The formation of dental plaque is a multi-stage process beginning with the adsorption of salivary protein to the cleaned surface of the tooth. In this way the so-called acquired enamel pellicle is formed [1], which after 1–2 days is inhabited by oral cavity bacteria. The colonizing bacteria create 1–20 layers, with Gram-positive cocci (Streptococcus spp.) prevailing. Towards the apex, the plaque is also inhabited by filamentous and fusiform bacteria and a very small number of Gram-negative cocci. The bacteria inhabiting this layer are classified as aerobes and facultative anaerobes (obligate anaerobes are very few) [2].

Further development of the plaque is characterized by an increase of the number of layers, up to approximately 200–300, as well as a rise in the number of anaerobes. Stage 3 is plaque maturation. The number of layers amounts to over 300, the thickness of the plaque is 0.5 mm and it is visible with the naked eye. The chemical composition of the plaque changes, spirochetes and flagellated bacteria occur. The population of Gram-negative bacteria also increases.

For over 100 years, research has been conducted aimed at an explanation of the influence of bacteria on the etiopathogenesis of periodontal diseases. The gingival sulcus is inhabited by over 500 bacteria species but, according to the research, only some of them play a significant role in the initiation and course of a periodontal disease [4]. A normal gingival sulcus contains a prevalent number of Gram-positive bacteria whereas in gingival pockets
Bacteria in the subgingival plaque prevail [5, 6]. In the 1980s, the research focused on identification of a single bacterial species which would induce periodontal diseases [6]. Contrary to this hypothesis there was another one suggested – a non-specific plaque theory stating that all the bacteria in dental plaque are involved in causing periodontal diseases [7]. In the 1990s, the specific plaque hypothesis presented by Socransky [8] discarded the previous two opposite hypotheses. According to Socransky’s theory, inflammation of periodontal tissues is induced by bacterial complexes comprising some specific species. These bacteria include Gram-negative anaerobes: Porphyromonas gingivalis (P.g.), Prevotella intermedia (P.i.), Tannerella forsythia (T.f.), Aggregatibacter actinomycetemcomitans (A.a), Capnocytophaga ochracea, Eikenella corrodens (E.c.), Campylobacter rectus (C.r.), Fusobacterium nucleatum (F.n.), and Treponema denticola (T.d.). The research conducted in the last 25 years has demonstrated that the presence of the above-mentioned bacteria indicates a positive correlation with clinical symptoms such as gingival inflammation, and clinical loss of the connective tissue attachment as well as increased depth of the gingival pocket [6]. Socransky [8] demonstrated that bacteria related to periodontal diseases form several complexes: red, orange, species associated with the orange complex, green, and the Aggregatibacter actinomycetemcomitans (A.a.) complex, which show different pathogenicity. Bacteria of the red complex demonstrate a powerful correlation with the depth of gingival pockets. The composition of complexes is presented in Table 1. The complexes are related to one another by numerous nutritional dependences as well as defense mechanisms.

Table 1. Periopathogenic complexes, their bacterial composition and pathogenicity [9–11]

<table>
<thead>
<tr>
<th>Type of complex</th>
<th>Composition</th>
<th>Pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange complex</td>
<td><em>Prevotella intermedia</em>&lt;br&gt;<em>Parvimonas micra</em>&lt;br&gt;<em>Fusobacterium nucleatum</em>&lt;br&gt;<em>Campylobacter rectus</em>&lt;br&gt;<em>Eubacterium nodatum</em></td>
<td>moderate to high pathogenicity</td>
</tr>
<tr>
<td>Species associated with the orange complex</td>
<td><em>Streptococcus gordonii</em>&lt;br&gt;<em>Streptococcus intermedius</em>&lt;br&gt;<em>Streptococcus sanguis</em></td>
<td>moderate to high pathogenicity</td>
</tr>
<tr>
<td>Red complex</td>
<td><em>Treponema denticola, Porphyromonas gingivalis</em>&lt;br&gt;<em>Tannerella forsythia</em></td>
<td>high pathogenicity</td>
</tr>
<tr>
<td>Green complex</td>
<td><em>Capnocytophaga gingivalis</em>&lt;br&gt;<em>Capnocytophaga ochracea</em>&lt;br&gt;<em>Capnocytophaga spatigera</em></td>
<td>moderate pathogenicity</td>
</tr>
<tr>
<td>A.a. complex</td>
<td><em>Aggregatibacter actinomycetemcomitans</em></td>
<td>very high pathogenicity</td>
</tr>
</tbody>
</table>

