

## Correlation between occlusal trauma and oral microbiota: a microbiological investigation

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**The occlusal trauma has been defined as an injury to the periodontium resulting from occlusal forces that exceeds the reparative capacity of the attachment apparatus. Currently, the effects of occlusal trauma on tooth support tissues, the onset and the progression of periodontal disease are still debated. In fact, researchers started evaluating the possible effects of occlusal discrepancies on incidence, progression, and treatment outcomes of periodontitis, but all the results underlined the more significant role played by microbiological flora. The results of this study show that after 60 days of treating the occlusal trauma, a significant reduction was achieved in the periodontal bacterial flora.**

The periodontal disease origins by subgingival micro-organisms that cause the destruction of deep periodontal tissues (1-4). The current microbiological knowledge in the field of periodontology is made more uncertain by the difficulties that have always hindered the definition of pathogenic periodontal bacteria (1). More than 400 species may be cultured from the periodontal pockets of different individuals and it is possible to recover 30-100 species (any one of which may be a pathogenic agent) from a single site. Many bacterial species present in the pockets are difficult to culture and identify (2).

The physical limitations of pockets make it difficult to obtain a consistently representative sample. Because sampling areas do not all appear to be equally active, the

time at which the sample is taken may be as critical as the sampling area in determining disease pathogenesis. The growth of opportunistic species may also be an outcome of disease rather than its cause. Because their levels may increase after or at the same time as those of the true pathogenic agents, it may be difficult to distinguish between them experimentally (3).

The culture method was extensively used in the past to characterize the gingival microflora that colonizes the periodontal region. This cannot be considered a reliable system because not all the micro-organisms present in periodontal pockets survive in standard transport media and they do not all display a good growth rate in the media normally used in laboratories (4).

This is due firstly to the metabolic properties of

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the strains, which are often extremely demanding in terms of nutrients and oxygen tolerance. The culture method, moreover, cannot discriminate between different strains of the same bacterial species that often exhibit different levels of virulence and thus different levels of pathogenicity (5). Up-to-date molecular biology investigation methods (real-time PCR) now allow us to identify periodontal pathogens with greater specificity and sensitivity than when using culture investigation methods (6) and also to quantify them reliably (7, 8). Such methods are being used to establish the role of pathogens in periodontal disease with greater clarity and also the significance of their surprising presence even in areas unaffected by periodontal damage (9, 10).

The bacterial species considered periodontal are probably saprophytes. These are commonly present in the oral cavity and can live for years as perfectly compatible members of the microbial flora. Individuals are continually colonized by pathogenic periodontal bacteria at the gingival margins and in the subgingival area yet may not show any signs of on-going or previous periodontal damage (1-4).

The subjects were observed frequently and despite accumulating plaque they did not suffer any evidence of periodontal tissue damage even years later or, conversely, patients who display severe periodontal changes despite maintaining excellent standards of oral hygiene.

The periodontal disease is also found to be extremely site specific even when plaque and tartar deposits extend throughout the mouth. For this reason, an environmental factor may be at play that is able to influence the microorganisms and force them to express damaging factors that remain hidden for a long time or select for the more virulent clonal varieties (3).

Traumatic forces may discharge themselves onto a tooth or group of teeth when they are subjected to non-centric pre-contact or interference during disclusive jaw movements or following extractions that distort the dental alignment (8). This study was carried out to assess the role of occlusal trauma as a primary or second primary factor in the etiology of periodontal disease.

It involved measuring quantitative and qualitative

changes in the subgingival bacterial flora that could be achieved after treating the occlusal trauma alone, without pharmacological or surgical treatment. The changes were considered in relation to the clinical parameters recorded such as probing depth (PD), bleeding on probing (BOP), presence of pus (Pus).

## MATERIALS AND METHODS

Fifteen patients were selected from those receiving treatment: 10 males and 5 females (aged between 41 and 62) that were nonsmokers and suffering from adult periodontitis. The subjects suffering from systemic conditions and those who had taken antibiotics during the previous 2 months were excluded from the study. The patients were informed about the study participation and completed an informed consent form according to the Helsinki declarations. In this study, it was considered the upper anterior group from canine to contralateral canine to standardize the sampling procedure and measurements. Each patient displayed a pocket depth of 3 mm on at least three teeth in the upper anterior group from canine to contralateral canine and typical signs and symptoms of occlusal trauma: dental mobility (mobility of at least 2 on Miller's scale) (1-4), persistent difficulty in chewing, fremitus, presence of dental wear facets.

