Proceedings

Detection of Aβ(1-40) Protein in Human Serum as A Causative Agent of Alzheimer's Disease by Strain Gauge Cantilever Biosensor Immobilizing Liposome Incorporating Cholesterol †

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† Presented at the Eurosensors 2017 Conference, Paris, France, 3-6 September 2017.

Abstract: We have successfully measured amyloid beta (Aβ) (1-40) protein added in human serum by a NiCr strain gauge cantilever biosensor immobilized with liposomes incorporating cholesterol. Importantly, we investigated the effect of incorporation of cholesterol in the liposome in order to suppress the interaction between the liposome and many different proteins included in human serum. It was revealed that incorporating cholesterol suppresses the interaction between the proteins other than Aβ in human serum and the liposome. Finally, we detected Aβ(1-40) in human serum with typical chronological behaviors due to Aβ aggregation and fibrillization. Furthermore, as a digital low-pass filtering procedure could reduce external noises, the cantilever sensor immobilized with liposome incorporating cholesterol can detect low-concentrated Aβ in human serum.

Keywords: cantilever; NiCr strain gauge; biosensor; liposome; amyloid beta; aggregation; fibrillization; interaction; human serum; digital low-pass filtering procedure

1. Introduction

Alzheimer's disease (AD) is one of dementia and the mechanism is not clear yet. However amyloid beta (Aβ) is the predominant agent related to AD. Also it is recognized that AD is caused by interaction between Aβ and cerebral nerve cells and accumulation of Aβ on them [1,2]. We have reported that our NiCr strain gauge cantilever microsensor immobilized with liposome as sensing biomolecule could detect 50 nM Aβ(1-40) [3], which is less than the reported value in AD patients [4]. Though our sensor can detect 50 nM Aβ(1-40), it's not enough because we are aiming to apply the sensor to diagnose mild cognitive impairment (MCI) patients. Thus it is essential to improve the sensitivity because Aβ concentration might be less than 1 nM in their plasma. Moreover we have to detect the Aβ in human serum of MCI patients. There are a lot of proteins in human serum except Aβ. Therefore it is important for the liposomes to have the high selectivity against Aβ. In this work, we tried to measure the Aβ in the human serum including many different proteins that would exhibit interactions with the phospholipids of the liposome.

2. Experimental Methods

Figure 1 shows the cantilever microsensor with the human serum added with Aβ(1-40) in a droplet-sealing structure. This cantilever biosensor can sense Aβ(1-40) by interaction between liposomes and Aβ(1-40). Especially we think the sensor can detect oligomer and protofibril as Aβ aggregation-intermediates that are more poisonous than Aβ fibril [3]. This time, we employed 1,2-

Ditetradecanoyl-sn-glycero-3-phosphocholine (DMPC) and that incorporated with cholesterol. DMPC liposomes were immobilized on the cantilever by Self-Assembled Monolayer (SAM). Non-aggregated Aβ was dissolved in 0.1% ammonia water and added with human serum. Non-aggregated Aβ solution was introduced to polydimethylsiloxane (PDMS) droplet-sealing structure. It is possible to measure Aβ(1-40) in target solution for more than 24 hrs. We investigated the dynamic behavior between DMPC liposomes and proteins other than Aβ in human serum. In addition, in order to measure less than 1 nM Aβ, we needed to eliminate noise due to temperature fluctuation and the other external one. Therefore we attempted to apply a digital low-pass filtering procedure to improve the sensitivity of this biosensor.
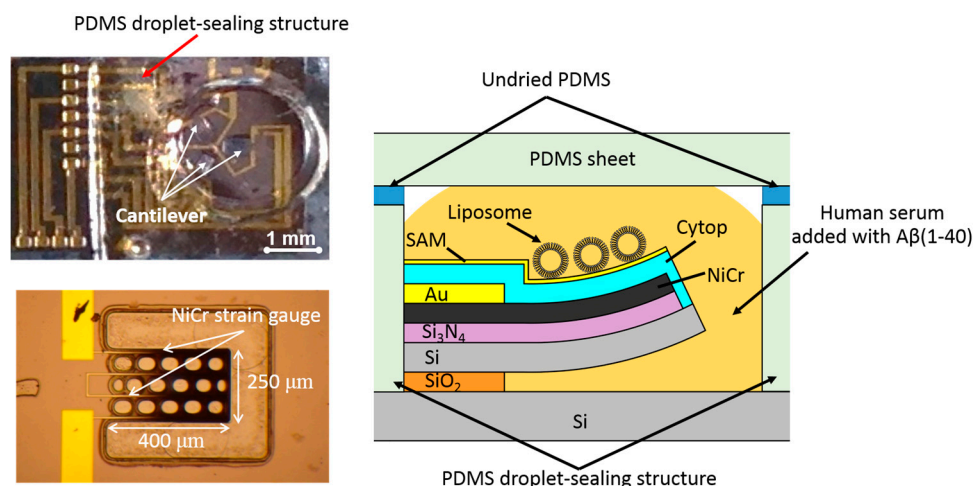
Figure 1. (**a**) Top surface; (**b**) a part of cantilever (**c**) a cross sectional illustration of the cantilever biosensor with PDMS droplet sealing structure in human serum added with Aβ(1-40).

