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Comparative evaluation of salivary microbial levels of red complex bacteria in patients wearing three different types of fixed lingual retainers: a clinical study

Abstract

Introduction: One of the major challenges for orthodontists is the long-term stability of orthodontic treatment. This has urged orthodontists to seek methods to ensure stable results following the completion of orthodontic treatment. Incisor crowding is reported to occur in follow-up stages of orthodontically treated patients. Maintenance of incisor alignment following orthodontic treatment has led to the development of retainers (removable and fixed). Fixed lingual retainers have been criticized for their potential to compromise the periodontal status, due to accumulation of plaque and calculus along the retainer wire.

Objectives: The aim of this study was to evaluate and compare salivary microbial levels of the "Red Complex Bacteria" after orthodontic treatment with fixed appliances, during the retention period using three different types of fixed lingual retainer wires.

Materials and methods: 30 patients who have completed orthodontic treatment and met the inclusion criteria were randomly divided into 3 groups with 10 patients each. Fixed lingual retainers (Retainium or Penta-One or Bond-a-Braid wire) were bonded to the lingual surfaces of the six anterior teeth. Saliva samples were collected at debonding (T0), 4 weeks after debonding (T1) and 8 weeks after debonding (T2). Saliva samples were stored at -80o Celsius followed by PCR testing, One- way ANOVA test followed by Tukey's post hoc Test was used to compare the mean PCR values of *P. gingivalis*, *T. denticola* and *T. forsythia* (Red Complex Bacteria) between 3 groups at different time intervals. Repeated measures of ANOVA test followed by Bonferroni's post hoc Test was used to compare the mean PCR values of various microorganisms between different time intervals in each group.

Results: The PCR test results demonstrated that the highest Red Complex Bacterial growth was observed in Group 3 (Bond-a-Braid wire) followed by Group 2 (Penta-One wire). Whereas the least Red Complex Bacterial growth was observed in Group 1 (Retainium wire) at different time intervals (T0, T1, T2). The maximum growth of *P. gingivalis* was observed at debonding (T0). The least growth of *P. gingivalis* was seen at 8 weeks after debonding (T2). Similarly, *T. denticola* and *T. forsythia* showed highest values at debonding (T0), while lowest values were observed at 8 weeks after debonding (T2).

Conclusion: The present study concluded that there is statistically significant difference ($p<0.001$) in the salivary microbial levels (Red Complex Bacteria) with different types of fixed lingual retainer wires at three time intervals (T0, T1 and T2).

Keywords: orthodontists, red complex bacteria and fixed lingual retainers

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Introduction

Fixed orthodontic items such brackets, bands, or retainers hamper oral hygiene, leading to plaque build-up and gingival irritation. Orthodontic success depends on good dental hygiene and caries management.[1] The orthodontic wire, brackets, and bands provide additional plaque-forming surfaces, increasing oral bacteria. Dental plaque can cause caries, gingivitis, and periodontitis. Knowing microbiological changes in orthodontic patients, especially retention, is critical.[2] The retention phase, in which dental motions are maintained following active treatment, is crucial for orthodontic success and preventing teeth from shifting. Stretching periodontal fibres may cause many orthodontic patients to relapse. Follow-up orthodontic patients describe incisor crowding. Retainers maintain incisor alignment after orthodontic treatment.[3] Removable and fixed retainers are used clinically. Clinicians must rely on patients' discipline and long-term compliance while using detachable retainers. This device won't affect oral hygiene. Bonding provided permanent interdental wire connections as retention devices. As they are put on the lingual tooth surfaces, patients accept and comply with fixed lingual retainers.[4]

Despite being typically safe, glued retainers can hinder oral hygiene efforts. Plaque and calculus can accumulate along fixed retainer wires, compromising periodontal health. Following splinting with fixed retainers, functional loads on anterior teeth shift, which may affect periodontal health. Long-term fixed retainer wear has unknown periodontal effects, however they certainly make dental hygiene more challenging.[5] Fixed retainers are associated to gingival recession, plaque retention, probing haemorrhage, and deeper probing. A more recent study found that the clinical periodontal health of subjects was not affected by bonded lingual retainers despite increased plaque accumulations in the lower incisor region.[6]

