

COMPARISON OF ANTIMICROBIAL ACTIVITY OF TWO COMMERCIAL TOOTHPASTES

Oana Botoacă¹, Cristina-Crenguța Albu^{2*}, Claudia Florina Bogdan-Andreescu^{1*}, Ștefan-Dimitrie Albu³, Andreea Grama⁴, Carmen Liliana Defta⁵

¹“Titu Maiorescu” University, Bucharest, Romania, Faculty of Dental Medicine, Department of Speciality Disciplines,

²“Carol Davila” University of Medicine and Pharmacy Bucharest, Romania, Faculty of Dentistry, Department of Genetics,

³“Carol Davila” University of Medicine and Pharmacy Bucharest, Romania, Faculty of Dentistry, Department of Periodontology,

⁴“Carol Davila” University of Medicine and Pharmacy Bucharest, Romania, Faculty of Dentistry.

⁵“Carol Davila” University of Medicine and Pharmacy Bucharest, Romania, Faculty of Dentistry, Department of Microbiology

Corresponding authors; *e-mail*: cristina.albu@umfcd.ro;

claudia.andreescu@prof.utm.ro

ABSTRACT

The bacteria within the oral cavity are incriminated for caries and periodontitis. The simplest method to reduce bacterial load is tooth brushing. The commercial toothpaste has therapeutic ingredients with antibacterial effects. **The aim of this study** was to evaluate the effect on the oral microbiome of two commercial toothpaste. One toothpaste has active ingredients 8% arginine and 1450 ppm fluoride and another 1100 ppm fluoride and 0.3% triclosan. **Material and method**: samples of dental plaque from 10 healthy subjects were collected before and after tooth brushing. The oral hygiene was scored using the oral hygiene index simplified. The Gram staining was used to identify bacterial genera. **Results**: The main bacterial strain identified was Gram-positive cocci. The filamentous Gram-negative bacteria were frequently identified mainly in subjects with poor and fair oral hygiene. In 17 out of 20 samples, there was a reduced bacterial community after tooth brushing. The paste with arginine and fluoride is more successful in decreasing filamentous Gram-negative bacteria. **Conclusion**: Both toothpastes provided antimicrobial activity. 1450 ppm fluoride in association with 8% arginine seems to be more effective than 1100 ppm fluoride in association with 0.3% triclosan in reducing the number of filamentous Gram-negative bacteria.

Key words: oral microbiome, toothpaste, antimicrobial effect, arginine, fluoride

INTRODUCTION

Oral microflora or oral microbiome or microbiota is defined as the sum of the microorganisms that exist in the human mouth [1]. The oral microbiome has multiple unique habitats, each one with its community of microorganisms [2]. On the teeth surfaces, microorganisms survive in a biofilm structured in an extracellular matrix of polymers from host and microbial origin called dental plaque [3], which mediates caries and periodontitis [4].

One particularity of oral microbiome is the

constant exposure to exogenous foreign substances: food and drink, dentifrices, smoking, medication, etc., which modified the composition of oral microflora [5]. In addition, the number and type of oral microbes varies with age and personal hygiene [6].

Toothpaste is a paste or gel designed to be used in association with a toothbrush to maintain and improve oral health [7]. Tooth brushing is the most common measure of oral hygiene and it is widely accepted as a highly effective practice for supragingival plaque

removal [8]. Correct tooth brushing is the key factor to microbial plaque control [9, 10] and, as a result, reduces the bacteria load within the oral cavity.

The tooth brushing affects mainly the community of microorganisms, which resides on the teeth surfaces and of interest are the bacteria implicated in common oral disease like caries and periodontitis [11, 12].

Tooth brushing combines two different plaque control methods: mechanical and chemical, with antimicrobial agents incorporated into toothpaste. A typical toothpaste has the following components: abrasive, humectant, binder, detergent, flavor, preservative, and therapeutic agent [13]. The therapeutic agent is usually antibacterial, and the conventional toothpastes contain triclosan and fluoride [14].

The aim of this study was to assess the effect of two commercial toothpastes over the bacterial species on the dental plaque.

MATERIAL AND METHODS

We performed an observational analytic study on 10 healthy subjects, 5 male and 5 female, with a range age between 19 and 59 years old, about the composition of oral flora before and after tooth brushing. The study was performed following the ethical standards of the Declaration of Helsinki on experimentation involving human subjects.

All the subjects who participated in this study provided preoperative written informed consent and agreement for the use of the tissue for research.

For each subject was performed a thorough anamnesis, a summary general clinical examination, and a detailed oral examination to identify possible conditions, that could interfere with the oral microbiome.

The exclusion criteria were: gingivitis, active periodontal disease, untreated dental caries, or any other oral condition that could

affect oral microbiome; fewer than 14 teeth; history of antibiotic usage in the past three months; long-term use of anti-inflammatory medication; smoking; pregnancy; the presence of systemic diseases that could affect oral health; and systemic medications that could disturb salivary function.

The participants in this study did not have untreated caries or active periodontitis. The oral hygiene was assessed using the oral hygiene index simplified (OHI-S), which is based on the amount of plaque and calculus present on teeth [15]. OHI-S was recorded based on the previously described method [16, 17], and oral hygiene was scored as poor, fair, or good.

The sampling of biological products from the participants in this study was carried out exclusively within the Microbiology Discipline of "Carol Davila", Faculty of Dental Medicine of the same trained periodontist. Each subject was scheduled in the morning on two different days, one week apart. The participants were asked not to consume any kind of food or drink before the sampling and also not to perform tooth brushing or use other auxiliary means of hygiene upon awakening.

There were no restrictions regarding diet throughout this study.

