DUAL NETWORK MICROFLUIDIC ELISA FOR THE QUANTIFICATION OF BIOMARKERS IN SERUM

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The sensitive and specific detection of proteins is at the core of many routine analyses in fundamental research, as well as in the biotechnological and pharmalogical industries. Direct applications for medical diagnostic, food control and environmental safety are also based on protein quantification. Despite such a wide use, these experimentations are still commonly achieved in microwell plates, which make them expensive and labor intensive to realize. Microfluidics promises to provide a drastic reduction in both the cost and time associated with such immunoassays.

Recently, we have achieved a proof-of-concept describing the evolution of our original stop-flow microfluidic ELISA [1] into a fully functional platform based on the novel concept of Dual Network [2]. This new system circumvents the recurrent problem of non-specific absorption without the need for long, complex and costly pre-treatments of the PDMS chip. Its principle relies on the complete separation of the immune complex formation phase and the subsequent immunodetection phase, using an adapted design with a combination of magnetic bead manipulation and embedded pressure valves.

The platform was tested for the quantification of a widely used biomarker, the cytokine TNF-alpha, in order to assess its potential for challenging applications such as the diagnostic of sepsis. Septic patients have been reported to display serum concentrations of TNF-alpha ranging from 20 to 100 pg/mL. To achieve the quantification of this low level of TNF-alpha, the thorough characterization and optimization of several assay parameters, namely the concentration of the detection antibody, the incubation and detection times and the magnetic bead displacement velocity was performed. The standard curve showed a sensitivity of 40 pg/mL (2.6 pM) for TNF-alpha in pure serum with excellent linearity up to at least 500 pg/mL and good reproducibility (Figure 1).

The total assay time for the realization of the 8 parallel reactions on a single chip was about an hour with possibilities for further automation. This represents a significant time reduction with regards to traditional ELISA with comparable sensitivity values. The Dual Network system also displayed performances similar to those of two of the most achieved microfluidic immunoassays described to date [3,4], with additional benefits due to the simple and inexpensive fabrication process, and the use of non-treated PDMS chips. These results proved our Dual Network platform suitable for fundamental and applied research studies, as well as demonstrate its potential for the early diagnosis of diseases and infections when low variations of biomarker concentrations have to be detected.

References:

[1] Herrmann M, Veres T and Tabrizian M, Lab Chip, **6(4)** (2006) 555-560.
[2] Herrmann M, Roy E, Veres T and Tabrizian M, Lab Chip **7(11)** (2007) 1546-1552.
[3] Cesaro-Tadic S, Dernick G, Juncker D, Buurman G, Kropshofer H, Michel B, Fattinger C and Delamarche E, Lab Chip **4** (2004) 563-569.

[4] Honda N, Lindberg U, Andersson P, Hoffmann S and Takei H, Clin Chem **51** (2005) 1955-1961.

Figures:

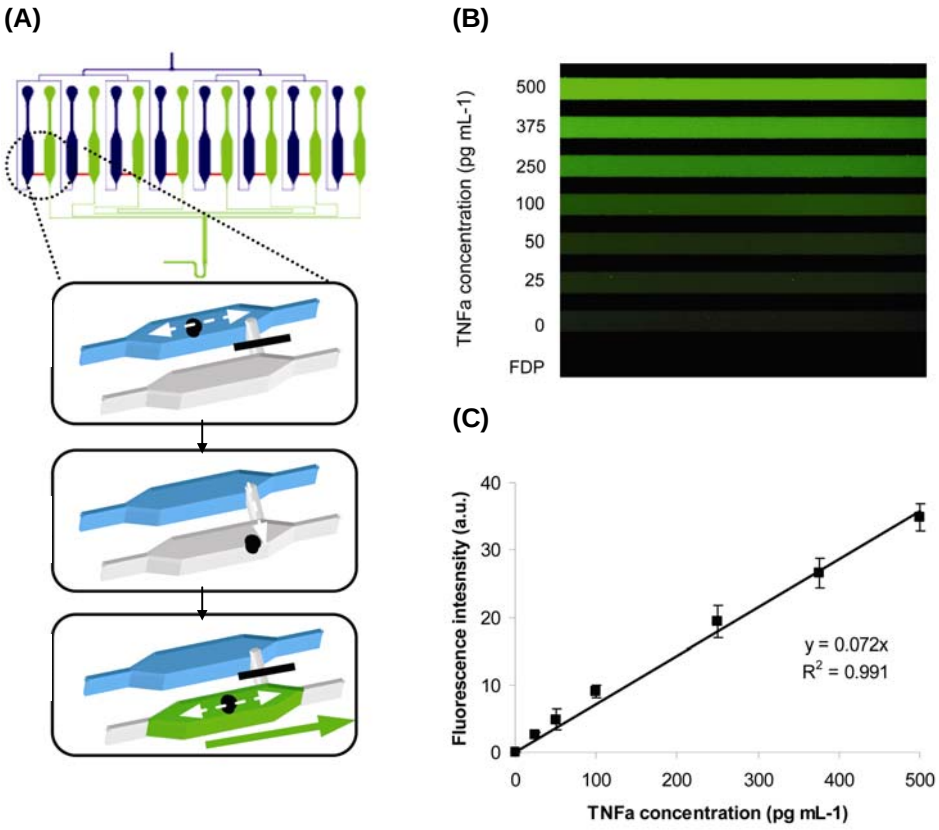

Figure 1: (A) Microfluidic layout for the Dual Network ELISA system with a close up on one pair of chambers showing the sequential steps of the assay (formation of immune complex, reactive bead transfer and detection). (B) Fluorescent photograph at the detection site for a typical assay for TNF-alpha. (C) Standard curve for the quantification of TNF-alpha in pure serum (3 repetitions).