RESEARCH PAPER

Assessment of salivary microbial levels and gingival status of patients wearing aligners and fixed orthodontic appliance: A Prospective Comparative Study

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ABSTRACT

Background: Fixed orthodontic appliances create numerous plaque-retentive surfaces that favour cariogenic and periodonto-pathogenic organisms, whereas clear aligners, being removable, are thought to allow better hygiene maintenance. Prospective comparisons of fixed appliances, self-ligating brackets, and clear aligners that examine both salivary microbial counts and gingival health together, particularly during the earliest days of treatment, remain limited.

Aim: To compare salivary *Streptococcus mutans* and *Lactobacillus* levels and gingival status among patients treated with fixed appliances, self-ligating brackets, and clear aligners.

Materials and Methods: Thirty patients undergoing orthodontic treatment at the Department of Orthodontics and Dentofacial Orthopaedics, Mahatma Gandhi Dental College and Hospital, Jaipur, were allocated to three groups of ten fixed appliances (Group 1), self-ligating brackets (Group 2), and clear aligners (Group 3). Gingival Index, Plaque Index, Bleeding on Probing, and gingival (probing) depth were recorded, and saliva was cultured on Mitis Salivarius and MRS agar for *S. mutans* and *Lactobacillus*, respectively, before treatment (T1), on day 3 (T2), and on day 10 (T3). Friedman, Wilcoxon signed-rank (Bonferroni-corrected), Kruskal–Wallis, and Cochran's Q tests were used as appropriate.

Results: The three groups were statistically indistinguishable at baseline. By T2 and T3, highly significant differences had emerged for every parameter ($p<0.001$ for most comparisons). The fixed appliance group showed the steepest rise in *S. mutans* and *Lactobacillus* counts, plaque and gingival scores, and bleeding on probing, followed by the self-ligating group; the clear aligner group changed the least, and its bleeding-on-probing and gingival-depth scores did not reach statistical significance over the ten-day period.

2: Within the limits of this short-term study, clear aligners were associated with a distinctly more favourable microbial and periodontal profile than fixed or self-ligating appliances during the initial phase of orthodontic treatment, supporting careful appliance selection in patients at elevated caries or periodontal risk.

Keywords: Clear aligners; Fixed orthodontic appliances; Self-ligating brackets; Streptococcus mutans; Lactobacillus; Gingival index; Plaque index; Bleeding on probing

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INTRODUCTION

Orthodontic care today is shaped as much by patient expectations of comfort and appearance as by the biomechanics of tooth movement, and this shift has driven a steady expansion of appliance options beyond the conventional metal bracket[1]. Whatever the appliance, though, introducing any device into the mouth changes its microbial landscape, and these changes are not merely cosmetic — they can determine whether a patient completes treatment with healthy gingivae and sound enamel or with white-spot lesions and early periodontal disease[2,3].

The oral cavity is host to a dense, largely commensal microbial community that, left undisturbed, keeps pathogenic colonisation in check[4]. Fixed appliances disturb this balance mechanically: brackets, bands, ligatures, and archwires add surface area and create undercuts that are difficult to clean, and biofilm accumulates preferentially at these sites. The clinical

consequence has been documented repeatedly, with enamel demineralisation reported in anywhere from a small fraction to nearly the entire treated population depending on hygiene and appliance design, and white-spot lesions appearing in more than a third of patients within the first year of fixed therapy[5]. Two organisms are chiefly implicated: *Streptococcus mutans* and *Lactobacillus* species, both acid-producing and both especially at home on the retentive surfaces that fixed appliances create[4]. *Lactobacillus* in particular is an acid-tolerant, environmentally versatile genus its probiotic and fermentative properties are well characterised even outside the mouth, in foods such as regional yoghurts[36] but inside the mouth its proliferation tracks closely with the surface area available for colonisation.

Clear aligners first marketed in the late 1990s and now a mainstream option for mild-to-moderate malocclusion, aligners are removable, which in principle lets patients brush and floss without the obstruction that fixed hardware presents. Their thermoplastic material itself does not appear to be the deciding factor: in-vitro comparisons of several PETG-based aligner polymers against enamel and stainless-steel brackets found no meaningful difference in early bacterial adhesion or 72-hour biofilm formation[6], suggesting that whatever clinical advantage aligners hold comes from removability rather than from the material surface.

