Original Research

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Analysis of the surface microbiome of removable monomeric and monomer-free plastics dentures treated with various hygiene products

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Abstract

AIM. The aim of this study is to investigate the differences in the surface microbiome of removable dentures depending on the base material and hygiene products.

MATERIALS AND METHODS. The study was attended by 30 patients aged 65 to 70 years using different hygiene products. The study included the determination of the prothesis hygiene index and PCR analysis of the material from the surface of the plate. Statistical analysis of the data included a description of accounting characteristics and assessment of the statistical significance of changes in the studied indicators.

RESULTS. Statistical analysis showed a pronounced, statistically significant negative dynamics for all microbiological indicators in the structure of the removable apparatus and an improvement in the hygiene of the plate and the oral cavity.

CONCLUSIONS. A study of patients using orthopedic structures with different bases, as well as the use of different hygiene products using a visual-index assessment and microbiological analysis will allow you to choose the most optimal hygiene option and device design.

Keywords: removable denture, PCR study, microbiome

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Анализ микробиома поверхности съемных протезов из мономерной и безмономерной пластмасс, обработанных различными средствами гигиены

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Резюме

ЦЕЛЬ. Изучение корреляции микробиома на поверхности съемных протезов в зависимости от материала базиса и средств гигиены.

МАТЕРИАЛЫ И МЕТОДЫ. В исследовании приняли участие 30 пациентов в возрасте от 65 до 70 лет. Исследование включало определение индекса гигиены протеза и ПЦР-анализ материала с поверхности протеза. Статистически анализ данных включал описание учетных признаков, оценку статистической значимости изменений изучаемых показателей.

РЕЗУЛЬТАТЫ. Статистический анализ показал выраженную, статистически значимую отрицательную динамику по всем микробиологическим показателям в структуре биопленки съемной конструкции и улучшение индекса гигиены протеза и полости рта.

ВЫВОДЫ. Исследование пациентов, использующих ортопедические конструкции с базисами их различных материалов, а также применение стандартного и специализированного средств гигиены с помощью наглядно-индексной оценки и микробиологического анализа позволит подобрать наиболее оптимальный вариант гигиены и материала протеза.

Ключевые слова: съемный протез, ПЦР-исследование, микробиом

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INTRODUCTION

According to the World Health Organization (WHO) data for 2022, oral diseases are among the most prevalent non-communicable conditions globally, affecting an estimated 3.5 billion people. The global number of cases has increased by one billion over the past 30 years, reflecting a generally low level of public awareness regarding preventive measures, treatment strategies, and methods for the restoration of dental defects and edentulous areas[1].

Despite the growing demand for the rehabilitation of dental arch defects through dental implant therapy, removable prosthodontics remains the treatment of choice in many clinical scenarios. According to the 2019 epidemiological survey of the Russian population, periodontal disease was observed in 78% of adults aged 35 to 44 years, with a mean number of natural teeth amounting to 28. In individuals aged 60 years and older, these figures reached 90% and 11 teeth, respectively. The mean DMFT (Decayed, Missing, and Filled Teeth) index in the 65+ age group was 23.0, with the "M" (missing) component accounting for approximately 78% of the total. Thus, around 42% of individuals over the age of 60 demonstrate a need for prosthodontic treatment [1].

One of the major challenges faced by prosthodontists during the fabrication of removable prosthetic appliances lies in the patient's adaptation to the prosthesis. Every edentulous area must therefore be thoroughly analyzed to determine the most optimal prosthetic design. Approximately 30–40% of patients report discomfort when wearing partial or complete removable dentures [2]. Rapid adaptation to removable prostheses and prevention of mucosal irritation – including the development of prosthetic stomatitis – largely depend on the patient's adherence to daily and effective denture hygiene protocols. According to the 2019 national epidemiological survey conducted in Russia, among individuals aged 65 years and older, stomatitis was diagnosed in 3.19% of cases, and candidiasis in 1.47% [1].

For the prevention of such complications, it is essential that denture bases be polished to a high-gloss finish. Nevertheless, the internal and external surfaces of denture base often retain microporosity inherent to the polymer structure. This microtopography significantly increases microbial adhesion to the prosthesis surface [3; 4].

