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Serebrov Kirill Dmitrievich

CLINICAL AND EXPERIMENTAL EVALUATION OF THE USE OF SOLUTIONS FOR CLEANING AND DISINFECTION OF REMOVABLE LAMINAR DENTURES

3.1.7. Dentistry

DISSERTATION

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Scientific supervisor:

Razumova Svetlana Nikolaevna,

Doctor of Medical Sciences, Professor

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INTRODUCTION

The prevalence of patients with complete edentulous of teeth among the elderly people in Russia is more than 25% (Voronov I.A. 2016) [3]. Patients of elderly and senile age (60-90 years) are the main group that uses removable dentures (Morozov A.N., et al., Osipova V.L. 2018) [12, 15]. Removable acrylic dentures are one of the possible options for rehabilitation of patients with partial or complete edentulism (Karpovich E.A. 2019) [7]. The use of removable dentures requires adequate hygiene of both the oral cavity and the dentures themselves. Insufficient cleaning leads to the development of oral diseases of infectious-toxic and chemotoxic genesis, which are manifested by inflammation of the oral mucosa (OM) (Midha S. et al. 2017, Serebrov K.D. 2023) [14, 26]. Regular disinfection of dentures, which should be performed by patients themselves, increases the lifespan of dentures and maintains a normal balance of oral microflora (Baba Y et al. 2018, de Arruda CNF et al.) [45, 58].

Acrylic resin, from which the denture base is made, is a rather porous material with a limited lifespan [10]. The denture base adsorbs pathogenic microorganisms on its surface, which form a microbial plaque (biofilm) (Konnov V.V. 2021) [9]. Since removable dentures have prolonged contact with the oral mucosa, microbes also infect the oral mucosa, causing inflammation (Maciel JG. 2024) [64, 76]. According to Ghazal ARA et al (2019) removable dentures with a service life of more than 5 years need to be replaced. According to Schmutzler A et al. (2021) microorganisms penetrate the denture base so deeply (absorption) that cleaning of removable dentures does not lead to an effective result [92]. Therefore, the use of dentures beyond the stated lifespan of the acrylic resin leads to bacterial contamination and the inability to perform any hygiene cleaning effectively (Ghazal ARA et al. 2019, Zhang K. 2022) [62, 104].

Today, there are a big number of personal care products for removable dentures. They are presented in the form of special devices for mechanical cleaning (brushes, wipes, pads), ready-made solutions or concentrates of various disinfectants, and (microwave ovens, ultrasonic baths) [83]. The most popular and easy to use method of disinfecting dentures is their immersion in a disinfectant solution. Solutions used for this purpose should be non-toxic for humans, have a pronounced bactericidal effect with a short

exposure time, and easy to use (Ahmedbeyli DR. 2021) [32]. However, the existing methods of denture cleaning have drawbacks (Razumova S.N. et al. 2021-2025, AlHamdan EM et al. 2021, Nishi Y et al. 2022) [18, 19, 20, 21, 35, 81]. Therefore, the problem of quality and effective denture cleaning remains a pressing issue. In this regard, the development of a protocol for disinfection of removable dentures is an important medical and social task.

For successful prevention of oral inflammatory diseases in patients with removable dentures, a clear protocol that is easily accessible at home and understandable for patients is necessary.

Degree of development of the research topic

The analysis of Russian and foreign literature and clinical practical experience testify to the necessity of searching for new methods of cleaning and disinfection of removable dentures. Studies similar to the topic of the thesis describe various methods of cleaning removable dentures. However, all existing techniques, despite a number of advantages, have disadvantages. Mechanical cleaning of dentures with toothbrush and toothpaste is a rather aggressive method in relation to the denture base. Due to the abrasiveness of the brush and paste, micropores and cracks are formed on the denture surface, which leads to faster contamination of the denture. Cleaning of dentures by immersing them in disinfectant solutions does not affect the entire quality composition of the biofilm. The lack of mechanical cleaning reduces the effectiveness of denture cleaning. The use of physical methods (microwave, ultrasound) for disinfection of dentures reduces microbial contamination, but requires special equipment and can lead to changes in the configuration of the denture. The use of protective varnishes requires multiple renewals due to the short-lived nature of the protective coating. This method is not available at home. Creating a method of denture cleaning that is understandable to patients and easy to perform at home will reduce the risk of oral diseases the configuration of the denture. The use of protective varnishes requires multiple renewals due to the short-lived nature of the protective coating. This method is not available at home. Creating a

clear technique for denture cleaning that is understandable to patients and easy to perform at home will reduce the risk of oral diseases.

Research aim

Increase of efficiency of cleaning and disinfection of removable dentures at complex use of chemical and mechanical methods.

Research objectives

1. Study by profilometry and scanning electron microscopy (SEM) the surface of acrylic dentures using different methods of denture care.
2. Develop a “Device for cleaning removable laminar dentures”.
3. Develop a protocol for cleaning and disinfection of removable dentures for use at home.
4. Study the effectiveness of our new developed algorithm of cleaning and disinfection of removable dentures.

Scientific novelty of the research

1. The “Device for cleaning removable laminar dentures” (patent application registration No. 2025101283 dated 22.01.2025), which allows to minimize the damage of denture bases and increase the efficiency of their cleaning, is developed.
2. A new protocol for cleaning and disinfection of removable dentures using the “Device for cleaning removable dentures” and the solution “Anolit ANK SUPER” at home is proposed.

Theoretical and practical significance

The significance of the study lies in the theoretical and practical justification of the application of the developed protocol for cleaning and disinfection of dentures, which contributes to increasing the service life of removable laminar dentures, increases the efficiency of their disinfection and cleaning. The developed “Device for cleaning of

removable plastic dentures" in combination with the solution "Anolit ANK SUPER"/ chlorhexidine digluconate 0,05% is an effective method of cleaning of removable dentures.

The developed algorithm of daily care of removable laminar dentures allows to maintain their cleanliness and should be included in the recommendations on their care.

Methodology and methods of research

The study was performed according to the principles and methods of evidence-based medicine. The design of the study included carrying out laboratory and clinical stages. At the preparatory stage, the literature was analyzed, patients were questioned, and the study design was created. At the laboratory stage, denture bases were studied by profilometry, scanning electron microscopy (SEM) and mass-spectrometry, an algorithm for cleaning dentures was developed, and a "Device for cleaning removable laminar dentures" was created. At the clinical stage the patients with removable dentures were examined, groups were formed, new dentures were made, the efficiency of dentures cleaning according to our proposed method was evaluated after 1, 3, 6 months and 1 year of their use by mass-spectrometry, and use of Ulitovsky-Leontiev index of denture cleanliness, statistical processing of the obtained results was carried out by mathematical statistics using the computer package STATISTICA 13.

Implementation of the research results

Practical and theoretical recommendations of the dissertation are used in the clinical practice of prosthodontist dentists of the Center for Dental and Maxillofacial Implantology of the clinical diagnostic center of RUDN, are used in the educational process for students of dentistry and residents, doctors of advansed training, who study at the department of dentistry.

They are used in the educational process for dental students and residents, doctors studying at the department of propaedeutics of dental diseases RUDN.

Provisions presented for defense

1. The developed “Device for cleaning removable laminar dentures” (patent application, registration No. 2025101283 from 22.01.2025), allows to maintain the hygiene of dentures at a good level for a long time.
2. The developed protocol of denture cleaning with the use of “Device for cleaning removable plastic dentures” and disinfectant solution “Anolit ANK SUPER” allows to increase the efficiency of denture cleaning.

Correspondence of the dissertation to the passport of scientific specialty

The dissertation corresponds to the passport of specialty 3.1.7. Dentistry (medical sciences), the field of research according to item 5. Study of etiology, pathogenesis, epidemiology, methods of prevention, diagnosis and cleaning of diseases of the oral mucosa. The conformity of the content of the dissertation to the specialty 3.1.7. Dentistry (medical sciences), for which it is submitted for protection, is confirmed by the approbation of the work, its scientific novelty and practical significance.

Degree of reliability of the results and approbation of the work

Approbation of the dissertation study was carried out at the meeting of the Department of Propaedeutics of Dental Diseases of the Medical Institute of the Federal State Educational Institution of Higher Education “Peoples' Friendship University of Russia named after Patrice Lumumba” (protocol No. 0300-36-04/8 of 24.03.2025). The degree of reliability of the obtained results of the dissertation research is confirmed by a sufficient number of observations (120 patients, 240 removable dentures), representativeness of the sample population of the research objects, use of modern high-precision methods of statistical data analysis based on the principles of evidence-based medicine, including the analysis of primary data of patients with division into main and control groups, assessment of the conformity of the type of distribution of the sample of the investigated parameters to the normal law, and conducting the analysis of the distribution of the sample of the investigated parameters to the normal law. The results were reported at the conferences: All-Russian Scientific and Practical Conference of

Young Scientists with International Participation, CH MA 2023, International Research Conference “IN THE WORLD OF SCIENCE AND EDUCATION”, International Research Center “Endless Light in Science” 2024, Interuniversity Conference for Young Scientists and Postgraduates “Actual Issues of Stomatology”, MI RUDN 2024.

Author's personal contribution

The author developed the research design under the scientific supervision. The author independently studied Russian and foreign literature on the topic of scientific research and conducted a patent search, conducted a questionnaire survey of patients and laboratory analysis of the surface structure of dentures with different methods of their cleaning. A patent application for “Device for cleaning removable laminar dentures” (registration No. 2025101283 dated 22.01.2025) was developed and filed. Clinical examination of patients, formation of groups for the study, fabrication of dentures, instructing patients on the processing of dentures, collection of material for mass spectrometric study to evaluate the effectiveness of the developed technique, statistical processing and analysis of the obtained data were carried out by the author independently.

Main scientific results

1. Modern methods of cleaning and disinfection of removable dentures in clinical and home conditions were analyzed, the results were published in [18, pp. 335-341], [24, pp. 99-100]. Personal participation of the author in obtaining these results: collection of material, interpretation, writing the article.
2. The existing methods of care of removable dentures are considered and their disadvantages are studied. The necessity of creating a new protocol for daily care of removable dentures at home was proved. The results were published in [23, p. 168]. Personal participation of the author in obtaining these results: collection of material, interpretation, writing the article.
3. Analysis of the effectiveness of using the solution “Anolit ANK SUPER” for disinfection of complete removable acrylic dentures, the results are published in [19, pp.

65-71]. Personal participation of the author in obtaining these results: collection of material, description, writing and design of the article.

4. Evaluation of efficiency and antimicrobial activity of the solution “Anolit ANK SUPER” against fungi and yeasts, anaerobic microorganisms, actinobacteria, cocci and bacilli, the results are published in [20, pp. 570-574]. Personal participation of the author in obtaining these results: collection of material, interpretation, writing the article.

5. The relationship between the increase in microbial contamination of complete removable acrylic dentures and the period of use has been proved, the results were published in [25, pp. 50-51]. Personal participation of the author in obtaining these results: collection of material, interpretation, writing the article.

6. Evaluation of the effectiveness of using disinfectant solutions for daily cleaning of removable dentures according to the index of DC Ulitovsky-Leontiev, the results were published in [21, pp. 385-389]. Personal participation of the author in obtaining these results: collection of material, interpretation of results, writing the article.

The dissertant analyzed foreign and Russian sources of specialized literature on the topic of the study. The results of laboratory and clinical studies were analyzed and statistical data processing was carried out. The “Device for cleaning removable laminar dentures” (patent application, registration № 2025101283 from 22.01.2025) and the method of cleaning removable dentures at home are developed. Conclusions are formulated and practical recommendations are given. In clinical and laboratory research the share of participation of the dissertant amounted to 100%, in statistical data processing 75%. The conclusions obtained in the course of the study are reliable, justified and derived from the results of research and statistical processing of materials. The hypothesis is built on known verifiable data and facts using 106 scientific literature sources, with which the results of the thesis research are in agreement. Statistical processing of the research results was carried out using the computer package STATISTIÑA 13. Data distribution corresponded to the normal law, descriptive statistics included calculation of arithmetic mean and standard deviation. Parametric criteria were used to evaluate differences between groups, including the t-test with Bonferroni correction for multiple comparisons. The critical level of statistical significance p was equal to 0.05.

Publications on the topic of research

On the subject of the dissertation research the degree candidate has published 4 scientific papers in the editions recommended by the Higher Attestation Commission at the Ministry of Science and Higher Education of the Russian Federation, 3 publications in the materials of conferences.

Structure and volume of the dissertation

The dissertation work is outlined on 129 pages of computer text and consists of an introduction, 4 chapters, conclusion, conclusions and practical recommendations, a list of references and an appendix. The work is illustrated with 36 figures and 31 tables. The list of literature contains 106 sources, including 30 domestic and 76 foreign authors.

CHAPTER 1. LITERATURE REVIEW

1.1. Current views on acrylic removable dentures disinfection

In the contemporary world life expectancy has increased from 66.2 years to 72.6 years for the last 24 years [28, 72, 97]. There is also a tendency towards an increase in the number of elderly people consequently. In the Russia Federation the number of edentulous patients of retirement age is more than 25% [1, 33, 80]. The most urgent task of orthopedic dentistry is optional recovery of lost dentition functions, including through removable dentures, both in the case of complete and partial edentulism [27]. Complete and partial removable dentures in dental practice are used in the edentulous patient's treatment [7, 36, 84]. Any removable denture has its own design features that vary on the characteristics of a particular clinical case (alveolar ridge prominence, mucous membrane pliability, величина dentition defect size etc.). But all removable dentures have common construction details: denture base, artificial teeth and clamps (if there are patients remaining teeth) [13, 86, 93]. Lower jaw denture base is located on the alveolar part of mandible and upper jaw denture base is located on the alveolar part of maxilla and the palate. There are metal and non-metal removable denture bases. Rubber and plastic-based dentures bases refers to non-metal ones. Rubber bases are older and their use has been reduced in recent years due to the fact that the process of rubber vulcanization is quite long and labor-intensive, rubber dentures have greater porosity and specific gravity than acrylic dentures. [37, 85]. Therefore, these dentures have been replaced by ones made of acrylic or nylon plastic, which are much easier for patients to care for. There are special indications for using a metal removable denture base and they include: patients' allergies to plastic dentures, but also bruxism. The removable dentures bases have their own maximum boundaries. The boundaries of removable dentures should pass along the transitional fold on the labial and buccal surfaces with an edentulous alveolar part of the upper and lower jaws, without affecting the mobile areas of the mucous membrane (frenulum). If these areas are covered with a base, this will lead to the development of decubitus ulcers. On the lingual side, the denture base runs along the transitional fold, without touching the lingual frenulum; on the hard palate, the removable denture base

should fall slightly short of line A. In this case, it is necessary to cover the alveolar tubercle to improve the fixation of the denture. [8, 36, 39].

One of the most popular types of removable dentures is the production of complete removable acrylic dentures. [26, 95]. The main material that the denture base is made of is polymethylmethacrylate (acrylic plastic). Polymethylmethacrylate (PMMA) — is a rigid amorphous polymer with high transparency, weather resistance and good physico-mechanical and electrical insulating properties. However, this material is also quite porous in its structure. [20, 42, 69]. Therefore, these dentures require daily hygienic treatment to prevent the development of inflammatory diseases of the oral cavity. Micropores on the surface of removable denture bases serve as retention points for various microorganisms. Microcracks also form on their surfaces with prolonged use of removable dentures due to constant mechanical impact. Polushkina et al. (2022) conducted a study and obtained data that with an unsatisfactory level of oral hygiene and the dentures themselves, pathogenic microorganisms can penetrate into the denture bases by 2–2.5 mm. [17]. The risk of developing inflammatory diseases of the oral cavity is extremely high with this level of development of microbiological imbalance. [34, 41, 88]. Homeostatic microflora in the human oral cavity is the result of mutual adaptation of the organism and the microbiota on the organs and tissues of the oral cavity. The biological balance between normal, opportunistic and pathogenic microflora of the oral cavity allows the body to function normally. The presence of foreign objects in the oral cavity, such as removable dentures with foreign microbiota on their surface, can lead to microbiological imbalance. [53, 54, 59]. Microorganisms enter the oral cavity with food, water and from the air, where constant humidity, optimal pH and temperature values, all this creates favorable conditions for the adhesion and reproduction of various microbial species [35, 105].

The presence of biofilm – structured microbial communities that are attached to the surface of removable dentures – is associated with serious systemic diseases, especially in older people [25]. Oral bacteria can cause bacterial endocarditis, aspiration pneumonia, and chronic obstructive pulmonary disease. There is ample evidence of the correlation between proper oral hygiene and overall systemic health [38].

Tulbah et al. (2022) conducted a study on the microbiota composition on the surface of complete removable acrylic dentures and the oral cavity itself. The following microorganisms were found on the oral mucosa of patients: bacteria, fungi, protozoa, viruses, etc. According to the authors, anaerobic bacteria predominate - streptococci, lactic acid bacteria (lactobacilli), bacteroids, fusobacteria, porphyromonas, prevotella, veillonella, and actinomycetes. Among the bacteria in the biofilm on the surface of the oral mucosa, streptococci predominate, making up 30–60% of the total microflora of the oral cavity; while certain organisms are located in different areas of the oral cavity, namely: Streptococcus mitis is found on the inner surface of the cheeks, Streptococcus salivarius is on the papillae of the tongue, and Streptococcus sanguis and Streptococcus mutans are on the surface of the teeth. The oral cavity is also inhabited by spirochetes of the genera Leptospira, Borrelia and Treponema, mycoplasmas (M. orale, M.salivarium), various protozoa - Entamoeba buccalis, Entamoeba dentalis, Trichomonas buccalis. During the study, the authors found that the permanent microflora on the surfaces of acrylic complete removable denture bases (CRD) is characterized by several features, namely: the presence of aerobic (7) and anaerobic (10) species [100]. The qualitative and quantitative composition of the microbial plaque on the surfaces of acrylic complete removable denture bases changes over time of using the dentures. The patterns that were identified by the authors during the study require further study in the context of improving the hygienic condition of removable dentures over time of their use. Destruction of microbial flora on the surface of denture bases significantly depends on the species composition of the microbiota. Reducing the level of contamination reduces the quantitative indicators of bacterial contamination of dentures [58, 106].

Dental plaque on dentures is a complex aggregate containing more than 108 organisms per milligram and including more than 600 species of prokaryotes according to the studies of McReynolds DE et al. (2023) [78, 99]. Different species of bacteria enter into symbiosis with each other, forming a symbiotic biofilm. Biofilms in patients with dentures, as well as the literature on the microbiota of removable denture biofilms, have been extensively studied. Brown JL et al. (2021) found that eighty-two bacterial species are present on the denture surface, including three types of Candida spp. (Candida

albicans; Candida glabrata; Candida tropicalis), which were identified in denture biofilm samples from patients with and without denture stomatitis. Twenty-six bacterial phylotypes were detected in "healthy" denture wearers, while 32 phylotypes were found exclusively in patients with stomatitis. The group of microorganisms in stomatitis was represented by Streptococcus spp. (23%), Atopobium spp. (16%) and Prevotella sp. (11%). C. albicans was identified as the main fungal species in this group of patients [47, 48, 74]. It can be concluded that various biofilms with associated pathogenic risks are present in both healthy individuals and people with stomatitis. In the study of R AN et al. (2023) using molecular hybridization methods, 16 species of actinomycetes and streptococci with high abundance, Veillonella parvula, Capnocytophaga gingivalis, Eikenella corrodens and Neisseria mucosa were recorded in denture biofilms [87]. Alqarni H et al. (2022) considered the presence of periodontal pathogens Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis as an important feature of the microflora of patients with removable dentures. The presence of V. parvula, E. corrodens and N. mucosa in the biofilm of dentures was also confirmed in the study [40]. To prevent inflammatory diseases of the oral cavity in individuals using partial and complete dentures, it is necessary to regularly perform both oral hygiene and hygiene of the dentures themselves. It is necessary to understand the etiology of the microorganisms that are the target of the medicinal treatment of the dentures in order to properly and effectively treat the denture.

According to Kaypetch R et al. (2023), Staphylococcus aureus and Candida albicans are the greatest interest from the point of view of the etiology of inflammatory diseases of the mucous membrane of the denture bed caused by microbial contamination of the dentures. In the study removable acrylic dentures, the following microorganisms were identified: Enterococcus faecalis, Pantoea agglomerans, Citribacter diversus, E. Coli. Thus, Candida albicans is found in 98% of cases on the adjacent surface of dentures, and in 68–94% of people using dentures, candidiasis occurs. The metabolic products of Candida albicans (lactic acid, etc.) cause pain, burning and hyperemia of the mucous membrane under the denture, damage to the corners of the mouth, and lead to intoxication of the body. In addition, when using removable dentures, it was found that the number of

Esherihia coli in the oral cavity increased from 10 to 34%, and the number of Staphylococus aureus - from 10 to 22% [72]. Stomatitis caused by a microbiotic film on the surface of dentures is an inflammatory reaction of the oral mucosa caused by insufficient hygienic treatment of removable dentures.

Denture-related stomatitis is an inflammatory reaction of the oral mucosa caused by inadequate hygienic treatment of removable dentures. Denture stomatitis is one of the most common problems in the elderly who wear partial or complete dentures, and this disease affects 30 to 77.5% of patients who wear dentures [31]. Although the onset and severity of the disease are influenced by various factors, the most common causative factors are poor fixation of dentures in the oral cavity, the age of the dentures, the age of the patients themselves, and poor denture hygiene [16]. In addition, microbial plaque on the denture surface serves as a catalyst for the development of oral diseases, and an association with systemic diseases such as pneumonia and diabetes has been reported [9, 18, 19, 20, 21, 73]. Thus, dentures plaque control has become an important aspect in denture-related stomatitis.

The regular disinfection and cleaning of removable dentures should be carried out to maintain the microbiotic balance of the oral cavity and prevent various inflammatory diseases.

1.2. Methods of disinfecting removable dentures

There are many methods for cleaning and disinfecting dentures. We have divided the methods of disinfection of removable dentures into 4 groups: chemical, mechanical, physical (using special apparatuses) and application of special protective varnishes. In 17% of scientific papers there is data on the use of toothbrushes and pastes (mechanical method) for cleaning dentures. On chemical methods of disinfection and cleaning of dentures 53% of papers were found. Physical methods of disinfection of removable dentures using special apparatuses were presented in 20% of publications. Disinfection of dentures by applying protective coatings was described in 10% of the literature sources studied [18].

1.2.1. Disinfection of complete removable dentures by mechanical methods

In a study by K. Wiatrak and et al. (2017) revealed that the use of toothpastes with active additives (terpinen-4-ol, γ-terpinen, p-cymene, α-terpinen and 1,8-cineol) reduces the risk of various oral diseases [102]. Based on the research of N.V. Novak et al. (2016), the strength of enamel is 3845±20 (HV50) and the hardness of plastic denture artificial teeth is 174±6 (HV50). When using toothbrushes and pastes with high hardness and abrasiveness, the roughness of denture surfaces will be much higher than tooth enamel [13], which is proved by the study of S.N. Razumova et al. (2021), which shows that the use of a hard brush and abrasive paste increases the roughness of tooth enamel after one year more than 7.5 times [22].

Studies published by E.A. Karpovich (2019) show that manual cleaning with paste leads to roughness on removable dentures made of different materials. The use of a soft toothbrush, a paste of low abrasiveness and regular cleaning of the denture with “Korega” tablets for a week is the most rational method of hygiene of removable dentures, according to the author [7].

C. Midha et al. (2017) proposed to use gel toothpaste containing 5% by weight of polyorganosilsesquioxane particles and promotes polishing of denture bases. The disadvantage of the proposed algorithm is low efficiency of denture disinfection, weak antimicrobial activity, especially against fungi of the genus Candida [14].

Julia Gruender et al (2021) conducted a study that evaluated the effectiveness of cleaning plaque from removable dentures using a brush. In this research paper, a total of 32 patients with acrylic removable dentures were examined. The microbial film on the surface of the denture bases was stained and the dentures were photographed from all sides. One group of patients cleaned the dentures with a toothbrush, while the other patients did not clean the removable dentures.

The authors obtained the results that the area of plaque on the dentures without any hygiene measures was larger in the denture bed than on the cheek/lingual surfaces. Manual brushing of the dentures with a brush was not significantly better than no denture treatment at all [65].

Yuya Baba et al. (2018) conducted a study that investigated the effectiveness of cleaning dentures with a toothbrush and immersing them overnight in saline solution (mechanical method) and cleaning dentures with a toothbrush and immersing them overnight in disinfectant solution (combined method) [44].

A total of 30 patients with complete secondary adentia participated in the study. The patients were divided into 2 groups of 15 patients each. In group 1, the patients cleaned the dentures with a toothbrush for 2 minutes after each meal and soaked them overnight in saline solution. In Group 2, patients brushed the dentures with a toothbrush for 2 minutes after each meal and soaked them overnight in disinfectant solution. In this algorithm, patients treated the dentures for 3 weeks. The study evaluated denture cleanliness, patient satisfaction with the denture cleaning method, and patients' oral health-related quality of life (OHRQOL). The study determined significant differences between denture cleaning methods in Candida albicans (C. albicans) content on the surface of complete removable acrylic dentures in adenosine triphosphate bioluminescence ($p = 0.00003$) and denture staining ($p = 0.003$). No significant differences in C. albicans content on the oral mucosa, participant satisfaction, ease of cleaning, comfort, aesthetics or impact on oral health were found for patients with complete tooth loss [47].

Based on the results, it can be concluded that denture cleaning with the combined method is a more effective way to clean complete removable dentures. However, according to the study, the quality of life of patients and the usability of dentures are not affected by these 2 methods.

The proposed methods of denture cleaning with toothbrushes and pastes have a number of disadvantages. Hygienic products do not have the significant cleaning and disinfecting properties required for denture materials. Patients do not understand the information about the abrasiveness of hygiene products, which contributes to the roughness of the denture surface and the deterioration of the hygienic condition of the dentures.

1.2.2. Disinfection of complete removable dentures by chemical methods

The chemical group of options for cleaning and disinfection of removable dentures is the most common. Various chemical compounds are used in medical practice. All disinfection preparations are divided into toxicity classes, which are categorized into four classes. The 1st class includes extremely hazardous substances, the 2nd - highly hazardous, the 3rd - moderately hazardous, the 4th - low hazardous.

All disinfectants for dentures should meet the following characteristics: have a bactericidal effect (exhibit a wide range of antimicrobial and fungicidal activity), be convenient to use, safe for the patient, have a cleaning effect and have a low active concentration [18, 19, 20, 21, 92, 98].

In an experiment conducted by P. Masetti et al. (2018), the effectiveness of using solutions of apple cider vinegar, chlorhexidine, sodium perborate and sodium hypochlorite for cleaning and disinfecting removable dentures was studied. The results showed that these substances showed high antimicrobial activity against Gram-negative and Gram-positive bacteria [76].

Kalivrajiyan E.S. et al. (2013) compared the effectiveness of a “new” solution (cetrimide, silver ions, chitosan succinate) and 1% chlorhexidine bigluconate solution for cleaning and disinfection of removable dentures. The results of the study showed that the “new” solution was more effective in removing microbial plaque from the surface of denture bases than chlorhexidine digluconate. The researchers also studied the effectiveness of the drug “Orthosol-Dent” for disinfection of partial and full removable dentures. The studies showed high antimicrobial and fungicidal efficacy of the preparation with a short exposure time, as well as its safety for denture elements [5].

A substance with silver ions can also be used as a disinfectant solution for cleaning removable dentures. This preparation has effective fungicidal and antimicrobial activity due to the combined action of its constituent substances. It is important to note that the substance does not adversely affect the materials from which partial and full removable dentures are made. The representative of this substance in the Russian dental market is Radosept Ag. This substance belongs to the fourth class of toxicity and it has no

allergenic, embryotoxic, tetratogenic, skin-resorptive properties. It also has a long shelf life, stable and resistant to neutralization.

A preparation with silver ions ("Radosept Ag+") was studied in the experiment of Golubev N.A. et al. (2013). The results showed that this substance has high antimicrobial and fungicidal efficacy. At short exposure to the denture base, it had no adverse effect on the materials used for the fabrication of removable dentures and did not cause allergic, embryotoxic or skin-resorptive reactions in patients [6].

Studies by Komolov R. V. et al. (2015) proved that the substance with silver ions has no active effect on spore-forming bacteria and yeast-like fungi of the genus Candida [8].

In a study conducted by J. Duyck et al. (2016), the effectiveness of storing removable dentures in a substance containing alkaline peroxide and active ingredients such as potassium carbonate, sodium perborate and sodium benzoate was studied. The results of the experiments proved that the use of this solution reduces the number of bacteria on the bases of arquil dentures and does not affect their structure [55].

Literature data describing the use of various phytotherapeutic agents for disinfection and cleaning of complete removable dentures were also studied. In the studies of Golubev N.A. et al. (2017) it was proposed to use a mixture consisting of 10% alcohol tincture (1:5), a collection of marigold flowers, common mountain ash fruit, peppermint leaves, herb Tavolga astringenta, roots of medicinal soapberry in equal proportions and zinc oxide. The prosthesis was soaked in the solution for 5-20 minutes, then thoroughly rinsed with running water. The researchers concluded that due to the complex composition of this mixture, it interacts unevenly with all elements of the prosthesis, and the lack of mechanical treatment does not provide a qualitative antibacterial effect [4, 28].

In the experiment conducted by Shashmurina V.R. et al. (2017) was described a method of disinfection of dentures using the preparation "Optimax". The substance consists of N,N-bis(3-aminopropyl)-dodecylamine (5%), functional impurities, non-ionogenic surfactants, corrosion inhibitor, dye and deionized drinking water. In the experiment it was revealed that this method of disinfection completely removes

microorganisms from the surface of removable dental prostheses, reduces the number of fungi of the genus Candida and slows down their growth.

