Versatile fabrication and integration method of optical oxygen sensors in organ-on-chips

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Abstract—Oxygen concentration is an essential parameter in *in vitro* cell cultures as it affects cell viability, cellular activity, cell differentiation, and can be a measure for cellular response. Organ-on-chip (OoC) devices provide an *in vitro* cell culture method, aiming to mimic the *in vivo* situation better than conventional cell culture methods. Oxygen control in these OoC devices is very meaningful especially for organs with inherently large oxygen concentration gradients, such as the gut. Here, we show a versatile fabrication method for optical oxygen sensor patches based on platinum tetrakis (pentafluorophenyl) porphyrin (PtTFPP) in polystyrene (PS). The PtTFPP/PS sensors show a linear Stern Volmer calibration plot. The proposed fabrication method allows abundant design freedom regarding both the sensor patches (size and shape) and integration (site and number of patches) in a typical two-channel OoC device.

Keywords—Optical oxygen sensor, Organ-on-chip, Platinum tetrakis (pentafluorophenyl) porphyrin, PtTFPP, Polystyrene

I. INTRODUCTION

The definition ‘Organ-on-chips’ (OoC) is used for *in vitro* microfluidic cell culture devices, which are often made of polydimethylsiloxane (PDMS), and considered promising alternatives for the conventional *in vitro* models or animal testing [1]–[3]. There is a lot of interest in oxygen control in OoC devices, as oxygen is a crucial molecule in the metabolism of human cells since both a deficiency as an abundance of oxygen can cause cell damage or even cell death [4], [5]. By measuring the (change of) oxygen concentration a wealth of information on cell viability, cellular activity, and cell responses can be provided, such as shown in [6].

The majority of the optical oxygen sensors are based on the quenching of the phosphorescence of metalloporphyrins by oxygen [4], [7], [8]. Platinum(II) porphyrins are known for their high quantum yields and long-live phosphorescence [9], beneficial for oxygen measurements. The sensing principle on which these kinds of sensors rely, is described in detail in [7]. Specifically, platinum tetrakis (pentafluorophenyl) porphyrin (PtTFPP) has a high photostability [7], [10] and is the most used oxygen sensitive dye [8].

Optical oxygen sensors based on metalloporphyrins for OoC devices are already shown in several different forms (described in more detail in [4], [11]), e.g. thin sensor films [12], [13], dye-coated optical fibers [14] or micro- and nanoparticles [15]. However, these sensor types all have their own disadvantages for measuring oxygen concentration during cell culture on specific sites; at or

along the membrane in a two-channel OoC. For example, sensor films [12], [13], or sensor spots [16] are often integrated in an OoC device with only one cell culture channel. They are spin coated on or imprinted in a substrate, making it complicated to integrate them (at the membrane) in a two-channel device. Dye-coated optical fibers have to be physically inserted into the OoC, and this method can be tedious when multiplexing is desired. Oxygen sensors can also be applied in a separate sensor module in series of the OoC [17], which inherently makes it difficult to monitor the oxygen concentration at specific sites along the OoC. Oxygen sensing micro- and nanoparticles are impractical for

(long-term) continuous oxygen measurements during cell culture under flow in an OoC. Microparticles can accumulate in the culture channel and nanoparticles will be taken up by mammalian cells.

Here, we present a versatile method to fabricate optical oxygen sensor patches, based on PtTFPP in a polystyrene (PS) matrix which accommodates abundant design freedom regarding the sensor patches (size and shape). Furthermore, we show the integration in a typical PDMS OoC, where the (several) sensor patches can be placed in the PDMS OoC at any site of interest.

II. EXPERIMENTAL

A. PtTFPP/PS patch fabrication

PS (Mw 280,000, Sigma-Aldrich) is dissolved in chloroform (Sigma-Aldrich) (7 %w/v), where after this mixture is added to PtTFPP (1 %w/v) (Frontier Scientific) and vortexed thoroughly. The PtTFPP/PS in chloroform mixture is pipetted on an in-house milled aluminum mold with 0.4 mm to 1 mm diameter cavities with a depth of approximately 200 μ m and 500 μ m. The chloroform is left to evaporate for 24 hours. Using Scotch tape, the PtTFPP/PS patches can be removed from the aluminum mold (see Fig. 1(A) step 1-4).

B. Integration of patches in a PDMS OoC

PDMS (Sylgard 184 Silicone elastomer kit, Dow Corning) was mixed (10:1 %w/w) and degassed for approximately 2 hours. The PtTFPP/PS patches were 'glued' into an in-house micromilled poly(methyl methacrylate) (PMMA) mold, using uncured PDMS (10:1 %w/w), which is then pre-cured for 30 minutes at 60°C. Subsequently, the PMMA mold was completely filled with PDMS (10:1 %w/w) and cured for 4 hours at 60°C (see Fig. 1(A) step 5). This process can be performed for both PDMS top and bottom layer, followed by assembly of the OoC (see Fig. 1(B) and (C)). Alternatively, a PDMS layer could also be bonded to a glass slide using oxygen plasma, thereby creating a one-channel device.

Fig. 2. Excitation and emission spectrum of PtTFPP embedded in a PS matrix, integrated in a PDMS chip. The Soret band of PtTFPP, usually dominantly present at 402 nm is partly cut off [19]. Two Q bands are found at respectively 512 and 545 nm. The emission peak is observed to be at 648 nm with a shoulder at around 700 nm.

