Local immobilization of quantum dots in a microchannel for the development of a point of care biosensor

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ABSTRACT

The objective of this work is to develop a point of care (POC) device that can be able to rapidly and selectively immobilize bio-labelled quantum dots (QDs) for the detection of biological entities, such as pathogens. The POC device is made of an array of biotin functionalized silica microdiscs, localised in a microchannel onto which streptavidin coated quantum dots (SA-QDs) will strongly attach. Preliminary results of such immobilizations in a microchannel are reported in this abstract.

KEY WORDS: Quantum dot, Surface functionalization, Selective Immobilization, Nanoparticle, Biotin-Streptavidin, Biosensor, Microchannel.

INTRODUCTION

Quantum dots (QDs) have attracted tremendous interest in recent years both for their significant properties and their application potential [1]. In particular, QDs offer a high quantum yield: a broad absorption spectra and a narrow, symmetric fluorescence spectra [2]. QDs have been applied in optical bio-sensing for detecting various bio-molecules of interest [3]. Several methods have been proposed to immobilize these nanocrystals onto a surface. However, immobilizations in microfluidic channels have so far been limited to DNA [4], cells, proteins and enzymes [5], and are often complex and time consuming. In contrast, the objective of this study is to develop a simple and fast procedure to immobilize bio-labelled QDs in a microfluidic channel. This microchannel will be part of a POC testing device aimed for the detection of biological entities, such as pathogens. The immobilization of QDs takes place at specific locations where optical microcomponents designed to detect QDs emissions will be situated.

FABRICATION and PROCEDURE

The POC testing device is intended to be used outside conventional lab environment, thus, operational procedures should be as simple as possible. The sample solution (for instance blood) will be directly loaded through a microchannel by capillary force. An array of silica microdiscs located in the microchannel will allow the targeted biomaterial to selectively attach and get detected.

A Pyrex microchannel is anodically bonded on the silica-patterned silicon surface (Fig.1). Both the Pyrex microchannel and the silica microdiscs are structured by using microfabrication techniques. The 200 μm in diameter silica microdiscs are made of a 2 μm thick thermal silicon dioxide layer patterned on the silicon by BHF etching. The 50 μm deep microchannels were HF-etched in Pyrex wafer by using a 400 nm thick polysilicon mask.

The immobilization procedure, performed in-situ, includes only three steps (Fig.2), associated to three entities: the (3-Aminopropyl)triethoxysilane) (APTES), the biotinamidohexanoyl-6-aminohexanoic acid N-hydroxysuccinimide ester (NHS-B) and the streptavidin coated QDs (SA-QDs). Each step is performed by introducing 500 nL of the appropriate reagent in the microchannel. After incubation, the solvent is flushed through the channel in order to remove all weak molecular bindings. The molecules used in this architecture were selected for their specific functionalities. 1) The silane group allows the anchoring of the APTES onto the silica surface. The APTES binds specifically on the SiO_2 surface due to the strong APTES- SiO_2 affinity. 2) *N*-hydroxysuccinimide (NHS) activated esters of biotin react specifically with amino functions of the surface [6]. In such reaction, NHS is substituted by the amino group. The NHS-B is covalently bound to the aminosilanized surface, via amid function. Therefore, we obtain a microdisc surface uniformly capped with Biotin. 3) Finally, the immobilization of the SA-QDs on the target surface is carried out by using the SA-Biotin affinity. The tetrameric protein, streptavidin, binds very tightly to the small molecule biotin ($Ka=10^{15}$ M^{-1}). The SA-QDs are then immobilized onto the biotin functionalized surface.

RESULTS and DISCUSSION

The process of immobilization is characterized using fluorescence microscopy through the Pyrex cover plate, in which the microchannel is etched. As shown in figure 3, three microdiscs have been exposed at three different concentrations of

SA-QDs. As expected, the fluorescence intensity of QD functionalized microdiscs increases with the increase of the QD concentration. In order to test the immobilization of SA-QDs on the silica microdiscs, the devices were sonicated for up to 35 minutes. No alteration of the emission intensity was observed, inferring the strong SA-QD binding to the biotin, itself covalently bound on the amine modified surface.

FIGURES

Figure 1: Top view of a microchannel HF-etched in Pyrex bonded on a silicon substrate bearing a 200 μm in diameter silica microdisc.

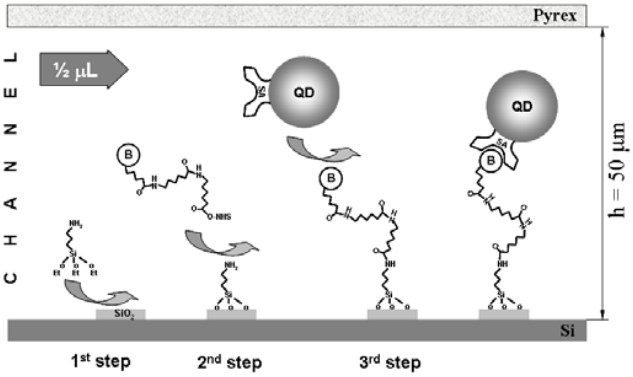

Figure 2: Schematic drawing of the in-situ immobilization procedure. Every step consists of the introduction of the reagent to incubate before washing by flushing solvent through the microchannel. First step: APTES, 1/2h incubation. Second step: NHS-Biotin, 3h incubation. Third step: SA-QD, 1h incubation.

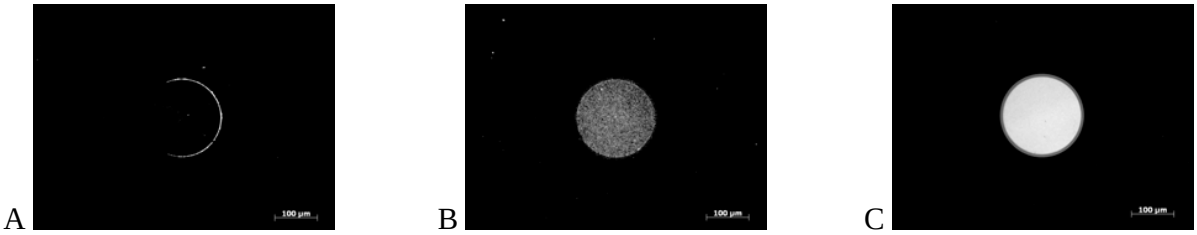

Figure 3: Fluorescence images of microdiscs exposed at different concentrations of SA-QDs. A: 3 nM, *B:* 9 nM, *C:* 60 nM.

CONCLUSION

The selective immobilization of QDs in a microchannel is an important step in the development of a microchannel-based QD biosensor. Such technology requires bio-labelled QDs to be immobilized at specific locations where the optical detection will occur. Thus, the immobilised bio-receptor conjugated QDs will be accessible to target pathogens in solution. When a pathogen is recognized by the bio-receptor, the optical signal of the pathogen-affected QD is expected to be altered.

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