The emergence of new materials and technologies for denture base fabrication has raised questions regarding their impact on oral hygiene. The traditional cleaning method involving toothpaste or soap and a brush may not fully satisfy patients – particularly those using injection-molded (thermally pressed) monomer-free dentures. Acrylic resin remains the most commonly used material for removable prostheses, though its significant drawback is the potential for allergic and toxic reactions due to high levels of residual monomer. This has driven the search for alternative materials that retain the strength and esthetics of acrylics while eliminating irritating components to the oral mucosa.

Thermoplastic materials have demonstrated such properties. Denture bases fabricated from thermoplastics using hot injection molding are monomer-free, do not irritate oral tissues, and exhibit a highly esthetic appearance mimicking natural gingiva. However, clinical experience has revealed several important drawbacks. Thermoplastic materials are porous and rely on mechanical retention between the denture base and artificial teeth. This combination promotes increased microbial adhesion, contributing to gingivitis, periodontitis, and secondary caries. In the absence of adequate hygiene, microbial biofilm can penetrate the denture base to a depth of 2.0–2.5 mm [5].

The most frequently isolated microorganisms from removable denture surfaces include *Staphylococcus aureus*, various streptococci (*S. mutans*, *S. mitis*, *S. sanguis*, *S. salivarius*), *Candida* species, and key periodontopathogens (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *P. endodontalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola*) [6].

In response, manufacturers have introduced modern denture cleansing agents, which are claimed by marketers to be more effective and accessible. Consequently, the effectiveness of traditional brushing with soap must be evaluated based on microbial profiling of denture surfaces [7; 8].

Thus, the selection of an optimal hygiene strategy for removable prostheses remains a highly relevant issue in contemporary dental practice.

AIM

The aim of this study is to compare the degree of microbial colonization on removable prostheses fabricated from monomer-containing versus monomer-free acrylic materials, under two hygiene protocols: conventional cleaning using toothpaste or soap, and cleaning with a specialized active-oxygen-based cleansing agent, currently considered one of the most accessible solutions for maintaining denture hygiene.

[1] Pan American Health Organization. Global oral health status report: Towards universal health coverage for oral health by 2030. Washington, D.C.: PAHO/WHO; 2022. Available at: https://www.paho.org/en/documents/global-oral-health-status-report-towards-universal-health-coverage-oral-health-2030 (accessed: 01.06.2025).

MATERIALS AND METHODS

The study involved 30 patients aged 65 to 70 years, all of whom were non-smokers, without diabetes mellitus, and had been using removable dentures for approximately 3.5 years. Prior to the study, all participants reported cleaning their dentures using baby soap and a hard-bristled toothbrush. Each patient wore a maxillary denture fabricated from thermoplastic resin and a mandibular denture made from monomer-containing acrylic resin.

The analysis of the prosthesis-associated microbiome was based on two comparative criteria: (1) the type of denture base material – monomer-containing versus monomer-free – and (2) the type of cleansing agent. Each patient served as their own control, simultaneously using both types of dentures and undergoing both hygiene protocols.

Microbiological samples were collected from the denture surface using sterile paper pins, three times per patient:

– **sample 1** was taken in the morning, following evening cleaning with soap;

– **sample 2** was collected after a single use of an active-oxygen-based cleansing agent;

– **sample 3** was obtained after 15 days of regular use of the oxygen-based denture cleanser.

Prosthetic hygiene was evaluated using the denture hygiene index described by Jeganathan, Thean, and Thong (as modified by Tarbet, 1982) [9], utilizing methylene blue staining. Prior to staining, the denture was rinsed in water to remove food debris. The appliance was then immersed in an erythrosine solution for 1 minute, rinsed to remove excess dye, and plaque presence was evaluated based on the intensity and distribution of staining on the mucosa-facing surface of the denture.

Microbiological diagnostics were performed using polymerase chain reaction (PCR). The principle of the method is based on the repeated amplification of target DNA fragments through thermal cycling. Detection of periodontopathogenic microorganisms was conducted using the “ProbaGS” kit (LLC “NPO DNA-Technology”) according to the manufacturer’s protocol. Amplification and detection were carried out on the DT-96 thermal cycler (LLC “NPO DNA-Technology”).

