



(RESEARCH ARTICLE)



## Parallelism between the efficiency of Sensodyne toothpaste and Lacalut toothpaste with hydroxyapatite in oral hygiene

Carmen Liliana Defta <sup>1</sup>, Ionuț Cristian Mănguță <sup>1</sup>, Cristina-Crenguța Albu <sup>1,\*</sup>, Ștefan-Dimitrie Albu <sup>1,\*</sup> and Oana Botoacă <sup>2</sup>

<sup>1</sup>"Carol Davila" University of Medicine and Pharmacy, 37 Dionisie Lupu Street, 1st District, 020021, Bucharest, Romania.

<sup>2</sup> Titu Maiorescu University, 22 Dâmbovnicului Street, 4th District, 040441, Bucharest, Romania.

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### Abstract

Currently, the concept of oro-dental prophylaxis and prevention is becoming more and more publicized among the population. These notions promote both the prevention of the development of dento-periodontal and oral structures diseases, diagnosing the diseases of the oral cavity and stopping their evolution, as well as the prevention of complications in the oro-maxillo-facial sphere. The purpose of this paper was to perform a comparative experimental study between the commensal oral flora and the composition of the biofilm after tooth brushing with Sensodyne Rapid Relief toothpaste and Lacalut toothpaste with hydroxyapatite. The study group included a number of 15 patients, of which 9 men and 6 women, aged between 20 and 71 years. The collection of biological preparations took place in the dental office, where the patients presented themselves for an individualized treatment plan, and the analysis and interpretation of the results were carried out in the Microbiology Discipline, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania. According to the results of our study, following dental brushing with Lacalut toothpaste with hydroxyapatite, its effectiveness was demonstrated on species such as G+ lanceolate cocci, curved G+ bacilli, G-spindle bacilli or G- filamentous formations, while the effect antibacterial for G+ shells in diplo and short chains was not as expected. Sensodyne Rapid Relief Toothpaste has been shown to have a positive effect on species of G+ lanceolate cocci, G- fusiform bacilli, round-headed G+ bacilli, curved G+ bacilli or G- filamentous formations. On the other hand, on G+ cocci in diplo and short chains and epithelial cells it did not show the desired efficiency, these being found in the highest percentage. This study once again shows the importance of microscopic examination in assessing the effect of some toothpastes on the weight of the oral flora.

**Keywords:** Bacterial plaque; Sensodyne Rapid Relief toothpaste; Lacalut toothpaste with hydroxyapatite; Microscopic examination; Oral hygiene

### 1. Introduction

Currently, the concept of oro-dental prophylaxis and prevention is becoming more and more publicized among the population [1, 2]. These notions promote both the prevention of the development of dento-periodontal and oral structures diseases, diagnosing the diseases of the oral cavity and stopping their evolution, as well as the prevention of complications in the oro-maxillo-facial sphere [3 - 5].

Prevention deals with measures designed to anticipate the occurrence of oro-dental pathology, while prophylaxis studies the necessary methods and their implementation in order to benefit from an appropriate preventive treatment [6 - 9].

\* Corresponding author: Cristina-Crenguta Albu and Ștefan-Dimitrie Albu

„Carol Davila" University of Medicine and Pharmacy, 37 Dionisie Lupu Street, 1st District, 020021, Bucharest, Romania.

However, a good part of the population is still not aware of the options for maintaining oral health or preventing related diseases in the oral cavity [10]. By not knowing these requirements, that is, by not performing the brushing technique properly and not using a toothpaste according to individual needs, patients are prone to accumulating a large amount of bacterial plaque, which over time can turn into dental tartar [11]. Also, not using a toothpaste that contains fluoride increases the incidence of carious pathology, with numerous repercussions on the dental tissues [12-14].

The aim of this work was to perform a comparative experimental study between the commensal oral flora and the composition of the biofilm after tooth brushing with Sensodyne Rapid Relief toothpaste and Lacalut toothpaste with hydroxyapatite.

## 2. Materials and methods

### 2.1. Materials

For the realization of this study, the following materials were used:

- Microbiology slides, sterile and degreased,
- Alcohol 90%,
- Physiological serum,
- Sterile, single-use consultation kit,
- Sterile exploratory probe,
- Oral aspirator,
- Sterile cotton roll,
- Biological samples collected from patients,
- Sensodyne Rapid Relief toothpaste and Lacalut toothpaste with hydroxyapatite,
- Gram kit for smear staining,
- Optical microscope with immersion objective (x100),
- Bunsen bulb,
- Cedar oil,
- Camera of the Samsung Galaxy A50 phone.