The wires used in this study were Retainium wire (Reliance Orthodontics Ltd., Itasca, Illinois, USA), Penta-One wire (Masel Orthodontics, Carlsbad, California, USA) and Bond-a-Braid wire (Reliance Orthodontics Ltd., Itasca, Illinois, USA).

a) Retainium wire is a single-strand nickel-free titanium flat ribbon wire with higher interproximal strength. Nickel-free, it prevents patient allergies.[7]

b) Penta-One wire is 0.0215" circular, 5-stranded stainless steel. It fractures less than thinner or 3-strand wires of the same thickness, yet it's elastic enough to enable some tooth motion.[8]

c) Bond-A-Braid wire is a flattened, soft, rectangular (8-braided) wire (0.027" x 0.011"). It's adaptable and prevents tooth movement from active force wires.[9]

Studies link periodontal pathogen levels in saliva and sub gingival plaque to gingival inflammation. Saliva, collected frequently with minimal patient discomfort, making it an ideal diagnostic tool. Saliva collection is a straightforward, safe, affordable, and non-invasive method for monitoring oral pathogen levels during orthodontic treatment and retention.[10] Saliva has promising indicators. It contains locally synthesised proteins, DNA and mRNA, and host and bacterial metabolites. Sub gingival pathogens include *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *T. forsythia*, and *P. intermedia*. The "Red Complex" includes *P. gingivalis, T. denticola*, and *T. forsythia*. The "Red Complex" correlates strongly with pocket depth and bleeding on probing, two important periodontal diagnostic measures. Putative periodontal pathogens in the gingival crevice aren't enough to trigger periodontal inflammation.[11]

Even in healthy people, these pathogens are present in the gingival crevice, albeit in low numbers. Detection of these potential periodontal infections relies on the procedures used. Many bacteria can't be cultivated using normal techniques; therefore cultivation studies underestimate microbial diversity. PCR is a fast, sensitive, and specific method for detecting bacterial infections.[12]

Many studies reveal alterations in bacterial levels and periodontal infections during orthodontic therapy. Few studies have examined periodontal pathogens in saliva following orthodontic treatment (during retention). This study evaluated and compared salivary microbial levels of "Red Complex Bacteria" after orthodontic treatment with fixed appliances, during the retention period utilising three commercially available fixed lingual retainer wires.[13]

The aim of the study was to evaluate and compare salivary microbial levels of *P. gingivalis, T. denticola* and *T. forsythia* in patients wearing three different types of fixed lingual retainers, after orthodontic treatment with fixed appliances.

The objectives of this study were

a) To determine the salivary levels of *P. gingivalis, T. denticola* and *T. forsythia* at debonding (T0), 4 weeks after debonding (T1) and 8 weeks after debonding (T2) using Polymerase Chain Reaction (PCR) technique.

b) To evaluate and compare the salivary levels of *P. gingivalis, T. denticola* and *T. forsythia* inpatients bonded with three different types of fixed lingual retainers (Retainium wire, Penta-One wire and Bond-a-Braid wire).

c) To compare the salivary microbial levels in samples collected at three different time points (At debonding, 4 weeks after debonding and 8 weeks after debonding).

Source of data

30 subjects were selected from the patients visiting Department of Orthodontics and Dentofacial Orthopaedics, M. R. Ambedkar Dental College and Hospital, Bengaluru.

Inclusion criteria

Healthy individuals with no systemic diseases, Individuals after completion of orthodontic treatment with fixed appliances. Patients with well-aligned anterior teeth and good to fair oral hygiene with a simplified oral hygiene index (OHI-S) score of zero to three months and no antibiotic use within the last 3 months.[14]

Exclusion criteria

Patients with any systemic disorders or with extensive caries and fixed or removable prostheses. Patients with poor oral hygiene with a simplified oral hygiene index (OHI-S) score of more than three. Moreover patients with poor periodontal health or patients using mouthwashes are excluded.[15]

Material and methods

Saliva sample collection

The patients were advised not to eat or drink anything, nor to brush their teeth up to 2 hours before the saliva sample was taken. The unstipulated whole saliva was collected by spitting method at debonding (T0), 4 weeks after debonding (T1) and 8 weeks after debonding (T2) in sterile Petri dishes.