The comparative literature supports such an advantage. In one of the larger prospective comparisons, 37.5% of patients treated with multibracket fixed appliances carried high-risk salivary *S. mutans* counts (>10^5 CFU/mL) after six months, against only 8% of aligner-treated patients corresponding to an odds ratio of roughly 7 for *S. mutans* and over 20 for *Lactobacillus* in the fixed-appliance group[7]. What remains less well characterised is how quickly this divergence appears, and how self-ligating brackets marketed as a hygienic improvement over conventional ligation compare with both extremes during the very first days of treatment, before longer-term adaptation or improved patient technique can narrow the gap. This study was designed to address that question by tracking salivary *S. mutans* and *Lactobacillus* levels alongside standard gingival indices in patients treated with fixed appliances, self-ligating brackets, and clear aligners, assessed before treatment and again at three and ten days afterward.

AIM AND OBJECTIVES

Aim: To assess salivary microbial levels and gingival status in patients wearing clear aligners, self-ligating brackets, and fixed orthodontic appliances.

Objectives:

1. To evaluate salivary levels of Streptococcus mutans and Lactobacillus in the three appliance groups.
2. To evaluate gingival status using standard gingival indices at three time points — before treatment (T1), three days after (T2), and ten days after (T3).

MATERIALS AND METHODS

Study design and setting

This prospective clinical study was carried out over six months (1 July – 31 December 2025) in the Department of Orthodontics and Dentofacial Orthopaedics, Mahatma Gandhi Dental College and Hospital, Jaipur. The study was conducted following institutional ethics committee approval, and written informed consent was obtained from every participant before enrolment.

Participants

Thirty patients already scheduled for orthodontic treatment were recruited. Inclusion required good general health and good baseline oral hygiene in patients undergoing fixed, self-ligating, or clear aligner therapy; patients were excluded if they were caries-prone, had used oral antimicrobial agents or antibiotics in the preceding three months, smoked, or had periodontal or systemic disease. Eligible patients were assigned to one of three groups of ten: Group 1, conventional fixed appliances with MBT prescription brackets; Group 2, self-ligating brackets; and Group 3, clear aligners. All participants received oral hygiene instruction before baseline recording, and fluoride mouth rinse was not permitted during the study period so that it would not confound the microbial findings. Patient data were coded serially rather than linked to name or identification number, to limit assessment bias.

Time points

Each participant was assessed three times: T1, immediately before appliance placement; T2, three days after placement; and T3, ten days after placement.

Clinical assessment

Gingival status was scored with the Gingival Index of Löe and Silness, which grades four gingival units around each tooth from 0 (no inflammation) to 3 (spontaneous bleeding, marked oedema)[8]; individual scores were averaged over all examined teeth, with 0.1–1.0 taken as mild gingivitis, 1.1–2.0 as moderate, and 2.1–3.0 as severe. Plaque was scored on the same 0–3 scale using the Silness and Löe index applied to the anterior index teeth[8]. Bleeding on probing was recorded with a William's periodontal probe walked gently around the sulcus, following the interdental bleeding criteria described by Carter and Barnes, and allowing 30–60 seconds after probing for bleeding to appear[9], consistent with the recognised if imperfect value of bleeding on probing as an early marker of periodontal inflammation[10]. Probing (gingival sulcus) depth was measured with the same probe, with 0–3 mm regarded as physiological.

Microbiological assessment

Saliva was collected by pipette into a sterile, serially coded container at each time point and transported to the microbiology laboratory within two hours. Samples were inoculated onto Mitis Salivarius agar for *Streptococcus mutans* and MRS agar for *Lactobacillus*, using the selective culture principles established for these media[11], and incubated at 37 °C for 36 hours. Colonies were scored using a standard chairside model chart: 0 for $<10^4$ CFU/mL, 1 for $<10^5$ CFU/mL, 2 for 10^5–10^6 CFU/mL, and 3 for $>10^6$ CFU/mL.

Statistical analysis

Because the sample size per group was small (n = 10) and the outcome variables were ordinal, non-parametric methods were used throughout. Within-group change across the three time points was analysed with the Friedman test, with Wilcoxon signed-rank post-hoc comparisons (Bonferroni-corrected) for pairwise contrasts, and Cochran's Q test was applied as a complementary check for the binary bleeding-on-probing outcome. Between-group comparisons at each time point were made with the Kruskal–Wallis test.

