Tobias Steege*, Mathias Busek, Stefan Grünzner, Andrés Fabían Lasagni and Frank Sonntag

Closed-loop control system for well-defined oxygen supply in micro-physiological systems

Abstract: To improve cell vitality, sufficient oxygen supply is an important factor. A deficiency in oxygen is called Hypoxia and can influence for example tumor growth or inflammatory processes. Hypoxia assays are usually performed with the help of animal or static human cell culture models. The main disadvantage of these methods is that the results are hardly transferable to the human physiology. Microfluidic 3D cell cultivation systems for perfused hypoxia assays may overcome this issue since they can mimic the in-vivo situation in the human body much better. Such a Hypoxia-on-a-Chip system was recently developed. The chip system consists of several individually laser-structured layers which are bonded using a hot press or chemical treatment. Oxygen sensing spots are integrated into the system which can be monitored continuously with an optical sensor by means of fluorescence lifetime detection.

Hereby presented is the developed hard- and software requiered to control the oxygen content within this microfluidic system. This system forms a closed-loop control system which is parameterized and evaluated.

Keywords: microfluidic, hypoxia, model-in-the-loop, lab-on-a-chip, perfusion

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1 Introduction

Microphysiological systems (MPS) are miniaturized, chip-sized platforms designed to emulate the physical environment for in-vitro cell cultures. They can be used as cellularized organoid systems to study cellular processes like migration, regeneration or proliferation. They are also capable to simulate the interaction of different organs as well as pharmacokinetics. That means the adsorption, distribution, metabolism and excretion of drugs and metabolites. MPS show wide application possibilities for various cell types and organ models. [1]

In this context, the Fraunhofer IWS has developed a tailored multilayer based MPS for hypoxia assays. Hypoxia is an important mechanism for many medical questions and can influence for example tumor growth. [2] The MPS combines active components such as valves and pumps as well as a cell culture chamber and an oxygenator. This closed circulation system is optimal for the examination of metabolites and the effect on tissue types. [3] The flow characteristics of the elements can be both mathematically described and validated using micro-Particle-Image-Velocimetry. [4] The medium-gas exchange inside the oxygenator element was mathematically described and validated with the help of SimulationX (ESI ITI GmbH, Dresden). [5]

However, these systems are growing steadily in their complexity. Therefore, new control and process technologies to operate various elements of such systems are needed. An ideal controlling unit for MPS should be scalable, user-friendly and independently working. [6] The existing control technology does not meet the requirements such as rapid prototyping, for example model-in-the-loop (MIL) of controlling software to mimick the physiological reaction of human organs in the MPS. The aim of this work is to develop a gas mixing system to regulate the oxygen concentration in the MPS by adjusting the gas mixture and the gas flow. In order to achieve this, a new software has to be designed which allows to create a closed-loop control system and the use of MIL prototyping.

2 Material and methods

2.1 Microfluidic system

The MPS developed at Fraunhofer IWS consists of two main assemblies, the pneumatic and the fluidic systems. These consist on several laser-structured and thermally bonded layers. Both assemblies are connected by a flexible, gas-permeable silicone membrane which allows the integration of pumps and valves as well as oxygenator elements. The integrated microfluidic actuators enable completely closed and perfused microfluidic circuits. Membrane deflection is achieved by applying vacuum or pressure. [7]

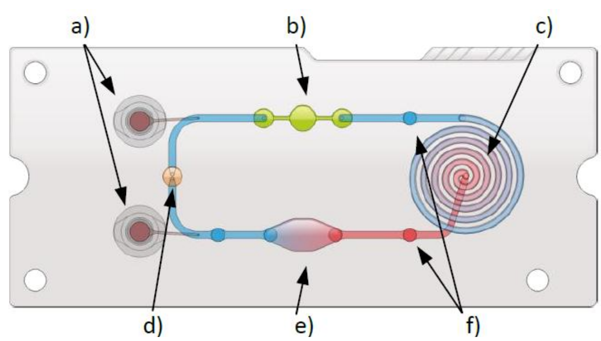

Figure 1: MPS layout: a) fluidic ports; b) micro pump; c) oxygenator; f) sensor spots; e) cell culture chamber; d) media exchange valve

In Figure 1, the MPS layout is shown. It includes in- and outlet ports, a valve for media exchange and a micro pump for fluid actuation. In previous fluidic designs, this pump was also used for gas exchange [8], which can cause gas bubble generation inside the channels. [9] To overcome this problem and enhance the oxygen transport capability, an additional oxygenator element was integrated in the MPS. Together with oxygen sensing spots this element can be used to control the concentration inside the MPS, as was reported in [3].

