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Microfluidic networks for surface plasmon resonance imaging real-time kinetics experiments

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ABSTRACT

The coupling of microfluidic devices with surface plasmon resonance imaging (SPRI) has emerged in recent years as a novel approach for the simultaneous monitoring of interactions of biomolecules arrayed onto gold substrates. In order to minimize a variety of effects which affect the final determination of kinetic parameters (non-specific interactions above all), difficult choices of appropriate references are often encountered in carrying out SPRI investigations. A common solution to these problems consists of laborious experimental setup involving the use of specially designed microchannels and tedious manipulation of the gold substrate that often produces surface degradation.

In this work, a discussion about appropriate choice of references in SPRI measurements is opened and the use of alternative microfluidic patterns coupled to the SPRI system is proposed as a solution to the above mentioned problems. Specifically, a Y-shaped SPRI flow cell has been constructed from masters in polyvinyl chloride and it has been identified as one of the most suitable experimental approach for obtaining appropriate referencing during SPRI experiments. The experimental set up has been tested in a real time study of the interaction between the Datura Stramonium Agglutinin and the asialofetuin and the obtained results demonstrate the suitability of such microfluidic network in SPRI investigations.

1. Introduction

The manipulation of fluids in the micro-environment has offered in recent years the possibility of solving outstanding system integration issues that are critical for biology and chemistry. In particular, microfluidic devices, which can be identified by the presence of one or more channels with at least one dimension less than 1 mm, have been used for many different applications [1,2] in the field of chemistry and biology [3] and the area of micro total analysis systems (μ-TAS), also called "lab-on-a-chip", or miniaturized analysis systems, is growing rapidly [4]. The main reason behind such rapid development is that there are many advantages over conventional instrumentation, as this miniaturized working environment is very suitable for lots of different applications such as cell patterning and separations [5], DNA analysis [6], enzyme reaction kinetics [7,8] and many others [9,10]. Microfluidic systems have also a very important application in the field of biosensor devices and, in recent years, they have often been coupled with techniques such as surface plasmon resonance imaging (SPRI) [11,12]. The latter expands the label-free capability of the standard SPR technique to rapidly evaluate the interaction between an analyte and its biospecific partner immobilized on the sensor surface [13,14] by allowing a multiplexed approach to the evaluation of biomolecular interactions and to the sensing of chemical and biological analytes [15–17]. To take fully advantage of the SPRI approach a precise control of both the patterning of biomolecules onto the sensor surface as well as the fluidic of the analyte solution is imposed. In this perspective, the use of microfluidic devices provides SPRI compatible convenient means for manipulating very small amounts of sample and for controlling the patterning of a variety of different biomolecules (i.e. DNA, RNA, peptides, proteins, carbohydrates) [18,19].

Nevertheless, SPR data are often affected by various artifacts and the choice of appropriate reference cells in the analysis of kinetic data represents a frequently addressed problem [20].

In SPRI, in order to overcome to some of the above mentioned inconveniences, experiments are usually conducted by following a multistep process where the arrayed surface is first obtained by microspotting [17,21] or by flowing into poly(dimethylsiloxane) (PDMS) made microchannels in contact to the functionalized sensor surface [22,23] a solution of the biomolecule to be arrayed. The interaction with the selected analytes requires the removal of the previous PDMS device and the use of a second set of microchannels that are attached to the surface perpendicular to the previously arrayed areas of the sensor surface. The intersections of the arrays

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with the microchannels define the regions of the sensor surface where the interactions take place, while the surrounding areas are used as reference, so to take into account non-specific interactions and/or changes in bulk refractive index.

A limitation of a similar double step approach is represented by the need to further manipulate the gold substrate after the anchoring of the biomolecular array.

We present here results on SPRI experiments aimed at optimizing the coupling to microfluidic devices. In particular, we discuss a microfluidic approach based on the use of a device carrying a Y-shaped microchannel, which tries to minimize the above mentioned limitation associated to the current use of PDMS made microfluidic devices in SPRI experiments. The study of the interaction between the lectin from Datura Stramonium Agglutinin (DSA) and the asialofetuin (AF) is reported to demonstrate the use of such microfluidic network in SPRI.

2. Experimental

2.1. Materials

Reagents were obtained from commercial suppliers and used without further purification. Triethanolamine (TEA), DSA and AF were purchased from Sigma-Aldrich; methoxy-polyethyleneglycol amine (mPEG, MW = 5000) was purchased from Nektar Therapeutics (USA). Phosphate buffered saline (PBS) solutions at pH = 7.4, (NaCl 137 mM, KCl 2.7 mM, phosphate buffered 10 mM, Amresco) were used in all the SPRI experiments.

Gold substrates (GWC Instruments, USA) for SPRI measurements were obtained by thermally evaporating a gold layer (450 Å) on to SF-10 glass slides. Chromium (50 Å) was used as the adhesion layer. 0.0097 g of dithiobis succinimidylpropionate (Lomant's reagent) were dissolved in 5 ml of dimethyl sulfoxide (DMSO) (Sigma-Aldrich) and the gold substrates were immersed in the obtained solution for 48 h. After two rinsing steps with ultra-pure water (Milli-Q element, Millipore) and absolute ethanol (Sigma-Aldrich), the modified gold substrates were then used for SPRI studies.