This study did not make any distinction between primary occlusal trauma and secondary occlusal trauma. Changes occurring in the periodontal tissue as a result of occlusal trauma were similar in all cases and independent of periodontal height.

The harmful effects of occlusal trauma were conservatively treated in 10 patients by means of intracoronal splinting of the dental elements from canine to contralateral canine. In the remaining five patients, a prosthetic solution was adopted that involved applying a provisional resin bridge from canine to contralateral canine. The treatment of the occlusal trauma was not preceded or followed by topical or systemic drug treatment or by surgical or non-surgical treatment of the affected periodontal tissues. Intracoronal splinting of the tooth elements was carried out using metal wire, achieved by interweaving three Ortosmail (Ortho Organizer, CA USA) 0.11" metal ligatures, and composite.

The intracoronal splinting cancelled the mobility of the tooth elements (mobility 0 on Miller's scale). A groove

was made on the palatal surface at the dental contact points to accommodate the metal wire. The subjects with partial edentulism were treated by means of a provisional resin bridge. This made it possible to correct the occlusal relationships between traumatized teeth and antagonistic teeth and distribute the functional loads more evenly. The stumps were prepared supragingivally in order not to alter the bacterial flora in the gingival sulcus. The patients were also advised to continue with their normal oral hygiene routine at home.

#### Clinical examination and bacteriological sampling

Changes of the clinical parameters PD, BOP and Pus were considered for each patient. The subgingival flora was examined for each patient on the four surfaces (midvestibular, distal vestibular, mid-palatal, distal palatal) of the tooth that displayed the greatest mobility or the deepest pocket if the mobility was the same.

Clinical parameters were measured, and the subgingival flora was sampled before treatment of the occlusal trauma (T0), after 20 days (T1) and lastly after 60 days of treatment (T2). It was not possible to include a control group in this study because all the teeth included in the splinting or prosthetic bridge displayed signs and symptoms of occlusal trauma, however slight. All the clinical measurements were conducted by two operators trained and calibrated with an excellent agreement intra- and inter-operator. Periodontal probing was carried out using a Williams probe, by calculating the distance between the base of the pocket and the gingival margin. BOP was considered positive (BOP+) or negative (BOP-) according to whether bleeding occurred on at least one of the four surfaces (mid-vestibular, distal vestibular, mid-palatal, distal palatal) of the tooth considered in each patient within 15 seconds of probing.

Similarly, each tooth studied was considered positive (Pus+) or negative (Pus-) for the presence of pus according to whether pus was present in at least one of the paper cones inserted into the four sites (midvestibular, distal vestibular, mid-palatal, distal palatal) for 10 seconds. The pus was distinguishable from the crevicular fluid due to its color and density. Microbiological sampling was carried out before measuring PD, BOP and Pus using a microbiological test based on real-time PCR (meridol® Perio Diagnostics, GABA International, Italy) in each of the four sites (mid-vestibular, distal vestibular, mid-

palatal, distal palatal) of the tooth examined, according to Ballini et al. (3). Total bacterial count, *Actinobacillus actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythensis* (Tf), *Treponema denticola* (Td), *Fusobacterium nucleatum* (Fn), *Prevotella intermedia* (Pi) were then measured in a specialized laboratory.

#### Statistical analysis

The significance of variations of PD, BOP and Pus

**Table I.** Changes of clinical parameters recorded (PD, BOP, Pus) during the observation period in the tooth studied for each patient.

