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Microbiota of complete removable dentures

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ABSTRACT

BACKGROUND: Complete dentures are a valid treatment option in edentulous patients. However, the average service life of complete dentures is 4–5 years, which is frequently due to microbial contamination.

AIM: To perform quantitative and qualitative assessment of microbiota on the surface of complete dentures after 1 and 4 years of use.

MATERIALS AND METHODS: The study included 40 fully edentulous patients (K08.1) who used acrylic complete dentures for no more than 5 years. There were two groups ($n=20$ each) based on the duration of use of complete dentures: 1 year in Group 1 and 4 years in Group 2. The microbiota composition was examined by mass spectrometry. The Statistica 13 package was used for statistical processing of the study findings. For multiple comparisons, the parametric t-test with Bonferroni correction was used to assess intergroup differences, with $p=0.05$ as the critical significance level.

RESULTS: Microbial contamination increased in all examined patients after using complete dentures for 1 to 4 years. Cocci and bacilli counts increased from 56 ± 5 (10^5 cells/g) ($p=0.03$) to 107 ± 8 (10^5 cells/g) ($p=0.04$). Anaerobe counts increased from 68 ± 6 (10^5 cells/g) ($p=0.0002$) to 102 ± 9 (10^5 cells/g) ($p=0.0002$). Actinobacteria counts increased from 30 ± 3 (10^5 cells/g) to 143 ± 12 (10^5 cells/g) ($p=0.003$). Gram-negative rod counts increased from 4 ± 1 (10^5 cells/g) ($p=0.0005$) to 24 ± 2 (10^5 cells/g) ($p=0.0006$). Yeast and mold counts increased from 977 ± 90 (10^5 cells/g) ($p=0.0003$) to $1,587\pm136$ (10^5 cells/g) ($p=0.003$).

CONCLUSION: Within 4 years, yeast and mold counts increased by 91%, actinobacteria counts by 61%, gram-negative rod counts by 500%, and anaerobe counts by 50% in all patients. The study findings indicate that microbial contamination of dentures is directly related to the duration of their use.

Keywords: complete dentures; oral microbiota in patients with complete dentures; hygiene of complete dentures; cleaning of complete dentures.

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Микробиота полных съёмных протезов

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АННОТАЦИЯ

Обоснование. Съёмное протезирование является актуальным способом лечения пациентов с адентией. Однако средний срок службы съёмных протезов составляет от 4 до 5 лет, зачастую это связано с их микробной обсеменённостью.

Цель исследования — изучить количественный и качественный состав микрофлоры на поверхности полных съёмных протезов через 1 и 4 года использования.

Материалы и методы. Обследовано 40 пациентов с диагнозом «полная адентия» (К08.1), использующих полные съёмные акриловые протезы не более 5 лет. Сформированы 2 группы ($n=20$ в каждой) в зависимости от длительности пользования протезами: 1-я — 1 год, 2-я — 4 года. Состав микробиоты определяли методом масс-спектрометрии. Статистическую обработку результатов исследования проводили с помощью пакета программ Statistica 13. Для оценки различий между группами использовали параметрический t-критерий с поправкой Бонферрони при множественных сравнениях, критический уровень статистической значимости p был равен 0,05.

Результаты. Установлено, что у всех обследованных пациентов увеличилась микробная обсеменённость протезов от 1 года до 4 лет использования. Концентрация кокков и бацилл выросла с 56 ± 5 (10^5 кл./г) ($p=0,03$) до 107 ± 8 (10^5 кл./г) ($p=0,04$). Концентрация анаэробов увеличилась с 68 ± 6 (10^5 кл./г) ($p=0,0002$) до 102 ± 9 (10^5 кл./г) ($p=0,0002$). Концентрация актинобактерий выросла с 30 ± 3 (10^5 кл./г) до 143 ± 12 (10^5 кл./г) ($p=0,003$). Концентрация грамотрицательных палочек выросла с 4 ± 1 (10^5 кл./г) ($p=0,0005$) до 24 ± 2 (10^5 кл./г) ($p=0,0006$). Количество грибов и дрожжей увеличилось с 977 ± 90 (10^5 кл./г) ($p=0,0003$) до 1587 ± 136 (10^5 кл./г) ($p=0,003$).

