



Analysis of the surface microbiome of removable orthodontic appliances cleaned with various hygiene products

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Abstract

AIM. The aim of this study is to investigate the differences in the microbiota of the surface of orthodontic plates depending on the used hygiene products.

MATERIALS AND METHODS. The study was attended by 36 patients aged 6 to 12 years, undergoing treatment on removable orthodontic equipment using various hygiene products. The study was carried out using the determination of the prosthesis hygiene index and microbiological analysis of the material from the surface of the plate. Statistically, the data analysis included a description of the accounting features, an assessment of the statistical significance of changes in the indicators under study.

RESULTS. In the structure of the biofilm of a removable plate, microbiological indicators before and after the experiment showed a pronounced, statistically significant negative dynamics. And also, an improvement in the hygiene index of the plate and oral cavity.

CONCLUSIONS. The use of various hygiene products with orthodontic patients and the study of the microbiological status of the surface of the plate after cleaning them will allow you to choose the most optimal option for cleansing the removable apparatus.

Keywords: removable orthodontic plate, microbiological analysis, biofilm, hygiene products

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Анализ микробиома поверхности съемных ортодонтических пластинок, обработанных различными средствами гигиены

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Резюме

ЦЕЛЬ. Изучить различия микробиома поверхности ортодонтических пластинок в зависимости от используемых средств гигиены.

МАТЕРИАЛЫ И МЕТОДЫ. В исследовании приняли участие 36 пациентов в возрасте от 6 до 12 лет с диагнозом K07.2 Аномалии соотношения зубных дуг, получающие лечение с использованием съемной ортодонтической аппаратуры с учетом применения различных средств гигиены полости рта. Исследование проведено с помощью определения индекса гигиены протеза и microbiологического анализа материала с поверхности пластинки. Статистический анализ данных включал описание учетных признаков, оценку статистической значимости изменений изучаемых показателей.

РЕЗУЛЬТАТЫ. В структуре биопленки съемной пластинки microbiологические показатели до и после эксперимента показали выраженную, статистически значимую отрицательную динамику. А также произошло снижение индекса гигиены пластинки и полости рта.

ВЫВОДЫ. Применение различных средств гигиены ортодонтическими пациентами и изучение microbiологического статуса поверхности пластинки после их чистки позволит выбрать наиболее оптимальный вариант очищения съемного аппарата.

Ключевые слова: съемный пластиночный аппарат, microbiологическое исследование, биопленка, средства гигиены

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INTRODUCTION

According to the World Health Organization (WHO), as of 2023, oral health problems are among the most prevalent non-communicable diseases globally, accounting for nearly 3.5 billion cases. Oral diseases affect approximately 45% of the world's population, indicating a significant deficiency in preventive measures. The prevention of pathological processes in the oral cavity includes oral hygiene education, pharmacological prophylaxis, and timely treatment interventions¹.

An epidemiological survey conducted in Russia in 2019 revealed that among six-year-old children, the prevalence of caries in permanent teeth, based on the DMFT index, was 2%. The components of the index were as follows: Decayed (D) – 0.02%, Missing (M) – 0.00%, and Filled (F) – 0.00%. At the age of 12, caries prevalence reached 72%, with a mean caries experience of 2.38%, the mean number of filled teeth at 0.52%, and extracted teeth at 0.02%. Among 15-year-olds, the prevalence of carious lesions increased to 82%, with the D component at 1.58%, F – 1.38%, and M – 0.04%. Periodontal health was reported in 90% of 12-year-olds and 74% of 15-year-olds.

In the city of Kirov, according to epidemiological dental assessments conducted in 2019 and 2022, the number of 12- and 15-year-old children diagnosed with periodontal tissue pathology increased. Among 12-year-olds, the proportion of children without periodontal lesions was higher than the national average, whereas in the 15-year-old group, the figure was lower, with more frequent occurrences of bleeding on probing and the presence of dental calculus. These indicators exceed the national average and reflect insufficient routine oral hygiene practices [1; 2].

Studies conducted across various regions of Russia have shown that among all dental pathologies, dentoalveolar anomalies (DAAs) occur in 41.5% to 69.9% of cases, with approximately every second patient using removable orthodontic appliances for occlusal correction. Over the past 20 years, the prevalence of DAAs has increased by approximately 25%. According to national epidemiological data, the incidence of dentoalveolar system disorders among children varies from 37.8 to 85%, depending on the developmental stage of the jaws and the phase of occlusion formation [3].