	PD	BOP	Pus	Time
Patient 1	7 mm	+	+	T0
	4 mm	-	+	T1
	3 mm	-	-	T2
Patient 2	7 mm	+	+	T0
	3 mm	-	-	T1
	3 mm	-	-	T2
Patient 3	4 mm	+	-	T0
	3 mm	+	-	T1
	2 mm	-	-	T2
Patient 4	4 mm	+	+	T0
	2 mm	-	-	T1
	2 mm	-	-	T2
Patient 5	3 mm	-	+	T0
	2 mm	-	-	T1
	1 mm	-	-	T2
Patient 6	10 mm	+	+	T0
	6 mm	-	-	T1
	4 mm	-	-	T2
Patient 7	5 mm	+	-	T0
	3 mm	-	-	T1
	2 mm	-	-	T2
Patient 8	3 mm	+	-	T0
	2 mm	-	-	T1
	1 mm	-	-	T2
Patient 9	4 mm	+	-	T0
	3 mm	-	-	T1
	2 mm	-	-	T2
Patient 10	3 mm	+	-	T0
	2 mm	-	-	T1
	2 mm	-	-	T2
Patient 11	8 mm	+	+	T0
	4 mm	-	-	T1
	3 mm	-	-	T2
Patient 12	3 mm	+	-	T0
	2 mm	-	-	T1
	2 mm	-	-	T2
Patient 13	10 mm	+	+	T0
	7 mm	-	+	T1
	5 mm	-	-	T2
Patient 14	9 mm	+	+	T0
	6 mm	-	+	T1
	4 mm	-	-	T2
Patient 15	5 mm	+	+	T0
	3 mm	-	-	T1
	2 mm	-	-	T2

was calculated by ANOVA followed by Tukey post-hoc test. The 2-sample t-test was used to compare the means between baseline bacterial quotas and bacterial quotas after 60 days from therapy (T2). All statistical procedures were performed using the Statistical Package for the Social Sciences (SPSS 11.0).

## RESULTS

The changes in probing pocket depths (PD), bleeding on probing (BOP), presence of pus (Pus) during the experimental study are illustrated in Table I. At each follow up visit and for each patient a significant improvement of clinical parameters from baseline was recorded (mean PD:  $p < 0.001$ ; % BOP- sites:  $p < 0.001$ ; % Pus sites:  $p < 0.002$ ). BOP and Pus were negative in T2 for all patients. The mean PD average reduction between T0 and T2 was 3.13 mm (Table II).

Subgingival flora load prior to treatment (T0), obtained from the sum of the four samples (mid-vestibular, distal vestibular, mid-palatal, distal palatal) taken from around the tooth studied, was compared for each subject with the loads present 20 days (T1) and 60 days after treatment (T2).

Eleven of the 15 treated patients experienced a significant reduction in the periodontal bacterial flora load following treatment of their occlusal trauma. They were suffering from severe periodontal disease (BOP+, Pus+, PD >4mm). Four patients with slight inflammation that had not yet reached the deep periodontal tissue experienced an inconsistent and non-significant reduction in the number of bacteria. Bacterial load at T0 was already limited and comparable to the levels that other Authors observed in healthy sites (1-4).

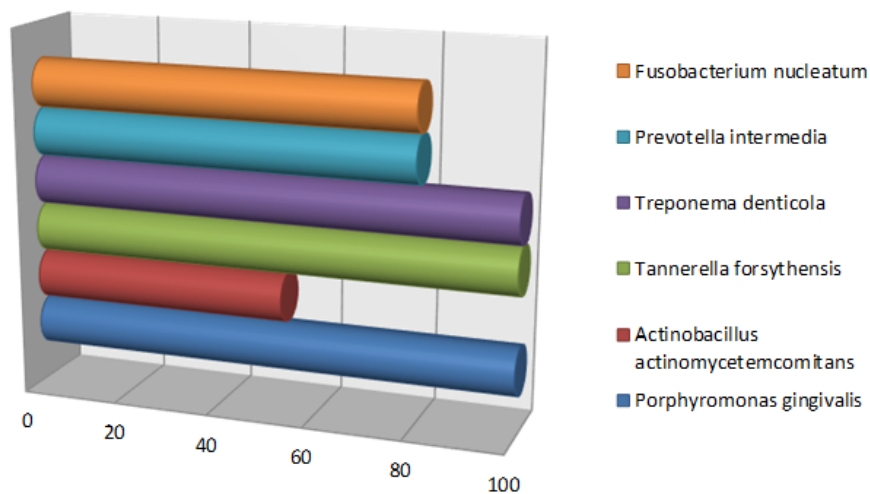
Although our treatment brought about an improvement from a clinical viewpoint, it did not lead to a drastic reduction in bacterial load, which was already in itself physiological. Eleven of the 15 patients treated were therefore considered when processing the results of microbiological testing. These were the subjects that displayed severe periodontal disease (BOP+, Pus+, PD > 4 mm). The average reduction in the number of each periodontal pathogen studied between T0 and T2 is shown in Figure 1. The counts of Aa fell by 53.1% ( $p < 0.127$ ), while Pg displayed an average reduction of 59.5% ( $p < 0.046$ ). The bacterial counts of Tf, Td, Fn, and Pi were reduced by 87.5% ( $p < 0.007$ ), 92.6% ( $p < 0.001$ ), 83.4% ( $p < 0.008$ ), and 84.3% ( $p < 0.227$ ), respectively.

