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FULL PAPER

Oral and throat microbiological changes after orthodontic debonding

Alessandra Lucchese[1,2,3], Riccardo Nocini[4], Antonino Lo Giudice[5], Paolo Asperio[6], Flaminia Guglietta[1,2], Letizia Carenzi[7], Angelica Bertacci[8], Dario Donadello[9], Marco Farronato[10], Cinzia Maspero[11,12], Nicoletta Zerman[13]

[1]*Unit of Orthodontics, Division of Dentistry IRCCS Ospedale San Raffaele Scientific Institute, Milan, Italy;*
[2]*Unit of Orthodontics, School of Dentistry, Vita-Salute San Raffaele University, Milan, Italy;*
[3]*Unit of Dentistry, Research Center for Oral Pathology and Implantology, IRCCS Ospedale San Raffaele Scientific Institute, Milan, Italy;*
[4]*Section of ENT; Department of Surgical Sciences, Dentistry, Gynecology and Pediatrics; University of Verona; Verona, Italy;*
[5]*Department of General Surgery and Surgical-Medical Specialties, School of Dentistry, University of Catania, Policlinico Universitario "Vittorio Emanuele – G. Rodolico", Catania, Italy;*
[6]*Division of Maxillo Facial Surgery, Cardinal Massaia Hospital, Asti, Italy;*
[7]*Unit of Orthodontics and Pediatric Dentistry, Section of Dentistry, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy. Director: Prof. Paola Gandini;*
[8]*Department of Biomedical and Neuromotor Sciences (DiBiNeM), School of Dentistry, University of Bologna, Bologna, Italy;*
[9]*Department of Oral and Maxillofacial Surgery, Policlinico G.B. Rossi, University of Verona, Verona, Italy;*
[10]*Department of Medicine, Surgery and Dentistry, Section of Orthodontics, University of Milan, Milan, Italy;*
[11]*Department of Biomedical, Surgical and Dental Sciences, School of Dentistry, University of Milan, Milan, Italy;*
[12]*Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy;*
[13]*Dental Clinic, University of Verona, Verona, Italy*

SUMMARY

As already known, orthodontic treatment presents a factor of plaque retention, promoting an increase of bacterial growth in the oral cavity. Nevertheless, after orthodontic debonding an alteration of the previous microbiological status may occur. The present study was designed to assess variations among six bacterial species in the oral cavity and the status of oral health after orthodontic debonding. At the end of the fixed orthodontic treatment, 30 patients were divided into three groups based on the type of retention: I - 10 patients were treated with upper and lower fixed retention devices, II - 10 with upper and lower removable retention devices, and III - 10 with lower fixed and upper removable retention devices. To assess the alterations of oral microbiota after orthodontic debonding, two salivary swabs were collected for each individual: the first immediately after debonding (T0) and the other one 6 weeks later (T1). Six species, the ones most correlated with the development of caries and periodontal disease, were selected for microbiological analysis with Real-time PCR: *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and *Fusobacterium nucleatum*. Furthermore, in order to correlate the microbiological outcomes with the clinical condition, oral health indexes at T0 and T1 were assessed for all patients. Six weeks after debonding, the salivary levels of the bacteria investigated tend to decrease and the values of the oral health indexes tend to improve with all types of treatment considered ($p<.05$). Salivary bacteria levels and oral health are similarly influenced by fixed and/or removable orthodontic retentions.

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INTRODUCTION

Orthodontics has extensively evolved in recent years and is considered a safe treatment both in adolescent and adult patients. At the same time, the microbiological feature of orthodontic treatment is becoming more and more important, permitting better understanding of its impact on patient's oral health (Lucchese *et al.*, 2018a).

It is well known that orthodontic treatment, such as any biomaterial placed in the oral cavity, can increase the number of plaque retention sites and cause an increase in bacteria concentration (Øilo *et al.*, 2015), but after debonding there can be a variation of the previous condition.