RESULTS

Table 1. Descriptive trend of clinical and microbial variables across the study period

Variable	T1	T2	T3	Overall trend
S. mutans	Score 1	Score 2	Score 3	Progressive increase
Lactobacillus	Score 1	Score 2	Score 3	Progressive increase
Gingival Index	Mild	Moderate	Severe	Progressive worsening
Plaque Index	Mild plaque	Moderate plaque	Heavy plaque	Plaque accumulation increased
Bleeding on probing	Positive	Reduced	Minimal	Declining bleeding tendency
Gingival depth	1 mm	2 mm	3 mm	Severity progressed

Table 1 summarises the direction of change across all three groups combined: scores for S. mutans, Lactobacillus, Gingival Index, Plaque Index, and gingival depth rose steadily from T1 to T3, while bleeding tendency declined as gingival health improved in the better-performing groups.

Across all three groups, baseline values were statistically indistinguishable (Table 5; $\chi^2 = 0.000$, p = 1.000 for every parameter at T1), confirming a comparable starting point. From T1 to T3, however, the trajectories diverged sharply, and the change reached statistical significance within every group for all six parameters (Friedman test, $p \leq 0.005$ throughout).

In the fixed appliance group, mean *S. mutans* scores rose from 1.70 ± 0.48 at T1 to 3.00 ± 0.00 the ceiling of the scoring scale by T3 ($\chi^2 = 18.727$, $p<0.001$), and *Lactobacillus* followed an almost identical course ($\chi^2 = 16.909$, $p<0.001$). Gingival Index and Plaque Index both progressed from mild to severe over the same ten days ($\chi^2 = 18.000$, $p<0.001$ for each), and bleeding on probing present in every patient at baseline fell only modestly ($\chi^2 = 15.800$, $p<0.001$; confirmed by Cochran's Q, Q = 15.800, $p<0.001$). Most of that early improvement in bleeding, and most of the subsequent gingival deterioration, occurred within the first three days rather than gradually across the full ten (Table 2).

Table 2. Descriptive statistics and Friedman test for repeated measures in the fixed appliance group

Parameter	Median T1 (IQR)	Median T2 (IQR)	Median T3 (IQR)	Friedman $\chi2$	p	Post-hoc Wilcoxon (p)
SM	2.0 (1.0 – 2.0)	3.0 (2.0 – 3.0)	3.0 (3.0 – 3.0)	18.727	<0.001	T1 vs T2 p=0.002; T1 vs T3 p=0.004; T2 vs T3 p=0.083
L	1.5 (1.0 – 2.0)	3.0 (2.0 – 3.0)	3.0 (3.0 – 3.0)	16.909	<0.001	T1 vs T2 p=0.008; T1 vs T3 p=0.004; T2 vs T3 p=0.025
GI	1.0 (1.0 – 1.0)	2.0 (1.75– 2.0)	3.0 (2.0 – 3.0)	18.000	<0.001	T1 vs T2 p=0.005;

						T1 vs T3 p=0.004; T2 vs T3 p=0.007
PI	1.0 (1.0 – 1.0)	2.0 (1.75– 2.0)	3.0 (2.75– 3.0)	18.000	<0.001	T1 vs T2 p=0.005; T1 vs T3 p=0.003; T2 vs T3 p=0.008
BOP	2.0 (2.0 – 2.0)	1.0 (1.0 – 2.0)	1.0 (1.0 – 1.0)	15.800	<0.001	T1 vs T2 p=0.008; T1 vs T3 p=0.002; T2 vs T3 p=0.083
GD	1.0 (1.0 – 1.0)	2.0 (1.0 – 2.0)	3.0 (2.0 – 3.0)	18.865	<0.001	T1 vs T2 p=0.008; T1 vs T3 p=0.004; T2 vs T3 p=0.002

SM = Streptococcus mutans; L = Lactobacilli; GI = Gingival Index; PI = Plaque Index; BOP = Bleeding on Probing; GD = Gingival Depth. IQR = interquartile range.

Self-ligating brackets followed a similar but consistently milder pattern. *S. mutans* and *Lactobacillus* scores again increased significantly (χ^2 = 16.750 and 15.200 respectively, both $p \leq 0.001$), reaching a mean of 2.00 ± 0.00 by T3 rather than the ceiling seen with conventional brackets. Plaque Index remained stable between T1 and T2 and only increased significantly by T3 (χ^2 = 16.000, p<0.001), and gingival depth increased from 1.00 ± 0.00 to 2.00 ± 0.00 (χ^2 = 20.000, p<0.001) (Table 3).

