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Microcontact printing of Alzheimer's β-amyloid monomers and fibrils

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ABSTRACT

Adsorption of the 40-residue Alzheimer's β-amyloid peptide (Aβ40) on a hydrophobic surface leads to formation of potentially disease-relevant aggregates. Existing techniques are limited in characterizing the adsorbed Aβ40 and producing potentially useful Aβ40 microstructures such as microarrays and microparticles. In this paper, a novel approach based on microcontact printing (μCP) to studying and utilizing adsorption of Aβ40 monomers and fibril fragments on hydrophobic surface of polydimethylsiloxane (PDMS) stamps has been developed. By transferring the adsorbed layer from the stamp to a glass substrate, this approach allows easy measurement of thickness of the adsorbed layer. It also enables characterization of the face of the adsorbed layer in contact with the stamp surface. This face exhibits significant higher thioflavin T fluorescence than the face exposed to water, suggesting β-sheet formation induced by the PDMS surface. The intrinsic stability of the adsorbed layer is evaluated by printing the layer on a water-soluble substrate and exposing it to water vapor or water. Stable particulate microstructures in water are obtained by chemically crosslinking the adsorbed peptides. Moreover, co-micropatterning of the different states of Aβ40 (monomers and fibril fragments) is demonstrated. This μCP-based approach is simple, versatile, and holds potential for various applications.

1. Introduction

Complex aggregation pathways of the Alzheimer's β-amyloid peptide (Aβ) play the central role in Alzheimer's disease and produce a variety of aggregated states [1–4]. In this work, we show that it is possible to deposit well-defined micropatterns of structurally distinct species formed by the 40-residue Alzheimer's β-amyloid peptide (Aβ40, amino acid sequence: DAEFRHDSGYEVHHQKLVFFA EDVGSNKGAIIGLMVGGVV) on a solid surface using a simple low-cost approach based on microcontact printing (μCP) with a polydimethylsiloxane (PDMS) stamp. The ability to print Aβ40 facilitates easy characterization of not only thickness and morphology of Aβ40 layer adsorbed on the PDMS stamp, but also the face of the layer in direct contact with the stamp surface. The layer thicknesses near molecular dimensions (~2.5 nm) make it possible to interpret distinct properties of PDMS-exposed and water-exposed faces of the layer in terms of molecular structure. Additionally, we demonstrate co-micropatterning Aβ40 monomers and fibril fragments, which could lay the basis for future investigations of interactions between live cells and different molecular structural states of Aβ40. Finally, our ability to print Aβ40 layers onto a water-soluble polyvinyl alcohol (PVA) film enables the analysis of Aβ40 layers after elimination of substrate support.

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There has been considerable effort to isolate different aggregated states of Aβ and understand the roles they play in Alzheimer's disease [4–7]. Aβ is a fragment of the amyloid precursor protein. *In vivo*, different Aβ isoforms exist with variable lengths between 39 and 42 residues [8]. The 40- and 42-residue variants (Aβ40 and Aβ42, respectively) represent the major disease-relevant isoforms [9,10]. Aβ amyloid fibrils (self-assembled nanofibers), deposited as the most abundant component of senile plaques in the brain, are considered to be the pathological hallmark of Alzheimer's disease (AD) [11,12]. Molecular structural measurements on Aβ fibrils have revealed that Aβ self-assembly can produce distinct structures which are highly sensitive to environmental conditions [13–15]. Recent work has further suggested that non-fibrillar Aβ aggregates, including low molecular weight oligomers, may play a special pathological role in Alzheimer's disease [7,16]. The observed complexity of Aβ aggregation pathways has made it difficult to isolate structurally homogeneous Aβ aggregate samples, understand fundamental Aβ aggregation pathways, or characterize biological effects of different Aβ aggregates.

Exposure of Aβ to hydrophobic surfaces has been identified as an effective means of promoting Aβ aggregation and driving formation of specific structures. It has been found that Aβ peptides can aggregate on Teflon, graphite, and lipid surfaces or air–water interfaces to form layers with high β-strand content [17–21]. Many methods for studying Aβ adsorption rely on spectroscopic or scattering-based techniques to characterize adsorbed peptide residing on the substrate surfaces [18,22–27]. Such methods do not readily allow distinct characterization of substrate-exposed and water-exposed faces of the adsorbed layer, and easy characterization of surface morphology and layer thickness simultaneously. Moreover, the intrinsic stability of an Aβ40 layer deposited on a hydrophobic surface cannot be analyzed without separating the layer from the substrate.