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).

Research indicates that around 30–40% of patients experience discomfort when wearing removable plate-type orthodontic appliances [4]. These devices are often perceived by the body as foreign objects, leading to irritation of the oral mucosa. Improved adaptation and a reduction in inflammatory reactions can be achieved through effective cleaning of the appliance. Prolonged use of such prostheses—particularly in the absence of adequate hygiene—allows microbial biofilms originating from dental plaque to penetrate up to 2–2.5 mm into the acrylic base of the plate [5].

The microbial species most commonly found in the biofilm on orthodontic appliances include *Staphylococcus aureus*, *Streptococcus* spp. (*S. mutans*, *S. mitis*, *S. sanguis*, *S. salivarius*), *Candida* yeasts, and various periodontopathogens such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Treponema denticola* [6].

Therefore, the selection of optimal hygiene products for cleaning orthodontic appliances remains a relevant and important issue in contemporary orthodontic practice.

AIM

The aim of this study is to perform a comparative evaluation of the microbiota associated with removable orthodontic appliances following two different hygiene protocols: mechanical cleaning using toothpaste and a toothbrush, and chemical cleaning using a specialized active oxygen-based agent. Additionally, the study seeks to assess the impact of appliance usage on the biochemical composition of oral fluid in orthodontic patients.

MATERIALS AND METHODS

The study was conducted with the participation of 36 patients aged 6 to 12 years undergoing treatment with removable orthodontic appliances. The patients were divided into two groups of 15 individuals each. The first group included orthodontic patients who used a specialized active oxygen-based hygiene product for appliance cleaning. The second group, serving as the control, included patients who used a medium-bristled toothbrush and conventional toothpaste for cleaning.

During the study, the prosthesis hygiene index (Tarbet modification) was recorded, oral fluid was collected for biochemical analysis, and biofilm samples were taken from the surface of the orthodontic appliances for microbiological evaluation using polymerase chain reaction (PCR). These parameters were assessed on

day 1 and day 30 of appliance cleaning using either conventional toothpaste/soap or oxygen-based cleansing tablets, respectively.

To assess the modified Tarbet index, the orthodontic appliance was removed from the oral cavity and immersed in water for one minute to eliminate food debris. The tissue-facing surface of the appliance was stained with erythrosine solution for one minute, after which the dye was rinsed off. The amount of plaque was evaluated based on the stained surface area and color intensity.

Unstimulated whole saliva samples were collected in the morning on an empty stomach, prior to toothbrushing, by passive drooling into sterile disposable tubes (5 mL per patient). The biochemical analysis of calcium ions (Ca^{2+}), phosphate (PO_4^{3-}), and total protein content in the saliva was conducted using photocolorimetric methods with reagent kits "Calcium-2-Olvex" and "FN-Olvex". The hydrogen ion concentration (pH) of the saliva was measured using a HI98103 Checker pH Tester (Hanna Instruments, Romania). Total antioxidant activity was determined by induced chemiluminescence, a method based on the assessment of free radical reaction activity in the sample [7]. Salivary pH was additionally measured using the "Expert-001" pH meter [8].

Microbial samples were collected from the appliance surface using sterile paper points, placed into saline solution, and transported to a microbiological laboratory. Serial tenfold dilutions were prepared using buffer solution and plated on meat-peptone agar (MPA) in Petri dishes, followed by incubation at 37°C and colony counting. DNA of periodontal pathogens was extracted using the "ProbaGS" kit (LLC "DNA-Technology"), in accordance with the manufacturer's protocol, and analyzed by real-time PCR using a DT-96 thermocycler (LLC "DNA-Technology"). The results were evaluated using the instrument's software. Based on the cycle threshold (Ct), the total microbial count was determined, expressed in colony-forming units per milliliter (CFU/mL), including the quantity of mesophilic aerobic and facultative anaerobic bacteria. Specific periodontal patho-

gens were identified, including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, and *Tannerella forsythia*. The microbial composition was categorized into microbial complexes according to the classification by S.S. Socransky [7; 9].