Bacterial DNA isolation from saliva

Target bacterial load: *P. gingivalis, T. denticola, T. forsythia*[16]

a) Label 2ml Centrifuge Tubes with your name and transfer 0.5ml saliva suspension, a suspension of bacteria, to your tubes.

b) Add 0.2ml DNA Release Buffer to the tube containing the Bacterial Suspension. Invert the tube several times to slowly mix. The DNA Release Buffer breaks open the bacterial cells releasing the DNA.

c) Add 0.02ml Protease to the tube to digest and remove the cellular material and protein and release the genomic DNA.

d) Close the cap. Briefly mix by inverting the tube 5-6 times and then place in a 50- 55°C water bath or heating block for 1 hour.

e) After 1 hour, add 0.1ml DNA Salt Solution to the tube and mix by inverting the tube several times. The salt solution aids in the precipitation of the DNA.

f) Centrifuge the tube for 5 minutes at 5,000xg to pellet the cell debris. Transfer the supernatant to your other labelled tube.

g) Add 0.8ml Precipitation Solution, close the tube and, whilst watching, slowly invert the tube several times to mix. White DNA strands may appear.

h) Remove the Precipitation Solution and wash the pellet with 0.5ml 70% ethanol and centrifuge as before. Remove the 70% ethanol and leave the open tube at room temperature for 10-15 minutes to dry. Resuspend in 30µl water and load 10-20µl on a 1% agarose gel to visualize the genomic DNA.

P. gingivalis Primer Sequence: Forward: AGG CAG CTT GCC ATA CTG CG and Reverse: ACT GTT AGC AAC TAC CGA TGT

T. denticola Primer Sequence Forward: TAA TAC CGA ATG TGC TCA TTT ACA T Reverse: TCA AAG AAG CAT TCC CTC TTC TTC TTA

T. forsythia Primer Sequence Forward: GCG TAT GTA ACC TGC CCG CA Reverse: TGC TTC AGT GTC AGT TAT ACC T

PCR protocol[17]

a) PCR was carried out in 0.2 ml PCR tubes in a Rotorgene thermal cycler.

b) The 10 ml salivary bacterial DNA extract and controls were amplified with 0.5 mM (3F &3R) primers.[17]

c) 200 mm of each dNTP (Promega), 10 mMKCl PCR buffer, 2 mm MgCl2 and 1.0 U Taq polymerase (Bioline).

d) Amplification conditions for both PCRs were as follows:

e) 5 min at 94 uC to denature the DNA, followed by 40 cycles of denaturation at 94 uC for 1 min, primer annealing at 55 uC for 1 min and strand extension at 72 uC for 2 min on a Rotorgene thermal cycler.

f) PCR products were separated on a 1.5 % agarose gel and DNA bands were visualized with ethidium bromide.

g) Primers and excess nucleotides were removed from the amplified DNA using a PCR clean-up kit (Bio Gene).

h) The amount of DNA in the cleaned-up product was quantified by comparing the intensity of the band to bands of known intensity in Ladder marker (Biogene).

The gel was stained with 0.5 µg/ml ethidium bromide, viewed under UV transilluminator and images were captured on a gel documentation system.

Results

One-way ANOVA test followed by Tukey's post hoc test showed that the difference in mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* at debonding (T0) were statistically significant at p<0.001. The PCR test results in Table IV demonstrate that the highest red complex bacterial growth at debonding (T0) was observed in Group 3 followed by Group 2. The least red complex bacterial growth was observed in Group 1 at debonding (T0) (Table 1).

One-way ANOVA test followed by Tukey's post hoc test revealed that the difference in mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* at 4 weeks after debonding (T1) were statistically significant at p<0.001 Table V demonstrates that the highest mean PCR values at 4 weeks after debonding (T1) were observed in Group 3 followed by Group 2. Group 1 showed the lowest mean PCR values at 4 weeks after debonding (T1) (Table 2).