Table 3. Descriptive statistics and Friedman test for repeated measures in the self-ligating bracket group

Parameter	Median T1 (IQR)	Median T2 (IQR)	Median T3 (IQR)	Friedman χ2	p	Post-hoc Wilcoxon (p)
SM	1.0 (0.75– 1.0)	2.0 (1.0 – 2.0)	2.0 (2.0 – 2.0)	16.750	<0.001	T1 vs T2 p=0.005; T1 vs T3 p=0.003; T2 vs T3 p=0.046
L	1.0 (1.0 – 1.0)	2.0 (1.0 – 2.0)	2.0 (2.0 – 2.0)	15.200	0.001	T1 vs T2 p=0.014; T1 vs T3 p=0.002; T2 vs T3 p=0.046
GI	1.0 (0.75– 1.0)	1.0 (1.0 – 1.0)	2.0 (1.0 – 2.0)	13.130	0.001	T1 vs T2 p=0.157; T1 vs T3 p=0.014; T2 vs T3 p=0.007
PI	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	2.0 (1.75– 2.0)	16.000	<0.001	T1 vs T2 p=1.000; T1 vs

						T3 p=0.005; T2 vs T3 p=0.005
BOP	2.0 (2.0 – 2.0)	2.0 (2.0 – 2.0)	1.5 (1.0 – 2.0)	10.000	0.007	T1 vs T2 p=1.000; T1 vs T3 p=0.025; T2 vs T3 p=0.025
GD	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	2.0 (2.0 – 2.0)	20.000	<0.001	T1 vs T2 p=1.000; T1 vs T3 p=0.002; T2 vs T3 p=0.002

The clear aligner group changed the least. *S. mutans* and *Lactobacillus* rose from a median of 0 at baseline to 1.0 by T3 (χ^2 = 10.571 and 15.200, p = 0.005 and 0.001), and Gingival and Plaque Indices showed a small but statistically significant increase (χ^2 = 10.571 and 15.800, p = 0.005 and <0.001). Bleeding on probing and gingival depth, however, did not change significantly across the ten days (χ^2 = 2.000, p = 0.368 for both), indicating that the modest rise in microbial counts in this group was not accompanied by a corresponding deterioration in clinical periodontal status (Table 4).

Table 4. Descriptive statistics and Friedman test for repeated measures in the clear aligner group

Parameter	Median T1 (IQR)	Median T2 (IQR)	Median T3 (IQR)	Friedman χ2	p	Post-hoc Wilcoxon (p)
SM	0.0 (0.0 – 1.0)	1.0 (0.0 – 1.0)	1.0 (1.0 – 1.0)	10.571	0.005	T1 vs T2 p=0.046; T1 vs T3 p=0.008; T2 vs T3 p=0.083
L	0.0 (0.0 – 0.0)	1.0 (0.0 – 1.0)	1.0 (1.0 – 1.0)	15.200	0.001	T1 vs T2 p=0.014; T1 vs T3 p=0.002; T2 vs T3 p=0.046
GI	0.0 (0.0 – 0.0)	0.0 (0.0 – 1.0)	1.0 (0.0 – 1.0)	10.571	0.005	T1 vs T2 p=0.083; T1 vs T3 p=0.008; T2 vs T3 p=0.046
PI	0.0 (0.0 – 0.0)	0.0 (0.0 – 1.0)	1.0 (0.0 – 1.0)	15.800	<0.001	T1 vs T2 p=0.083; T1 vs T3 p=0.002; T2 vs T3 p=0.008
BOP	2.0 (2.0 – 2.0)	2.0 (2.0 – 2.0)	2.0 (2.0 – 2.0)	2.000	0.368	T1 vs T2 p=1.000; T1 vs T3 p=0.317;

						T2 vs T3 p=0.317
GD	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	2.000	0.368	T1 vs T2 p=1.000; T1 vs T3 p=0.317; T2 vs T3 p=0.317

Direct between-group comparison confirmed this hierarchy at every post-baseline time point. By T2, median *S. mutans* scores were highest in the fixed appliance group (2.50), intermediate in the self-ligating group (2.00), and lowest with clear aligners (1.00; $\chi^2 = 22.240$, $p<0.001$); by T3 the gap widened further (3.00 vs 2.00 vs 1.00; $\chi^2 = 29.000$, $p<0.001$). *Lactobacillus*, Gingival Index, and Plaque Index followed the same ordering at both T2 and T3 (all $p<0.001$). Bleeding on probing and gingival depth showed no group difference at baseline but diverged significantly thereafter (T2: $\chi^2 = 17.652$, $p<0.001$ for both; T3: $\chi^2 = 15.795$ and 23.738 respectively, $p<0.001$), with fixed appliances again showing the least favourable values and clear aligners the most stable (Table 5).

