Liquid Blood Phantoms to Validate NIRS Oximeters: Yeast Versus Nitrogen for Deoxygenation

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Abstract

Liquid blood phantoms are a tool to calibrate, test and compare near-infrared spectroscopy (NIRS) oximeters. They comprise a mixture of saline, blood and Intralipid, which is subsequently oxygenated and deoxygenated to assess the entire range of tissue oxygen saturation (StO_2) from 0% to 100%. The aim was to investigate two different deoxygenation methods: yeast versus nitrogen (N_2) bubbling. The phantom was oxygenated with pure O_2 in both experiments, but deoxygenated by bubbling N_2 in the first and by addition of yeast and glucose in the second experiment. A frequency domain NIRS instrument (OxiplexTS) was used as reference and to monitor changes in the reduced scattering coefficient (μ_s') of the phantom. Both deoxygenation methods yielded comparable StO_2 values. The deoxygenation was slower by a factor 2.8 and μ_s' decreased faster when bubbling N_2. The constant bubbling of N_2 mechanically stresses the Intralipid emulsion and causes a decrease in μ_s', probably due to aggregation of lipid droplets. Deoxygenation by N_2 requires a more complex, air tight phantom. The gas flow cools the liquid and temperature needs to be monitored more closely. Consequently, we recommend using yeast for phantom deoxygenation.

1 Introduction

Since a few decades several near infrared spectroscopy (NIRS) devices have been developed and approved for clinical applications [1, 2]. Devices from different companies rely on different algorithms delivering varying StO_2 values for the same measurement [1, 3]. Absolute values to be used as treatment guidelines require different thresholds for each NIRS device [4–7]. Therefore, we recently developed a liquid phantom to compare and validate different NIRS devices [4–6, 8]. To achieve this, a liquid phantom with optical properties similar to the targeted human tissue is sequentially deoxygenated and oxygenated (i.e., StO_2 runs from 100% to 0% and back to 100%) while StO_2 is measured by different NIRS devices simultaneously.

The aim was to compare two deoxygenation methods with each other: (i) deoxygenation by bubbling nitrogen (N_2) versus (ii) deoxygenation by addition of yeast. Both methods are compared regarding the StO_2 values they deliver and on the phantom's scattering property.

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2 Methods

The setup employed was previously utilized for similar studies [5, 8]. Essentially, a black container was filled with a liquid phantom, mimicking the optical properties of neonatal brain. This container was equipped with four windows, i.e. holes in the black wall were covered watertightly with a silicone layer, which mimicked the optical properties of skull bone in the near infrared region: absorption coefficient μ_a (830 nm) = 0.11 cm^{-1}, reduced scattering coefficient μ_s' (830 nm) = 8.3 cm^{-1}, thickness = 2.5 mm. Sensors were placed on these windows and their near-infrared light passed through the windows into the phantom.

2.1 Liquid Phantom and Measurement Procedure

The black container was filled with liquid ingredients to resemble the optical properties of a neonatal brain. The ingredients were phosphate buffered saline (PBS), Intralipid (IL), human blood, sodium bicarbonate buffer (SBB); their quantities are listed in Table 1. PBS matches the osmolarity and ion concentration of the human body, whereas the IL adds scattering to the mixture. Human blood was added as erythrocyte concentrate containing haemoglobin. The SBB stabilized the liquid phantom's pH-level at ~7.4. The container was placed on a heating plate with a magnetic stirrer. The liquid phantom's temperature was kept constant at 37 °C throughout the measurements. The magnetic stirrer was set to a speed of 500 rpm, ensuring homogeneity of the liquid phantom during the measurement. The liquid phantom setup is depicted in Fig. 1a.

With four windows available, the StO_2 measured by three devices of interest were compared to the StO_2 measured by the reference device occupying the fourth window. To test the devices on the full measurement range, the liquid phantom was sequentially fully oxygenated and deoxygenated. To achieve StO_2 = 100%, O_2 was bubbled into the liquid phantom through a tube attached to the liquid container at a flow rate of 2 l/min during ~10 s. Once the liquid phantom was fully oxygenated, two different methods were applied to completely deoxygenate (StO_2 = 0%) it.

2.1.1 Deoxygenation Through N_2 Bubbling

For deoxygenation 1, the liquid phantom was deoxygenated by bubbling N_2 through a tube attached to the liquid phantom container. N_2 washes-out O_2 in a diffusion process and consequently reduces the blood's oxygenation. This process is nonlinear and time consuming. To completely deoxygenate the liquid phantom, the N_2 bubbling was performed in steps of increasing N_2 flow at time points of 50, 94, 112 and 136 minutes after starting N_2 bubbling. After complete deoxygenation, N_2 bubbling was stopped.