Experiments conducted by P.M.W. Zago et al. (2019) proved that most denture disinfection solutions contain strong oxidizing agents, the main one being hydrogen peroxide. With continuous application of these solutions, dentures may deform and pores may form on their surface, which serve as additional retention sites. These pores participate in the retention of pathogenic microorganisms. Substances with cationic surfactants, such as quaternary ammonium compounds, are safe for denture bases but have less pronounced antimicrobial activity [104].

It is recommended to use them in conjunction with other substances to achieve good results in denture cleaning. Methods of cleaning and disinfection of dentures proposed by F. Valentini-Mioso et al. (2019), includes rinsing the denture in running water, brushing with a paste mixture (sodium hypochlorite, polyvinylpyrrolidone, sodium silicate) and immersion for 15-20 minutes in a container with water and 3% hydrogen peroxide solution in a ratio of 8:1. However, this method of disinfection does not ensure full contact of the agent with the denture base, which leads to the formation of pores on the plastic of the base. These micropores eventually become retention areas for microbial biofilm, which worsens oral hygiene [101].

G.D.S. Ribeiro Rocha et al. (2020) studied the effectiveness of peroxides, chlorhexidine and chlorine dioxide for cleaning and disinfection of removable acrylic dentures and found that alkaline peroxides were ineffective against Candida spp., while chlorhexidine and chlorine dioxide significantly reduced the number of colony-forming units of microorganisms [57, 89].

In an experiment by Volchkova et al. (2020), the effectiveness of Protefix, an antiseptic tablet dissolved in water, was studied. The preparation contains the active substances sodium bicarbonate, potassium carbonate, sodium perborate, citric acid, sodium lauryl sulfate, peppermint and indigo C1 dye. The authors recorded that the preparation has high efficacy with short exposure time (10-15 min) and is easy to use for the patient [2].

As a result of research by Morozov A.N. et al. (2019), it was proved that the modified solution “Dentaseptin Ag+” has high efficacy as a disinfectant of removable dental prostheses made of acrylic plastic. In comparison with 0.05% solution of chlorhexidine bigluconate, as well as solutions “Radosept Ag+” and “Dentaseptin”, the solution “Dentaseptin Ag+” showed itself more active against Gram-negative and Gram-positive bacteria, as well as fungi of the genus Candida. From the above, it can be concluded that the various recommended chemical compounds with different antimicrobial and cleaning ability indicates that there is no universal denture cleaning agent that is easy to use [12].

There are also special substances for cleaning imported partial and full removable dentures. These substances are antiseptic soluble tablets for preparing solutions, for example, “Protefix”, which have a high cleaning ability and a short exposure time - 10-15 min. The composition of the specialized preparation “Protefix” includes: sodium bicarbonate 41,77% potassium carbonate 35,18% sodium perborate 16,63% citric acid 5,65% sodium lauryl sulfate 0,47% peppermint (peppermint oil + peppermint oil) 0,3% indigo dye Cl 73015 0,07%. The algorithm of the method of cleaning a removable dental prosthesis consists in immersing it in a container of water in which an antiseptic tablet has been dissolved in advance. Nevertheless, the above-described method of cleaning of schematic dentures has a number of disadvantages: these means are not relatively cheap and therefore are not accessible to poor social strata, and they also have in their composition contain a strong oxidizing agent that has a destructive effect on the surface of denture bases [103].

Victor G Morelli et al (2023) conducted a study in which they evaluated the antibacterial efficacy of different cleaning agents for removable dentures. The study design was as follows: strains of Candida albicans, Candida glabrata, Streptococcus mutans and Staphylococcus aureus were specifically seeded on denture samples. After the bacterial biofilm on the surface of the dentures finally matured the samples were immersed for 3 minutes in the following solutions: Polydent, Polydent for partial dentures, Efferdent, Steradent, Corega Tabs and distilled water. Dentures that were soaked in distilled water acted as the control group. The amount of residual bacteria on the surface

of the dentures was determined by the number of colony forming units and biofilm biomass. In parallel, to investigate the cleaning ability of denture disinfectants, artificially contaminated removable dentures were treated with each cleaning agent. Data were analyzed using the Kruskal-Wallis method followed by Dunn's post hoc test or ANOVA followed by Tukey's post hoc test (α= 0.05). The scientists reached the following conclusions: none of the denture cleaning and disinfection solutions reduced C. albicans biofilm. Such solutions for disinfecting removable dentures Efferdent and Corega Tabs contributed to the reduction of C. glabrata biofilm, while Steradent was effective against S. aureus biofilm. The use of Polident solution resulted in reduced biofilm formation rates of S. Mutans. These denture disinfection and cleaning tablets showed good cleaning properties, removing the artificial layer with carbohydrates, proteins and fats, but they were not effective in removing aggregated mature biofilm [49, 79, 84, 90].

Adriana Barbosa Ribeiro et al (2022) conducted a study comparing the effectiveness of different techniques for the care of removable dentures. In this randomized double-blind controlled clinical trial, 108 participants were divided into 4 groups of 27 patients each. Group 1 patients cleaned removable dentures with a 0.25% sodium hypochlorite solution. Group 2 patients cleaned the removable dentures with 0.15% triclosan solution. Group 3 patients used denture disinfection tablets. Group 4 patients used denture disinfection tablets and palatal mucosa cleaning solution. Before the study and after 10 days, the results of microbial contamination of the patients' dentures were measured. Kruskal-Wallis and Dunn tests (between groups) and Wilcoxon test (between periods) were used to compare the results (α = 0.05).

The researchers obtained the following results: after applying the above described denture hygiene methods and comparing with the initial level of microbial contamination, a significant level of oral hygiene of the patients and the dentures themselves was observed. The tested denture disinfection methods allow patients to better maintain the hygiene level of removable dentures [45].

Yasuhiro Nishi et al (2022) investigated the effect of different denture cleaning techniques on the number of microorganisms of the genus Candida. A total of 77 patients

(21 men, 56 women; mean age 84.4 years) were examined. Inclusion criteria: all patients used removable dentures, and the age of the patients ranged from 68 to 102 years [77].

The purpose of the experiment was explained to the participants using a document approved by the Clinical Research Ethics Committee of Kagoshima University Hospital (No. 18-89), and written consent was obtained from all participants. Patients with complete upper and lower removable dentures made of acrylic plastic without a metal base. Patients cleaned them independently (based on interviews with participants and caregivers) and did not have oral diseases such as stomatitis (according to clinical examinations and medical records). A total of 152 dentures (75 upper and 77 lower complete dentures used by the patients were examined. In the G-Power software (Heinrich Heine University, Düsseldorf, Germany), the sample size of the multiple regression analysis with seven explanatory variables on denture cleaning algorithms was 103 denture bases with a medium level effect ($f2 = 0.15$). The sample size for this experiment was sufficient.

The denture cleaning algorithms used were evaluated by interviewing participants and caregivers using a prepared questionnaire. The questionnaire examined whether patients used a denture brush other than a regular toothbrush, the frequency with which they brushed their dentures with a denture toothbrush or denture brush, the denture cleaner used (if any), the frequency with which they used denture cleaner, and the amount of time patients soaked their dentures in denture cleaner. The results were confirmed when compared to the observation records of a full-time dental hygienist who reviewed the denture cleaning methods used by the participants in the week prior to the evaluation. Denture cleaning preparations and denture brush information were visually confirmed by the inspector during the oral examination. Information on the age and sex of the participants was provided by medical records.

To collect plaque swabs, participants were told to remove their full removable dentures, which were then cleaned slightly with running water to remove saliva and then air-dried. Microbial plaques on the removable dentures were accumulated before lunch by one expert dentist other than the inspector, who recorded denture cleaning information using a sterile swab dipped in phosphate buffered saline (PBS) (Fukifuki Check II®,

Eiken Chemical, Tochigi, Japan). A sterile gauze swab was applied twice to the surface of the right lateral half of the studied removable denture. This was based on previous results displaying that the distribution of microbial plaque on the surfaces of upper complete dentures varied between different areas of the denture but was bilaterally symmetrical [68], and that Candida spp. were not always found in all defined areas of the denture under study. Each sterile gauze swab was combined with 10 mL of PBS in a plastic vial, the resulting samples were transferred to the laboratory for seeding and incubation for 5 h after sampling, and then the samples were examined for identification of Candida spp. All samples were diluted in 0.9% NACL solution and inoculated onto CHROMagar Candida plates (CHROMagarTM Candida, Kanto Chemical, Tokyo, Japan). The agar dishes were subjected to aerobic incubation at 37°C for two days. After the incubation period, colonies were formed which showed characteristics of Candida spp. (C. albicans, C. naked, C. tropicalis and other Candida spp. species) were presumptively identified. The colors and morphological features of these colonies were studied in more detail and the number of each colony-forming species was calculated [96]. The number of colony-forming units (CFU) of Candida spp. for each experimental denture was estimated by the number of cells per ml.

Dentures worn by females contained significantly lower numbers of Candida spp. than male participants ($p<0.001$). Dentures cleaned daily with denture cleanser contained significantly lower amounts of Candida spp. than those who used the cleanser once or twice a week or no cleanser ($p<0.001$). A significant difference was observed in the amount of Candida spp. between the different denture cleaning products ($p<0.01$) and the number of Candida species. The level of denture contamination was significantly lower in dentures cleaned with Pika® (Rohto Pharmaceutical Co., Ltd., Osaka, Japan), which contains Candida spp.-dissolving enzyme, than in dentures cleaned with the enzyme-containing preparation Polident® ($p<0.01$). Dentures that were soaked overnight contained significantly less Candida spp. than those soaked for less than 30 minutes ($p<0.01$).

The data obtained by the authors of correlation and multivariate linear regression analysis of denture cleaning regimes showed a correlation between the frequency of

treatment and disinfection of dentures and the number of Candida spp. on the surface of the denture base, patients. According to the data obtained, it can be hypothesized that the frequency of denture care products use is directly related to the amount of Candida spp. and the adhesion of microorganisms to dentures with a long period of use. In addition, daily use of denture cleaning and disinfection products seems to reduce the amount of Candida spp. on the denture surface and reduce adhesion to the denture surface [67].

In a study by Ramage G et al (2019) concluded that it is difficult to affect the matured C. albicans biofilm with periodic treatment with denture cleaner and disinfectant [85]. Therefore, biofilms should be removed from dentures before they finally mature. A strong correlation was found between the frequency of use of denture care techniques and the amount of Candida spp. on the surface of removable dentures in the described experiment.

In summary, in patients who used denture care products daily in this study, Candida spp. fungi were probably on the denture surface, but the solutions prevented the final maturation of the bacteria. Moreover, Ramage G et al (2022) demonstrated in their study that regular denture care use was more effective in reducing the total number of microorganisms on the denture surface than irregular treatment, and it has also been shown that denture cleaning protocols can induce changes in the quantitative and qualitative composition of the microflora. A denture hygiene procedure that reduces microbial contamination of dentures is considered more important than a procedure that removes them from the denture surface. In addition, even when dentures from both jaws were studied separately, the results of multivariate linear regression analysis were similar, and the effect of the frequency of denture care product use was most revealing; however, the number of Candida spp. detected was significantly higher on upper jaw dentures than on lower jaw dentures [82].

Previous studies have shown the prevalence of Candida spp. to be higher among elderly patients requiring nursing care than among elderly people who can clean dentures independently [70]. The prevalence of Candida spp. on denture surfaces was extremely high among nursing home residents using dentures [93]. These data indicate that getting rid of Candida spp. is a major concern when cleaning and disinfecting dentures. This is

important to consider when creating chemical and mechanical denture cleaning methods that are effective against the above microorganisms. Elderly people find it difficult to take good care of their dentures using toothbrushes; recently, a combination of chemical and mechanical cleaning methods (microwave, ultrasonic, or LED) has been shown to effectively clean C. albicans from the surface of dentures [C. albicans, D. albicans, and C. albicans in dentures]. Albicans from the surface of dentures [94]. These mixed disinfection techniques are considered optimal for use in the elderly. However, optimal application patterns for these combined techniques have not yet been described. Although daily use of these techniques may be effective, cost-effectiveness and the effort required to perform them must be considered.

The soaking time and temperature of peroxide solution for cleaning removable dentures were previously investigated, and the results showed that the most effective technique was immersion in the solution at room temperature for 8 h or at 65 °C for 5 min. Hwang et al (2022) reported that storing dentures overnight in disinfecting tablet solutions effectively reduced the level of C. albicans on the denture surface [66]. These results are similar to those obtained by Yasuhiro Nishi et al. who showed a relationship between the soaking time of removable dentures and the amount of Candida spp. On their surface in a multivariate analysis. However, most patients who used denture cleaning products soaked their dentures at room temperature overnight (approximately 8 hours). Thus, there is no unity in the amount of intergroup data on soaking time in the described study, and the results obtained were cross-sectional in nature. Nevertheless, based on their findings, the authors recommend soaking dentures daily overnight in denture cleaner. Although new denture cleaning methods may be developed in the future, further research is needed to establish the most effective denture cleaning regimen from the currently available options [81].

Rattiporn Kaypetch et al (2022) conducted a study to evaluate the efficacy of two new denture cleaning and disinfection preparations (GE and TM) compared to the three most popular denture cleaning agents (in the authors' opinion). The GE and TM preparations were compared with 0.5% sodium hypochlorite solution, NaClO solution and 0.12% chlorhexidine gluconate solution; CHX and Polident®; POL. The authors

evaluated the effects of the investigated agents for cleaning and disinfecting removable dentures with respect to microbial biofilm formation, stain removal and their effect on the physical properties of dentures.

The antimicrobial efficacy of the above-described agents was measured using microbial killing tests. This test was performed against major oral pathogens such as: Streptococcus mutans, Staphylococcus aureus, Escherichia coli and Candida albicans. Confocal laser scanning microscopy (CLSM) was performed to determine the strength of the antimicrobial effect of denture cleaning agents on microbial biofilms formed on the denture surface within 72 hours.

The authors also evaluated the stain removal properties of stains from staining beverages (such as tea or coffee) and changes in the physical parameters of removable dentures. Using a colorimetric test to assess the metabolic activity of cells (MTT), the toxicity of cleaning residues released by full removable acrylic dentures was studied.

All tested methods for cleaning and disinfecting complete removable acrylic dentures effectively killed bacteria and Candida albicans in the oral cavity of patients [24]. In addition, the authors obtained results that after soaking complete removable dentures in Polident®, GE and TM solutions for 180 minutes or more allowed the active ingredients of the solutions to effectively penetrate the denture biofilms and inhibit their growth, just as they did with a 10-minute immersion in 0.5% NaClO solution. However, immersion in 12% chlorhexidine gluconate solution for 20 minutes showed less activity against biofilms. The NaClO solution was the most effective in removing stains from artificial denture teeth. At the same time, exposure of complete removable acrylic dentures in 12% chlorhexidine gluconate solution increased staining of artificial teeth, and denture teeth immersed in this solution showed clinically unacceptable discoloration ($\Delta E > 5.5$). However, the difference in color of denture teeth stained and immersed in POL, GE, and TM cleaners was within the clinically acceptable range. There was no significant difference in stain removal performance between POL, GE and TM cleaners. GE and TM cleaners did not affect the surface roughness and color of the materials, moreover, the residues of both cleaners did not show cytotoxicity.

In summary, based on the results obtained, it can be concluded that two new denture cleaners containing natural components, GE and TM, demonstrated effective antimicrobial activity, biofilm removal and stain removal without toxicity or compromising the physical properties of acrylic materials [71].

Movchan O. et al (2022) conducted a study on the contamination of removable acrylic dentures. The aim of this study was to determine the bacterial contamination of acrylic dentures at different time intervals of their use, as well as to study the changes in the quantitative and qualitative composition of microbiota on the surface of removable dentures at different methods of their hygienic treatment. Smears from the inner surface of complete removable acrylic dentures were Gram stained and studied using a microscope, as well as sown on blood agar, Endo agar, yolk-salt agar, Sabouraud agar. Microbial species were identified using LAHEMA test system and determined in colony forming units (CFU). The data obtained by the authors in the course of their research indicate the accumulation of certain microbial species under the acrylic base of removable denture bases during prolonged use. Therefore, this type of denture needs regular decontamination procedures. Movchan O et al. studied the change in the quantitative and qualitative composition of the microbial biofilm (bacterial contamination) of acrylic denture bases with different treatment methods.

Patients in group “A” used Sideex solution, a two-component system consisting of a liquid component (glutaraldehyde solution) and a powdered activator that are mixed before use to produce a working activated solution. The liquid component is a cleaning agent. It is a colorless solution with a specific odor, which is a 2.2-2.7% aqueous solution of glutaraldehyde, which is the active agent (pH=3.0 - 4.5). The powdered activator is a pale yellow powder containing alkaline components and dye. The activated working solution is a fluorescent green solution with a specific odor, containing 2.2-2.7% glutaric aldehyde; pH=8.2-9.2. (UK). Preparation of the active solution is as follows: powder-activator is added to the container containing the liquid component and these components are mixed together. Cleaning is carried out by fully immersing the denture in the solution, the solution should cover the denture by at least 1 cm. Dentures are soaked in the solution for 15 minutes, then rinsed thoroughly in the same solution for 1-3 minutes. Sideex

activated solution is used for sterilization and disinfection of metal, glass, polymeric (plastic, rubber, etc.) medical devices.

The dentures of patients from group “B” were disinfected with 0.2% chlorhexidine bigluconate solution. The dentures were placed in the solution overnight for 14 days, changing the solution every two days. The disinfection of acrylic denture bases was more effective in group “B”, because there was a more significant reduction in the species composition of microbiota (qualitative index of microbial contamination reduction amounted to 72.0%). The authors have clearly shown that disinfection of acrylic bases of removable dentures in group “A” has a small effect on the species composition of the microbiota, although it significantly ($p<0.05$) reduces the quantitative indicators of microbial flora. As for group “B”, in this group the number of aerobic organisms decreased from 31 lg CFU/mL to 8.7 lg CFU/mL and anaerobic organisms decreased from 42.7 lg CFU/mL to 14.6 lg CFU/mL [80].

Amaya Arbeláez MI et al (2020) conducted a study to investigate the effect of long-term daily chemical disinfection of removable acrylic dentures on Candida albicans biofilm formation on denture bases. Amaya Arbeláez MI et al. selected large size (14 × 1.2 mm) acrylic resin (Tokuyama Rebase Fast II) and acrylic denture base samples (Vipi Wave) for the study. These samples were stored in 50 mL of distilled water for 2 days at 37 °C. The acrylic resin specimens were then immersed in five different disinfectant solutions: 0.5% sodium hypochlorite solution; 3.8% sodium perborate solution; 2% chlorhexidine gluconate solution; apple cider vinegar containing 4% maleic acid; and distilled water (control group). During the study, the specimens were soaked in the test solutions daily for 8 hours and then transferred to distilled water at 37°C for another 16 hours. The surface topography of denture bases and Candida albicans biofilm formation were evaluated at baseline (before treatment) and after 1, 3 and 6 months of denture disinfection. Surface topography was evaluated using arithmetic mean roughness (Ra) and scanning electron microscope (SEM), and biofilm formation was evaluated using the colony-forming unit (CFU/mL) method and Alamar Blue (cell metabolism) dye assay. The results were evaluated using three-way analysis of variance (ANOVA) and post-hoc tests ($\alpha = 0.05$).

The results obtained by the authors during the study showed a statistically significant effect of sample type ($p = 0.029$) and exposure time ($p < 0.001$) on sample roughness. Generally, acrylic samples (Tokuyama Rebase Fast II) had higher roughness than acrylic samples (Vipi Wave). In addition, the roughness of the samples after 1, 3, and 6 months of immersion in cleaning solutions was higher than before treatment. Regarding microbiological analyses, no statistically significant differences ($p > 0.055$) were found in the CFU/mL biofilm content between samples from both groups, time periods and disinfectant solutions. Considering the cell metabolism within the biofilms, the results showed that it was statistically significantly higher ($p < 0.05$) at baseline than after 1, 3 and 6 months of storage. SEM images showed that all disinfectant solutions provided surface changes in both groups of dentures after 1, 3 and 6 months of exposure [43].

Based on the results, it can be concluded that the roughness of the denture bases in both groups was affected by disinfection with all cleaning agents, it increased with time, and this effect was more noticeable in the Tokuyama Rebase Fast II acrylic resin denture group. This surface change was also observed in the SEM images. Although immersion in cleaning agents did not affect the number of cells in biofilms, their content gradually decreased after 1, 3, and 6 months of treatment [91].

1.2.3. Disinfection of complete removable dentures by physical methods

The 3rd group of denture disinfection methods includes denture cleaning with the help of special apparatuses. For this method of denture treatment 20% of works were found.

A study by M.A. Brondani et al. (2018) showed that disinfection of removable dentures prewashed in running water in a microwave oven with a power of at least 800 W is a sufficiently effective method of denture cleaning [40, 46]. However, a subsequent study by R.M.B. da Costa and co-authors (2022) revealed that high power can lead to changes in denture configuration. To avoid this, the authors recommend using a power of 500 W for 3 minutes or 450 W for 5 minutes [50, 51].

Ultrasonic disinfection is also an effective method. T.J. Mason (2015) suggested soaking dentures in an ultrasonic bath with Corega Tabs dissolved in water for 15 minutes

and then rinsing them with running water. This method allows to achieve a qualitative disinfection of dentures. To apply these techniques at home, special equipment and skills are required [77].

S. Papadiochou et al. (2018) developed a method of denture treatment in an ultrasonic glass cuvette using concentrated solutions of hydrochloric acid, alkali, and chloramine B. The disadvantage of this method is its duration and the aggressive effect of chemical solutions on the metal parts of dentures [82].

Osipova V.L. (2018) suggested placing dentures in a solution containing sodium carbonate, sodium tripolyphosphate, chlorhexidine bigluconate, polyvinylpyrrolidone, sodium lauryl sulfate, sodium silicate and water at room temperature. This should be followed by ultrasonic treatment of the denture at 20-24 kHz for 3 min followed by rinsing with running water. The combination of ultrasonic treatment and chemical agents allows the removal of soft and hard deposits on the denture and provides high antimicrobial, antiviral and antifungal efficacy [15].

Chizhov Yu. V. et al. (2021) analyzed three methods of cleaning and disinfection of removable plate prostheses: using ultrasonic disperser UZDN-A with a complex of individual reagents; using ozone sterilizer Ozon-Stom; using ultrasonic washing machine Retona and an original complex of domestic reagents. As a result, the authors came to the opinion that the use of the washing machine type and the original complex of domestic reagents for cleaning removable prostheses is the most preferable [29].

Anna Clara Gurgel Gomes et al (2024) compared the effectiveness of different methods of disinfecting and cleaning complete removable dentures. This study was a blinded cross-over randomized clinical trial (RCT) were recruited between October 2018 and November 2021. Inclusion criteria were participants wearing a complete maxillary denture (CMPD). Participants with broken or repaired removable maxillary dentures were excluded from this study.

After obtaining written consent, the medical records of the study participants were reviewed for common risk factors associated with microbial contamination of the MHCP and hence biofilm on the denture surface. The following data were still examined: time of hospitalization, gender, age, diagnosis, and intake of antibiotics, antifungal, and steroid

medications. When antibiotics and antifungal drugs were taken, the name of the drug and the date of swabbing for the study were recorded. In addition, two local risk factors associated with denture microbial biofilm density and the prevalence of oral infections were considered in this RCT (randomized clinical trial): patient age and nighttime denture wear.

Stratified block randomization of samples was performed using open Epi random software. To create similar groups, participants with baseline characteristics that could affect prognosis were randomly assigned to one of 17 study groups according to the denture cleaning protocol, i.e., three control groups and 14 experimental groups. In the control groups, patients brushed with a new soft toothbrush (Colgate Clean) with distilled water, neutral liquid hand soap (Lifebuoy Original) or toothpaste (Colgate Total® 12). The experimental groups used cleaning solutions of 1% sodium hypochlorite, 0.12% and 2% chlorhexidine bigluconate, alkaline peroxide solution tablets (Corega Tabs), or microwave irradiation (Model Sensor Crisp 38) with or without pre-cleaning of the removable denture. Also, in addition to 2% chlorhexidine bigluconate solution, which is considered an effective denture disinfectant, the denture care protocols included the same chlorhexidine solution at a concentration of 0.12%, as this mouthwash is more commonly used in the medical field.

Before using the above cleaning methods, all toothbrushes were sterilized in a 650 W microwave oven for 6 min. At the end of each experiment, the denture was immersed in a 200 mL container of distilled water for 3 min. In addition, if oral lesions (e.g., denture stomatitis, fibrous hyperplasia, medial rhomboid glossitis, etc.) were detected, the participant was duly referred to the clinic of the Bauru School of Dentistry, University of São Paulo, Bauru, Brazil.

To investigate the efficacy of cleaning protocols, the primary outcomes of this study were the area of biofilm coverage of dentures and the quantitative composition of microbial cells on the denture surface. The secondary outcome was the prevalence of the main risk factors investigated and their influence on the efficacy of the cleaning protocols.

Before and after use of disinfection methods, the biofilm of the inner surface of the HIFU was stained with a solution of 1% neutral red (Sigma-Aldrich Inc), which

actively produces biofilm. This dye is easily peeled off and has no antimicrobial effect. Denture surfaces were then photographed with a digital camera (Canon EOS Rebel T6i) mounted on a stand (CS-4). The conditions for determining cleaning quality were identical for all dentures (location, lighting, angle, and photographer). The photographs were then transferred to a computer to measure the total internal surface area and areas corresponding to the stained area using image processing software.

For quantitative microbiological seeding, the collected material was cultured in duplicate before and after application of purification methods. Microbiologic samples were obtained by vigorously rubbing sterile swabs into the surface of the HPVF baseplates for 1 min. Each swab was placed in a test tube containing 5 ml of 0.9% NaCl 0.9% solution. Then, to separate the collected material from the swabs, the tubes were placed in a tube holder inside an ultrasonic cleaning tank (Cristófoli) with cold water (6 to 10ºC) and treated with ultrasound for 20 min. Each tube was then shaken vigorously for 1 min before serial 10-fold dilutions using an aliquot (25 µl each). After each dilution, the samples were seeded in blood agar (New Prov Produtos para Laboratório) in order to culture the main strains of oral and non-oral bacteria (including the most important respiratory pathogens). Samples were also immersed in Sabouraud's dextrose agar (Sigma-Aldrich Inc) to detect Candida spp. Blood agar plates were incubated at 37°C in a capnophilic atmosphere (5% CO2) for 24-48 h, and Sabouraud's dextrose agar plates were incubated at 37°C for 48 h. Viable colonies were then quantified and colony forming units per milliliter (CFU/ml) were determined.

The diseases most commonly observed in the participants were, among others, disorders of the circulatory, genitourinary and respiratory systems. The Kruskal-Wallis test was applied to these data to assess whether differences could be detected between the study groups given the diagnoses prior to the application of the different proposed denture cleaning methods. The same test was applied after the evaluation of the cleaning methods to assess whether the hypotheses influenced their effectiveness.

The distribution of the data corresponded to an abnormal distribution. In addition, there was a lack of normality and homogeneity in the percentages of biofilm coverage on the denture surface. Thus, the Wilcoxon test was used by the authors to analyze each

cleaning method of removable dentures before and after disinfection. Comparisons between groups at different evaluation periods were made using the Kruskal-Wallis test followed by the Bonferroni post hoc test (α=.05). Pearson's correlation coefficient was used to test the correlation between quantitative variables (logarithmic values of 10 CFU/mL and percentage area of biofilm coverage of the denture). Analysis was performed using two statistical programs (SigmaPlot 12.0 and Systat Software Inc).

The authors noted no statistically significant differences between the study groups for criteria such as age and gender of the participants. No significant differences were found in patients with different durations of denture use, type of overnight denture storage, and medication use before denture cleaning was performed.

The most common comorbidities were: diseases of the circulatory system (n=85), diseases of the respiratory system (n=74). When evaluating quantitative microbiological culture data (log10 CFU/mL values), and data on the percentage area of biofilm coverage of dentures, using the Kruskal-Wallis test, the authors found no significant differences between the study groups before denture treatment (p=0.213 and p=0.281 respectively) and after (p=0.327 and p=0.060 respectively) application of denture cleaning protocols. Thus, there was homogeneity between the groups at the start with respect to data related to microbial colonization of removable dentures and they did not distort the disinfection methods evaluated in this study.

In all dentures studied, the authors observed a decrease in the area of denture sites contaminated by microbial biofilm ($p<0.001$) after hygiene measures. The group of dentures cleaned with a toothbrush and sodium hypochlorite solution had the highest level of cleanliness compared to the other groups ($p<0.05$). The method of cleaning dentures with toothbrush and toothpaste was the least effective. By its cleaning and antibacterial parameters, it was close to the group of dentures that were soaked in distilled water (control group) ($p<0.05$).

According to the results of the study, all dentures in all groups were not damaged and none of the participants had any complaints when using the dentures that passed the study.

The main objective of this study was to compare different methods of cleaning and disinfecting complete removable maxillary dentures in order to identify the most effective method of disinfection for patients at home. The aim was to identify a method that is suitable for all denture wearers and that will eliminate microbial biofilm after the first application.

The cleaning activity of denture disinfection methods was evaluated without regard to demographics, risk factors, and ICD-11 comorbidities. The results were calculated by statistical analysis and homogeneity was confirmed between all subject groups for all risk factors and comorbidities. In this study, 56.2% of the participants were female and their mean age was 71, 72 years. The mean age of PSPHF was more than five years, as observed previously, and night denture wear was observed in most (65.5%) of the participants in this study.

