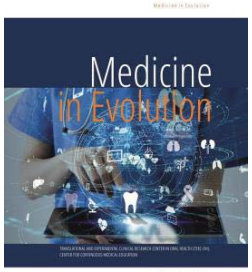
Bacterial colonization of removable orthodontic appliances



Luca M.M.[1], Nikolajevic-Stoican N.[1], Dinu Ş.[1], Buzatu R.[2], Dance F.[1], Popa M.[1]

[1]*Department of Pediatric Dentistry, Faculty of Dental Medicine, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania*
[2]*Department of Dental Aesthetics, Faculty of Dental Medicine, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania*

Correspondence to:
Name: Buzatu Roxana
Address: Bd. Revoluţiei 1989, no. 9, Timişoara, Romania
Phone: +40 721236147
E-mail address: roxana.buzatu@umft.ro

Abstract

Currently, orthodontic treatment represents an interest in biological and microbiological changes at the level of orthodontic devices. This paper presents a synthesis of an investigation into the existence of scientific evidence supporting the hypothesis that the presence of orthodontic appliances influences the oral microflora. The study was carried out in the Paediatric Dentistry Department of the Victor Babeş Timişoara University of Medicine and Pharmacy in the time interval 01.09.2021-01.03.2022, the patients being users of removable orthodontic appliances. Orthodontic appliances significantly influence the oral microflora, independent of the type of appliance, although removable appliances have been shown to have a lower impact on bacteria compared to fixed appliances. Orthodontic appliances are currently considered niches with bacteria, which determine the difficulty of performing oral hygiene, having a direct impact on the development of oral microflora at the level of saliva and supragingival dental plaque

Keywords: orthodontic appliance, elastomeric appliance, microflora, oral hygene

INTRODUCTION

Nowadays, although the continuous development of preventive techniques is advanced, more and more children are faced with poor hygiene, which over time leads to a loss of dental hard tissue due to the presence of bacteria, especially in the case of children who are users of orthodontic appliances [1]. The pathological condition given by the bacterial microflora has a major impact on the quality of life of patients, therefore prevention and awareness of the effects is very important. Removable orthodontic appliances influence the oral microflora, significant changes appear only 15 days after starting the treatment, and the initial increase of microorganisms was followed by a progressive decrease towards physiological values [2].

The composition of the oral microbial flora is influenced by several factors such as: diet, age, oral hygiene, presence of carious lesions, pregnancy, periodontal disease, genetic factors [3]. The oral cavity represents a unique environment in the human body, which is characterized by the presence of saliva, hard surfaces, temperature fluctuations and large variations in nitrogen and carbon intake. It is colonized by a complex microbiota that develops as biofilms on all mucosal and dental surfaces [1].

Bacteria in the oral cavity occupy the ecological niche that is provided by the surface of the teeth and the gingival epithelium. There is a dynamic balance between plaque bacteria and the host's defence system [1].

Saliva has a major influence on bacterial plaque, namely by mechanical cleaning of exposed oral surfaces, by buffering the acids produced by bacteria in the oral cavity and by controlling bacterial activity. Salivary secretions maintain the oral tissues in a physiological state and are considered protective in nature [4].

In the oral ecosystem, saliva contributes in many ways. The absorption of salivary glycoproteins is the result of the formation of the salivary film which results in the facilitation of bacterial adhesion. Saliva is a rich source of proteins and carbohydrates, it inhibits the growth of exogenous organisms, because non-specific defence factors, such as lysozyme and lactoferrin, and specific IgA, salivary leukocyte protease inhibitor (SLPI) are present at its level. Buffer capacity plays a major role in maintaining pH, and the acidity of saliva can favour the growth of cariogenic bacteria [5].

Orthodontic treatments are increasingly used not only to correct malocclusions, but also to improve all functions. Known for their benefits, they also have some disadvantages, namely: they produce a series of disorders in the oral cavity, also injuries. Their presence in the oral cavity modifies the oral ecosystem, as it provides:

- a space for the accumulation and retention of food;
- a different physico-chemical environment;
- surfaces for adhesion and attachment of the oral microflora forming the biofilm [2].