Fig. 3. The two excitation spectra of PtTFPP embedded in a PDMS matrix (PtTFPP/PDMS), and PtTFPP embedded in PS and integrated in a PDMS chip (PtTFPP/PS). The excitation spectrum of PtTFPP/PDMS is similar as seen in literature [19]. The excitation spectrum of PtTFPP/PS differs from reported before [10].

C. Excitation and emission spectra

The excitation and emission spectra of PtTFPP/PS were measured using a FluoroMax-4 photo spectrometer with lifetime extension (Horiba Scientific). The excitation spectrum was measured with emission at 650 nm and emission spectrum was measured with excitation at 540 nm.

To study the effect of the polymer matrix in which the PtTFPP is embedded, we studied the excitation spectra of both PtTFPP in a PDMS matrix (PtTFPP/PDMS) and PtTFPP in a PS matrix, integrated in a PDMS chip (PtTFPP/PS in PDMS). The PtTFPP/PDMS was obtained by dissolving PDMS in toluene (Sigma Aldrich) adding 1 %w/v PtTFPP, and subsequent curing at 60°C.

D. Calibration of PtTFPP/PS sensor

PtTFPP/PS sensors were calibrated for gaseous oxygen ranging from instrumental air (210 hPa partial oxygen pressure) to nitrogen (0 hPa partial oxygen pressure). A microscope with color CCD camera (FLIR Grasshopper 3, U232S6C) and a Chroma 49012-ET-FITC/EGFP long pass filter was used to record images. The images were processed using the software ImageJ (version 1.52a).

III. RESULTS AND DISCUSSION

A. Excitation and emission spectra

The excitation and emission spectra of the PtTFPP/PS in PDMS are shown in Fig. 2. The usually intense Soret band at 402 nm seen in PtTFPP is partly cut off, compared to results shown in literature [18], [19]. The two Q bands are present around 512 and 545 nm. The emission spectrum is comparable as shown before as a peak is observed at 648 nm with a shoulder at around 700 nm; this is slightly shifted with regard to values found in literature [20].

To clarify the difference of our excitation spectrum of PtTFPP/PS in a PDMS chip compared to literature [10], we compare in Fig. 3 PtTFPP/PS in PDMS (solid line) with PtTFPP/PDMS (dashed line). The excitation spectrum of PtTFPP/PDMS resemble absorption spectra as observed in

literature [19], [20], which is also shown for PtTFPP/PS [10], [18]. So, the integration of PtTFPP/PS sensors in PDMS specifically causes an effect on the excitation spectrum.

B. Calibration of PtTFPP/PS sensor

When calibrating the PtTFPP/PS sensors for gaseous oxygen, a linear Stern-Volmer relationship was obtained (Fig. 4), with a Stern-Volmer coefficient (K_{sv}) of 0.0168 hPa⁻¹ and a $R^2=0.998$. Such linear Stern-Volmer plot is earlier shown in literature [10].

We are aware that intensity based sensing is prone to measuring errors, since it is affected by various factors such as: the stability of the light source, background luminescence, concentration and distribution of the dye in probes or patches, photobleaching, and scattering [7], [8]. Lifetime measurements as presented in [18] for PtTFPP/PS sensors, can overcome the mentioned disadvantages of intensity based oxygen measuring [4]. For lifetime measurements only a modulated excitation source is required, and a detector which can sample within the lifetime of the PtTFPP. Future experiments will focus on

characterizing the oxygen sensor patches in an organ-on-chip during cell culture employing lifetime measurements.

C. Fabricated device

Fig. 5 shows two example PDMS chips with integrated PtTFPP/PS sensor patches (1 mm diameter) to illustrate the flexibility of the integration method. Fig. 5A shows a PDMS top channel containing three channels and 15 integrated sensor patches. Fig. 5B shows a PDMS OoC containing two parallel channels with sensor patches in both top and bottom PDMS layer, of which a cross section is shown in Fig. 5C.

The sensor patches can be placed anywhere in the PDMS chip as desired by gluing or pouring PDMS in the mold up to a certain height, partly curing, and subsequently adding the sensor patches. We fabricated patches ranging from 0.4–1 mm in diameter. The only limitation of the size and shape of the sensor patches is the minimal diameter mill available for milling the cavities in the aluminum mold.

The sensor patches can be used to study or confirm the effect of oxygen modulation within an OoC such as shown in [5], [13], [21]. This oxygen modulation can be important for cell differentiation on-chip [4] or e.g. co-culture with microbiota [22]. Furthermore, the sensor patches can be used to measure oxygen concentration during cell culture on-chip, providing valuable information about e.g. cell viability, activity and cellular response [4], [5].

IV. CONCLUSION

In conclusion, we showed a versatile fabrication method for optical oxygen sensor patches based on PtTFPP in a PS matrix. The only limitation regarding the size and shape of the sensor patches is the minimal diameter mill available. The PtTFPP/PS sensors show a linear Stern-Volmer plot. We also showed the integration of the sensor patches in both channels of a two-channel PDMS OoC. The integration method is flexible, allows multiplexing and integration of the sensors in the PDMS OoC at any desired site.

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