A Microfluidic Chip for Infrared CH_2-stretch Ratio Measurements of Suspended Mammalian Cells

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In this contribution we present a sensor system for measurements of the CH_2-stretch ratio of suspended mammalian cells. An infrared transparent CaF_2 microfluidic chip with three equal SU-8 structured sample chambers of 20 µm in height was fabricated. Cell samples were suspended in PBS (phosphate buffered saline) and introduced into the fluid chambers. The IR absorption of two epithelial kidney cell lines MDCK (Madin-Darby Canine Kidney) and Caki-1 was measured and we successfully distinguished normal MDCK from malignant Caki-1 by comparing their CH_2-stretch ratio.

Introduction

To increase the overall survival rate of cancer patients, an early detecting and accurate staging of primary tumours are highly important. While commonly used screening techniques are based on morphological features, e.g. visual inspection of suspicious tissue or cell samples after staining, these methods still result in a high number of false positives and negatives [1].

Previously, a four-wavelength mid-IR sensor system for measuring the CH_2-symmetric/ CH_2-antisymmetric stretch ratio of dried mammalian cell samples has been designed and realized as an alternative screening method. It was shown that the CH_2-stretch ratio is significantly increased for epithelial kidney carcinoma cells compared to the normal cell type [2].

Here we present a method for measurements of the CH_2-stretch ratio of suspended mammalian cells. Instead of the elaborate and time consuming preparation steps required for dried cell measurements to obtain a confluent cell monolayer attached to an infrared transparent CaF_2 slide, the cell sample is now suspended in PBS and directly introduced into an IR transparent sample chip.

Theory

Instead of recording the whole IR spectrum of cell samples with an IR spectrometer, the IR absorbance at only four specific wavelengths is measured with a sensor setup based on IR-LEDs, narrow bandpass filters and a photodiode detector. The IR absorbance at 3.33 µm and 3.57 µm is used for base-line correction, while the absorption peaks due to lipid CH_2-stretches in the cell membrane are located at the wavelengths 3.42 µm (CH_2-antisymmetric stretch) and 3.51 µm (CH_2-symmetric stretch). The baseline corrected absorbance at 3.42 µm and 3.51 µm is used to calculate the CH_2-symmetric/CH_2-antisymmetric stretch ratio of a cell sample (Fig. 1).

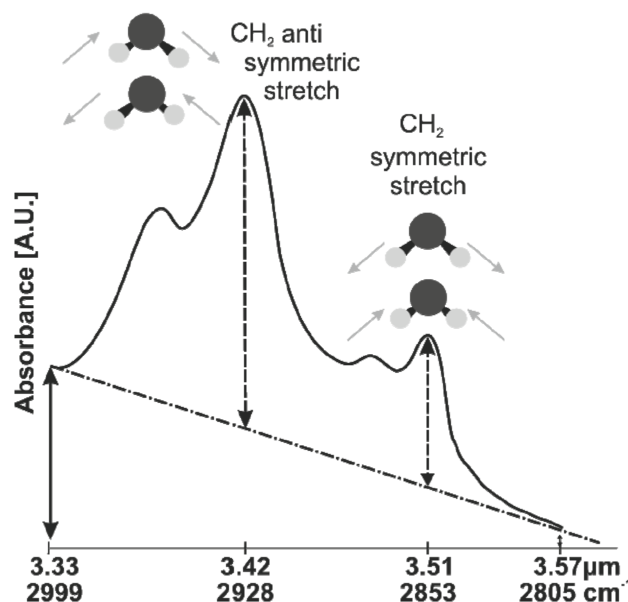

Fig. 1: Illustration of the cell type discrimination method. The infrared absorbance at four specific wavelengths is measured and the baseline corrected absorbance at 3.42 µm and 3.51 µm is used to derive the CH_2-stretch ratio.

This CH_2-stretch ratio of mammalian cells increases when the concentration of membrane stabilizing agents such as cholesterol in the plasma membrane decreases or is reallocated. This stretch ratio increase was shown for MDCK epithelial kidney cells exposed to the cholesterol reducing agent methyl-β-cyclo-dextrin [3].

