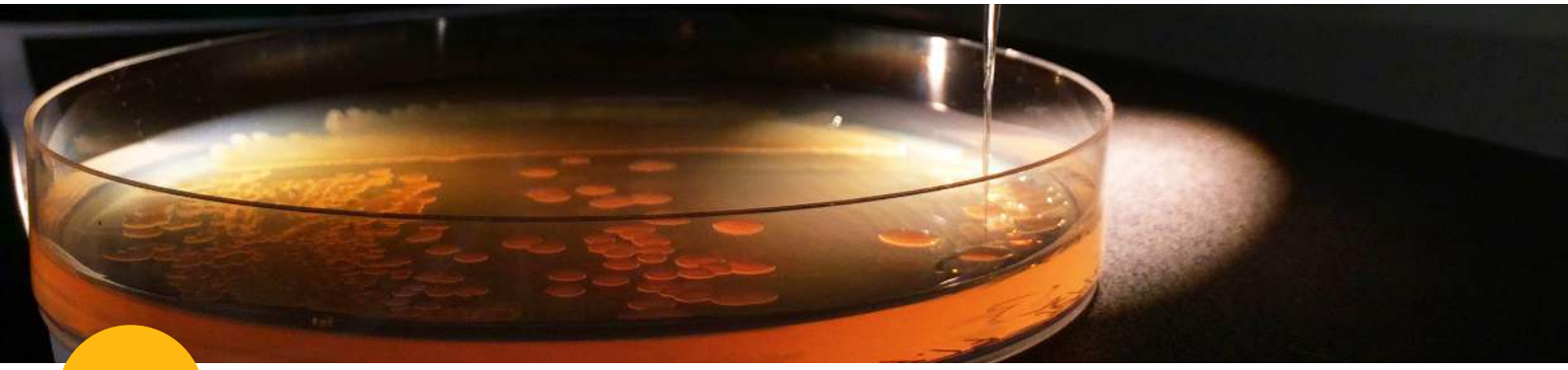

Unisense microsensors in medical biofilm research

O_2 and N_2O microprofiles in sputum samples from cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection

Introduction

With Unisense microsensors and the Unisense MicroProfiling System you can complete microprofiles in biofilms with extreme positioning accuracy (precision < 10 µm) and high spatial resolution to obtain valuable information about the microenvironment.

Kolpen et al. (2014) used the Unisense O_2 and N_2O microsensors to measure microprofiles in sputum samples from cystic fibrosis patients with chronic Pseudomonas aeruginosa infection. P. aeruginosa is the major cause of chronic lung infection of cystic fibrosis patients where the bacteria live as biofilm aggregates in the lungs. The biofilms can persist for years in the airways of the patient despite an active immune response and antibiotic therapy. The measurements in the paper by Kolpen et al. provided new insights about the microenvironment and growth of the P. aeruginosa biofilm which may lead to new treatment strategies.

Laboratory setup

The authors used Unisense O_2 and N_2O microsensors with a tip diameter of 25 µm (OX-25 and N_2O-25). The microsensors were connected to a Unisense amplifier and mounted on the motorized Unisense MicroProfiling System (Figure 1). The glass vials containing the freshly expectorated sputum samples were kept at 37 °C during the measurement. The sensors were first positioned manually at the upper surface of the sputum sample using the micromanipulator (MM33) to define the surface of the sample as the zero depth. Then the motorized MicroProfiling System was used to do automated microprofiles vertically through the sputum samples.

The SensorTrace Profiling software controlled the movement of the microsensors and the authors used a step size of 100 or 200 µm, a waiting time between measurements of 3 seconds for the O_2 profile and 5 seconds for the N_2O profile, and a delay time between each cycle of profile measurements of 10 seconds. Data acquisition and analysis were done with the SensorTrace Profiling software that logs the measured analyte concentration together with the depth position of the micromanipulator.

