Extended Abstracts of the 2015 International Conference on Solid State Devices and Materials, Sapporo, 2015, pp828-829

Detection of Amyloid-Beta Proteins during Fibrillization Process by Liposome-Immobilized Microcantilevers in Microfluidic Channel

Masayuki Sohgawa[1], Ziyang Zhang[2], Yuta Abe[1], Takashi Abe[1], Kaoru Yamashita[2], and Minoru Noda[2]

[1] Niigata Univ.
8050 Ikarashi 2-no-cho, Nishi-ku, Niigata, 950-2181, Japan
Phone: +81-25-262-7819 E-mail: sohgawa@eng.niigata-u.ac.jp
[2] Kyoto Inst. Tech.
1, Hashigami-cho, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

Abstract

We have fabricated a microfluidic channel for a long-term stable supply of target solution for a continuous measurement and a characterization of the fibrillization process in order to characterize amyloid beta (Aβ) as a causative protein of Altzheimer diseases at different stages of fibrillization on a model cell membrane as a label-free detection. The microfluidic channel was integrated with a liposome-immobilized microcantilever sensor for detection of liposome-Aβ interaction by measuring the resistance change of NiCr strain gauge embedded in the cantilever. Finally, it is found that the characteristic of chronological resistance change depends clearly on the states of Aβ during fibrillization. It is available by the developed device to distinguish the states of Aβ (monomer, aggregation-intermediate, or fibril) and evaluate the aggregation/fibrillization process of Aβ proteins.

1. Introduction

In recent super-aged society, prevention and treatment of dementia including Alzheimer diseases (AD) becomes important to increase the human health span. It is considered that accumulation of Amyloid beta (Aβ) proteins on brain cells have a critical role in initial stage of the AD. Liposomes are synthesized from phospholipid bilayer similar to cell membrane, so they have an ability to interact with biological proteins [1]. In our previous works, a microcantilever with NiCr strain gauge film was fabricated by surface micromachining and liposomes were immobilized intact on its surface to detect interaction with biological proteins including Aβ as resistance change by deflection [2,3]. For sustained measurement of interaction between liposomes and Aβ, a polydimethylsiloxane(PDMS)-based sealed structure has been used to prevent evaporation of the carrier fluid, however, continuous supply of the sample was difficult. In this work, we have prepared a microfluidic channel and measured the resistance change of the microcantilever caused by interaction between liposomes and Aβ with more long-term stability, thereafter investigated the states of Aβ on a model cell membrane of liposome during its fibrillization.

2. Fabrication of a Microcantilever with Microchannel

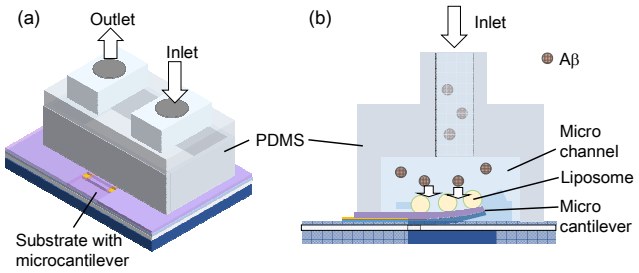

Fig. 1 Schematic illustrations of Aβ detection by using a liposome-immobilized microcantilever with microchannel, (a) bird's eye view and (b) cross-sectional view.

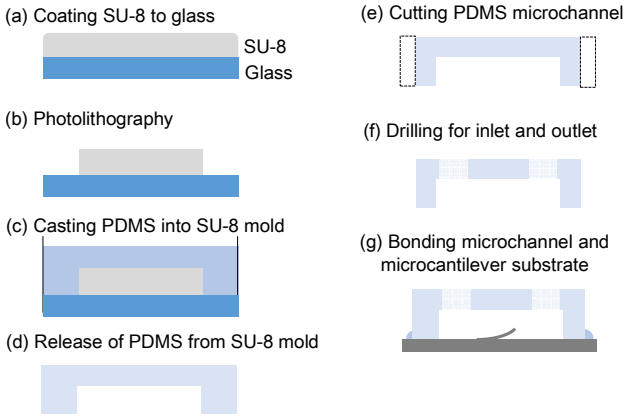

Fig. 2 Fabrication process of PDMS microchannel.

Figure 1 shows schematic illustrations of proposed structure in this work. The microcantilever was fabricated by surface micromachining of silicon-on-insulator (SOI) substrate. To detect microcantilever deflection as resistance change, strain-sensitive NiCr thin film with low temperature coefficient of resistance was deposited on the cantilever. The microchannel was fabricated by casting of PDMS in a mold formed by negative photoresist (SU-8) on a glass substrate. After fabrication of the microcantilever and microchannel, they were bonded by self-adhesion the PDMS surface induced by plasma ashing. The fabrication process is summarized in Fig. 2.

Figure 3 shows a photograph of the prepared sample of the microcantilever in PDMS microchannel. The microchannel have little leak and water in the channel was retained for more than several days. Sample solution was

flown into microchannel from reservoir by using a syringe pump. The maximum flow rate in the microchannel is about 5 μL/min.