The research demonstrated that the highest pathogenicity was shown by the Aggregatibacter actinomycetemcomitans complex and species included in the red complex such as *Treponema denticola, Porphyromonas gingivalis*, and *Tannerella forsythia*. Whereas the role of the orange complex is to prevent formation of the red complex, bacteria included in the red complex are believed to be marker bacteria of chronic periodontitis. Ximénez-Fyvie et al. [9] analyzed the difference between the composition of supragingival and subgingival plaque in patients with periodontal diseases. A total of 1170 plaque samples were collected for the qualitative and quantitative analysis of 40 bacterial species. A DNA probe as well as a DNA-DNA hybridization technique were used in this study to identify bacterial genomes. All the analyzed bacteria were found – 40 species. The Aggregatibacter actinomycetemcomitans were the most commonly isolated bacteria from both the plaque types, the supragingival and subgingival, although in the subgingival plaque *A. naeslundii* was found more often. The prevalent complexes in the supragingival plaque compared to the subgingival plaque were the green and yellow complexes as well as the complex with Aggregatibacter actinomycetemcomitans, whereas in the subgingival plaque the red and orange complex prevailed. According to the authors mentioned above, the supragingival plaque may constitute a source of infection as well as secondary infection of the periodontal tissues (Table 1).

For many years research has been conducted to develop a test that would allow effective and fast detection of bacteria and their metabolites in the gingival pockets. The available tests are based on methods such as microscopic observation of the colonies, bacterial cultures grown in a medium, immunological, enzymatic, and genetic methods [6]. Until now, no perfect method has been de-
Evaluation of Pathogenic Bacteria Occurring in Subgingival Plaque in Patients Using Orthodontic Appliances

A review of the available studies presented in Table 2 shows that interest in periopathogens occurring in gingival pockets during orthodontic treatment began at the end of the 1990s. Paolantonio et al. [13] tried to establish whether the use of a fixed orthodontic appliance may affect the increase of the number of Aggregatibacter actinomycetemcomitans in the subgingival plaque. The authors qualified 24 patients with a similar malocclusion for the study to collect their plaque samples. The orthodontic appliance was placed only on a single dental arch, the other one was the control arch. The samples were collected before the orthodontic appliance was placed on the arch, after 4, 8, and 12 weeks of treatment. Then the appliances were removed and after another 4 weeks further tests were carried out. The authors found increased inflammation indices as well as deteriorated oral hygiene during the orthodontic treatment. Where- as A.a., which before treatment was isolated from the gingival pockets of the dental arch with a fixed orthodontic appliance only in one patient, was found in 19 patients after 4 weeks, in 20 patients after 8 weeks, and in 7 patients after 4 weeks of the appliance removal. In the control arch, A.a. was isolated from one place during the study. The authors concluded that the appliance on an arch promoted an increase of the number of the A.a. bacteria in the gingival pockets of orthodontically moved teeth. According to the authors, the increase of the number of the A.a. bacteria did not affect its occurrence in other locations of the oral cavity.

The development of molecular biology methods have made it possible to replace bacteria isolation with the use of culture on media with methods that are faster and more accurate, and show more sensitivity and species specificity. Ximénez-Fyvie et al. [10] compared the microbiological composition of supragingival and subgingival plaque in adults. The study groups were comprised of patients with normal periodontal tissues as well as patients with periodontitis. Forty bacterial species from the microbial plaque were analyzed with the use of specific genome DNA probes. The molars, with the third molars excluded, underwent clinical evaluation for change in gingiva color, accumulation of dental plaque, bleeding on probing, and the grade of clinical loss of connective tissue attachment as well as the depth of the gingival pocket. The study demonstrated that in both study groups the supragingival plaque contained P.g., Tannerella forsythia (T.f.) and Treponema denticola (T.d.). Whereas the subgingival plaque in subjects with a periodontal disease showed increased numbers of P.g., and Tannerella forsythia (T.f.), as well as Prevotella, Fusobacterium, Campylobacter genera, in the samples of subgingival and supragingival plaque collected from subjects with a periodontal disease, an increase of the number of the following bacteria was found: P.g., T.f., and T.d. Sallum et al. [14] evaluated the periodontium and microbiological composition of gingival pockets in 10 patients (aged 18 ± 1.8 years of age) at the final stage of treatment with fixed appliances. The patients showed clinical symptoms of gingivitis. The authors analyzed 5 periopathogens (P.g., T.f., A.a., P.i., P.n.) both, in the supragingival plaque as well as the subgingival plaque. In regard of the periopathogens mentioned above, after 30 days of the fixed appliance removal, the most apparent was the decrease of the number of A.a. and T.f. The authors demonstrated improvement of the clinical condition of the gingiva in a month after.
the appliance was removed as well as a statistically significant difference in reducing the size of gingival pockets from 2.5 mm ± 0.51 to 1.92 mm ± 0.42 in patients treated with fixed orthodontic appliances. After 30 days of the appliance removal, the GI dropped from 100% to 23.3%.