According to the results obtained by the authors on the composition of microbiological cultures and the area of their biofilm coverage on the surface of dentures, the authors concluded that the methods of cleaning and disinfection of removable dentures differed in their effectiveness [23]. When summarizing the results of biofilm coverage area and microbiological (blood agar) and mycological (Sabouraud agar) cultures. The following denture disinfection methods had the highest cleaning efficacy: immersion in 1% sodium hypochlorite solution (whether or not a toothbrush was used in this protocol), denture treatment with 2% chlorhexidine solution, and denture treatment with microwave radiation [60, 61, 62, 63].

The authors concluded that all tested cleaning and disinfection methods for complete removable acrylic dentures resulted in significant reductions in both the area of contamination and the quantitative and qualitative composition of biofilm on the surface of the patients' dentures. In summary, the best results were observed in the group where dentures were treated with 1% sodium hypochlorite solution. A single immersion in this solution for 10 min, even in the absence of denture brushing, proved to be a practical, simple, and affordable option for cleaning patients' complete dentures [52, 75].

Thus, the combination of hardware and chemical methods of denture cleaning is quite effective, but requires special equipment and skills to operate it.

1.2.4. Disinfection of complete removable dentures with special varnishes

The 4th group of methods for disinfection and treatment of dentures includes the use of special protective antibacterial varnishes. This technique was described in 10% of the studied literature. I.A. Voronov (2016) developed a method of coating prosthesis with silicon carbide using plasma spraying ("Shell"). This coating significantly reduces the adhesion of pathogenic organisms to prostheses and reduces the rate of microbial biofilm formation [3]. Barilo A.S. et al. (2017) proposed a special varnish with antibacterial properties, one of the components of which was decamethoxin. The authors showed high sensitivity of V. subtilis, B. cereus, as well as Escherichia and Klebsiella to the action of decamethoxin [1]. A. Feldmann et al. (2022) found that the use of a varnish based on wine vinegar and 3% hydrogen peroxide solution reduces the adhesion of pathogenic microorganisms to denture bases and decreases their roughness [56]. All existing antibacterial varnishes require multiple renewals. Based on the literature we have studied, we can conclude that all methods and techniques of cleaning and disinfection of complete removable acrylic dentures have their drawbacks. Therefore, the problem of high-quality cleaning of dentures remains relevant.

CHAPTER 2. RESEARCH MATERIALS AND METHODS

2.1. Research Design

To attain the objectives of the study, a research design was developed that included several steps (Figure 2.1).

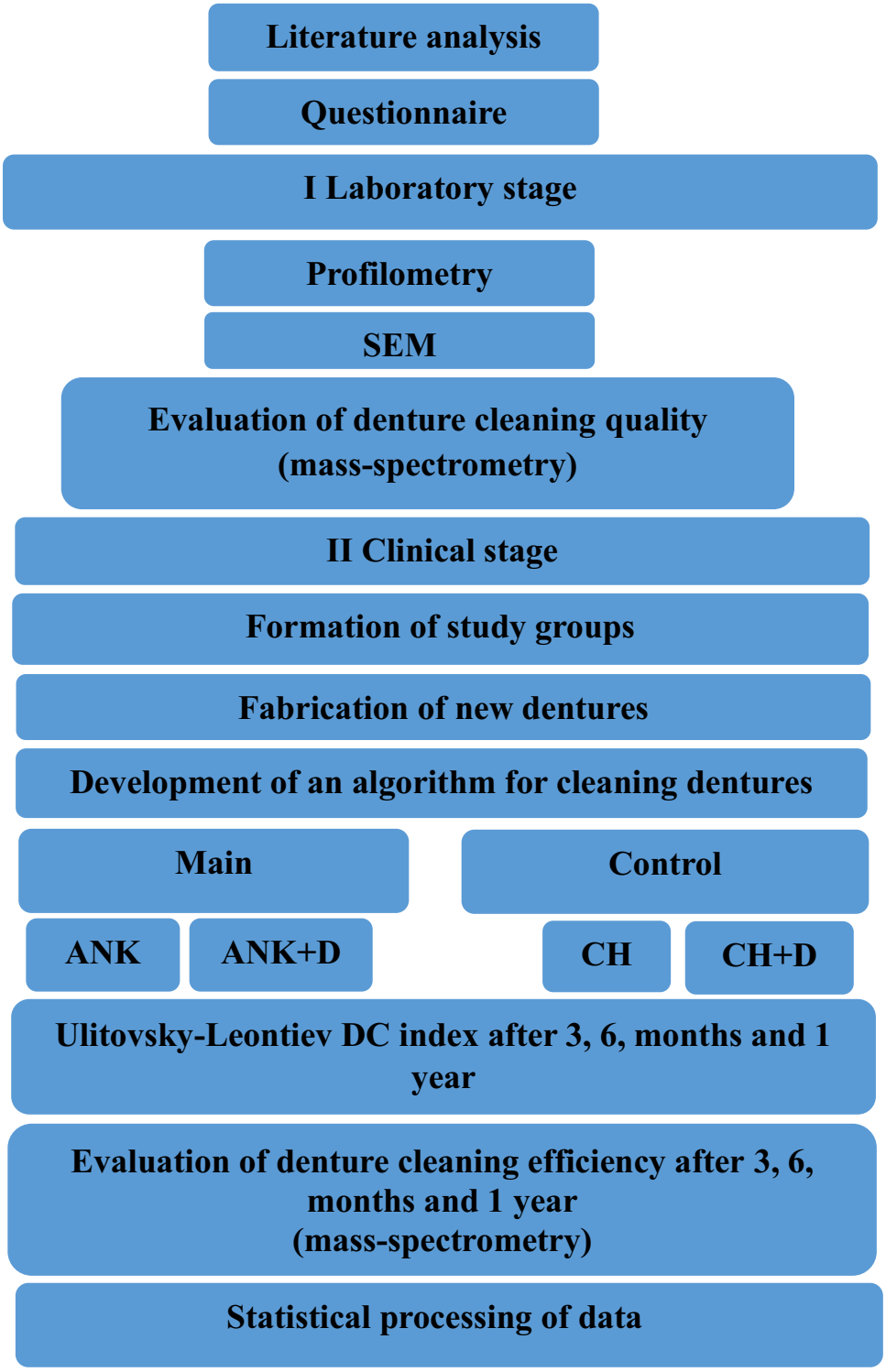


Figure 2.1-Design of the study

2.2. Questionnaire

In order to determine the most frequently used by patients options for cleaning removable dentures, we developed a questionnaire containing, in addition to the passport part, somatic status, questions about the care of complete removable dentures.

For example: “How long ago were your dentures made? How do you clean the denture at home? How often do you clean your denture?”. The questionnaire is presented in Appendix 1. 180 people participated in the questionnaire.

2.3. Profilometry method for the evaluation of denture surface roughness (DSR)

The surface roughness of old dentures was evaluated using the profilometry method. The essence of the method is the examination of the cross-sectional profile of the surface. Profilometry is an optical method based on the measurement of roughness parameters using a 3D optical profilometer Senso neox (“Sensofar”, Spain) at a 3D magnification of 50 on the principle of simultaneous transformation of the surface profile. The DSR is a representation of the microrelief of the denture base. The micro and nano-geometry of the denture surface was evaluated.

The DSR was studied using a standardized confocal profilometry protocol. This profilometer complies with the ISO 25178 standard, being an objective tool for surface characterization. Before starting the study, the device was calibrated to evaluate metrological performance using a calibration standard to correct for systematic errors and for compliance with the calibrated value. The arithmetic mean of the absolute values of profile deviations within the base length was taken as the point of change of surface roughness Sa (height parameter). The effect of different methods of cleaning removable dentures and their influence on the microrelief were studied.

2.4. Detection of defects by SEM on denture surfaces

The surfaces of denture bases were studied using a LEO-1430 VP scanning electron microscope (Carl Zeiss, Germany) under high vacuum conditions with a 4 QBSD back-reflected electron detector at room temperature, accelerating voltage 20 kV and

working distances 15-22 mm. Scanning electron microscopy (SEM) is one of the most effective and informative methods of surface structural analysis used today. Thanks to this technique it is possible to carry out a quantitative morphological assessment of the studied surface.

The essence of the scanning electron microscope application is as follows: the electron microscope beam is alternately moved across the entire scanning object, and the detector, which is located next to it, simultaneously reads the number of electrons that hit it. On the basis of the received signal from the detector, a point-by-point representation of the surface is constructed, namely: the more electrons hit the microscope detector at a certain point in time, the brighter the point in the picture will be displayed. The width of the electron beam of the microscope is 20-40 nanometers, which makes it possible to achieve high magnifications and examine even the smallest defects in the surface topography of dentures.

2.5. Ulitovsky-Leontiev Index of Denture Cleanliness (DC)

The Index of Denture Cleanliness (DC) was developed by Ulitovsky S.B. and Leontiev A.A. (2008) (Table 2.1) to objectively assess and monitor the level of cleanliness of removable dentures during the use.

The definition of DC consists in detecting plaque on certain parts of the denture using a point-rating system. The data are presented in Table 2.1.

Table 2.1 - Evaluation criteria of the Ulitovsky-Leontiev DC index

Indicators	**Criteria for assessing the Ulitovsky-Leontiev DC index**
1,0–1,9	High level of cleanliness of removable dentures
2,0–2,9	Good level of cleanliness of removable dentures
3,0–3,9	Satisfactory level of cleanliness of removable dentures
4,0–4,9	Poor level of cleanliness of removable dentures
5,0–5,5	Very poor level of cleanliness of removable dentures

The cleanliness of complete removable acrylic dentures on the upper and lower jaw was assessed by visual determination of plaque and subsequent staining of the denture.

A visual assessment of the denture was performed first. If no areas on the denture with soft, pigmented plaque were found and there were no stains on the surface, the denture was stained with erythrosine solution 5%.

The staining technique consisted of the following: the surface of the complete removable acrylic denture was stained with erythrosine 5% solution for 60 seconds. After this time, the dye was thoroughly washed off the surface of the denture, dried thoroughly, and its surface was visually evaluated.

If no evidence of plaque was detected after the procedures, the denture was given a score of 1. If only plaque was detected in certain areas, a score of 2 was assigned. If there was plaque visible to the naked eye on any of the surfaces, a score of 3 was given. If individual spots or a single plaque was detected on any of the surfaces, the denture was given a score of 4. If individual spots and a single plaque are seen on the vestibular surface of a full removable acrylic denture, a score of 5 is given. If heavy plaque is detected on the vestibular outer surface of the removable denture (the side of the denture that does not face the mucosa) and/or if individual spots and single plaque are visualized on the inner surface of the removable denture, 6 points are awarded. Heavy contamination of the vestibular-external and oral-external surfaces of the denture (the side of the denture that does not face the mucosa) and heavy contamination of the vestibular-internal or oral-internal surfaces of the removable denture (¼ to ½ of the denture surface was covered with plaque) were rated 7 points. Dentures were given a score of 8 if there was extensive contamination of the vestibular-external and oral-external surfaces and extensive contamination of the vestibular-internal or oral-internal surfaces of the removable denture (plaque covering ¼ to ½ of the inner surface area of the denture), as well as the presence of single deposits of tartar. If the visual examination of the denture revealed abundant mineralized plaque on any surface of the denture, the denture was given a score of 9 points. The maximum score of 10 points was given only if the plaque covered more than ¾ of the denture surface.

Thus, the S.B. Ulitovsky - A.A. Leontiev DC index is the sum of all the above-mentioned indices divided by the number of indices, the resultant index of which is calculated according to the following formula: Ulitovsky - Leontiev PE index = $\sum(a_1+. ... + a_x)$ xx, where: $\sum$ is the sum of quantitative evaluations of criteria; a1 is the number of points for the first criterion; a_x is the number of points for x criterion; x is the number of criteria used in the index.

2.6. Examination of denture and oral cavity contamination by mass-spectrometry

The microbiological evaluation of the biofilm of dentures and the oral cavity itself was carried out using the mass-spectrometry method.

The mass-spectrometry method is based on the direct extraction by chemical reactions of higher fatty acids from the submitted sample. Fatty aldehydes of phospholipids and long-chain fatty acids are found in the cell wall. This fatty acid composition in all bacteria is species specific and can be used to differentiate between different microorganisms. In addition, many microorganisms have their own markers. These markers are individual for taxa of different levels (family, genus or species). Thanks to them it is possible to differentiate the organisms in the clinical samples obtained. The basic concept of the above analysis is the direct extraction of higher fatty acids from the sample to be examined (in our study, these are cytosmear from the surfaces of complete removable acrylic dentures) by means of a chemical procedure. Then the isolated fatty acids are separated on a high resolution capillary column chromatograph and their composition is analyzed in dynamic mode on a mass spectrometer. Then on the computer with the help of special programs and data obtained from the mass spectrometer, the quantitative and qualitative composition of the microbiota obtained from the surfaces of the denture bases was determined.

Material was collected using sterile cotton cytosmear. Cytosmear were taken from the inner surface of complete removable acrylic dentures for both upper and lower jaws. Immediately after Tabbing, the experimental specimen was placed in a sterile container and numbered according to the study protocol.

In the laboratory, the tubes with the obtained biofilm samples were sorted (Figure 2.2).

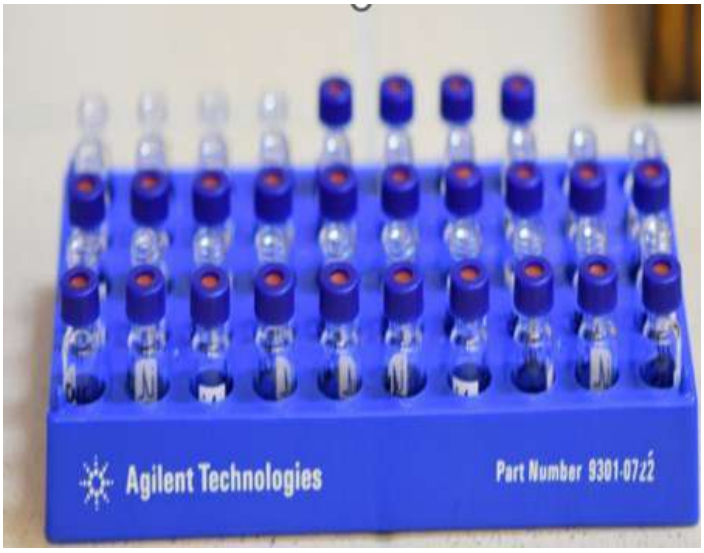


Figure 2.2 - Sorted tubes by groups

Then, all tubes with their contents were weighed on a special ultrathin scale to determine the weight of the obtained microbiota samples from the denture surface. This is necessary in order to know exactly the required volume of methanol that will be added to the obtained samples for acid methanolysis in the future (Figure 2.3).

Figure 2.3 - Microbiological weighing scales for weighing

After that, methanol (equal in volume to the contents of the tube) was added to the tubes with the denture microbiota samples. The tubes were then placed in an Orbital shaker to mix the methanol and the resulting microbiological samples from the surface of the dentures. This is a necessary condition for the subsequent acidic methanolysis and extraction of fatty acids and aldehydes from complex lipids of microorganisms and other cells of the liquid in the form of methyl esters and dimethyl acetals (Figure 2.4).

Figure 2.4 - Medical Digital Orbital Shaker

The tubes were then placed in front of a thermostat to create a controlled and contaminant-free environment. This is achieved by controlling the following parameters: temperature, humidity or gas content (carbon dioxide, nitrogen, oxygen). Also in this thermostat the processes of temperature control, derivatization and evaporation of samples take place. At this stage of the study, fatty acids and aldehydes are released from complex lipids of microorganisms obtained from cytosmear as methyl esters and dimethylacetals (Figure 2.5).

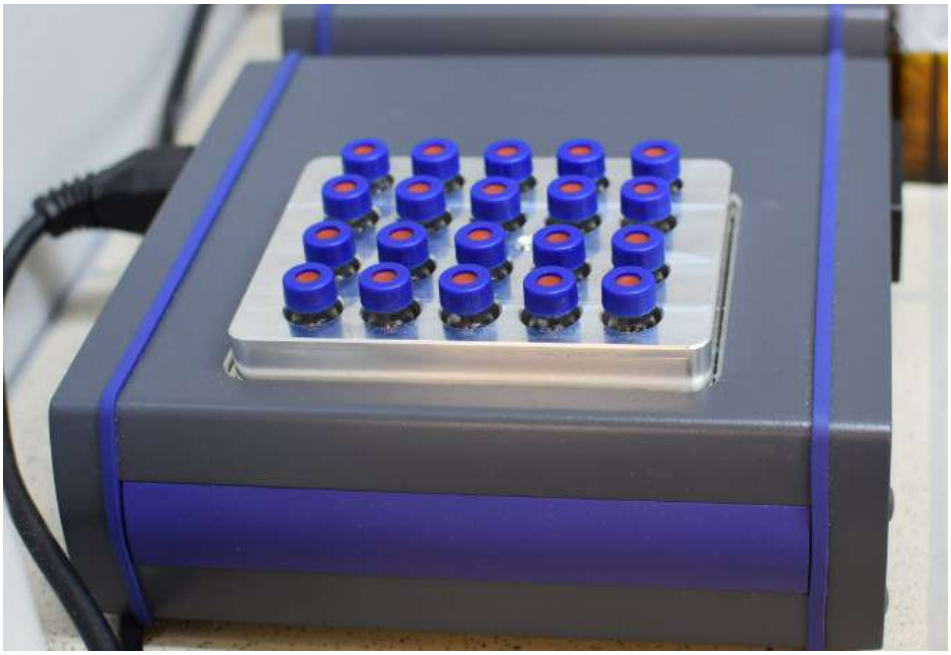


Figure 2.5 - Solid state thermostat TT-20

The tubes were then immersed in a Maestro-αMS Gas Chromatography-Mass Spectrometer (Figure 2.6).

Figure 2.6 - Maestro-αMS Gas Chromatography-Mass Spectrometer

After that, the quantitative and qualitative composition of the microbiota obtained from the surfaces of the denture bases was determined on a computer using special programs and data obtained from the mass spectrometer (Figure 2.7).

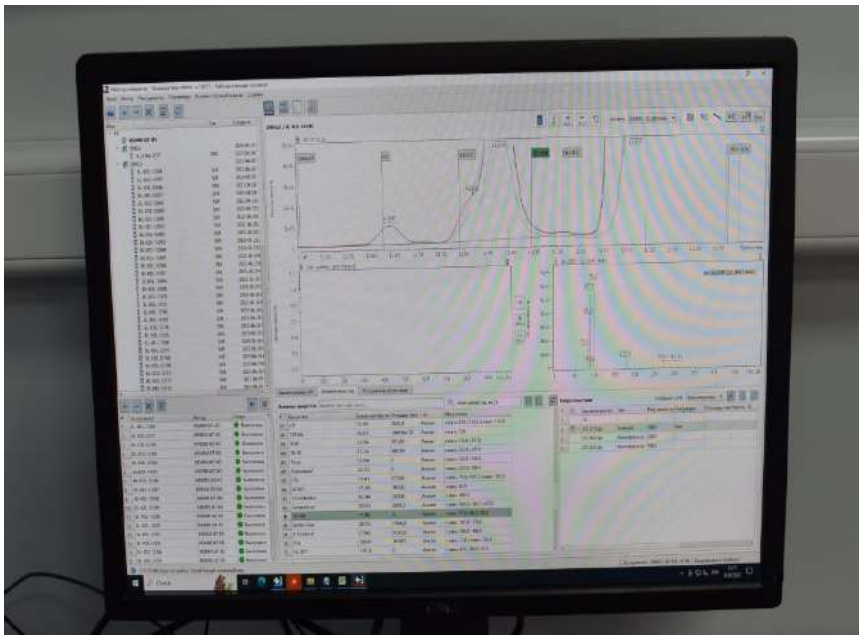


Figure 2.7 - Processing of the obtained data

2.7. Characteristics of the disinfectants used

"Anolit ANK SUPER" (ANK) - disinfectant solution (DS) of a wide range of action, actively affects bacteria, mycobacteria, viruses, fungi, spores and prions (Appendix 2, 3, 4). Is harmless to humans, environmentally friendly, safe in all forms of application (irrigation, immersion, wiping, immersion). Safety is achieved due to the composition and concentration of active ingredients (AI): metastable aqueous solution of electrochemically activated oxidants (hydrochloric acid, hydrogen peroxide, ozone and singlet oxygen). Human phagocytes produce similar in composition and properties active substances that perform a protective function and are responsible for the destruction of pathogens.

No species of microorganisms over time can develop sensitization and resistance to the solution "Anolit ANK SUPER". This is due to metastability (variability of AI forms).

Store (DR) “Anolit ANK SUPER” in a closed container, which will preserve its disinfectant, detergent and sterilizing properties for 6 months from the date of manufacture. This preparation is registered, certified and meets the unified sanitary-epidemiological and hygienic requirements for products (goods) subject to sanitary-epidemiological supervision (control).

Aqueous solution of chlorhexidine digluconate 0.05% (CH) is a topical antiseptic with bactericidal action. This drug is active against Gram-positive and Gram-negative bacteria. It has bactericidal, bacteriostatic, fungicidal and virulicidal action. CH solution has an effect on Gram-positive and Gram-negative bacteria (Treponema spp., Neisseria gonorrhoeae, Ureaplasmaspp., Bacteroides fragil is, Chlamydia spp.), protozoa (Trichomonas vaginalis), viruses and fungi. On bacterial spores this drug is active only at elevated temperature. This solution very rarely causes allergic reactions in patients, skin and tissue irritation, does not have a damaging effect on objects made of glass, plastic and metals. This solution is used as a therapeutic and prophylactic agent for various infections, for antiseptic cleaning and disinfection.

2.8. Experimental selection of disinfectant exposure time

To determine the optimal exposure time of dentures in the solution, 60 dentures were studied, the period of use was 3.5 years and more. Patients were randomly divided into two groups. In group 1 (main, n=30) the dentures were cleaned and disinfected with the tested solution “Anolit ANK SUPER”. In group 2 (n=30), dentures were cleaned with 0.05% chlorhexidine solution.

Dentures were immersed in the solutions for 5, 20 minutes, and 8 hours. After each exposure, material was collected for mass-spectrometry.

2.9. Clinical stage

2.9.1. Characteristics of the studied groups

N=60 (100%) patients (male n=30 (50%)), female (n=30 (50%)), aged 45 to 80 years with the diagnosis of complete secondary absence of teeth (K08.1) participated in the clinical study. Patients were divided into 4 groups of 15 patients each. The CH group

included n=15 patients, n=30 dentures were cleaned with chlorhexidine digluconate solution 0.05%. The CH +D group included n=15 patients, n=30 dentures were studied, cleaned with “Device for cleaning removable laminar dentures” moistened with CH solution. ANK group included n=15 patients, n=30 dentures cleaned with “Anolit ANK SUPER” solution were studied. The ANK+D group included n=15 patients, n=30 dentures cleaned with “device” wetted with “Anolit ANK SUPER” solution.

Inclusion criteria for the study: age of patients from 45 to 80 years, presence of complete removable acrylic dentures on the upper or lower jaw, atrophy of the maxillary alveolar ridge of the 1st degree and alveolar ridge of the lower jaw of the 2nd degree, thickness of the mucosa and alveolar ridges of the upper and lower jaw not less than 2-3 mm, mobility of the mucosa of the 1st type according to Keller, absence of allergic reactions to the components of the material from which the denture is made, absence of concomitant somatic diseases.

Omission criteria: age of patients less than 45 and more than 80 years, absence of complete removable acrylic dentures (patients with partial removable acrylic dentures or complete removable acrylic dentures that had been previously repaired in the dental laboratory were not included in the study), inability of patients to clean the dentures independently.

Exclusion criteria: if the patient did not show up within the designated time frame for denture cleanliness assessment, they were excluded from the study. The data are presented in tables 2.2, 2.3.

Table 2.2 - Age of patients in CH, CH+D, ANK, ANK+D groups

Cleaning method	**Number of patients**	**Mean age, median (lower quartile; upper quartile) Me (lq uq)**
ANK+D	15	71 (66; 74)
ANK	15	72 (66; 75)
CH+D	15	72 (64; 78)
CH	15	69 (58; 76)

Table 2.3 - Description of groups for the study (CH, CH +D, ANK, ANK+D)

Groups	Solution	Number of people at all N (%)	Number of people in group n (%)	Cleaning time (Minutes)
Control	Chlorhexidine digluconate solution 0.05%(CH)	60 (100)	15 (100)	20 (2 times in a day)
	Chlorhexidine digluconate solution 0.05%+ device (CH +D)		15 (100)	6 (2 times for 3 minutes)
Main	Anolit ANK SUPER solution (ANK)		15 (100)	6 (2 times in a day)
	Anolit ANK SUPER solution + device (ANK +D)		15 (100)	2 times for 3 minutes)

2.10. Statistical methods of research

Statistical processing of the study results was carried out using the computer package Statistica 13, Maestro program and MBA (microbiological analyzer). Data were tested for conformity to the normal law, threshold value or baseline level of statistical significance p=0.05. If the data conformed to the normal law and the variances did not differ according to Levene's criterion, parametric methods were applied and the central values were described as arithmetic mean plus standard deviation M±s, otherwise, when the data did not conform to the normal law, non-parametric methods were used with description of central values as median and quartile segment Me (25q; 75q), which contains 50% of the sample values, to the left and right of the median. Differences were statistically significant when data were compared pairwise at $p < 0.05$.

Comparative evaluation of data within each group was performed (6 comparisons) with a Bonferroni correction critical value of p=0.0083. Comparison of group data after 1 month, 3 months, after 6 months, after 12 months at the level of statistical significance p=0.05.

Chapter 3. RESULTS OF RESEARCH

3.1. Questionnaire results

The results of the patient questionnaire n=180 (100%) showed that the patients had been using removable dentures for 3.5 years or more.

When asked “What method do you use to clean your denture at home?” n=72 (40%) patients responded that they treat under running water with a toothbrush, n=72 (40%) soak the dentures in DS, n=27 (15%) patients responded that they clean under running water with a sponge, n=9 (5%) rinse under running water with a “tissue cloth”. The questionnaire data is presented in Table 3.1.

Table 3.1 - Results of patient questionnaires

Method of cleaning dentures	**Number of patients**	
	At al N (%)	**n (%)**
Toothbrushing under running water	180 (100%)	72 (40%)
Immersion in disinfectant solutions		72 (40%)
Sponge under running water		27 (15%)
«Tissue cloth» under running water»		9 (5%)

Upper and lower jaw dentures were included and studied in the study.

3.2. Denture surface profilometry results

The results of profilometry showed that the cleaning method of removable dentures affects the structure of the denture base. The change in denture surface roughness (DSR) was studied on n=60 (100%) samples. The results are presented in Table 3.2 and Figures 3.1-3.4.

Table 3.2 - Profilometry data

Type of cleaning	Roughness in micrometers (μm)	P
TB	5,83 ± 0,25	0,000001
DS	1,58 ± 0,3	0,000001
Sponge	1,48 ± 0,26	0,000001
«Tissue cloth»	1,57 ± 0,05	0,000001

The change in DSR cleaned with TB over a long period of time was studied on n=24 (40%) specimens and was 5.83 ± 0.25 μm (p=0.000001). The surface of the dentures is rougher and patients find it more difficult to maintain a satisfactory level of hygiene. The data are presented in Figure 3.1.

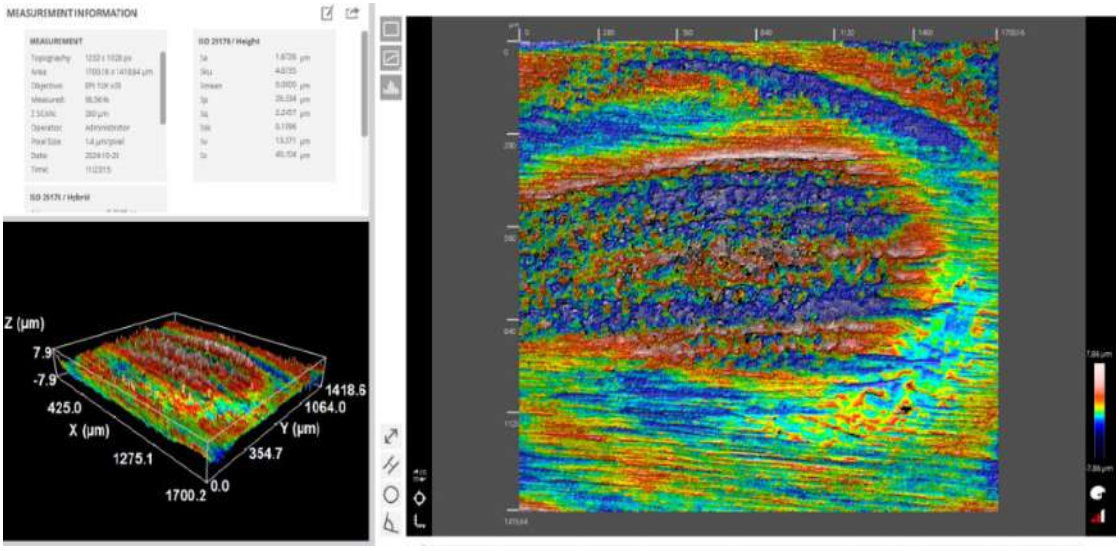


Figure 3.1 - Profilometry data of the denture cleaned with TB

The DSR cleaned by immersion in DS was studied on n=24 (40%) specimens and was 1.58 ± 0.3μm (p=0.000001), which was 3.5 times lower than that of the TB cleaning. The data are presented in Figure 3.2.

