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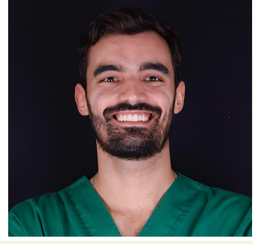

# Nitric oxide metabolites and their association with periodontal disease – a closer look to the crevicular fluid.


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## Introduction

Nitric oxide (NO) is a versatile signalling molecule involved in several critical physiological mechanisms, including neural communication, regulation of vascular tone and blood pressure, protection against cellular damage, inhibition of platelet aggregation, and immune responses. The main enzymatic source of NO is the endothelial nitric oxide synthase (eNOS), which is essential for maintaining vascular function [1–4]. However, under low-oxygen (hypoxic) conditions, the body may not produce enough NO through this enzymatic pathway. In such cases, an alternative biochemical route—the reduction of nitrate to nitrite and subsequently to NO—becomes particularly important for sustaining NO-dependent processes. The efficiency of this alternative NO pathway is influenced by multiple factors, including dietary intake of nitrates and nitrites, and the composition of the oral microbiota. The presence of conditions such as periodontal disease (PD), can alter the oral microbiome, thereby potentially impacting nitrate metabolism and NO production. Emerging evidence suggests that disturbances in the nitrogen cycle within the oral cavity may have broader implications for systemic health and be reflected in the salivary levels of NO metabolites such as nitrite [5]. However, quantifying nitrite in saliva is challenging due to the many confounding factors involved, as saliva reflects the state of the rest of the organism and can therefore be affected by other systemic conditions. Consequently, this study focuses on quantifying nitrite in crevicular fluid (CF) rather than saliva, as it is believed that the nitrite concentration in the periodontal pocket/gingival sulcus is more directly related to periodontal disease.

## AIMS

- Quantification of nitrite in crevicular fluid: collection of crevicular fluid samples in a standard location (in the case of the group with periodontal health) and also in the worst location (in the case of the group with periodontal disease).
- Analysis of the microbiome of the gingival sulcus (in the case of the periodontal health group) and also of the periodontal pocket (in the case of the periodontal disease group).
- Analysis of the tongue surface microbiome.

## Materials and Methods

PARTICIPANTS' RECRUITMENT AND CF SAMPLING:

Participants were required to be over 35 years old, in good health, and not taking any chronic medication, including diabetes, hypertension, asthma, antibiotics, or other chronic medication. The site selected for the CF collection was the mesio-vestibular location of the right upper first molar. Sample collection was conducted in triplicate to confirm the reliability of the results. Following informed consent and completion of a clinical and dietary questionnaire, CF samples were collected using Periopapers.

QUANTIFICATION OF NITRITES:

The quantification of nitrite in CF samples was carried out during consultation using the nitrite biosensors immediately after the specimens' collection. The nitrite biosensors are made of carbon screen-printed electrodes from DropSens, modified in-house with specific enzymes (Fig. 1), as described by Monteiro and collaborators [6-7]. Figure 2 depicts the protocol workflow, encompassing sample collection, volume assessment, nitrite extraction and quantification, as outlined below:

- After fluid collection from the crevicular pocket with a periopaper (Fig. 2A), the latter is placed in the periotron to quantify the volume of sample collected (Fig.2B).
- Then, according to a previously optimized procedure for extracting nitrite, the Periopaper is placed in a microtube containing 60 μL of distilled water, after which the mixture was subjected to five minutes of agitation in a vortex (Fig. 2C).
- For nitrite quantification the biosensors were connected with a portable potentiostat (Sensit Smart, PalmSens) controlled through the PSTouch software on a tablet (Fig.2D).


**Figure 1** – Schematic representation of the modified screen-printed electrode used as the foundation of the nitrite biosensor.

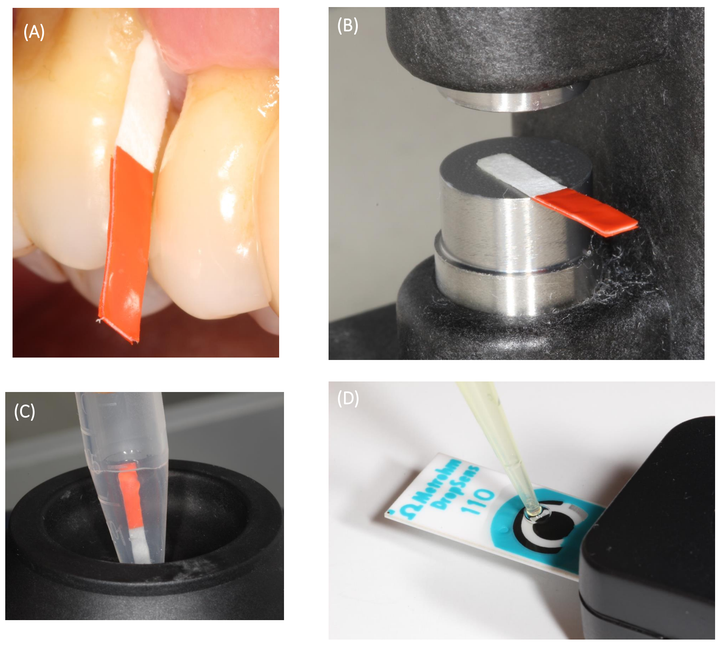


**Figure 2** – **(A)** CF collection with Periopapers (Ora Flow). **(B)** Volume measurement with Periotron (Ora Flow). **(C)** Periopaper submerged in 60 microliters of water and agitated for 5 minutes. **(D)** Sample deposition on the surface of the electrode.