2.2 Controlling unit

The pneumatically driven micro pumps are operated by a controlling unit based on an embedded Linux system. It can control up to four MPS, each consisting of various pumps and valves. Furthermore, several interfaces (Ethernet, CAN) give the opportunity to connect the system for example to a laboratory information management system.

The controlling unit is extended by a gas blending module based on pulsed mixing in this work. For each input, a pressure sensor together with a pressure regulator is used to adjust the pressure. The gases are combined in a mixing chamber in a pulsed manner via a switching a valve, wherein either the number of pulses or the width of the individual gas pulses can be varied. The gas mixture in the chamber can be determined for two gases with the following equation:

$$c(t) = \frac{\int_0^{t_1} q_1 dt + \int_{t_1}^{T} q_2 dt}{V} * 100\% \quad (1)$$

The main advantage of this solution is that it can be easily scaled up for multiple outputs. Furthermore, the mixing chamber can be used as a gas humidifier at the same time. This additional feature is very important, because the used silicone membrane is known to be also permeable for water vapour and therefore media will evaporate through the oxygenator over time if the process gas is not humidified. [10]

2.3 Optical oxygen sensing

The commercially available OPAL-system (Colibri Photonics, Potsdam) is used for non-invasive oxygen measurements in conjunction with an optical module from Fraunhofer IOF. Measurement is based on dynamic fluorescence decay, which is described elsewhere [11]. The oxygen sensitive fluorescence dye is integrated in 50 µm big CPOx beads (Colibri Photonics, Potsdam), which are immobilized by means of partial inclusion in polydimethylsiloxane (PDMS) at the channel bottom. Those spots can be used to measure the oxygen concentration in the gas and liquid phase. The optical module includes an adjustable high-power LED, filters and a focusing lens for excitation. Emitted light is collected by the same focusing lens, transferred to the beam splitter, filtered and finally detected with a photomultiplier. A 3-axis laboratory automation robot can be used to move the sensor to the sensing spots, where the fluorescence decay is measured. Based on the decay value the oxygen content expressed in vol.-% air saturation can be calculated. In this context 100 vol.-% in the gas phase is equivalent to an oxygen content of 20.95% at atmospheric pressure air.

3 Results

The software approach was not to implement the controlling unit directly in the software, but to model the close loop control unit using mathematical concepts inside a simulation program. Therefore an interface was developed which is able to connect to the simulation program with Scilab (Scilab Enterprises, Versailles), so that there is no difference between the test environment and the implemented controller.

This leads to particular requirements for the simulation and the interface regarding the use of standardized protocols for the data exchange between different program modules. Such requirements are:

1. modular design
2. configuration files for different hardware options
3. multi-client capability
4. support for extension modules
5. processing instructions from the clients

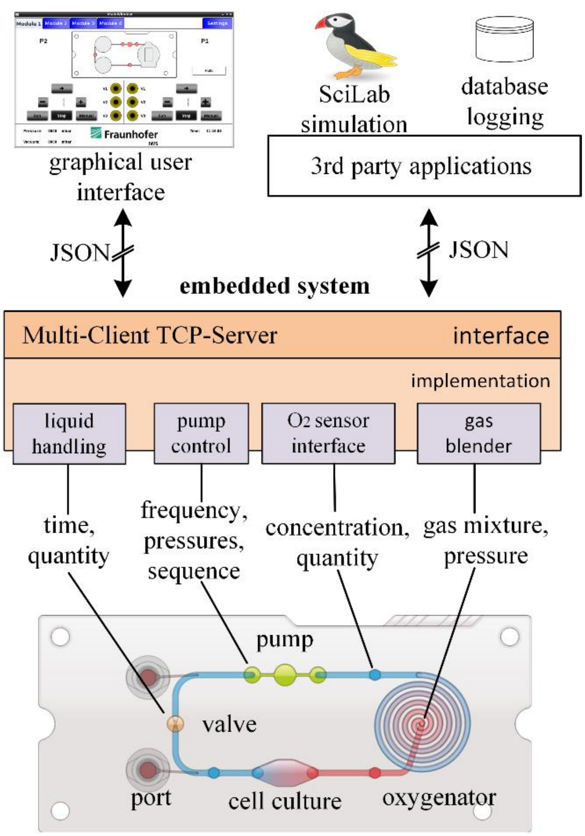

Figure 2: software schema of the controlling unit

Figure 2 shows the structure of the software schematically. As can be seen, the individual elements of the chip (oxygenator, micro pump, sensor, reservoirs) are controlled via the associated modules (sensor interface, pump controller, gas mixing module, liquid handling scheduler).