Table 1 Mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* at debonding (T0) in 3 different fixed lingual retainer wire groups

Comparison of mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* at debonding [T0] using One- way ANOVA test followed by Tukey's post hoc test									
Organism	**Groups**	**N**	**Mean**	**SD**	**Min**	**Max**	**P-value a**	**Sig. diff**	**P-value b**
P. gingivalis	Group 1	10	17.183	1.976	14.54	20.71		G1 VS G2	<0.001 *
	Group 2	10	25.598	3.261	20.51	29.79	<0.001*	G1 vs G3	<0.001 *
	Group 3	10	30.319	0.999	28.55	31,67		G2 vs G3	<0.001 *
T. denticola	Group 1	10	11.259	1.246	9.67	12.67		G1 vs G2	<0.001* *
	Group 2	10	16.326	1.85	13.78	18.54	<0.001*	G1 vs G3	<0.001*
	Group 3	10	24.254	2.398	20.31	28.19		G2 VS G3	<0.001* *
T. forsythia	Group 1	10	34.097	1.886	30.65	37.24		G1 VS G2	<0.001*
	Group 2	10	48.246	3.926	41.19	53.17	<0.001*	G1 VS G3	<0.001*
	Group 3	10	63.547	4.198	53.82	68.42		G2 VS G3	<0.001 *

*Statistically Significant **Note** a. P-Value derived by One-way ANOVA Test b. P-value derived by Tukey's post hoc test

Table 2 Mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* at 4 weeks after debonding (T1) in 3 different fixed lingual retainer wire groups

Comparison of mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* 4 weeks after debonding [T1] using One- way ANOVA test followed by Tukey's post hoc test									
Organism	**Groups**	**N**	**Mean**	**SD**	**Min**	**Max**	**P-value a**	**Sig. diff**	**P-value b**
P. gingivalis	Group 1	10	9.963	1.386	7.71	12.43		G1 vs G2	<0.001 *
	Group 2	10	23.764	2.691	19.73	28.1	<0.001*	G1 vs G3	<0.001 *
	Group 3	10	29.131	2.158	24.55	32.1		G2 VS G3	<0.001 *
T. denticola	Group 1	10	6.544	0.786	5.32	7.51		G1 vs G2	<0.001 *
	Group 2	10	15.298	1.759	13.02	17.74	<0.001*	G1 vs G3	<0.001* *
	Group 3	10	21.19	2.255	17.72	24.52		G2 vs G3	<0.001*
T. forsythia	Group 1	10	27.447	2.432	23.18	31.92		G1 VS G2	<0.001 *
	Group 2	10	45.552	3.202	39.42	49.01	<0.001*	G1 VS G3	<0.001* *
	Group 3	10	60.523	4.961	50.3	66.75		G2 vs G3	<0.001 *

*Statistically Significant **Note** a. P-Value derived by One-way ANOVA Test. P-value derived by Tukey's post hoc test

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One-way ANOVA test followed by Tukey's post hoc test showed that the difference in mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* at 8 weeks after debonding (T2) were statistically significant at p<0.001. The test results in Table VI demonstrate that the highest mean PCR values were observed in Group 3 followed by Group 2 at 8 weeks after debonding (T2). Group 1 showed the lowest mean PCR values at 8 weeks after debonding (T2) (Table 3).

The mean PCR values of *P. gingivalis* between 3 different time intervals (T0, T1, T2) in each group using repeated measures of ANOVA test were statistically significant at p<0.001 for Group 1 and 2; and they were statistically significant at p=0.02 for Group 3. Bonferroni's post hoc test results were statistically significant at p<0.001 for Group 1 and 2. The mean PCR values using Bonferroni's post hoc test were statistically significant at p=0.04 for Group 3. According to the results obtained in Table VII, the maximum growth of *P. gingivalis* was observed at debonding (T0). The least growth of *P. gingivalis* was seen at 8 weeks after debonding (T2) (Table 4).