The multiplex assay included primers for the identification of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, and *Tannerella forsythensis*, enabling the simultaneous detection of key periodontopathogenic bacterial DNA within a single sample. Following amplification, the total microbial load was quantified, expressed as the number of colony-forming units per milliliter (CFU/mL) of mesophilic aerobic and facultative anaerobic bacteria.

To visualize microbial associations relevant to periodontitis, microorganisms were classified in accordance with the microbial complex system proposed by S.S. Socransky [10; 11].

Statistical analysis involved descriptive statistics of recorded parameters and evaluation of the statistical significance of observed differences. Data processing was carried out using Microsoft Excel software.

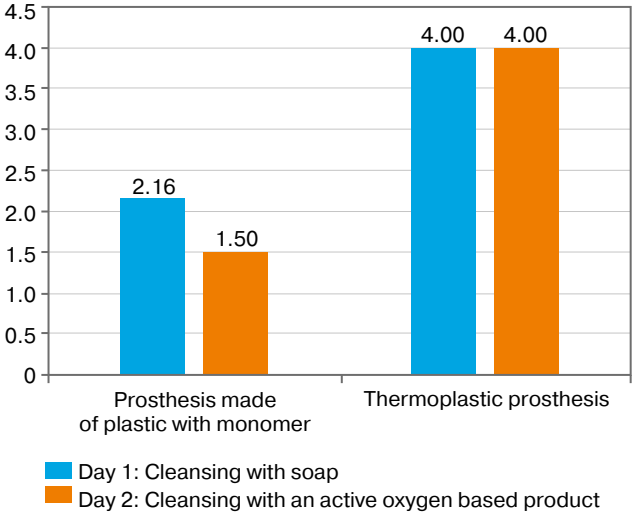

Fig. 1. Denture Hygiene Index, in points

Рис. 1. Индекс гигиены протезов, в баллах

To support the findings, scanning electron microscopy (SEM) images of the denture surfaces were obtained at ×8 magnification before and after the application of active oxygen-based denture cleansers.

RESULTS

The mean caries intensity among the examined patients, as measured by the DMFT index, was 26.67 ± 5.05, with the following component values: Decayed (D) = 8.3 ± 3.1, Missing (M) = 11.2 ± 3.0, and Filled (F) = 7.2 ± 2.2. The high DMFT score in this cohort was primarily attributed to the “Missing” component.

For dentures fabricated from monomer-containing acrylic resin, the mean denture hygiene index (modified Tarbet index) following cleaning with baby soap was 2.16 ± 0.25. After using the specialized active-oxygen-based cleanser, the index value significantly decreased to 1.50 ± 0.06, indicating improved denture hygiene. Both the intensity and surface area of staining were markedly reduced, with the stained region covering approximately one-quarter of the prosthesis surface.

In contrast, hygiene assessment of dentures made from monomer-free material yielded inconclusive results. Regardless of the cleansing method used, the hygiene index remained at 4.0 ± 0.4 in both cases, corresponding to heavy plaque accumulation and staining over more than three-quarters of the denture surface. However, a notable visual observation was made: following the application of the active-oxygen-based cleanser, the methylene blue stain appeared more vivid and was more difficult to remove from the denture base (Fig. 1).

It is well established that the surface layer of monomer-free denture base materials becomes coated over time with biofilm in the oral cavity, effectively “sealing” the micropores of the material [5]. The difficulty in removing the dye was likely due to its penetration into micropores that were “opened” after the biofilm layer was

partially removed by the active-oxygen-based cleansing agent. However, a single application of the cleanser was insufficient for the active components to penetrate deeply into the pore structure and achieve thorough decontamination of the denture base. The study was extended to further assess the microbial composition of dentures fabricated from monomer-containing acrylic resin and thermoplastic "medium-stiffness nylon", using polymerase chain reaction (PCR) for precise identification. In addition, the effectiveness of the specialized cleansing agent was evaluated over a prolonged application period of two weeks.