To monitor the cleanliness of the appliance surface, a photoprotocol was performed using a light electron microscope ($\times 8$ magnification) before and after cleaning with oxygen-releasing tablets.

RESULTS

In the first and second groups, the DMFT+dmft index was 4.25 ± 0.48 and 5.25 ± 0.63 , respectively, which is considered a moderate level and falls within the relative norm for the 6–12 age group.

At the beginning of the study, the prosthesis hygiene index (modified Tarbet) in the two groups averaged 3.5 ± 0.29 and 3.75 ± 0.25 points, respectively. After 30 days of appliance cleaning, the index decreased to 1.8 ± 0.48 points in the group using toothpaste/soap and to 0.8 ± 0.25 points in the group using oxygen-releasing tablets, indicating superior efficacy of the latter. Visually, the intensity of staining on the appliances was significantly reduced, not exceeding 25% of the plate surface.

Biochemical analysis of oral fluid in the control and experimental groups did not reveal statistically significant differences. In Group 1, the salivary pH was 7.28 ± 0.24 at baseline and 7.03 ± 0.02 at the end of the experiment ($p=0.07$); in Group 2, it was 7.61 ± 0.26 and 7.59 ± 0.27 , respectively ($p=0.64$) (Fig. 1). The differences in both groups were not statistically significant. A slight shift toward acidity was observed in the control group, whereas Group 2 maintained a consistently alkaline environment in the oral cavity. Total protein levels in oral fluid decreased steadily in both groups, indicating reduced salivary viscosity and, consequently, improved oral self-cleansing, associated with regular hygiene of orthodontic appliances regardless of the cleansing method used.

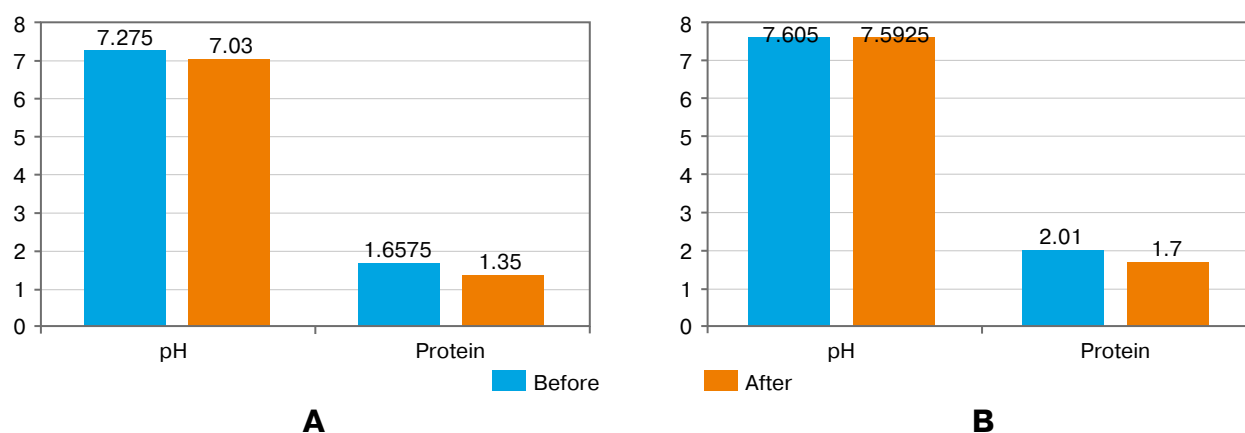


Fig. 1. Changes in biochemical parameters in the control group (A) and the group of patients using an oxygen hygiene product (B)

Рис. 1. Изменения биохимических показателей в контрольной группе (A) и группе пациентов, использующих кислородсодержащее средство гигиены (B)

The total microbial count (TMC) in the control group increased threefold after one month of cleaning with toothpaste: prior to the intervention, the microbial load was $(1.00 \pm 0.1) \times 10^7$ CFU/mL, while after the intervention it reached $(3.03 \pm 2.33) \times 10^7$ CFU/mL. In contrast, the experimental group showed a statistically significant ($p \leq 0.05$) twofold reduction in TMC. At baseline, the microbial count was $(5.50 \pm 2.6) \times 10^5$ CFU/mL, and after one month of appliance cleaning with the specialized hygiene agent, it decreased to $(3.25 \pm 2.25) \times 10^4$ CFU/mL, demonstrating the effectiveness of the active oxygen-based product (Fig. 2).

