



## Research Article

## Alleviating Salivary Microbiome Dysbiosis Associated with Dental Malocclusion Utilizing Low-level Laser Therapy

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## ABSTRACT

Dental malocclusions have been found to disrupt the balance of oral bacteria, contributing to the development of dental caries and periodontal diseases. Utilizing Low-Level Laser Therapy (LLLT) can potentially restore biodiversity and ecological equilibrium in the oral microbiome, promoting optimal oral health. We propose that administering LLLT with a diode laser emitter emitting visible red light at a wavelength of  $970 \pm 10$  nm and power output of 100mW, can have a positive impact on the oral microbiome. With an exposure area of  $6\text{cm}^2$  for a duration of 30 seconds per session (4 sessions per week), it may help prevent dental caries and periodontitis associated with dental malocclusion. We believe that LLLT has the potential to improve the biodiversity of the oral microbiome and promote good oral hygiene and integrity. This non-invasive treatment can serve as an effective therapeutic adjunct by stimulating the oral mucosa.

**Keywords:** Dental Malocclusion; Dental Caries; Periodontitis; Low Level Laser Therapy

## 1 INTRODUCTION

### 1.1 Oral microbiome and dental malocclusion

The oral microbiome is distinctive for each individual, similar to fingerprints, and reflects the overall state of the oral cavity. A balanced and diverse microbiome consisting of various bacteria such as *Streptococcus*, *Staphylococcus*, *Veillonella*, *Actinomyces*, *Corynebacterium*, *Prevotella*, *Porphyromonas*, *Lactobacillus*, *Propionibacterium*, *Fusobacteria*, and *Eubacteria* indicates a healthy oral cavity<sup>(1–3)</sup>. Each bacterial species plays a unique role in maintaining oral symbiosis which is crucial for determining oral health. However, dental malocclusion can disrupt this balance and lead to dysbiosis of the salivary microbiome<sup>(1–3)</sup>.

Malocclusion, which can arise from misalignment or discrepancies in the occlusion of upper and lower teeth, is associated with an unfavorable environment for the balance of the oral microbiome. This imbalance increases the likelihood of developing dental caries and periodontal

diseases. As a result of malocclusion and its secondary effects, dysbiosis in the salivary microbiome may occur<sup>(4,5)</sup>.

Dental malocclusion can cause an imbalance in the oral microbiome, shifting it from a mutually beneficial relationship with its host to a parasitic one. This dysbiosis of the salivary microbiome is associated with common oral diseases such as dental caries and periodontal disease<sup>(4,5)</sup>.

In dental caries, the prevalent bacteria that are increased in numbers include *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus acidophilus*<sup>(6,7)</sup>. In periodontal diseases like gum disease, the upregulated microorganisms consist of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Tannerella forsythia* and *Treponema denticola*<sup>(8)</sup>. There is an established connection between the composition of saliva microbiome, dental misalignment, and systemic illnesses. An imbalance in oral microbial diversity has a direct impact on various organs in our body, resulting in severe medical conditions<sup>(9,10)</sup>. The occurrence of chronic

inflammation and recurrent bacterial invasions precipitates the emergence of bacteremia, the formation of organ abscesses, and the manifestation of prominent systemic maladies, including Diabetes and Cardiovascular Diseases (CVD)<sup>(11,12)</sup>.

In order to effectively mitigate the potential adverse consequences of dental malocclusion, it is imperative to foster a diverse and symbiotic salivary microbiome. This approach can help decrease the prevalence of harmful bacteria in the mouth and establish a healthier oral milieu. Enhancing our comprehension of dysbiosis in different areas of the oral microbiome is crucial for preventing conditions such as periodontitis and caries, as well as systemic illnesses like cancer. By examining investigations on microbial imbalances associated with these diseases, we can proactively take measures towards maintaining optimal oral health and overall well-being.

### 1.2 Low Level Laser Therapy

Low-Level Laser Therapy, also known as LLLT, is an innovative treatment that utilizes red and/or near-infrared light at low levels. This therapy offers a multitude of benefits including cell and tissue stimulation, accelerated healing processes, and effective relief from pain and inflammation<sup>(13)</sup>. Considered a type of light therapy in academic circles, LLLT employs non-ionizing light sources like lasers, LED lights, and broadband light across both visible and infrared ranges. It's important to note that this form of therapy operates on a non-thermal basis by stimulating natural chromophores within the body. As a result, it triggers photophysical and photochemical reactions that have remarkable effects at various biological levels<sup>(14)</sup>.

LLLT administered through low-intensity lasers targets specific components of cells known as photoacceptors or chromophores<sup>(13)</sup>. These include cytochrome c oxidase, opsins, and flavoproteins. When these photoacceptors are stimulated by LLLT, it results in increased enzymatic activity, leading to enhanced electron transport, greater oxygen consumption, heightened mitochondrial membrane potential, and ultimately accelerated production of ATP. This process also triggers the generation of signaling molecules such as reactive oxygen species, nitric oxide, and cyclic AMP. Consequently, key cellular processes including cell division and migration are activated through the involvement of transcription factors, structural proteins, and various enzymatic actions<sup>(15,16)</sup>.