Table 3 Mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* at 8 weeks after debonding (T2) in 3 different fixed lingual retainer wire groups

Comparison of mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* 8 weeks after debonding [T2] using One- way ANOVA test followed by Tukey's post hoc test									
Organism	**Groups**	**N**	**Mean**	**SD**	**Min**	**Max**	**P-value [a]**	**Sig. diff**	**P-value [b]**
P. gingivalis	Group 1	10	9.459	1.135	7.62	10.84	<0.001*	G1vs G2	<0.001*
	Group 2	10	20.691	2.243	17.3	24.44		G1vs G3	<0.001*
	Group 3	10	26.785	3.257	23.39	33.79		G2 vs G3	<0.001*
T. denticola	Group 1	10	5.686	0.797	4.69	6.84	<0.001*	G1vs G2	<0.001*
	Group 2	10	13.509	1.567	11.42	15.45		G1vs G3	<0.001*
	Group 3	10	18.387	1.864	15.82	21.14		G2 vs G3	<0.001*
T. forsythia	Group 1	10	21.986	1.913	19.65	25.65	<0.001*	G1vs G2	<0.001*
	Group 2	10	42.336	2.587	37.19	47.12		G1vs G3	<0.001*
	Group 3	10	54.908	4.614	45.31	61.15		G2 vs G3	<0.001*

*Statistically Significant **Note** a. P-Value derived by One-way ANOVA Test. P-value derived by Tukey's post hoc test

Table 4 Mean PCR values of *P. gingivalis* at debonding (T0), 4 weeks after debonding (T1) and 8 weeks after debonding (T2) in 3 different fixed lingual retainer wire groups

Comparison of mean PCR values of *P. gingivalis, T. denticola* and *T. forsythia* 8 weeks after debonding [T2] using One- way ANOVA test followed by Tukey's post hoc test									
Organism	**Groups**	**N**	**Mean**	**SD**	**Min**	**Max**	**P-value [a]**	**Sig. diff**	**P-value [b]**
P.gingivalis	Group 1	10	9.459	1.135	7.62	10.84	<0.001*	G1vs G2	<0.001*
	Group 2	10	20.691	2.243	17.3	24.44		G1vs G3	<0.001*
	Group 3	10	26.785	3.257	23.39	33.79		G2 vs G3	<0.001*
T. denticola	Group 1	10	5.686	0.797	4.69	6.84	<0.001*	G1vs G2	<0.001*
	Group 2	10	13.509	1.567	11.42	15.45		G1vs G3	<0.001*
	Group 3	10	18.387	1.864	15.82	21.14		G2 vs G3	<0.001*
T. forsythia	Group 1	10	21.986	1.913	19.65	25.65	<0.001*	G1vs G2	<0.001*
	Group 2	10	42.336	2.587	37.19	47.12		G1vs G3	<0.001*
	Group 3	10	54.908	4.614	45.31	61.15		G2 vs G3	<0.001*

*Statistically Significant **Note** a. P-Value derived by One-way ANOVA Test b. P-value derived by Tukey's post hoc test

The mean PCR values of *T. denticola* between different time intervals (T0, T1, T2) in each group using repeated measures of ANOVA test followed by Bonferroni's post hoc test were statistically significant at p<0.001. Table VIII showed the highest mean PCR values of *T. denticola* at debonding (T0) in each group. The lowest mean PCR values were observed at 8 weeks after debonding (T2) in each group (Table 5).

The mean PCR values of *T. forsythia* between different time intervals (T0, T1, T2) in each group using repeated measures of ANOVA test followed by Bonferroni's post hoc test were statistically significant at p<0.001. In Table IX, the test results demonstrated the highest amount of *T. forsythia* at debonding (T0). The lowest amount of *T. forsythia* was observed at 8 weeks after debonding (T2) (Table 6).

Table 5 Mean PCR values of *T. denticola* at debonding (T0), 4 weeks after debonding (T1) and 8 weeks after debonding (T2) in 3 different fixed lingual retainer wire groups

Comparison of mean PCR values of *T. denticola* between different time intervals in each group using Repeated measures of ANOVA test followed by Bonferroni's post hoc test									
Groups	**Time**	**N**	**Mean**	**SD**	**Min**	**Max**	**P-value [a]**	**Sig. diff**	**P-value [b]**
	TO	10	11.259	1.246	9.67	12.67		TO vs TI	<0.001*
Group I	TI	10	6.544	0.786	5.32	7.51	<0.001*	TO vs T2	<0.001*
	T2	10	5.686	0.797	4.69	6.84		TI vs T2	<0.001*
	TO	10	16.326	1.850	13.78	18.54		TO vs TI	<0.001*
Group 2	TI	10	15.298	1.759	13.02	17.74	<0.001*	TO vs T2	<0.001*
	T2	10	13.509	1.567	11.42	15.45		TI vs T2	<0.001*
	TO	10	24.254	2.398	20.31	28.19		TO vs TI	<0.001*
Group 3	TI	10	21.190	2.255	17.72	24.52	<0.001*	TO vs T2	<0.001*
	T2	10	18.387	1.864	15.82	21.14		TI vs T2	<0.001*