### 2.2. Methods

#### 2.2.1. Selection of the patient group

The study group included a number of 15 patients, of which 9 men and 6 women, aged between 20 and 71 years.

In addition to the collection of biological samples, each patient also benefited from the preparation of a thorough anamnesis, in order to be sorted according to sex, age, environment of origin (rural or urban), educational level, pre-existing pathologies and degree of oral hygiene, in after completing a questionnaire. They were also asked to complete the consent form in accordance with the legal provisions.

The collection of biological preparations took place in the dental office, where the patients presented themselves for an individualized treatment plan, and the analysis and interpretation of the results were carried out in the Microbiology Discipline, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

#### 2.2.2. Collection of pathological products

To perform the collection of biological products, dental probes from the disposable, sterile consultation kit were used (Fig. 1). Sampling took place from the occlusal and proximal tooth surfaces and the gingival sulcus, examining whether there was sufficient plaque to test the plausibility of the study.

Collections were made, the first from the bacterial plaque before tooth brushing, and the second and third one hour after using Sensodyne Rapid Relief toothpaste and Lacalut toothpaste with hydroxyapatite (Fig. 2, 3).



**Figure 1** Disposable consultation kit



**Figure 2** Sensodyne Rapid Relief toothpaste



**Figure 3** Lacalut toothpaste with hydroxyapatite

#### 2.2.3. Performing smears

Each selected microbiology slide was marked with the patient's identification data (initial name/surname) and the bacterial plaque sample before or after brushing.

Work steps:

- Showing off
  - a glass slide is selected, which is degreased with sanitary alcohol and positioned on the work table;
  - obtain the bottle of physiological serum and apply a drop on the middle of the blade;
  - the biological preparation is scraped with the tip of the exploratory dental probe and mixed with the physiological serum;
  - eccentric movements are made to be able to spread the sample in a fine layer.

- Drying
  - the slide is placed to dry until the physiological serum completely evaporates, and the biological product takes the form of a whitish film.
- Fixation
  - this stage consists of passing the blade through the flame of the Bunsen bulb 4-5 times;
  - the fixing is checked by touching the blade with the back of the free hand, without exceeding the temperature of 56 °C, eliminating the risk of smear damage.
- Coloring
  - it is done using the Gram technique for smear staining;
  - description of the Gram technique: Gentian violet penetrates the bacterial cell of Gram-positive (G+) microorganisms, resulting in a bluish-violet coloration. Gram-negative (G-) bacteria do not capture the dye, it being removed by the alcohol-acetone mixture. Next, they are stained with Fuchsin, resulting in a pink-red hue [11].

The special Gram kit (Fig. 4) contains:

- Gentian violet,
- Lugol's solution,
- Alcohol-acetone mixture,
- Fuchsin diluted 1/10.