At the first appointment, each subject received a sample of Elmex Sensitive Professional (Colgate-Palmolive) toothpaste and a new disposable toothbrush or a brush head for the rotary one. At the second appointment, after one week, the subjects received a sample of Colgate Total Original (Colgate-Palmolive) toothpaste. On each of these days, the subjects were monitored for brushing their teeth, as well as for correct, incorrect, or absent flossing.

The sampling of dental plaque was performed using single-use consultation kits. The OptraGate (Ivoclar, Vivadent) retractor

was used to remove the soft parts and expose all the teeth and the isolation was performed with sterile cotton wool rolls placed in the oral vestibules.

The exploratory probe was used to collect sufficient material relevant to the study from the axial and occlusal surfaces of the teeth, as well as from their related gingival sulci.

Therefore, four samples were done for the same subject, two in the first appointment, before and two minutes after tooth brushing with Elmex Sensitive Professional (Colgate-Palmolive), and another two, one week later, in the second appointment, before and two minutes after tooth brushing with Colgate Total Original (Colgate-Palmolive).

The collected dental plaque was placed in a drop of saline solution on a sterile microscope slide and then displayed with the dental explorer on the entire slide surface.

The following working technique was applied to the collected samples: fixation of specimen by passing three times the slide through the flame of a Bunsen burner, cooling, immersion in 50% alcohol, drying, Gram staining with 2% crystal violet for one minute, rinsing with tap water, flooding with Lugol's solution for one minute, spilling without washing, decolorizing with acetone alcohol for 10 seconds, washing, staining with fuchsine 1/10 for one minute and final washing.

The Gram staining technique was chosen

to differentiate the Gram-positive bacteria, which stain violet under this procedure, and keep color, from the Gram-negative bacteria, which stain pink [18].

The examination was done with a 100× oil immersion objective microscope with cedar-wood oil to change the refractive index, to obtain 1000x magnification [19].

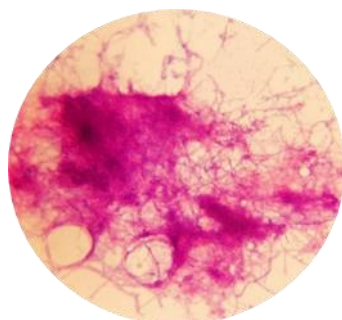
RESULTS AND DISCUSSIONS

The summative description of the studied group is presented in Table 1.

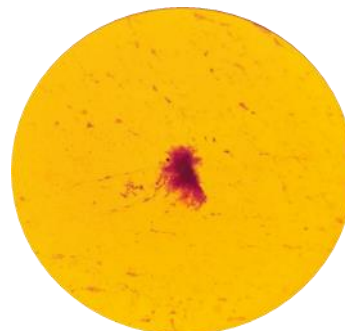
Table 1. Demonstration of patient data.

Subject	Age	Gender	OHI-S
1	24	F	good
2	59	M	fair
3	21	M	poor
4	54	F	good
5	38	M	poor
6	48	F	poor
7	51	M	poor
8	19	M	poor
9	27	F	good
10	26	F	fair

Figures 1-20 reveal the structure of the dental plaque before (a) and after (b) tooth brushing for selected subjects in order of examination.

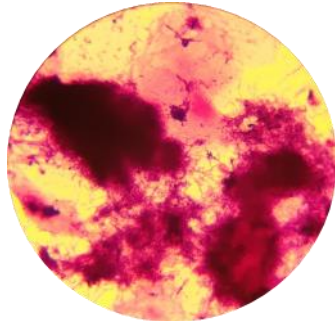


(a)

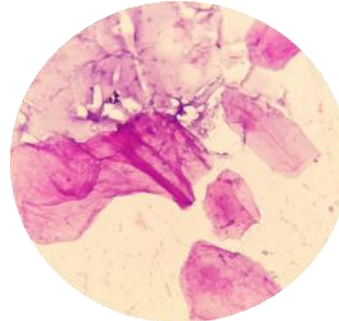


(b)

Figure 1. Patient no. 1. (a) before tooth brushing with Elmex: frequent filamentous Gram-negative bacteria, abundant Gram-positive cocci in diplo and in small chains; (b) after tooth brushing with Elmex: filamentous Gram-negative bacteria, rare Gram-positive cocci in diplo, one epithelial cell.

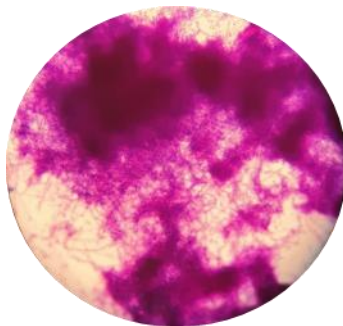


(a)

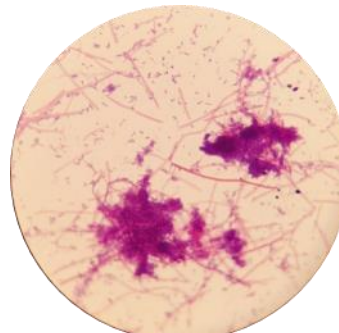


(b)

Figure 2. Patient no. 2. (a) before tooth brushing with Elmex: present epithelial cells, frequent filamentous Gram-positive bacteria, Gram-positive cocci in diplo and in small chains, frequent Gram-negative fusiform bacilli, rare Gram-positive curved bacilli; (b) after tooth brushing with Elmex: present epithelial cells, rare Gram-positive cocci in diplo and Gram-positive lanceolate cocci.