The introduction of an orthodontic appliance into the oral cavity will induce a change in the number and composition of the oral microflora. In addition to the growth of cariogenic bacteria, such as Streptococcus mutans, Lactobacilli, there is also an increase in oral yeasts. Colonization with Candida albicans is of interest among orthodontists due to the possible cariogenic effect of the yeast [6,7].

The modification of the oral microbiota is closely related to the application of orthodontic appliances. Their introduction into the oral cavity greatly inhibits oral hygiene and causes an increase in the number of retentive areas for bacterial plaque. Changes at the oral level are followed by changes such as an increase in bacterial concentration, change in buffer capacity, acid pH, and salivary flow. According to some studies, the influence that orthodontic appliances have on the oral cavity, the increase in the number of bacteria and also

the viability of S. mutans and lactobacilli species has been studied. However, the changes that occurred between the number of bacteria from the beginning of orthodontic treatment and during it vary [8].

Aim and objectives

The aim of the paper is to highlight any correlation between the qualitative and quantitative changes in the oral microflora and orthodontic appliances. Predisposing factors for the appearance of changes are bacterial plaque, carious lesions, periodontal disease as well as other infections that can have an impact on the oral health of patients wearing orthodontic appliances. The results of this work could motivate practitioners on periodic controls regarding oral health, and patients on the importance of oral hygiene to prevent the risks of carious lesions and juvenile periodontal disease.

MATERIAL AND METHODS

The study was carried out in the Paediatric Dentistry Department of the Victor Babeş Timişoara University of Medicine and Pharmacy in the time interval 01.09.2021-01.03.2022, the patients being users of removable orthodontic appliances.

The patients were not informed in advance about the exact date on which the collection was taking place in order not to influence the results by instituting hygiene of the oral cavity and of the orthodontic appliance, different from the usual practice, noted in the patient's record as patients with unsatisfactory hygiene.

1. Criteria for including patients in the study:

- patients aged between 8-14 years, regardless of gender, with unsatisfactory hygiene, without oral lesions, users of removable orthodontic appliances.

2. Criteria for excluding patients from the study

- patients younger or older than the 8-14 years range, patients not wearing appliances, with oral lesions, patients wearing removable appliances but with very good hygiene.

Two kits were used for the collection of microbiological samples, one of the pharyngeal exudate type and a Micro-IDent® and micro-IDent®plus kit.

The pharyngeal exudate kit contains an ethylene-sterilized, cotton-tipped plastic applicator, length 13x165 mm, used for collection from the device and oral cavity, immersed in a liquid of modified Stuart culture medium that allows preservation and transport and a clear plastic tube with a label so that it can be identified in the laboratory. This culture medium allows the identification of pathogens such as Neisseria gonorrhoeae, Haemophilus influenzae, S. mutans, C. albicans, Lactobacillus, etc.

The Micro-IDent® and micro-IDent®plus kit produced by HAIN is presented in a blue plastic box containing 4 sterile plastic containers, coded with the colours yellow, blue, green and red. The 4 containers contain endodontic paper cones, 4% taper, yellow code 15. The micro-IDent® and micro-IDent®plus test system used can identify from 5 and 11 species of periodonto-pathogenic bacteria as well as their concentrations. Simple testing includes the following pathogenic species: Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi). The test of 11 pathogenic species can identify: Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi), Parvimonas micra (Pm), Fusobacterium nucleatum (Fn), Campylobacter rectus (Cr), Eubacterium nodatum (En), Eikenella corrodens (Ec), Capnocythophaga sp. (Cs).