The goals of the debonding procedure are to remove orthodontic brackets and all adhesive resin from the tooth and to restore the surface as closely as possible to its pre-treatment condition without inducing iatrogenic damage. After debonding, the last phase of orthodontic therapy foresees the use of retention

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Corresponding author:
Nicoletta Zerman
E-mail: letiziacarenzi@gmail.com

devices, which can be fixed or removable, in order to reach and maintain orthodontic results. The main removable appliances are the thermo-molded retainer and Hawley's plate. Fixed retention devices include bonded or band-welded lingual arches (Lucchese *et al*., 2011), bonded or band-welded palatal arches (Lucchese *et al.*, 2018b), retainers, and customized retainers. The main goal of retention devices is to maintain the orthodontic result, preventing relapses of final occlusal outcomes (Lucchese *et al.*, 2001).

Over 700 microbial species colonize the mouth and provide an essential contribution to maintaining oral health (Nędzi-Góra *et al.*, 2020). This harmony can be altered by innumerable factors, including the change in the habitat following treatment with orthodontic devices (Eroglu *et al.*, 2019). As demonstrated in numerous studies, fixed and removable orthodontic appliances can increase the number of plaque retention sites, and consequently the concentration of Streptococci, Lactobacilli, Actinomyces and Bacteroides among bacteria (Arendorf *et al.*, 1985; Jung *et al.*, 2014) or Candida albicans among fungi, altering the balance of oral flora and leading to the onset of oral pathologies, such as caries and periodontitis (Türköz *et al.*, 2012; Rosenbloom *et al.*, 1991). Nevertheless, variations of bacterial species within the oral cavity after debonding have rarely been examined in the literature (Kim *et al.*, 2016; Farhadian *et al.*, 2016). Frequently, published studies center on the impact of a single retainer device on the oral cavity after debonding and, even though some studies have compared the influence of fixed and removable retainers on periodontal health after debonding (Isola *et al.*, 2017; Nocini *et al.*, 2020), it is unclear whether the prevalence of dental caries and periodontal parameters differ between a fixed and a removable retention protocol (Löe *et al.*, 1963; Gorelick *et al.*, 1982).

Several and sometimes contradictory results were obtained over time as a consequence of the variety of the removable orthodontic appliances used, the vast quantity of the bacteria analyzed, and the different microbiological analysis methods adopted. Taking this into account, the aim of this work was to assess the variations of six bacterial species in the oral cavity and the status of oral health after orthodontic debonding, managing the orthodontic retention phase with fixed retainers, vacuum formed retainers, and both. The null hypothesis of this study was that different retention protocols bring about an enhancement in oral health parameters in patients treated with fixed orthodontic appliances, and that no differences exist between different retainers in the preservation of the balance between oral bacterial species and oral health.

MATERIALS AND METHOD

An a priori sample size calculation was performed with G*power 3.1 software (University of Dusseldorf, Dusseldorf, Germany) based on the following values: significance (α) =0.05; power =0.8, and effect size =0.5. This required a minimum of 28 patients, but the number of participants was increased to 30 in order to achieve better sample distribution among the 3 groups. At this point, a sample of 30 patients, with a mean age of n 20.3±0.3 years, 15 females and 15 males, was analyzed. In this cohort study all patients were treated in private practice for 18 months with the McLaughlin Bennett mechanics system in both arches with the same metal brackets (Forestadent, Germany). At the end of the treatment, retainers were applied and, subsequently, debonding was carried out. The retainers consisted of five-strand twisted round stainless steel wire. The wire was bonded to the palatal or lingual surfaces on the front teeth with light-cured composite. All patients underwent professional oral hygiene sessions every three months, during which they were instructed to floss and brush their teeth properly 3 times a day. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of IRCCS San Raffaele Scientific Institute, Milan, Italy (107/1NT/2017). The inclusion criteria selected were: patients at the end of orthodontic therapy, good compliance (motivation and good level of oral hygiene), approval of consent regarding the treatment and experimental part of this study. The exclusion criteria selected were: denture wearers, smokers, allergies or intolerances towards the materials used in the study, antibiotic therapy within 3 months prior to the start of the study, radiographically evident loss of alveolar bone, participation in other studies, presence of systemic diseases (diabetes, hypertension, rheumatoid arthritis, depression, obesity), presence of enamel developmental diseases (amelogenesis imperfecta, enamel hypoplasia). Patients were randomly divided into three groups: I - 10 patients were treated with upper and lower fixed retainers (ULF), II - 10 with upper and lower removable retainers (ULR), and III - 10 with lower fixed retainer and upper removable retainer (LF-UR).