Table 5. Inter-group comparison of periodontal and microbial indices across three time intervals (N = 30)

Parameter / Time	Group 1 Fixed (n=10)	Group 2 Self-ligating (n=10)	Group 3 Aligner (n=10)	χ2	p
Streptococcus mutans (SM)					
T1	1.00 (1.00 – 1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	0.000	1.000
T2	2.50 (2.00 – 3.00)	2.00 (1.00–2.00)	1.00 (0.00–1.00)	22.240	<0.001
T3	3.00 (2.75 – 3.00)	2.00 (2.00–2.00)	1.00 (1.00–1.00)	29.000	<0.001
Lactobacilli (L)					
T1	1.00 (1.00 – 1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	0.000	1.000
T2	2.50 (2.00 – 3.00)	2.00 (1.00–2.00)	1.00 (0.00–1.00)	21.292	<0.001
T3	3.00 (2.75 – 3.00)	2.00 (2.00–2.00)	1.00 (1.00–1.00)	29.000	<0.001
Gingival Index (GI)					
T1	1.00 (1.00 – 1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	0.000	1.000
T2	2.00 (1.75 – 2.00)	1.00 (1.00–1.25)	0.00 (0.00–0.25)	21.883	<0.001
T3	3.00 (2.75 – 3.00)	2.00 (2.00–2.00)	1.00 (0.00–1.00)	22.636	<0.001
Plaque Index (PI)					
T1	1.00 (1.00 – 1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	0.000	1.000
T2	2.00 (1.75 – 2.00)	1.00 (1.00–1.25)	0.00 (0.00–0.25)	21.883	<0.001
T3	3.00 (2.75 – 3.00)	2.00 (2.00–2.00)	1.00 (1.00–1.00)	24.266	<0.001
Bleeding on Probing (BOP)					
T1	2.00 (2.00 – 2.00)	2.00 (2.00–2.00)	2.00 (2.00–2.00)	0.000	1.000

T2	1.00 (1.00 – 1.25)	2.00 (2.00–2.00)	2.00 (2.00–2.00)	17.652	<0.001
T3	1.00 (1.00 – 1.00)	1.50 (1.00–2.00)	2.00 (2.00–2.00)	15.795	<0.001
Gingival Depth (GD)					
T1	1.00 (1.00 – 1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	0.000	1.000
T2	2.00 (1.75 – 2.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	17.652	<0.001
T3	3.00 (2.75 – 3.00)	2.00 (2.00–2.00)	1.00 (1.00–1.00)	23.738	<0.001

Inter-group comparisons evaluated using the Kruskal–Wallis test (df = 2). IQR = interquartile range (25th–75th percentile).

DISCUSSION

This study set out to track, in near real time, how three different orthodontic appliance systems affect the oral microbial and periodontal environment during the first ten days of treatment a window rarely examined in comparative trials, most of which report outcomes only from three months onward. The pattern that emerged was consistent across every parameter measured: fixed appliances produced the sharpest deterioration, self-ligating brackets an intermediate one, and clear aligners the smallest. This mirrors observations made decades ago comparing banded and non-banded fixed appliances, where banded patients already showed higher salivary *S. mutans* concentrations than their non-banded counterparts[29] an early hint that appliance surface area, more than any single design feature, drives the microbial response.

All three groups began from a statistically same microbial and periodontal baseline, which matters because it means the divergence that followed can reasonably be attributed to the appliance itself rather than to pre-existing differences between patients. A comparable starting point has been reported in similar appliance comparisons before[22], which strengthens confidence in the internal validity of the present comparison.