According to PCR analysis, a significant increase in the number of periodontal pathogens was observed in the control group. The microbial load on the appliances used by control group patients before and after the intervention, respectively, was as follows: *Aggregatibacter actinomycetemcomitans* $[(2.80 \pm 2.41) \times 10^3$ and $(3.25 \pm 1.00) \times 10^4$ CFU/mL, $p < 0.05$], *Porphyromonas gingivalis* $[(2.80 \pm 2.41) \times 10^3$ and $(7.75 \pm 2.25) \times 10^4$ CFU/mL, $p < 0.05$], *Porphyromonas endodontalis* $[(3.00 \pm 2.34) \times 10^3$ and $(5.25 \pm 2.75) \times 10^4$ CFU/mL, $p < 0.05$], *Prevotella intermedia* $[(2.80 \pm 2.41) \times 10^3$ and $(2.80 \pm 2.41) \times 10^4$ CFU/mL, $p < 0.05$], *Tannerella forsythia* $[(2.76 \pm 2.43) \times 10^2$ and $(2.78 \pm 2.42) \times 10^4$ CFU/mL, $p < 0.05$], and *Treponema den-*

ticola $[(2.76 \pm 2.43) \times 10^2$ and $(3.25 \pm 2.25) \times 10^2$ CFU/mL, $p = 0.65$]. An increase in *Candida albicans* colonization was also observed $[(3.03 \pm 2.33) \times 10^3$ and $(5.05 \pm 2.60) \times 10^3$ CFU/mL, $p = 0.14$], possibly due to the observed decrease in salivary pH. In parallel, a marked decline in the quantity of *Lactobacillus* spp. – representatives of the normal oral microbiota – was noted, which is considered a negative factor for oral health (Fig. 3).

In contrast, PCR analysis in the experimental group demonstrated a reduction in the levels of periodontal pathogens alongside an increase in *Lactobacillus* spp. counts. Specifically, the microbial concentrations before and after the intervention were as follows: *Aggregatibacter actinomycetemcomitans* $[(5.50 \pm 2.60) \times 10^2$ and $(3.25 \pm 2.25) \times 10^2$ CFU/mL, $p < 0.05$], *Porphyromonas gingivalis* $[(5.50 \pm 2.60) \times 10^2$ and $(7.75 \pm 2.25) \times 10^1$ CFU/mL, $p < 0.05$], *Porphyromonas endodontalis* $[(3.25 \pm 2.25) \times 10^2$ and $(7.75 \pm 2.25) \times 10^1$ CFU/mL, $p < 0.05$], *Prevotella intermedia* $[(5.50 \pm 2.60) \times 10^2$ and $(3.25 \pm 2.25) \times 10^1$ CFU/mL, $p < 0.05$], *Tannerella forsythia* $[(3.00 \pm 2.34) \times 10^2$ and $(7.75 \pm 2.25) \times 10^1$ CFU/mL, $p < 0.05$], *Treponema denticola* $[(2.75 \pm 2.43) \times 10^3$ and $(5.28 \pm 2.73) \times 10^1$ CFU/mL, $p < 0.05$], and *Candida albicans* $[(2.80 \pm 2.60) \times 10^3$ and $(5.50 \pm 2.49) \times 10^2$ CFU/mL, $p < 0.05$] (Fig. 4).