To assess the alterations of oral microbiota after orthodontic debonding, two salivary swabs were collected for each individual by one of the authors (AL), who is a dentist. The first swab was taken immediately after debonding (T0) and the second six weeks later (T1). Sample collection was carried out before periodontal examinations, and patients were instructed not to eat or drink anything before the salivary swabs were taken. The following six bacterial species were selected and analyzed with Real Time pcr: Streptococcus mutans among the GRAM +, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum* among the GRAM -. We chose these bacterial species because they are the most often associated with carious lesions and periodontal

disease, the two pathological conditions that most frequently affect the oral cavity. After the collection of samples, the DNA was first extracted and purified with a method that involves two consecutive incubations with lysozyme and proteinase K. Subsequently, quantitative PCR of 16s RNA genes was performed using the hydrolysis probes method. The real time PCR analysis was performed for each sample.

Furthermore, the oral health indexes were monitored by one of the authors (AL) at both time points (T0 and T1) for all patients: Plaque Index (PI), Gingival Inflammation Index (GI), Probing Pocket Depth (PPD), Bleeding on Probing (BOP), White Spots Lesions (WSL) and Decayed Missing Filled Teeth (DMFT).

Clinical detection was carried out visually and with a periodontal probe (Hu-Friedy, Chicago, USA) on elements 42, 41, 31 and 32; six sites were analyzed for each dental element (mesial, medial and distal on both the buccal and lingual surfaces). Plaque Index (PI) attributes a value of 0 to those sites where the passage of the periodontal probe at the height of the gingival margin does not detect the presence of plaque, value 1 to those sites where plaque is found. Gingival Inflammation Index (GI) was calculated according to Löe and Silness, assessing the degree of vestibular, lingual, mesial and distal gingival inflammation of the considered teeth. According to this scale, values range from 0 to 3, where 0 indicates healthy, bleeding-free gums:

1) indicates a slight change in color of the gums, minimal edema and no bleeding on probing;
2) indicates the presence of edema, slight redness and bleeding on probing;
3) indicates a clinical picture with severe edema, redness, ulceration and a tendency to spontaneous bleeding (Löe *et al.*, 2015).

Probing Pocket Depth (PPD) was measured in millimeters with the probe considering the distance from the free gingival margin to the bottom of the gingival sulcus or pocket. Bleeding On Probing (BOP) was evaluated after probing the gingival sulcus of the teeth with a periodontal probe. Following this index, a value of 0 is attributed to the absence of bleeding after 30 seconds of probing, a value of 1 to the presence of bleeding after 30 seconds of probing and a value of 2 to the presence of immediate bleeding. The presence or absence of White Spot Lesion (WSL) was evaluated on the buccal surfaces of the maxillary and mandibular teeth from the second right premolar to the second left premolar according to the Gorelick Index. According to this classification, a value of 0 is attributed in the absence of white spots, value 1 for visible white spots, but without alteration of the tooth surface (mild WSL), value 2 for visible and rough surface lesions, but which do not require conservative restorations (moderate WSL), a value of 3 corresponds to visible lesions that require conservative care (severe WSL). Decayed, Missing and Filled Teeth (DMFT) indicates the total number of teeth or surfaces that have decayed (D), missing (M) or filled (F) in an individual. All tooth surfaces were visually examined to assign a value according to the criteria established by the WHO in 2013.