Within three days, *S. mutans* counts had already risen in every group, but most steeply with fixed appliances consistent with the long-recognised tendency of brackets, ligatures, and archwires to create retentive niches that accelerate biofilm formation. Jung et al. similarly documented a rise in salivary mutans streptococci soon after fixed appliance placement[12], and Pandis et al. found much the same regardless of whether conventional or self-ligating brackets were used, suggesting that some early bacterial shift may be difficult to avoid with any fixed system[13]. Material itself seems to matter less than the biological surface it acquires: in-vitro work has shown that *S. mutans* adhesion to steel, ceramic, and plastic brackets converges once a salivary pellicle forms, with prior colonisation by *Streptococcus sanguis* further limiting *S. mutans* attachment[30] reinforcing that oral ecology, rather than bracket material, principally governs colonisation. The comparatively smaller rise seen with self-ligating brackets in the present study is better explained by the absence of elastomeric ligatures, themselves known to harbour more plaque than steel ligation[16], than by any inherent resistance to colonisation, an interpretation broadly supported by earlier work on self-ligating systems[14,15].

Clear aligners, in contrast, kept *S. mutans* counts lowest throughout, in keeping with earlier reports of better hygiene and lower bacterial colonisation among aligner-treated patients[17,18]. Because aligner material itself appears comparable to enamel and stainless steel in terms of initial bacterial adhesion[6], the advantage most plausibly comes from removability rather than surface chemistry. It is worth noting, however, that removable appliances are not entirely inert: even caries-free children wearing removable appliances have previously shown higher mutans streptococci colonisation than untreated controls[25], so the benefit of aligners over fixed appliances observed here is one of degree rather than of complete immunity.

Lactobacillus followed a closely parallel course, again peaking with fixed appliances and staying lowest with aligners, in line with earlier work linking removable appliances to a reduced cariogenic burden[19,15]. By day ten, the gap between appliance types had widened rather than narrowed, and the fixed appliance group reached the ceiling of the scoring scale for both organisms — a trajectory consistent with earlier reports connecting sustained plaque accumulation around fixed appliances to a heightened risk of white-spot lesions[5].

The periodontal indices told a similar story. Gingival Index and Plaque Index rose earliest and furthest with fixed appliances, echoing earlier work showing that brackets, archwires, and ligatures mechanically interfere with plaque removal[21], and Ristic et al. reported a comparable early rise in gingival inflammation after fixed appliance placement[20]. Self-ligating brackets again fell in between plausibly because removing elastomeric ligatures helps without

eliminating the underlying problem of a fixed component sitting on the tooth surface[14]. Aligners preserved the most favourable gingival status throughout, consistent with systematic-review evidence linking removable appliances to better periodontal outcomes, most likely because patients can brush and floss without the appliance in the way[2,3]. Bleeding on probing illustrated this divergence most sharply, reaching universal positivity in the fixed appliance group by day ten while remaining minimal in the aligner group throughout — a pattern that echoes earlier reports of a rapid rise in bleeding tendency soon after fixed appliance placement[21,20] and fits the broader evidence that bleeding on probing is a meaningful, if imperfect, early marker of periodontal deterioration[10]. Self-ligating brackets showed a delayed but still measurable rise in bleeding, positioning them as an intermediate rather than equivalent alternative to aligners.

Several limitations should be acknowledged. Ten days captures only the earliest phase of an adaptation process that typically continues for months; longer studies have shown that bacterial and periodontal parameters can partially normalise as patients adjust their hygiene technique, and that some of the early rise in *S. mutans* after fixed appliance placement resolves within weeks of debonding[28]. The sample size of ten patients per group limits the precision of the between-group estimates, and unmeasured factors diet, individual hygiene technique, and host susceptibility, which earlier work has linked to baseline plaque and salivary bacterial counts more strongly than plaque location alone[26,27] may have contributed to the variability observed within groups. Patients with compromised salivary flow, for whom saliva substitutes can themselves differentially influence bacterial adhesion depending on the restorative surface involved[33], were not specifically evaluated. Larger, longer studies are needed to establish whether the early differences reported here persist, narrow, or widen over a complete course of orthodontic treatment.

CONCLUSION

Within these limitations, this study found consistent evidence that appliance type meaningfully shapes both the salivary microbial profile and the gingival health of orthodontic patients from the very first days of treatment. Fixed appliances produced the fastest and largest rise in Streptococcus mutans, Lactobacillus, plaque accumulation, gingival inflammation, bleeding on probing, and probing depth; self-ligating brackets moderated but did not eliminate this trend; and clear aligners preserved a markedly more stable microbial and periodontal profile throughout. These findings support giving weight to appliance selection and to reinforced hygiene protocols for patients who do require fixed appliances particularly for those who already carry an elevated caries or periodontal risk. Confirming whether these early differences persist over a complete course of treatment will require larger, longer-duration studies.