Central to the μCP technique is the transfer of an ink material from a stamp bearing micrometer-sized relief figures to a substrate through conformal contact [28]. Moreover, the ability to print onto a water-soluble sacrificial film makes it possible to use μCP to produce particulate microstructures with potential applications for drug delivery and cell tracking [29–31]. The most commonly used stamp material is PDMS. Its hydrophobic nature allows adsorption of a thin layer of proteins and peptides on the stamp surface due to the hydrophobic interaction, which also permits easy transfer of the ink from the stamp to the substrate [32–35]. While the printed proteins or peptides were mostly used to direct patterning and growing of cells, three Aβ-derived peptides containing nine amino acids were printed on mica to study re-organization of the peptides confined on a surface [36,37]. To the best of our knowledge, μCP of Aβ40 has been not reported.

2. Experimental

2.1. Materials

Dimethyl sulfoxide (DMSO), thioflavin T (ThT), and glutaraldehyde were purchased from Sigma–Aldrich. Glass slides and coverslips, and rhodamine isothiocyanate (RITC) were purchased from VWR. The Sylgard® 184 polydimethylsiloxane (PDMS) kit was purchased from Dow-Corning. Polyvinyl alcohol (PVA) (Mw: 3000 Da, 88% hydrolyzation) was purchased from Scientific Polymer Products Inc. (Ontario, NY, USA).

2.2. Fabrication of PDMS stamps

PDMS stamps were fabricated using the method as described [28]. Briefly, the resin and curing agent were mixed at 10:1 weight ratio before poured over a master prepared by photo-lithography. After being cured for 24 h in a 37 °C oven, the stamp was peeled off from the master and cut into 1 cm × 1 cm blocks. Stamps with two surface features were used in this work. One carried 7 μm-diameter circular pillars with 20 μm center-to-center distance and 3.4 μm depth. The other carried 7 μm-wide stripes with 15 μm edge-to-edge distance and 6.5 μm depth.

2.3. Aβ40 monomer purification and fibril fragment preparation

Aβ40 was synthesized according to standard FMOC solid phase synthesis by the Peptide Synthesis Facility at the Florida State University and purified by standard HPLC procedure. The purity of these Aβ40 preparations was determined by matrix-assisted laser desorption/ionization–mass spectrometry (MALDI–MS) to be >90%. Lyophilized Aβ40 peptides, stored desiccated at −80 °C until use. Aβ40 monomers were purified using the method as described before [38]. Briefly, 1 mg Aβ40 peptide was dissolved in 500 μL DMSO at the concentration of 2 mg/mL before 1 min sonication followed by a 10 min centrifugation at 16000 rpm. Then the solution was injected into a Superdex 75 HR 10/300 column (Amersham Pharmacia, Piscataway, NJ) equilibrated with MilliQ water, at a flow rate of 0.5 mL/min. Collected fractions were quantified by UV absorbance at 280 nm ($\varepsilon = 1490\ M^{-1}\ cm^{-1}$) [39]. The collected fractions (fraction 27–28, Fig. S1 in Supplementary material) were stored at 4 °C until use. Normally, the size-exclusion chromatography (SEC) purified monomer was stable under 4 °C up to a week as the monomer retained its characteristic single peak elution profile in SEC [5,40]. All the samples were made within a week after the monomer purification. To obtain a fibril sample, Aβ40 monomer (0.5 mg/mL) was incubated in MilliQ water at 37 °C for 3–5 days under quiescent conditions. To speed up fibril formation, the sample was seeded with 10% (v/v) preformed Aβ40 fibrils [41]. The concentration of final fibril fragment solution is 0.5 mg/mL. Aβ40 fibril fragments were prepared by sonicating the fibril solution for 15 min in a bath ultrasound cleaner.

2.4. Preparation of PVA-coated glass slides

The glass slides were coated with a thin PVA by spin-coating a 1 mL 10 wt% aqueous PVA solution at 1000 rpm for 15 s.