## Results & Discussion

To date, 19 patients have been enrolled in the clinical study; however, two were excluded from the analysis due to **biosensor-related** issues. Of the remaining participants, 10 were female, with ages ranging from 35 to 86 years, and a mean age of 54.3 years. The group with periodontal health included 12 participants, while 5 participants were classified as having periodontal disease (Fig. 3).

According to our preliminary results (Fig. 4), the concentration of nitrite tended to be higher in the group of patients with periodontal health compared to the PD group. In particular, the majority of samples from PD-affected sites, both less and most affected locations, showed values below the 0.5 μM detection limit. The most affected PD locations (yellow) are absent in the higher nitrite concentration ranges (> 20 μM), further highlighting the contrasting behaviour. The > 40 μM category includes 5 samples, all from periodontally healthy individuals. This suggests that higher nitrite concentrations are more frequent in the healthy group.

These preliminary results contradict some conclusions previously published by other research groups that have associated higher values within the group with periodontal disease [8-9]. This may suggest that NO metabolism in periodontal disease is dysregulated or that nitrite is rapidly consumed in inflammatory environments. Measuring nitrite levels in fluids like CF or saliva can help in diagnosing and tracking diseases such as periodontal disease. However, these levels don't always directly indicate how active or how much nitric oxide synthase (NOS) is present in the tissues [10]. According to the literature, the expression of iNOS is increased in the presence of inflammatory stimuli. The presence of NO in periodontal tissues can be beneficial in terms of its antimicrobial activity or its participation in the immune response, but it can also be deleterious due to its cytotoxic effect on periodontal tissues [11]. Therefore, the role of NO and its metabolites has yet to be clarified. In the specific case of our study, since nitrite concentrations tend to be higher in the periodontally healthy group and since there are few studies evaluating its concentration in the crevicular fluid, it is necessary to increase the size of the study groups. Additionally, it is important to analyse other factors, such as the oral microbiota in the crevicular fluid or the diet, to gain further clarity.

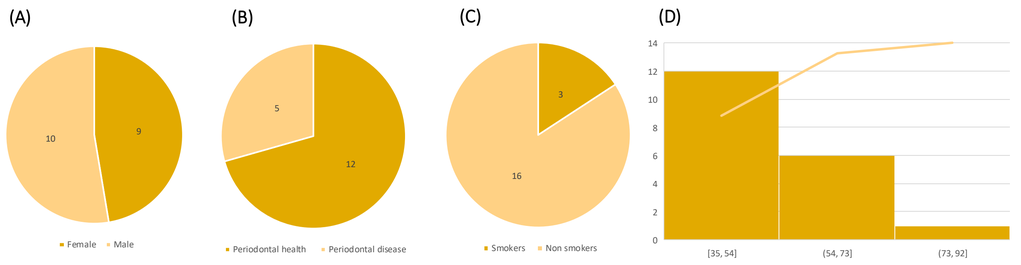


**Figure 3** – (A) - Distribution of participants by gender. (B) - Distribution of participants by study groups. (C) - Distribution of participants by smoking habits. (D) - Distribution of the participants by age.

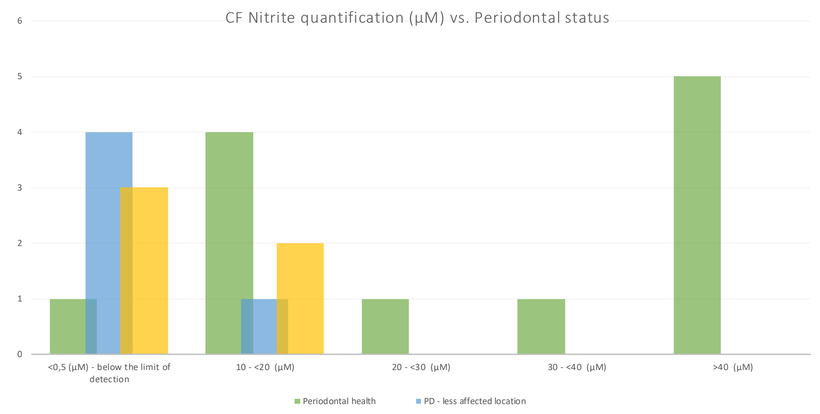


**Figure 4** – Nitrite concentrations in the gengival crevicular fluid samples from patients with and without periodontitis.

## Conclusions

- The preliminary results suggest that nitrite concentrations are higher in periodontally healthy individuals, which contradicts previous findings associating elevated nitrite levels with periodontal disease [8-9].
- The low nitrite values in PD-affected sites may indicate a reduced local nitric oxide production or increased degradation, or it could reflect microbial or environmental factors suppressing nitrite accumulation.
- A significantly larger sample size is required to validate these findings and to clarify the role of nitrite in periodontal health and disease at the level of the gingival crevicular fluid.
- To better understand the role of NO in periodontal disease, future research will incorporate data on additional influencing factors, such as oral microbiota composition and dietary intake.

## References


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