**Figure 4** Gram kit

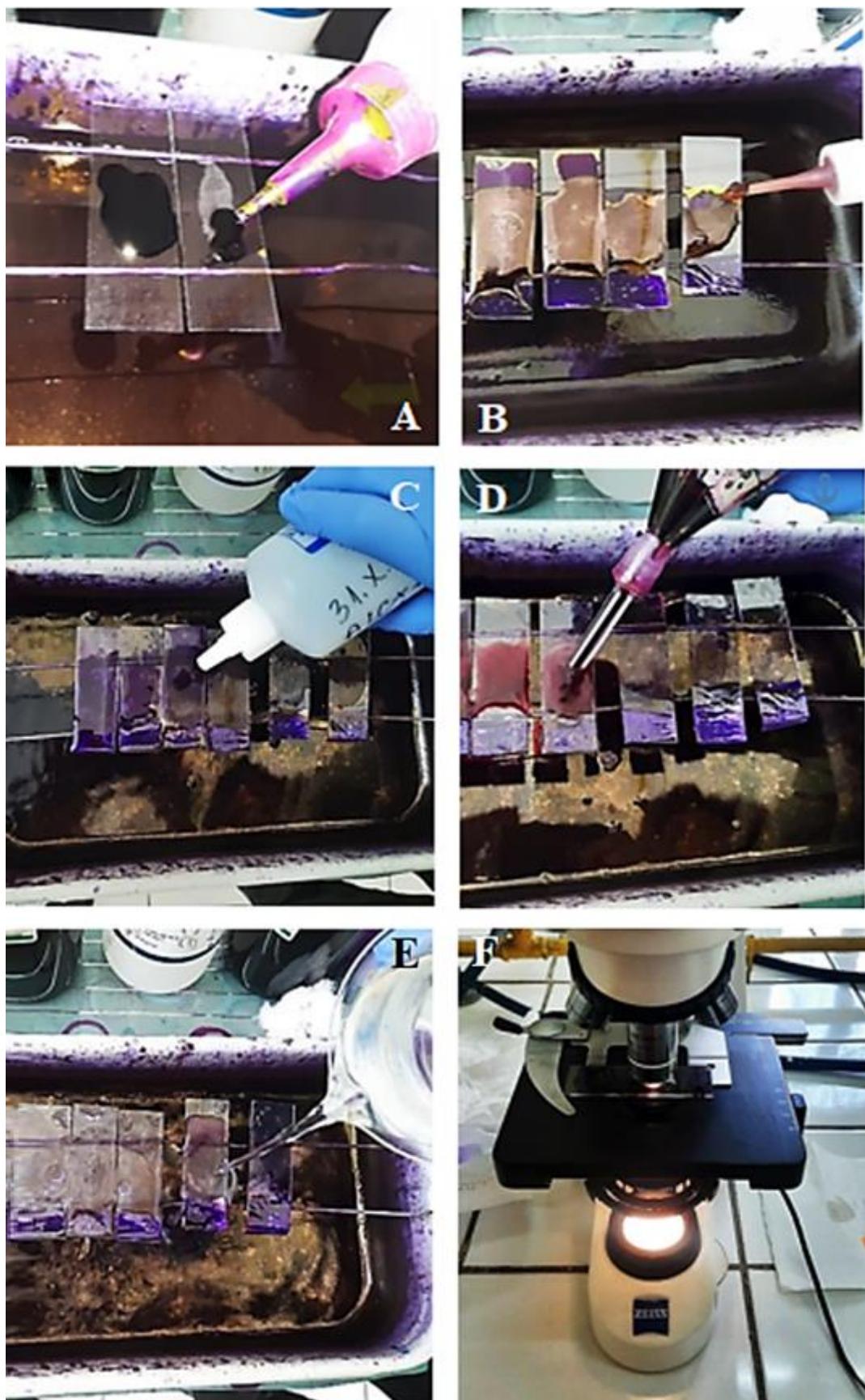
The working technique for staining the smears was as follows:

- apply a few drops of Gentian violet on the smear and wait 2-3 minutes (Fig. 5A) wash the solution with tap water;
- apply Lugol's solution and leave for 1-2 minutes (Fig. 5B);
- Lugol is removed by washing;
- decolorize the smear with the alcohol-acetone mixture and wait 1 minute (Fig. 5C);
- wash the blade with tap water;
- recolor the preparation with Fuchsin 1:10 for 2-3 minutes (Fig. 5D);
- wash the blade under running water for the last time (Fig. 5E);
- the slides are arranged in a stand so that they can dry.

- Examining the smears with the optical microscope

After staining and drying the slides, the stage of examining them under a microscope follows (Fig. 5F).

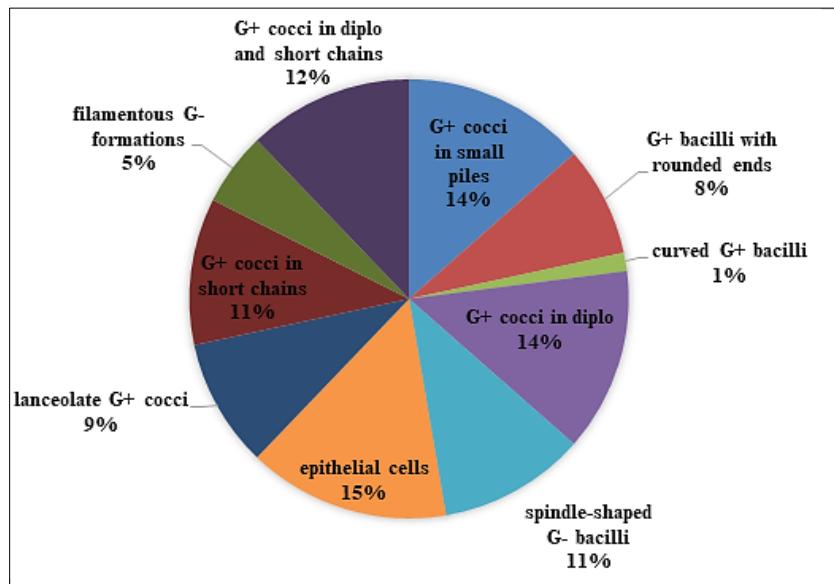
- immersion objective (x100) is used;
- a drop of cedar oil is applied to the smear, as it has the role of magnifying the refractive index, enlarging the image up to 1000 times;
- fix the slide on the plate of the optical microscope and bring the x100 objective into contact with the oil on the surface of the slide;
- raise the tube until the necessary frames are recorded with the help of the macroscope;
- microvisca is used for superior image clarity.