Заключение. За 4 года у всех пациентов концентрация грибов и дрожжей выросла на 91%, концентрация актинобактерий — на 61%, концентрация грамотрицательных палочек — на 500%, концентрация анаэробных микрорганизмов — на 50%. Полученные результаты показывают, что обсеменённость протезов напрямую связана с длительностью использования.

Ключевые слова: съёмные протезы; микробиота полости рта пациентов со съёмными протезами; гигиена съёмных протезов; методы гигиенической обработки съёмных протезов.

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BACKGROUND

Edentulism, or complete tooth loss, is a prevalent condition. Demographic trends indicate that its incidence will continue to rise as life expectancy increases [1]. Tooth loss negatively impacts quality of life, leading to impaired speech, aged facial appearance, and, potentially, temporomandibular joint dysfunction, as well as dietary restrictions. Prosthetic rehabilitation is essential to restore masticatory function, diversify dietary options, and normalize speech. One widely used treatment option is the fabrication of complete removable acrylic dentures [2]. This type of prosthetic rehabilitation is one of the most common globally. It effectively restores function, does not require high-end equipment, and is generally suitable for most edentulous patients [3]. However, treatment success depends not only on the quality of the prosthesis, but also on maintaining adequate denture hygiene.

Despite their advantages, complete removable acrylic dentures have limitations. These include the potential for allergic reactions and insufficient retention due to advanced bone and mucosal atrophy [4]. The denture base is made from acrylic resin, a porous material requiring meticulous daily cleaning and disinfection. Due to the low thermal conductivity of the acrylic base and the resulting "greenhouse effect," the tissue surface of the denture creates favorable conditions for the proliferation of both pathogenic and opportunistic microorganisms [5], increasing the risk of inflammatory oral diseases. Denture stomatitis, frequently associated with microbial plaque on the denture surface, is among the most common conditions. Effective disinfection requires a thorough evaluation of oral hygiene and microbial contamination of dentures [6,7]. The composition of the microbiota in the denture-bearing area depends on several factors: oral hygiene status, denture type and material, duration of denture use, the time interval between tooth extraction and prosthetic treatment, and the patient's immune status [8]. Regardless of the type of removable dentures, their use leads to excessive microbial growth in the oral cavity and increases the risk of colonization by pathogenic species. As with natural dentition, a salivary glycoprotein-based acquired pellicle, containing salivary amylase, albumin, mucin, lysozyme, and immunoglobulins, forms on the denture surface once placed in the oral cavity [9]. The primary colonizers of dental enamel include gram-positive *Streptococcus* spp. (*S. oralis*, *S. mutans*, *S. mitis*, *S. gordonii*, *S. sanguinis*, and *S. parasanguinis*) as well as *Veillonella* spp., *Neisseria* spp., *Rothia* spp., *Abiotrophia* spp., *Gemella* spp., and *Granulicatella* spp. [10]. In 2019, Morse et al. investigated the cultivable flora on denture surfaces and reported predominant colonization by *S. mutans*, *S. mitis*, *S. salivarius*, and *S. sanguis*, along with gram-positive *Actinomyces* spp. (*A. israelii*, *A. naeslundii*, *A. odontolyticus*), *Lactobacillus* spp., and *Veillonella* spp. [11]. Understanding the qualitative and quantitative

microbial composition is critical for developing effective denture disinfectants.

AIM: To assess the quantitative and qualitative composition of the microbiota on the surface of complete removable dentures after 1 and 4 years of use.

METHODS

Study Design

This was an observational, single-center, prospective, cross-sectional, controlled, blinded, randomized study.

Eligibility Criteria

Inclusion criteria: age 40 to 80 years, use of complete removable acrylic dentures for no longer than 5 years, and absence of allergic reactions.

Study Setting

The study was conducted at the Department of Propedeutics of Dental Diseases of the RUDN University, in the clinical and diagnostic center of the same institution (Moscow).

Study Duration

The study was carried out from October 2023 to July 2024.