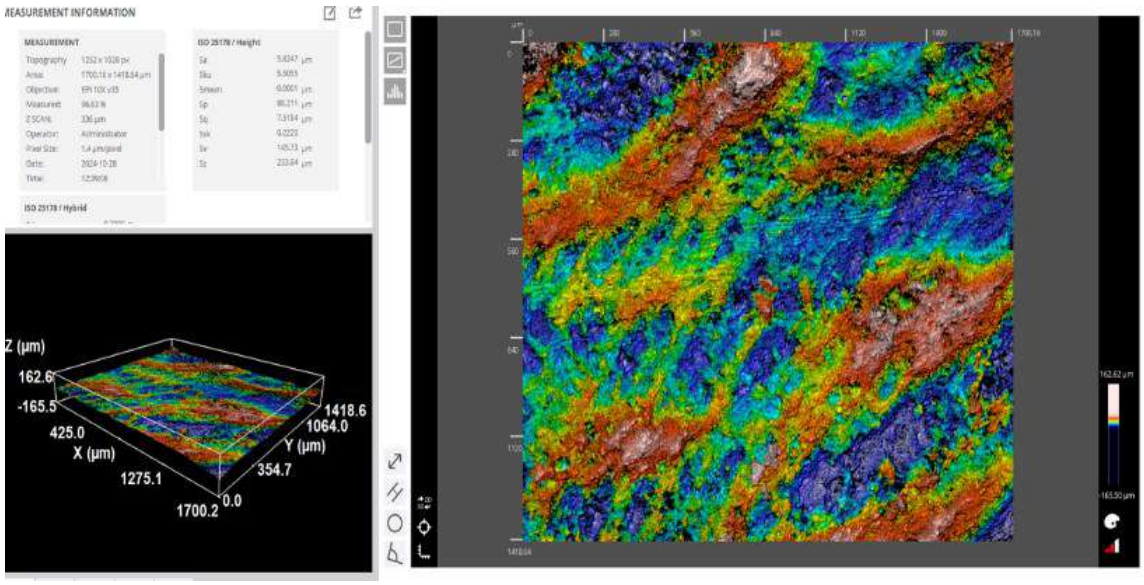


Figure 3.2 - Profilometry data of the denture cleaned with DS.

The DSR of the sponge cleaned under running water was studied on n=9 (15%) specimens. The SPP was 1.48 ± 0.26 µm (p=0.000001), which is 4 times lower than that of the denture cleaned with DS. The results of the study are presented in Figure 3.3.

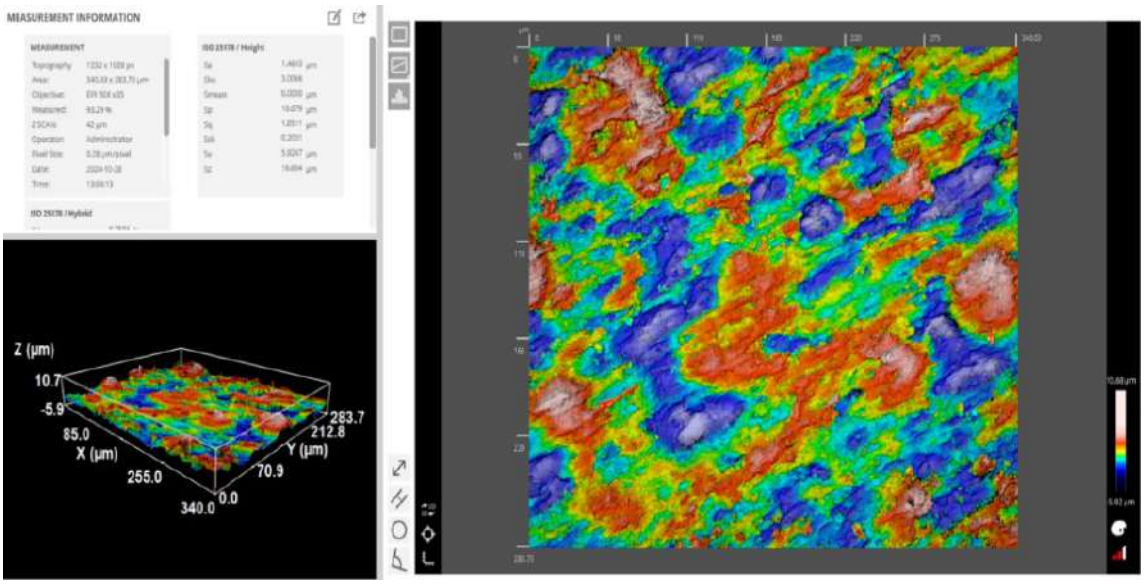


Figure 3.3 - Profilometry data of denture cleaned with sponge

DSR cleaned under running water with a tissue cloth was studied on n=3 (5%) images and was determined to be 1.57 ± 0.05 μm (p=0.000001), which is 3.5 times lower than that of the denture cleaned with a sponge. The data are presented in Figure 3.4.

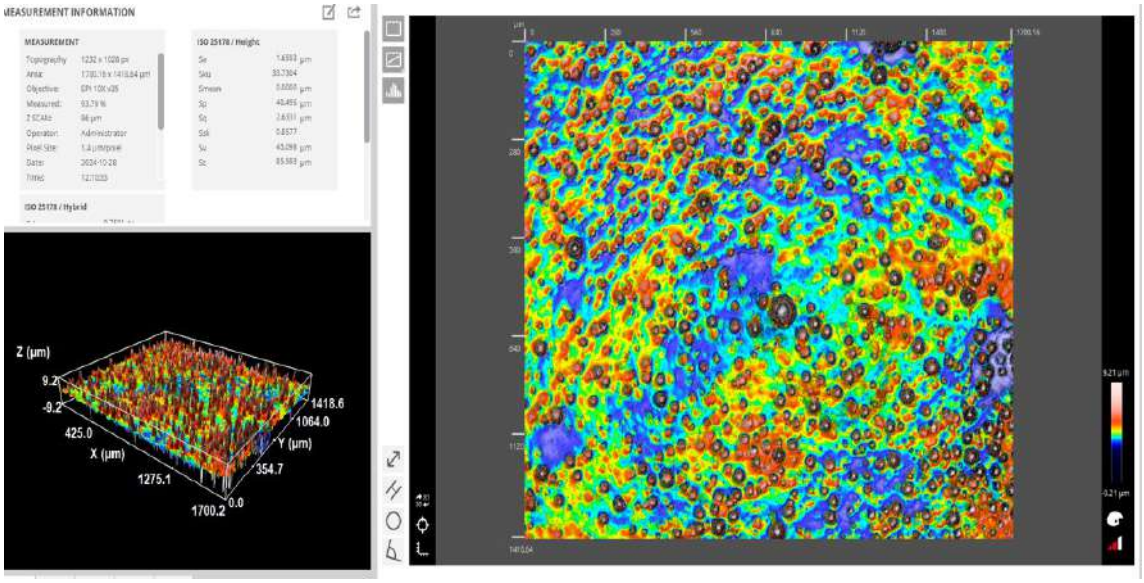


Figure 3.4 - Profilometry data of the denture cleaned with a “tissue cloth”

The use of sponges, tissue cloth and immersion of dentures in DS does not have a negative effect on the denture base in contrast to toothbrushing. The most aggressive method of denture cleaning with respect to the denture base is toothbrushing. Consequently, dentures cleaned with a toothbrush have the highest roughness compared to dentures cleaned with other methods. At the same time, there were no statistically significant differences (p=0.37) in the roughness of dentures cleaned with a sponge, “tissue cloth” and immersion in DS.

3.3. Denture surface SEM results

The SEM results showed that on all old dentures of both upper and lower jaws, irrespective of the cleaning method (immersion in DS, cleaning with a sponge or tissue cloth), various defects in the form of cracks, chipping and micropores are present on the surface of the bases. Such relief changes serve as additional retention sites for pathogenic microorganisms. The development of microbial colonies on the surface of denture bases

can lead to the development of inflammatory diseases of the oral cavity. The surface of the denture bases was evaluated with a microscope under magnification of 100 and 300 times.

During the cleaning dentures by TB according to SEM data, a large number of defects were detected on their surface. Micropores and multiple scratches, chipping and inhomogeneity of relief were visualized in the field of view. Photographs of the surface of the dentures treated with TB are shown in Figure 3.5 (A, B).

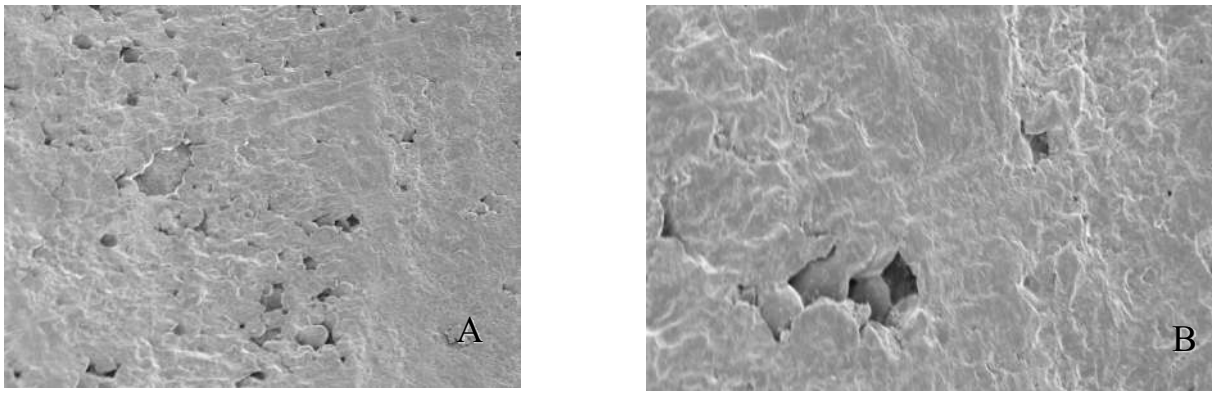


Figure 3.5 - Scanning electron microscopy of denture surface cleaning with TB: A) 100x magnification, B) 300x magnification.

The evaluation of the denture surface during cleaning by immersion in DS did not reveal any significant damage or changes in the relief of the denture bases. Minor defects of the denture bases were caused by the service life of the dentures. Photographs of the surface of the dentures cleaned by immersion in DS at magnifications of 100 and 300 times are shown in Figure 3.6 (A, B).

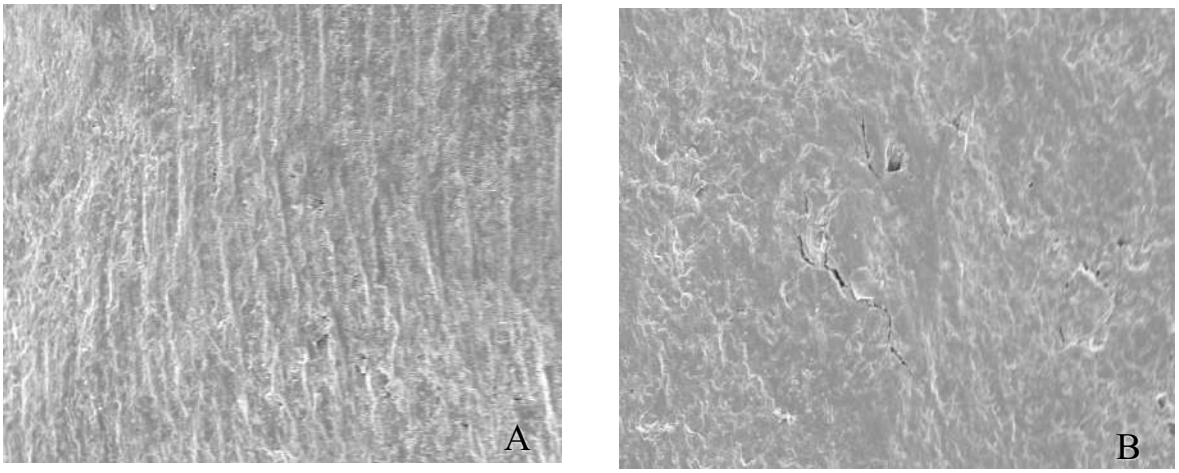


Figure 3.6 - Scanning electron microscopy of denture surface cleaned by immersion in DS: A) 100x magnification, B) 300x magnification

Analysis of the denture surface during sponge cleaning showed that no defects are detected on the surface, the surface is quite smooth. The micropores visible in the photographs are due to the porosity of the acrylic plastic from which the dentures are made. The data are presented in Figure 3.7 (A, B).

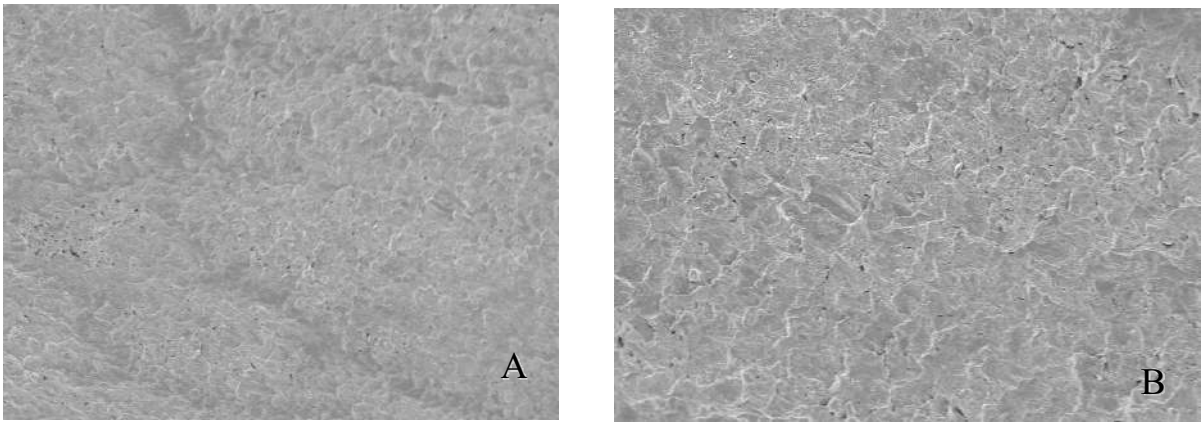


Figure 3.7 - Scanning electron microscopy of denture surface during sponge cleaning: A) magnification of 100 times, B) magnification of 300 times

When dentures were cleaned with a “tissue cloth” under running water, the SEM data show no clinically significant defects on the surface, and no damage to the bases was detected. The micropores visible in the photographs are due to the porosity of the acrylic plastic from which the dentures are made and their service life. The data are presented in Figure 3.8 (A, B).

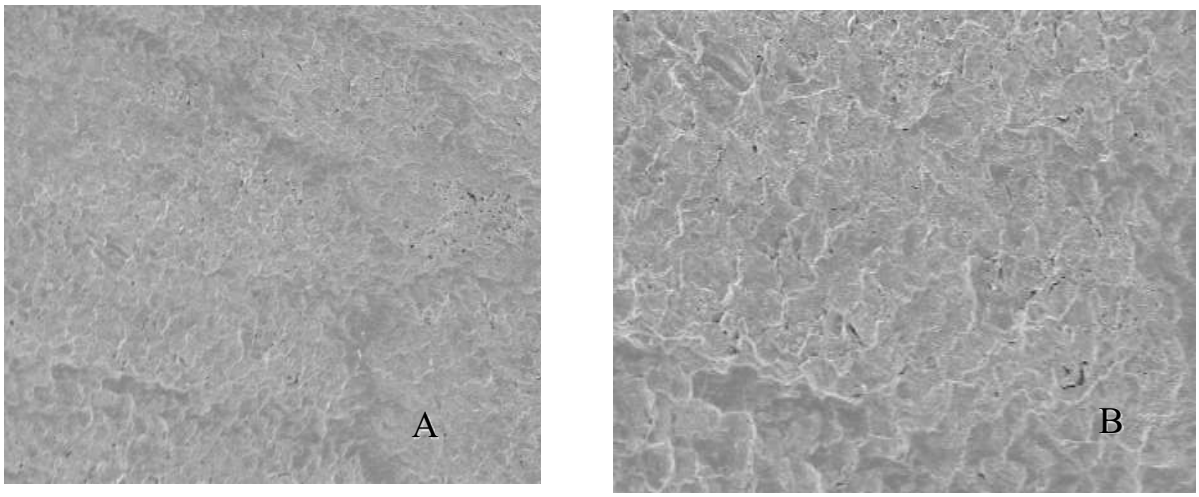


Figure 3.8 — Scanning electron microscopy of the surface of prostheses during "cloth napkin" treatment: A) magnification by 100 times, B) magnification by 300 times

All studied dentures n=60 (100%), used for more than 3.5 years, have n=60 (100%) damage on the surface. According to SEM data, long-term cleaning of dentures with TB leads to multiple damages. Microcracks and pores appear on their surface, which serves as retention areas for pathogenic microorganisms. Dentures cleaned with DS, sponge and tissue cloth have a smoother surface and the least change in the reliefs of the bases.

3.4. DC index of dentures used for 3.5 years and more

Examination of “old” dentures revealed pigmented plaque on the surface of the dentures. When the dentures were visually evaluated, the PE index was found to be at a “poor” level of cleanliness. When the dentures were cleaned with the TB index was 4.6±0.5 (p=0.207837), when immersed in DS 4.5±0.5 (p=0.207837), when cleaned with a sponge 4.1±0.5 (p=0.207837); “tissue cloth” 4.2±0.5 (p=0.207837). The data are presented in table 3.3.

Table 3.3 - DC index

Method of cleaning	DC values	P
TB	4,6±0,5	0,207837
DS	4,5±0,5	0,207837
Sponge	4,1±0,5	0,207837
«Tissue cloth»	4,2±0,5	0,207837

Note: there are no statistically significant differences, p>0.08

The results showed that the index of DC used for more than 3.5 years corresponds to a “poor” level of cleanliness regardless of the processing method. The data are presented in Figures 3.9-3.12.

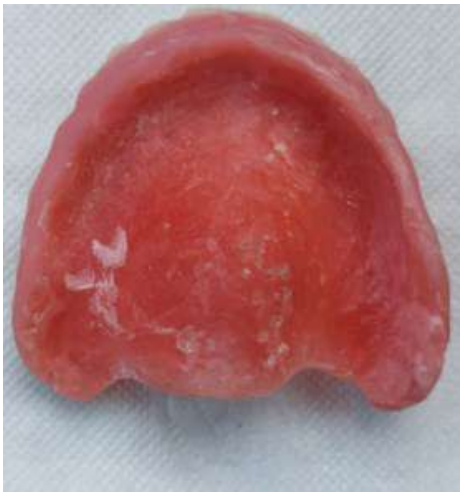


Figure 3.9 - Visual assessment of the DC cleaned by TB

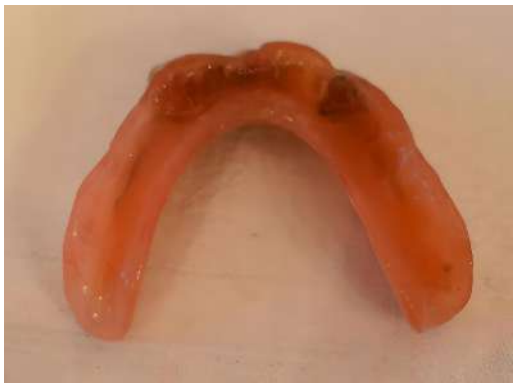


Figure 3.10 - Visual assessment of DC cleaned in the DS

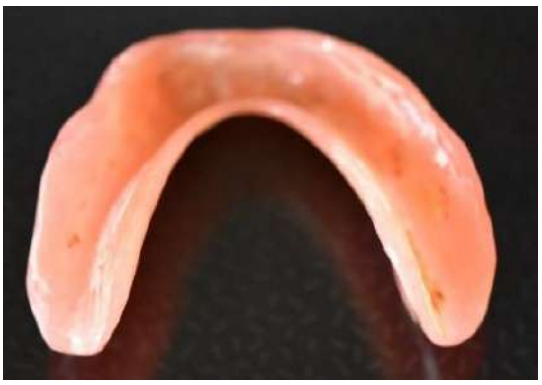


Figure 3.11 - Visual assessment of DC cleaned with sponge

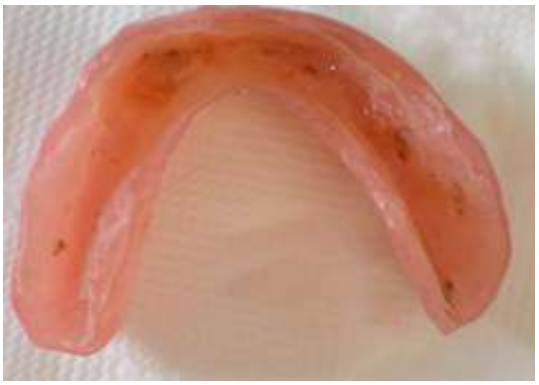


Figure 3.12 - Visual evaluation of DC cleaned with a “tissue cloth”

3.5. Results of mass-spectrometry of oral mucosa and denture surfaces

The results of examining the oral mucosal contamination of patients' oral mucosa n=60 (100%) and full removable acrylic dentures n=120 (100%) by mass-spectrometry showed that the following microorganisms were detected on the surface: Fungi and yeasts (campesterol-producing microscopic fungi, Aspergillus spp, Candida spp), anaerobic organisms (Lactobacillus spp, Peptostreptococcus anaerobius, Clostridium perfringens, Clostridium ramosum, Prevotella spp, Clostridium difficile), actinobacteria (Streptomyces spp, Corynebacterium spp), cocci and bacilli (Bacillus megaterium, Streptococcus mutans, Staphylococcus epidermidis).

On the oral mucosa of n=60 (100%) the content of fungi and yeasts was 1826.0±70.0 (105 cells/gram), and microscopic fungi producing campesterol 634±25 (105 cells/gram). Aerobic mold fungi from the ascomycetes department (Aspergillus spp)

were found in the amount of 657±26 (105 cells/gram). Candida spp were detected in the amount of 239±10 (105 cells/gram). Anaerobic organisms were detected 183±9.0 (105 cells/gram), lactobacillus spp were detected 56±3 (105 cells/gram). Peptostreptococcus anaerobius was found 42±2 (105 cells/gram), Clostridium perfringens anaerobic spore-forming bacteria was 25±2 (105 cells/gram) and Clostridium ramosum bacteria content was 33±2 (105 cells/gram). Prevotella spp microorganisms were detected in the amount of 6±1 (105 cells/gram), Clostridium difficile - 12±1 (105 cells/gram), Actinobacteria - 167.0±30.0 (105 cells/gram). The level of microorganisms Streptomyces spp. was 98±18 (105 cells/gram), Corynebacterium spp. - 32±6 (105 cells/gram). Cocci and Bacillus spp. were detected at 140±11.0 (105 cells/gram), with Bacillus megaterium at 72±8 (105 cells/gram). Streptococcus mutans was detected at a count of 45±7 (105 cells/gram) and the microbial content of Staphylococcus epidermidis was 17±4.0 (105 cells/gram). The data are presented in Figure 3.13.

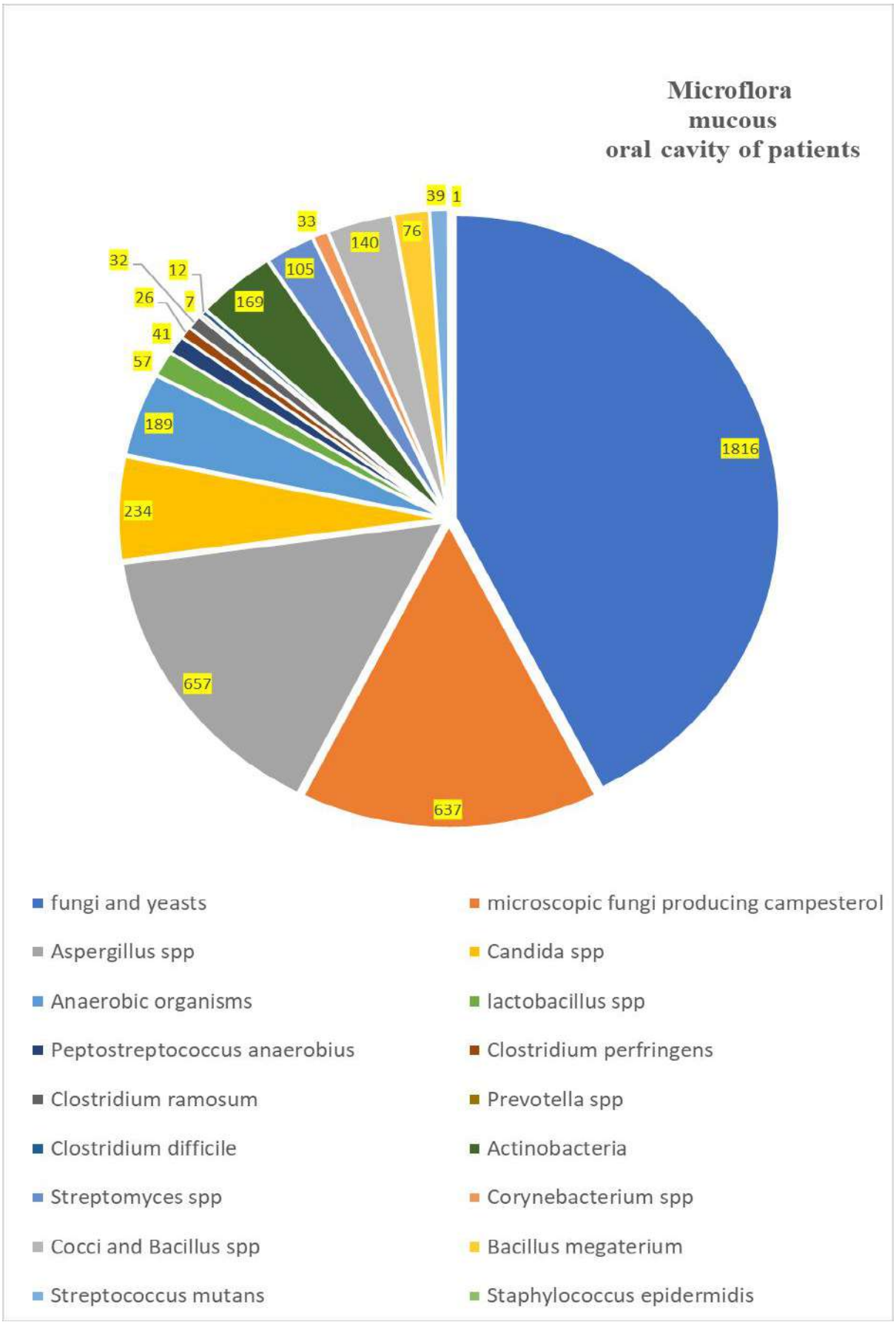


Figure 3.13 - Microbial content in the oral cavity

On the surface of the dentures cleaned with TB, 2145±77.0 (105 cells/gram) fungi and yeasts (p=0.001173), anaerobes -172±9.0 (105 cells/gram) (p=0.000001), cocci and bacilli 254±11 (105 cells/gram) (p=0.000507), actinobacteria 275±30 (105 cells/gram) (p=0.028995) were found. The data are presented in table 3.4.

Table 3.4 - Content of microorganisms on the surface of dentures

Method of cleaning	**Type of microorganisms**			
	Fungi and yeasts	**Anaerobic organisms**	**Cocci and Bacillus spp.**	**Actinobacteria**
TB	2145±77,0	172±9,0	254±11	275±30
P	**0,001173**	**0,000001**	**0,000507**	**0,028995**
The DS immersion	1845±77,0	166±9,0	135±11	178±30
P	**0,000000**	**0,000012**	**0,000079**	**0,025709**
Sponge	1645±77,0	157±9,0	124±11	165±30
P	**0,003608**	**0,008009**	**0,002785**	**0,001831**
«Tissue cloth»	1890±77,0	155±9,0	189±11	171±30
P	**0,000081**	**0,005632**	**0,003357**	**0,028995**

Note:

Actinobacteria: there are statistically significant differences between sponge and DS, sponge and TB, tissue cloth and TB at $p<0.05$.

Anaerobes: differences are statistically significant in pairwise comparison between all groups at $p<0.008$.

Fungi and yeasts: differences were statistically significant in pairwise comparison between all groups at $p<0.008$, except DS and TB.

Cocci: differences were statistically significant in pairwise comparison between all groups at $p<0.008$, except DS and TB.

On the surface of dentures cleaned by immersion in DS, 1845±77.0 (105 cells/gram) fungi and yeasts (p=0.000000), anaerobic organisms 166±9.0 (105 cells/gram) (p=0.000012), cocci and bacilli 135±11 (105 cells/gram) (p=0.000079), actinobacteria 178±30 (105 cells/gram) (p=0.025709) were detected.

On the surface of dentures cleaned under running water with sponge, 1645±77.0 (105 cells/gram) fungi and yeasts (p=0.003608), anaerobic organisms 157±9.0 (105 cells/gram) (p=0.008009), cocci and bacilli 124±11 (105 cells/gram) (p=0.002785), actinobacteria 165±30 (105 cells/gram) (p=0.001831) were detected.

On the surface of dentures cleaned under running water with a tissue cloth, 1890±77.0 (105 cells/gram) fungi and yeasts (p=0.000081), anaerobic organisms 155±9.0 (105 cells/gram) (p=0.005632), cocci and bacilli 189±11 (105 cells/gram) (p=0.003357), actinobacteria 171±30 (105 cells/gram) (p=0.028995) were found.