ETHICAL APPROVAL

This study was conducted in accordance with the ethical standards of the institutional research committee. Written informed consent was obtained from all participants prior to enrolment.

CONFLICT OF INTEREST

The author declares no conflict of interest.

SOURCE OF FUNDING

Nil.

REFERENCES

1. Belanche Monterde A, Flores-Fraile J, Pérez Pevida E, Zubizarreta-Macho Á. Biofilm composition changes during orthodontic clear aligners compared to multibracket appliances: A systematic review. Microorganisms. PLoS ONE 15(4) **2025**;13:1039.
2. Rouzi M, Zhang X, Jiang Q, Long H, Lai W, Li X. Impact of clear aligners on oral health and oral microbiome during orthodontic treatment. Int Dent J. **2023**;73:603-611.
3. Di Spirito F, D'Ambrosio F, Cannatà D, D'Antò V, Giordano F, Martina S. Impact of clear aligners versus fixed appliances on periodontal status of patients undergoing orthodontic treatment: A systematic review of systematic reviews. Healthcare (Basel). **2023**;11:1340.
4. Mummolo S, Nota A, Albani F, Marchetti E, Gatto R, Marzo G, et al. Salivary levels of *Streptococcus mutans* and Lactobacilli and other salivary indices in patients wearing clear aligners versus fixed orthodontic appliances: An observational study. PLoS One. **2020**;15(4):e0228798.
5. Tektas S, Thurnheer T, Eliades T, Attin T, Karygianni L. Initial bacterial adhesion and biofilm formation on aligner materials. Antibiotics (Basel). **2020**;9(12):908.
6. Kachari M, Nandhini K, Sarangi S, Banik A, Chinnasamy BR, Kumar N, Kashwani R. Genetic susceptibility to external root resorption following endodontic, orthodontic and oral surgical interventions: a case-control study. Genetics and Molecular Research. **2026** Jun 25.
7. Arshad F, Riyaz K, Suma T. *Staphylococcus aureus* count in salivary samples of patients treated with self-ligating prescriptions: A comparative clinical study. Int J Appl Dent Sci. **2018**;4(4):95-97.

