A SIMPLE POINT-OF-CARE TEST FOR DRUG MONITORING IN WHOLE BLOOD OF PATIENTS WITH AUTOIMMUNE DISEASES

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ABSTRACT

We present here a novel "whole blood in – result out" self-powered microfluidic test for detecting adalimumab (ADM) drug within 20 minutes that can enable therapeutic drug monitoring (TDM) at the point-of-care (POC). A validated ELISA assay was downscaled to perform in 15 µL of whole (citrated) blood without compromising its specificity and sensitivity. All liquid manipulations are integrated on a single microfluidic platform, namely the (i)SIMPLE chip[1], that functions in a completely autonomous fashion upon single finger-press activation.

KEYWORDS: Point-of-care, microfluidics, therapeutic drug monitoring, adalimumab

INTRODUCTION

Autoimmune diseases, like inflammatory bowel disease and rheumatoid arthritis, involve chronic inflammation. Possible treatments include tumor necrosis factor alpha (TNFα) inhibitors, such as the fully human monoclonal ADM, which can (i) maximize clinical response, while minimizing loss of response [2] and (ii) reduce treatment cost. Several ADM assays are currently available, including classical ELISA and lateral flow tests [3]–[5], but they suffer from long time-to-result or require off-chip sample preparation, respectively, emphasizing that true POC tests for TDM are not yet available on the market.

EXPERIMENTAL

The (i)SIMPLE microfluidic platform can both push and pull liquids without any external pumps while being inexpensively made from plastics and filter paper, and easy to use/fabricate (Fig. 1A). For integrating the validated MA-ADM28B8/MA-ADM40D8-HRP ELISA on the (i)SIMPLE chip, we first downscaled the assay time in buffer. Next, this protocol was used for testing whole citrated blood samples spiked with ADM, both in normal and 75% reduced volumes. The performance of the downscaled ELISA was then tested in flow by immobilizing capture antibody on the polymer surface of plain microfluidic channels connected to external pumps. The designed (i)SIMPLE device (Fig. 1B) can perform all steps and incubations upon a single finger-press activation in 15 µL of citrated whole blood.

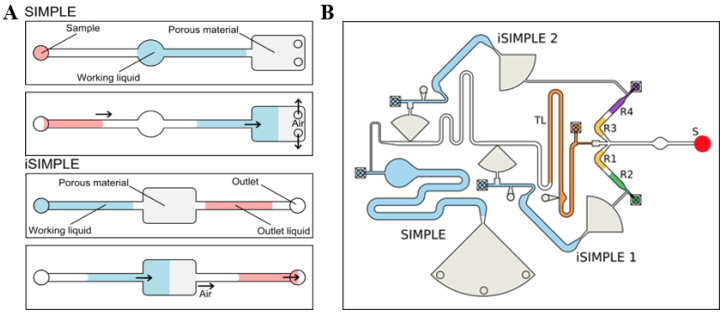

Figure 1: A) The Self Imbibing Microfluidic Pump by Liquid Encapsulation (SIMPLE) consists of a working liquid (blue) that is brought into contact with a porous material (grey) by a finger press, after which it starts wicking into the material. The underpressure draws the sample (red) into the material. The infusion SIMPLE, (i)SIMPLE works in reverse, pushing the sample through the channel; B) Digital design of the integrated (i)SIMPLE chip for ADM detection. The sample (S) is pulled into the detection channel once the chip is activated. The wash (R1-R3) and reagent steps (R2-R4) flow sequentially over the chip. The readout is performed at the widened white area on the right.

RESULTS AND DISCUSSION

After reproducing the original ELISA in buffer, we downscaled the assay to 2 min incubation for both target and conjugate antibody (i.e. detection antibody conjugated with HRP), compared to original 16 and 2 hours, respectively. Then, we successfully performed the short assay in citrated whole blood samples spiked with ADM, both in normal and reduced volume (Fig. 2). Next, the downscaled assay was tested in flow on the plain channels, where we achieved similar sensitivity (Fig. 3A). Alternatively, we also integrated a plasmapheresis filtration system with the (i)SIMPLE platform, capable of on-chip extraction of plasma in a few minutes, starting from a whole (citrated) blood sample (Fig.3B-D).