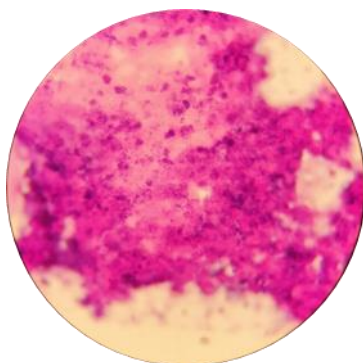


(a)

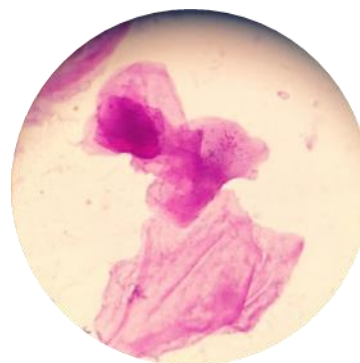


(b)

Figure 3. Patient no. 3. (a) before tooth brushing with Elmex: dominant Gram-negative flora, present filamentous bacteria, fusiform bacilli, rare cocci in diplo, frequent cocci in big clusters; (b) after tooth brushing with Elmex: rare filamentous Gram-negative bacteria, rare fusiform bacilli, present cocci in diplo and rare cocci in diplo in cluster.



(a)



(b)

Figure 4. Patient no. 4. (a) before tooth brushing with Elmex: present polymorphonuclear cells, present cocci in diplo, rare lanceolate cocci in diplo; (b) after tooth brushing with Elmex: present Gram-positive cocci in diplo in very short chains, present epithelial debris.

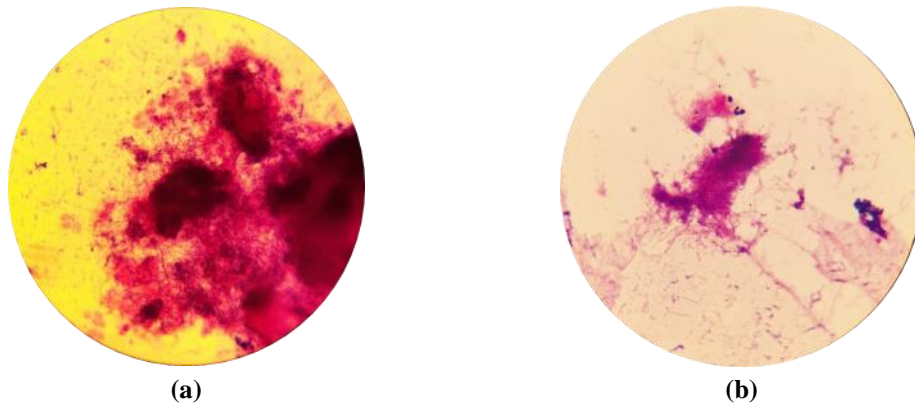


Figure 5. Patient no. 5. (a) before tooth brushing with Elmex: one polymorphonuclear cell, Gram-positive cocci in short chains, filamentous Gram-negative bacteria; (b) after tooth brushing with Elmex: present very rare Gram-negative bacilli in chains, very rare cocci in diplo, frequent filamentous Gram-negative bacteria, rare cocci in big clusters.

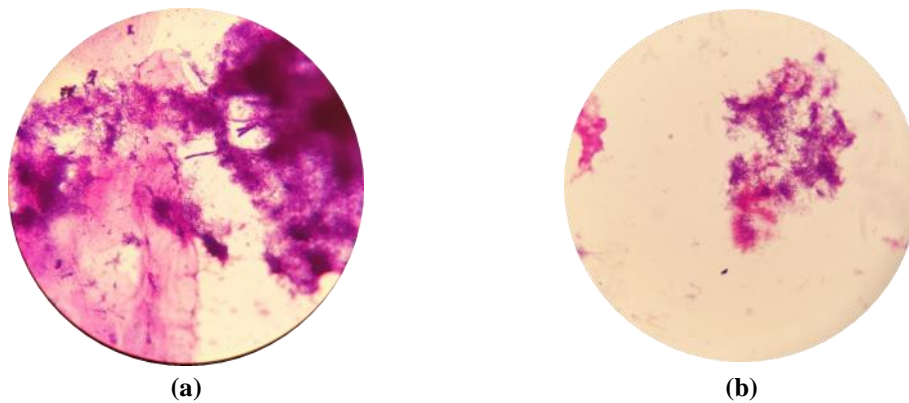


Figure 6. Patient no. 6. (a) before tooth brushing with Elmex: frequent Gram-positive lanceolate cocci, frequent Gram-positive cocci in diplo, frequent Gram-negative short bacilli, frequent Gram-positive curved bacilli, Gram-positive bacilli with rounded ends; (b) after tooth brushing with Elmex: cocci in diplo in small and big clusters, rare Gram-negative fusiform bacilli.

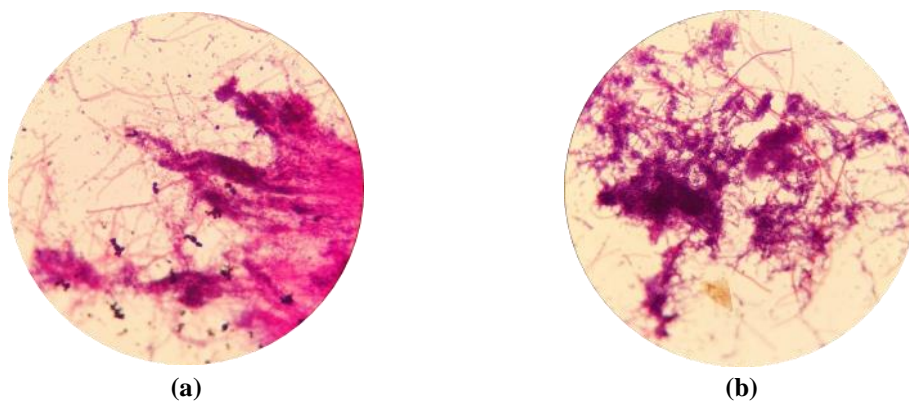
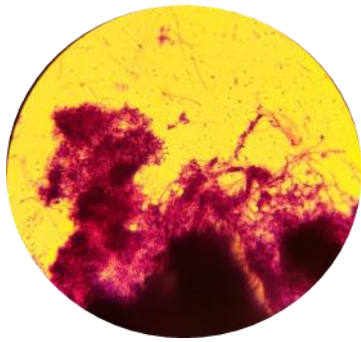
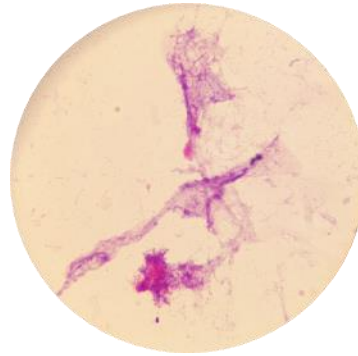