**Figure 5** A. Application of Gentian violet; B. Application of Lugol's solution; C. Decolorization with alcohol-acetone mixture; D. Recoloration with Fuchsin; E. Washing with water; F. Examination with the optical microscope under oil immersion ( $\times 100$ ) objective

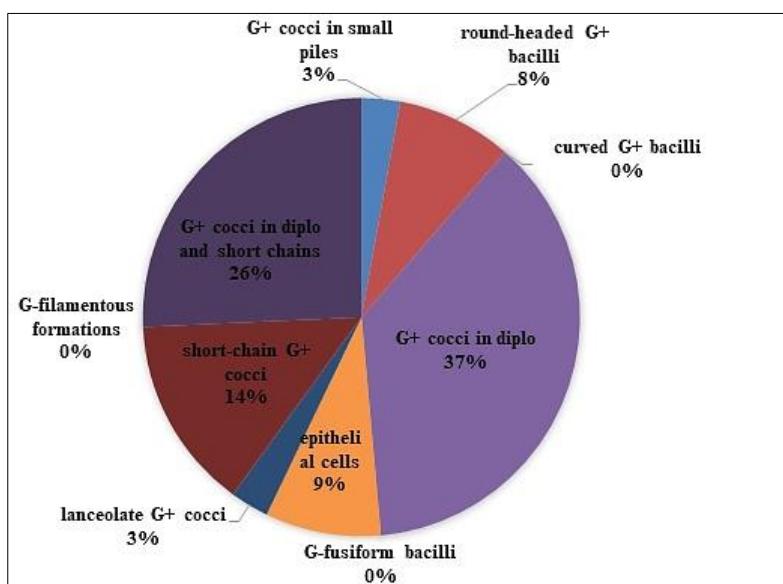
### 3. Results and Discussion

The study of the distribution of bacteria in the dental plaque of the study participants indicated that: the predominant percentage is represented by epithelial cells (15%), G+ cocci in small piles and G+ cocci in diplo (14%), G+ cocci in diplo and short chains (12 %), spindle-shaped G- bacilli and G+ cocci in short chains (11%), lanceolate G+ cocci (9%), G+ bacilli with rounded ends (8%), filamentous G- formations (5%) and curved G+ bacilli (1 %), (Fig. 6).



**Figure 6** Distribution of bacteria in dental plaque

In the article published by Ambarkova (2018), the idea that the oral environment is not a single and uniform environment, but is colonized by various bacterial species in different areas, was emphasized. For example, species such as Fusobacterium, Prevotella and anaerobic spirochetes are found in the gingival sulcus. The oral cavity of newborns is rapidly colonized by Streptococcus salivarius, while with the appearance of the first teeth, Streptococcus mutans and Streptococcus sanguinis also appear. During puberty, the existence of Bacteroides and Spirochaetes species predominates. In a healthy oral cavity, Streptococcus, Peptostreptococcus and Veilonella represent more than 80% of the oral microflora [9, 11, 15].