The microbiological collection was carried out using the two kits: pharyngeal exudate and Micro-IDent® from the level of orthodontic devices as follows:

- From the removable orthodontic devices, it was sampled the internal and external base and from the anchoring elements as springs and bows (Fig. 1)

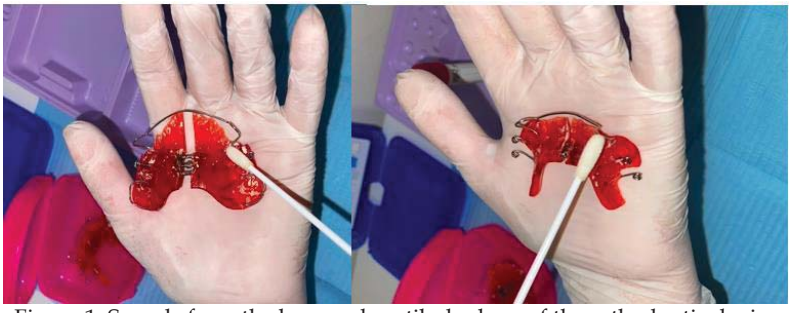


Figure 1. Sample from the base and vestibular bow of the orthodontic device

- Both faces and dental indentations were sampled from the elastomeric removable devices (Fig. 2).

Figure 2. Sample from the elastomeric orthodontic device

The pharyngeal exudate kit was used by brushing the applicator on the described surfaces for 15 seconds (Fig. 1,2), to be immersed in the transport medium from the provided container. The patient's initials were written on the test tube, to be sent within a maximum of 2 hours after collection to the Timişoara Bioclinica Laboratory.

The Micro-IDent® kit was used by brushing paper cones on the described surfaces for 15 seconds and with the help of color-coded containers they were collected from different surfaces as follows: red - bows and springs, yellow – internal base, blue – external base, green – front area of the device (Fig. 3).

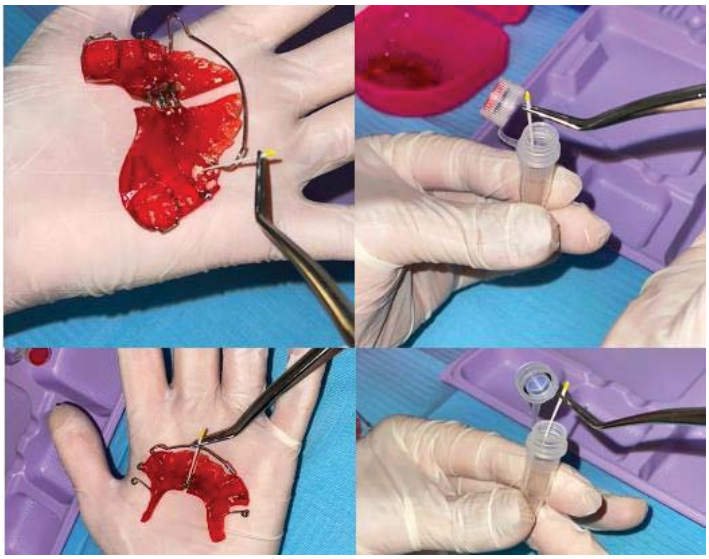


Figure 3. Sample from the base and screw of the orthodontic device

The elastomeric devices were sampled using the following coding: red – maxillary surface, yellow – mandibular surface, blue – maxillary anterior area, green – mandibular anterior area (Fig. 4).

Figure 4. Sample from the elastomeric orthodontic device

Test results were sent within 7 days for the exudate and 10 days for the Micro-IDent® test.

The patients were instructed about the importance of achieving hygiene of the orthodontic device and the oral cavity.

RESULTS

The results obtained after the sampling were received from the Timişoara Bioclinica Laboratory in the form of microbiological analysis (Table 1, Table 2).