*Statistically Significant **Note** a. P-Value derived by One-way ANOVA Test. P-value derived by Tukey's Post hoc Test

Table 6 Mean PCR values of *T. forsythia* at debonding (T0), 4 weeks after debonding (T1) and 8 weeks after debonding (T2) in 3 different fixed lingual retainer wire groups

Comparison of mean PCR values of *T. forsythia* between different time intervals in each group using repeated measures of ANOVA test followed by Bonferroni's post hoc test									
Groups	**Time**	**N**	**Mean**	**SD**	**Min**	**Max**	**P-value [a]**	**Sig. viff**	**P-value [b]**
	TO	10	34.097	1.886	30.65	37.24		TO *vs* T1	<0.001*
Group I	T1	10	27.447	2.432	23.18	31.92	<0.001*	TO *vs* T2	<0.001*
	T2	10	21.986	1.913	19.65	25.65		T1*vs* T2	<0.001*
	TO	10	48.246	3.926	41.19	53.17		TO *vs* T1	<0.001*
Group 2	T1	10	45.552	3.202	39.42	49.01	<0.001*	TO *vs* T2	<0.001*
	T2	10	42.336	2.587	37.19	47.12		T1*vs* T2	0.001*
	TO	10	63.547	4.198	53.82	68.42		TO *vs* T1	0.08
Group 3	T1	10	60.523	4.961	50.3	66.75	<0.001*	TO *vs* T2	<0.001*
	T2	10	54.908	4.614	45.31	61.15		T1*vs* T2	<0.001*

*Statistically Significant **Note** a. P-Value derived by One-way ANOVA Test. P-value derived by Tukey's post hoc test

Discussion

Orthodontic success depends on good dental hygiene and caries management. The orthodontic wire creates additional plaque-forming surfaces, which boosts oral bacteria. Dental plaque can cause caries, gingivitis, and periodontitis. Knowing microbiological changes in orthodontic patients, especially retention, is critical.[18] The retention phase, in which dental motions are maintained following active treatment, is crucial for orthodontic success and preventing teeth from shifting. After orthodontic treatment, fixed or removable retainers maintain stability. Fixed retainers are made of different-sized and-material wires and glued to the teeth with composite resin.[19] Removable retainers might be vacuum-formed or acrylic splints with clasps. Long-term fixed retainer usage makes dental hygiene harder, according to periodontal studies. Fixed retainers are linked to gingival recession, plaque retention, probing haemorrhage, and deeper probing. All are likely caused by long-term tissue irritation by the fixed retainer or microorganisms around it.[20]

Fixed orthodontic therapy affects mouth flora by increasing bacterial retention regions. Orthodontic appliances improve oral hygiene. 5 weeks after debonding, the number of germs decreased significantly, according to studies. After debonding, the simplified oral hygiene index, plaque index, and gingival index were also examined.[21] All periodontal indicators dropped after debonding, and dental hygiene improved. Another study investigated the periodontal consequences of detachable or fixed retainers at baseline (before debonding) and 1, 3, and 6 months later, reporting an improvement in gingival health in both groups.[22]

A study examined the plaque index, gingival index, and calculus index of upper and lower lingual retainers versus upper and lower vacuum formed retainers over 12 months. After 12 months of retention, lingual retainers were related with increased plaque, calculus, and gingival inflammation. Both groups' oral hygiene improved after removing permanent appliances.[23] The divergent outcomes of these trials comparing detachable and fixed retainers were attributed to the short follow-up period and the greater motivation of patients given toothpastes and toothbrushes during oral hygiene training. In adult subgingival plaque samples from varying depths, *T. forsythia* and *P. gingivalis* were strongly correlated. Deeper periodontal pockets had more of both species. *P. gingivalis* wasn't found without *T. forsythia*. *P. gingivalis* and *T. denticola* were strongly associated in subgingival plaque samples.[24]

