MICROFLUIDICS FOR ALZHEIMER'S DISEASE: SCREENING AND DIFFUSION TO STUDY AMYLOID-β AGGREGATION

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ABSTRACT

Amyloid beta (Aβ) peptide aggregation is a key event in neurodegenerative Alzheimer's disease, but the complete biochemical process is still partly unknown due to its complexity. Very few studies have exploited the advantages of microsystems to assess this question, in spite of their potential ability to shed light on such multi-parameters, dynamic phenomena. In this work, we propose two approaches, relying on microfluidics, aiming at progressing on our comprehension of Aβ aggregation mechanisms. In a first part of this study, we use concentration gradients combined with an original on-chip triggering of the reaction to demonstrate a threshold in Aβ concentration necessary to initiate the aggregation. Secondly, we work on a reaction-diffusion configuration with fluorescently labeled peptides. In stable (non-aggregating) conditions, it enables measuring the diffusion coefficient of different model Aβ peptides. In the presence of metallic ions, inducing aggregation, we measure the first kinetic steps of the process, and we show that surfaces play a crucial role.

KEYWORDS

Amyloid aggregation, Alzheimer's disease, microfluidic screening, reaction-diffusion.

INTRODUCTION

This paper reports microfluidic methods to study the aggregation of Amyloid-beta (Aβ), a pivotal process in Alzheimer's disease. In fact the aggregation produces many intermediates with different cell toxicity. These ones aggregate on neuron where they form β-sheets which provoke neuronal death. In spite of its importance, the reaction is however not fully understood yet because the aggregation is dynamic and multi-parameters: it is influenced by concentration of Aβ, metallic ions, pH…[1]. In spite of their potential for dynamic, complex biochemical processes, microsystems have hardly been used in the context of Aβ aggregation. To our knowledge, very few studies have used microfluidics for Aβ aggregation: Meier et al. [4] have shown the importance of surfactants for the aggregation in micro-droplets and Lee et al. [5] have functionalized channel surfaces with Aβ seed then flow Aβ solution with drugs to see their impact. In this context, we propose in this work a novel microfluidic approach to progress on this question. Two complementary systems have been developed: one exploits the screening ability of microsystems and is based on the generation of concentration gradients [2]; the other relies on reaction-diffusion flow [3] to study the first kinetic steps.

EXPERIMENTAL RESULTS

In our first approach, we try to determine whether there is a concentration threshold of Aβ for the aggregation to occur, a relevant biochemical issue. For detection, we use fluorescence microscopy with Thioflavin T (ThT), which becomes strongly fluorescent in the presence of fibrillar aggregates. To screen Aβ concentration we have primarily adapted a simple linear gradient chip with nine output and tested it with Aβ42 (the full native peptide, 42 amino-acids). This experiment led to aggregation but we observe a clogging of chip. It thus seems necessary to have peptide in stable condition in the gradient chip for the concentration set, and trigger the aggregation afterwards. For that purpose, we designed a chip with gradient part and trigger part (Fig. 1.a). In addition, more than a linear gradient, we can modulate the flow ratio between gradient part and trigger part to screen more conditions (Fig. 1.b). Primarily we have tested our chip with full peptide, Aβ42, and we triggered the aggregation with pH (for some concentration conditions, amyloid Aβ42 is stable at high pH but aggregates for intermediate pH values). As expected, aggregation occurs only after the trigger part, which demonstrates that the device is functional: our original design enables on-chip initiation of aggregation. Anyway, the aggregates obtained are amorphous and this pathway is not the one we want study, since it is hypothesized not to be the one involved in Alzheimer's disease. Then we used a peptide fragment (Aβ14-23, constituted of amino-acids 14 to 23 of the full peptide), since this model peptide aggregates with fibrillar pathway. Furthermore, the aggregation of Aβ14-23 is more easily controlled than for the full peptide: it is triggered by Zn^{2+} [6]. We have tested our chip in the condition of table 1.c; and we obtained the minimum Aβ14-23 concentration to observe aggregation (Fig. 1.e). This threshold value (around 40 µM), obtained with only 100 µL of sample and a single experiment, is consistent with classical biochemistry approach, which demonstrates the relevance of our system. Let us note however that at the intersection between gradient and trigger part, we observe some accumulation of peptide on the wall. This process may perturb the mixing process and consequently makes difficult a complete, quantitative interpretation of the result. Two points have still to be improved in that purpose: achieving efficient mixing at the triggering point and fully eliminating adhesion of peptide.