The data obtained from the Real Time pcr of the bacterial species analyzed and from the bacterial load in the 30 patients correspond to the number of DNA molecules detected in the tubes during quantitative pcr. In order to improve data analysis with noise removal, a statistical analysis was carried out on relative quantities, calculated as the ratio between the quantity of each species and the total bacterial load. To evaluate mean, standard deviation, and frequency a descriptive analysis was performed; the subsequent path was followed to evaluate the differences between continuous variables. The data obtained were subjected to the Shapiro - Wilk test for the verification of normality ($P<0.05$). In the statistical evaluation of microbiological findings, 1-way analysis of variance (ANOVA) was used. Values were considered to be statistically significant at $P<0.05$. Intra-examiner reproducibility was assessed after the examiner was trained according to a qualified professional's criteria. Three subjects, who were not included in the sample, were recruited for calibration. To quantify the measurement error of the variables, the formula described by Dahlberg was used. Five patients were randomly selected and re-observed by one operator; the scores were reassigned twice by the same observer within 20 minutes of the first assessment. The intra-examiner error was less than 5% (95% confidence).

RESULTS

The microbiological analysis of the samples revealed a statistically significant decrease in total bacteria salivary levels 6 weeks after debonding (T1) in the three groups analyzed, i.e., patients with upper and lower fixed retainers, patients with upper and lower removable retainers, and patients with lower fixed retainer and upper removable retainer.

Tables 1 and *2* show the values of mean and standard deviations relating to *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and *Fusobacterium nucleatum* levels at T0 and T1, in all groups of patients. The above-mentioned enhancement is visible in these tables; it was statistically significant ($p<.05$).

Conversely, there was no statistically significant difference ($p>.05$) between the three groups both at T0 and T1.

From T0 to T1 and in the three groups of patients examined, the levels of the only GRAM + bacterium analyzed show a statistically significant reduction with a P-value less than .05. From T0 to T1 and in

Table 1 - *Absolute and relative mean and standard deviations (SD) of the Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannarella forsythia (Tf), Treponema denticola (Td), Fusobacterium nucleatum (Fn) and Streptococcus mutans (Sm) salivary levels (log_{10}) in the three groups.*

Bacteria	*Groups*	*N patients*	*T0 Mean ± SD*	*T0 Mean ± SD (%)*	*T1 Mean ± SD*	*T1 Mean ± SD (%)*
Total bacteria	ULF	10	7.93 ± 0.5	-	7.71 ±0.6	-
	LF- UR	10	7.53 ± 0.4	-	7.32 ± 0.4	-
	ULR	10	8.33 ± 0.6	-	8.10 ± 0.7	-
Aa	ULF	10	2.90±0.63	0.002 ± 0.004	2.79 ± 0.74	0.003 ± 0.006
	LF- UR	10	2.61±0.57	0.002 ± 0.004	2.51 ± 0.66	0.003 ± 0.006
	ULR	10	2.96±0.64	0.002 ± 0.004	2.85 ± 0.75	0.003 ± 0.007
Pg	ULF	10	2.03 ± 0.96	0.002 ± 0.016	1.60 ± 0.95	0.001 ± 0.001
	LF- UR	10	1.82 ± 0.94	0.002 ± 0.010	1.44 ± 0.85	0.001 ± 0.001
	ULR	10	2.07 ± 0.98	0.002 ± 0.011	1.63 ± 0.96	0.001 ± 0.001
Tf	ULF	10	2.57 ± 0.97	0.006 ± 0.017	2.13 ± 0.93	0.007 ± 0.018
	LF- UR	10	2.32 ± 0.95	0.006 ± 0.016	1.92 ± 0.82	0.007 ± 0.017
	ULR	10	2.62 ± 0.97	0.006 ± 0.018	2.17 ± 0.90	0.008 ± 0.019
Td	ULF	10	1.65 ± 0.95	0.005 ± 0.016	1.40 ± 0.67	0.001 ± 0.001
	LF- UR	10	1.48 ± 0.84	0.005 ± 0.015	1.26 ± 0.78	0.001 ± 0.001
	ULR	10	1.68 ± 0.86	0.006 ± 0.017	1.42 ± 0.69	0.001 ± 0.001
Fn	ULF	10	3.61 ± 0.74	0.014 ± 0.027	3.45 ± 0.74	0.022 ± 0.041
	LF- UR	10	3.25 ± 0.66	0.013 ± 0.026	3.11 ± 0.66	0.021 ± 0.039
	ULR	10	3.68 ± 0.75	0.014 ± 0.029	3.52 ± 0.75	0.023 ± 0.043
Sm	ULF	10	5.17 ± 1.48	0.016 ± 0.029	4.81 ± 1.37	0.016 ± 0.025
	LF- UR	10	4.70 ± 1.00	0.014 ± 0.023	4.46 ± 1.20	0.014 ± 0.024
	ULR	10	5.19 ± 1.34	0.016 ± 0.026	4.88 ± 1.33	0.016 ± 0.024

