MICROFLUIDIC FLOW REACTORS WITH INTEGRATED MICRO-HEATERS AND FLUORESCENT TEMPERATURE SENSORS FOR REACTION MONITORING

Christian Höra[1], Zhe Shu[2], Erik Beckert[2], Stefan Nagl[1], Detlev Belder[1]
[1]*Universität Leipzig, GERMANY and*
[2]*Fraunhofer-Institut für Angewandte Optik und Feinmechanik (IOF), GERMANY*

ABSTRACT

We present the combined integration of fluorescent temperature sensor layers and resistive micro-heaters in microfluidic reactor chips and their application for temperature control and monitoring during an on-chip enzymatic conversion. The production process of the chips consists of wet chemical etching, photolithography, blade coating and soft lithography. These multifunctional chips allow both temperature control and microscopic imaging of the local temperature in microfluidic flow reactors. They are thus able to determine the temperature dependency of chemical and biological reaction on the microscale. This is demonstrated by the tryptic cleavage of modified peptides yielding a fluorescent product at different temperatures.

KEYWORDS: flow reactor, microscopic temperature control, fluorescent temperature sensor, microheater, enzymatic reaction

INTRODUCTION

For efficient process control precise temperature measurement is crucial. Integrated heaters are widely used for temperature adjustment on microchips, however, the local temperature on a microchip could so far be determined only with distinct point measurements, invasively, or with elaborate experimental setups such as IR microscopy. Temperature-dependent fluorescent probes were added to the working liquid in microchannels or embedded into PDMS. Recently we have described the integration and application of homogeneous oxygen sensor layers into micro flow reactor platforms via wet etching, spin coating and soft lithography. [1] Herein we describe a multistep method that allows the integration of fluorescent temperature sensors and of inkjet-printed resistive microheaters into glass-PDMS micro flow reactors of ca. 150 µm width and 40 µm depth using wet etching, blade coating and soft lithography.

THEORY

Luminescent molecules often display a temperature-dependent emission, with a degree that is dependent on the molecule itself and its environment. A strong temperature dependence of their emission near ambient temperatures is found for certain metal-ligand complexes due to their interorbital transitions. For effective use they have to embedded into a thin layer of a suitable inert matrix in direct contact with the liquid. These may be produced by coating a layer of probe compound and matrix polymer in a solvent (sensor cocktail), and controlled solvent evaporation to form the final sensor layer.

Resistive or Joule heaters work via routing electrical current through a conductor. The amount of released heat is set by the electrical current times the voltage drop across the structure. Localization may be achieved by microfabrication of suitable conductors via inkjet printing or other methods.

EXPERIMENTAL

The stages of microchip production are shown schematically in figure 1 a-k. A flow reactor structure was introduced into a glass slide via wet chemical etching. In the next step, the temperature sensor layer was integrated into the resulting channels. Therefore, a solution consisting of 1 % (w/w) of the $Ru(phe)_3$ probe, 99 % polyacrylonitrile (PAN) that was overall 8 % (w/w) in DMSO was brought onto the etched microchannel structure on glass with an automatic film applicator (Fig. 1b). The coated glass slide was immediately heated to 70 °C for 10 minutes on a heating plate (Fig. 1c). During this procedure the solvent is evaporating, leaving a thin sensor layer. The excess film covering the glass slide outside the channels was removed with a razor blade (Fig. 1d-e). A PDMS cover layer (250 µm) was applied via plasma bonding. (Fig. 1f-g). The PDMS cover plate was punched with a needle to supply access to the microfluidic structure and PDMS slabs containing the fluidic connections were introduced via plasma bonding (Fig. 1h). Silver nanoparticle-based microheaters were printed on a PET foil, sintered and implemented on the chip by enclosure with thermocurable PDMS prepolymer (Fig. 1i-j).

Sensor film thicknesses were obtained via tactile surface profilometry using microchannels without top cover (Fig. 1e) and the temperature sensor was calibrated in a dedicated chamber attached to a thermostat via fluorescence intensity measurements.

Temperature and reaction monitoring were performed on an inverse fluorescence microscope with LED excitation and CCD camera detection. Analytes were monitored by 365 nm UV LED excitation and readout of their fluorescence in the blue spectral range, the temperature sensor signal was observed by excitation with blue light from an LED and detection in the yellow to red spectral range.