Intervention

The quantitative and qualitative composition of the microbiota was determined by mass spectrometry of microbial markers. Swabs were collected from the inner surfaces of the maxillary and mandibular complete removable acrylic dentures without prior hygiene treatment. The samples were placed in labeled test tubes and transported in a cooled container to the laboratory. Upon arrival, the test tubes were sorted and checked for integrity. Prior to analysis, they were incubated in a thermostatic device to maintain optimal conditions for bacterial viability. The samples were then analyzed using the Maestro- α MS gas chromatograph-mass spectrometer (Interlab, Russia).

Main Study Outcome

Microbial contamination of all denture surfaces increased over time.

Additional Study Outcomes

In addition to microbial growth, denture hygiene deteriorated with prolonged use.

Subgroup Analysis

The study included 40 fully edentulous patients (K08.1) using acrylic complete removable dentures for no longer than 5 years. They were divided into 2 groups: Group 1 ($n=20$) used dentures for 1 year; Group 2 ($n=20$) used dentures for 4 years.

Outcomes Registration

Swabs were taken from denture surfaces, and the samples were analyzed using mass spectrometry to assess main and additional outcomes.

Statistical Analysis

Statistical analysis was performed using the Statistica 13 software. The data were normally distributed; therefore, descriptive statistics included calculation of the mean and standard deviation (mean \pm SD). Intergroup differences were assessed using parametric tests, including the t-test with Bonferroni correction for multiple comparisons. A *p* value of 0.05 was considered significant.

RESULTS

Participants

Microbial contamination was assessed on the surfaces of complete removable acrylic dentures. In Group 1, biofilm was examined after 1 year of denture use; in Group 2, it was examined after 4 years.

Primary Results

The analysis of surface microbiota in patients who used removable dentures for 1 year and 4 years revealed the following (Table 1).

Both groups showed the presence of the following cocci and bacilli: *Bacillus cereus*, *Bacillus megaterium*,

Enterococcus spp., *Streptococcus* spp., *S. mutans* (anaerobic), *Staphylococcus aureus*, and *Staphylococcus epidermidis*. These species were detected both in patients who used dentures for 1 year and those who used them for 4 years.

In Group 1, the concentration of cocci and bacilli was 56 ± 5 ($\times 10^5$ CFU/g) (*p*=0.00). In Group 2, it increased to 107 ± 8 ($\times 10^5$ CFU/g) (*p*=0.00) (Fig. 1).

Fungal and yeast species (*Aspergillus* spp., *Candida* spp., campesterol) were identified in both groups. The concentration in Group 1 was 977 ± 90 ($\times 10^5$ CFU/g) (*p*=0.0003). In Group 2, it increased to $1,587 \pm 136$ ($\times 10^5$ CFU/g) (*p*=0.0003) (Fig. 2).

The following actinobacteria were identified in both groups: *Actinomyces* spp., *A. viscosus*, *Corynebacterium* spp., *Nocardia* spp., *N. asteroides*, *Mycobacterium* spp., *Pseudonocardia* spp., *Rhodococcus* spp., *Streptomyces* spp., and *S. farmamarensis*. Their concentration in Group 1 was 30 ± 3 ($\times 10^5$ CFU/g) (*p*=0.0002). In Group 2, it increased to 143 ± 12 ($\times 10^5$ CFU/g) (*p*=0.0002) (Fig. 3).

Gram-negative rods identified in both groups included the following: *Alcaligenes* spp./*Klebsiella* spp., *Kingella* spp., *Flavobacterium* spp., *Moraxella* spp./*Acinetobacter* spp., *Porphyromonas* spp., *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*. Their concentration was 4 ± 1 ($\times 10^5$ CFU/g) in Group 1 (*p*=0.0005) and 24 ± 2 ($\times 10^5$ CFU/g) in Group 2 (*p*=0.0005) (Fig. 4).