Figure 7. Patient no. 7. (a) before tooth brushing with Elmex: frequent lanceolate cocci in diplo, frequent cocci in diplo and in clusters, Gram-negative filamentous bacteria, rare Gram-negative fusiform bacilli; (b) after tooth brushing with Elmex: frequent Gram-negative fusiform bacteria, numerous cocci in diplo, cocci in clusters, rare curved bacilli, frequent filamentous bacteria.

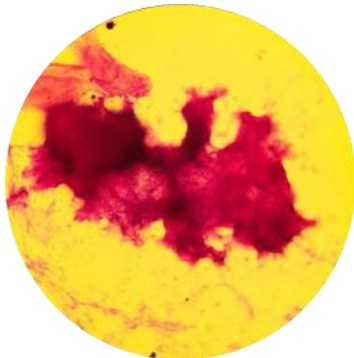


(a)

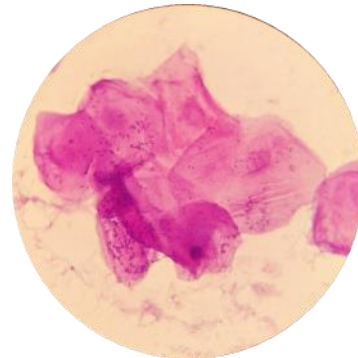


(b)

Figure 8. Patient no. 8. (a) before tooth brushing with Elmex: Gram-negative filamentous bacteria, abundant Gram-negative fusiform bacilli, frequent cocci in diplo, frequent lanceolate cocci in diplo; (b) after tooth brushing with Elmex: cocci in diplo in clusters, rare lanceolate cocci in diplo.

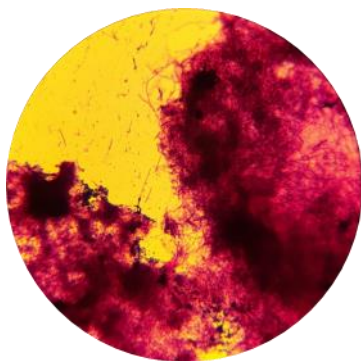


(a)

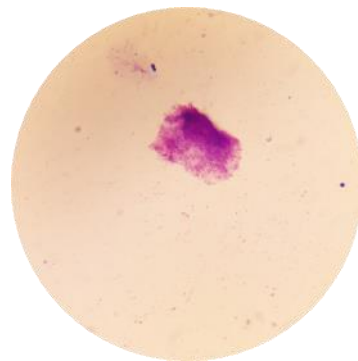


(b)

Figure 9. Patient no. 9. (a) before tooth brushing with Elmex: cocci in diplo, two epithelial cells; (b) after tooth brushing with Elmex: present epithelial cells, isolated cocci in diplo and lanceolate.



(a)



(b)

Figure 10. Patient no. 10. (a) before tooth brushing with Elmex: short Gram-negative bacilli, rare Gram-positive bacilli with rounded ends, frequent filamentous Gram-negative bacteria, cocci in diplo; (b) after tooth brushing with Elmex: rare epithelial cells, attached cocci in diplo, two per cell, one fusiform Gram-negative bacillus.

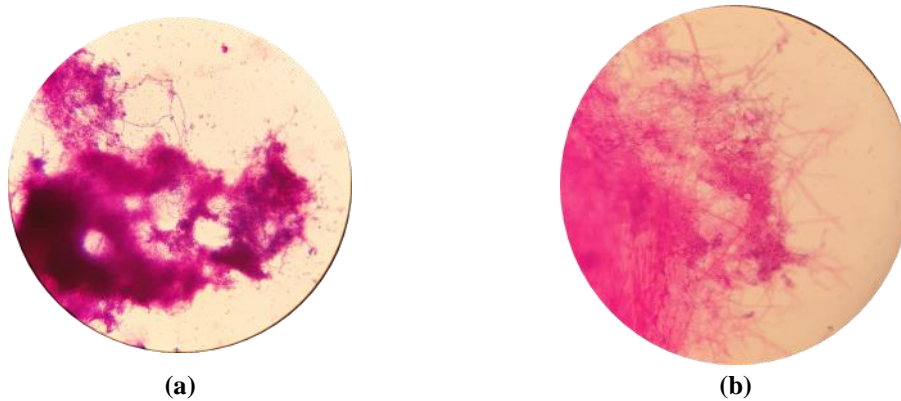


Figure 11. Patient no. 1. (a) before tooth brushing with Colgate: frequent filamentous Gram-negative bacteria, frequent cocci in diplo and lanceolate cocci in diplo, frequent cocci in diplo in small and big clusters, present Gram-positive bacteria, present Gram-positive curved bacilli; (b) after tooth brushing with Colgate: frequent filamentous Gram-negative bacteria, Gram-positive cocci in rare clusters, rare lanceolate cocci.