## DISCUSSION

The results of this study show that after 60 days of treating the occlusal trauma, a significant reduction was achieved in the periodontal bacterial flora (*Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Fusobacterium nucleatum*, *Prevotella intermedia*), which corresponds to an improvement in PD, BOP and Pus. It was not always possible to restore the microbial load to within a physiological range because in some cases the periodontitis treated were so severe that surgical treatment was required in any case. After 20 days of treatment, despite an initial improvement in clinical parameters, the pocket microflora underwent inconsistent changes. This can probably be explained through the agonist-antagonist relations that are present between the bacterial species involved. The

**Table II.** Descriptive statistics of PD changes during the observation period for all patients ( $n=15$ ) corresponds to the number of patients treated.

	Mean±DS	Error std.	C.I. 95%
<b>T0</b> <b>(n=15)</b>	5.6667±2.6	0.67377	4.2216-7.1118
<b>T1</b> <b>(n=15)</b>	3.4667±1.6	0.42389	2.5575-4.3758
<b>T2</b> <b>(n=15)</b>	2.5333±1.1	0.29059	1.9101-3.1566



**Fig. 1.** Microbiological findings in periodontal specimens: frequency with which pathogens were identified.

treatment to resolve the occlusal trauma brought about a reduction in the bacterial load without the need for topic and systemic pharmacological treatment or surgical or non-surgical treatment of the affected periodontal tissues. The two bacterial species, *Tannerella forsythensis* and *Treponema denticola*, which displayed the most pronounced reduction in average bacterial load, i.e. 87.5% and 92.6% respectively, are the species that should be considered the most important microbial risk factors for differentiating between subjects with periodontitis and subjects with healthy periodontal tissue (4).

The reorganization of the micro-circulation in the periodontal tissues and the removal of the environmental stress probably induce a significant change in factors specific to the local environment such as temperature, osmotic pressure, the concentration of certain metabolites (iron, calcium and magnesium) and the concentration of oxygen. Each of these factors are able to influence the subgingival species, their virulence and the expression of the most aggressive clonal types (1, 5, 6, 11-17).

Increased growth in the periodontal tissues stimulates the local immune defences that force the bacterial flora into quiescence. Grant et al. (9) compared the subgingival microflora associated with mobile teeth and immobile teeth in 35 patients

receiving maintenance treatment and 15 patients with untreated periodontitis. Dental mobility was found to be a risk factor for an increase in the number of periodontal bacteria, particularly *Campylobacter rectus* and *Peptostreptococcus micros*.

According to the specific studies (1-4), the most common periodontal bacteria are present in diseased and healthy sites, in each individual, from an early age (18). Even though this etiopathogenic factor can easily be found and identified in all patients, periodontal disease remains an extremely localised condition in most cases. A second site-specific factor must come into play that brings about initial inflammation in the periodontal tissues to generate a locus minoris resistentiae where periodontal species can express their virulence.

Occlusal trauma is able to bring about an extension in the periodontal ligament space (8) and hence determine the apical migration of the bacteria and their proliferation (19-23). Lastly, *Actinobacillus actinomycetemcomitans* has been isolated in 53.3% of the sites examined. Although this is considered one of the main periodontal pathogens (4), more recent studies agree that *Actinobacillus actinomycetemcomitans* performs a pathogenic role in some subjects, but not in all (2). Now that more sensitive detection methods are available, its role in



periodontal disease must probably be at least partly reviewed. Some authors considers that a disturbance must arise in the environment (24-27), including mesenchymal compartment (28-36), that allows the pathogenic species to flourish so that the periodontal bacteria can reach the threshold levels required to bring about disease, even if probiotics seems to be a promising tool (12, 21, 37-44). In conclusion we believe that treatment of the occlusal trauma interrupts the disturbance in the environment to bring about a consequent aggression from the pathogenic species.

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