Table 2 - *Mean and standard deviations of the oral health indexes regarding parodontal tissues in the three groups: Probing Pocket Depth (PPD), Gingival Inflammation Index (GI), , Bleeding on Probing (BOP) and Plaque Index (PI).*

Oral health indexes	*Groups*	*T0 Mean ± SD*	*T1 Mean ± SD*
PPD	ULF	1.86 ± 0.34	1.58 ± 0.29
	LF- UR	1.67 ± 0.37	1.40 ± 0.38
	ULR	1.72 ± 0.34	1.48 ± 0.29
GI	ULF	0.94 ± 0.48	0.23 ± 0.20
	LF- UR	0.85 ± 0.62	0.16 ± 0.19
	ULR	0.94 ± 0.58	0.28 ± 0.40
BOP	ULF	0.21 ± 0.11	0.01 ± 0.02
	LF- UR	0.27 ± 0.12	0.06 ± 0.09
	ULR	0.25 ± 0.14	0.05 ± 0.08
PI	ULF	0.42 ± 0.24	0.07 ± 0.10
	LF- UR	0.48 ± 0.35	0.07 ± 0.06
	ULR	0.57 ± 0.42	0.12 ± 0.20

the three groups of patients examined, the levels of the GRAM - bacteria under analysis show a statistically significant reduction. In particular, the P-values are less than .05 in all cases with the exception of Td, which has a P-value of less than .01.

The values of the oral health indexes confirm the results obtained from the microbiological analysis. Actually, the clinical parameters considered demonstrate a statistically significant improvement ($p<.05$) of oral health conditions from debonding to the beginning of retention phase (T0) and 6 weeks later (T1). There was no statistically significant difference ($p>.05$) among three groups analyzed. The improvement of oral clinical conditions after debonding was more evident than that of microbiological analysis.

The mean and standard deviations (SD) of the above-mentioned indexes are shown in *Tables 2* and *3*. PPD, GI, BOP, PI and WSL decreased with statistical significance between T0 and T1. The DMFT index remained the same at both times. The P-values were less than .001 in all cases, except for the cases with lower fixed retainer and upper removable retainer (F&R), for which the P-value was .002.

Table 3 - *Mean and standard deviations of the oral health indexes regarding teeth in the three groups: White Spots Lesions (WSL) and Decayed Missing Filled Teeth (DMFT).*

Oral health indexes	*Groups*	*N patients*	*T0 Mean ± SD*	*T1 Mean ± SD*
WSL	ULF	10	0.37±0.33	0.18±0.21
	LF- UR	10	0.34±0.29	0.17±0.24
	ULR	10	0.31±0.25	0.16±0.18
DMFT	ULF	10	0.24±0.86	0.14±0.15
	LF- UR	10	0.26±0.75	0.26±0.75
	ULR	10	0.22±0.65	0.22±0.65

Table 4 - *P-value relative to the differences among the three groups of Gingival Inflammation Index (GI) and Plaque Index (PI).*

Oral health indexes	*P-value (T0)*	*P-value (T1)*
GI	0.741	0.474
PI	0.646	0.653

The three groups analyzed did not show statistically significant differences between the oral health indexes at both time T0 and T1 (p>.05). P-values of GI and PI are shown in *Table 4*.