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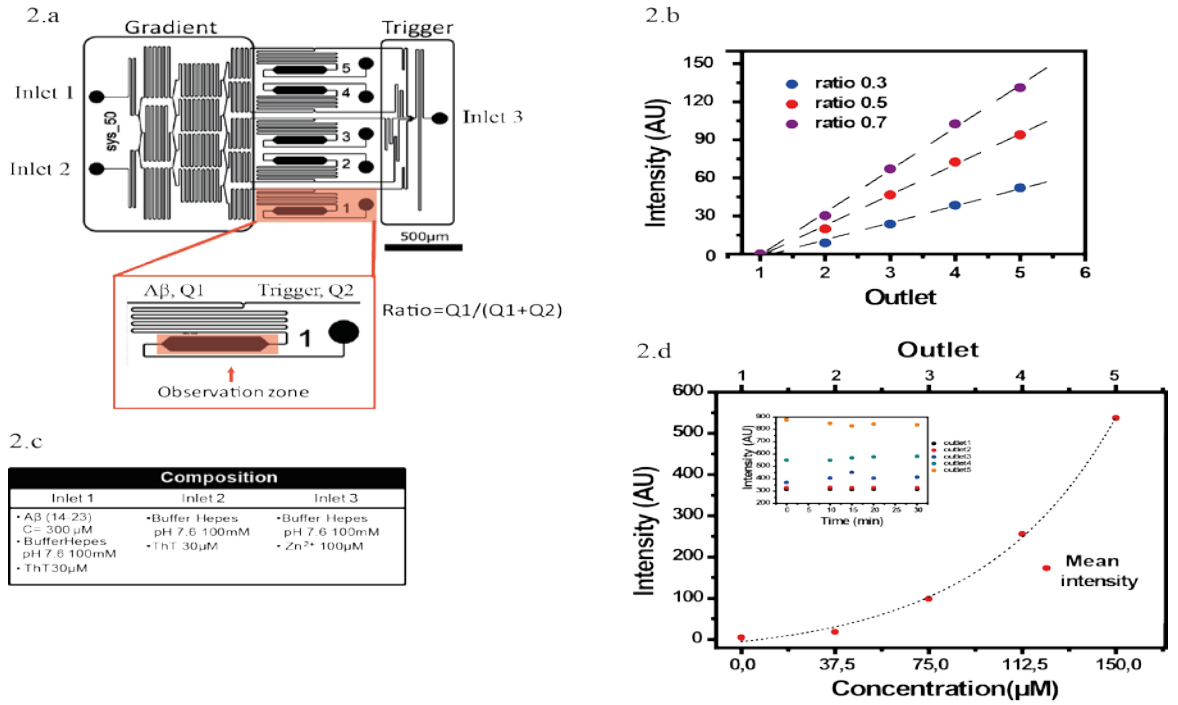


Figure 1: (a) Design of gradient/trigger chip. (b) System's calibration by fluorescein for different flow rate ratios gradient/trigger. (c) Experimental condition with Aβ14-23. (d). Mean fluorescence intensity as function of Aβ14-23 concentration. Inset shows time evolution, demonstrating stationarity.