2.1.2 Deoxygenation Through the Addition of Yeast

Before deoxygenation 2, reoxygenation was performed by bubbling O_2 into the liquid phantom through another tube. Subsequently, 3 g of yeast and 3 ml of glucose were added. Glucose fed the yeast, which consumed the O_2 and deoxygenated the haemoglobin. The preparation of the second measurement cycle took <20 min.

2.2 Measurement Devices

Three different commercially available tissue oximeters were tested, because they are often deployed in clinics. All devices were equipped

Table 1 List of ingredients for the liquid phantom and the resulting haematocrit and haemoglobin concentration

PBS (mL)	Blood (mL)	IL (mL)	SBB (mL)	Glucose (mL)	Yeast (g)	Htc (%)	c_{tHb} (μM)
2500	34	74	20	3	3	0.87	47

PBS phosphate buffered saline, *IL* intralipid, *SBB* sodium bicarbonate buffer, *Htc* haematocrit, c_{tHb} total haemoglobin concentration

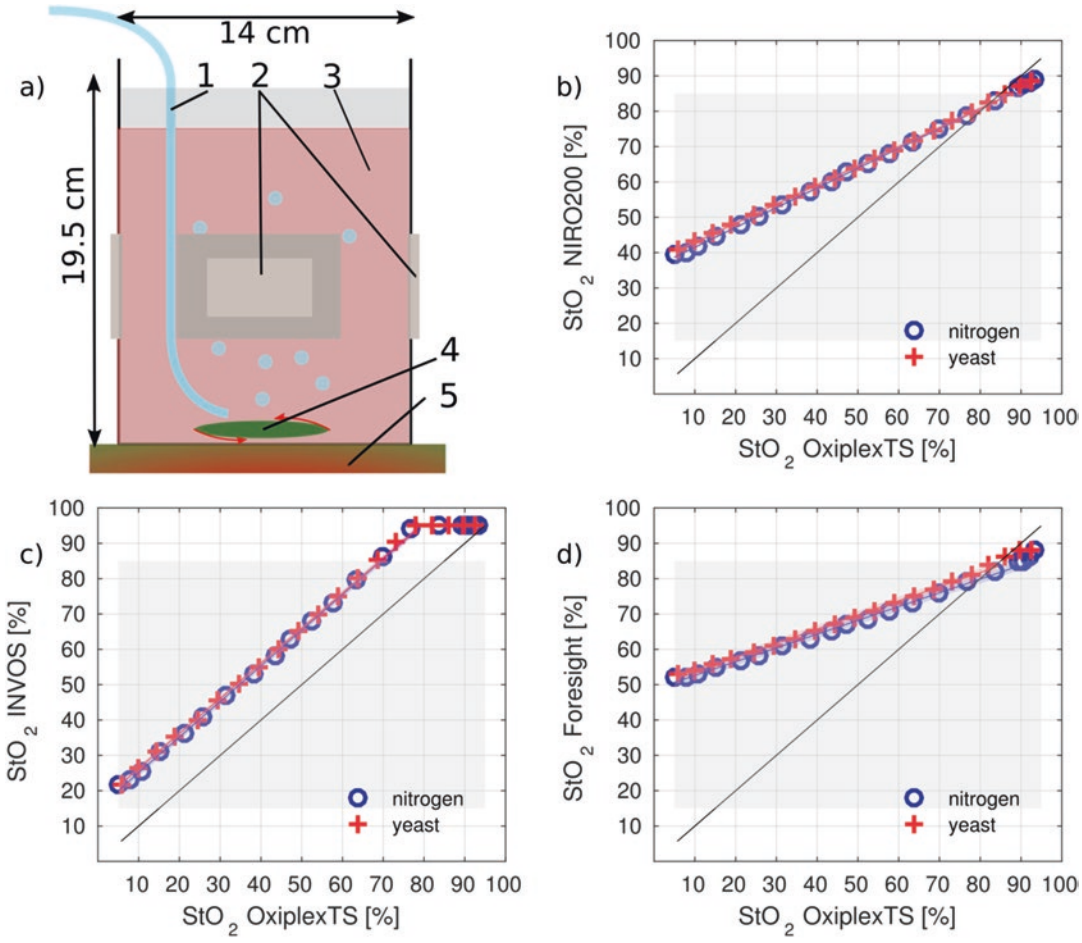

Fig. 1 (**a**) Liquid phantom setup employing a tube (1) to bubble O_2 or N_2. The container is equipped with four windows (2), a stirring bar (4) and a heating plate (5) to control the liquid phantom's (3) temperature and homogeneity. The measured StO_2 values of (**b**) NIRO-200, (**c**) INVOS and (**d**) FORE-SIGHT are shown on the *y*-axis with the respective OxiplexTS values on the *x*-axis. StO_2 is very similar between the two deoxygenation methods: N_2 bubbling and yeast

with paediatric sensors and were acquiring StO_2 in the phantom simultaneously.

