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# Substantiation Of The Antimicrobial Activity Of Drugs (Foams) For Topical Use Based On The Results Of The Evaluation Of The Growth Curves Of Microbial Populations In An In Vitro Experiment

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**Abstract.** In recent years, according to WHO statistics, there has been an increase in dental morbidity, which is why it is given great importance in scientific research both in Russia and abroad.

The World Society of Dentists takes such common diseases of the oral cavity as gingivitis, stomatitis, periodontitis, caries seriously also because of the presence of these diseases among people with chronic diseases and metabolic disorders in the body, which negatively affect the process of timely detection of dental disorders and, consequently, the appointment of adequate treatment tactics. Chronic diseases of the oral cavity and their high prevalence indicate the lack of effectiveness of existing therapeutic and preventive measures.

That is why this article focuses on conducting an experimental cultural microbiological study and justifies the need to identify the microbiota of the oral mucosa of patients for topical use of drugs (foams) when using orthodontic structures.

## 1. Introduction

Persons who are patients of the orthodontic department and constitute one of the most likely risk groups, the condition of the mucous membrane and its reactions to orthodontic treatment require special attention, since morphofunctional disorders caused by anomalies of the dentofacial system are themselves powerful pathogenetic factors that cause the initiation and development of inflammatory diseases [1-3]. Orthodontic equipment installed in the oral cavity does not in itself cause the appearance of local periodontitis [4]. The cause of the occurrence is a diet with excessive consumption of sugars and poor oral hygiene. The orthodontic device only accumulates soft plaque, which, with inadequate personal hygiene, leads to the development of local inflammation. Irrational orthodontic treatment can lead to the occurrence of local inflammation: the use of inappropriate structures of orthodontic devices for the patient, the use of excessive forces of their action [5-7].

In this regard, it is necessary to take into account the general somatic status of the patient when selecting orthodontic structures with a mandatory study of the microbiology of the oral mucosa.

The aim of the study was to conduct a microbiological research of the culture and identification of the microbiota of the oral mucosa of patients at sites adjacent to orthodontic designs

**2. Matherials and methods**

For the isolation and cultivation of microorganisms, 5% blood agar with hemin and menadion was used, for the isolation of anaerobes, the crops were placed for 7 days in Himedia anaerostats (Great Britain-India) with an oxygen-free gas mixture (80% nitrogen, 10% hydrogen, 10% carbon dioxide). A palladium catalyst was used to reduce the oxygen residues.

The identification of microbiota taxa (genus, species) was carried out based on the results of the study of morphological, cultural and biochemical properties. The results of the quantitative study were expressed in terms of the decimal logarithm of the microbial number-CFU/cm2 (colony-forming units per 1 $cm^2$).

To determine the sensitivity of microbial strains, the method of serial dilutions of antibacterial drugs developed at the Department of Microbiology, Virology, and Immunology of the Moscow State Medical and Stomatological University named after A.I. Evdokimov using the RTS-1 bioreactor (BioSan, Latvia) was used.

To conduct the experiment in the bioreactor, special sterile test tubes of two types were used: (TubeSpin®, 50 ml, with a membrane filter) - for aerobic bacteria and fungi; (TubeSpin®, 50 ml, according to the Falcon type with a lid without a membrane) – for anaerobic bacteria. 15 ml of culture medium was added to each tube and a pre-prepared bacterial suspension was introduced using an automatic pipette. The studied dilutions of dosage forms (foams) were introduced in an amount of 5 ml into each tube with a microbial suspension. A study of 3 samples (variants) of "Splat", "Sugar Stop" and "Cito Stop" was carried out [10].

Using the software bioreactor asked the necessary parameters for automatic cultivation with the construction of growth curves from each sample on the monitor, which then was printed out in the form of graphs and evaluated the nature suppress the growth of crops, depending on the degree of dilution of the studied formulations (foams) and dosage forms. The effect of inhibition of microbial growth recorded in the elongation phase of adaptation and the lag time of the onset of the logarithmic growth phase, increasing its duration and lower amplitude curve in the stationary growth phase (plateau) compared with the growth control of microbial culture without drug [8-11].

The cultivation process in all parallels is semi-periodic. The cultivation time is 48 hours. Statistical processing: construction of a regression dependence (second-order parabola), with an estimate of the Fisher criterion and the Pearson correlation coefficient.

**3. Results and discussion**

To identify the antimicrobial activity of the patented dosage forms (foams), a study was conducted with etiologically significant strains of representatives of the oral microbiota: Streptococcus sanguis, periodontopathogenic anaerobes Prevotella intermedia, yeast fungi Candida albicans.