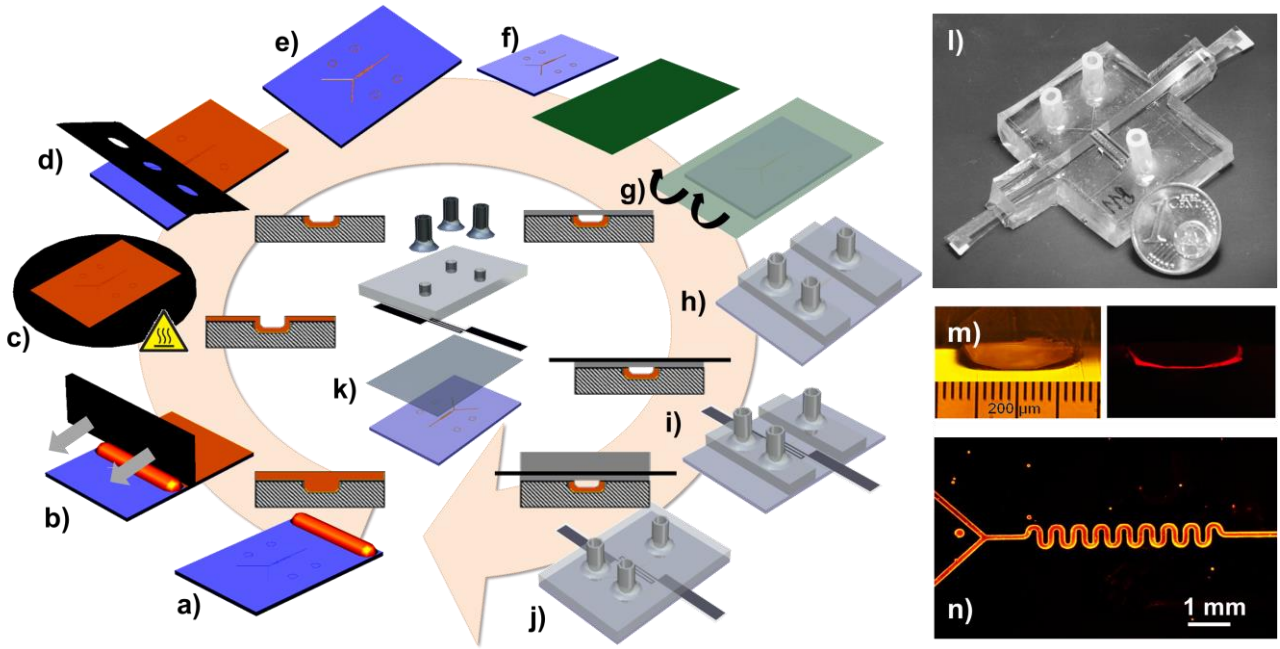

Fig. 1: (a-k) schematic of the microchip production; a) wet-etched flow reactor structure, b) blade coating, c) curing at 70 °C, d) removal of excess polymer, e) flow reactor with integrated temperature sensor, f,g) plasma bonding to PDMS cover and removal of the PET support, h) plasma bonding to PDMS slabs and attachment of fluidic contacts, i) attachment of microheater foil, j) enclosure in PDMS, k) explosion drawing of the chip, l) photograph of microchip, m) right: fluorescence micrograph of a channel cross-section (20x obj.), left: transmitted light image, n) fluorescence micrograph of the integrated sensor layer (1x obj.).

RESULTS AND DISCUSSION

Reproducible thin and even layers of sensor polymer could be produced in the recesses of wet-etched glass slides. Sensor layer thickness was investigated and could be adjusted by the quantity of PAN in the sensor cocktail (Fig. 2a) from ca. 0.5 to 2.7 µm using solutions that were 2 to 10 % (w/w) PAN in DMSO. The coating steps could be applied repeatedly and led to sensor layer thicknesses from 1.6 to 6.5 µm (Fig. 2b).

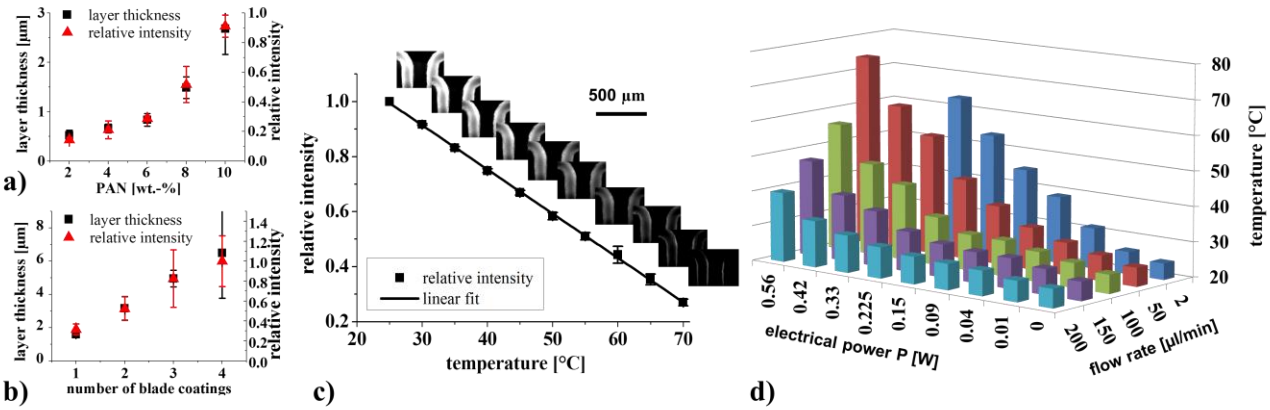

Fig. 2: a) Thickness of sensor layer depending on the quantity of PAN in the sensor cocktail, b) thickness of sensor layer in depending on number of coatings (8 wt.% PAN), c) Calibration curve of the sensor layer with pasted intensity micrographs (20x obj), d) Flow reactor temperature created by microheater depending on flow rate observed by the integrated sensor layer.