Table 1. Results of exudate analyses

Patient	Exsudate results
Patient 1	Candida albicans
Patient 2	Stenotrophomonas maltophilia
Patient 3	Streptococcus mutans, Candida albicans
Patient 4	Staphylococcus aureus, Candida albicans
Patient 5	Lactobacillus acidophilus, Staphylococcus aureus

Table 2. Results of Micro-IDent® analyses. Legend: Very high = +++, Raised = ++, Low = +, Undetectable = –

Patient	Micro-IDent® results
Patient 1	Actinobacillus actinomiycetemcomitans (Aa) – Porphyromonas gingivalis (Pg) – Prevotella intermedia (Pi) – Bacteroides forsythus (Bf) ++ Treponema denticola (Td) + Peptostreptococcus micros + Fusobacterium nucleatum/periodonticum ++ Campylobacter rectus + Eubacterium nodatum – Eikenella corrodes ++ Capnocytophaga spec. +
Patient 2	Actinobacillus actinomiycetemcomitans (Aa) ++ Porphyromonas gingivalis (Pg) + Prevotella intermedia (Pi) – Bacteroides forsythus (Bf) + Treponema denticola (Td) ++ Peptostreptococcus micros + Fusobacterium nucleatum/periodonticum + Campylobacter rectus + Eubacterium nodatum – Eikenella corrodes ++ Capnocytophaga spec. +
Patient 3	Actinobacillus actinomiycetemcomitans (Aa) + Porphyromonas gingivalis (Pg) ++ Prevotella intermedia (Pi) + Bacteroides forsythus (Bf) ++ Treponema denticola (Td) – Peptostreptococcus micros – Fusobacterium nucleatum/periodonticum + Campylobacter rectus + Eubacterium nodatum + Eikenella corrodes + Capnocytophaga spec. +
Patient 4	Actinobacillus actinomiycetemcomitans (Aa) - Porphyromonas gingivalis (Pg) – Prevotella intermedia (Pi) + Bacteroides forsythus (Bf) + Treponema denticola (Td) ++ Peptostreptococcus micros + Fusobacterium nucleatum/periodonticum ++ Campylobacter rectus + Eubacterium nodatum – Eikenella corrodes + Capnocytophaga spec. –
Patient 5	Actinobacillus actinomiycetemcomitans (Aa) – Porphyromonas gingivalis (Pg) – Prevotella intermedia (Pi) ++ Bacteroides forsythus (Bf) ++ Treponema denticola (Td) ++ Peptostreptococcus micros + Fusobacterium nucleatum/periodonticum + Campylobacter rectus – Eubacterium nodatum + Eikenella corrodes ++ Capnocytophaga spec. –

DISCUSSIONS

Following the completion of the study, an increased presence of Candida albicans yeast was observed, in a percentage of 37%, which is part of the normal bacterial flora of the oral cavity but has potential as an opportunistic pathogen in the following situations: low immunity, poor oral hygiene, general diseases [9,10]. Streptococcus aureus has a high pathogenic potential, causing suppurative infections or septicaemia [11]. Patients with Streptococcus aureus need drug treatment, carried out after carrying out the antibiogram type analysis to establish resistance to the different classes of antibiotics. Streptococcus mutans is the main bacterium responsible for the development of carious processes, and the quantifiable number on an exudate type analysis denotes the presence of large numbers of colonies also at the level of the orthodontic device, diagnosing the patient with increased cariogenic potential [12] (Fig. 15).

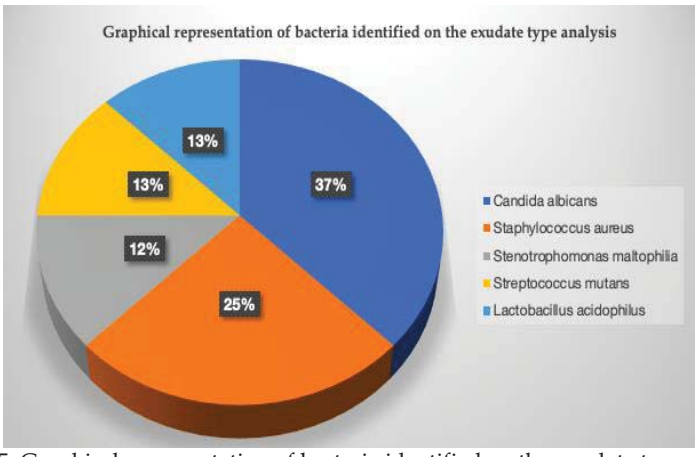


Figure 5. Graphical representation of bacteria identified on the exudate type analysis