According to the results of cultivation of the clinical S. sanguis isolate, the adaptive phase was observed in the control tube up to 6 hours of cultivation. The initial period of the exponential phase (F-1) - the period of accelerated development (P-1), had a long flow pattern. Analyzing the development curve quite clearly, there is a gradual increase in the optical density at intervals of 6-10 hours and 10-12 hours, which corresponds to the degree of predominance of the processes of initial cell growth, and then the initial fission. The logarithmic "jump" or true logarithmic period is characterized by the intensity of the cell development rate according to the geometric progression principle (P-2), which reached its peak value by the 16th hour of the experiment (indicator α), with an optical density index (OD) of 4.5±0.3 mcf. P-3 is a period of negative acceleration, in which the bacterial cells become less active, and the generation period lengthens. According to the results of the end of this period and the exponential phase as a whole, by the 22nd hour of the experiment, bacterial cells reached the maximum microbial concentration (M-concentration, indicator β) - 5.26±0.3 mcf. The steady-state equilibrium of the culture (F-3) lasted for 8 hours, with no signs of an increase in the biomass, and no change in the optical density. The average OD value in this phase is 5.28±0.3 mcf (22-30 h). Starting from the 31st hour of the experiment, the process of bacterial cell death was observed along a trend close to the descending logarithmic curve (Table 1).

**Table 1.** Table of key points of development in the periodic type of cultivation of S. sanguis.

| | Parallel | S. sanguis | | foam 1 (Splat) | | foam 2 (Sugar Stop) | | foam 3 (Cito Stop) | |
|---|---|---|---|---|---|---|---|---|---|
| | optical density* / time phase / period | OD (mcf) | time (hour) | OD (mcf) | time (hour) | OD (mcf) | time (hour) | OD (mcf) | time (hour) |
| **I** | **Adaptive phase** | 0.02 | 6 | 0.05 | 8 | 0.12 | 8 | 0.02 | 10 |
| **II** | **Exponential phase** | 5.26 | 22 | 4.25 | 22 | 4.57 | 24 | 4.14 | 28 |
| **II. 1** | **Accelerated development Period (P1)** | 0.74 | 12 | 0.64 | 14 | 1.0 | 16 | 0.73 | 18 |
| **II. 2** | **Logarithmic development period (P2), indicator α** | 4.5 | 16 | 3.42 | 18 | 4.14 | 20 | 3.57 | 24 |
| **II. 3** | **Negative acceleration period (P3), indicator β** | 5.26 | 22 | 4 | 20 | 4.57 | 24 | 4.14 | 28 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **III** | **Stationary phase (OD-average)** | 5.28 | 22-30 | 4.24 | 20-30 | 4.57 | 24-28 | 4.17 | 28-34 |
| **IV** | **The Death Phase** | x | 30-48 | x | 30-48 | x | 28-48 | x | 34-48 |

* Method error ±0.3 mcf

When culturing the microbial population with the test sample "Splat" and "Sugar Stop", the process of prolonging the adaptive period up to 8 hours of cultivation was noted. To a greater extent, the boundaries of the periods of initial growth and cell division were marked for the "Sugar Stop" sample (14-16 hours), and the "Splat" sample almost immediately passed into an intense logarithmic increase in optical density due to an increase in microbial biomass. For both samples, the rate of generation of new populations was identical, and it also coincided with the generative nature of cell development in the control sample. Due to the prolongation of the adaptive period, the time of change and transition of bacterial activity to negative acceleration was later, and the duration of the P-3 period was shortened. Maximum peak true logarithmic acceleration: sample "Splat" - 3.42±0.3 mcf (18 h); "Sugar Stop" - 4.14±0.3 mcf (20 h). Maximum microbial number (M-concentration): Sample "Splat" - 4.00±0.3 mcf (20 h); "Sugar Stop" - 4.57±0.3 mcf (24 h). The development of cells in the stationary period was with a slight variation in the optical density only for the "Sugar Stop" sample, and the total average OD (∑ F-3 "Splat" and "Sugar Stop") was 4.40±0.3 mcf (20-30 h), which was statistically significant relative to the control sample.

When culturing the microbial population with the test sample "Cito Stop", a longer process of extension of the adaptive phase was observed relative to all previous samples (up to 10 hours). At the interval of 10-18 hours, a low-intensity, gradual increase in the optical density was observed, which is comparable to the gradual increase in the main processes of the initial microbial generation. The exponential phase started relatively later than all the other images, and the rate of cell doubling was an order of magnitude lower than in the comparison samples. The maximum peak value of the true logarithmic acceleration (indicator α) is 3.57±0.3 mcf (24 hours); the maximum microbial number (M-concentration, indicator β) is 4.14±0.3 mcf (28 hours). The stationary phase was shortened, with an average OD of 4.17±0.3 mcf (28-34 h).

The test sample "Cito Stop" showed the greatest significant statistical difference relative to the control sample, as well as in comparison with the samples "Splat" and "Sugar Stop".