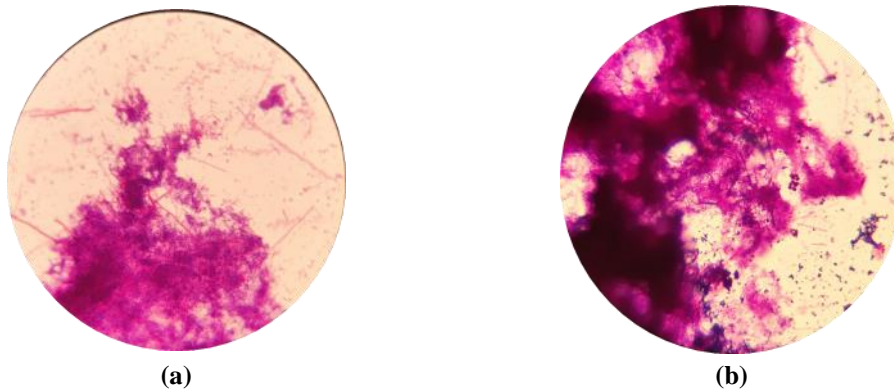


Figure 12. Patient no. 2. (a) before tooth brushing with Colgate: frequent filamentous bacteria with granular structure, Gram-negative fusiform bacilli, Gram-positive cocci in diplo in clusters and short chains, present lanceolate cocci in diplo; (b) after tooth brushing with Colgate: large Gram-positive formations, present Gram-positive lanceolate cocci in diplo, frequent Gram-negative filamentous bacteria.

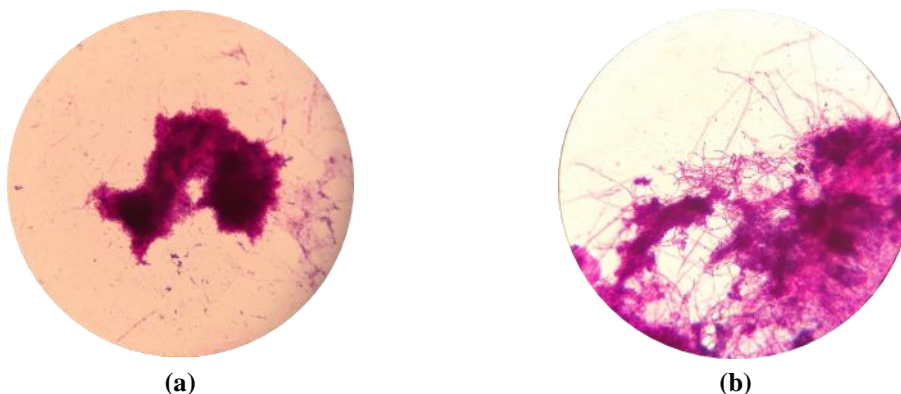


Figure 13. Patient no. 3. (a) before tooth brushing with Colgate: frequent Gram-negative fusiform bacilli, frequent cocci in diplo in small and big clusters, rare Gram-positive bacilli with rounded ends, present Gram-negative filamentous bacteria; (b) after tooth brushing with Colgate: frequent Gram-negative filamentous bacteria, very rare cocci in diplo.

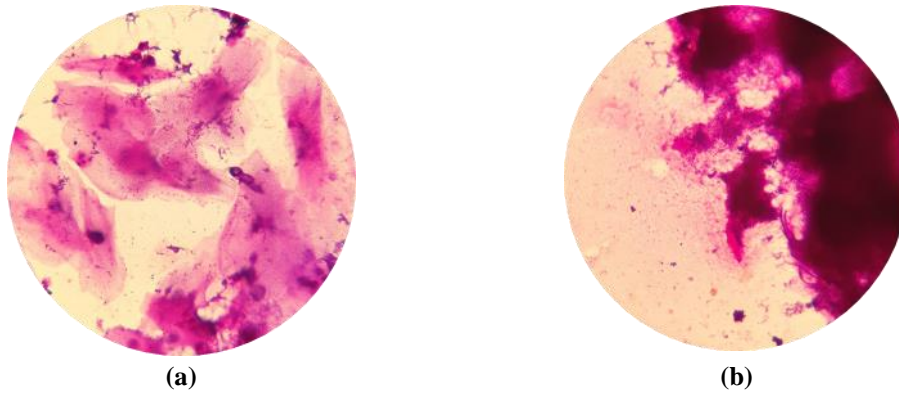


Figure 14. Patient no. 4. (a) before tooth brushing with Colgate: frequent epithelial cells, frequent cocci in diplo in clusters and short chains, very rare polymorphonuclear cells, lanceolate cocci in diplo, very rare fusiform bacilli, very rare Gram-positive bacilli with rounded ends; (b) after tooth brushing with Colgate: relatively frequent cocci in diplo, lanceolate cocci in diplo and rare curved bacilli.

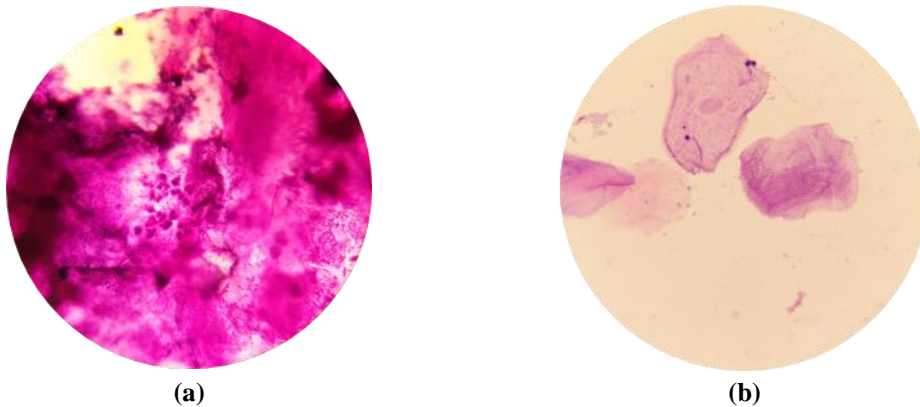


Figure 15. Patient no. 5. (a) before tooth brushing with Colgate: frequent epithelial cells, frequent Gram-positive cocci in diplo in clusters and short chains, rare Gram-positive bacilli with rounded ends, present polymorphonuclear cells; (b) after tooth brushing with Colgate: frequent Gram-positive cocci in diplo, rare Gram-positive lanceolate cocci in diplo.