Different results were presented by Narango et al. [15]. They examined similar parameters but they conducted the study before and after 3 months of fixation of the orthodontic appliance elements on the teeth in a 30-subject study group and 30-subject control group. The authors did not find a statistically significant difference in the depth of gingival pockets or the clinical loss of the connective tissue attachment during the study periods. An increase of gingival indices was found as well as the plaque index 3 months after the treatment was started. Additionally, changes within the microflora of the subgingival plaque were found with an increased number of the following bacteria: Porphyromonas gingivalis, Prevotella intermedia/Prevotella nigrescens, Tannerella forsythia, and Fusobacterium species.

Ristic et al. [16], in a prospective study, compared clinical parameters (PI, GI, GBI, PD) and microbiological analysis in 32 patients aged 12–18, treated with fixed appliances. A comparative analysis was performed before the treatment, 3 weeks, 1 month, 3 and 6 months after the treatment with a fixed appliance was started. The authors demonstrated an increase of clinical and bacteriological parameters after commencement of the treatment. Rather surprising was the observation they made, that beginning with treatment month 3, gingival indices and plaque indices started to decrease in value. The investigators observed an increase of the number of periopathogens but they did not find a clinical loss of connective tissue attachment.

Another study by Lee et al. [17] presented an increased number of periopathogens in the subgingival plaque in patients during orthodontic treatment (17 subjects in the study group, 19 subjects in the control group). The study demonstrated an increased T.f., T.d., and P.n. in subjects during orthodontic treatment. According to the authors, treatment with a fixed appliance may affect the increase of periopathogens in the subgingival plaque.

There are few published studies which evaluate the clinical parameters and provide microbiological evaluation to compare the flora of the gingival pockets of teeth with fixed bands and teeth with brackets. In one of the studies, the authors [18] assessed the periodontium in 33 patients aged 12–18. The subjects had been using orthodontic appliances for at least 6 months. The authors investigated clinical condition and evaluated subgingival flora in premolars with brackets compared to molars with cemented bands. The bands on the investigated teeth were cemented subgingivally or supragingivally. The study did not provide a comparison with a control group nor the inclusion criteria for the gingival pockets. The authors demonstrated that all the patients in the study showed 45.0% of gingival pockets smaller than 2 mm, 50.8% with a depth of 3 mm, 4.2% of pockets with a value exceeding 4 mm and one case exceeding 5 mm. The difference between the deeper pockets around the teeth with bands and more shallow pockets around those with brackets was statistically significant. The PI measurement did not indicate a statistically significant difference between the groups, whereas the GI measurement showed higher values in the case of teeth with cemented bands.

Demling et al. [19] tried to clarify the influence of supragingival flora on the subgingival flora. Apart from clinical parameters (API and SBI), they evaluated the surfaces of 28 orthodontic bands in patients treated with fixed appliances. The investigators demonstrated that 16.1% ± 9.2 of the supragingival surface and 3.6% ± 4.4 of the subgingival surface was covered with biofilm. The difference was statistically significant. The biggest amount of biofilm in the subgingival zone was observed within the areas of notches in the orthodontic bands. The authors drew a conclusion that most probably the immunological response of the gingival fluid mediators inhibits bacterial adhesion to the subgingival surface of the bands.

Thornberg et al. [20] conducted an analysis of 190 subgingival plaque samples in orthodontically treated patients in 5 study periods (the mean age of the subjects was 13 years and 6 months). The subgingival plaque samples were collected before the treatment was started, after 3 months of treatment, after 6 months and after 12 months of treatment and after approximately 3 months of the fixed appliance removal. The authors analyzed the composition of the subgingival flora for periopathogenic bacteria: Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Eikenella corrodens, Fusobacterium nucleatum, Treponema denticola, and Campylobacter rectus. Six of the eight periopathogens (P.i., T.f., E.c., F.n., T.d., C.r.) increased considerably in treatment month 6 but the authors stated that their levels returned to the baseline values after 12 months.

Research by Kloehn and Pfeifer [21], Boyd et al. [22], and Sallum et al. [14] demonstrated that after the orthodontic appliance is placed, many patients developed generalized gingivitis regardless of the type of their malocclusion, the type of
appliance, its components or the type of the adhesive. The occurrence of new retentive sites promotes increased bacterial plaque accumulation. There are available studies referring to monitoring the indices of periodontium condition (Plaque Index, Gingival Index, PD), whereas the studies investigating the microflora of gingival pockets are fewer and the research comparing the condition of the periodontium and the microflora of the gingival pockets in patients who are using fixed and removable orthodontic appliances is almost non-existent [14, 23, 24].