Anaerobes found in both groups included: *Bacteroides fragilis*, *Bifidobacterium* spp., *Blautia coccoides*,

Table 1. Microbial concentration on denture surfaces (mean \pm SD), $\times 10^5$ CFU/g

Groups	Cocci and bacilli	Fungi and yeasts	Actinobacteria	Gram-negative rods	Anaerobes
Group 1 (1 year)	56 ± 5	977 ± 90	30 ± 3	4 ± 1	68 ± 6
Group 2 (4 years)	107 ± 8	1587 ± 136	143 ± 12	24 ± 2	102 ± 9
<i>p</i> -value	0.0	0.0003	0.0002	0.0005	0.0002

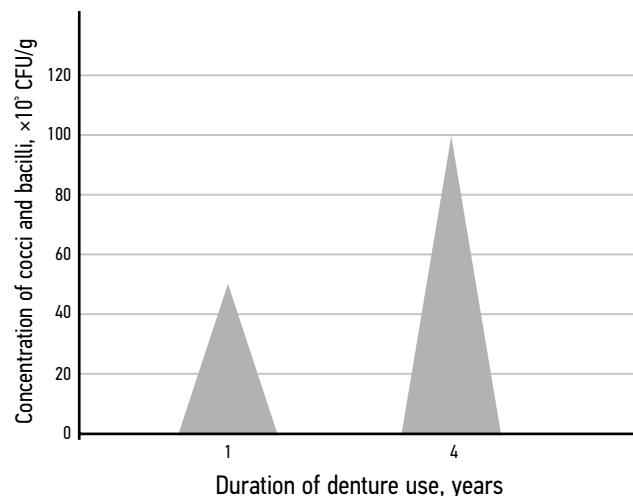


Fig. 1. Concentration of cocci and bacilli on denture surfaces in Groups 1 and 2.

Clostridium spp. (*C. tetani* group), *C. difficile*, *C. histolyticum*/*Streptococcus pneumoniae*, *C. perfringens*, *C. propionicum*, *C. ramosum*, *Eubacterium* spp., *Eggerthella lenta*, *Fusobacterium* spp./*Haemophilus* spp., *Lactobacillus* spp., *Peptostreptococcus anaerobius*,

Prevotella spp., *Propionibacterium* spp. (*P. acnes*, *P. freudenreichii*, *P. jensenii*), *Ruminococcus* spp., and *Veillonella* spp. Their concentration in Group 1 was 68 ± 6 ($\times 10^5$ CFU/g) ($p=0.0002$). In Group 2, it was 102 ± 9 ($\times 10^5$ CFU/g) ($p=0.0002$) (Fig. 5).

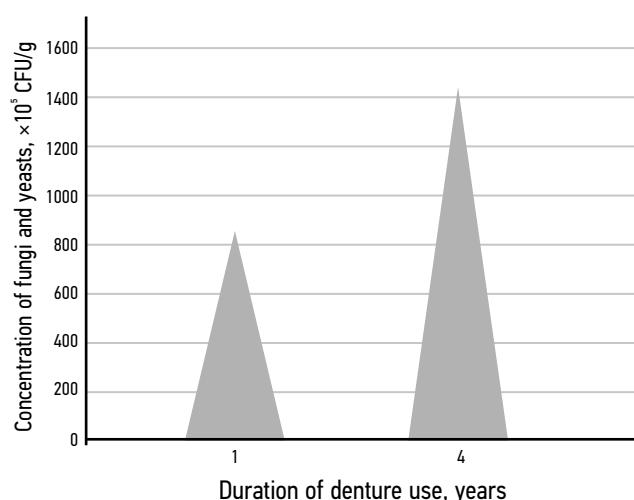


Fig. 2. Concentration of fungi and yeasts on denture surfaces in Groups 1 and 2.

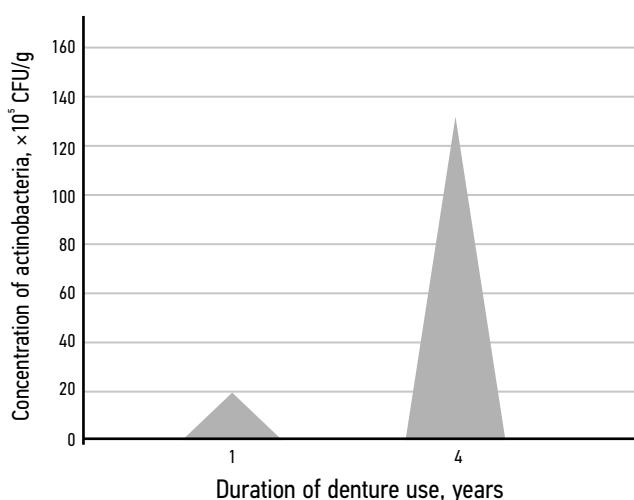


Fig. 3. Concentration of actinobacteria on denture surfaces in Groups 1 and 2.