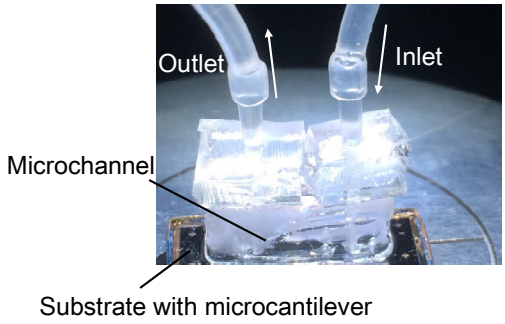

Fig. 3 A photograph of microchannel bonded on the substrate with the microcantilever.

3. Detection of Aβ protein and Discussion

Non-aggregated Aβ(1-40) powder was dissolved in ammonia water (0.1 %) to prepare 10-μM Aβ(1-40) solution. Then it was introduced into microfluidic channel, and the fibrillization process of Aβ on the lipid membrane was investigated through monitoring the resistance change for 24 h.

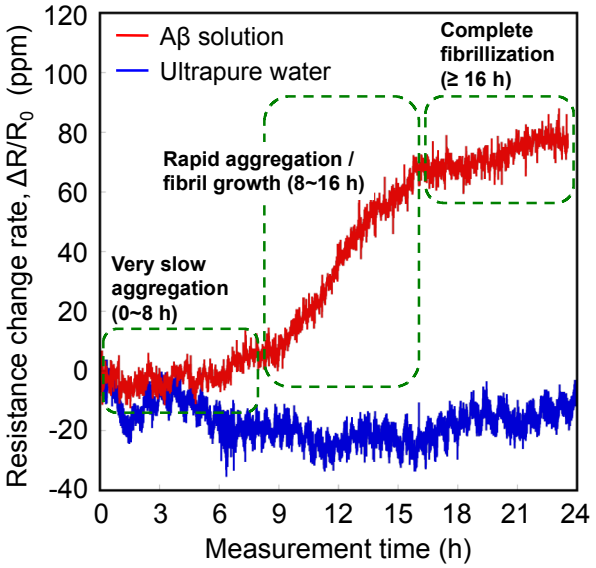

Fig. 4 Resistance change rate of the cantilever sensor in Aβ(1-40) solution (10 μM) and ultrapure water as a function of measurement time.

Fig. 4 exhibits the resistance change caused by liposome-Aβ interaction during the fibrillization process of Aβ on the lipid membrane. It is clearly observed from Fig. 4 that the resistance keeps stable for 24 h in water. It is indicated that a long-time stable measurement is achieved owing to the microfluidic channel. In Aβ(1-40) solution, the resistance remains almost stable for only 8 h from the start. After 8-hour measurement, the resistance significantly increases with time. However, after measuring for 16 h, the increase in resistance over time becomes very slight. It is clear that the characteristic of chronological resistance change is closely related to the Aβ fibrillization process. As we know, Aβ monomers do not interact with lipid membrane and aggregation of Aβ enhances its interaction ability [4]. It is also reported that Aβ aggregation-intermediates are more toxic to cell membrane than fibrils themselves [5]. Combined with the above results, Table I summarizes the characteristics of Aβ(1-40) during its fibrillization on lipid membrane.

Table I Interaction strength, main state, and fibrillization process of Aβ(1-40).

Aβ(1-40)			
Measurement time	**Interaction strength**	**Main state**	**Fibrilization process**
0-8 h	Low	Monomer	Very slow aggregation
8-16 h	High	Aggregation-intermediate	Rapid aggregation/ fibril growth
≥16 h	Low	Full-grown fibril	Complete fibrillization

4. Conclusion

A long-term stable supply of target solution is realized by using the newly fabricated microfluidic channel, leading to characterization of Aβ fibrillization process by the liposome-immobilized microcantilever sensor. By the developed integrated device, it is found that the characteristic of chronological resistance change depends closely on the states of Aβ during fibrillization. We believe that the developed biochip is effective for a long-term measurement to investigate the states of Aβ and its aggregation/fibrillization process.

Acknowledgements

This research was partly supported by a Grant-in-Aid for Scientific Research (KAKENHI Grant No. 25249048) from the Japan Society for the Promotion of Science (JSPS).

References

[1] N. Yoshimoto, M. Yoshimoto, K Yasuhara, T. Shimanouchi, H. Umakoshi, and R. Kuboi, *Biochem. Eng. J.* **29** (2006) 174.
[2] M. Sohgawa, T. Fujimoto, K. Takada, K. Yamashita, and M. Noda, *IEEE Sensors 2013* (2013) 846.
[3] Z. Zhang, T. Akai, M. Sohgawa, K. Takada, K. Yamashita, and M. Noda, *Extended Abstracts of the 2014 International Conference on Solid State Devices and Materials* (2014) 304.
[4] J. A. Kotarek, and M. A. Moss, *Anal. Biochem.* **339** (2010) 30.
[5] H. A. Lashuel, D. Hartley, B. M. Petre, T. Walz, and P. T. Lansbury Jr, *Nature* **418** (2002) 291.