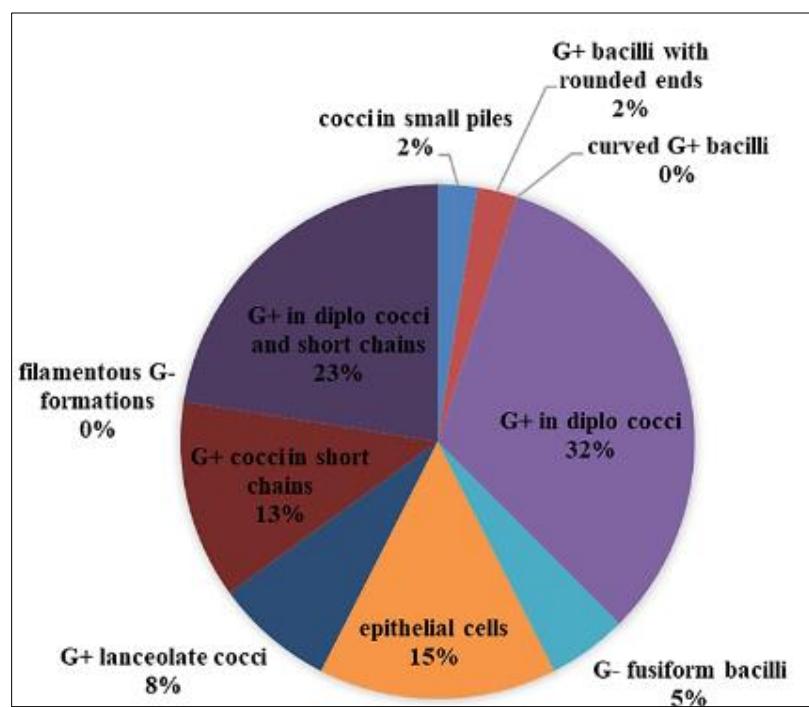


**Figure 7** Bacterial distribution after using Lacalut toothpaste with hydroxyapatite

Following the use of Lacalut toothpaste with hydroxyapatite, its effectiveness in the context of tooth brushing was ascertained by the partial or total reduction of the following biological products: G+ cocci in small piles (3%), lanceolate G+ cocci (3%), curved G+ bacilli (0%), G-fusiform bacilli (0%) and G-filamentous formations (0%). It can be seen that this toothpaste was partially effective on round-headed G+ bacilli (8%), epithelial cells (9%) and short-chain G+ cocci (14%). However, Lacalut toothpaste with hydroxyapatite did not show a potentiated antimicrobial effect on the following bacterial species, failing to reduce them quantitatively: G+ cocci in diplo and short chains (26%) and G+ cocci in diplo (37%), (Fig. 7).

Considering the study published by Simonovska (2019), the effectiveness of Lacalut toothpaste against caries-producing bacterial species can be stated. Patients were reported to have predominant colonies of *Streptococcus mutans* and *Lactobacilli*, and after tooth brushing, their numbers decreased considerably. This study was also the first research to examine the antimicrobial effect of Lacalut toothpaste with hydroxyapatite, which provides a superior degree of mineralization and protection against caries through an optimal concentration of organic and inorganic fluorides in its composition [16, 17].

Following the use of Sensodyne Rapid Relief toothpaste, the following biological products were identified in a relatively high percentage: G+ in diplo cocci (32%), G+ in diplo cocci and short chains (23%), epithelial cells (15%) and G+ cocci in short chains (13%). This proves that Sensodyne Rapid Relief toothpaste was not beneficial in reducing the bacterial plaque composed of G+ cocci, but it did contribute to the significant reduction of G+ lanceolate cocci (8%), G- fusiform bacilli (5%), G+ bacilli with rounded ends (2%) and G+ cocci in small piles (2%). Moreover, following the use of this toothpaste, maximum efficiency was observed on curved G+ bacilli and filamentous G- formations, which were completely reduced (0%), (Fig. 8).



**Figure 8** Bacterial distribution after using Sensodyne Rapid Relief

According to the study carried out by Camargo (2017), Sensodyne Rapid Relief toothpaste did not show an optimal antimicrobial effect, especially for the *Streptococcus mutans* strain, as no quantitative reduction of this bacterium was observed, even if a concentration of quite high. As for *Candida albicans*, it was less resistant to the toothpaste [18-20].

#### 4. Conclusion

According to the results of our study, at the level of the bacterial plaque of the patients examined, the existence of epithelial cells predominates, which denotes the presence of an old, mature plaque correlated with an average level of oral hygiene (15%), G+ cocci in small piles (14 %), G+ in diplo cocci (14%) and G- fusiform bacilli (11%).

Following dental brushing with Lacalut toothpaste with hydroxyapatite, its effectiveness was demonstrated on species such as G+ lanceolate cocci, curved G+ bacilli, G- spindle bacilli or G- filamentous formations (with values between 0-3%), while the effect antibacterial for G+ shells in diplo and short chains was not as expected (37%).

Sensodyne Rapid Relief Toothpaste has been shown to have a positive effect on species of G+ lanceolate cocci, G-fusiform bacilli, round-headed G+ bacilli, curved G+ bacilli or G- filamentous formations (values between 0 and 8%) . On the other hand, on G+ cocci in diplo and short chains and epithelial cells it did not show the desired efficiency, these being found in the highest percentage (32%).

This study once again shows the importance of microscopic examination in assessing the effect of some toothpastes on the weight of the oral flora.

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## Compliance with ethical standards

### Acknowledgments

We gratefully acknowledge the patients for giving us permission to conduct this study.

### Disclosure of conflict of interest

The authors declare no conflict of interest.

### Statement of informed consent

Informed consent was obtained from the patients included in the study.

### Funding

No funding was received for this study.

### Authors' contributions

Authors CLD, ICM and OB contributed to this work in conceptualization, methodology, software, and formal analysis. CCA and SDA contributed in software, formal analysis, and data curation. CLD, ICM and OB contributed in validation, supervision, project administration. All authors read and approved the final version of the manuscript.

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