According to the results of cultivation of the clinical P. intermedia isolate, the lag phase was observed in the control tube up to 4th hour of cultivation. At the interval of 4-10 hours, there was a gradual increase in generative activity, which was marked by a period of initial accelerated cell development. After 10 hours of cultivation, the optical density index rushed up, and the trend of increasing biomass was comparable to the geometric regenerative progression. By the 18th hour of cultivation, the maximum optical density was reached at the end of the true exponential jump period (index α) – 6.00±0.3 mcf (18th hour). During the next two hours, the cells developed according to the principle of negative acceleration, with a gradual decrease in the rate of generation and the predominance of the processes of inhibition of enzymatic

activity. By the 20th hour of the experiment, the maximum bacterial concentration (beta index) was reached-6.24±0.3 mcf (20th h). The stationary equilibrium phase, characterized by the balance of newly formed and dying bacterial cells, was observed for 8 hours (20-28 hours). The average optical density index in this phase is 6.26±0.3 mcf. The phase of death was traced by a linear type of acceleration, with a gradual predominance of autolysis and exposure to toxic metabolic products (Table 2).

**Table 2.** Table of key points of development in the periodic type of P. intermedia cultivation.

| | Parallel | P. intermedia | | foam 1 (Splat) | | foam 2 (Sugar Stop) | | foam 3 (Cito Stop) | |
|---|---|---|---|---|---|---|---|---|---|
| | optical density* / time phase / period | OD (mcf) | time (hour) | OD (mcf) | time (hour) | OD (mcf) | time (hour) | OD (mcf) | time (hour) |
| **I** | **Adaptive phase** | 0.01 | 4 | 0.08 | 6 | 0.02 | 6 | 0.07 | 8 |
| **II** | **Exponential phase** | 6.24 | 20 | 5.21 | 18 | 4.56 | 22 | 4.09 | 22 |
| **II. 1** | **Accelerated development Period (P1)** | 0.74 | 10 | 0.54 | 10 | 0.55 | 10 | 0.48 | 12 |
| **II. 2** | **Logarithmic development period (P2), indicator α** | 6 | 18 | 4.74 | 16 | 4.44 | 20 | 3.41 | 18 |
| **II. 3** | **Negative acceleration period (P3), indicator β** | 6.24 | 20 | 5.21 | 18 | 4.56 | 22 | 4.09 | 22 |
| **III** | **Stationary phase (OD-average)** | 6.26 | 20-28 | 5.28 | 18-24 | 4.53 | 22-26 | 4.1 | 22-26 |
| **IV** | **The Death Phase** | x | 28-48 | x | 24-48 | x | 26-48 | x | 26-48 |

* Method error ±0.3 mcf

When culturing the microbial population with the "Splat" sample under study, the pattern of prolonged changes in the adaptive phase of population development was not observed. Overcoming the process of adaptation to the formed conditions of cultivation was similar to the control image, which was also observed in the period of accelerated development at the interval of 6-10 (P-1). The exponential jump in the increase in optical density in the true logarithmic period was comparable in the rate of bacterial growth

with the process of cultivation in the control tube, however, the maximum peak generation was reached two hours earlier, with an OD of 4.74±0.3 mcf (16th hour). The M-concentration was reached two hours later by the 18th hour of the experiment, with an optical density of 5.21±0.3 mcf. The stationary phase was less prolonged, relative to the control, with an average OD of 5.28±0.3 mcf (18-24 hours).

When culturing the microbial population with the test sample "Sugar Stop", the adaptive phase lasted up to 6th hour of cultivation. A pronounced boundary between the periods of accelerated development and true logarithmic development was not observed, and from the 8th hour of the experiment, bacterial generation passed into intensive development. In this sample, a diauxian transition pattern (14-16 hours) was observed, associated with the appearance of one or more transient (i.e. temporary) growth phases in the culture. This is due to the fact that the bacteria being in the environment, find several alternative sources of food. The observed division of the phase of exponential development into two separate periods contributed to a lower set of biomass by cells, which was reflected in the optical density indicators: α – 4.44±0.3 mcf (20 h); β - 4.56±0.3 mcf (22 h); stationary phase - 4.53±0.3 mcf (22-26 h).

When culturing the microbial population with the test sample "Cito Stop", the extension of the adaptive phase to 8th hour of the experiment was noted. The rate of bacterial generation was significantly lower than in the previous samples, which contributed to a later transition of the microbial population to the phase of exponential progression. The achievement of the peak concentration of biomass at the peak of the true logarithmic period was noted for a period earlier, relative to the "Sugar Stop" sample (by 18th hour), which was also reflected in the subsequent change in the rate of cell growth and in the generation of the gross microbial number when the M-concentration was reached. The optical density index at the point β is 4.09±0.3 mcf (22nd hour).