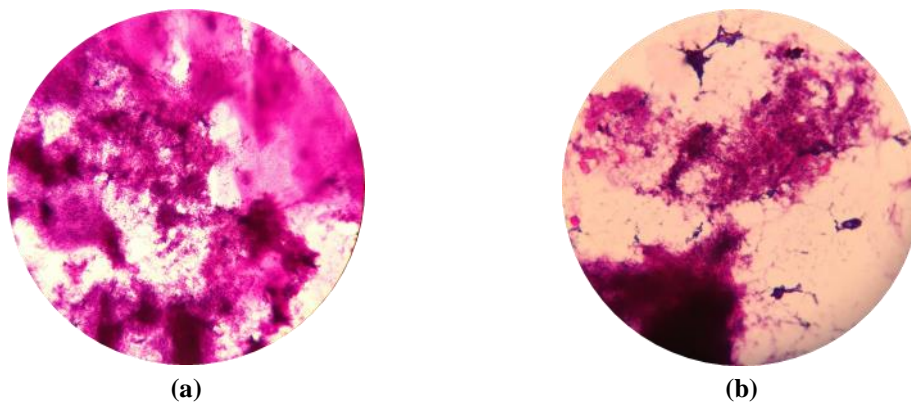


Figure 16. Patient no. 6. (a) before tooth brushing with Colgate: cocci in diplo in small and big clusters, frequent epithelial cells, very rare Gram-positive bacilli with rounded ends; (b) after tooth brushing with Colgate: frequent Gram-negative flora, frequent short bacilli, lanceolate cocci in diplo, cocci in diplo in small clusters.

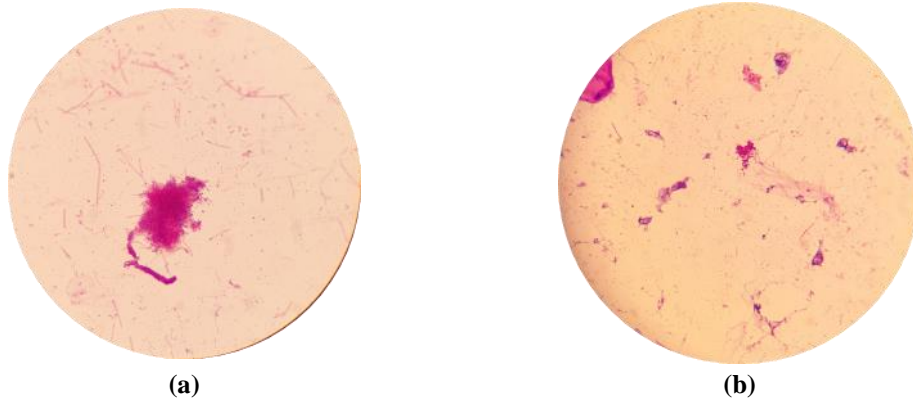


Figure 17. Patient no. 7. (a) before tooth brushing with Colgate: frequent fusiform bacilli, Gram-positive curved bacilli, rare Gram-positive bacilli with rounded ends, lanceolate cocci in diplo, cocci in small and big clusters, very rare epithelial cells; (b) after tooth brushing with Colgate: very rare Gram-negative fusiform bacilli, cocci in diplo, cocci in diplo in short chains and very small clusters.

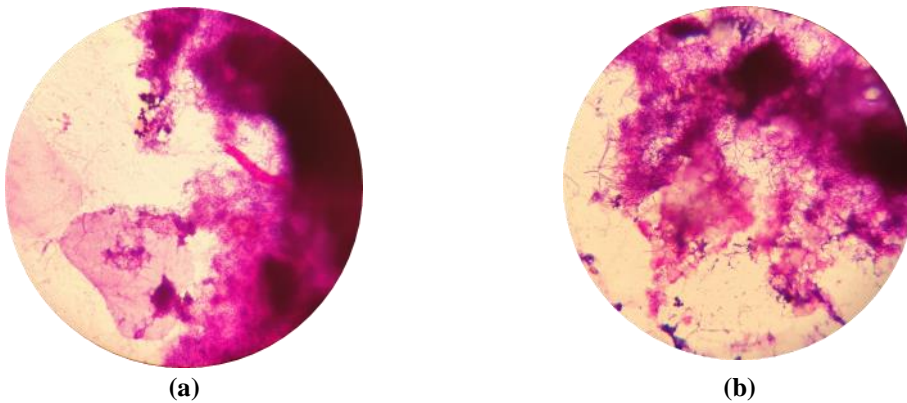


Figure 18. Patient no. 8. (a) before tooth brushing with Colgate: frequent lanceolate cocci in diplo, cocci in diplo in short chains, Gram-positive cocci in diplo and in small clusters, Gram-negative filamentous bacteria, Gram-negative short bacilli, rare epithelial cells; (b) after tooth brushing with Colgate: present filamentous bacteria, lanceolate cocci in diplo, cocci in diplo, Gram-negative fusiform bacilli, Gram-negative short curved bacilli.

