Fullerene-filtered light spectrum and fullerenes modulate emotional and pain processing in mice. Symmetry 13: 11.
18. Srivastava A, Saha S, Sahu C (2020) Early and accurate detection of bacterial isolates from dental plaque in subjects with primary, mixed, and permanent dentition by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry technique. J Indian Sc Periodontol 24: 104-108.
19. Rams TE, Sautter JD, Getreu A, van Winkelhof AJ (2016) Phenotypic identification of *Porphyromonas gingivalis* validated with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Microb Pathog 94: 112-116.
20. Rams TE, Sautter JD, Hsiao CY, van Winkelhof AJ (2018) Phenotypic identification of periodontal *Prevotella intermedia/nigrescens* group isolates validated by MALDI-TOF mass spectrometry. Anaerobe 54: 201-204.

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Conflict of interests: No conflict of interests is declared.