QUANTIFICATION OF AMYLOID-β SECRETION IN ISOLATED OPTOFLUIDIC CHAMBER ARRAY

Liz Y. Wu, Yeonho Choi, Soon Gweon Hong, Helen Wu, Megan Dueck, and Luke P. Lee
Biomolecular Nanotechnology Center, Berkeley Sensor & Actuator Center
Department of Bioengineering, UC Berkeley, USA

ABSTRACT
Amyloid-β (Aβ) secretion plays a critical role in the pathogenesis of Alzheimer’s disease (AD) and intercellular neuronal communication is potentially an important factor influencing Aβ secretion levels. However, there is no existing system that can isolate a specific number of neuronal cells, encapsulate their Aβ secretions, and measure the dynamics of Aβ secretion directly to investigate how the number and density of cells affects the secretion activity. We have designed an optofluidic system to isolate one or more neuronal cells in nanoliter-volume culture chambers, where Aβ secretions are confined and detected. Since surface enhanced Raman scattering (SERS)-based detection is ideal for real-time and label-free Aβ quantification, 2D nanocrescent SERS array is integrated on the bottom floor of the cell culture chambers for Aβ secretion detection. This system enables, for the first time, the systematic characterization of Aβ secretion for different cell population densities.

KEYWORDS: Amyloid beta, Cell culture, SERS, Nanocrescent

INTRODUCTION
Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that affects more than 24 million people worldwide. Previous studies strongly suggest that Aβ secretion plays a critical role in the pathogenesis of AD; therefore, a great amount of research has been devoted to understand the dynamics of Aβ secretion by neuronal cells under different physiological perturbations. However, platforms for systematic studies of Aβ kinetics or high throughput screening of therapeutic Aβ inhibitor are requisite. Here, we develop an optofluidic system to isolate a specific number of neuronal cells (down to a single cell) in nanoliter-volume culture chambers, where the Aβ molecules secreted from the cells are confined to be detected.
We are integrating a 2D nanocrescent surface enhanced Raman scattering (SERS) array into the base of the device for real-time, label-free Aβ quantification. Nanocrescent structures generate strong electromagnetic field enhancement within the gap between their tips. This enhancement increases the Raman scattering intensity of nearby molecules, enabling the detection of Aβ.

**EXPERIMENTAL**

The PDMS device is made by a 2-layer SU-8 mold that defines 50 μm-high main channels and 5 μm-high gap channels that separate the trap structure from the substrate below. The PDMS device was bonded to a glass substrate patterned with a nanocrescent array. SH-SY5Y cells (a neuroblastoma cell line) suspended at approximately 10^6 cells mL^{-1} were loaded into the device at a flow rate of 10 μL min^{-1}. After trapping the cells at the top U-shaped structure of each nanoliter chamber, flow was gradually increased to ~35 μL min^{-1} until the cells were pushed into the chamber. Vertical pressure was applied to the PDMS device by screwing down an acrylic board on top of the PDMS device to seal the 5 μm channels for isolation of the nanoliter chambers. Figure 2 a-d shows the illustrations and phase contrast images of a single neural cell loading. To load additional cells, we can either repeat the above process or load a higher density cell suspension at a higher flow rate.

![Figure 2. Device operation and cell culture](image)

(a) (b) (c) (d) (e) (f)

(a) the cell loading begins with trapping a SY5Y cell at the top U-shape structure of the nanoliter chamber (b) increased flow rate expands the 5 μm gap and the cell is able to enter the chamber (c) vertical pressure is applied to press down the PDMS structure and seal the chamber (d) image of a SY5Y neuronal cells cultured for 24 hours (e) multiple cells cultured in the device (f) image of the device saturated with red food dye (top), blue dye was loaded after pressure application. No mixing occurs, confirming an effective seal (bottom)

The fabrication of the gold nanocrescent array starts with the formation of a closed-packed polystyrene (PS) bead array on the glass substrate. Oxygen plasma is used to decrease the size of the beads. A thin film of gold is evaporated onto the PS bead array with the substrate tilted at an angle to the evaporation source. This is followed by ion milling at a different angle, which etches the gold layer, leaving behind a 2D gold nanocrescent structure in the shadow of the PS beads. The SERS signal from the substrate is tested by incubating the nanocrescent array with 1 μM Aβ solution and measuring the SERS signal (JY-Horiba Labram spectrometer). For specific Aβ binding, a cysteamine/sialic acid conjugated solution made via EDC chemistry is
incubated in the device for 2 hrs, and the thiol groups of the cysteamine bind to the gold surface to form a self-assembled monolayer of sialic acid.

RESULTS AND DISCUSSION

Figure 2d shows an SY5Y cell (a neuronal cell line often used in Aβ secretion research) cultured 24 hours in the single cell culture chamber. The inner volume of each culture chamber is 1 nL, providing a sufficient amount of media to keep a cell alive for at least 24 hours. Fig. 2e shows multiple cells cultured in the isolated chamber. We visually demonstrated the effectiveness of the sealing process by saturating the device first with red food dye, followed by applying vertical pressure, then subsequently loading blue dye and confirming that no mixing occurred (Fig. 2f).

SEM images and optical characterization of the nanoplasmonic array are shown in Fig. 3. The localized surface plasmon resonance peak is tunable by adjusting the shape of the nanocrescent probe to optimize the SERS enhancement. Simulation of the EM field intensity of the 2D nanocrescent structure showed strong field enhancement in the sub-10nm gap between the tips (Fig. 3b). The scattering spectra and the SERS signals of 1 μM Aβ for the substrates etched at different ion milling angles are plotted in Fig. 3c and 3d.

CONCLUSIONS

We have designed an optofluidic device that can isolate single neuronal cells in nanoliter cell culture chambers and detecting secreted molecules. So far, we have characterized the SERS detection of Aβ molecules using nanocrescent arrays separately. By integrating nanocrescent arrays into microfluidic cell culture chambers, we anticipate that we will be able to quantify Aβ molecules secreted from individual or small populations of neuronal cells.

REFERENCES