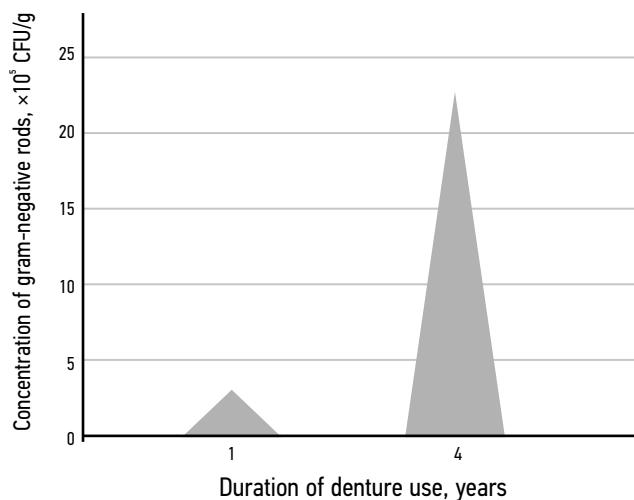


Fig. 4. Concentration of gram-negative rods on denture surfaces in Groups 1 and 2.

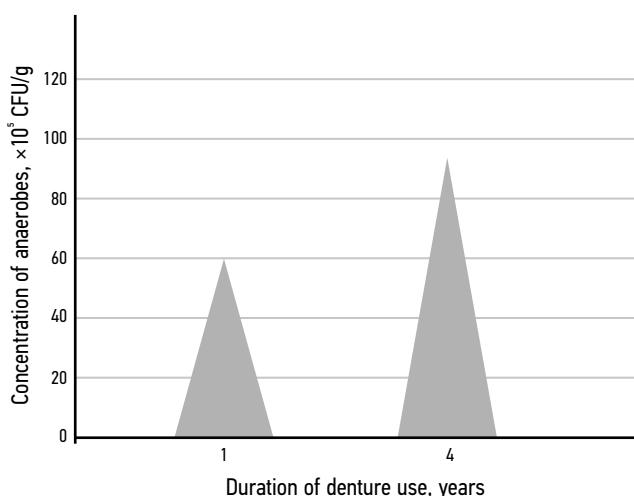


Fig. 5. Concentration of anaerobes on denture surfaces in Groups 1 and 2.

Secondary Results

The observed increase in microbial counts over time suggests a deterioration in denture hygiene with prolonged use.

Adverse Events

No adverse events were reported.

DISCUSSION

Our findings demonstrate that fungi and yeasts dominate the microbial composition of denture surfaces. Long-term use (up to 4 years) leads to increased colonization not only by cocci and fungi but also by actinobacteria, gram-negative rods, and anaerobes. These findings are consistent with previous studies. For example, Vecherkina et al. reported a predominance of cocci and extensive *Candida* growth in cases of critically low pH (81% of cases) [12]. Bugorkov et al. identified *Staphylococcus* spp. and *Candida* spp. as the dominant pathogens [13]. An et al. concluded that *Candida* spp. play a key role in inflammatory oral diseases, with a significant increase in their counts after 5 years of denture use [14].

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However, our data also indicate a significant rise in cocci, bacilli, actinobacteria, and anaerobes after 4 years of denture use.

CONCLUSION

The study findings indicate that microbial contamination of dentures correlates directly with duration of use. The longer a denture is worn, the more extensive the microbial colonization. Over a 4-year period, fungi and yeast counts increased by 91%, actinobacteria by 61%, gram-negative rods by 500%, and anaerobes by 50%.

ADDITIONAL INFORMATION

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