- *FORE-SIGHT Elite* tissue oximeter (CAS Medical Systems, Inc., CASMED, Branford USA). The FORE-SIGHT system employs five wavelengths (690, 730, 770, 810, and 870 nm). The small sensor has two source-detector separations (SDS): 1.0 and 2.5 cm.
- *NIRO-200* tissue oximeter (Hamamatsu Photonics K.K., Japan). The NIRO-200 employs three wavelengths (735, 810 and 850 nm). The small sensor has two SDS: 3.0 and 4.0 cm.
- *INVOS 5100C* tissue oximeter (Medtronic, Minneapolis, USA). The INVOS applies two wavelengths (730 and 810 nm). The Oxyalert Neonatal NIRSensor has detectors at a SDS of 3.0 and 4.0 cm.
- *OxiplexTS* (ISS, Champaign, Illinois USA) frequency-domain NIRS device works with two frequency-modulated light sources (690 and 830 nm), measures absolute μ_a and μ_s' and therefore was our reference. A rigid sensor was employed with four SDS (2.5, 3.0, 3.5 and 4.0 cm).

3 Results

The complete deoxygenation process took 144 min for the N_2 bubbling and 52 min for the yeast. Thus, yeast is deoxygenating the phantom 2.8 times faster than N_2.

For each deoxygenation, the StO_2 measured by the OxiplexTS was related to the corresponding simultaneous values of the other tissue oximeters in the scatter plots shown in Fig. 1b–d. The values drawn in the scatter plots show data from the decreasing slope. Depicted are the StO_2 values of the OxiplexTS in the range from 95% to 5%, with linear fits based for this range. The StO_2 was not affected by the deoxygenation method (Fig. 1).

The absolute μ_s' at 690 and 830 nm and the StO_2 are shown in Fig. 2. Both were measured with OxiplexTS. μ_s' (830 nm) decreased during the N_2 cycle from 5.4 to 4.9 cm^{-1} (8.4% reduction in 163 min, 3.1% per hour). A reduction from 5.0 to 4.9 cm^{-1} (2.3% reduction in 71 min, 1.9% per hour) was observed during the yeast cycle. The decrease during the yeast cycle was corrected for the additional scattering induced by incorporating the yeast. The reduction in μ_s' was five times smaller for yeast than for N_2 bubbling.

4 Discussion and Conclusion

We compared two deoxygenation methods for liquid blood phantoms and observed a good agreement in measured StO_2. Especially INVOS and NIRO-200 demonstrate a nearly perfect overlap between both methods, whereas FORE-SIGHT Elite displays consistently but only slightly lower StO_2 values when employing N_2 bubbling.

In Fig. 2 we see a higher noise level for StO_2 and μ_s' at low StO_2. This originates from the high μ_a at 690 nm due to the high concentration of deoxyhaemoglobin, which absorbs strongly at 690 nm. This leads to low light levels and higher noise of the OxiplexTS.

The decrease in μ_s' is five times larger for deoxygenation 1 than for deoxygenation 2. The influence of employing N_2 on μ_s' might be explained by two reasons: (i) the deoxygenation cycle took 2.8 times longer; and (ii) the continuous bubbling of N_2 may cause mechanical stress leading to an aggregation of lipid droplets and hence a drop in μ_s'. Additionally, yeast is less costly and does not create optical inhomogeneities in the phantom through bubble formation.

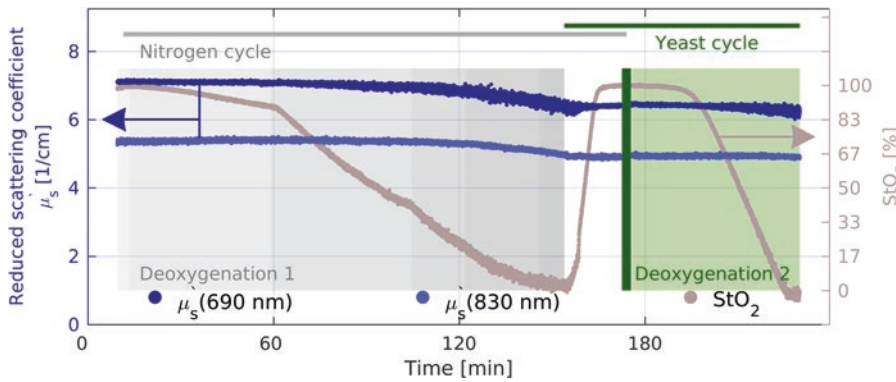

Fig. 2 Reduced scattering coefficient μ_s' (left axis) and StO_2 (right axis) measured with the OxiplexTS are shown. The shading in deoxygenation 1 depicts the increases in nitrogen flow as the phantoms StO_2 was decreasing. For the deoxygenation 2, a vertical bar marks the time point when yeast was added

In conclusion, although both methods provide comparable StO_2 results, we recommend using yeast for phantom deoxygenation due to higher speed and phantom stability.

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