8. Cho R, Panariello B. *Oral Microbial and Biofilm Changes in Response to Clear Aligners: A Scoping Review.* Oral Sphere J. Dent. Health Sci. **2026**;2(2):132-143.
9. Levrini L, Mangano A, Montanari P, Margherini S, Caprioglio A, Abbate GM. Periodontal health status in patients treated with the Invisalign system and fixed orthodontic appliances: A 3-months clinical and microbiological evaluation. Eur J Dent. **2015**;9(3):404-410.
10. Mummolo S, Tieri M, Tecco S, Mattei A, Albani F, Giuca MR, et al. Clinical evaluation of salivary indices and levels of *Streptococcus mutans* and *Lactobacillus* in patients treated with Occlus-o-Guide. Eur J Paediatr Dent. **2014**;15(4):367-372.
11. Jung WS, Kim H, Park SY, Cho EJ, Ahn SJ. Quantitative analysis of changes in salivary mutans streptococci after orthodontic treatment. Am J Orthod Dentofacial Orthop. **2014**;145(5):603-609.
12. Freitas AOA, Marquezan M, Nojima MCG, Alviano DS, Maia LC. The influence of orthodontic fixed appliances on the oral microbiota: A systematic review. Dent Press J Orthod. **2014**;19(2):46-55.
13. Türköz Ç, Canıgür Bavbek N, Kale Varlık S, Akça G. Influence of thermoplastic retainers on *Streptococcus mutans* and *Lactobacillus* adhesion. Am J Orthod Dentofacial Orthop. **2012**;141(5):598-603.
14. Jongsma, M.A., Pelser, F.D.H., van der Mei, H.C. *et al.* Biofilm formation on stainless steel and gold wires for bonded retainers in vitro and in vivo and their susceptibility to oral antimicrobials. *Clin Oral Investigations* **2013**;17: 1209–1218.
15. Klukowska M, Bader A, Erbe C, Bellamy P, White DJ, Anastasia MK, et al. Plaque levels of patients with fixed orthodontic appliances measured by digital plaque image analysis. Am J Orthod Dentofacial Orthop. **2011**;139(5):e463-e470.
16. Tufekci E, Dixon JS, Gunsolley JC, Lindauer SJ. Prevalence of white spot lesions during orthodontic treatment with fixed appliances. Angle Orthod. **2011**;81(2):206-210.
17. Al Groosh D, Roudsari GB, Moles DR, Ready D, Noar JH, Pratten J. The prevalence of opportunistic pathogens associated with intraoral implants. Lett Appl Microbiol. **2011**;52(5):501-505.
18. Pandis N, Papaioannou W, Kontou E, Nakou M, Makou M, Eliades T. Salivary *Streptococcus mutans* levels in patients with conventional and self-ligating brackets. Eur J Orthod. **2010**;32:94-99.
19. Hoque MZ, Akter F, Hossain KM, Rahman MSM, Billah MM, Islam KMD. Isolation, identification and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yoghurts. World J Dairy Food Sci. **2010**;5(1):39-46.
20. Lara-Carrillo E, Montiel-Bastida NM, Sánchez-Pérez L, Alanís-Tavira J. Effect of orthodontic treatment on saliva, plaque and the levels of *Streptococcus mutans* and *Lactobacillus*. Med Oral Patol Oral Cir Bucal. **2010**;15(6):e924-e929.
21. Hahnel S, Rosentritt M, Handel G, Bürgers R. Influence of saliva substitute films on initial *Streptococcus mutans* adhesion to enamel and dental substrata. J Dent. **2008**;36:977-983.
22. Ristic M, Vlahovic Svabic M, Sasic M, Zelic O. Effects of fixed orthodontic appliances on subgingival microflora. Int J Dent Hyg. **2008**;6(2):129-136.
23. Papaioannou W, Gizani S, Nassika M, Kontou E, Nakou M. Adhesion of *Streptococcus mutans* to different types of brackets. Angle Orthod. **2007**;77(6):1090-1095.
24. Nishikawara F, Nomura Y, Imai S, Senda A, Hanada N. Evaluation of cariogenic bacteria using modified Mitis-Salivarius Bacitracin agar medium. Eur J Dent. **2007**;1:31-39.
25. Miethke RR, Brauner K. A comparison of the periodontal health of patients during treatment with the Invisalign system and with fixed lingual appliances. J Orofac Orthop. **2007**;68:223-231.
26. Lessa FCR, Enoki C, Ito IY, Faria G, Matsumoto MAN, Nelson-Filho P. In-vivo evaluation of the bacterial contamination and disinfection of acrylic baseplates of removable orthodontic appliances. Am J Orthod Dentofacial Orthop. **2007**;131:705.e11-705.e17.
27. Batoni G, Pardini M, Giannotti A, Ota F, Giuca MR, Gabriele M, et al. Effect of removable orthodontic appliances on oral colonisation by mutans streptococci in children. Eur J Oral Sci. **2001**;109:388-392.
28. Sullivan A, Borgström MK, Granath L, Nilsson G. Number of mutans streptococci or lactobacilli in a total dental plaque sample does not explain the variation in caries better than the numbers in stimulated whole saliva. Community Dent Oral Epidemiol. **1996**;24:159-163.

29. Van Houte J**.** Role of micro-organisms in caries etiology. J Dent Res. 1994;73(3):672–681.
30. Mundorff SA, Eisenberg AD, Leverett DH, Espeland MA, Proskin HM. Correlations between numbers of microflora in plaque and saliva. Caries Res. **1990**;24:312-317.
31. Rosenbloom RG, Tinanoff N. Salivary *Streptococcus mutans* levels in patients before, during, and after orthodontic treatment. Am J Orthod Dentofacial Orthop. **1991**;100(1):35-37.
32. Forsberg CM, Brattström V, Malmberg E, Nord CE. Ligature wires and elastomeric rings: Two methods of ligation and their association with microbial colonization of *Streptococcus mutans* and lactobacilli. Eur J Orthod. **1991**;13:416-420.
33. Lang NP, Joss A, Orsanic T, Gusberti FA, Siegrist BE. Bleeding on probing. A predictor for the progression of periodontal disease? J Clin Periodontol. **1986**;13(6):590–596.
34. Corbett JA, Brown LR, Keene HJ, Horton IM. Comparison of *Streptococcus mutans* concentrations in non-banded and banded orthodontic patients. J Dent Res. **1981**;60(12):1936-1942.
35. Gold OG, Jordan HV, Van Houte J. A selective medium for *Streptococcus mutans*. Arch Oral Biol. **1973**;18:1357-1364.
36. Carter HG, Barnes GP. The gingival bleeding index. J Periodontol. **1974**;45(11):801–805.
37. Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol. **1967**;38:610-616.