Micro-IDent® analysis for the identification of germs associated with periodontitis revealed the majority presence of bacteria in the green and orange complexes that are recognized for inducing periodontal disease. The bacteria in the red complex are responsible for the production of gingival hypertrophy and the appearance of new blood vessels, with gingival bleeding as a clinical sign. The bacteria from all the complexes present in different numbers in the patients included in the study, quantitatively and qualitatively influence the bacterial biofilm, all of which are etiological factors of periodontal disease [13] (Fig. 16).

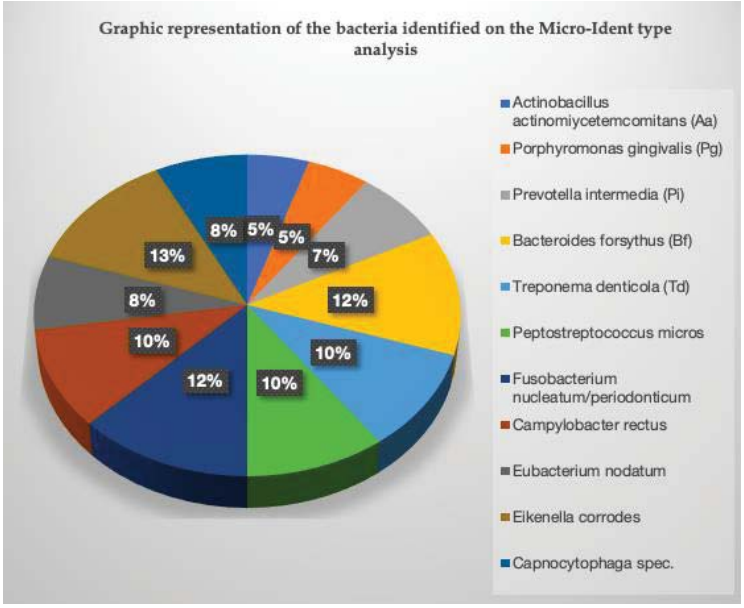


Figure 6. Graphical representation of bacteria identified on the Micro-Ident type analysis

Scientific publications have demonstrated that the presence of removable orthodontic appliances in the oral cavity of patients could change the nature of dental plaque and the colonization of oral microorganisms, leading to an increase in the microbial population, especially Streptococcus and Lactobacillus [14,15,16,17]. These may be associated with intraoral adverse effects on enamel and periodontal tissues. The use of appliances creates a favourable environment for the accumulation of microflora components and food residues, which, over time, can cause caries or aggravate any pre-existing periodontal disease. They can interfere with the practice of oral hygiene and can cover considerable parts of the tooth surfaces, so an increase in microflora has been reported in relation to orthodontic treatment [18,19,20].

According to a study conducted in 2011 by Asli Topaloglu-Ak et al. who investigated the effects of mobile, removable and fixed orthodontic appliances among 69 patients aged 6-17 years on mutant Streptococcus, Candida albicans and Lactobacillus sp. The introduction of any foreign object into the oral cavity, causes changes in the microbiological environment, as it provides multiple adhesion surfaces for the colonization of C. Albicans. The study concluded that orthodontic appliances are capable of increasing the bacterial and fungal population. Their long-term use increases the risk of periodontal disease, carious lesions and has a negative effect on the oral flora. In order to preserve oral health, periodic checks at short intervals, evaluation of oral hygiene to prevent caries are recommended [21].

The oral environment has the ability to adapt when the orthodontic appliance is inserted into the oral cavity. Several studies have shown an increase in stimulating flow, salivary pH and the buffering capacity of saliva, which lead to an increase in its anti-cariogenic property. There are studies that show that orthodontic appliances can increase the number of surfaces where plaque is accumulated by changing the bacterial flora, which leads to an increased risk of carious lesions [22,23,24].