Thus, the number of fungi and yeasts in the sponge cleaning is 1.3 times less compared to the TB cleaning, in the DS and tissue cloth cleaning 1.1 times less compared to the TB cleaning. The number of anaerobes is 1.1-1.3 times less when cleaned with DS, sponge and cloth compared to TB cleaning. The number of actinobacteria decreases 1.6 times in DS, sponge and wipe cleaning compared to TB cleaning. The number of cocci and bacilli decreases in the cleaning with tissue cloth in 1.3 times, in the cleaning with DS in 1.9 times, in the cleaning with sponge in 2 times in comparison with TB.

Thus, the questionnaire survey showed that the most common methods of denture cleaning are immersion in DS n=24 (40%) and brushing n=24 (40%). However, toothbrushing leads to surface changes in the denture bases (cracks and micropores), which were detected by profilometry and SEM. No damage was detected on the surface of dentures cleaned with sponge, tissue cloth and DS immersion. The sponge cleaning method was the most gentle for acrylic dentures, while the aggressive cleaning of dentures with DS was the most aggressive. Similar results were obtained when the contamination of dentures was examined by mass-spectrometry. The most ineffective method of denture

cleaning in terms of the number of microorganisms on the surface of the dentures was cleaning them with TB. The most effective way of cleaning in relation to the microbiome on the surface of dentures is their cleaning with a sponge.

3.6. Determining the optimal time for immersion of removable dentures in DS

To choose the optimal time of disinfection in DS, from the functional and practical point of view, we have studied the quantitative and qualitative composition of microflora by mass-spectrometry of 60 complete removable dentures of the upper and lower jaw, the period of use of which was 3.5 years and more, after their immersion for 5, 20 minutes and 8 hours.

CH and “Anolit ANK SUPER” were used as DS. Patients were randomly divided into two groups. In group 1 (main, n=30), dentures were cleaned and disinfected with the tested solution “Anolit ANK Super”. In group 2 (control, n=30), dentures were cleaned with 0.05% chlorhexidine digluconate solution.

The results of the study showed that the use of CH 0.05% and “Anolit ANK SUPER” solution for disinfection of removable acrylic dentures reduces the number of microorganisms on their surface.

Before cleaning, 1846±81.0 (105 cells/gram) of fungi and yeasts were found on the surface of dentures of both groups, after 5 minutes exposure in CH 0.05% solution, the number of fungi and yeasts decreased to 1608±65.0 (105 cells/gram) (p=0.00), and after 5 minutes exposure in “Anolit ANK SUPER” solution, the number of fungi and yeasts decreased to 312±24.0 (105 cells/gram) (p=0.003). After 20 minutes exposure in CH 0.05% solution, the number of fungi and yeast decreased to 967±27.0 (105 cells/gram) (p=0.00), and after 20 minutes exposure in “Anolit ANK SUPER” solution, the number of fungi and yeast decreased to 215±35.0 (105 cells/gram) (p=0.04). After 8-hour exposure in the CH 0.05% solution, the number of fungi and yeast decreased to 825±126.0 (105 cells/gram) (p=0.00), and in the “Anolit ANK SUPER” solution, the number of fungi and yeast decreased to 125±33.0 (105 cells/gram) (p=0.03). The data are presented in Figure 3.14.

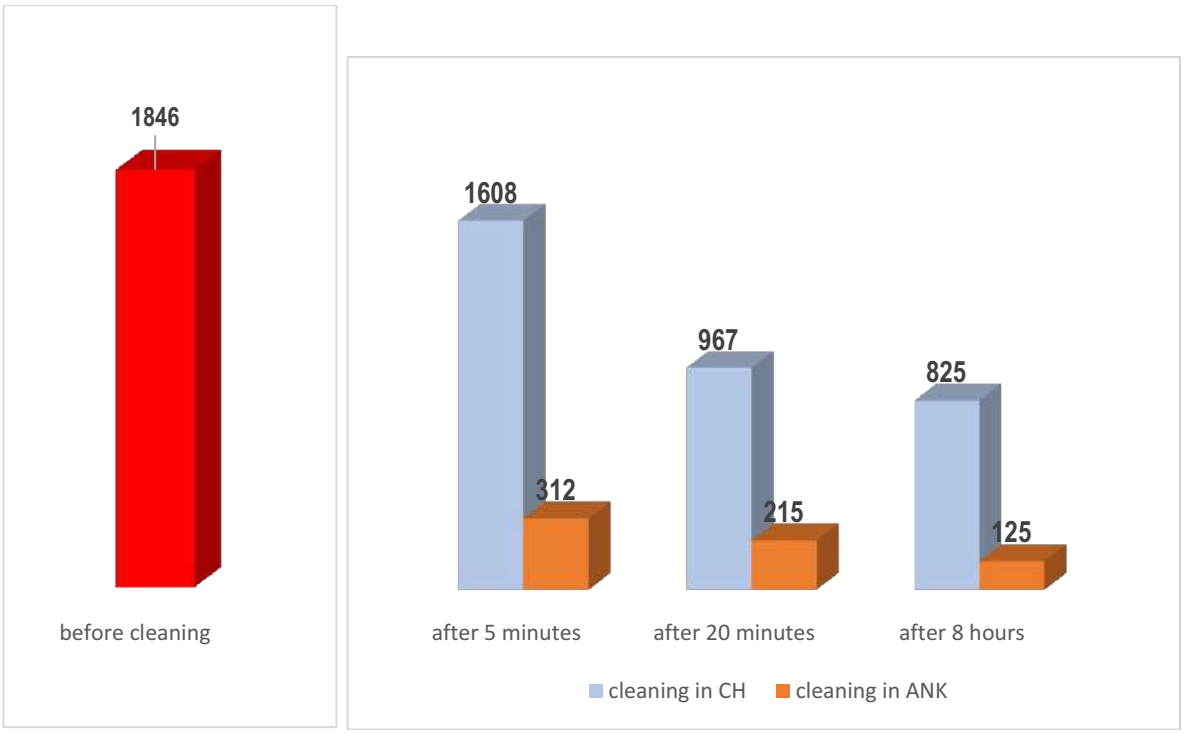


Figure 3.14 - Fungus and yeast content before and after cleaning

Note: - differences are statistically significant in pairwise comparison of data after 5 minutes of cleaning in CH and Anolit, after 20 minutes of cleaning in CH and Anolit, after 8 hours of cleaning in CH and Anolit at $p < 0.05$

Before cleaning of dentures 154±9.0 (105 cells/gram) anaerobic microorganisms were detected, after 5 minutes exposure in CHH solution 0.05% anaerobic microorganisms decreased to 101±9.0 (105 cells/gram) (p=0.00), and after 5 minutes exposure in "Anolite ANK SUPER "ANK" solution the number of anaerobic microorganisms decreased to 15±1.0 (105 cells/gram) (p=0.005). After 20-minute exposure in 0.05% CH solution, the number of anaerobic microorganisms decreased to 90±5.0 (105 cells/gram) (p=0.00), and after 20-minute exposure in "Anolit ANK SUPER "ANK" solution, the number of anaerobic microorganisms decreased to 12±3.0 (105 cells/gram) (p=0.004). After 8-hour exposure in CH 0.05% solution, the number of anaerobic microorganisms decreased to 58±4.0 (105 cells/gram) (p=0.00), and after 8-hour exposure in "Anolit ANK SUPER "ANK" solution, the number of anaerobic microorganisms decreased to 10±3.0 (105 cells/gram) (p=0.006).

The data are presented in Figure 3.15.

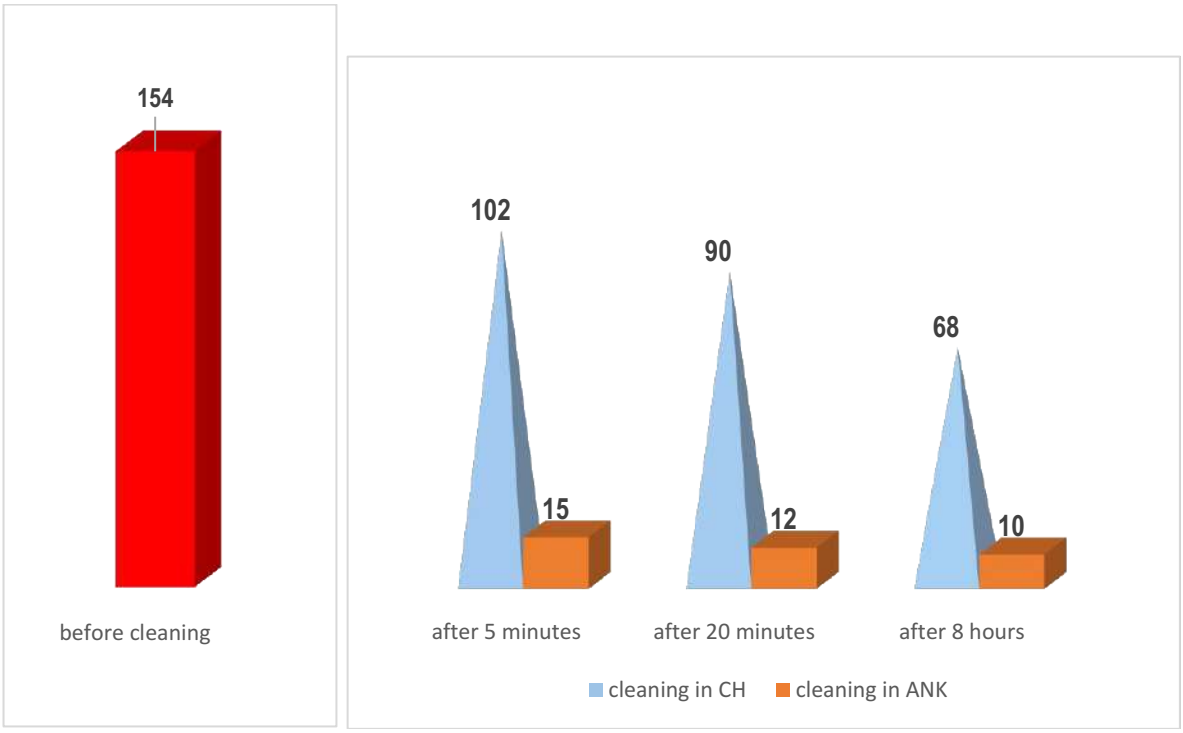


Figure 3.15 - Content of anaerobes before and after cleaning

Note: - differences are statistically significant in pairwise comparison of data after 5 minutes of cleaning in CH and Anolit, after 20 minutes of cleaning in CH and Anolit, after 8 hours of cleaning in CH and Anolit at $p < 0.05$.

Before cleaning of the dentures, 163±30.0 (105 cells/gram) actinobacteria were detected, after 5 minutes exposure in CHH 0.05% actinobacteria solution decreased to 143±7.0 (105 cells/gram ($p=0.00$), and after 5 minutes exposure in "Anolite ANK SUPER "ANK" solution, the number of actinobacteria decreased to 16±4.0 (105 cells/gram) ($p= 0.00002$). After a 20-minute exposure in 0.05% CH solution, the number of actinobacteria decreased to 102±5.0 (105 cells/gram) ($p=0.00$), and after a 20-minute exposure in "Anolit ANK SUPER "ANK" solution, the number of actinobacteria decreased to 12±3.0 (105 cells/gram) ($p=0.006$). After 8-hour exposure in 0.05% CH solution, the number of actinobacteria decreased to 30±2.0 (105 cells/gram) ($p=0.00$) , and after 8-hour exposure in "Anolit ANK SUPER "ANK" solution, the number of actinobacteria decreased to 8±3.0 (105 cells/gram) ($p=0.03$). The data are presented in Figure 3.16.

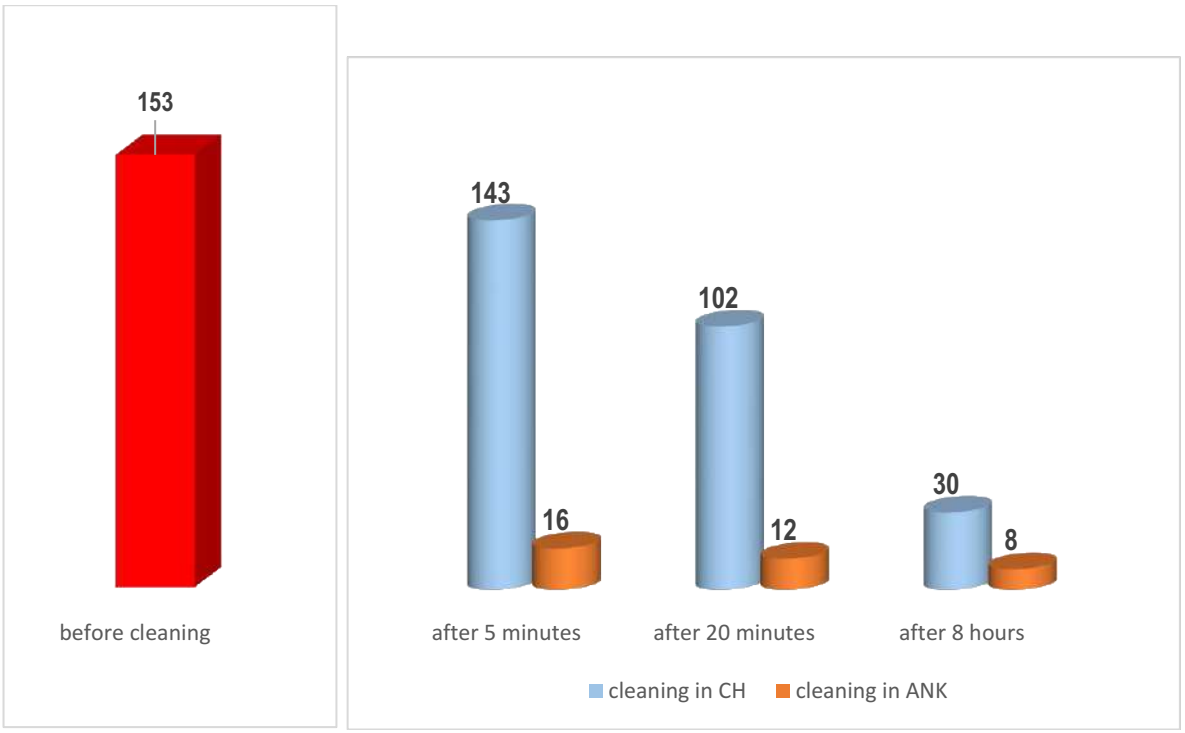


Figure 3.16 - Actinobacteria content before and after cleaning

Note: - differences are statistically significant in pairwise comparison of data after 5 minutes of cleaning in CH and Anolit, after 20 minutes of cleaning in CH and Anolit, after 8 hours of cleaning in CH and Anolit at $p < 0.05$

Before cleaning of dentures 135±11.0 (105 cells/gram) cocci and bacilli were detected, after 5 minutes exposure in CH 0.05% solution the number of cocci and bacilli decreased to 107±8.0 (105 cells/gram) (p=0.00), and after 5 minutes exposure in "Anolit ANK SUPER "ANK" solution the number of cocci and bacilli decreased to 15±5.0 (105 cells/gram) (p=0.03). After 20 minutes exposure in 0.05% CH solution, the number of cocci and bacilli decreased to 56±4.0 (105 cells/gram) (p=0.00), and after 20 minutes exposure in "Anolit ANK SUPER "ANK" solution, the number of cocci and bacilli decreased to 8±3.0 (105 cells/gram) (p=0, 007)After 8-hour exposure in 0.05% CH solution, the number of cocci and bacilli decreased to 12±2.0 (105 cells/gram) (p=0.00), and after 8-hour exposure in "Anolit ANK SUPER "ANK" solution, the number of cocci and bacilli decreased to 3±3.0 (105 cells/gram) (p=0.009). The data are presented in Figure 3.17.

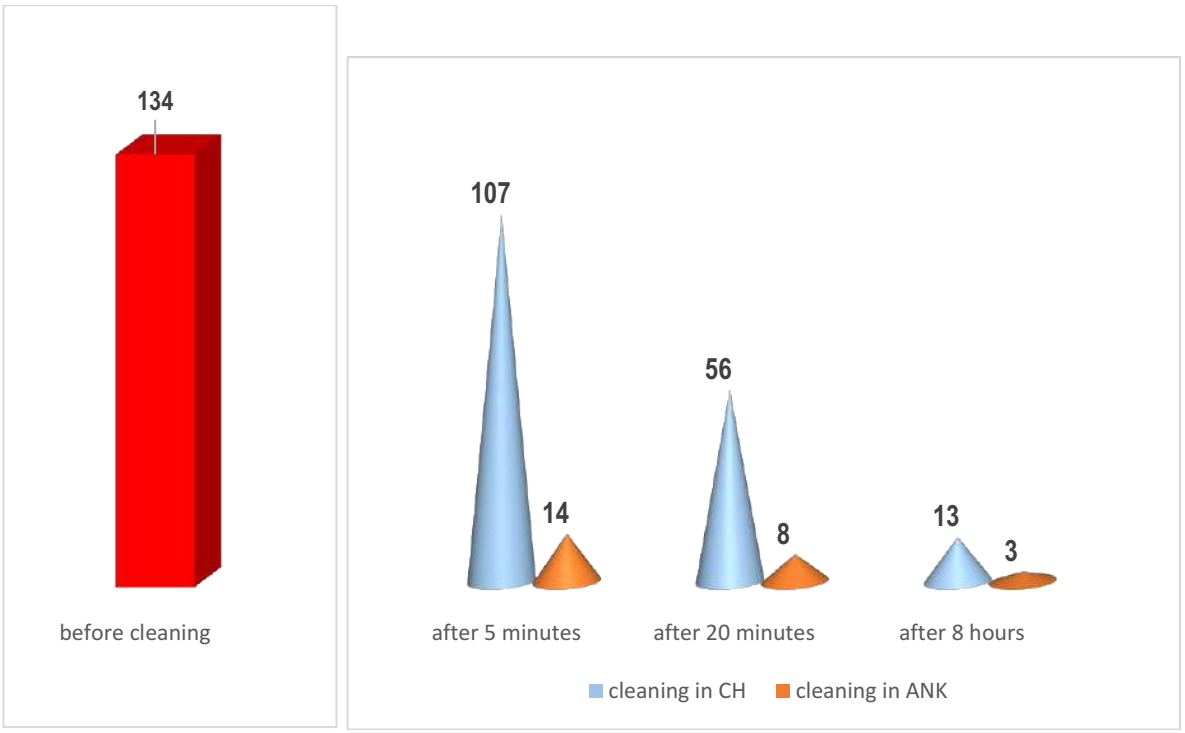


Figure 3.17 - Cocci and bacilli content before and after cleaning

Note: - differences are statistically significant in pairwise comparison of data after 5 minutes of cleaning in CH and Anolit, after 20 minutes of cleaning in CH and Anolit, after 8 hours of cleaning in CH and Anolit at $p < 0.05$.

The results of the study showed that the use of CH 0.05% and "Anolit ANK SUPER" solution for disinfection of removable acrylic dentures reduces the number of microorganisms on their surface. The optimum time of immersion of dentures in solutions "Anolit ANK SUPER" and CH according to the practical principle of application was 20 minutes, which is 1.4 times more effective according to the functional principle than immersion of dentures in DS for 5 minutes.

3.7. Development of algorithms for cleaning dentures

Based on the study of the surface of dentures and evaluation of the effectiveness of the cleaning methods used, we have developed a "Device for cleaning removable laminar dentures" (registration number 2025101283 from 22.01.25). The "device for cleaning removable dentures" is a cover that the patient can put on the toothbrush (handle or

working part) by himself. This case has the following parameters: length -10 cm, the first layer is made of foam base 0.3-0.5 cm thick and 1-2 cm in diameter, and the outer layer is made of microfiber 0.5 cm thick and 2-4 cm in diameter.

To solve the problems of the study, we hypothesized that the quality of denture cleaning would be improved by combining the use of the solution “Anolit ANK SUPER” and “Device for cleaning removable laminar dentures”. Four variations of denture cleaning protocols were developed to confirm the hypothesis:

1) Complete removable acrylic dentures were immersed in a container of CH solution without mechanical cleaning for 20 minutes 2 times a day. The volume of the container should allow complete immersion of the dentures.

2) Complete removable acrylic dentures were cleaned daily with a “Device for cleaning removable laminar dentures” soaked in CH solution for 3 minutes 2 times a day.

3) Complete removable acrylic dentures were immersed in a container with “Anolit ANK SUPER” solution without mechanical cleaning for 20 minutes 2 times a day. The volume of the container should allow complete immersion of the dentures.

4) Complete removable acrylic dentures were cleaned daily with a “Cleaning device for removable laminar dentures” soaked in “Anolit ANK SUPER” solution for 3 minutes 2 times a day.

CHAPTER 4. CLINICAL STUDIES

All patients were examined prior to prosthodontic cleaning. On examination, the oral cavity was free of inflammatory diseases. All patients had complete absence of teeth, atrophy of the maxillary alveolar ridge of the 1st degree and alveolar ridge of the mandible of the 2nd degree, thickness of the mucosa and alveolar ridges of the upper and lower jaw not less than 2-3 mm, mobility of the mucosa of the 1st type according to Keller, absence of allergic reactions to the components of the material from which the denture was made.

Patients N=60 (100%) were randomly divided into 2 groups (control and main) of n=30 people each. In each group 60 dentures were studied. Patients in the control group were divided into the CH subgroup n=15 and the CH+D subgroup n=15. Patients in the main group were divided into ANK subgroup n=15 and ANK+D subgroup n=15. Patients cleaned with dentures daily 2 times a day (morning/evening): in the CH subgroup immersed for 20 minutes in chlorhexidine digluconate 0.05% solution; in the CH +D subgroup cleaned the dentures with a “Device for cleaning removable laminar dentures” moistened with CH solution for 3 minutes; in the ANK subgroup, the dentures were immersed for 20 minutes in “Anolit ANK SUPER” solution; in the ANK+D subgroup, the dentures were cleaned with “Device for cleaning removable laminar dentures” soaked in “Anolit ANK SUPER” solution for 3 minutes.

4.1. Results of the Ulitovsky-Leontiev cleanliness assessment of new dentures

The results of the DC evaluation of new dentures before the study showed that the Ulitovsky-Leontiev index was “high” (1.0 point) according to the scoring and rating system. DC were evaluated after 3, 6 months, and 1 year. The data are presented in Tables 4.1-4.4 and Figure 4.1.

In the CH subgroup after 3 months, visual assessment of the DC index recorded a “high” level of cleanliness - 1.1±0.3 points (p=0.000000). After 6 months, visual assessment by DC index showed a “high” result, the values corresponded to 1.3±0.4 points (p=0.000043). After 1 year, the DC index corresponded to a “high” level, with values corresponding to 1.3±0.4 points (p=0.000000). The data are presented in Table 4.1.

Table 4.1 - Values of the DC index in the CH subgroup

Period of use	DC values	P
3 months	1,1±0,3	0,000000
6 months	1,3±0,4	0,000043
1 year	1,3±0,4	0,000000

Differences are statistically significant in pairwise comparison of values at $p<0.017$ (total three comparisons 0.05/3=0.0167 rounded p=0.017).

In the CH +D subgroup after 3 months, the DC index was recorded at a “high” level of cleanliness of -1.1±0.5 points on visual assessment (p=0.000000). After 6 months the DC index was at 1,2±0,3 points (p=0,000000), which corresponded to the value “high”. After 1 year of denture cleaning with sponge soaked in CH solution, the DC index was recorded at “high” - 1,2±0,2 points (p=0,008093). The data are presented in table 4.2.

Table 4.2 - DC index values in the CH +D subgroup

Period of use	DC values	P
3 months	1,1±0,5	0,000000
6 months	1,2±0,3	0,000000
1 year	1,2±0,2	0,008093

Differences were statistically significant when values were compared pairwise at $p<0.017$ (total three comparisons 0.05/3=0.0167 rounded p=0.017).

In the ANK subgroup, after 3 months of denture cleaning in “Anolit ANK SUPER” solution, visual assessment of the DC index showed a “high” level of 1.1±0.4 points (p=0.001742). After 6 months, the DC index corresponded to a “high” level of 1.2±0.4 points (p=0.122589). After 1 year, the DC index remained at the level of “high” - 1.2±0.3 points (p=0.000145). The data are presented in table 4.3.

Table 4.3- PE index values in the ANK subgroup

Period of use	DC values	P
3 months	1,1±0,4	0,001742
6 months	1,2±0,4	0,122589
1 year	1,2±0,3	0,000145

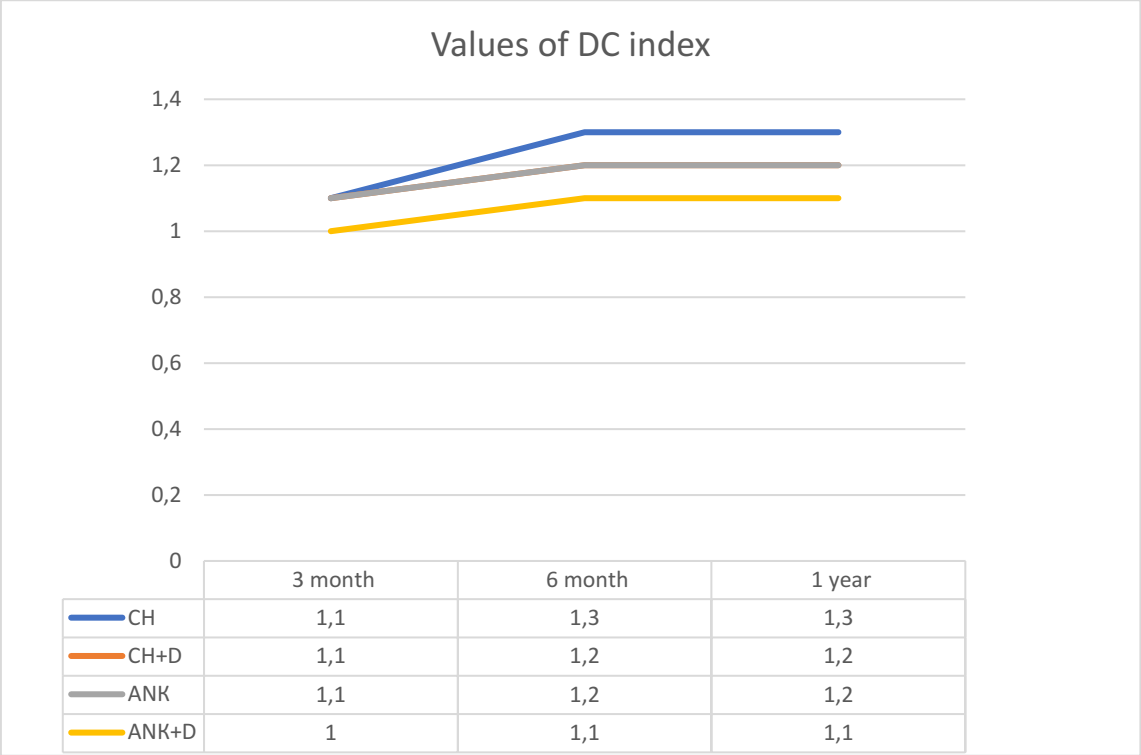
Differences are statistically significant in pairwise comparison of values between data of 3 months and 1 year, 6 months and 1 year at $p<0.017$. There were no differences between 3 and 6 months (total of three comparisons 0.05/3=0.0167 rounded p=0.017).

In the ANK+D subgroup, after 3 months of cleaning, the DC index was recorded at a “high” level of cleanliness, 1.0±0.5 points (p=0.083422). After 6 months, the DC index corresponded to a “high” level of cleanliness - 1.0±0.3 points (p =0.093769). After 1 year, the DC index of the denture was recorded at a “high” level of cleanliness - 1.1±0.2 points (p=0.198892). The data are presented in Table 4.4.

Table 4.4 - DC index values in the ANK+D subgroup

Period of use	DC values	P
3 months	1,0±0,5	0,198892
6 months	1,1±0,3	0,093769
1 year	1,1±0,2	0,083422

There were no differences statistically significant in pairwise comparison of values between data at 3 months and 1 year, 6 months and 1 year, $p>0.017$ (total of three comparisons 0.05/3=0.0167 rounded p=0.017).



	3 month	6 month	1 year
CH	1,1	1,3	1,3
CH+D	1,1	1,2	1,2
ANK	1,1	1,2	1,2
ANK+D	1	1,1	1,1

Figure 4.1 - DC index values after 3 months, 6 months and 1 year

4.2. Cleanliness results of dentures fabricated 3.5 and more years ago according to the Ulitovsky-Leontiev technique

The cleanliness of previously fabricated dentures in each group was evaluated using the Ulitovsky-Leontiev technique after 3, 6 months and 1 year. The data are presented in Tables 4.5-4.8 and Figure 4.2.

The results showed that in the CH subgroup before the study, the DC index was recorded at a “poor” level of cleanliness, 4.4±0.3 points (p=0.207837). At cleaning of dentures by soaking daily for 20 minutes in CH solution after 3 months at visual estimation the DC index was fixed on “bad” level of cleanliness - 4,1±0,3 points (p=0,000000). After 6 months, visual assessment by DC index showed a “satisfactory” result, the values corresponded to 3.9±0.4 points (p=0.000043). After 1 year, the DC index corresponded to a “satisfactory” level - 3.6±0.4 points (p=0.000000). The data are presented in Table 4.5.

Table 4.5 - DC index values in the CH subgroup

Period of use	DC values	P
Before cleaning	4,4±0,3*	0,207837
3 months	4,1±0,3	0,000000
6 months	3,9±0,4	0,000043
1 year	3,6±0,4	0,000000

Differences are statistically significant in pairwise comparison of values at p<0.017 (total of three comparisons 0.05/3=0.0167 rounded p=0.017)

Note: *- no statistically significant differences before cleaning p=0.207837> 0.008.