Experimental

Chip Fabrication

To allow for CH_2-stretch ratio measurements of cell samples in suspension a microfluidic chip was designed and realized. The chip consists of two bonded 1 mm thick infrared transparent CaF_2 wafers with 20 µm high SU-8 chamber structures in between. With this chamber height the strong infrared absorbance of water can be reduced while an even distribution of approximately 15 µm large epithelial kidney cells throughout the sample chamber is guaranteed. A sample chip consists of three chambers, each with a diameter of 3.5 mm allowing multiple 1.5 mm diameter spots to be measured and a volume of about 0.3 µL.

The in-house fabrication consisted of the following steps (Fig. 2): SU-8 50 was spun on the cleaned top and bottom CaF_2 wafers and UV-exposed such that unexposed SU-8 was still enclosed by exposed SU-8 (Fig. 2 (a) – (b)). Then a copper layer was deposited on it and patterned (Fig. 2 (c) – (d)). SU-8 which was not protected by the metal layer was removed with developer to form the chamber structures on both wafers (Fig. 2 (e)) before the Cu layer was etched away (Fig. 2 (f)). Eventually, inlet and outlet holes of 600 µm diameter were drilled in the bottom wafer before the wafers were aligned and bonded (Fig. 2 (g) – (h)). The soft, thermoplastic SU-8 from the top wafer filled the gaps by capillary forces when heated during wafer bonding. At 150 – 200 °C, unexposed SU-8 is thermally cross-linked, serving as an adhesive layer between the

wafers in order to increase bonding strength [4]. Dicing of the wafer yielded single chips as shown in Fig. 3.

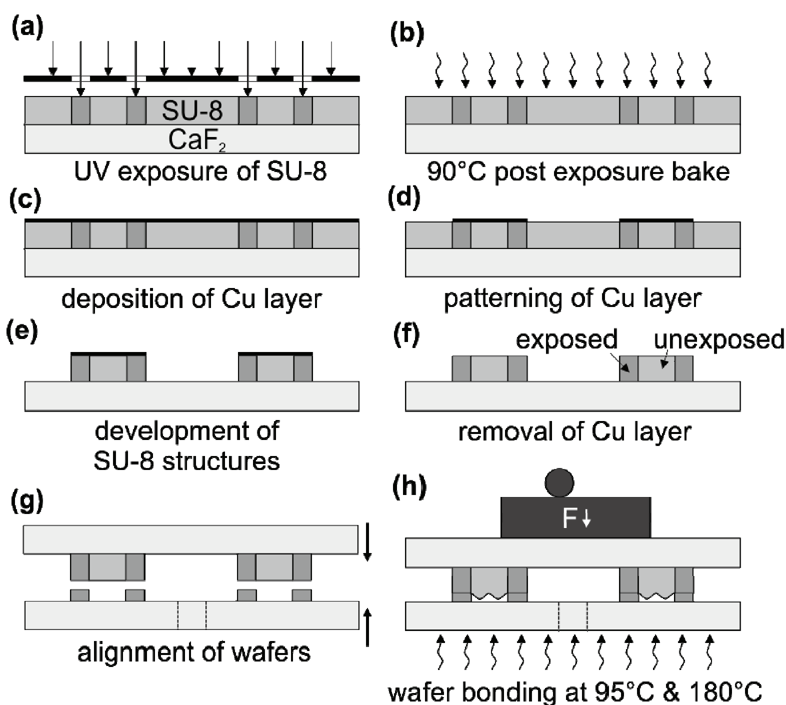

Fig. 2: Patterning of SU-8 on bottom and top wafer to form fluidic chamber structures comprising unexposed SU-8 enclosed by UV-exposed SU-8 sidewalls ((a) – (f)), followed by wafer bonding and thermal cross-linking of unexposed SU-8 ((g) – (h)).