The temperature sensor was calibrated in dedicated chambers between 25 and 70 °C and the fluorescence of the sensor layer followed a linear relationship towards temperature with a decrease of 1.6 % per K. (Fig. 2c). The lowest detectable temperature difference in this setup was 0.3 K.

The local temperature created in the flow reactor by the integrated micro heaters was examined at various flow rates using hydrodynamically injected 150 µM PBS buffer, pH 7.5. The response of the sensor layer was recorded at an area of 0.35 mm^2 and using the calibration fit could be converted into temperature. Figure 2d shows the dependency of the flow rates, electrical energy and the resulting temperature in the microreactor. As expected, the heat transfer to the fluid increased

at lower flow rates and higher electrical currents. At a flow rate of 2 µL/min that was used for the following experiments the temperature (T) could be related to electrical power (P) by function 1.

$$T\,[°C] = (119.04 \pm 3.80)\ \cdot P\,[W] + \ (24.69 \pm 0.05) \qquad (1)$$

These reactor chips were then used to determine the temperature dependency of a model reaction, the tryptic cleavage of modified peptides yielding the fluorescent product Coumarin 120. This fluorophore was found to have temperature-independent emission in the observed temperature range (deviations < 0.8 %).

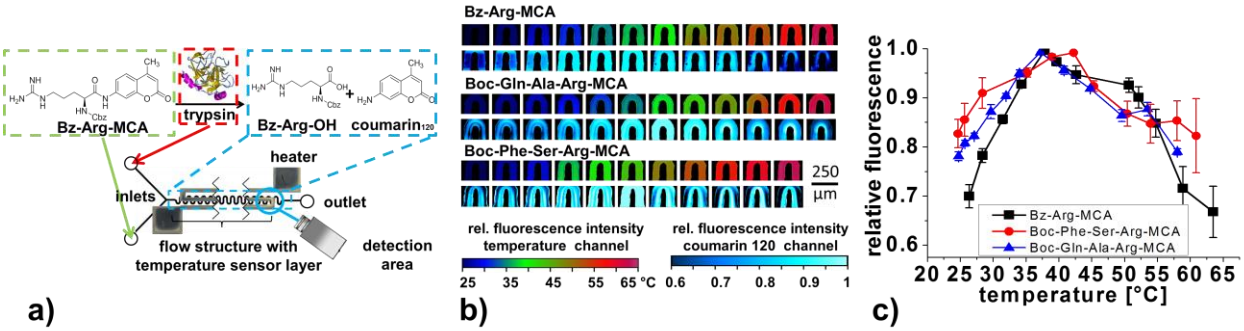

Fig. 3: a) schematic of the enzymatic conversion and the microchip layout, , b) false-colored fluorescence micrographs of the temperature channel and the coumarin channel, b) plot of temperature dependent tryptic cleavage of various peptides modified with coumarin 120.

The substrates were introduced into the microreactor at one inlet as a 500 µM solution in PBS buffer pH = 7.5 and 20.8 µM bovine trypsin in PBS was introduced into the second inlet (Fig. 3a). At a flow rate of 2 µl/min these solutions mixed effectively and the reaction turnover was monitored at an area of 0.35 mm^2 close to the outlet of the reactor. (Fig. 3b) The reaction was run continuously for three minutes, recorded and then the temperature was gradually increased.

With this system three different coumarin labeled peptides were investigated. All three showed a strongly temperature dependent reaction turnover. The temperature-fluorescence plots showed a similar trend for the different substrates with local maxima between 37.2 and 42 °C. For the proteins Bz-Arg-MCA and Boc-Gln-Ala-Arg-MCA maximum turnover was observed at 37.8 respectively 37.2 °C, for Boc-Phe-Ser-Arg-MCA the maximum was observed at 42.2 °C.

CONCLUSION

Functional flow reactor microchips with integrated microheaters and fluorescent temperature sensor layers were successfully developed and used for temperature-resolved monitoring of enzymatic transformations. The use of fluorescent temperature sensor layers comprising a temperature-sensitive luminophore in an inert matrix is attractive since it allows to determine the local temperature spatially resolved, in real-time with submicron resolution using optical microscopy in the visible range and the matrix is in direct contact with the fluid in the microreactor. These microchips may also be used for other enzymatic conversions such as PCR or ELISA techniques or in other areas such as miniaturized chemical synthesis or bioprocess control.

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[1] L. Gitlin, C. Höra, R. Meier, S. Nagl, D. Belder, *submitted*

CONTACT

*Prof. D. Belder, tel: +49 341 97 - 36091; belder@uni-leipzig.de