Studies show an increased colonization for the fungal pathogen C. Albicans being frequently detected in patients wearing orthodontic devices. These devices increase the rate of oral carriage of the bacteria, and during treatment lead to a change in their number, our results also coincide with other studies investigating yeast growth [9,10,25].

Orthodontic appliances significantly influence the oral microflora, independent of the type of appliance, although removable appliances have been shown to have a lower impact on bacteria compared to fixed appliances. Orthodontic appliances are currently considered niches with bacteria, which determine the difficulty of performing oral hygiene, having a direct impact on the development of oral microflora at the level of saliva and supragingival dental plaque [26,27]. Compared to patients not wearing orthodontic appliances, patients during treatment reported the existence of qualitative and quantitative changes in supra- and subgingival bacterial plaque throughout the period of orthodontic treatment [28].

CONCLUSIONS

Following this study carried out on 5 patients aged between 8-14 years, we came to the conclusion that the quantitative and qualitative changes in the microbial biofilm present among patients wearing orthodontic removable devices appear from the first week of wearing and become more and more increased during the treatment, with a higher colonization of the following species in order: the orange complex (Fusobacterium, Prevotella and Campylobacter spp), green complex (Eikenella corrodens A. actinomycetemcomitans, Capnocytophaga spp.), and then that of red complex (P. gingivalis, T. forsythia and T. denticola). Patients treated with orthodontic devices are advised to improve their oral hygiene and to attend periodic check-ups, especially in the first months of treatment.

The increased need for orthodontic treatments these days also involves the awareness of family and patients about the need to achieve good hygiene because the specificity of the materials orthodontic removable devices are made of, have an increased porosity and thus, in the absence of compliance with maintenance indications, they contribute negatively to the development and multiplication of a large number of potentially pathogenic and cariogenic bacteria.

REFERENCES

1. Lucchese A, Bondemark L, Marcolina M, Manuelli M. Changes in oral microbiota due to orthodontic appliances: a systematic review. J Oral Microbiol. 2018 Jul 3;10(1):1476645.
2. Lucchese, A.; Bonini, C.; Noviello, M.; et al. The Effect of Removable Orthodontic Appliances on Oral Microbiota: A Systematic Review. Appl. Sci. 2021, 11, 2881
3. Majumdar S, Singh AB. Normal Microbial Flora of Oral Cavity. J Adv Med Dent Scie Res 2014;2(4):62-66.
4. Ogodescu, A. S., Morvay, A. A., Luca, M. M., et al, I. (2014). Quantification of Biofilms formed by Candida spp. on Two Types of Plastic Materials used in Pediatric Dentistry and Orthodontics. Revista de Materiale Plastice, 51(4), 424-7.
5. Forssten, S. D., Björklund, M., & Ouwehand, A. C. (2010). Streptococcus mutans, caries and simulation models. Nutrients, 2(3), 290-298.
6. Patil, S., Rao, R. S., Amrutha, N., et al. (2013). Oral microbial flora in health. World J Dent, 4(4), 262-6
7. Ahirwar SS, Gupta MK and Snehi SK: Dental caries and lactobacillus: role and ecology in the oral cavity. Int J Pharm Sci & Res 2019; 10(11): 4818-29. doi: 10.13040/IJPSR.0975-8232.10(11).4818-29.
8. Saloom, H. F., Mohammed-Salih, H. S., & Rasheed, S. F. (2013). The influence of different types of fixed orthodontic appliance on the growth and adherence of microorganisms (in vitro study). Journal of clinical and experimental dentistry, 5(1), e36.
9. Tsui C, Kong EF, Jabra-Rizk MA. Pathogenesis of Candida albicans biofilm. Pathog Dis. 2016 Jun;74(4):ftw018.
10. Byadarahally Raju, S., & Rajappa, S. (2011). Isolation and identification of Candida from the oral cavity. International Scholarly Research Notices, 2011.

