QUANTIFICATION OF AMYLOID FIBRILIZATION BY SIMULTANEOUS DUAL MODE DETECTION WITH OPTICAL SCATTERING IMAGE AND DIELECTRIC RELAXATION SPECTROSCOPY

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ABSTRACT

We demonstrate the dual mode quantification method of Amyloid-β fibrilization using dark-field optical imaging and dielectric relaxation spectroscopy. Optically transparent sub-micron gap microfluidic electrode is designed to observe the change of hydrodynamic radius during the process of Amyloid-β fibrilization and correlated the hydrodynamic radius via dual mode measurements.

KEYWORDS: Amyloid-β, dark-field image, dielectric relaxation spectroscopy

INTRODUCTION

Since the precise role of Amyloid-β aggregates in causing the neurodegenerative disease is still unknown, systematic elucidation of oligomerization and aggregation of Amyloid-β peptides is essential for the treatment and potential prevention of Alzheimer’s disease. It is critical to find solutions for the effective characterization methods to find the pathway of Amyloid-β fibrilization from soluble Amyloid-β peptide to insoluble fibrils, as well as analytical biophotonic and bioelectronic systems to determine of the neurotoxic activity of protofibrillar intermediates in vitro and in vivo; however, current characterization methods of Amyloid fibrilization are primary based on Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM), which are difficult to perform in-situ long-term monitoring as well as multiple mode measurements.

Figure 1 The schematics of the quantification for Amyloid beta fibrilization: Amyloid polymerization steps in (a) neurons and (b) corresponding dielectric spectroscopic response due the change of hydrodynamic radius during amyloid fibrillization.
Here we characterize the process of Amyloid-β fibrilization by optically transparent microfluidic electrodes, which allow to capture both optical and electrical (i.e. impedance) measurements of Amyloid-β fibrilization (Fig. 2). The motivation of monitoring the dynamics of oligomerization and aggregation of Amyloid-β is to gain new insights for drug discovery and impedance-based early AD diagnostics.

EXPERIMENTAL
The devices are fabricated with Indium-Tin Oxide (ITO) coated glasses and Polystyrene (PS) beads for transparent electrodes and dielectric spacer, respectively (Fig. 2). The gap size is controlled by the different size of PS beads, and calculated by a simple equation based on capacitance measurements \( C = \varepsilon A/d \), \( C \): capacitance \([\text{F}]\), \( \varepsilon \): permittivity \([\text{F/m}]\), \( A \): area \([\text{m}^2]\), \( d \): gap distance \([\text{m}]\). In this experiment, 800 nm gap devices are created by 50 nm PS beads with high repeatability (standard deviation = 100 nm). After sample solution of Amyloid-β monomer is loaded into the device, it is placed on the microscope system and connected to the dielectric analyzer for in-situ dual optical (dark-field image) and electrical (dielectric relaxation spectroscopy) measurements (Fig. 2).

RESULTS AND DISCUSSION
As incubation time is increased, soluble Amyloid-β peptide transform into large-size insoluble oligomers or plaque. As a result, these fibrillized Amyloid are clearly resolved as bright dots in dark field image, and the number of dots consistently increases (Fig. 3a). In addition, this fibrilization process is shown as increasing scattering intensity which is measured from each dark field image (Fig. 3b). In DRS measurement, more importantly, the resonance peak in permittivity is shifted to the lower frequency from 1,900 Hz to 850 Hz (Fig. 3b) because the aggregated Amyloid fibril structure have longer hydrodynamic radius than Amyloid-β monomers (Fig. 4a). Moreover, these DRS results were fitted with Cole-Cole equation method to characterize the oligomerization and aggregation of amyloid based on the hydrodynamic radius. As shown in Fig. 4b, the calculated radius is consistently
increased from 19 nm to 21 nm with respect to incubation time, which is strong evidence and quantification data for amyloid fibrillization.

![Figure 3](image1.png)

Figure 3 The representative (a) dark-field images and (b) total scattering intensity change with respect to incubation time

![Figure 4](image2.png)

Figure 4 The representative (a) permittivity change, and (b) calculated effective hydrodynamic radius as a function of incubation time. The concentration of Amyloid beta is 100 μM. The bar in dark-field image corresponds 10 μm

CONCLUSIONS

In conclusion, we developed simultaneous dual mode of optical and electrical monitoring method for Amyloid-β fibrillization using a transparent microfluidic electrode and we have plan to show the correlations of optical electrical data. Systematic studies on the dynamics of oligomerization and aggregation of Amyloid-β peptides are in progress. The elucidation of Amyloid-β aggregation’s mechanism might provide new insight for new drug discovery and impedance-based early Alzheimer’s Disease diagnostics.

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