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Reversible magnetic clamp of a microfluidic interface for the seric detection of food allergies on allergen microarrays

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ABSTRACT

To provide a robust platform for fluid handling, most microfluidic devices usually involve irreversible bonding methods to achieve a leak free interface between the microchannels and the holding substrate. Such an approach induces a major drawback when biological interactions are performed on a microarray format as it is difficult to recover the biochip for further fluorescence scanner analysis. This work describes an automated microfluidic platform using a reversible magnetic clamp for multiplexed immunodiagnostics. The microfluidic device is composed of a magnetic PDMS layer (containing iron powder) coated by PDMS, which is reversibly clamped to an epoxysilane glass slide containing an array of various antigens. The microfluidic device was validated for *in vitro* diagnosis of food allergies on an allergen microarray after serum interaction. The statistical analysis of spot intensities (signal to noise ratios) on the microarray displayed excellent reproducibility. In addition to the reduction of volumes provided by miniaturization, this approach is versatile, is easy-to-produce and provides an effective platform for multiplexed immunodiagnosis based on conventional fluorescent detection schemes.

1. Introduction

Immunoassays are routine tests mainly devoted to *in vitro* diagnosis and biological or pharmaceutical R&D. They exploit the specificity of antigen-antibody recognition for the detection of biological analytes of interest in patient samples [1]. The best-known assay is the enzyme-linked immunosorbent assay (ELISA) in which the detection of an antigen–antibody complex is performed on a microtiter plate [2]. Most of the analytical methods currently used for clinical diagnosis involve manual processing that, in most detrimental cases, might be the sources of contamination and non-reproducible errors that lead to false positive or negative results. In this context, microfluidic systems have demonstrated many advantages to improve the reproducibility of bioanalytical protocols while providing additional advantages regarding miniaturization, integration and automation [3]. Bringing together microfluidics and immunoassays lead to promising tools for automatic, sensitive and selective measurements of biological components.

Ensuring the compatibility of microfluidic devices with well-established biological methods and standard substrates is essential in the development of novel analytical platforms. This is particularly the case of microarray technologies that cover a large range of applications in biomolecular analysis. Indeed, in order to provide a robust platform for fluid handling, most microfluidic devices usually involve irreversible bonding methods to achieve a leak-free interface between the microchannels and the holding substrate. Such approach give rise to a major drawback when biological interactions are performed on a microarray format as it is difficult to recover the biochip for further fluorescence scanner analysis. Several methods to reversibly seal microfluidic device were described in the literature, including sealing by self-adhesion properties of the material, vacuum seal by aspiration or sealing by magnetism. Each process has advantages and drawbacks regarding implementation, cost and target applications. The sealing by self-adhesion is described to be weak. Hence, surface treatment needs to be achieved to enhance the contact strength between the microfluidic device and the substrate [4]. On another side, the reversible clamping directed by vacuum requires complex setups and the addition of a microchannel network for the sealing by aspiration of the substrate and the microfluidic device [5]. Finally, the sealing by magnetism is described to be easy to implement, biocompatible and inexpensive [6–8].

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Here, we describe the design, fabrication and validation of a generic microfluidic interface allowing multiplexed detection of food allergies on a biochip format using a reversible magnetic clamp. The microfluidic device is composed of a magnetic polydimethylsiloxane (PDMS) layer (containing iron powder), which is reversibly sealed to an epoxysilane glass slide containing an array of food allergens. The channel design integrates two independent inlets to separate the required buffers (saturation and washing buffers, labeled secondary antibody) from sample (serum, blood) and avoid potential contaminations during the sequential injections. Despite the integration of magnetic particles within the microfluidic chip, the fabrication process was optimized to permit optical observations during the analytical workflow. The geometry and dimensions of the channels were optimized through hydrodynamic simulations performed with COMSOL software to provide a uniform spreading of the reactant and analytes on the capture area. The functionality of the device was validated for *in vitro* diagnosis of food allergies on a microarray of spotted allergens after interaction with the serum of a well characterized patient.

2. Material and methods

2.1. Microfluidic device design and fabrication

The microfluidic design (Fig. 1a) consists of a y-shape structure comprising two independent inlets (one for injecting buffers and secondary labeled antibody solutions and the other for serum injection) connected to a larger incubation chamber (15 mm long × 1.5 mm wide × 0.2 mm high) where the capture spots are located. The geometry and dimensions of the three channels between the inlets and outlet were optimized through hydrodynamic simulations using COMSOL Multiphysics software. In particular, the main chamber and the inlet channels were designed to provide a 25 μL/min flow rate for 50 mBar input pressures which matches the operating pressures of the external handling system. Considering this working pressure, the differences in the input channel dimensions were used to lower the risks of contaminations by diffusion between the serum and the other liquids in the time frame of the analysis workflow.