3. Results and Discussion

Firstly, we applied a digital low-pass filtering procedure to eliminate noises. Figure 2 shows resistance change rate using 1,2-Dipalmitoyl-sn-glycero-3-phosphorycholine (DPPC) incorporated with cholesterol after adding 50 nM Aβ(1-40) solution. As shown in Figure 2, the external noise and due to the temperature fluctuation are successfully eliminated by a digital low-pass filtering procedure.
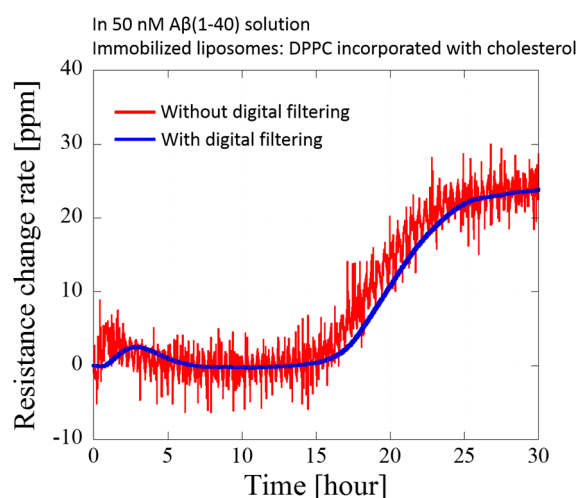

Figure 2. Resistance change rate of microcantilever sensor immobilized with DPPC liposomes incorporated with cholesterol in 50 nM Aβ(1-40) solution with and without digital filtering procedure.

Secondly, we investigated interaction between proteins in human serum and liposomes of DMPC and DMPC incorporating cholesterol. As shown in Figure 3, resistance change rate of the cantilever immobilized with DMPC liposomes increased throughout measuring time. On the other hand, resistance of that immobilized with DMPC incorporated with cholesterol was averagely stable. We think that cholesterol can reduce the interaction between the proteins in human serum and DMPC liposomes due to its stabilizing effect on the membrane [5]. This suggests that we can suppress the interaction between the proteins in human serum and liposomes by the incorporation of cholesterol. In other words, the cantilever sensor would be able to detect the dynamic state of Aβ in human serum.
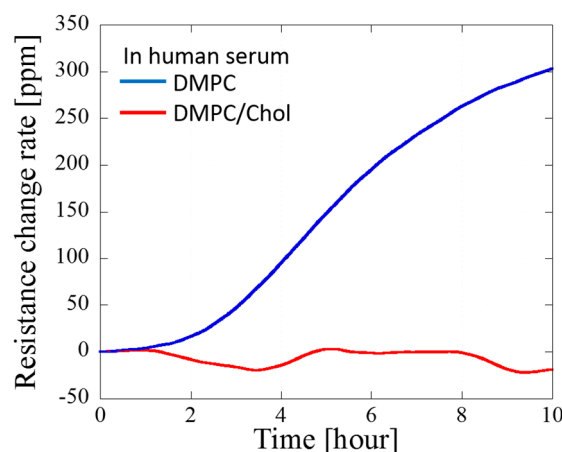
Figure 3. Resistance change rate of the cantilever immobilized with DMPC liposomes and DMPC/Cholesterol liposomes in human serum.

Finally, we tried to detect 1 μM Aβ(1-40) dissolved in human serum. The result is shown in Figure 4. From 0 to 11 h, the resistance of the cantilever increased because monomeric Aβ(1-40) interacted DMPC [6] and DMPC did not interact proteins in human serum. After that, until 15 h, the resistance rapidly increased. This indicates that cholesterol enhanced the interaction between DMPC liposomes and Aβ(1-40) [7]. Furthermore, after 15 h, the increasing ratio of the resistance change rate of the cantilever becomes decreased. Although membrane fluidity is increasing by interacting proteins, the decrease suggests that a series of aggregation and fibrillization become matured and saturated. Therefore, we consider that the micro cantilever biosensor can detect the typical chronological behaviors of Aβ(1-40). Consequently, we expect that the cantilever biosensor can detect low Aβ concentration in human serum by utilizing incorporation of cholesterol and the digital filtering procedure.
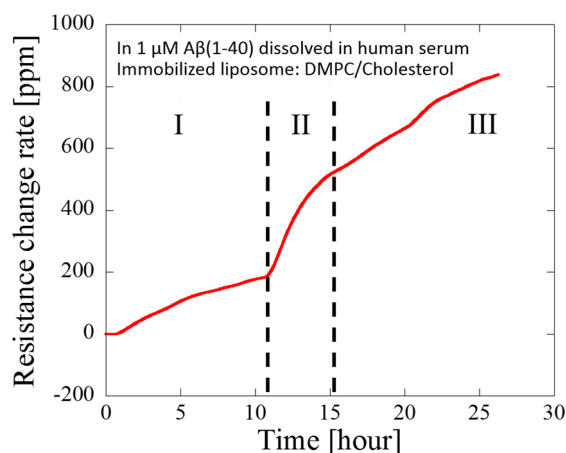
Figure 4. The change in resistance of the cantilever biosensor immobilized with DMPC/Cholesterol in 1 μM Aβ(1-40) dissolved in human serum: (I) monomeric Aβ(1-40), (II) aggregation of Aβ(1-40) and (III) mature Aβ(1-40) fibril.

4. Conclusions

We attempted to detect Aβ(1-40) protein added in human serum using a NiCr strain gauge cantilever biosensor immobilized with liposomes incorporating cholesterol so as to apply the sensor for a diagnosis of AD and especially MCI patients. It is possible to eliminate external noises by a digital filtering procedure. It shows that incorporation of cholesterol can suppress the interaction between DMPC liposomes and proteins in human serum. In addition, we detected the dynamic behavior of Aβ(1-40) through aggregation and fibrillization in human serum by the sensor immobilized DMPC liposomes incorporated with cholesterol. We conclude that the cantilever biosensor immobilized with liposomes incorporating cholesterol can detect low-concentrated Aβ in human serum.

Conflicts of Interest: The authors declare no conflict of interest.

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