2.2. Microfluidics background and PDMS channels fabrication

Fluids behaviour in the microfluidic environment is different from the one observed in the macroscopic world [2], resulting very suitable and advantageous for SPRI investigations. In particular, behaviour of fluids in the microfluidic regime can be rationalized by quantifying the relative contribution of a variety of competing physical phenomena by using dimensionless numbers. While the Péclet dimensionless number (*Pe*) expresses the relative importance of convection to diffusion, the Reynolds number (*Re*) helps in quantifying the relative contribution of inertial (f_i) and viscous (f_v) forces. In the microfluidic environment viscous forces typically overwhelm inertial forces, and the resulting flows are therefore laminar. In this scenario, the T-Sensor is a μ-TAS component that combines microfluidic separation and detection functions and it has been applied also for quantitative analysis of molecular interactions [24]. Fluids coming from two separate microchannels are put in contact in a single and wider microchannel and interact during parallel flow until they exit the microstructure. Interdiffusion occurs during the time that they are in contact and the occurred diffusion at a certain point down the single channel where the two solutions are put in contact can be obtained from:

$$l = (Dt)^{0.5} \quad (1)$$

where l is the distance (perpendicular to the flow direction) that spherical particles will diffuse in time t, while D is the diffusion coefficient which is, for a given temperature and solvent viscosity, a function of the diameter and so, ultimately, of the molecular weight (MW) of the particles.

All the above mentioned details have to be considered if a diffusion-controlled mixing of miscible liquids in the microfluidic regime has be obtained by putting in contact two different fluids in a Y-shaped microchannel [25,26]. As long as the latter has appropriate dimensions and a right choice of flow rate is made [27], the fluids flow in a laminar mode and the mass transport is diffusion rather than convection limited. As a consequence, the boundary between the two miscible fluids can be regarded as a dynamic interface that can be manipulated and put to practical use [2].

A Y-shaped master in polyvinyl chloride (PVC) was specially designed for this work and poly(dimethylsiloxane) (PDMS) flow cells were fabricated from such master as described elsewhere [28]. The overall length of the Y-shaped channel was 1 cm and the two branches had identical dimensions (80 μm depth, 0.4 cm length, 0.5 mm width) that differ from the one of the single channel (80 μm depth, 0.7 cm length, 1 mm width). At the ends of each channel circular reservoirs (diameter 400 μm) were pierced into the PDMS by using a piercing tool of appropriate size. C-Flex tubes (Upchurch Scientific) were inserted in such reservoirs in order to connect the PDMS microfluidic cell to a Masterflex L/S (Cole-Parmer, USA) peristaltic pump, operating at 500 μl/min in order to minimize any mass transport effect. An image of the device carrying the Y-shaped flow cell is shown in Fig. 1. An Re value of about 100 can be calculated for the experimental condition maintained during the SPRI experiments. Such value indicates that our Y-shaped microchannel ensures laminar flow conditions during SPRI experiments.

2.3. SPRI experiments and data analysis

All the SPRI experiments were carried out by using an SPR imager apparatus (GWC Technologies, USA) elsewhere described in detail [29]. SPR images were analyzed by using the V++ software (version 4.0, Digital Optics Limited, New Zealand) and the software package Image J 1.32j (National Institutes of Health, USA). SPRI provides data as pixel intensity units on a 0–255 scale. SPRI curves used for real-time kinetic experiments were obtained by plotting versus time the difference between the integrated areas of preselected regions of interest (ROIs) of successive SPR images of the chip. As specified by the manufacturer, raw data were converted in percentage of

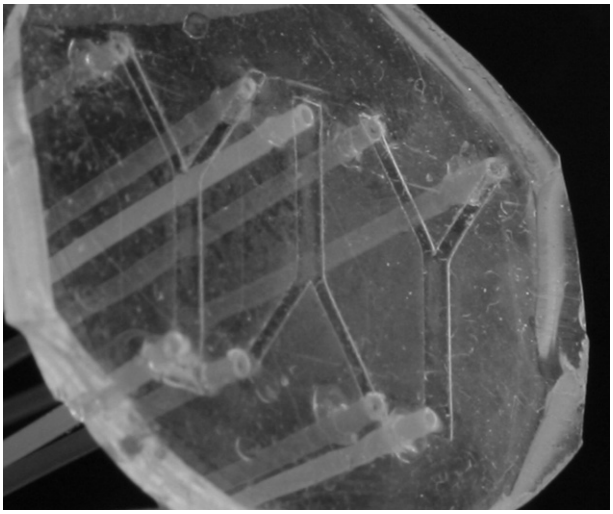

Fig. 1. The image shows the Y-shaped flow cell built as described in the text. The PDMS-made flow cell is put in contact to the SPRI gold sensor surface. Solutions are then flowed inside the pipes by a peristaltic pump.