In the subgroup of CH+D before the study, the DC index was recorded at a “poor” level of cleanliness - 4.5±0.5 points (p=0.207837). At cleaning of dentures with the “Device for cleaning removable laminar dentures” wetted in the solution of CH after 3 months at visual estimation the DC index was fixed at the “good” level of cleanliness - 2,7±0,2 points (p=0,000000). In 6 months, the DC index was at the mark of 2,1±0,3 points (p=0,000000), that corresponded to the value “good”. After 1 year of cleaning of dentures “Device for cleaning removable laminar dentures”, wetted in CH solution, the DC index was recorded at “high” - 1.9±0.5 points (p=0.008093). The data are presented in Table 4.6.

Table 4.6 - DC index values in the CH +C subgroup

Period of use	DC values	P
Before cleaning	4,5±0,5*	0,207837
3 months	2,7±0,2	0,000000
6 months	2,1±0,3	0,000000
1 year	1,9±0,5	0,008093

Differences are statistically significant in pairwise comparison of values at p<0.017 (total of three comparisons 0.05/3=0.0167 rounded p=0.017).

Note: *- no statistically significant differences before cleaning p=0.207837> 0.008.

In the ANK subgroup before the study, the DC index was recorded at a “poor” level of cleanliness - 4.5±0.5 points (p=0.207837). After 3 months of cleaning of dentures by soaking for 20 minutes daily in “Anolit ANK SUPER” solution, visual assessment of the DC index showed a “satisfactory” level of 3±0.4 points (p=0.001742). After 6 months, the DC index corresponded to a “good” level of 2.4±0.4 points (p=0.122589). After 1 year, the DC index remained at the level of “good” - 2.1±0.3 points (p=0.000145). The data are presented in table 4.7.

Table 4.7- DC index values in the ANK subgroup

Period of use	**DC values**	**P**
Before cleaning	4,5±0,5*	0,207837
3 months	3±0,4	0,001742
6 months	2,4±0,4	0,122589
1 year	2,1±0,3	0,000145

Differences are statistically significant in pairwise comparison of values between data of 3 months and 1 year, 6 months and 1 year at p<0.017. There are no differences between 3 and 6 months (total of three comparisons 0.05/3=0.0167 rounded p=0.017).
Note: *- no statistically significant differences before cleaning p=0.207837> 0.008.

In the ANK+D subgroup before the study, the DC index was recorded at a “poor” level of cleanliness - 4.6±0.5 points (p=.207837). After 3 months of denture cleaning with the device wetted with “Anolit ANK Super” solution the DC index was recorded at “good” level of cleanliness - 2±0,5 points (p=0,083422). After 6 months the DC index corresponded to a “high” level of cleanliness - 1,6±0,3 points (=0,093769p). After 1 year, the DC index of the denture was recorded at a “high” level of cleanliness - 1.5±0.2 points (p=0.198892). The data are presented in Table 4.8.

Table 4.8 - DC index values in the ANK+D subgroup

Period of use	DC values	P
Before cleaning	4,6±0,5*	0,207837
3 months	2±0,5	0,198892
6 months	1,6±0,3	0,093769
1 year	1,5±0,2	0,198892

There are no statistically significant differences in pairwise comparison of values between data of 3 months and 1 year, 6 months and 1 year, p>0.017 (total three comparisons 0.05/3=0.0167 rounded p=0.017).

Note: *- no statistically significant differences before cleaning p=0.207837> 0.008.

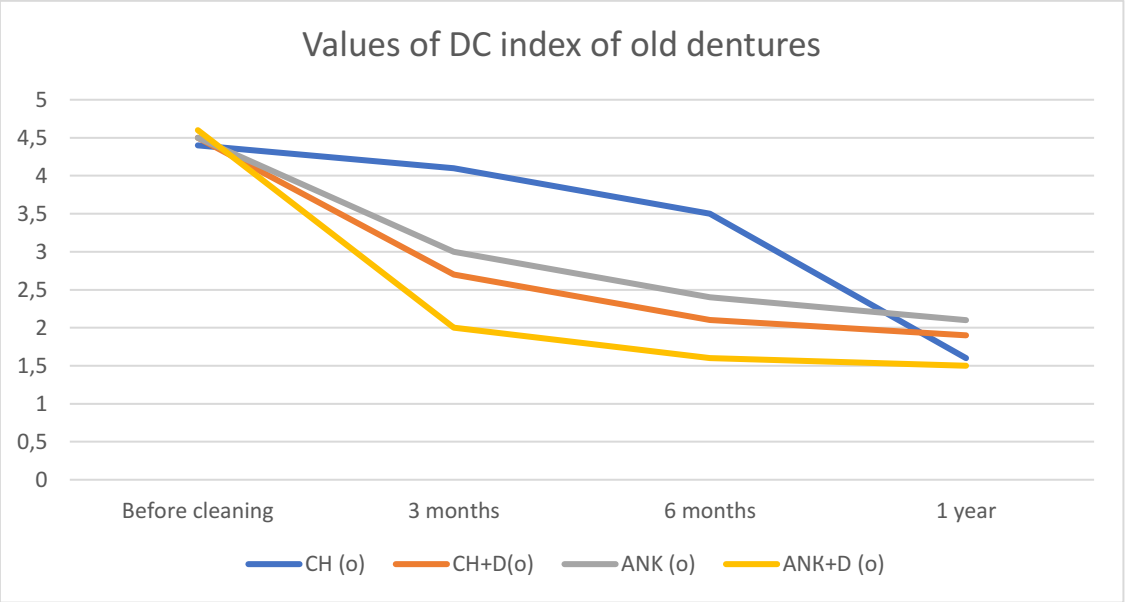


Figure 4.2 - PE index values after 3 months, 6 months and 1 year

4.3. Results of quantitative and qualitative assessment of microflora on the surface of new dentures by mass-spectrometry

4.3.1. Fungi and yeasts

The results showed that fungi and yeasts were isolated from the surface of the dentures: microscopic fungi ((producing campesterol), a toxin that leads to the development of mycosis), Candida spp.

In the CH subgroup, after 1 month, the number of fungi and yeast 160±24 (105 cells/gram) (p=0.000074), after 3 months - 160±24 (105 cells/gram) (p=0.000074), after 6 months - 170±24 (105 cells/gram) (p=0.000074), after 1 year - 170.0±24.0 (105 cells/gram) (p=0.000074).

In the CH+D subgroup, the number of fungi and yeasts after 1 month was 157±24.0 (105 cells/gram) (p=0.000074), after 3 months was 157±24 (105 cells/gram) (p=0.000074), after 6 months was 165±24 (105 cells/gram) (p=0.000074), and after 1 year was 165±24 (105 cells/gram) (p=0.000074).

In the ANK subgroup, the number of fungi and yeasts after 1 month 158±24 (105 cells/gram) (p=0.000074), after 3 months - 158.0±24.0 (105 cells/gram) (p=0.000074), after 6 months - 168±24 (105 cells/gram) (p=0.000074, after 1 year - 168±24 (105 cells/gram) (p=0.000074).

In the ANK+D subgroup, the number of fungi and yeasts was 153±24 (105 cells/gram) after 1 month (p=0.000074), 153±24 (105 cells/gram) after 3 months (p=0.000074), 160±24 (105 cells/gram) after 6 months (p=0.000074), and 160±24 (105 cells/gram) after 1 year (p=0.000074). The data are presented in Table 4.9.

Table 4.9 - Average values of fungi and yeast content

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	160±24#	157±24#*	158±24#	153±24#*	0,000074
After 3 months	160±24#	157±24#*	158±24#	153±24#*	0,000074
After 6 months	170±24#	165±24#*	168±24#	160±24#*	0,000074
After 1 year	170±24#	165±24#*	168±24#	160±24#*	0,000074

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH+C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

In the CH subgroup, the number of microscopic fungi producing campesterol after 1 month was 30±7 (105 cells/gram), after 3 months was 30±7 (105 cells/gram) (p=0.004409), after 6 months was 32±7 (105 cells/gram) (p=0.004409), and after 1 year was 32±7 (105 cells/gram) (p=0.004409).

In the CH+D subgroup, the number of microscopic fungi producing campesterol after 1 month was 27±2 (105 cells/gram) (p=0.004409), after 3 months - 27±2 (105 cells/gram) (p=0.004409), after 6 months - 29±2 (105 cells/gram) (p=0.004409), after 1 year - 29±2 (105 cells/gram) (p=0.004409).

In the ANK subgroup, the number of microscopic fungi producing campesterol after 1 month was 28±3 (105 cells/gram) (p=0.004409), after 3 months was 28±3 (105 cells/gram) (p=0.004409), after 6 months was 30±3 (105 cells/gram) (p=0.004409), and after 1 year was 30±3 (105 cells/gram) (p=0.004409).

In the ANK+D subgroup, the number of microscopic fungi producing campesterol after 1 month was 20±2 (105 cells/gram) (p=0.004409), after 3 months - 20±2 (105

cells/gram) (p=0.004409), after 6 months - 21±2 (105 cells/gram) (p=0.004409), after 1 year - 21±2 (105 cells/gram) (p=0.004409). The data are presented in Table 4.10.

Table 4.10 - Average values of content of microscopic fungi synthesizing campesterol

Time shedule	**CH**	**CH+D**	**ANK**	**ANK+D**	**p**
After 1 month	30±7#	27±2*#	28±3#	20±2*#	0,004409
After 3 months	30±7#	27±2*#	28±3#	20±2*#	0,004409
After 6 months	32±7#	29±2*#	30±3#	21±2*#	0,004409
After 1 year	32±7#	29±2*#	30±3#	21±2*#	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH+C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

The number of Candida spp. microorganisms in the CH subgroup after 3 months 39±10 (105 cells/gram) (p=0.000074), after 6 months 40±10 (105 cells/gram) (p=0.000074), after 1 year 40±10 (105 cells/gram) (p=0.000074).

In the CH+D subgroup, the number of Candida spp. microorganisms after 1 month was 33±5 (105 cells/gram) (p=0.0000740), after 3 months was 33±5 (105 cells/gram) (p=0.000074), after 6 months was 35±5 (105 cells/gram) (p=0.000074), and after 1 year was 35±5 (105 cells/gram) (p=0.000074).

In the ANK subgroup, the number of Candida spp microorganisms after 1 month was 37±3 (105 cells/gram) (p=0.000074), after 3 months was 37±3 (105 cells/gram) (p=0.000074), after 6 months was 38±3 (105 cells/gram) (p=0.000074), after 1 year was 38±3 (105 cells/gram) (p=0.000074).

In the ANK+D subgroup, the number of Candida spp microorganisms after 1 month was 30±1 (105 cells/gram) (p=0.000074), after 3 months was 30±1 (105 cells/gram) (p=0.000074), after 6 months was 31±1 (105 cells/gram) (p=0.000074), and after 1 year was 31±1 (105 cells/gram) (p=0.000074). The data are presented in table 4.11.

Table 4.11 - Level of microorganisms Candida spp.

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	39±10#	33±5#*	37±3#	30±1#*	0,000074
After 3 months	39±10#	33±5#*	37±3#	30±1#*	0,000074
After 6 months	40±10#	35±5#*	38±3#	31±1#*	0,000074
After 1 year	40±10#	35±5#*	38±3#	31±1#*	0,000074

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

4.3.2. Anaerobic microorganisms

Anaerobic microorganisms were detected on all dentures: opportunistic Gram-positive anaerobic spore-forming spore-forming cocci Peptostreptococcus anaerobius. Peptostreptococcus anaerobius are not representatives of the normal microflora of the human body. These microorganisms are mainly localized in the oral cavity, colon and vagina of healthy women; anaerobic, immobile, thin, spore-forming, Gram-positive bacteria Clostridium ramosum; Clostridium perfringens, this type of microorganism is the causative agent of various human food poisoning and gas gangrene. Clostridium perfringens produces at least 13 toxins known to science. The targets for the 13 toxins are

biological membranes in various tissues. Tissue damage is caused through the action of enzymatic processes. These enzymatic processes are responsible for catalyzing hydrolytic cleavage and disruption of cell permeability, followed by tissue edema and autolysis characteristic of gas gangrene. Therefore, solutions for denture disinfection must have an effective antibacterial effect against Clostridium perfringens.

In the CH subgroup, the number of anaerobic microorganisms after 1 month -75±9 (105 cells/gram) (p=0.004409), after 3 months - 75±9 (105 cells/gram) (p=0.004409), after 6 months - 76±9 (105 cells/gram) (p=0.004409), after 1 year - 76±9 (105 cells/gram) (p=0.004409).

In the CH +D subgroup, the number of anaerobic microorganisms after 1 month was 73±4 (105 cells/gram) (p=0.004409), after 3 months was 73±4 (105 cells/gram) (p=0.004409), after 6 months was 74±4 (105 cells/gram) (p=0.004409), and after 1 year was 74±4 (105 cells/gram) (p=0.004409).

In the ANK subgroup, the number of anaerobic microorganisms after 1 month was 74±4 (105 cells/gram) (p=0.004409), after 3 months was 74±4 (105 cells/gram) (p=0.004409), after 6 months was 75±4 (105 cells/gram) (p=0.004409), and after 1 year was 75±4 (105 cells/gram) (p=0.004409).

In the ANK+D subgroup, the number of anaerobic microorganisms after 1 month was 70±4 (105 cells/gram) (p=0.004409), after 3 months was 70±4 (105 cells/gram) (p=0.004409), after 6 months was 72±4 (105 cells/gram) (p=0.004409), and after 1 year was 72±4 (105 cells/gram) (p=0.004409). The data are presented in Table 4.12.

Table 4.12. - Average values of anaerobic microorganisms content

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	75±9#	73±4#*	74±4#	70±4#*	0,004409
After 3 months	75±9#	73±4#*	74±4#	70±4#*	0,004409
After 6 months	76±9#	74±4#*	75±4#	72±4#*	0,004409
After 1 year	76±9#	74±4#*	75±4#	72±4#*	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

In the CH subgroup, the number of Peptostreptococcus anaerobius after 1 month 40±2 (105 cells/gram) (p=0.004409), after 3 months 40±2 (105 cells/gram) (p=0.004409), after 6 months 42±2 (105 cells/gram) (p=0.004409), after 1 year 42±2 (105 cells/gram) (p=0.004409).

In the CH +D subgroup, the number of Peptostreptococcus anaerobius after 1 month was 35±2 (105 cells/gram) (p=0.004409), after 3 months was 35±2 (105 cells/gram) (p=0.004409), after 6 months was 37±2 (105 cells/gram) (p=0.004409), and after 1 year was 37±2 (105 cells/gram) (p=0.004409).

In the ANK subgroup, the number of Peptostreptococcus anaerobius was 37±2 (105 cells/gram) after 1 month (p=0.004409), 37±2 (105 cells/gram) after 3 months (p=0.004409), 39±2 (105 cells/gram) after 6 months (p=0.004409), and 39±2 (105 cells/gram) after 1 year (p=0.004409).

In the ANK+D subgroup, the number of Peptostreptococcus anaerobius was 32±2 (105 cells/gram) after 1 month (p=0.004409), 32±2 (105 cells/gram) after 3 months

(p=0.004409), 33±2 (105 cells/gram) after 6 months (p=0.004409), and 33±2 (105 cells/gram) after 1 year (p=0.004409). The data are presented in Table 4.13.

Table 4.13. - Peptostreptococcus anaerobius content level

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	40±2#	35±2#*	37±2 #	32±2#*	0,004409
After 3 months	40±2#	35±2#*	37±2 #	32±2#*	0,004409
After 6 months	42±2#	37±2#*	39±2 #	33±2#*	0,004409
After 1 year	42±2#	37±2#*	39±2#	33±2#*	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

In the CH subgroup, the number of Clostridium ramosum after 1 month 18±1 (105 cells/gram) (p=0.004409), after 3 months 18±1 (105 cells/gram) (p=0.004409), after 6 months 19±1 (105 cells/gram) (p=0.004409), after 1 year 19±1 (105 cells/gram) (p=0.004409).

In the CH+D subgroup, the number of Clostridium ramosum after 1 month was 15±1 (105 cells/gram) (p=0.004409), after 3 months was 15±1 (105 cells/gram) (p=0.004409), after 6 months was 16±1 (105 cells/gram) (p=0.004409), and after 1 year was 16±1 (105 cells/gram) (p=0.004409).

In the ANK subgroup, Clostridium ramosum counts were 16±1 (105 cells/gram) after 1 month (p=0.004409), 16±1 (105 cells/gram) after 3 months (p=0.004409), 17±1 (105 cells/gram) after 6 months (p=0.004409), and 17±1 (105 cells/gram) after 1 year (p=0.004409).

In the ANK+D subgroup, the number of Clostridium ramosum after 1 month was 10±1 (105 cells/gram) (p=0.004409), after 3 months was 10±1 (105 cells/gram) (p=0.004409), after 6 months was 11±1 (105 cells/gram) (p=0.004409), and after 1 year was 11±1 (105 cells/gram) (p=0.004409). The data are presented in table 4.14.

Table 4.14 - Clostridium ramosum levels

Time shedule	**CH**	**CH+D**	**ANK**	**ANK+D**	**p**
After 1 month	18±1	15±1	16±1	10±1	0,004409
After 3 months	18±1	15±1	16±1	10±1	0,004409
After 6 months	19±1	16±1	17±1	11±1	0,004409
After 1 year	19±1	16±1	17±1	11±1	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

In the CH subgroup, the number of Clostridium perfringens after 1 month was 14±1 (105 cells/gram) (p=0.004409), after 3 months was 14±1 (105 cells/gram) (p=0.004409), after 6 months was 15±1 (105 cells/gram) (p=0.004409), and after 1 year was 15±1 (105 cells/gram) (p=0.004409).

In the CH +D subgroup, the number of Clostridium perfringens after 1 month was 10±1 (105 cells/gram) (p=0.004409), after 3 months was 10±1 (105 cells/gram) (p=0.004409), after 6 months was 12±1 (105 cells/gram) (p=0.004409), and after 1 year was 12±1 (105 cells/gram) (p=0.004409).

In the ANK subgroup, the number of Clostridium perfringens after 1 month was 12±1 (105 cells/gram) (p=0.004409), after 3 months was 12±1 (105 cells/gram) (p=0.004409), after 6 months was 13±1 (105 cells/gram) (p=0.004409), and after 1 year was 13±1 (105 cells/gram) (p=0.004409).

In the ANK+D subgroup, the number of Clostridium perfringens after 1 month was 8±1 (105 cells/gram) (p=0.004409), after 3 months was 8±1 (105 cells/gram) (p=0.004409), after 6 months was 10±1 (105 cells/gram) (p=0.004409), and after 1 year was 10±1 (105 cells/gram) (p=0.004409). The data are presented in table 4.15.

Table 4.15 - Clostridium perfringens levels

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	14±1 #	10±1 #	12±1 #	8±1#	0,004409
After 3 months	14±1 #	10±1 #	12±1#	8±1 #	0,004409
After 6 months	15±1#	12±1 #	13±1 #	10±1 #	0,004409
After 1 year	15±1 #	12±1#	13±1#	10±1 #	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

4.3.3. Actinobacteria

The presence of actinobacteria was detected on all the dentures. Streptomyces microorganisms (Latin Streptomyces) is a genus of actinobacteria in the family Streptomycetaceae of the order Streptomycetales. This genus of microorganisms is the largest of the entire family Streptomycetaceae. This family includes 668 species. Streptomyces spp. is capable of causing human bacteremia; Corynebacterium (Latin:

Corynebacterium) is a genus of Gram-positive bacilliform bacteria. Most species of Corynebacterium existing in nature are not pathogenic to humans, but there are a number of exceptions. By the quantitative composition of Corynebacterium spp. it is possible to assess changes in homeostatic balance.

In the CH subgroup, the number of actinobacteria after 1 month was 13±1 (105 cells/gram) (p=0.004409), after 3 months was 13±1 (105 cells/gram) (p=0.004409), after 6 months was 14±1 (105 cells/gram) (p=0.004409), and after 1 year was 14±1 (105 cells/gram) (p=0.004409).

In the CH +D subgroup, the number of actinobacteria after 1 month was 11±1 (105 cells/gram) (p=0.004409), after 3 months was 11±1 (105 cells/gram) (p=0.004409), after 6 months was 12±1 (105 cells/gram) (p=0.004409), and after 1 year was 12±1 (105 cells/gram) (p=0.004409).

In the ANK subgroup, the number of actinobacteria was 12±1 (105 cells/gram) after 1 month (p=0.004409), 12±1 (105 cells/gram) after 3 months (p=0.004409), 13±1 (105 cells/gram) after 6 months (p=0.004409), and 13±1 (105 cells/gram) after 1 year (p=0.004409).

In the ANK+D subgroup, the number of actinobacteria after 1 month was 9±1 (105 cells/gram) (p=0.004409), after 3 months was 9±1 (105 cells/gram) (p=0.004409), after 6 months was 9±1 (105 cells/gram) (p=0.004409), and after 1 year was 9±1 (105 cells/gram) (p=0.004409). The data are presented in table 4.16.

Table 4.16 - Mean values of actinobacteria content

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	13±1 #*	11±1 #	12±1#	9±1 #	0,004409
After 3 months	13±1 #*	11±1#	12±1 #	9±1 #	0,004409
After 6 months	14±1 #*	12±1#	13±1 #	10±1 #	0,004409
After 1 year	14±1 #*	12±1 #	13±1 #	10±1 #	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

In the CH subgroup, the number of streptomycetes after 1 month 7±1 (105 cells/gram) (p=0.004409), after 3 months 7±1 (105 cells/gram) (p=0.004409), after 6 months 8±1 (105 cells/gram) (p=0.004409), after 1 year 8±1 (105 cells/gram) (p=0.004409).

In the CH +D subgroup, the number of streptomycetes after 1 month was 5±1 (105 cells/gram) (p=0.004409), after 3 months was 5±1 (105 cells/gram) (p=0.004409), after 6 months was 6±1 (105 cells/gram) (p=0.004409), and after 1 year was 6±1 (105 cells/gram) (p=0.004409).

In the ANK subgroup, the number of streptomycetes after 1 month was 6±1 (105 cells/gram) (p=0.004409), after 3 months was 6±1 (105 cells/gram) (p=0.004409), after 6 months was 7±1 (105 cells/gram) (p=0.004409), and after 1 year was 7±1 (105 cells/gram) (p=0.004409).

In the ANK+D subgroup, the number of streptomycetes after 1 month was 3±1 (105 cells/gram) (p=0.004409), after 3 months was 3±1 (105 cells/gram) (p=0.004409), after

6 months was 4±1 (105 cells/gram) (p=0.004409), and after 1 year was 4±1 (105 cells/gram) (p=0.004409). The data are presented in Table 4.17.

Table 4.17 - Mean values of the content of Streptomyces spp.

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	7±1#	5±1 #*	6±1 #*	3±1 #*	0,004409
After 3 months	7±1#	5±1 #*	6±1 #*	3±1 #*	0,004409
After 6 months	8±1#	6±1#*	7±1 #*	4±1 #*	0,004409
After 1 year	8±1#	6±1 #*	7±1 #*	4±1 #*	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1,3,6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1,3,6 months and 1 year at $p<0.0083$

In the CH subgroup, the number of Corynebacterium spp. after 1 month was 6±1 (105 cells/gram) (p=0.004409), after 3 months was 6±1 (105 cells/gram) (p=0.004409), after 6 months was 7±1 (105 cells/gram) (p=0.004409), and after 1 year was 7±1 (105 cells/gram) (p=0.004409).

In the CH +D subgroup, the number of Corynebacterium spp. after 1 month was 4±1 (105 cells/gram) (p=0.004409), after 3 months was 4±1 (105 cells/gram) (p=0.004409), after 6 months was 5±1 (105 cells/gram) (p=0.004409), and after 1 year was 5±1 (105 cells/gram) (p=0.004409).

In the ANK subgroup, the number of Corynebacterium spp. was 5±1 (105 cells/gram) after 1 month (p=0.004409), 5±1 (105 cells/gram) after 3 months (p=0.004409), 6±1 (105 cells/gram) after 6 months (p=0.004409), and 6±1 (105 cells/gram) after 1 year (p=0.004409).

In the ANK+D subgroup, the number of Corynebacterium spp. after 1 month was 2±1 (105 cells/gram) (p=0.004409), after 3 months was 2±1 (105 cells/gram) (p=0.004409), after 6 months was 3±1 (105 cells/gram) (p=0.004409), and after 1 year was 3±1 (105 cells/gram) (p=0.004409). The data are presented in table 4.18.

Table 4.18 - Level of Corynebacterium spp.

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	6±1 #	4±1 #*	5±1# *	2±1 #*	0,004409
After 3 months	6±1 #	4±1 #*	5±1 #*	2±1 #*	0,004409
After 6 months	7±1 #	5±1 #*	6±1 #*	3±1 #*	0,004409
After 1 year	7±1 #	5±1# *	6±1 #*	3±1 #*	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1,3,6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1,3,6 months and 1 year at $p<0.0083$

4.3.4. Cocci and bacilli

The presence of cocci and bacilli was also detected on the surface of all dentures: Bacillus megaterium is a bacilliform, Gram-positive, mostly aerobic, spore-forming bacterium that inhabits a wide variety of habitats. Bacillus megaterium reaches a length of about 100 μm and a diameter of 0.1 μm, which is quite large for bacteria. Cells are often found in pairs and chains, where cells are connected by polysaccharides on cell walls.

In the CH subgroup, the number of cocci and bacilli after 1 month was 48±5 (105 cells/gram) (p=0.004409), after 3 months was 48±5 (105 cells/gram) (p=0.004409), after

6 months was 49±5 (105 cells/gram) (p=0.004409), and after 1 year was 49±5 (105 cells/gram) (p=0.004409).

In the CH +D subgroup, the number of cocci and bacilli after 1 month was 43±5 (105 cells/gram) (p=0.004409), after 3 months was 45±5 (105 cells/gram) (p=0.004409), after 6 months was 47±5 (105 cells/gram) (p=0.004409), and after 1 year was 47±5 (105 cells/gram) (p=0.004409).

In the ANK subgroup, the number of cocci and bacilli after 1 month was 46±5 (105 cells/gram) (p=0.004409), after 3 months - 46±5 (105 cells/gram) (p=0.004409), after 6 months - 48±5 (105 cells/gram) (p=0.004409), after 1 year - 48±5 (105 cells/gram) (p=0.004409).

In the ANK+D subgroup, the number of cocci and bacilli after 1 month was 43±5 (105 cells/gram) (p=0.004409), after 3 months was 43±5 (105 cells/gram) (p=0.004409), after 6 months was 44±5 (105 cells/gram) (p=0.004409), and after 1 year was 44±5 (105 cells/gram) (p=0.004409). The data are presented in table 4.19.

Table 4.19 - Cocci and bacilli levels

Time shedule	**CH**	**CH+D**	**ANK**	**ANK+D**	**p**
After 1 month	48±5#	45±5 #*	46±5# *	43±5 #*	0,004409
After 3 months	48±5#	45±5 #*	46±5 #*	43±5 #*	0,004409
After 6 months	49±5#	47±5 #*	48±5 #*	44±5 #*	0,004409
After 1 year	49±5#	47±5# *	48±5 #*	44±5# *	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

In the CH subgroup, the number of Bacillus megaterium after 1 month was 17±3 (105 cells/gram) (p=0.004409), after 3 months was 17±3 (105 cells/gram) (p=0.004409), after 6 months was 20±3 (105 cells/gram) (p=0.004409), and after 1 year was 20±3 (105 cells/gram) (p=0.004409).

In the CH +D subgroup, the number of Bacillus megaterium after 1 month was 13±3 (105 cells/gram) (p=0.004409), after 3 months was 15±3 (105 cells/gram) (p=0.004409), after 6 months was 13±3 (105 cells/gram) (p=0.004409), and after 1 year was 15±3 (105 cells/gram) (p=0.004409).

In the ANK subgroup, Bacillus megaterium counts after 1 month 15±3 (105 cells/gram) (p=0.004409), after 3 months 17±3 (105 cells/gram) (p=0.004409), after 6 months 15±3 (105 cells/gram) (p=0.004409), after 1 year 17±3 (105 cells/gram) (p=0.004409).

In the ANK+D subgroup, the number of Bacillus megaterium megaterium after 1 month was 10±3 (105 cells/gram) (p=0.004409), after 3 months was 10±3 (105 cells/gram) (p=0.004409), after 6 months was 12±3 (105 cells/gram) (p=0.004409), and after 1 year was 12±3 (105 cells/gram) (p=0.004409). The data are presented in table 4.20.

Table 4.20 - Bacillus megaterium content level

Time shedule	**CH**	**CH+D**	**ANK**	**ANK+D**	**p**
After 1 month	17±3 #	13±3 #*	15±3 #	10±3 #*	0,004409
After 3 months	17±3 #	13±3 #*	15±3 #	10±3#*	0,004409
After 6 months	20±3 #	15±3 #*	17±3#	12±3#*	0,004409
After 1 year	29±3#	15±3 #*	17±3#	12±3#*	0,004409

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at p<0.0083

4.4. Results of quantitative and qualitative microflora composition assessment by mass-spectrometry on the surface of dentures with service life of 3.5 and more years

4.4.1. Fungi and yeasts

In the CH subgroup, the number of fungi and yeasts of removable dentures after 1 month was 1661±77 (105 cells/gram) (p=0.007191), up to 1568±77 (105 cells/gram) after 3 months (p=0.007191), 1234±77 (105 cells/gram) after 6 months, and 966±24 (105 cells/gram) after 1 year (p=0.007191).