Of particular interest is the gas mixing module and the O_2 sensor interface, which forms the control circuit. As shown in the overview, all software modules can be accessed via the graphical user interface (GUI) or 3rd party application programs. An interface protocol based on JSON was implemented for this purpose. The JSON format has following advantages:

1. compact data format
2. text-based data exchange
3. supported by many programming languages and simulation programs

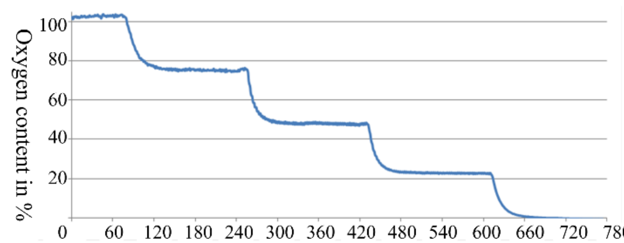

Figure 3: stepwise deoxygenation of the process gas stream inside the oxygenator element

The proof-of-principle was carried out by applying different process gas mixtures to the oxygenator and measuring the oxygen content at the outlet of the oxygenator. These spots were calibrated with compressed air and nitrogen, as previously reported in [3]. As shown in Figure 3 the oxygen content of the process gas stream, was sequentially reduced from 100% to 0% in 25% steps while the oxygen content at the outlet of the oxygenator was measured continuously.

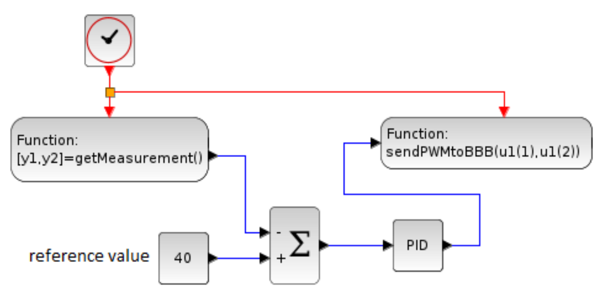

Figure 4: Scilab model with PID controller and connection interface to the controlling unit

Afterwards, an interface to the simulation program Scilab was implemented, which is particularly suitable for the control unit design and MIL based approaches. [12] Corresponding functions for querying the current oxygen content in the medium and setting the gas mixture were implemented. Within the simulation program a proportional–integral–derivative (PID) controller for the system consisting of a sensor, an oxygen generator and a gas mixer were modeled (Figure 4).

In Figure 5 the time response of the implemented PID controller with an desired oxygen concentration set to 40%, monitored at an oxygen spot in the channel, is shown. As can be observed, the PID can reduce the oxygen level inside system to the desired value in less than a minute. Due to the inertia of the system, a short overshoot occurs.

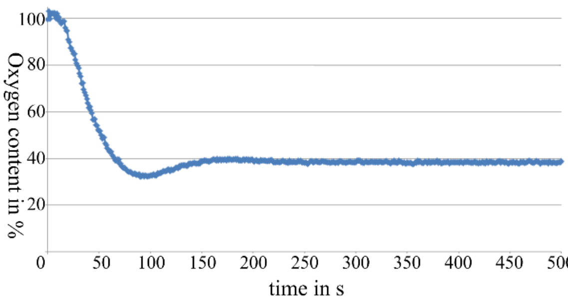

Figure 5: time behavior of the oxygen level inside the media using a PID controller

4 Conclusion and outlook

A microphysiological system with an integrated pump and an oxygenator was presented, which could be driven by different process gases generating well-defined oxygenation levels in one or more cell chambers. The pneumatic micro pump as well as the oxygenator were operated by a controlling unit with a gas blending extension module.

It was shown, that the new controlling software allowed to control various MPS software models comfortably. Furtheremore, an interface based on a JSON protocol was implemented for the communication between different modules. The system was designed as a multi-client server, this makes it possible to connect several programs simultaneously to the control unit, like a GUI, a simulation program or a logging application.

A workflow using this principle is shown, by controlling the oxygen level inside a MPS using a PID controller to ensure long time stability. For measuring the oxygen content, a commercial available optical sensor was used. The gas exchange was realized via the integrated oxygenator element on the MPS. A MIL approach was chosen, whereby a PID controller was implemented with the simulation tool Scilab. The results show that this approach is promising for the long-term stability of studies with defined, freely selectable oxygen levels.

Next, the mathematical models of the individual elements will be included in the simulation. This would permit to simulate different scenarios within the research experiment, like the different stages of hypoxia of a heart attack.

Author's Statement

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