A systematic analysis of the literature showed that the clinical parameters of periodontium condition in patients using orthodontic appliances most commonly investigated in the research of

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Clinical parameters</th>
<th>Bacterial species</th>
<th>Diagnostic method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>Ximénez-Fyvie et al. [10]</td>
<td>40 bacterial species</td>
<td>DNA probes</td>
<td>differences between supra and subgingival plaque were in the proportions and levels of Actinomyces, „orange” and „red” complex species</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Asai et al. [26]</td>
<td>T.d., T.v., T.m.</td>
<td>Real-time PCR</td>
<td>T.d. cells were detected in plaque samples from deep pockets</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Lee et al. [17]</td>
<td>PI, GI, PD, CAL, BOP</td>
<td>A.a., T.d., P.g., T.f., P.n., P.i.</td>
<td>16S rDNA PCR</td>
<td>T. forsythia, T. denticola, and P. nigrescens were significantly more common in the samples obtained from the orthodontic patients</td>
</tr>
<tr>
<td>7.</td>
<td>Naranjo et al. [15]</td>
<td>PI, GI, PD, CAL, BOP,</td>
<td>P.g., P.i., P.n., T.f., Fusobacterium sp.</td>
<td>Microbial culture</td>
<td>bracket placement influences the colonization of important periodontopathic bacteria</td>
</tr>
<tr>
<td>8.</td>
<td>Ristic et al. [16]</td>
<td>PD, CAL</td>
<td>P.i., A.a., P.g., F.n.</td>
<td>PCR</td>
<td>total number of periodontopathic anaerobes increased during 3 months treatment with fixed appliances</td>
</tr>
<tr>
<td>9.</td>
<td>Thornberg et al. [20]</td>
<td>-</td>
<td>A.a., P.g., P.i., T.f., E.c., F.n., T.d., C.r.</td>
<td>DNA probes</td>
<td>for 6 (P.i., T.f., E.c., F.n., T.d., C.r.) of the 8 pathogens, the percentages of subjects with high pathogen counts increased after 6 months of fixed appliance treatment</td>
</tr>
<tr>
<td>10.</td>
<td>Choi et al. [27]</td>
<td>-</td>
<td>A.a., T.f., C.r., E.c., P.g., P.i., P.n., T.d.</td>
<td>16 rRNA PCR</td>
<td>reduction of sites positive for C.r. and E.c. after appliance removal</td>
</tr>
<tr>
<td>11.</td>
<td>Demling et al. [19]</td>
<td>BOP, PD, PI</td>
<td>A.a., P.g.</td>
<td>PCR</td>
<td>prevalence of A.a. and P.g. remained unchanged after 3 months of fixed appliance treatment</td>
</tr>
<tr>
<td>12.</td>
<td>Demling et al. [28]</td>
<td>API, SBI</td>
<td>Biofilm occurrence on the orthodontic bands</td>
<td>Electron microscopy</td>
<td>no mature subgingival biofilm was found on the orthodontic bands</td>
</tr>
<tr>
<td>13.</td>
<td>Atassi and Awaratani [29]</td>
<td>PI, OPI, GBI</td>
<td>-</td>
<td>Clinical examination</td>
<td>PI and OPI were high with mean scores</td>
</tr>
<tr>
<td>14.</td>
<td>Kim et al. [18]</td>
<td>PI, BOP</td>
<td>37 species</td>
<td>DNA-DNA hybridization</td>
<td>deeper pockets were found around orthodontic bands</td>
</tr>
<tr>
<td>15.</td>
<td>van Gastel et al. [30]</td>
<td>PD, BOP</td>
<td>Aerobe/anaerobe ratio</td>
<td>Bacterial cultures</td>
<td>relatively more anaerobes at T2 (bracket removal) compared to T1 (baseline)</td>
</tr>
</tbody>
</table>
1970s were as follows: PI, GI, BOP, and PD. Other parameters assessed in the studies included CAL, GBI (Gingival Bleeding Index), OPI (Ortho-Plaque Index), etc (Table 2).

### Conclusion

As the above literature review implies, many questions still remain unanswered but the increase of periopathogens during treatment with orthodontic appliances is probable. The literature review demonstrated that they were often the red complex bacteria, which were perceived as the ones showing high pathogenicity. Many authors stated that changes in periodontal tissues might occur during orthodontic treatment. There are few publications referring to young children (9–14 years of age); the number of comparisons of patients treated with fixed and removable orthodontic appliances are also scarce. The comparison of periodontal tissue condition and oral hygiene in children treated with fixed and removable orthodontic appliances would allow implementation of preventive and follow-up measures during the therapy as well as provide an answer to a question whether subgingival microbial flora diagnostic testing is justified to prevent periodontal complications.

### References


Address for correspondence:
Agnieszka Elżbieta Osmólska-Bogucka
Department of Orthodontics
Warsaw Medical University
Nowogrodzka 59
02-006 Warsaw
Poland
E-mail: agabog@mp.pl

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