

# Effect of Nitrate and Nitrite on In-Vitro Oral Biofilms

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#### Introduction

Gingivitis is associated with an increase in dental plaque mass and an increase in the proportion of Gram-negative anaerobes. Prebiotics have the potential to modify plaque composition<sup>1</sup>.

Dietary nitrate supplementation has been shown to modify oral microbiome composition by increasing the relative abundance of the health-associated and aerobic genera *Neisseria* and *Rothia* <sup>2</sup>.

This study investigated the effect of nitrate and nitrite on the composition of in-vitro biofilms derived from human saliva.

## **Methods**

- Pooled saliva from 6 volunteers as inoculum
- Biofilms grown using the Calgary Biofilm Device in a mucin-containing complex medium
- Medium was supplemented with 0.5 mM, 5 mM and 10 mM sodium nitrate or nitrite
- · Biofilms were harvested after 7 days treatment
- 16S rRNA gene community profiling using the Illumina MiSeq platform for the V1-V2 region
- · Data analysed in mothur

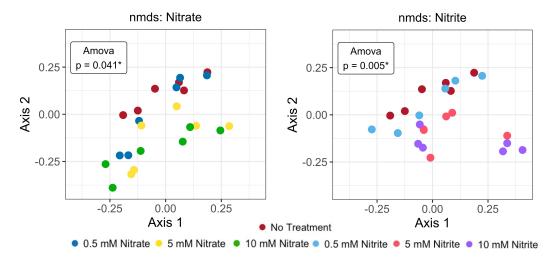


Figure 1 Shifts in bacterial community structure of in-vitro biofilms after supplementation.

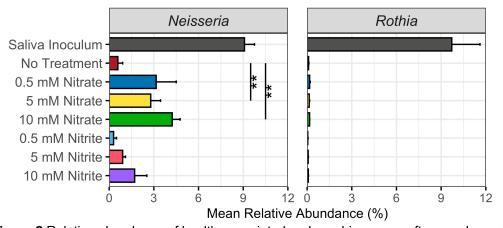


Figure 2 Relative abundance of health-associated and aerobic genera after supplementation.

## Results

- Treatment with 5 mM and 10 mM nitrate (p = 0.021 and p = 0.015) or nitrite (p = 0.01 and p = 0.004) led to significant changes in the bacterial community composition compared to no treatment (Figure 1)
- Health-associated genus Neisseria was significantly increased in biofilms treated with 5 mM or 10 mM nitrate (Figure 2)
- Rothia grew poorly in the biofilm model, although present in the saliva inoculum, and no treatment effects were seen (Figure 2)

### **Conclusions**

Supplementation with nitrate and nitrite can modify the composition of the bacterial biofilm community by increasing relative abundance of *Neisseria* spp. This supports the hypothesis that electron acceptors such as nitrate could be used as prebiotics to maintain oral health by encouraging growth of health-associated species.

## **Conflict of Interest**

This project is supported by GlaxoSmithKline.

## References

<sup>1</sup>Jockel-Schneider Y, Schlagenhauf U, Stolzel P, Gossner S, Carle R, Ehmke B, Prior K, Hagenfeld D. 2021. Nitrate-rich diet alters the composition of the oral microbiota in periodontal recall patients. J Periodontol doi:10.1002/JPER.20-0778

<sup>2</sup>Velmurugan S, Gan JM, Rathod KS, Khambata RS, Ghosh SM, Hartley A, Van Eijl S, Sagi-Kiss V, Chowdhury TA, Curtis M, Kuhnle GG, Wade WG, Ahluwalia A. 2016.

Dietary nitrate improves vascular function in patients with hypercholesterolemia: a randomized, double-blind, placebo-controlled study. Am J Clin Nutr 103:25-38.

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