Low-level laser therapy has gained widespread use in the management of pain, wound healing, and reduction of inflammation. Its ability to initiate favorable changes at the sub-cellular and cellular levels makes it an effective treatment option. LLLT can reduce pro-inflammatory cytokines, decrease oxidative stress, and influence macrophage phenotype<sup>(13,14)</sup>. Additionally, a study conducted on healthy mice showed that NIR LLLT treatment three times per week using

a wavelength of 808 nm resulted in significant alterations to the diversity of the gut microbiome after 14 days<sup>(13,17)</sup>.

Promising results have been observed in the initial studies investigating the effects of LLLT on the human gut microbiome. These studies reveal that LLLT can lead to an increase in beneficial bacteria such as *Akkermansia muciniphila*, *Bifidobacterium species*, and *faecalibacterium species*, which are known to contribute to a healthy gut microbiome. This indicates that different types of bacterial and fungal cells present in the microbiome may respond positively to LLLT thanks to photoacceptors/chromophores being present<sup>(13,18,19)</sup>.

Research has shown that low-level laser therapy can have an impact on the gut microbiome in rats, potentially through its anti-inflammatory properties and redox signaling. This implies that LLLT not only reduces pro-inflammatory cytokines but also has the ability to promote a shift in cell polarization from a pro-inflammatory state to an anti-inflammatory state<sup>(20–22)</sup>.

Based on the information presented earlier about the importance of a varied oral microbiome for overall dental and systemic health, the correlation between the oral microbiome and dental malocclusion, and the possible effect of LLLT on gut microbiome, we propose a hypothesis that posits LLLT may contribute to preventing dysbiosis and supporting the recovery and upkeep of a healthy oral microbiome. This could potentially decrease the vulnerability to dental caries and periodontal diseases in individuals with dental malocclusion.

We strongly recommend low-level laser therapy as a promising treatment option for individuals with dental misalignment. The use of a diode laser device, applied in a rotary motion throughout the oral cavity, can effectively target and treat the affected areas. It is suggested to undergo at least four sessions per week for optimal results. To ensure proper coverage and safety, it is important to maintain a distance of 3 cm between the laser probe and tissue during each session. For further guidance on specific devices, irradiation parameters, and treatment protocols, please refer to Table 1 provided below.

### 1.3 Analysis of Oral Microbiome

Research has shown that the microbial composition of saliva is influenced by various factors, including local oral conditions and socio-environmental/socioeconomic factors. It plays a crucial role in determining periodontal health and can provide important insights into the overall condition of the oral environment.

To evaluate the potential benefits of LLLT on the oral cavity, the saliva of the subject can be analysed to assess changes in the composition of the salivary microbiome before and after LLLT treatment. This can be done by collecting saliva samples at baseline, after each LLLT session, and at follow-up visits. These samples can be analyzed using

**Table 1:** The device information, irradiation parameters, and treatment parameters to enhance the biodiversity of the oral microbiome

Number & Type of Emitters (laser or LED)	Laser – Diode
Wavelength	970±10 nm
Pulse mode & Laser power	CW 2.5 W
Irradiance	200 mW/cm <sup>2</sup>
Fluence (Radiant exposure)	6 J/cm <sup>2</sup>
Time	30 seconds
Number of points irradiated	16
Area irradiated (cm <sup>2</sup> )	6cm <sup>2</sup>
Number and frequency of treatment sessions	4 sessions per week

techniques such as next-generation sequencing or microbial culturing to determine the diversity and abundance of different bacterial species. Additionally, the levels of pro-inflammatory and anti-inflammatory cytokines in the saliva can be measured using a multiplex immunoassay. This comprehensive evaluation will provide valuable insights into the impact of LLLT on the oral microbiome and inflammatory response associated with dental malocclusion<sup>(23,24)</sup>.

The analysis of the oral microbiome typically requires DNA sequencing to identify the various microorganisms present. However, this task can be challenging due to the lower concentration of microbial DNA relative to host DNA in the oral cavity.

To fully comprehend the role of these microbes, it is imperative to have knowledge about their composition despite the significant impact they can have. A simple and effective method to isolate microbial cells from host cells known as microbial enhancement "based on the size difference between the host cells and the bacterial cells"<sup>(25)</sup>.

This method involves filtration or centrifugation techniques to separate microbial cells from host cells, allowing for a more accurate analysis of the oral microbiome. Microbial enrichment methods are valuable for studying microbial communities and increasing the diversity of microorganisms. These methods contribute to our understanding of microbial symbioses, mucocutaneous niches, and the overall microbiome. Future research should explore the potential of these methods in whole-genome sequencing and probe design for metagenomes<sup>(26–29)</sup>. In analyzing oral microbiome diversity after LLLT therapy in dental malocclusion subjects, techniques like DNA sequencing and microbial enrichment can provide comprehensive insights into the oral microbiome's response to low-level laser treatment.

## 2 CONCLUSION

Maintaining good oral hygiene practices, such as regular brushing and flossing, is crucial for reducing bacteria in the mouth and preventing dental issues. The composition of the oral microbiome plays a significant role in maintaining overall oral health. Low-level laser therapy can reduce inflammation in the oral mucosa and positively influence

the composition of the microbiome. After LLLT treatment, there is a decrease in pathogenic species and an increase in beneficial bacteria, particularly in patients who respond well to LLLT. This indicates that LLLT may enhance biodiversity within the oral microbiome, promoting better oral health and tissue integrity. As a non-invasive therapeutic option, LLLT could be valuable alongside other treatments while providing psycho stimulation to the oral mucosa.

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