Similar results were seen in another study. Red Complex members were discovered in considerable numbers in adult periodontitis lesions, especially deep pockets or advanced lesions.[25]

Detection of these potential periodontal infections relies on the procedures used. Many bacteria cannot be cultivated using normal techniques; therefore, cultivation studies underestimate microbial diversity.[26] Anaerobic culturing only recovers portion of the sample's microscopic count. The culturing approach focuses on detecting viable organisms (living bacteria) and requires quick sample processing to maximise bacterial survival, along with strict transport conditions. Due to this method's low sensitivity; little amounts of a pathogen in a sample may go unnoticed. PCR is used to detect bacterial infections. PCR analysis is used in periodontal research because it is more sensitive and selective than traditional culture.[27] It is a sensitive and specific approach for detecting, identifying, and differentiating organisms. PCR is the best method for amplifying genes and RNA transcripts. This is the most used method for studying DNA. It identifies periodontal infections in subgingival swabs and saliva.[28] This study compared salivary microbial levels of "Red Complex Bacteria" (*P. gingivalis, T. denticola*, and *T. forsythia*) in individuals using three types of fixed lingual retainers after orthodontic treatment with fixed equipment.[28] 30 individuals, who finished orthodontic treatment at M. R. Ambedkar Dental College and Hospital, Bengaluru, participated in the study. Before entering the trial, they were evaluated for inclusion/exclusion and gave informed consent.

Retainium wire is a single-strand nickel-free titanium flat ribbon wire with higher interproximal strength and reduced wear rate. Nickel-free, it prevents patient allergies. Penta-One wire is a 5-stranded 0.0215" circular stainless steel wire. It fractures less than thinner or 3-strand wires of the same thickness, yet it's elastic enough to enable some tooth motion. Bond-A-Braid wire is a flattened, dead soft, rectangular (0.027" x 0.011") eight-braided wire. It's adaptable and prevents tooth movement from active force wires. In an evaluation of bonded lingual retainer wires, smaller diameter multi-stranded stainless steel wires bonded to the six lower anterior teeth are preferred. Flexible wire allows periodontal patients' teeth to move physiologically.[29] Flat braided wires are not as commonly used in bonded retainers as circular wires. Also examined is the amount of tiny wires in multi-stranded cables. One study recommended a 0.0215" five-stranded twisted wire to prevent stress fracture, while another utilised a 0.0175" multi-stranded wire.[30,31]

Three groups of 10 patients were randomly selected. Six anterior teeth received fixed lingual retainers (Retainium, Penta-One, or Bond-a-Braid wire). Saliva was collected at debonding (T0), 4 weeks after (T1), and 8 weeks after (T2) fixed lingual retainers were installed. The saliva samples taken were stored at -80° Celsius. PCR test was performed to isolate the bacterial DNA from the saliva with the target bacterial load- *P. gingivalis, T. denticola* and *T. forsythia*. One-way ANOVA followed by Tukey's post hoc Test was used to compare *P. gingivalis, T. denticola*, and *T. forsythia* PCR data between 3 groups at different time intervals. The mean PCR values of various microorganisms at different time intervals in each group were compared using repeated measures ANOVA and Bonferroni's post hoc test.[30] All tests were significant at $p<0.001$ Group 3 had the most Red Complex Bacterial growth, followed by Group 2. Group 1 had the least Red Complex Bacterial growth (T0, T1, T2). Several studies comparing fixed lingual retainer wires found that single-strand wires develop less oral bio film than multi-strand wires. In vivo study compared periodontal indices between ordinary plain retainer and braided retainer. The conventional retainer demonstrated better plaque, gingival, and calculus indices than the braided kind. Similar study compared gingival health, plaque accumulation, tooth stability, and integrity of multistrand and round wire bonded lingual retainers. Multistrand wire retainers gathered more plaque on the lower anterior teeth than round wire retainers. In vivo bio film growth was compared on single- and multi-strand retention wires. Single-strand retention wires had less bio film than multi-strand wires. This study found that *P. gingivalis* grew fastest after debonding (T0). 8 weeks after debonding, *P. gingivalis* growth slowed (T2). *T. denticola* and *T. forsythia* had maximum values at debonding (T0) and lowest values 8 weeks afterwards (T2). The removal of orthodontic appliances reduces *A. actinomycetemcomitans*, *P. gingivalis, T. denticola*, *T. forsythia* (Red Complex Bacteria), and *P. intermedia*, improving clinical periodontal parameters. After 3 months, most clinical and microbiologic indicators normalise. From debonding (T0) to 8 weeks following debonding, the levels of Red Complex Bacteria decreased dramatically in the present study (T2). The present investigation quantifies *P. gingivalis, T. denticola*, and *T. forsythia* (Red Complex Bacteria) in patients with a fixed lingual retainer using PCR. Due to several constraints, the number of patients and saliva sample collecting time were limited. Further investigations with longer sample collection times, more patients, and microbiologic plaque sample comparisons are needed.