In the CH +D subgroup after 1 month - 1683±77 (105 cells/gram) (p=0.007191), after 3 months - 1289±77 (105 cells/gram) (p=0.007191), after 6 months - 615±77 (105 cells/gram) (p=0.007191), after 1 year - 215±77 (105 cells/gram) (p=0.007191).

In the ANA subgroup, after 1 month - 1702.0±77 (105 cells/gram) (p=0.007191), after 3 months - up to 1374±77 (105 cells/gram) (p=0.007191), after 6 months - 876±77 (105 cells/gram) (p=0.007191), after 1 year - 455±24 (105 cells/gram) (p=0.007191).

In the ANK+D subgroup, after 1 month it was 1712.0±77 (105 cells/gram) (p=0.007191), after 3 months it was 1230±77 (105 cells/gram) (p=0.007191), after 6 months it was 549±77 (105 cells/gram) (p=0.007191), and after one year it was 157±15 (105 cells/gram) (p=0.007191). The data are presented in table 4.21.

Table 4.21 - Mean values of fungi and yeast content

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	17±3 #	13±3 #*	15±3# *	10±3 #*	0,007191
After 3 months	17±3 #	13±3 #*	15±3 #*	10±3 #*	0,007191
After 6 months	20±3 #	15±3 #*	17±3 #*	12±3 #*	0,007191
After 1 year	29±3 #	15±3# *	17±3#*	12±3 #*	0,007191

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

4.4.2. Anaerobic microorganisms

In the CH subgroup, the number of anaerobic microorganisms after 1 month was 144±9 (105 cells/gram) (p=0.007191), after 3 months was 133±9 (105 cells/gram) (p=0.007191), after 6 months was 115±9 (105 cells/gram) (p=0.007191), and after 1 year was 109±5 (105 cells/gram).

In the CH +D subgroup after 1 month - 145±10 (105 cells/gram) (p=0.007191), after 3 months - 122±9 (105 cells/gram), after 6 months - 107±9 (105 cells/gram) (p=0.007191), after 1 year - 109±3 (105 cells/gram) (p=0.007191).

In the ANK subgroup after 1 month - 147±10 (105 cells/gram) (p=0.007191), after 3 months - 130±9 (105 cells/gram) (p=0.007191), after 6 months - 112±9 (105 cells/gram) (p=0.007191), after 1 year - 109±10 (105 cells/gram).

In the ANK+D subgroup, after 1 month - 140±10 (105 cells/gram) (p=0.007191), after 3 months - 101±9 (105 cells/gram) (p=0.007191), after 6 months - 99±9 (105

cells/gram) (p=0.007191), after 1 year - 89±3 (105 cells/gram) (p=0.007191). The data are presented in table 4.2.

Table 4.22 - Content of anaerobic microorganisms

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	144±9#	145±10#*	147±10#*	140±10#*	0,007191
After 3 months	133±9#	122±9#*	130±9#*	101±9#*	0,007191
After 6 months	115±9#	107±9#*	112±9#*	99±9#*	0,007191
After 1 year	109±5#	97±3#*	109±10#*	89±3# *	0,007191

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

4.4.3. Actinobacteria

In the CH subgroup, the number of actinobacteria after 1 month was 153±30 (105 cells/gram) (p=0.007191), after 3 months was 132±30 (105 cells/gram) (p=0.007191), after 6 months was 116±30 (105 cells/gram) (p=0.007191), and after 1 year was 102±12 (105 cells/gram) (p=0.007191).

In the CH +D subgroup, 147±30 (105 cells/gram) after 1 month (p=0.007191), 78±20 (105 cells/gram) after 3 months (p=0.007191), 56±3 (105 cells/gram) after 6 months (p=0.007191), and 44±2 (105 cells/gram) after 1 year (p=0.007191).

In the ANK subgroup, 152±30 (105 cells/gram) after 1 month (p=0.007191), 117±15 (105 cells/gram) after 3 months (p=0.007191), 90±7 (105 cells/gram) after 6 months (p=0.007191), and 83±2 (105 cells/gram) after 1 year (p=0.007191).

In the ANK+D subgroup, the number of actinobacteria after 1 month was 146±30 (105 cells/gram) (p=0.007191), after 3 months was 56±6 (105 cells/gram) (p=0.007191), after 6 months was 46±3 (105 cells/gram) (p=0.007191), and after 1 year was 38±1 (105 cells/gram) (p=0.007191). The data are presented in table 4.23.

Table 4.23 - Mean values of actinobacteria content

Time shedule	CH	CH+D	ANK	ANK+D	p
After 1 month	153±30#	147±30#*	152±30#*	146±30#*	0,007191
After 3 months	132±30#	78±20#*	117±15#*	56±6#*	0,007191
After 6 months	116±30#	56±3#*	90±7#*	46±3#*	0,007191
After 1 year	102±12#	44±2#*	83±2#*	38±1#*	0,007191

Note: * - differences are statistically significant in pairwise comparison of data after 1, 3, 6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH +C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1, 3, 6 months and 1 year at $p<0.0083$

4.4.4. Cocci and bacilli

In the CH subgroup, the number of cocci and bacilli after 1 month was 118±11 (105 cells/gram) (p=0.007191), after 3 months was 113±3 (105 cells/gram) (p=0.007191), after 6 months was 110±11 (105 cells/gram) (p=0.007191), and after 1 year was 104±3 (105 cells/gram).

In the CH +D subgroup after 1 month - 110±11 (105 cells/gram) (p=0.007191), after 3 months - 101±3 (105 cells/gram) (p=0.007191), after 6 months - 76±3 (105 cells/gram) (p=0.007191), after 1 year - 58±11 (105 cells/gram) (p=0.007191).

In the ANK subgroup, after 1 month - 115±11 (105 cells/gram) (p=0.007191), after 3 months - 98±3 (105 cells/gram) (p=0.007191), after 6 months - 98±3 (105 cells/gram) (p=0.007191), after 1 year to 76±3 (105 cells/gram) (p=0.007191).

In the ANK+D subgroup, after 1 month to 104±8 (105 cells/gram) (p=0.007191), after 3 months to 78±3 (105 cells/gram) (p=0.007191), after 6 months to 51±3 (105 cells/gram) (p=0.007191), and after 1 year to 50±10 (105 cells/gram) (p=0.007191). The data are presented in table 4.24.

Table 4.24 - Cocci and bacilli levels

Time shedule	**CH**	**CH+D**	**ANK**	**ANK+D**	**p**
After 1 month	118±11#	110±11#*	115±11#	104±8#*	0,007191
After 3 months	113±3#	101±3#*	98±3#	78±3#*	0,007191
After 6 months	110±11#	76±3#*	87±3#	51±3#*	0,007191
After 1 year	104±3#	58±11#*	76±3#	50±10#*	0,007191

Note: * - differences are statistically significant in pairwise comparison of data after 1,3,6 months and 1 year in the groups of CH and CH +C, CH and ANK, CH and ANK+C, CH +C and ANK+C, CH+C and ANK+C, ANK and ANK+C at $p<0.05$.

- differences are statistically significant when comparing data after 1,3,6 months and 1 year at $p<0.0083$

CHAPTER 5. CONCLUSION

The number of elderly patients has more than tripled since 2000. The older the patient, the harder it is for them to maintain a good level of removable denture hygiene. A good level of denture hygiene serves as a preventive measure against the development of inflammatory oral diseases. There are various methods and recommendations for the care of removable dentures.

According to the results of questionnaires and analysis of literature data, it was found that mostly patients clean dentures with a brush or immerse them in DS, less often they treat them with a sponge or “tissue cloth”.

We evaluated the surface roughness of dentures with a service life of 3.5 or more years using profilometry. The results showed that the daily use of TB for denture cleaning resulted in an increase in surface roughness of the denture bases to 5.83+0.25 ($p=0.000001$). The DSR is 3.9 times lower with daily sponge application 1.48+0.26 ($p=0.000001$), 3.7 times lower with “cloth wipe” application 1.57+0.05 ($p=0.000001$), and when they are immersed in DS 1.58+0.3 ($p=0.000001$).

After we studied the surface of dentures under the electron microscope, we found that the change in the microrelief of the surface of denture bases in the form of pores and microcracks is caused by their cleaning with TB. Microcracks and pores are formed on their surface. When dentures were cleaned with DS, sponge and tissue cloth, the surface was smoother, clinically significant changes were not found, probably, single pores and microcracks are caused by the duration of use of dentures and fatigue of the construction material.

Dentures cleaned with TB, DS, sponge and “tissue cloth” for 3.5 years and more were evaluated by the DC index according to the Ulitovsky-Leontiev method. The results showed that regardless of the method of denture cleaning, the level of cleanliness corresponds to “bad”. The index corresponded to the value of 4.6±0.5 ($p=0.207837$) when using TB, 4.5±0.5 ($p=0.207837$) when using DS, 4.1±0.5 ($p=0.207837$) when cleaned with a sponge and 4.2±0.5 ($p=0.207837$) when cleaned with a cloth wipe.

Cytosmear from the surface of dentures with a service life of 3.5 years or more were studied by mass-spectrometry. The results showed that the following

microorganisms were detected on the surface: fungi and yeasts (by cleaning with TB 2145±77.0 (105 cells/gram), (p=0.001173)), DS - 1845±77.0 (105 cells/gram) (p=0.000000), sponge - 1645±77.0 (105 cells/gram) (p=0.003608), tissue cloth - 1890±77.0 (105 cells/gram) (p=0, 000081), anaerobes TB - 172±9.0 (105 cells/gram) (p=0.000001), DS - 166±9.0 (105 cells/gram) (p=0.000012), sponge - 157±9.0 (105 cells/gram) (p=0.008009), tissue wipe - 155±9.0 (105 cells/gram) (p=0, 005632), Actinobacteria (TB - 275±30 (105 cells/gram) (p=0.028995), DS - 178±30 (105 cells/gram) (p=0.025709), sponge - 165±30 (105 cells/gram) (p=0.001831), tissue wipe - 171±30 (105 cells/gram) (p=0, 028995), cocci and bacilli (TB - 254±11 (105 cells/gram) (p=0.000507), DS - 135±11 (105 cells/gram)(p=0.000079), sponge - 124±11 (105 cells/gram) (p=0.002785), tissue wipe - 189±11 (105 cells/gram) (p=0.003357). The number of fungi and yeasts in the sponge cleaning was 1.3 times less compared to the TB cleaning, in the DS and tissue cloth cleaning was 1.1 times less compared to the TB cleaning. The number of anaerobes is 1.1-1.3 times less when cleaned with DS, sponge and cloth compared to TB cleaning. The number of actinobacteria decreases 1.6 times in DS, sponge and wipe cleaning compared to TB cleaning. The number of cocci and bacilli decreases in case of cleaning with a tissue cloth by 1.3 times, in case of cleaning with DS by 1.9 times, in case of cleaning with a sponge by 2 times in comparison with CL.

Antibacterial efficacy of CH and “Anolit ANK SUPER” solutions by mass-spectrometry was determined by comparing the optimal time of immersion of removable dentures in DS for 5, 20 minutes and 8 hours. The experimental results showed that the number of detectable microorganisms after exposure in DS decreased systematically. For example, when dentures were immersed in CH, the number of fungi and yeasts decreased after 5 minutes of exposure by 12.9% (p=0.00), after 20 minutes by 47.6% (p=0.00) and after 8 hours by 55.3% (p=0.00). When dentures were immersed in “Anolit ANK SUPER”, the number of fungi and yeasts decreased after 5 minutes of exposure by 83%, after 20 minutes by 88%, and after 8 hours by 93%. Data on all microorganisms are presented in Figure 5.1.

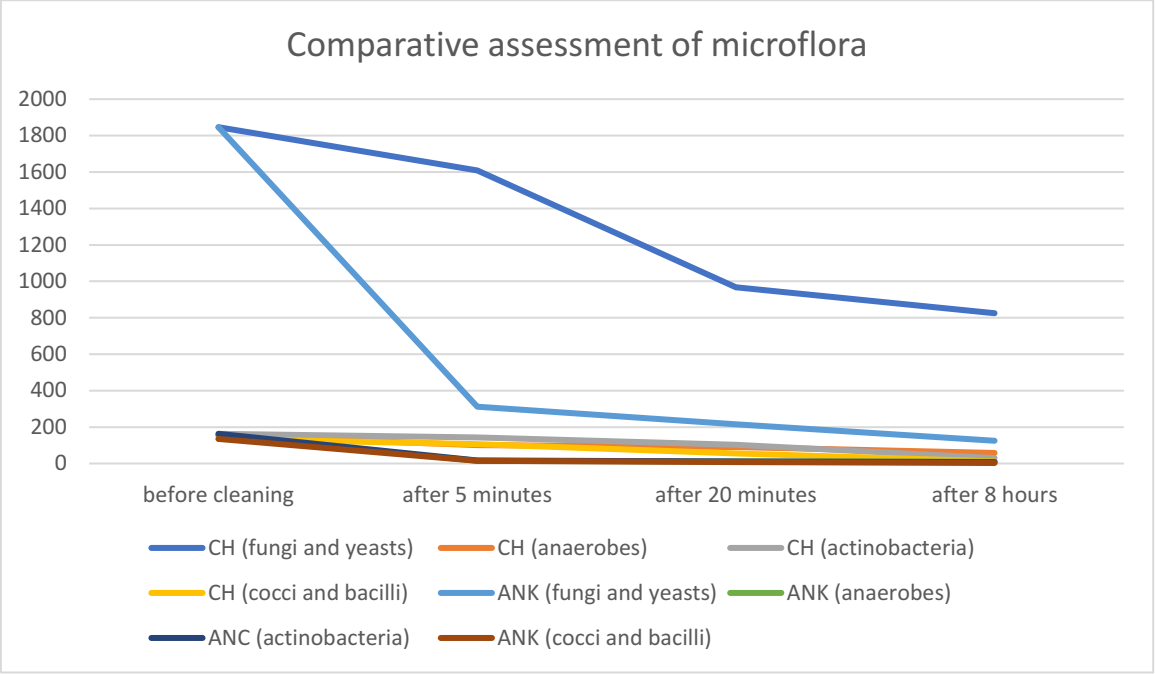


Figure 5.1- Comparative assessment of microflora

In order to develop an algorithm for cleaning removable dentures, based on the study of the denture surface and assessment of the effectiveness of the cleaning methods used, we developed a “Device for cleaning removable laminar dentures” (registration number 2025101283 from 22.01.25), which is a cover that the patient can independently put on the toothbrush (handle or working part), 10 cm long, consisting of two layers - inner foam and outer microfiber.

The algorithm we developed for denture cleaning involved a combination of using the solution “Anolit ANK SUPER” and “Device for cleaning removable laminar dentures” in comparison with CH. Four protocol variants were tested to confirm the hypothesis. Dentures without mechanical cleaning (n=60) were immersed in a container of ANK (n=30) and CH (n=30) for 20 minutes 2 times a day. Dentures were cleaned daily for 3 minutes 2 times a day with the “Device for Cleaning Removable Laminar Dentures” soaked in ANK (n=30) and CH (n=30) solution.

To evaluate the effectiveness of the developed algorithm of denture cleaning, n=60 (100%) patients with complete absence of teeth were prosthetized and n=120 (100%) complete removable dentures were fabricated. We compared the DC values according to the Ulitovsky-Leontiev method after 3, 6 months and 1 year. The results showed that

dentures without mechanical cleaning (n=60) immersed in DS for a year had a “high” DC level: in the ANK subgroup the mean index value was 1.17 (p=0.000000), in the CH 1.23 (p=0.000000). Dentures cleaned daily for a year with the “Device for cleaning removable laminar dentures” (n=60) in combination with DS had a mean DC index “high”: in the ANK+D subgroup 1.06 (p=0.000000), in the CH+D subgroup 1.13 (p=0.000000). Thus, dentures cleaned with the technique we developed showed a “high” level of DC (p=0.000000) throughout the study period. The data are presented in Figure 5.2.

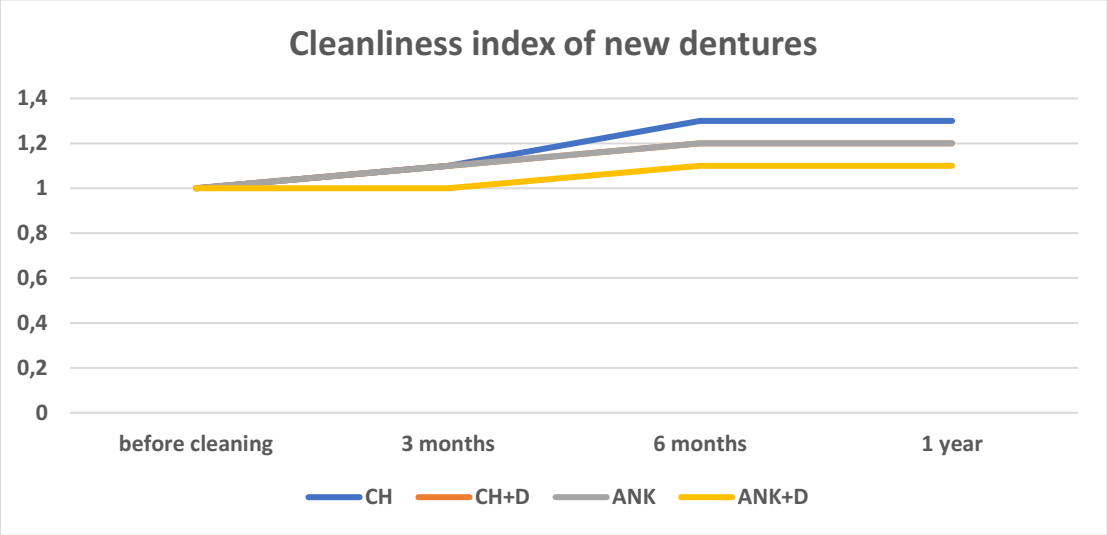


Figure 5.2- Cleanliness index of new dentures

The evaluation of the DC of old dentures cleaned for a year using the algorithm we developed showed that the PE level changed from “poor” (p=0.000000) to “high” in the ANK+D subgroups 1.5±0.2 (p=0.198892) and CH +D to 1.9±0.5 (p=0.008093). Comparison of the cleanliness index of old and new dentures is presented in Figure 5.3.

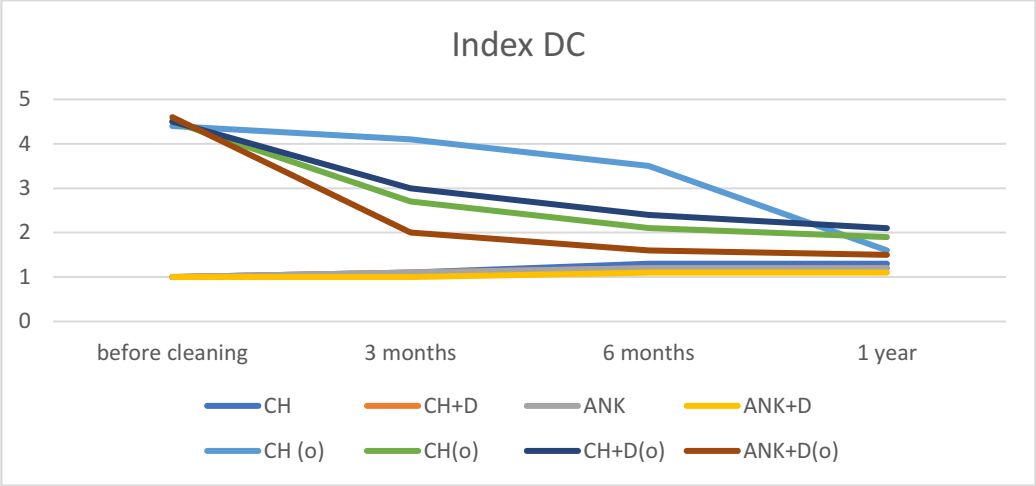


Figure 5.3- DC index

The results of the evaluation of the quantitative and qualitative composition of the microbiota by mass-spectrometry of new dentures processed according to our developed algorithm for 1 year showed a slight increase in the number of fungi and yeasts in the CH +D subgroup by 5% from 157±24 (105 cells/gram) to 165±24 (105 cells/gram) (p=0.000074), in the ANK+D subgroup by 4% from 153±24 (105 cells/gram) to 160±24 (105 cells/gram) (p=0.000074). The data are presented in Figure 5.4.

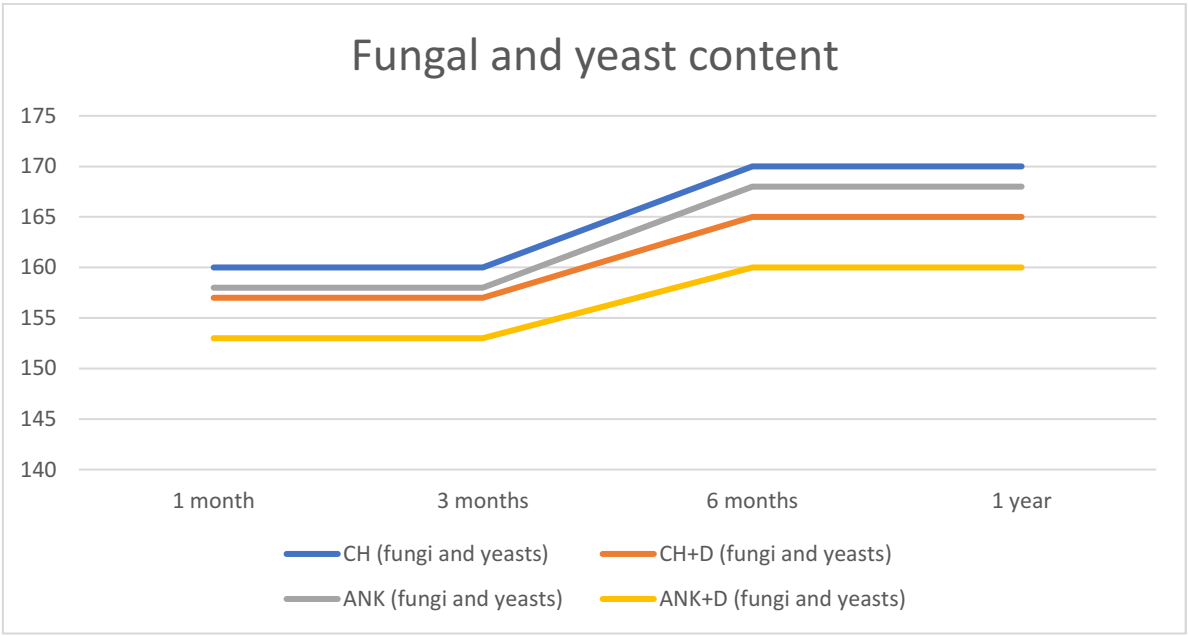


Figure 5.4- Fungi and yeast content

There was a slight increase of 1.4% from 73±4 (105 cells/gram) to 74±4 (105 cells/gram) (p=0.004409) of anaerobic microorganisms in the CH +D subgroup and 2.7% from 70±4 (105 cells/gram) to 72±4 (105 cells/gram) (p=0.004409) in the ANK+D subgroup. The data are presented in Figure 5.5.

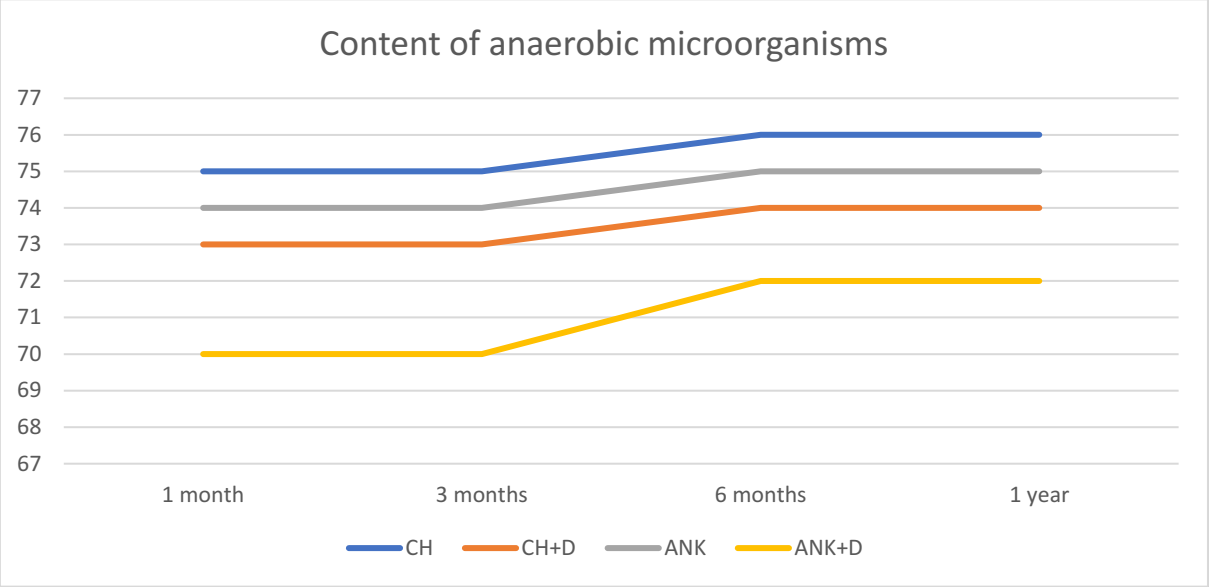


Figure 5.5- Content of anaerobic microorganisms

Actinobacteria content at 1 year increased by 8.3% in the CH +D subgroup from 11±1 (105 cells/gram) to 12±1 (105 cells/gram) (p=0.004409), and by 10% in the ANK+D subgroup from 9±1 (105 cells/gram) to 10±1 (105 cells/gram) (p=0.004409). The data are presented in Figure 5.6.

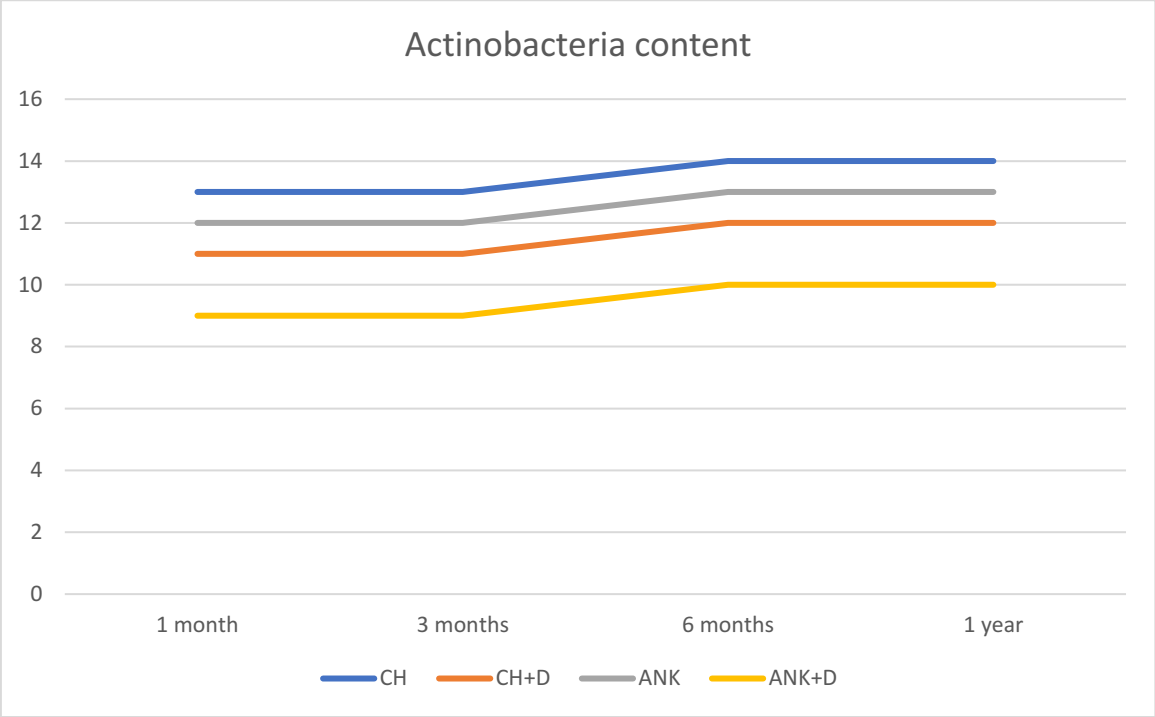


Figure 5.6- Actinobacteria content

The number of cocci and bacilli on the surface of the new dentures increased by 4% from 45±5 (105 cells/gram) to 47±5 (105 cells/gram) in the CH +D subgroup (p=0.004409), and by 2% from 43±5 (105 cells/gram) to 44±5 (105 cells/gram) in the ANK+D subgroup (p=0.004409). The data are presented in Figure 5.7.

Thus, the increase in pathogenic microflora growth on the base beds of new removable denture bases during the study year ranged from 1% to 10% depending on the microorganism species. The lowest growth of pathogenic microflora on the denture bases was found in the ANK+D subgroup.