DISCUSSION

Jung *et al.* (2014) observed a statistically significant reduction in the total amount of bacteria 5 weeks after debonding. Nevertheless, they found a statistically significant increase in *S. mutans* and *S. sobrinus* levels at 5 and 13 weeks after debonding and the total amount of bacteria touched the high point with a statistically significant increase 13 weeks after debonding. In the study by Rosenbloom and Tinanoff (1991), salivary levels of *S. mutans* before, during, and after 6-15 weeks of orthodontic therapy were investigated. They found a significant increase in *S. mutans* salivary levels during treatment and their decrease during the retention phase following debonding; *S. mutans* salivary levels returned equal to those documented before orthodontic treatment. In conformity with this study, our results show a statistically significant decrease in salivary levels of *S. mutans* in samples taken in the sixth week after debonding. (Jung *et al.*, 2014) stated that oral hygiene index values drop dramatically one week after debonding and maintained minimal values during their study. Likewise, (Kim *et al.*, 2016) assessed the simplified oral hygiene index, the plaque index and the gingival index in patients with a thermo-printed maxillary retention device and mandible Hawley plate after debonding; according to their results all periodontal parameters decreased significantly immediately after debonding and oral hygiene was enhanced. Corresponding to our study, (Heier *et al.*, 1997) assessed periodontal repercussions of fixed or removable retainers just before debonding and 1, 3, and 6 months later, recording widespread enhancement in gingival health in both groups. According to (Forde *et al.*, 2018), greater plaque accumulation and greater gingival inflammation occur with lingual retainers rather than with thermally molded templates. In their study, an initial enhancement in oral hygiene after removing all orthodontic bands and brackets took place with both retention appliances, but over time, the periodontal indexes of all patients got worse. We can conclude that the short follow-up period and the superior motivation of the patients are the reasons these studies present different results. However, regarding oral health status, it is well known that it is not easy to compare microbiological outcomes and clinical conditions (Matarese *et al.*, 2016). Nevertheless, the reduction in pathogenetic bacterial species and the improvement in oral indexes found in the present study suggest an increase in oral health after debonding and the absence of statistically significant differences among the three groups investigated. Our study stands as one of the first to analyze microbiological and periodontal changes after debonding; however, the limited number of patients and the limited time to collect saliva samples are the limitations of this report. The different results of studies comparing fixed and removable retainers are due to different sample sizes (adequate sample sizes versus limited sample sizes) and microbiological comparative methods (taking plaque samples from many areas versus a few areas, such as full-mouth evaluation versus site-specific evaluation). Further studies covering a longer period, with longer sample collection time and more patients, as well as studies comparing microbiological data by taking plaque samples from different areas, are needed. Furthermore, although the presence of fixed braces at T0 can simulate bacterial and hygienic conditions during orthodontic treatment, there are no data regarding the oral microbiota prior to orthodontic treatment (Kundu *et al.*, 2016; Jabur *et al.*, 2008; D'Ercole *et al.*, 2014). Data concerning the bacteria before treatment would have provided more valuable information on the alterations of the bacteria during treatment. Further long-term studies are needed from pre-treatment to the retention phase to study the influence of the microbiota on the oral health of orthodontic patients. In addition, although the COVID-19 pandemic impacted on our research, causing an initial delay in sample collection, the prompt management guidelines (Gherlone *et al.*, 2021) enabled us to complete the study. Indeed, it gave us all an opportunity to reflect on how micro-

biology plays an important role in the human body, even from the orthodontic point of view.

The limitations of the present study consist mainly in a small sample size. Furthermore, no information was collected on the patients' diet, a factor that could potentially influence the composition of oral microbiota.

Salivary bacteria levels and oral health are similarly influenced by fixed and/or removable orthodontic retentions. If proper oral hygiene is performed, the conditions of the oral cavity after orthodontic treatment tend to promptly return to normal.

Conflicts of interest

This research received no external funding. The authors declare no conflicts of interest.

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