Fig. 3: Photograph of the 11x15x2 mm³ microfluidic sample chip. Each chip consists of three sample chambers of 3.5 mm diameter and a volume of about 0.3 µL.

Suspended Cell Measurements

Two MDCK and two Caki-1 cell samples were investigated in order to measure and compare their CH_2-stretch ratios in suspension. Of each cell line two different cell generations (cell passages) were prepared. Cells were removed from the culture flask by

means of trypsination, washed with PBS two times, and resuspended in PBS. Before cell sample measurements, chambers were filled with PBS and the IR absorbance was recorded as reference. Consequently, the PBS was removed and cell suspension was filled into the chambers (Fig. 4). The time between subsequent measurements of both normal MDCK and malignant Caki-1 samples was approximately five weeks. Of each sample five spots were measured.

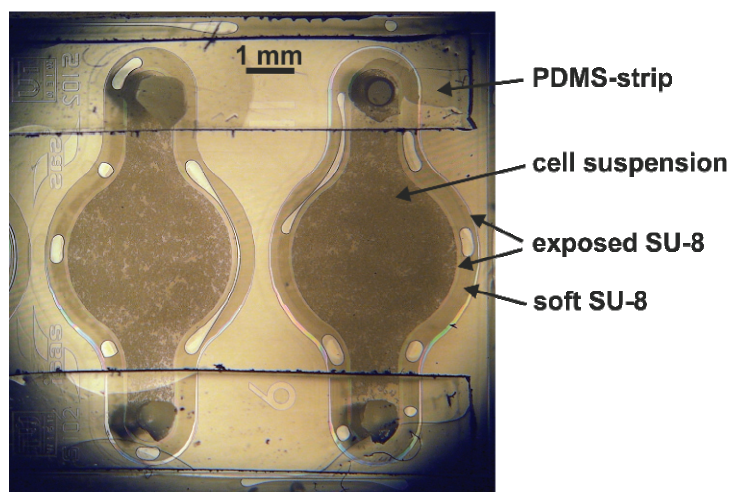

Fig. 4: Two sample chambers containing mammalian cells suspended in PBS. The inlets and outlets were sealed with PDMS strips to avoid evaporation.

Each bar in Fig. 5 represents the average value of the CH_2-stretch ratio and standard deviation of all spots recorded for one of the four samples. The MDCK cell samples yielded a CH_2-stretch ratio of 0.45 ± 0.01 and 0.44 ± 0.01, respectively. Malignant Caki-1 showed CH_2-stretch ratios of 0.49 ± 0.01 and 0.48 ± 0.01.

Compared to previously conducted CH_2-stretch ratio measurements on dried samples, suspended cell measurements all yielded lower stretch ratio values due to changes in the protein hydration levels [5].

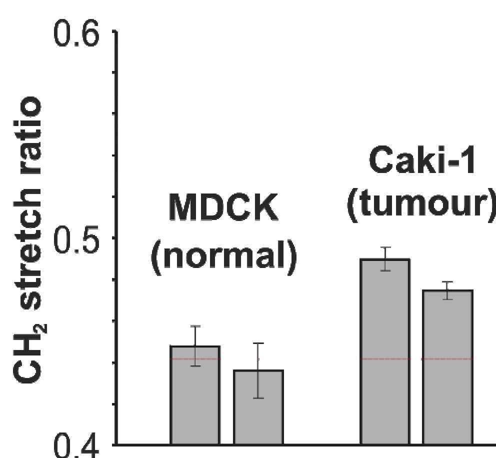

Fig. 5: Two MDCK and Caki-1 cell samples were measured. Each bar represents the average value and standard deviation of the CH_2-stretch ratio of multiple spots.

Conclusions

Suspended viable cell measurements offer much shorter preparation times (≈ 1/2 hour) compared to dried cells. The measurement results show the feasibility to distinguish normal (MDCK) from tumor (Caki-1) epithelial kidney cell lines. Furthermore, a miniaturization of the sensor system could enable the realization of devices for point-of-care diagnostics, since the CH_2-stretch ratio is a promising indicator for the detection of tumor cells.

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