Conclusion

Orthodontic treatment requires good oral hygiene. The fixed lingual retainer wire creates new plaque-forming sites, changing the oral micro biome. This study evaluated and compared salivary microbial levels of "Red Complex Bacteria" after orthodontic treatment with fixed appliances, during the retention period utilising three commercially available fixed lingual retainer wires. Retainium, Penta-One, and Bond-a-Braid wires were employed in this study (Reliance Orthodontics Ltd., Itasca, Illinois, USA). In this study, 30 orthodontic patients were randomly assigned into 3 groups of 10 each. Six anterior teeth received fixed lingual retainers (Retainium, Penta-One, or Bond-a-Braid wire). Saliva was collected at debonding, 4 weeks after, and 8 weeks later (T2). Saliva samples were frozen at -80°C. *P. gingivalis, T. denticola*, and *T. forsythia* were isolated from saliva using PCR. Group 3 (Bond-a-Braid wire) had the highest Red Complex Bacterial growth, followed by Group 2. (Penta-One wire). Group 1 (Retainium wire) had the least Red Complex Bacterial growth over time (T0, T1, T2). This study found that *P. gingivalis* grew fastest after debonding (T0). 8 weeks after debonding, *P. gingivalis* growth slowed (T2). *T. denticola* and *T. forsythia* had maximum values at debonding (T0) and lowest values 8 weeks afterwards (T2). This work quantifies *P. gingivalis, T. denticola*, and *T. forsythia* (Red Complex Bacteria) in patients with a fixed lingual retainer using PCR. This study had a tiny sample size and only collected saliva samples 3 times. Thus, longer investigations with longer sample collecting times and higher sample sizes are needed, as well as plaque sample comparison studies.

Study finding overview

The current standard of care of practice recommends using wires made of flexible, multi-stranded stainless steel that have a smaller diameter and are bonded to the first six teeth in the arch. Those individuals who have periodontal problems should consider employing wire that is more flexible in order to facilitate the physiological movement of their teeth.

However, according to the results of our research, the most significant growth of red complex bacteria was detected in Group 3 (Bond-a-Braid wire), followed by Group 2 (Penta-One wire). At a

variety of time intervals, Group 1 (Retainium wire) had the lowest levels of red complex bacterial growth.

However, additional research must be conducted that covers a longer time period, has a longer time for sample collection, and includes a greater number of patients. Additionally, research must be conducted that compares microbiologic data by taking samples of plaque.

Therefore, the type of wire that is required for usage as a fixed lingual retainer is determined by a number of different criteria at the same time.

Declaration

Ethics approval and consent to participate: The institutional review board of the Department of Orthodontics & Dentofacial Orthopedics, M. R. Ambedkar Dental College and Hospital, Bengaluru India approved the study protocol. All the patients provided informed consent for their data to be used in this study **EC Number: 1014/844A/MDS 2022**

Consent for publication

Applicable, Reference Number: **1014/844A/MDS 2022**

Author contributions

Conceptualization: SAH. Data curation: AP. Formal analysis: RSN, AP, And SAH. Methodology: VK, AN. Writing- original draft: SAH, AJ. Writing- review & editing: SAH&AJ.

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Conflicts of interest

The Authors declare that there are no conflicts of interest.

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