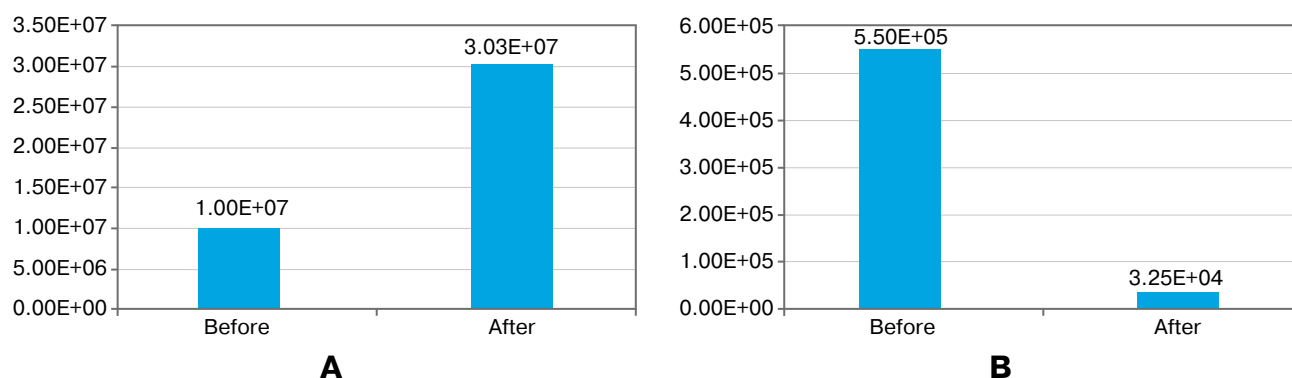


Fig. 2. Total microbial number in control (A) and experimental (B) groups before and after examination, in CFU/ml

Рис. 2. Общее микробное число в контрольной (A) и экспериментальной (B) группах до и после эксперимента, в КОЕ/мл

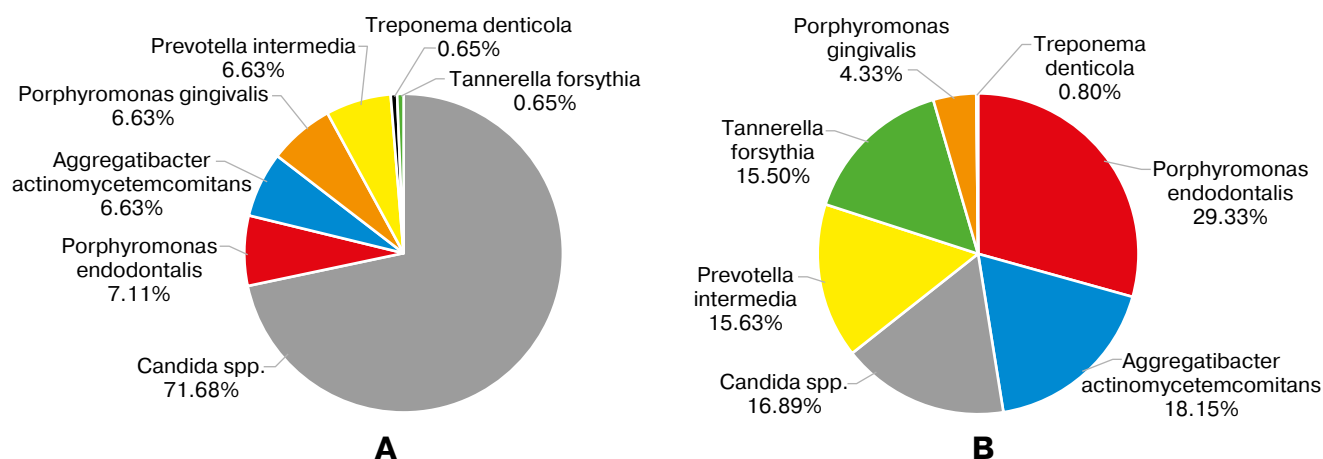


Fig. 3. PCR studies data in the control group before (A) and after (B) investigation