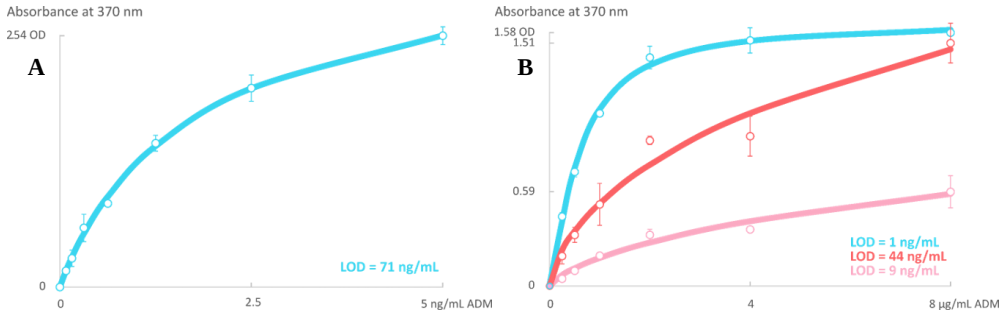

Figure 3: A) The original ELISA protocol detects ADM from 0 to 5 ng/mL in 1/2000 diluted buffer samples. B) Downscaled ELISA in buffer (blue), in whole citrated blood samples with original volumes (pink) and volumes reduced by 75% (red). The expected ADM concs in whole blood are 0.25-8 µg/mL. The curves were fitted with a four-parameter logistic fit; error bars represent one standard deviation (n=3).

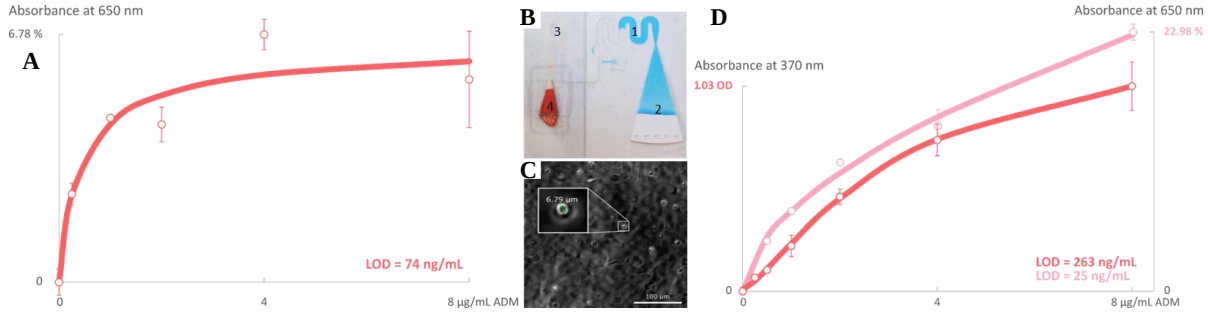

Figure 2: A) Calibration curve on-chip in flow with external pumps in whole citrated blood. B) SIMPLE-based plasmapheresis: working liquid (1), porous material (2), analytical channel (3) and filtration unit (4). The chip was activated by depositing a droplet of whole blood on the inlet. Once the blood was absorbed into the filter material, the SIMPLE pump created enough underpressure to elute the plasma into the analytical channel. C) Bright field microscopy visualizing the red blood cells (RBCs) still present in the plasma, 381 ± 91 RBCs/mm³, which is comparable to standard centrifugation method (340 ± 47 RBCs/mm³). D) Calibration curve for ADM ELISA in plasma samples in microtiter plates (red; measured on spectrophotometer) and on-chip (pink; measured on handheld reflectometer). All curves were fitted with a four-parameter logistic fit; error bars represent one standard deviation (n=3).

CONCLUSION

We presented a one-press-activated POC microfluidic chip capable of autonomously handling whole blood samples to detect ADM. Additionally, we demonstrated the compatibility of the same platform with on-chip plasmapheresis, revealing thus flexibility of the (i)SIMPLE chip to accommodate complex bioassays (i.e. other monoclonal therapeutic antibodies besides ADM) and utilize a wide range of biological samples

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