Reduction of the dead volumes, and calculation of the hydrodynamic resistance of the devices were performed in order to ensure that required pressures and flow rates be compatible with the external liquid handling systems (see section below). The width in the main incubation chamber was also set to provide a uniform spreading of the reactants according to the spot distribution in the microarray.

The aluminium master mold (aluminium 2017A) required for the microfluidic device manufacturing was micro-machined using a 500-μm-diameter mechanical tool. The microfluidic device is composed of one layer of magnetic PDMS covered by a layer of native PDMS (Fig. 1b). It was obtained in two steps. PDMS prepolymer and curing agent (Sylgard 184, Dow Corning) were mixed manually in a 10:1 ratio and iron powder (Sigma Aldrich, hydrogen reduced, 50 μm diameter, 50/50, *w*/*w*) was further added to the PDMS prepolymer mixture [9]. The magnetic PDMS prepolymer solution was degassed and carefully poured onto the master. A doctor blade technique was used to remove excess of magnetic PDMS and provide uniform layer, with no material left on the top of the protruding structures of the master mold. Then the magnetic PDMS layer was cured for 1 h at 65 °C leading to a layer close to 200 μm height of magnetic PDMS. Then a second layer of PDMS, without iron powder, was casted on the top of the magnetic layer (12 h, 65 °C) (Fig. 1b). Finally, the PDMS microfluidic device was removed from the mold and inlets and outlet were punched. The homogeneity of the magnetic layer thickness and the spatial distribution of the iron particles were investigated through SEM and optical imaging (Fig. S1).

2.2. Allergen array manufacturing and serum

Total food extracts (egg white and yolk, crab sticks, cashew nut, wheat, salmon, bass, cod, tuna, shrimp, cow milk, goat milk, peanut, mustard, buckwheat, sesame, soya, hazelnut, walnut) used in this study and sera of rabbits immunized against peanut were obtained from Pharma-DEV lab (AOMC team, Toulouse). The food extracts were dissolved at a concentration of 0.5 mg/mL in phosphate buffer (25 mM NaH_2PO_4, pH 7.4) and were spotted in triplicate on epoxysilane slide (Nexterion® Slide E, Schott Nexterion, Jena, Germany) using a conventional microarrayer (Q-Array Mini, Genetix) equipped with pins (SMP3, ArrayIt) delivering around 2 nL of food extract solutions per spot with a medium size of 150 μM at 300 μM pitch. The quality of the spotting was optimal at 45% relative humidity and constant temperature of 20 °C. After deposition food allergen microarrays were dried overnight under ambient conditions.

Human serum was obtained from Rangueil Hospital (Toulouse). Their specific IgE level was determined in serum by *in vitro* measurement of allergen-specific IgE by the ImmunoCAP® assay [10] (Thermo Scientific). For the patient tested in this study, the values determined by ImmunoCAP® specific IgE were cow and goat milk, 100 kUA/L, egg yolk 13.8 kUA/L and peanut 4 kUA/L.

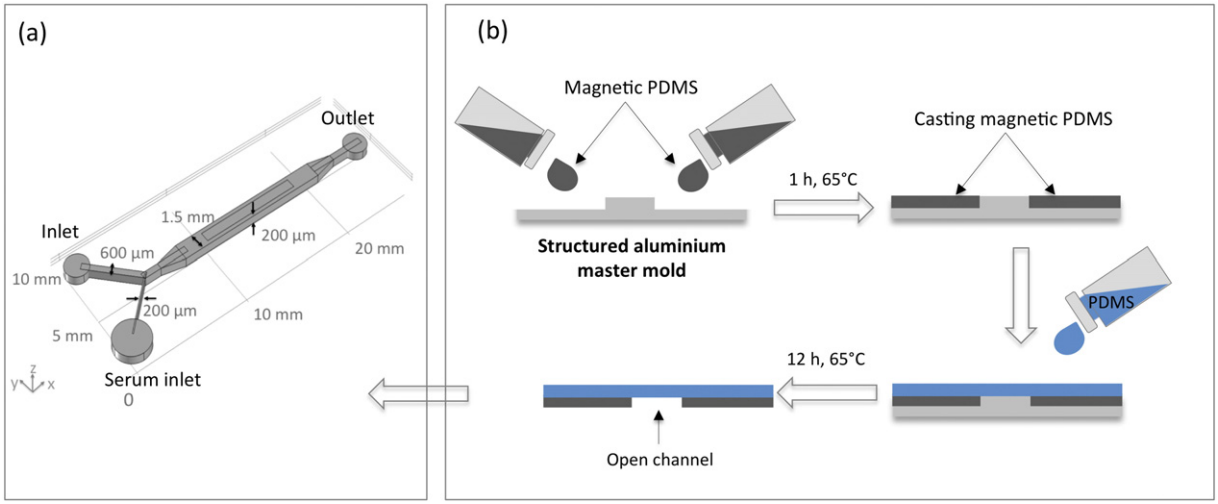

Fig 1. a) Design and b) fabrication of the magnetic microfluidic interface.