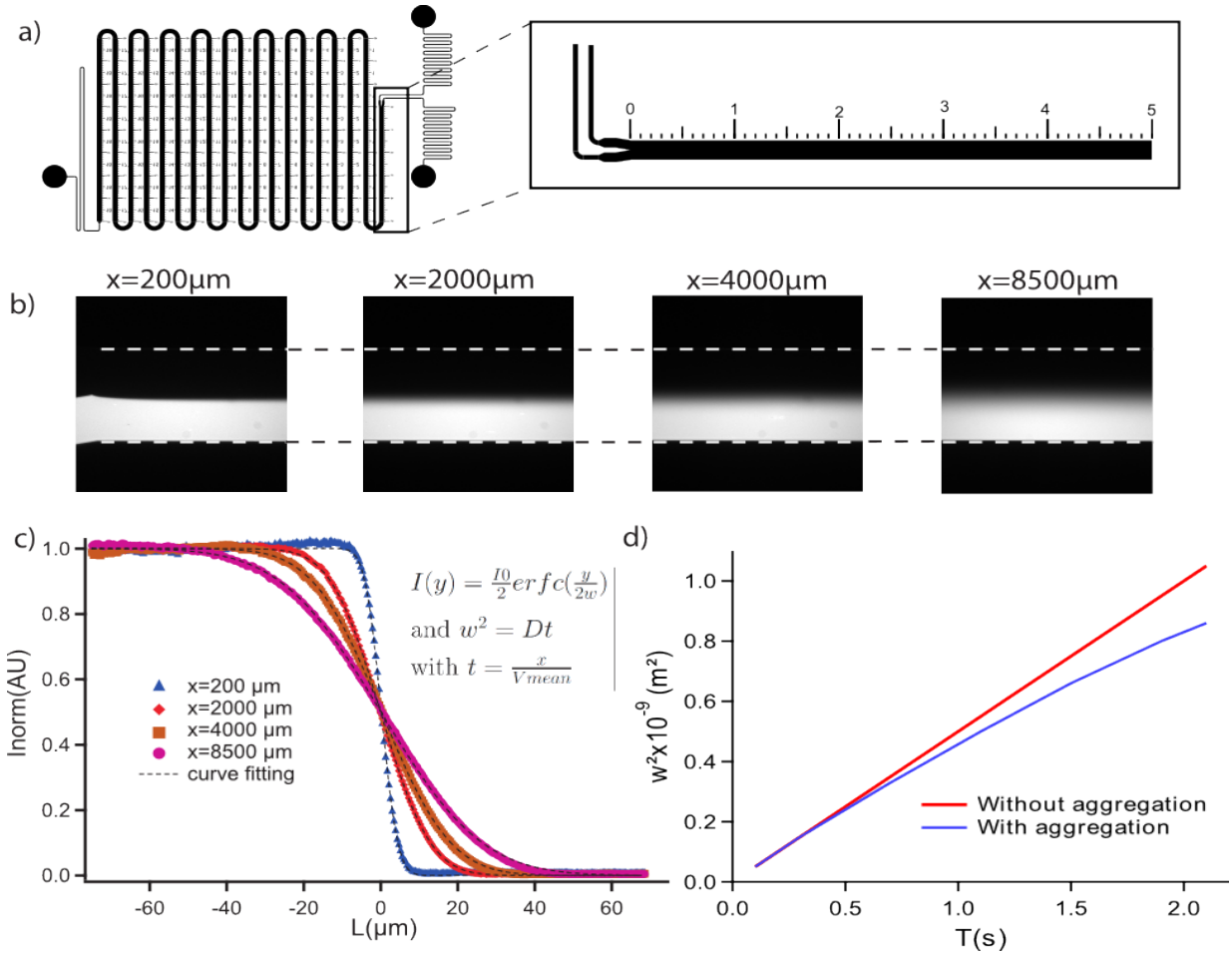
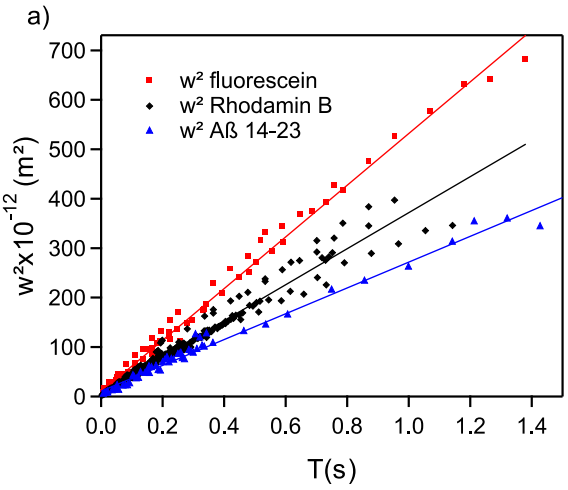


Figure 2: (a) Design of Diffusion-reaction Chip. (b) Fluorescence images of rhodamin B diffusion along the channel. (c) Intensity profile extracted from (2.b), text shows the function used to determine the diffusion coefficient. (d) Expected evolution of the square of the diffusion width (T is the mean residence time) with and without aggregation.

In our second approach we have studied the aggregation kinetics by using diffusion in laminar co-flows [3]: we measure the diffusion of peptide in non-aggregating and in aggregating conditions. This method should enable determining the local diffusion coefficient (D) and consequently have some information about temporal evolution of the mean size of aggregate (Fig. 2.d). For that purpose, we designed a chip (Fig. 2.a) with co-flow between Aβ solution and a buffer solution (inducing or not aggregation). Measurements are illustrated in Fig. 2.b for Rhodamine B. Evolution of the intensity profile can be related to the diffusion coefficient (Fig. 2.c).



b)

Probes	$D_{meas}(m^2/s)$	D_{MNR} (m^2/s)
Fluorescein	5.23×10^{-10}	X
Rhodamin B	3.64×10^{-10}	3.1×10^{-10}
Aβ14-23	2.60×10^{-10}	2.2×10^{-10}

Figure 3: (a) Graph presenting w^2 as a function of time for 3 probes : fluorescein, rhodamin B and Aβ14-23. (b)Recapitulating table of measurement of diffusion coefficient with our chip and with NMR

To calibrate the chip we have made some experiment on standard fluorescent probes (Rhodamin B and Fluorescein), then for fluorescently labeled Aβ 14-23 in non aggregating conditions. The measured values of D (Fig. 3) are in agreement with our NMR measurements and with the literature. Work is currently in progress to measure diffusion in presence of aggregation: a local determination of D (that should decrease during aggregation) will constitute a stationary measurement of the reaction kinetics (Fig. 2.c).

CONCLUSION

We use two microfluidic approaches to study amyloid beta aggregation: concentration gradient combined with on-chip triggering of the reaction enables determining a threshold in peptide concentration to initiate the aggregation; and a reaction-diffusion chip is designed to assess the very first steps of the process.

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