MAGNETIC SENSOR PARTICLES: A NEW TOOL FOR THE DETERMINATION OF OXYGEN IN MICROFLUIDICS

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ABSTRACT
We present the application of multifunctional nanoparticles in microfluidic devices. The particles have an oxygen sensing function and can be collected by magnetic forces at any desired position in the microfluidic channels. The nanoparticles are prepared by a simple precipitation technique. Read-out of the in situ generated sensor spots was performed applying luminescence lifetime and ratio metric imaging. The formation of multiple sensor spots in one channel for the parallel measurement of various parameters is shown and the respiration of E. coli culture assembly illustrated.

KEYWORDS: Magnetic Particles, Optical Sensor, Imaging, Microfluidics.

INTRODUCTION
A variety of microfluidic devices with integrated optical oxygen sensors have been developed in the recent years [1]. The sensing principle is based of indicator dyes embedded in a host polymer. Such sensors have been integrated in different formats such as sensor layers or nano sensor beads [2]. While layer integration means an additional step for the processing of the microfluidic chip, nano sensor particles can be simply added to the fluid in the microfluidic channels and therefore take no additional effort of chip development.

SENSOR CONCEPT
Magnetic nano sensor particles [3] represent a new approach to combine the advantages of sensor layers and sensor beads: they can be collected inside a microfluidic channel from outside by a magnet. (Fig. 1) This allows the in-situ generation of sensor spots with increased brightness compared to non-magnetic nano sensor particles. These sensor spots can be moved to any desired position, enabling the measurement at different areas of the devices’ channels. Furthermore the application of magnetic sensor particles enables parallel monitoring of multiple analytes, when multiple sensor spots with sensitivity to different analytes are generated inside one microfluidic channel. Potential methods for magnetic separation are illustrated in Figure 1.

EXPERIMENTAL
Magnetic sensor particles were prepared as recently described by our group using a simple precipitation technique [3]. Luminescent indicator dyes, lipophilic magnetic nanoparticles and a host polymer were dissolved in THF. Upon addition of water, the spherical particles are formed spontaneously by precipitation of the polymer. Sensor spots were read-out with a microscope using luminescent lifetime or ratio metric imaging using a color camera [4]. Plain luminescence intensity images are usually unsuitable for directly measuring oxygen concentrations.

Figure 1: Scheme of a polymeric magnetic particle with incorporated luminescent indicator dye and magnetic nano particle (top left) and in-situ sensor spot generation inside a microfluidic channel (top right). After growing adherent cells or a biofilm in a microfluidic channel the magnetic sensor particles are injected, collected by magnetic fields and read-out by a microscope or an optical fibre.

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Intensity fluctuations originate from inhomogeneities of the light source, of the sensitivity of the detection system or of the dye distribution within the sensor layer and have to be corrected. In this work two referencing schemes were used: ratiometric imaging and lifetime imaging by rapid lifetime determination (RLD). Ratiometric imaging is based on calculating the ratio of the red channel (PrTFPP-emission; oxygen-sensitive signal) to the green channel (MY-emission; reference signal) of a color camera. In lifetime imaging applying RLD the luminescent probe is excited by a light pulse and emission is measured at two different measuring windows (D₁ and D₂) at different delay times (t after the excitation pulse. Images were obtained using an epifluorescence microscope (Zeiss Axiovert 25 CFL), equipped with a 50 W mercury arc lamp for luminescence excitation. For luminescence lifetime imaging a blue ultrabright light emitting diode (Luxeon, 470 nm, 5 W) was used as triggered light source. A monochrome CCD camera Sensimod, PCO, Kehlheim, Germany) and a color CCD camera (AVT Marlin F-201C) were connected to the microscope.

RESULTS AND DISCUSSION

Magnetic sensor particles were optimized regarding different read-out systems and characterized for their application in microfluidics. The particles were used to generate in-situ sensor spots in the channels of microfluidic devices. The accumulation characteristics depending on the type of magnet used were investigated as well as the behavior under varying flow rates (Fig. 2). Sensor spots are formed within 10 of 500 µl/min. In addition, the generation of multiple separated sensor spots within one microfluidic channel was successfully performed. The response of the in-situ generated sensor spots towards oxygen in the microfluidic channels was successfully shown (Fig. 3). In addition, the respiration of microorganism inoculated in a microfluidic system was demonstrated. Figure 4 shows an overlay of transmitted light image and lifetime image of _E. coli_ colonies in a microfluidic channel using magnetic nano sensor particles separated on top of the cell assembly.

Figure 2: Formation of a sensor spot in a microfluidic channel (width 3800 µm, height 400 µm) by application of a permanent magnet (placed below chip). After the collection water is injected to the channel and non-bound particles are flushed away resulting in a dot-shaped spot (left). The generation of multiple sensor spots applying different magnets at various positions (right).

Figure 3: Calibration curve of magnetic sensor particles obtained by lifetime imaging (left) and response curve (right) inside a microfluidic channel, when oxygenated and deoxygenated solutions were pumped through the channel.
CONCLUSION
Magnetic nano sensor particles are valuable tools to measure oxygen inside microfluidic channels in terms of simplicity of integration and flexibility. Potential applications are the measurement of cell metabolism or bio-catalytic transformations, the elucidation of oxygen gradients or modeling of transport phenomena in microfluidic channels.

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