According to the PCR analysis, after cleansing with soap, the surface of the monomer-based dentures was predominantly colonized by pathogenic microorganisms associated with mucosal inflammation, periodontitis, and periodontal disease. *Candida* species – classified as opportunistic pathogens – are part of the oral microbiome in approximately 30–75% of the global population. Their proliferation is closely linked to the host's immune responsiveness [12]. In elderly individuals, age-related immunosenescence and comorbidities may compromise immune defense mechanisms, placing those over the age of 60 in a high-risk group for the development of denture-induced candidal stomatitis (Fig. 2).

Results of PCR-Based Microbial Quantification after Single Application of the Cleansing Agent

PCR analysis of microbial content on denture surfaces treated with the active-oxygen-based cleansing solution revealed a significant reduction in pathogenic microbiota, particularly on dentures fabricated from monomer-containing acrylic resin. In contrast, a single application of the cleanser was insufficient to eradicate microorganisms embedded in the porous structure of thermoplastic materials. The specialized hygiene agent demonstrated notable efficacy against key periodontopathogenic microorganisms responsible for periodontitis and periodontal disease; however, it showed limited activity against *Candida* spp. (Fig. 3).

On dentures made of monomer-containing acrylic resin, the microbial counts before and after one application of the cleanser were as follows:

Aggregatibacter actinomycetemcomitans: [$7.67 \pm 6.49 \times 10^3$ and $1.03 \times 10^2 \pm 4.33 \times 10^1$] CFU/mL ($p < 0.05$);

Porphyromonas gingivalis: [$8.39 \pm 8.32 \times 10^4$ and $1.57 \times 10^3 \pm 6.42 \times 10^2$] CFU/mL ($p < 0.05$);

Porphyromonas endodontalis: [$6.33 \times 10^2 \pm 4.74 \times 10^2$ and $1.02 \times 10^2 \pm 4.40 \times 10^1$] CFU/mL ($p < 0.05$);

Prevotella intermedia: [$5.70 \pm 4.88 \times 10^2$ and $5.00 \pm 2.24 \times 10^0$] CFU/mL ($p < 0.05$);

Tannerella forsythia: [$3.67 \pm 3.27 \times 10^1$ and $5.00 \pm 2.24 \times 10^0$] CFU/mL ($p < 0.05$);

Candida albicans: [$6.23 \pm 4.78 \times 10^3$ and $7.24 \pm 6.57 \times 10^3$] CFU/mL ($p = 0.47$).

In contrast, PCR results for dentures made from monomer-free (thermoplastic) material demonstrated less pronounced microbial reductions after a single cleaning cycle:

Aggregatibacter actinomycetemcomitans: [$1.39 \times 10^4 \pm 5.68 \times 10^3$ and $5.37 \pm 2.19 \times 10^2$] CFU/mL ($p < 0.05$);

Porphyromonas gingivalis: [$5.72 \pm 2.33 \times 10^2$ and $5.33 \pm 2.18 \times 10^2$] CFU/mL ($p = 0.73$);

Porphyromonas endodontalis: [$1.35 \times 10^2 \pm 5.51 \times 10^1$ and $5.38 \pm 2.20 \times 10^2$] CFU/mL ($p = 0.67$);

Prevotella intermedia: [$1.37 \times 10^2 \pm 5.58 \times 10^1$ and $8.33 \pm 3.40 \times 10^0$] CFU/mL ($p < 0.05$);