11. Klaus, K., Eichenauer, J., Sprenger, R. et al. Oral microbiota carriage in patients with multibracket appliance in relation to the quality of oral hygiene. Head Face Med 12, 28 (2016).
12. Contaldo, M., Lucchese, A., Lajolo, C., Rupe, C., Di Stasio, D., Romano, A.,... & Serpico, R. (2021). The oral microbiota changes in orthodontic patients and effects on oral health: An overview. Journal of Clinical Medicine, 10(4), 780.
13. Michael G. Newman, Fermin A. Carranza, Henry Takei: Clinical Periodontology, ediţia 13, 2018
14. Pathak, A. K., & Sharma, D. S. (2013). Biofilm associated microorganisms on removable oral orthodontic appliances in children in the mixed dentition. Journal of Clinical Pediatric Dentistry, 37(3), 335-340.
15. Jing, D., Hao, J., Shen, Y., et al. (2019). Effect of fixed orthodontic treatment on oral microbiota and salivary proteins. Experimental and therapeutic medicine, 17(5), 4237-4243.
16. Perkowski, K., Balraza, W., Conn, D. B., et al. (2019). Examination of oral biofilm microbiota in patients using fixed orthodontic appliances in order to prevent risk factors for health complications. Annals of Agricultural and Environmental Medicine, 26(2).
17. Condò, R., Casaglia, A., Condò, S. G., et al. (2012). Plaque retention on elastomeric ligatures. An in vivo study. Oral & implantology, 5(4), 92.
18. Sarul, M., Kozakiewicz, M., & Jurczyszyn, K. (2021). Surface evaluation of orthodontic wires using texture and fractal dimension analysis. Materials, 14(13), 3688.
19. Sifakakis, I., & Eliades, T. (2017). Adverse reactions to orthodontic materials. Australian Dental Journal, 62, 20-28.
20. Hsu, K. L., Balhaddad, A. A., Garcia, I. M., et al. (2020). Assessment of surface roughness changes on orthodontic acrylic resins by all-in-one spray disinfectant solutions. Journal of Dental Research, Dental Clinics, Dental Prospects, 14(2), 77.
21. Topaloglu-Ak, A., Ertugrul, F., Eden, et al. (2011). Effect of orthodontic appliances on oral microbiota—6 month follow-up. Journal of Clinical Pediatric Dentistry, 35(4), 433-436.
22. Bechir, A., Pacurar, M., Bechir, E. S., et al (2014). Aesthetic importance of resin based dental materials used for orthodontic appliances. Mater Plast, 51, 57-61.
23. Matei, M., Dimofte, A. R., Ionuta, G., et al. (2020). The role of plastics in orthodontics orthodontic appliances. Romanian Journal of Oral Rehabilitation, 12(4), 292-299.
24. Collares, F. M., Rostirolla, F. V., Macêdo, É. D. O. D. D., et al. (2014). Influence of mouthwashes on the physical properties of orthodontic acrylic resin. Brazilian Journal of Oral Sciences, 13, 203-208.
25. Chang, H. S., Walsh, L. J., & Freer, T. J. (1999). The effect of orthodontic treatment on salivary flow, pH, buffer capacity, and levels of mutans streptococci and lacto bacilli. Australian orthodontic journal, 15(4), 229-234.
26. Vital, S. O., Haignere-Rubinstein, C., Lasfargues, J. J., & Chaussain, C. (2010). Caries risk and orthodontic treatment. International orthodontics, 8(1), 28-45.
27. Hägg, U., Kaveewatcharanont, P., Samaranayake, Y. H., & Samaranayake, L. P. (2004). The effect of fixed orthodontic appliances on the oral carriage of Candida species and Enterobacteriaceae. The European Journal of Orthodontics, 26(6), 623-629.
28. Dallel, I., Merghni, A., Ben Tanfouss, S., et al. (2019). The effect of orthodontic appliances on oral microflora: A case-control study. Oral Science International, 16(1), 29-34.