The studied samples "Sugar Stop "and "Cito Stop" showed a significant statistical difference relative to the sample "Splat". All the samples under study showed a significant decrease in the optical density at key points relative to the control sample.

According to the results of cultivation of a clinical isolate of C. albicans, in the control sample, the adaptive phase lasted up to 4th hour of the experiment. The period of accelerated population development was observed at the interval of 4-6 hours of cultivation, followed by an intensive increase in biomass in the exponential phase of development. By the 10th hour of the experiment, the maximum peak optical density index was reached at the end of the true logarithmic period (the α index at the end of the P-2 period) - 4.54±0.3 mcf. Slowing the enzymatic activity of the population, and as a result, reducing the rate of generation of new cells, promoted the change of the trend curve, followed by a transition into a period of negative acceleration. This period was observed in the interval from 10th hour to 14th hour, with the achievement of M-concentration at OD-5.34±0.3 mcf. The stationary phase was not marked by factors contributing to an additional increase in cell biomass, against which the optical value of the turbidity of the cultured broth did not change. The average optical density in this phase is 5.41±0.3 mcf (14th-22nd hour).

Starting from 23rd hour, a long phase of fungal population death was observed along the linear line of curve formation.

When culturing the fungal population with the "Splat" sample under study, there was no statistically significant pattern of lag phase prolongation. The cells developed like a culture in a control sample, with a gradual decrease in the rate of generation of new populations in the exponential phase. A significant slowdown in generative activity was observed by the 14th hour of the experiment, when the index α-4.41±0.3 mcf was reached. From 14th to 16th hour, a phase of negative acceleration was observed, followed by the release of the culture into a short stationary equilibrium. The average optical density in this phase is 4.64±0.3 mcf (16-20 h).

When culturing the fungal population with the studied samples "Sugar Stop" and "Cito Stop", the extension of the adaptive phase was observed up to 6th hour from the beginning of the experiment. The period of accelerated development of these samples was also observed with varying degrees of intensity of the generation intensity, which was significantly prolonged relative to the control sample and the "Splat" sample under study. There was a picture of a lower rate of growth of new cells in the "Cito Stop" sample, which contributed to a longer passage of the process for the formation of M-concentration (indicator β). The end of the true logarithmic rise for these samples (indicator α): the sample "Sugar Stop" - 3.84±0.3 mcf (14th hour), "Cito Stop" - 3.89±0.3 mcf (16th hour). The stationary phase of development of these samples was about 7 hours, without signs of an increase in the biomass and optical value, with a total average OD (∑ F-3 "Sugar Stop" and "Cito Stop") - 4.16±0.3 mcf (16th-24th hour), which was statistically significant relative to the control sample and the test sample "Splat" (Table 3).

**Table 3.** Table of key points of development in the periodic type of cultivation of C. albicans.

| | Parallel | C. albicans | | foam 1 (Splat) | | foam 2 (Sugar Stop) | | foam 3 (Cito Stop) | |
|---|---|---|---|---|---|---|---|---|---|
| | optical density* / time phase / period | OD (mcf) | time (hour) | OD (mcf) | time (hour) | OD (mcf) | time (hour) | OD (mcf) | time (hour) |
| **I** | **Adaptive phase** | 0.05 | 4 | 0.01 | 4 | 0.11 | 6 | 0.03 | 6 |
| **II** | **Exponential phase** | 5.34 | 14 | 4.65 | 16 | 4.11 | 16 | 4.25 | 20 |
| **II. 1** | **Accelerated development Period (P1)** | 0.45 | 6 | 0.32 | 6 | 0.85 | 10 | 0.47 | 10 |
| **II. 2** | **Logarithmic development** | 4.54 | 10 | 4.41 | 14 | 3.84 | 14 | 3.89 | 16 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **period (P2), indicator α** | | | | | | | | |
| **II. 3** | **Negative acceleration period (P3), indicator β** | 5.34 | 14 | 4.65 | 16 | 4.11 | 16 | 4.25 | 20 |
| **III** | **Stationary phase (OD-average)** | 5.41 | 14-22 | 4.64 | 16-20 | 4.1 | 16-24 | 4.22 | 20-24 |
| **IV** | **The Death Phase** | x | 22-48 | x | 20-48 | x | 24-48 | x | 24-48 |

* Reliability of the method in terms of optical density OD: ±0.3 mcf

**4. Conclusions**

Thus, the conducted experimental studies showed that all the studied samples showed a significant decrease in the optical density at the key points of the analysis of the growth curve of the microbial population relative to the control sample. The studied samples "Sugar Stop "and" Cito Stop "showed a significant statistical difference also with respect to the sample "Splat", which indicates a more pronounced antimicrobial activity of the claimed samples.

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