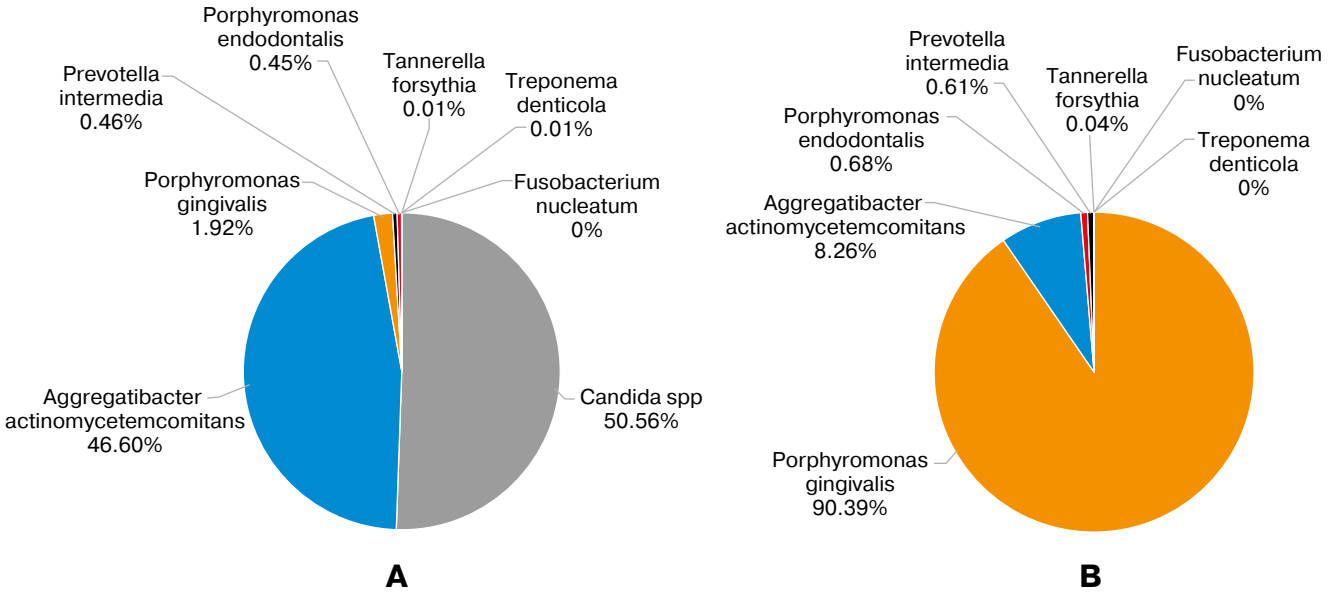

Fig. 2. PCR analysis of microbiota with prosthetic dentures cleaned before investigation: *A* – thermoplastic prosthesis; *B* – prosthesis made of plastic with monomer

Рис. 2. Результаты ПЦР-анализа микробиоты с протезов до начала исследования: *A* – протез из безмономерной пластмассы; *B* – протез из пластмассы с мономером

Tannerella forsythia: [3.33 ± 1.36 × 10^0 and 1.67 × 10^0 ± 6.80 × 10^1] CFU/mL ($p < 0.05$);

Candida albicans: [7.30 ± 2.98 × 10^3 and 5.38 ± 2.20 × 10^2] CFU/mL ($p < 0.05$).

After 15 days of using the oxygen-based cleansing tablets, a significant improvement in denture surface cleanliness was observed for both monomer-containing acrylic and thermoplastic materials. PCR diagnostics confirmed the visual assessment, demonstrating a reduction in the quantity of pathogenic microorganisms to undetectable levels.

Comparative Efficacy of Cleansing Agents on Monomer-Based Dentures

When comparing the two hygiene protocols, it was found that dentures fabricated from monomer-containing acrylic resin were more amenable to cleansing. A positive trend in biofilm reduction was observed after the first application of the specialized active-oxygen-based cleanser. By day 15, microbiological analysis confirmed the complete eradication of periodontopathogenic microorganisms from the denture surface.

The microbial counts before and after 14 days of using the oxygen-based cleanser on monomer-based dentures were as follows:

Aggregatibacter actinomycetemcomitans: [(7.67 ± 6.49) × 10^3 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$);

Porphyromonas gingivalis: [(8.39 ± 8.32) × 10^4 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$);

Porphyromonas endodontalis: [6.33 × 10^2 ± 4.74 × 10^2 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$);

Prevotella intermedia: [(5.70 ± 4.88) × 10^2 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$);

Tannerella forsythia: [(3.67 ± 3.27) × 10^1 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$).

Although *Candida albicans* was initially present, its quantity was substantially reduced following the use of the specialized cleanser. At baseline, the total count of *Candida* spp. was (9.81 ± 1.65) × 10^3 CFU/mL. After 14 days, only *C. albicans* remained, with a significantly reduced level of (1.67 ± 1.67) × 10^0 CFU/mL (Fig. 4).