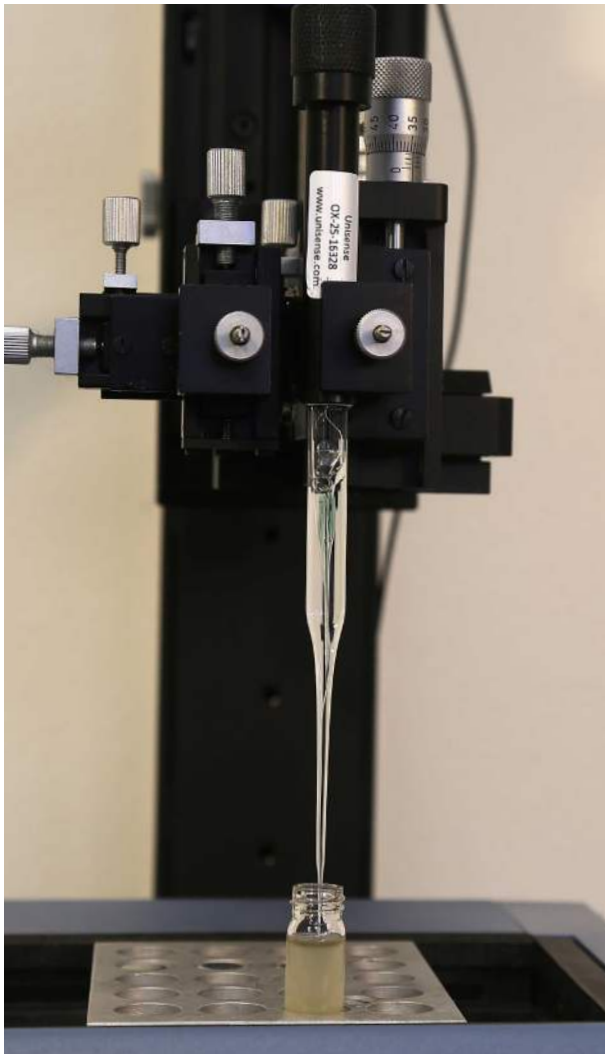

Figure 1: *MicroProfiling setup with a Unisense microsensor (25 µm tip diameter) mounted in the Unisense micromanipulator (MM33) measuring in a sputum sample. Photo: Kindly provided by Mette Kolpen.*

Results and conclusion

Based on the O_2 and N_2O microsensor profiling measurements, the authors demonstrated that sputum samples from patients with chronic P. aeruginosa infection consist of an upper oxygenated zone and a lower anoxic zone below around 3 mm from the sputum surface (Figure 2). N_2O production from the bacteria was mainly confined to the lower anoxic part and a maximum median concentration of 41.8 µM N_2O was found. Significantly less N_2O was found in control sputum samples from cystic fibrosis patients without infection. N_2O is an intermediate in the denitrification pathway and the data indicated that P. aeruginosa may acquire energy for growth from denitrification when O_2 is absent.

Using the Unisense microsensors to obtain O_2 and N_2O concentration gradients with high spatial resolution, the authors could explore the microenvironment in the sputum and they demonstrated N_2O production in clinical samples from infected cystic fibrosis patients for the first time.

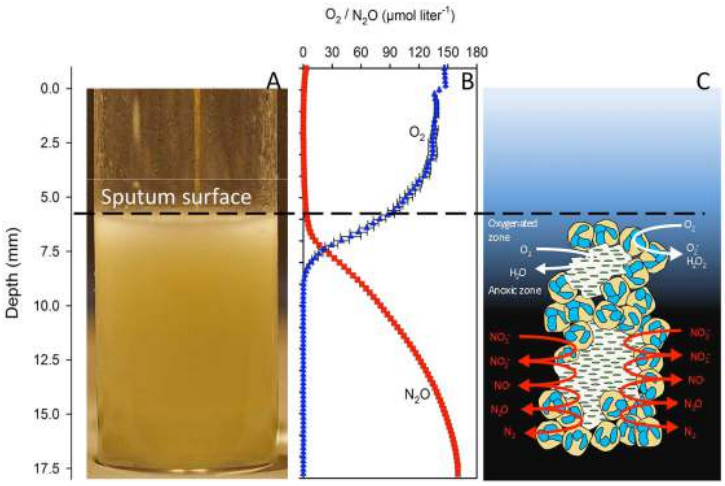

Figure 2: *A) Unisense microsensor inserted into a sputum sample from a cystic fibrosis patient with chronic lung infection. B) O_2 microprofiles (mean and SD of three profiles measured in the beginning of experiment) and N_2O microprofile measured 6-7 h after the beginning. C) Schematic model showing the metabolic mechanisms in the oxygenated and anoxic zones in the sputum sample.*
Figure adapted from Kolpen et al. 2014.

System Components	Product
Sensors	OX-25, N_2O-25
Amplifier	Microsensor Multimeter
Positioning Equipment	MicroProfiling System
Software	SensorTrace Suite (including Profiling software)

The results are published in Kolpen et al. (2014) Nitrous oxide production in sputum from cystic fibrosis patients with chronic *pseudomonas aeruginosa* lung infection. PLOS ONE Vol 9, Issue 1.

Related publications

Kolpen et al. (2016) Reinforcement of the bactericidal effect of ciprofloxacin on Pseudomonas aeruginosa biofilm by hyperbaric oxygen treatment. International Journal of Antimicrobial Agents 47 (2016) 163-167.

Kolpen et al. (2014). Nitric oxide production by polymorphonuclear leucocytes in infected cystic fibrosis sputum consumes oxygen. Clinical and Experimental Immunology. 177. 310-319.

Cowley et al. (2015) Pediatric cystic fibrosis sputum can be chemically dynamic, anoxic, and extremely reduced due to hydrogen sulfide formation. mBio Volume 6, Issue 4.

Fox et al. (2016) Anaerobic bacteria grow within Candida albicans Biofilms and induce biofilm formation in suspension cultures. Current Biology 24, 2411-2416.

Pabst et al. (2016) Gel-entrapped Staphylococcus aureus bacteria as models of biofilm infection exhibit growth in dense aggregates, oxygen limitation, antibiotic tolerance, and heterogenous gene expression. Antimicrobial Agents and Chemotherapy, Volume 60, Number 10.

Madsen et al. (2015) Facultative Control of Matrix Production Optimizes Competitive Fitness in Pseudomonas aeruginosa PA14 Biofilm Models. Applied and Environmental Microbiology, Vol 81, Number 24.

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