Towards lab-on-card, label-free enzyme immunoassays based on electrical impedance spectroscopy

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A high throughput solid-phase enzyme immunoassay (EIA) platform based on lab-on-card (LOC) microfluidics is proposed. In addition, a first step towards label-free detection of immunosorbent assays based on Electrical Impedance Spectroscopy (EIS) is made. The reported LOCs are developed by alternately stacking PET layers and adhesive layers. LOCs can be equipped with aluminium interdigitated electrodes and coated with polystyrene to perform EIS. Furthermore, the end product of a colorimetric ELISA test was flushed into a LOC and subsequently the optical density was measured. The preliminary tests provide a stable base for the label-free EIA based on EIS.

Introduction

ELISA tests are an established value in analytical biochemistry. It is an accurate quantifying method. However, it is labour intensive and requires skilled personnel. In this work, a high throughput and automated ELISA platform is developed. To achieve this, the platform is based on lab-on-card (LOC) microfluidics. Throughput can be increased even further when Electrical Impedance Spectroscopy (EIS) is used to monitor the presence of the target. Indeed, EIS is label free and does not require the enzyme-linked antibody. Similar measurements with impedance have been reported by others [1], however not yet in a true microfluidic setup. To validate the system, the screening of humoral autoimmune response in Multiple Sclerosis is taken as an application [2].

Materials & Methods

The lab-on-card microfluidics reported in this work are developed with a rapid-prototyping technique. The LOC is built by alternately stacking PET-layers with a thickness of 100 µm and double sided adhesive with a thickness of 50 µm. The adhesive layers can be structured with microfluidic channels. In this work a Speedy 100R (provided by Trotec Laser GmbH) laser cutter was used. The PET layers can be coated and hence are the functional layers. An example of a card equipped with interdigitated electrodes is presented in figure 1a and a card with multiple sensing zones is presented in figure 1b. The latter is designed to mimic the layout of a standardized microplate. The measurement chambers in both LOC designs are coated with Polystyrene(PS). This was dissolved in toluene and subsequently spincoated onto a PET-substrate, either with or without electrodes, to a thickness of 600 nm. The aluminium was deposited with a home-build DC-pulsed sputtering system with an unbalanced magnetron (provided by the Kurt J. Lesker Company).

Results & Discussion

The binding capacity of PS was tested with contact angle measurements after coating them with SPAG-16 or THIO. A 5° drop in contact angle for a 10 µg/ml SPAG-16 and THIO coating was observed. The ELISA protocol from [2] was performed and the resulting optical density(OD) was measured. Next, the end product of the ELISA is flushed into a LOC containing wells of 20 µl. Here the ODs were measured, and compared to a measurement in a standard well plate. The results are presented in Figure 1. The ODs of the LOC are lower since the light travels through less fluid, hence less absorption occurs.

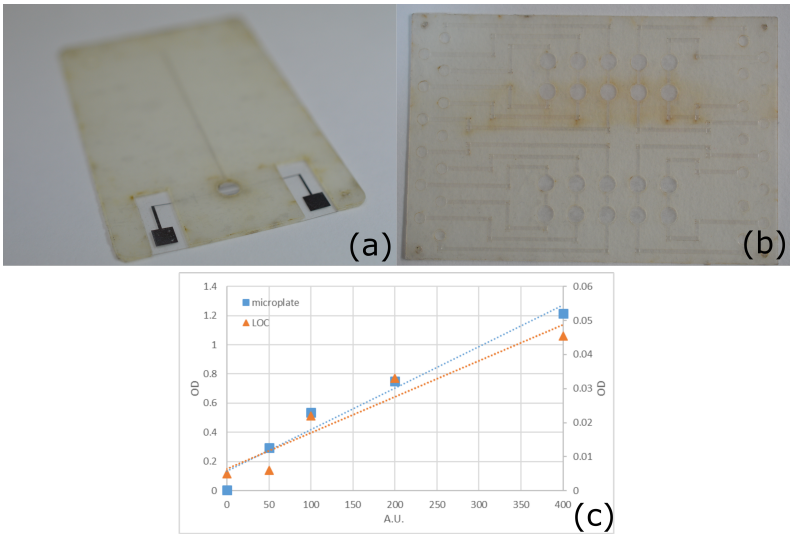

Figure 1: **(a)** shows a LOC with interdigitated electrodes for EIS measurements.
(b) displays a LOC that mimics a ninety-six-well plate. It is used for OD measurements.
(c) presents the comparison of an OD measurement of a serially diluted sample, measured in a microplate and in the LOC presented in (b).

Conclusions

The preliminary data of the OD measurements show that LOC microfluidics have the same sensitivity as typical microplate measurements. This ensures that EIA tests can happen at this scale and that experiments based on impedance are promising.

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References

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