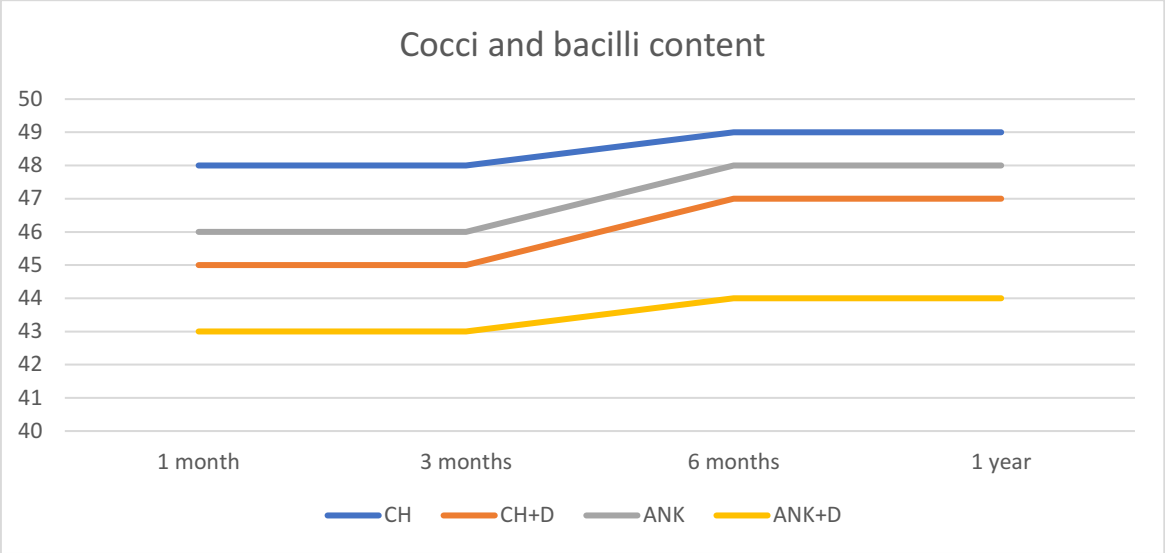


Figure 5.7- Cocci and bacilli content

The results of mass-spectrometry study of the surface of dentures made 3.5 and more years ago and cleaned by our proposed method showed a decrease in all microorganisms. The content of fungi and yeasts in the CH subgroup decreased by 48% from 1846±77 to 966±24, in the CH +D subgroup by 88% from 1867±77 to 215±35, in the ANK subgroup by 76% from 1886±77 to 455±24, and in the ANK+D subgroup by 90% from 1896±77 to 205±15. A comparison of fungi and yeast content on the old and new dentures is shown in Figure 5.8.

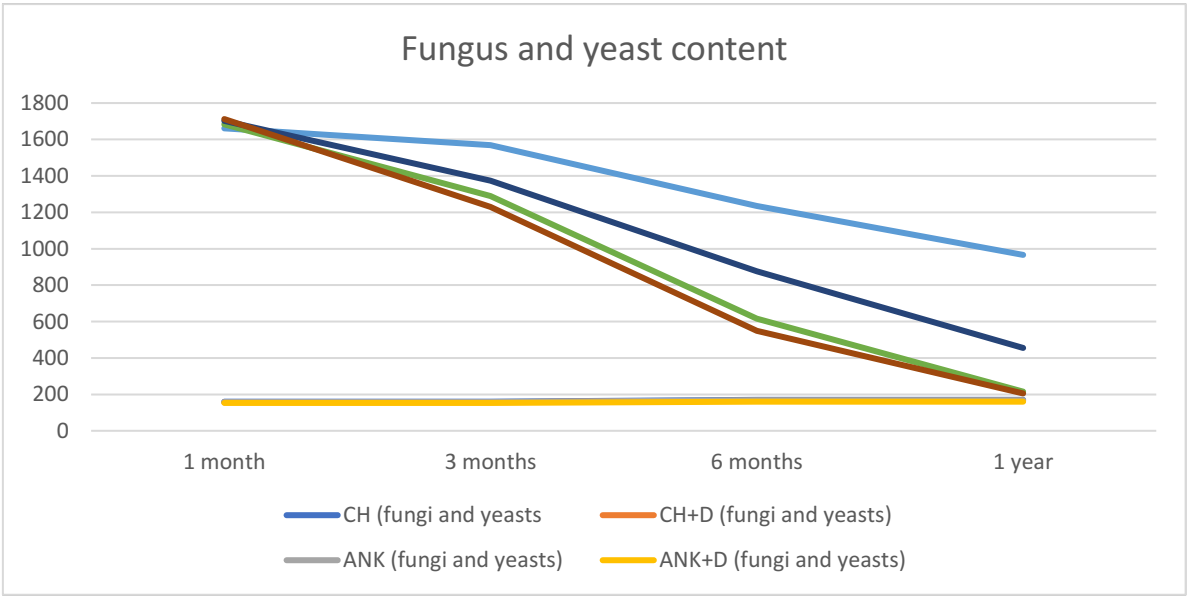


Figure 5.8- Fungus and yeast content

The number of anaerobic microorganisms decreased 33% from 180±9 to 121±5 in the CH subgroup, 46% from 187±10 to 102±3 in the CH +D subgroup, 38% from 175±10 to 109±10 in the ANK subgroup, and 45% from 174±10 to 95±3 in the ANK+D subgroup. A comparison of anaerobic microorganisms on old and new dentures is shown in Figure 5.9.

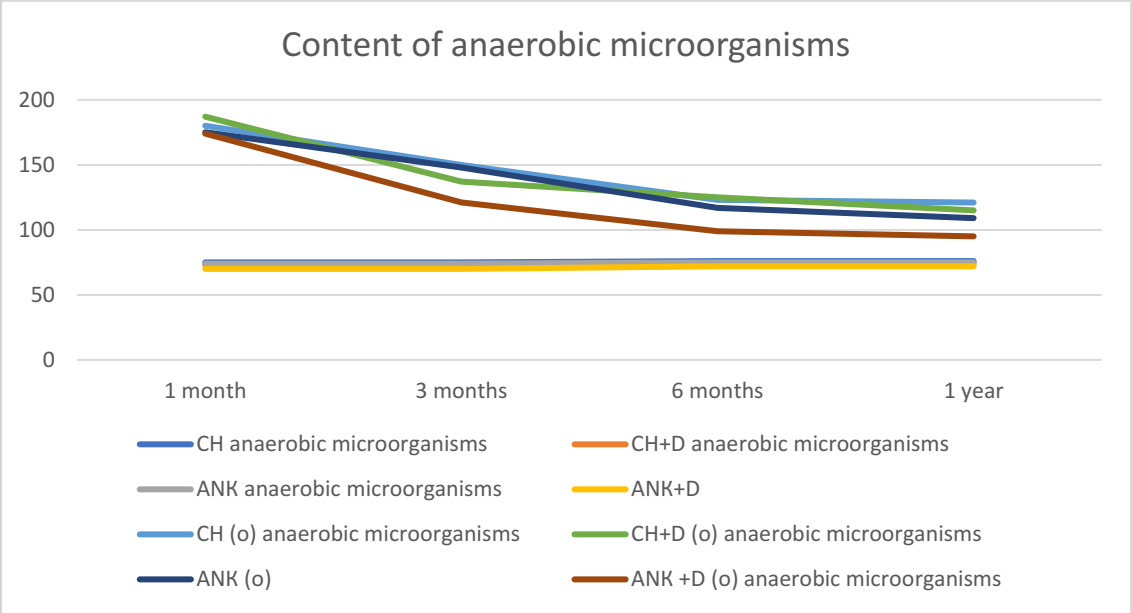


Figure 5.9- Content of anaerobic microorganisms

The number of actinobacteria in the CH subgroup decreased by 33% from 153±30 to 102±12, in the CH +D subgroup by 88% from 157±30 to 19±2, in the ANK subgroup by 49% from 163±30 to 83±2, and in the ANK+D subgroup by 90% from 176±30 to 18±1. A comparison of actinobacteria content on old and new dentures is shown in Figure 5.10.

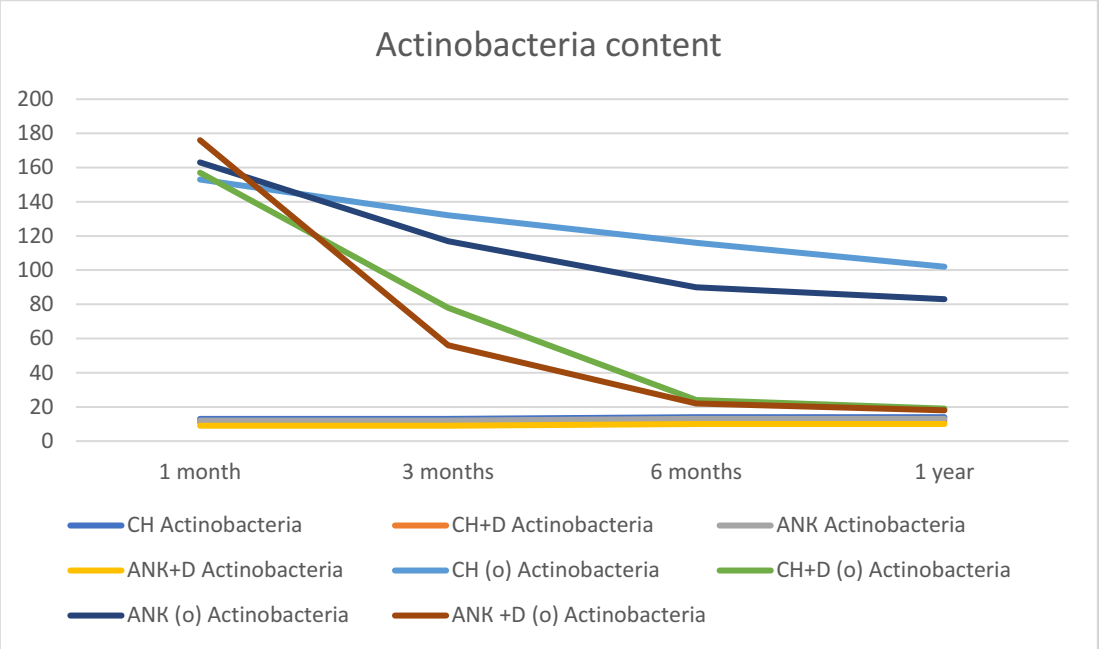


Figure 5.10- Actinobacteria content

The content of cocci and bacilli in the CH subgroup decreased by 22% from 134±11 to 104±3, in the CH +D subgroup by 57% from 135±11 to 58±11, in the ANK subgroup by 43% from 133±11 to 76±3, and in the ANK+D subgroup by 59% from 134±8 to 55±10. The comparison of coccus and bacilli content on old and new dentures is presented in Figure 5.11.

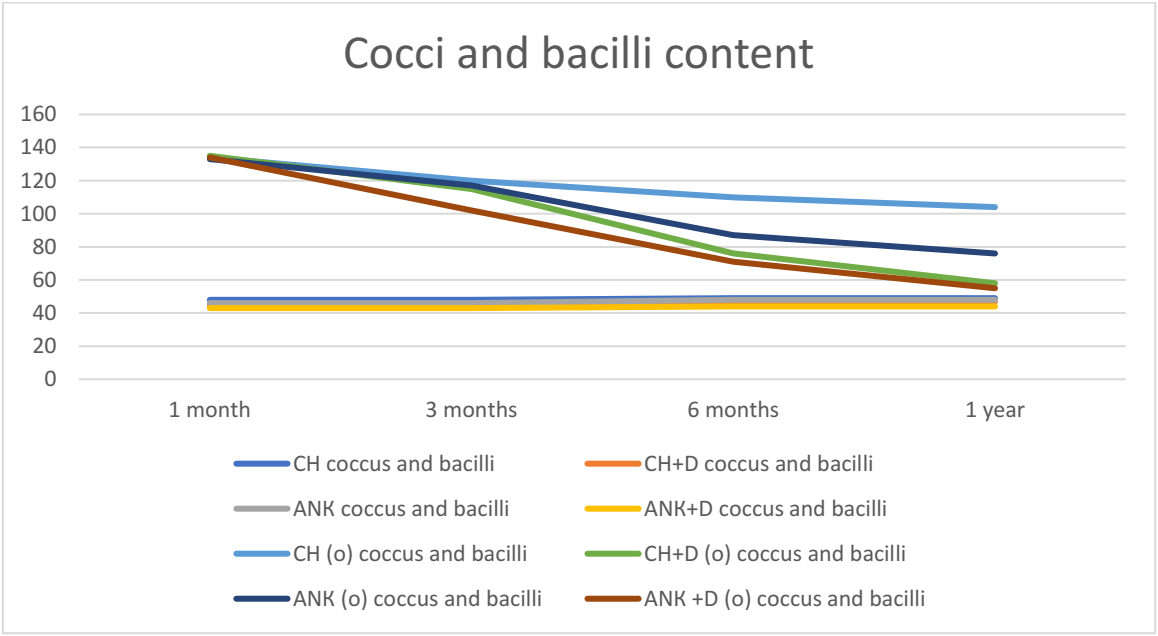


Figure 5.11- Cocci and bacilli content

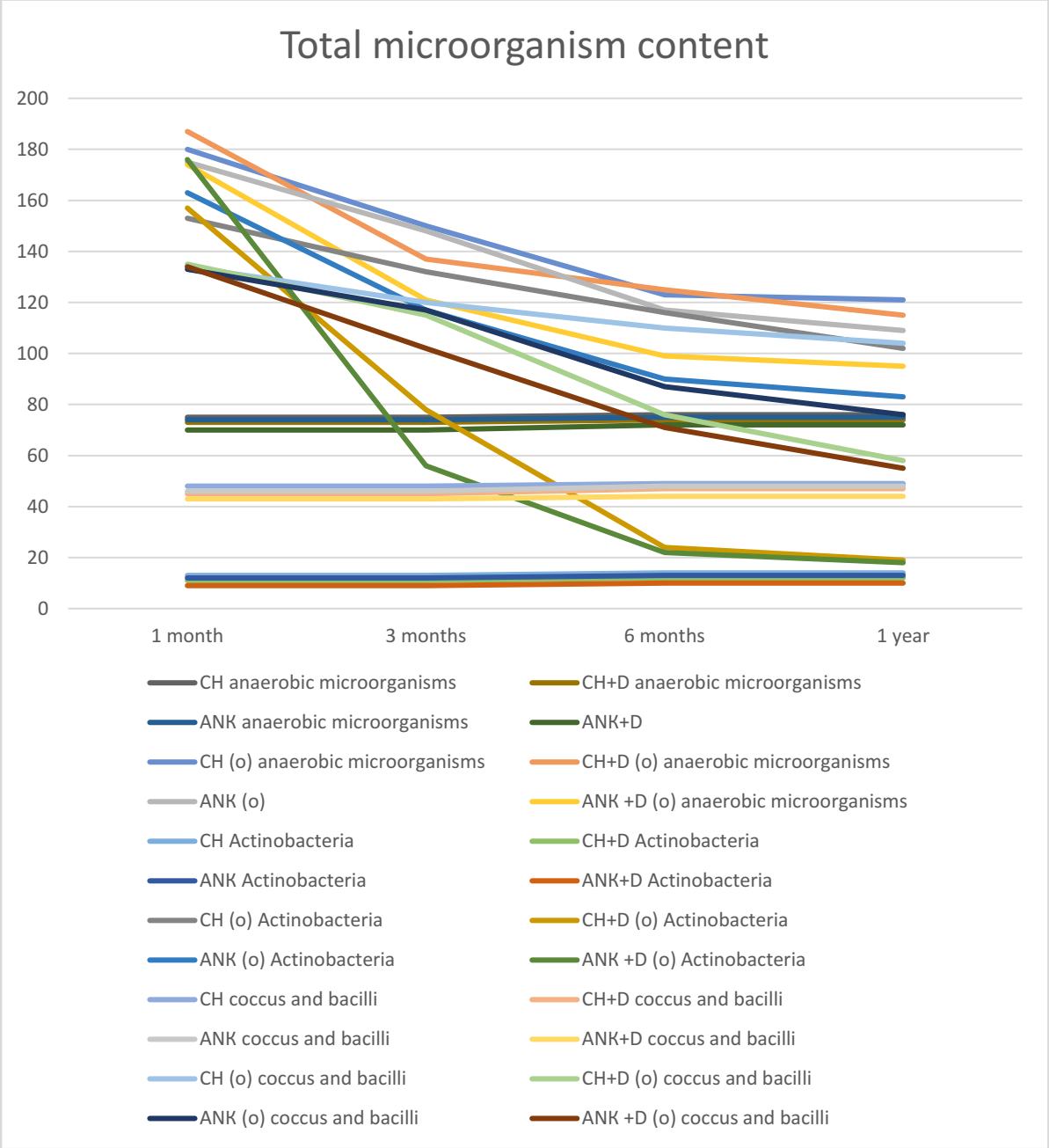


Figure 5.12- Total microorganism content

Thus, the number of pathogenic microflora on the base of removable denture bases made 3.5 and more years ago decreased from 49% to 89% during the year of the study. The most effective cleaning of removable dentures in terms of the amount of pathogenic microflora on the base bed was recorded in the ANK+D subgroup. Data on the total content of microorganisms on the surface of old and new dentures are presented in Figure 5.12.

CONCLUSIONS

1. The results of profilometry and scanning electron microscopy (SEM) of the surface of acrylic dentures showed that the use of ZSH leads to changes in the surface topography of denture bases in the form of pores and microcracks, the surface roughness was 5.83+0.25 (p=0.000001). The use of disinfecting solutions (p=0,000001), sponges (p=0,000001), “cloth wipes” (p=0,000001), reduce the level of surface roughness in 3,9 times and do not lead to clinically significant changes.

2. The “Device for cleaning removable laminar dentures” (patent application registration No. 2025101283 dated 22.01.2025) was developed; it is a two-layer cover, the outer layer is made of microfiber with the length of 10 cm, thickness of 0.5 cm and diameter of 2-4 cm.

3. A protocol for cleaning and disinfection of removable dentures for individual use at home has been developed, which includes the use of the developed “Device for cleaning removable dentures” in combination with a disinfectant solution (“Anolit ANK SUPER”/ chlorhexidine digluconate 0.05%).

4 The efficiency of application of the algorithm of cleaning and disinfection of removable dentures developed by us during a year of their use is confirmed by the decrease in the number of microorganisms detected by mass-spectrometry on the surface of old dentures (3.5 and more years): the number of fungi and yeasts decreased by 8.5 times (p=0.004409), actinobacteria by 9 times (p=0.004409); anaerobes by 1.8 times, cocci by 2.4 times (p=0.004409). In the study of new dentures, it was found that the lowest microflora growth was determined in the ANK+D subgroup in average 1.05 times (from 1% to 10%) depending on the microorganism species (p=0.000074).

5. Dentures cleaned with our developed methodology showed a “high” DC level during the whole study period when new dentures were cleaned (ANK+D - 1.06 (p=0.000000), CH +D - 1.13 (p=0.000000)). The level of DC of old dentures at application of the algorithm of cleaning and disinfection of removable dentures developed by us, after a year changed from “bad” (p=0,000000) to “high” in subgroups ANK+D - 1,5±0,2 (p=0,198892) and CH +D - 1,9±0,5 (p=0,008093).

PRACTICAL RECOMMENDATIONS

1. Include the “Device for cleaning removable dentures” in the algorithm of processing removable dentures.

2. to use “Device for cleaning removable dentures” in combination with disinfectant solutions “Anolit ANK SUPER”, chlorhexidine digluconate 0,05%.

3. Dentures should be cleaned daily 2 times a day for 3 minutes with “Device for cleaning removable plastic dentures” in combination with disinfectant solutions (“Anolit ANK SUPER”, chlorhexidine digluconate 0.05%).

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LIST OF ABBREVIATIONS

CH-chlorhexidine diglucanate solution 0.05%

RDS-Roughness of the denture surface

TB-Toothbrush

DC-Denture cleanliness

SEM - scanning electron microscopy

DR-disinfecting solution

APPENDIX

1) Patient Questionnaire

Question	ANSWER
NAME	
Gender	
Year of birth	
How long ago did you have your denture made?	
1 year ago	
2 years	
3 years	
4	
5	
More than 5 years	
Other	
Presence of chronic diseases	
Endocrine	
Infectious	
Cardiovascular diseases	
Gastrointestinal diseases	
How do you clean your denture at home?	
With a toothbrush and toothpaste	
Under running water	
With a foam sponge	
Cloth napkin	
Soaking in disinfectant	
How often do you clean your denture?	
Once a day at night	
1 time a day in the morning	
2 times a day in the morning and evening	
After every meal	
Complaints	
Itchy gums	
Redness of gums	
Bad breath	

Does the denture fit well?	
Good fixation of the upper jaw	
Good retention of the lower jaw	
The lower jaw is not fixed	
The lower jaw is not fixed	

2) Certificate of Registration “Anolit ANK SUPER”

ЕВРАЗИЙСКИЙ ЭКОНОМИЧЕСКИЙ СОЮЗ

Федеральная служба по надзору в сфере защиты прав потребителей и благополучия человека
заместитель Главного государственного санитарного врача Российской Федерации
Российская Федерация

(уполномоченный орган государства - члена Евразийского экономического союза)

СВИДЕТЕЛЬСТВО

о государственной регистрации продукции

№ RU.77.99.88.002.E.000777.03.21 от 11.03.2021 г.

ПРОДУКЦИЯ

средство дезинфицирующее "Анолит АНК СУПЕР", вырабатываемое установками типа "СТЭЛ-АНК-СУПЕР". Область применения: в соответствии с инструкциями по применению средства: от 15.03.2013 г. № ДА 005-13 с дополнением от 28.02.2021 г., № ДА 004-13 от 15.03.2013 г., № ДА 003-13 от 15.03.2013 г., от 09.06.2014 г. №ДА 006-14. Изготовлена в соответствии с документами: ТУ 9392-001-30133733-2012.

ИЗГОТОВИТЕЛЬ

ООО "Делфин Аква", 390017, Рязанская обл., г.о. город Рязань, г. Рязань, ш. Ряжское, д.20, оф.115 (адрес места осуществления деятельности по изготовлению продукции: 115088, г. Москва, Южнопортовый 2-й проезд, д. 35, стр. 1), Российская Федерация.

ЗАЯВИТЕЛЬ

ООО "Делфин Аква" 390017, Рязанская обл., г.о. город Рязань, г. Рязань, ш. Ряжское, д.20, оф.115, Российская Федерация. ОГРН: 1117746614260

СООТВЕТСТВУЕТ

Единым санитарно-эпидемиологическим и гигиеническим требованиям к продукции (товарам), подлежащей санитарно-эпидемиологическому надзору (контролю)

СВИДЕТЕЛЬСТВО ВЫДАНО НА ОСНОВАНИИ

взамен свидетельства о государственной регистрации № RU.77.99.88.002.E.010872.12.15 от 17.12.2015 г.; экспертных заключений: ИЛЦ ГУП МГЦД №051-14 от 08.07.2014 г., ФБУН "ЦНИИ эпидемиологии" Роспотребнадзора ИЛЦ № 374-исх от 15.03.2013 г.; этикетки; рецептуры; ТУ; инструкций по применению средства: от 15.03.2013 г. № ДА 005-13 с дополнением от 28.02.2021 г., № ДА 004-13 от 15.03.2013 г., № ДА 003-13 от 15.03.2013 г., от 09.06.2014 г. №ДА 006-14

СРОК ДЕЙСТВИЯ не ограничен

Заместитель руководителя — И.В. Брагина

(должность руководителя (уполномоченного лица) уполномоченного органа государства - члена Евразийского экономического союза) — М.П. — (подпись) — (Ф. И. О.)

№0425726

3) Safety Data Sheet

ПАСПОРТ БЕЗОПАСНОСТИ ХИМИЧЕСКОЙ ПРОДУКЦИИ

Внесен в Регистр Паспортов безопасности

РПБ № 30133773.20.59916 от «11» декабря 2019 г.

Действителен до «11» декабря 2024 г.

Ассоциация «Некоммерческое партнерство Координационно-информационный центр государств-участников СНГ по сближению регуляторных практик» (Ассоциация «НП КИЦ СНГ»)

Заместитель директора _____ Регистр Паспортов безопасности Ассоциация «НП КИЦ СНГ» Г.М. Муратова /

м.п.

НАИМЕНОВАНИЕ

техническое (по НД)	Средство дезинфицирующее «АНОЛИТ АНК СУПЕР» вырабатываемое установками типа «СТЭЛ-АНК-СУПЕР»
химическое (по IUPAC)	не имеет
торговое	Средство дезинфицирующее «Анолит АНК СУПЕР», вырабатываемое установками типа «СТЭЛ-АНК-СУПЕР»
синонимы	не имеет

Код ОКПД 2 20.20.14.000 **Код ТН ВЭД** 380894

Условное обозначение и наименование нормативного, технического или информационного документа на продукцию (ГОСТ, ТУ, ОСТ, СТО, (M)SDS)

ТУ 9392-001-30133733-2012 Дезинфицирующее средство «АНОЛИТ АНК СУПЕР», вырабатываемый установками типа «СТЭЛ-АНК-СУПЕР»

ХАРАКТЕРИСТИКА ОПАСНОСТИ

Сигнальное слово Осторожно

Краткая (словесная): Малоопасная по степени воздействия на организм продукция в соответствии с критериями ГОСТ 12.1.007-76 (4 класс опасности). В форме аэрозоля вызывает раздражение органов дыхания (верхних дыхательных путей) и слизистых оболочек глаз. При случайном разливе в большом количестве средство может загрязнять объекты окружающей среды

Подробная: в 16-ти прилагаемых разделах Паспорта безопасности

ОСНОВНЫЕ ОПАСНЫЕ КОМПОНЕНТЫ	ПДК р.з., мг/м^3	Класс опасности	№ CAS	№ EC
Хлоркислородные и гидропероксидные оксиданты, в том числе хлорноватистая кислота	не установлена	не установлен	нет 7790-92-3	нет

ЗАЯВИТЕЛЬ ООО «Делфин Аква», Москва
(наименование организации) (город)

Тип заявителя производитель, поставщик, продавец, экспортер, ~~импортер~~
(ненужное зачеркнуть)

Код ОКПО 30133773 **Телефон экстренной связи** 8(495) 993-46-46

Руководитель организации-заявителя _____ /М.А. Левачева /
(подпись) (расшифровка)

м.п.

4) Declaration of Conformity

ДЕКЛАРАЦИЯ О СООТВЕТСТВИИ

Общество с ограниченной ответственностью «Делфин Аква» (ООО «Делфин Аква»)

наименование организации или фамилия, имя, отчество индивидуального предпринимателя, принявших декларацию о соответствии

Зарегистрирован(а) Межрайонной инспекцией Федеральной налоговой службы №2 по Рязанской области, дата регистрации 12.02.2021 года, ОГРН: 1117746614260

сведения о регистрации организации или индивидуального предпринимателя (наименование регистрирующего органа, дата регистрации, регистрационный номер)

Российская Федерация, 390017, Рязанская обл., г.о. город Рязань, г.Рязань, ш. Ряжское, д.20, оф.115, телефон: +74959934646, почта: info@delfin-aqua.com

адрес, телефон, факс

в лице Генерального директора Левачевой Марии Александровны

(должность, фамилия, имя, отчество руководителя организации, от имени которой принимается декларация)

заявляет, что

Средство дезинфицирующее "Анолит АНК СУПЕР", вырабатываемое установками типа "СТЭЛ-АНК-СУПЕР". Выпускаемая по ТУ 9392-001-30133733-2012.

Серийный выпуск. Код ОКПД 2 20.20.14, код ТН ВЭД ЕАЭС 3808 94

(наименование, тип, марка продукции, на которую распространяется декларация, сведения о серийном выпуске или партии (номер партии, номера изделий, реквизиты договора (контракта), накладная, код ОК 005-93 и (или) ТН ВЭД ТС или ОК 002-93 (ОКУН)

Изготовитель: Общество с ограниченной ответственностью «Делфин Аква» (ООО «Делфин Аква»). Адрес места нахождения: Российская Федерация, 390017, Рязанская обл., г.о. город Рязань, г.Рязань, ш. Ряжское, д.20, оф.115. Адрес места осуществления деятельности по изготовлению продукции: Российская Федерация, 115088, Москва, 2-й Южнопортовый проезд, д. 35, стр.1

наименование изготовителя, страны и т.п.))

соответствует требованиям ГОСТ 12.1.007-76 пп. 1.2, 1.3; Нормативные показатели безопасности и эффективности дезинфекционных средств, подлежащие контролю при проведении обязательной сертификации № 01-12/75-97 пп. 1.1–1.7, 2.1–2.9, 5.1

(обозначение нормативных документов, соответствие которым подтверждено данной декларацией, с указанием пунктов этих нормативных документов, содержащих требования для данной продукции)

Декларация принята на основании:

Свидетельства о государственной регистрации № RU.77.99.88.002.E.000777.03.21 от 11.03.2021 года, выданного Федеральной службой по надзору в сфере защиты прав потребителей и благополучия человека на основании экспертных заключений: ИЛЦ ГУП МГЦД №051-14 от 08.07.2014 г., ФБУН «ЦНИИ эпидемиологии» Роспотребнадзора ИЛЦ №374-исх от 15.03.2013 г., ФБУН НИИДезинфектологии Роспотребнадзора от 18.02.2021г.; ТУ, рецептуры, этикетки, инструкций по применению средства: от 15.03.2013 г. №ДА 005-13 с дополнением от 28.02.2021 г., № ДА 004-13 от 15.03.2013 г., № ДА 003-13 от 15.03.2013 г., № ДА 006-14 от 09.06.2014 г.

(информация о документах, являющихся основанием для принятия декларации)

Регистрационный номер декларации о соответствии: РОСС RU Д-RU.PA01.B.74396/21

Дата регистрации декларации о соответствии 09.04.2021 г.

Декларация о соответствии действительна до 08.04.2026 г.

М.П. _____ Левачева М. А.

(подпись) (инициалы, фамилия)