Efficacy of Long-Term Cleansing on Monomer-Free (Thermoplastic) Denture Bases

PCR analysis of biofilm samples from dentures fabricated using monomer-free thermoplastic material also demonstrated high cleansing efficacy after a 14-day hygiene protocol. Microbial loads before and after two weeks of using the active-oxygen-based cleanser were as follows:

Aggregatibacter actinomycetemcomitans: [1.39 × 10^4 ± 5.68 × 10^3 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$);

Porphyromonas gingivalis: [(5.72 ± 2.33) × 10^2 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$);

Porphyromonas endodontalis: [1.35 × 10^2 ± 5.51 × 10^1 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$);

Prevotella intermedia: [1.37 × 10^2 ± 5.58 × 10^1 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$);

Tannerella forsythia: [(3.33 ± 1.36) × 10^0 and (0.00 ± 0.00) × 10^0] CFU/mL ($p < 0.05$).

These objective data demonstrate complete eradication of periodontopathogenic microorganisms. Additionally, the quantity of *Candida* spp. was reduced by nearly 60%. Following soap-based cleaning, *Candida albicans* levels were (1.51 ± 0.54) × 10^4 CFU/mL, while after two weeks of specialized cleansing, the level dropped to (6.67 ± 1.67) × 10^0 CFU/mL.

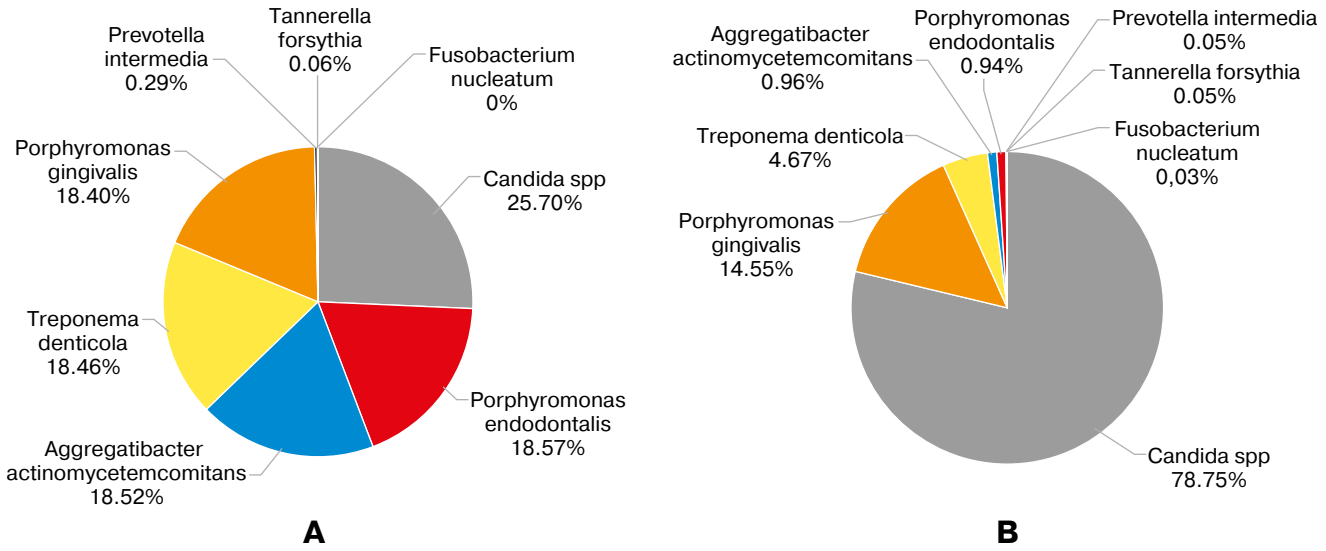

Fig. 3. PCR analysis of microorganisms from the surface of the prosthesis one-time cleaned with oxygen-containing tablets: *A* – thermoplastic prosthesis; *B* – prosthesis made of plastic with monomer

Рис. 3. Результаты ПЦР-анализа микроорганизмов с поверхности протеза, однократно очищенного кислородсодержащими таблетками:
A – протез из безмономерной пластмассы; *B* – протез из пластмассы с мономером

Although dentures made of thermoplastic material require a longer cleansing period due to their microporous structure, regular use of specialized active-oxygen-based cleansers ensures excellent hygiene outcomes. These agents release active oxygen upon dissolution in water, which penetrates even the smallest pores of the thermoplastic base, disrupting biofilms and removing plaque. Importantly, the cleanser is non-abrasive and does not compromise the microstructure of the prosthesis (Fig. 5).