Рис. 3. ПЦР исследование в контрольной группе до (A) и после (B) исследования

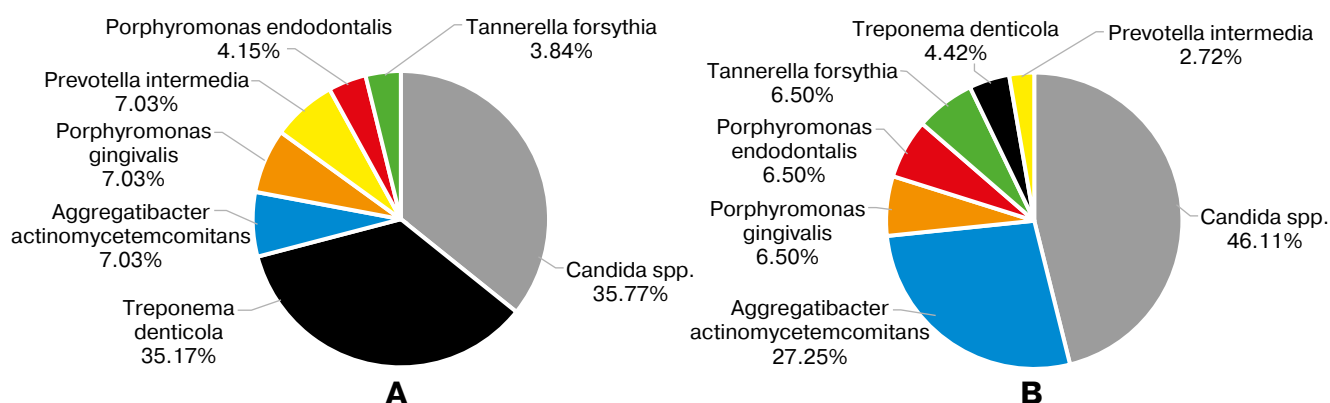


Fig. 4. PCR studies data in the experimental group before (A) and after (B) investigation

Рис. 4. ПЦР исследование в экспериментальной группе до (A) и после (B) исследования

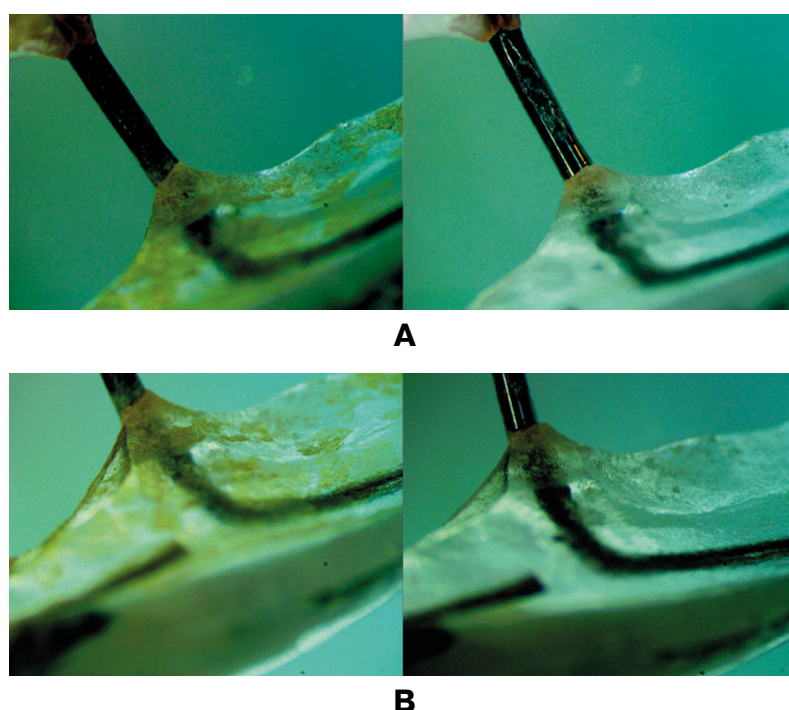


Fig. 5. Photos of an orthodontic structure taken on a scanning electron microscope (magnification x8) before (A) and after (B) cleaning with oxygen-containing tablets

Рис. 5. Фото ортодонтической конструкции, сделанные на сканирующем электронном микроскопе (увеличение x8) до (A) и после (B) чистки кислородсодержащими таблетками

The photodocumentation performed before and after treatment of the orthodontic appliances with the oxygen-releasing agent, using a scanning electron microscope at $\times 8$ magnification, visually confirmed a reduction in biofilm coverage and plaque accumulation (Fig. 5).

CONCLUSIONS

1. More effective plaque removal from removable orthodontic appliances, as assessed by the Tarbet index, was achieved using an active oxygen-based cleansing agent compared to conventional cleaning with a toothbrush and toothpaste.

2. Biochemical analysis demonstrated that the absence of significant differences in salivary pH and the reduction in total protein content in both the experimental and control groups were attributable not to the composition of the hygiene products, but rather to the overall improvement in appliance and oral hygiene practices.

3. Microbiological examination of biofilm from the appliance surface revealed that maintaining hygienic conditions is more effective with regular use of an active oxygen-based cleansing agent, resulting in a twofold reduction in total microbial count and a 75% decrease in biofilm surface area. Therefore, the use of such specialized products should be recommended as part of routine care for removable orthodontic appliances.

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