DIGITAL QUANTIFICATION OF ALZHEIMER'S BIOMARKER IN BIOLOGICAL SAMPLES

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ABSTRACT

This manuscript reports a digital ELISA integrated on a digital microfluidics (DMF) platform for the ultrasensitive detection of Alzheimer's disease (AD) biomarker Tau protein over a wide range of relevant physiological concentrations. High-affinity antibodies combined with optimal assay conditions led to astonishing limits of detection in the low-attomolar range in buffer samples, which constitutes the first step toward developing a blood test for AD diagnosis.

KEYWORDS: Alzheimer, Tau protein, Digital ELISA, Digital Microfluidics

INTRODUCTION

Nearly 36 million people worldwide have been diagnosed with AD or a related dementia[1]. Several studies have proven that AD patients display elevated levels of total protein Tau in biological fluids and, consequently, this protein has become a very important AD biomarker[2]. However the use of Tau as biomarker for early AD diagnosis has been limited by its very low concentration in blood plasma, below the detection limit of conventional ELISA assays, requiring thus collection of cerebrospinal fluid (CSF) samples for reliable detection. Therefore, a method for measuring Tau protein in blood would advance diagnosis of AD by eliminating the need for invasive, expensive, time-consuming procedures. Consequently, research efforts are underway to provide reliable and sensitive quantification of Tau protein in serum and plasma.

Recently, electrowetting-on-dielectric-based DMF has been introduced by our research group as a novel platform to perform single-molecule detection on superparamagnetic particles[3]. A millimeter-sized array, containing 62.500 hydrophilic-in-hydrophobic (HIH) microwells with dimensions tailored to accommodate single particles, is micropatterned on the top surface of the microfluidic chip. In the presence of a magnet, high-throughput seeding of superparamagnetic particles is achieved. Because the fluorescent signal, generated on such trapped magnetic particles during a bioassay, remains confined in femtoliter-sized droplets, the quantification of single target molecules becomes attainable. In this work, the described platform is challenged for the implementation of a Tau-specific digital ELISA.

EXPERIMENTAL

Figure 1A and B depict the double-plated DMF device that was used in the experiments. The top plate with the HIH patterned microwells and the bottom plate containing the actuation electrodes were fabricated with standard microfabrication techniques as described previously [3] and were mounted in a custom-made holder that houses electrical connections for DMF droplet actuation (Figure 1C).

Before seeding the magnetic particles on the platform, Tau protein was captured on their surface with Tau-specific antibodies. The generated immunocomplexes were subsequently labeled with an enzymatic reporter capable of generating a fluorescent product. Since a reproducible and high loading efficiency is important for final experiments, first it was tested whether the immunocomplex formed on the particles interferes with the seeding efficiency. To do that, the loading efficiency was evaluated on three different arrays for (a) bare particles, (b) particles with capture antibody on their surface and (c) particles with the formed immunocomplex, and for different number of seeding cycles.

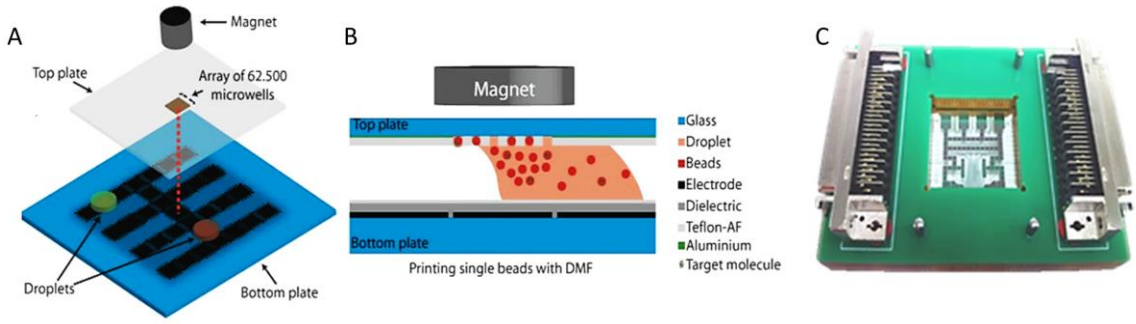

Figure 1. Printing of single superparamagnetic particles for performing automated single-molecule detection by means of digital microfluidics (DMF). (A) Schematic representation of a double plate DMF chip consisting of a top and bottom plate. (B) Side view of the DMF device showing how suspended particles in a droplet are attracted towards the array with the magnet. Reproduced from Witters et al. (2013)[3]. *(C) Picture of the in-house fabricated EWOD-based DMF platform showing the double plate DMF chip integrated in the chip holder (6 x 4.5 x 0.5 cm).*

Next, the performance of two different high-affinity detection antibodies, ADx201 and ADx202, (with K_D in pM range)[4] was evaluated, and optimization of different assay parameters (i.e. antibody concentration and incubation time with the capture particles) was performed. Finally, the optimal assay conditions were employed to build a calibration curve over a wide range of relevant physiological concentrations expected in different biological sample matrices.