The photographic documentation obtained before and after treatment of removable dentures with the specialized cleansing agent – performed using scanning electron microscopy at ×8 magnification – visually confirmed the results of the PCR analysis and the Tarbet denture hygiene index across all study groups (Fig. 6).

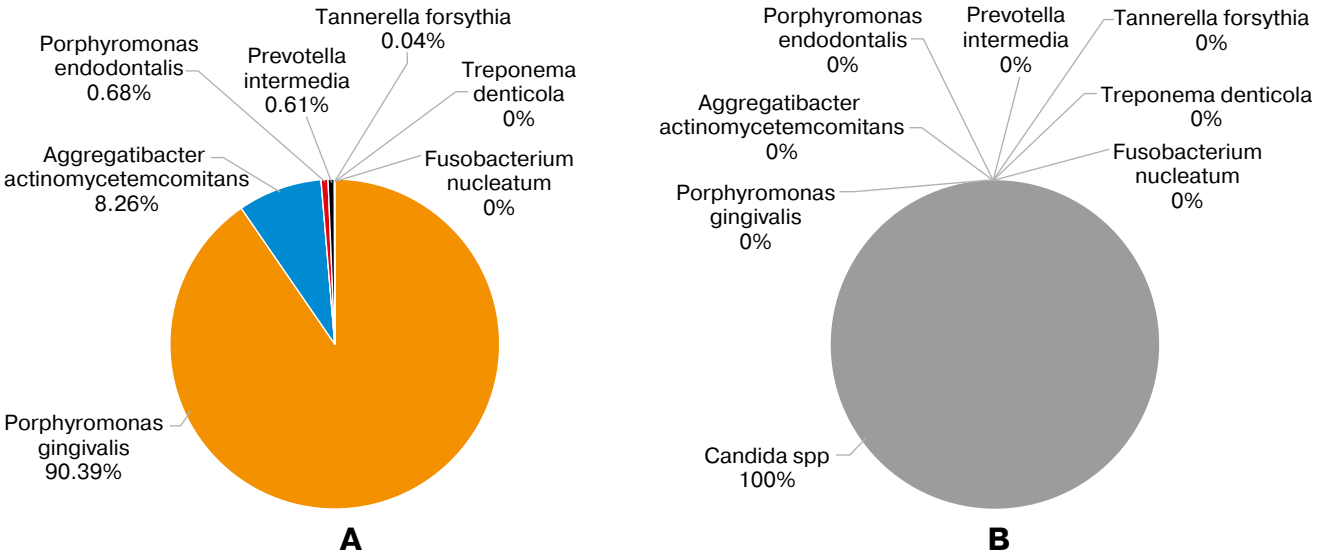

Fig. 4. Results of cleaning dentures microbial biofilm made of monomer plastic with oxygen product: *A* – thermoplastic prosthesis; *B* – prosthesis made of plastic with monomer

Рис. 4. Результаты очищения микробной биопленки средством на основе активного кислорода протезов из пластмассы с мономером: *A* – протез из безмономерной пластмассы; *B* – протез из пластмассы с мономером