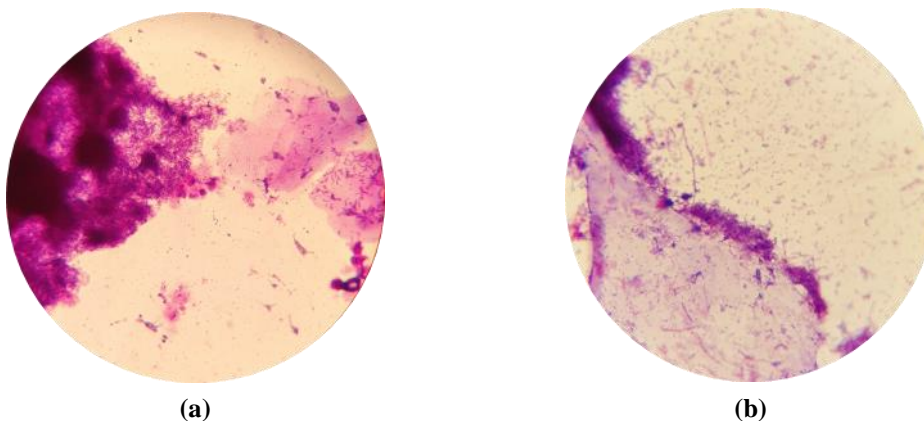


Figure 19. Patient no. 9. (a) before tooth brushing with Colgate: present epithelial cells, lanceolate cocci in diplo in short chains, frequent Gram-positive short bacilli with rounded ends, present Gram-negative short bacilli, Gram-positive short bacilli with rounded ends; (b) after tooth brushing with Colgate: rare Gram-positive cocci in short chains, Gram-positive cocci in big clusters, very rare Gram-negative filamentous bacteria.

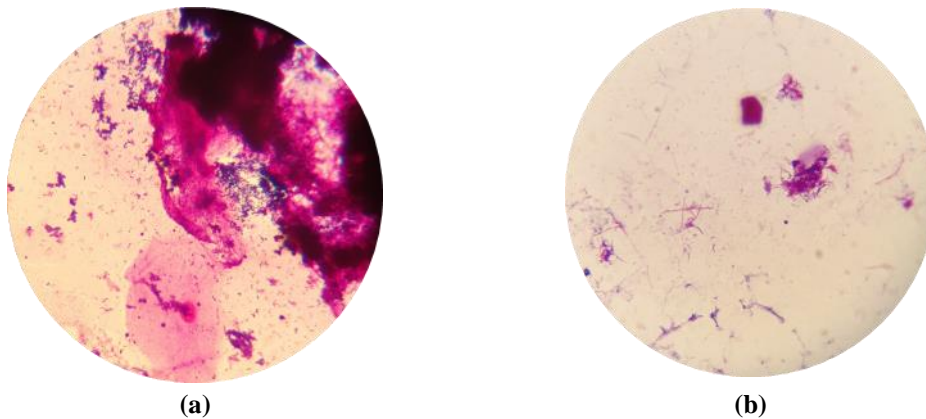


Figure 20. Patient no. 10. (a) before tooth brushing with Colgate: Gram-positive cocci in diplo in small and big clusters, frequent Gram-negative bacilli in big clusters, rare Gram-positive curved bacilli, very rare fusiform bacilli, very rare Gram-positive bacilli with rounded ends; (b) after tooth brushing with Colgate: Gram-negative fusiform bacilli, rare Gram-positive cocci in diplo in small clusters, very rare Gram-positive bacilli with rounded ends.

The bacteria identified in collected samples are presented in Table 2.

Table 2. The bacterial strains identified in collected samples.

Bacterial strain	Before tooth brushing with Elmex	After tooth brushing with Elmex	Before tooth brushing with Colgate	After tooth brushing with Colgate
filamentous Gram-negative bacteria	6	4	4	4
filamentous Gram-positive bacteria	1	-	1	-
Gram-negative bacilli	-	1	1	-
Gram-negative short bacilli	2	-	1	1
Gram-negative fusiform bacilli	4	4	4	3
Gram-positive bacteria	-	-	1	-
Gram-positive bacilli with rounded end	2	-	6	1
Gram-positive bent bacilli	2	1	3	1
Gram-positive cocci in diplo	7	7	4	4
Gram-positive cocci in chains	3	-	3	1
Gram-positive cocci in clusters	3	5	7	4

Gram-positive lanceolate cocci in diplo	4	2	4	5
polymorphonuclear cells	1	-	1	-
epithelial cells	1	3	4	-
epithelial debris	-	1	-	-

In this study, we investigated the effects of two commercial toothpaste of the same producer Colgate-Palmolive, which leads with GlaxoSmithKline Romanian market of oral hygiene products [20].

The Elmex Sensitive Professional toothpaste is indicated in treatment of dentine hypersensitivity [21] and has the following components: 8% arginine as active ingredients, and calcium carbonate, aqua, sorbitol, sodium monofluorophosphate (1450 ppm fluoride), bicarbonate, sodium lauryl sulfate, aroma, cellulose gum, sodium bicarbonate, tetrasodium pyrophosphate, titanium dioxide, benzyl alcohol, sodium saccharin, xanthan gum, limonene [22]. Fluoride is the antibacterial ingredient, and it is the active ingredient for caries prevention according to the producers. Arginine helps hard tissue remineralisation [23].

This study used the Colgate Total Original toothpaste, which provides 12-hour antibacterial protection for teeth, tongue, cheeks, and gums [24], with active ingredients: sodium fluoride 0.24% (1100 ppm fluoride), triclosan 0.3% [25]. The Inactive ingredients: hydrated silica, water, glycerin, sorbitol, sodium lauryl sulfate, copolymer PVM/MA, flavor, cellulose gum, propylene glycol, sodium hydroxide, carrageenan, sodium fluoride, triclosan, sodium saccharin, artificial dyes. CI 77891 [25]. This paste contains two antibacterial ingredients: fluoride and triclosan.