RESULTS AND DISCUSSION

As can be seen in Figure 2A, the seeding process of magnetic particles was not affected by the formation of the immunocomplex on the particle surface. Loading efficiencies of close to 100% were achieved for all three types of particles, even with only 2 seeding cycles.

The results from the evaluation of the 2 different detection antibodies are presented in Figure 2B. As it can be seen, ADx202 gives a significantly lower signal for the control sample (0 fM Tau protein), defining the background. Since the aim is to develop an ultrasensitive assay, this antibody was selected for further experiments.

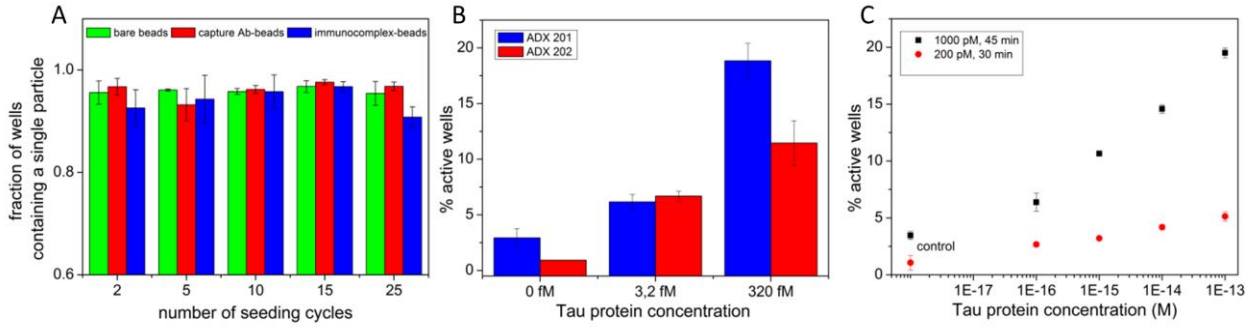

Figure 2. Tau-specific digital ELISA optimization. (A) Loading efficiency of single superparamagnetic particles that are bare (red), modified with antibody (blue) and with the immunocomplex formed on their surface (green) in femtoliter-sized microwells after different seeding cycles. (B) Bar chart of the percentage of active wells for the respective concentration of target when using two different antibodies: ADx201 (blue) and ADx202 (red). Error bars represent the standard error of two repetitions. (C) Plot of the percentage of active wells versus the target concentration when using different assay conditions: 30 min incubation with 200 pM detection antibody (red) and 45 min incubation with 1 nM detection antibody (black). Error bars represent the standard error of three repetitions.

Figure 2C shows how the ratio of active beads increases when increasing both the concentration of detection antibody and the incubation time with the capture particles. An incubation time of 45 min with a 1 nM solution of the antibody resulted in the highest signal-to-noise ratio for the detection of Tau protein.

Finally, with the latter assay conditions, a Tau-specific digital ELISA was performed for a wide range of Tau protein concentrations (100 aM - 100 pM). The obtained fluorescent images are shown in Figure 3. The high-affinity antibodies combined with optimal assay conditions gave limits of detection in the low-attomolar range in buffer samples. For higher Tau concentrations (picomolar levels) more than 70% of the seeded particles captured a target molecule and thus total fluorescence levels were measured instead (analogue quantification approach, Figure 3, 6-8).

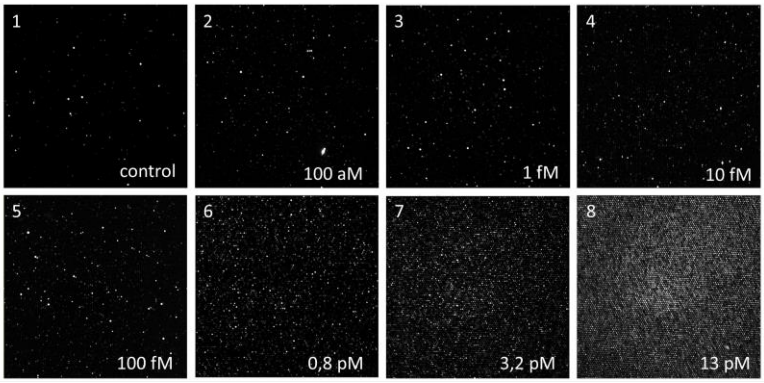

Figure 3. Fluorescent images of the digital (1-5) and analogue (6-8) quantification of Tau protein on the microwell array. The displayed field of view corresponds to ~1/6 of the total array surface (~10000 wells).

CONCLUSION

A digital ELISA integrated on a DMF platform for the ultrasensitive detection of Alzheimer's biomarker Tau protein is reported. This platform enables Tau quantification over a wide range of relevant physiological concentrations expected in different sample matrices, including CSF and blood plasma. Moreover, the employed DMF-based platform offers the possibility of integrating all sample manipulations on chip, which could allow for the performance of miniaturized and fully automated tests for early diagnosis of Alzheimer's disease (AD) in patients.

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