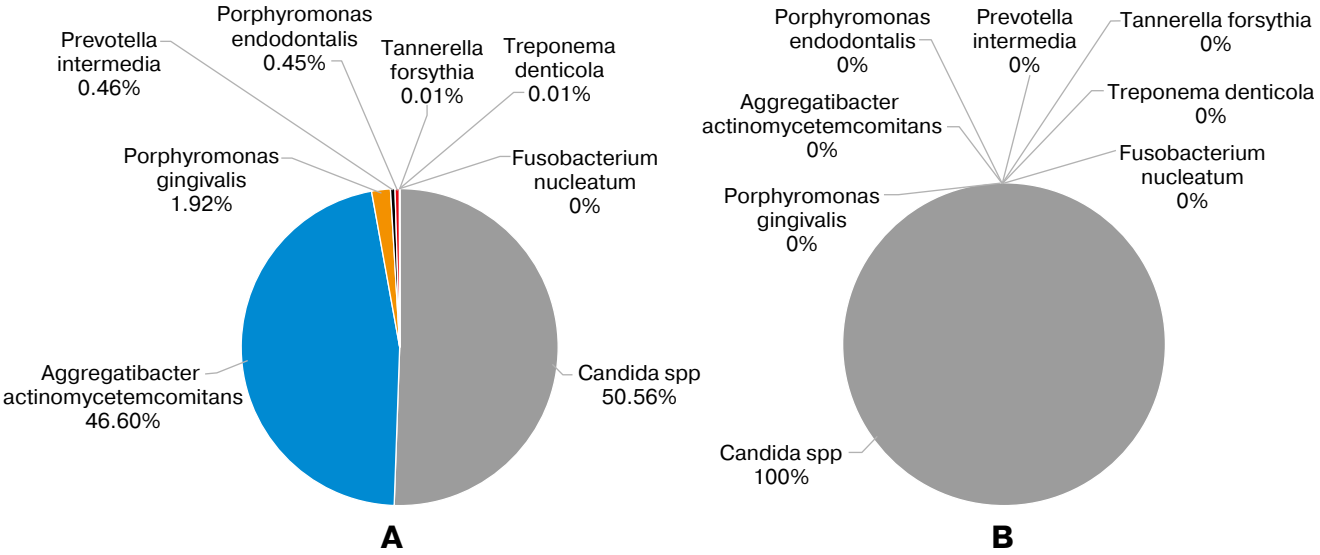

Fig. 5. Results of cleaning dentures made of thermoplastic material from biofilm with oxygen product: *A* – thermoplastic prosthesis; *B* – prosthesis made of plastic with monomer

Рис. 5. Результаты очищения биопленки средством на основе активного кислорода протезов из термопластичного (безмономерного) материала: *A* – протез из безмономерной пластмассы; *B* – протез из пластмассы с мономером

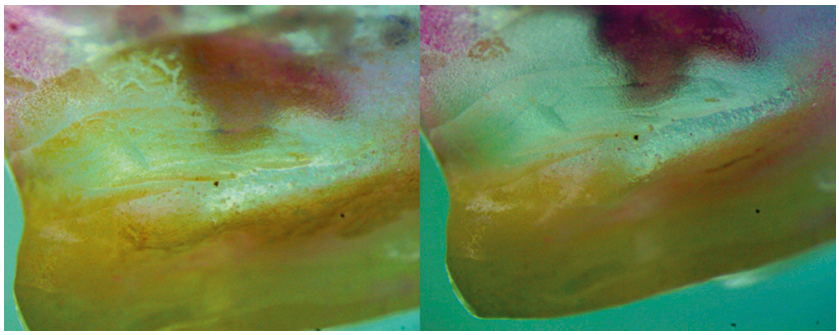

Fig. 6. Photos of an orthopedic structure taken on a scanning electron microscope (magnification x8) before and after cleaning with oxygen-containing product

Рис. 6. Фото ортопедической конструкции, сделанные на сканирующем электронном микроскопе (увеличение x8) до и после чистки кислородсодержащим средством

CONCLUSIONS

1. The prosthetic hygiene index (Tarbet index) indicates superior cleanliness of dentures fabricated from monomer-containing acrylic resin.

2. A comparative analysis of monomer-containing and monomer-free acrylic denture materials demonstrated that the surface of monomer-based dentures harbors a greater number of periodontopathogenic microorganisms.

3. Structural changes in the biofilm – characterized by a reduction in periodontopathogens and *Candida* spp. – are observed only after two weeks of using oxygen-releasing cleansing tablets

4. Monomer-free acrylic resin appears to be a more favorable material for removable prostheses due to the absence of residual monomer, which reduces porosity and limits colonization by periodontopathogenic microorganisms.

5. All removable prosthetic appliances require thorough daily cleaning with agents based on active oxygen.

Despite the positive outcomes observed, the long-term efficacy of such cleansing agents warrants further investigation.

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