The anti-caries and antimicrobial effect of

fluoride depends on concentration [26], at least 1000 ppm of fluoride prevents dental caries in the permanent and primary dentition [27].

A recent study [28] investigated the antimicrobial effect of arginine in association with 1000 ppm natrium fluoride. It demonstrated that the incorporation of 2% arginine in natrium fluoride toothpaste significantly enhances the antimicrobial effect against caries-generating, while 4% and 8% arginine in natrium fluoride toothpaste were ineffective in enhancing the antimicrobial effect of natrium fluoride.

Triclosan is a broad-spectrum antimicrobial agent, which is widely used in personal care products, including toothpaste [29]. It has proven effectiveness in controlling dental plaque formation and maintaining gingival health [30, 31]. 0.3% triclosan in combination with 1450 ppm fluoride in toothpaste is more effective than a regular 1450 ppm fluoride toothpaste in controlling biofilm formation and improving the periodontal clinical conditions in a study done over two years [32].

In brief, both toothpaste have antimicrobial agents: Elmex Sensitive Professional 1450 ppm fluoride and Colgate Total Original 1100 ppm fluoride and 0.3% triclosan.

The oral flora from dental plaque shows diversity even for the same individual. In one week, the bacterial load was different for each subject.

The main bacterial strain identified in collected samples was Gram-positive cocci (*Streptococcus*), as cocci in diplo, cocci in chains, and lanceolate cocci in diplo, facts confirmed by different studies [33-35].

Also, filamentous Gram-negative bacteria were frequently identified - in seven subjects, four with poor OHI-S, two with fair OHI-S, and one with good OHI-S. The filamentous Gram-negative bacteria were identified in four out of seven subjects after tooth brushing with Elmex Sensitive Professional and in all four subjects after tooth brushing with Colgate Total Original. Filamentous genera are abundant in mature plaque [36, 37], they are attached in the final stage of the plaque formation [38].

Gram-negative fusiform bacilli were recognized in seven subjects, five with poor OHI-S and two with fair OHI-S. These bacilli facilitate infection with multiresistant bacteria [39] and are prevalent in cancer patients [40].

Gram-negative short bacilli were frequent in samplings from a subject with poor oral hygiene and rare in two subject with fair and good OHI-S.

Gram-positive bacilli, bent or with rounded ends, were rare or very rare, and often, they were not identified after tooth brushing, no matter the toothpaste.

Both toothpastes reduced the bacterial load after tooth brushing for almost all subjects. There were three exceptions: two for Elmex Sensitive Professional for a 38-year-old male with poor oral hygiene and a 27-year-old female with good oral hygiene. One week later, both of them had a more diverse flora, and the bacterial load reduced after tooth brushing with Colgate Total Original. The third exception was a 48-year-old female subject with poor oral hygiene, with more bacterial diversity after tooth brushing with Colgate Total Original.

In this study, three subjects had good oral

hygiene and, all of them, showed less microbial diversity in the first week. This fact is a surprise, because a diverse microbial community is associated with a healthy oral state [41], successful oral aging [42], and consequently proper oral hygiene. The bacteria from dental plaque are the key factor in the initiation of caries and periodontal disease [43]. An increased microbial diversity is a characteristic that markedly distinguishes periodontal disease from caries [44].

In addition, two of them had predominant Gram-positive microbiota, and one abundant Gram-positive cocci, but with frequent filamentous Gram-negative bacteria. It is well-known that the main genera in immature plaque are Gram-positive cocci and bacilli, and in mature plaque increases the number of Gram-negative cocci, bacilli, and filamentous bacteria [45].

The filamentous Gram-positive bacteria were identified in a 59-year-old subject with fair OHI-S and they were not detected after tooth brushing. This bacterial strain is related to both periodontitis [46] and deep caries [47].

Elmex Sensitive Professional is more effective on Gram-negative flora, mainly filamentous Gram-negative bacteria, and less effective on Gram-positive flora, especially cocci. Colgate Total Original showed effectiveness on Gram-positive flora and less efficient on filamentous Gram-negative bacteria, in three cases were frequent filamentous Gram-negative bacteria before and after tooth brushing. According to these findings, it could be presumed, that a concentration of 1450 ppm fluoride in association with 8% arginine is more effective than 1100 ppm fluoride in association with 0.3% triclosan in reducing the number of filamentous Gram-negative bacteria.

Within the limitation of this study, due to

the small number of participants, it seems reasonable to investigate the antimicrobial effect of different concentrations of arginine in association with different concentrations of fluoride, on oral microbioma.

In collected samples were also identified epithelial cells, polymorphonuclear cells, and in one case epithelial debris. The presence of epithelial cells on teeth surfaces is normal, as a result of desquamation [48], but the

CONCLUSIONS

The composition of oral microbiome is modified by tooth brushing with commercial toothpaste. Both toothpaste reduce de bacterial load with dental plaque, but 1450 ppm fluoride in association with 8% arginine is more effective than 1100 ppm fluoride in association with 0.3% triclosan in reducing

presence of polymorphonuclear cells indicates inflammation. The polymorphonuclear cells appear in two subjects, in both cases, on the first sample before any brushing and in the third sample, before tooth brushing with Colgate Total Original, and this time in association with frequent epithelial cells. It is possible that inflammation to increase the desquamation of epithelial cells as a defense mechanism [49].

the number of filamentous Gram-negative bacteria.

Gram-positive cocci is the prevalent bacteria genera in collected samples. The filamentous Gram-negative bacteria and Gram-negative fusiform bacilli are associated with poor and fair oral hygiene.

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