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Easy Detection of Amyloid β-Protein Using Photo-Sensitive Field Effect

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Abstract

This article describes a novel method for the detection of amyloid-β (Aβ) peptide that utilizes a photo-sensitive field-effect transistor (p-FET). According to a recent study, Aβ protein has been known to play a central role in the pathogenesis of Alzheimer's disease (AD). Accordingly, we investigated the variation of photo current generated from p-FET with and without intracellular magnetic beads conjugated with Aβ peptides, which are placed on the p-FET sensing areas. The decrease of photo current was observed due to the presence of the magnetic beads on the channel region. Moreover, a similar characteristic was shown when the Raw 264 cells take in magnetic beads treated with Aβ peptide. This means that it is possible to simply detect a certain protein using magnetic beads and a p-FET device. Therefore, in this paper, we suggest that our method could detect tiny amounts of Aβ for early diagnosis of AD using the p-FET devices.

Keywords : p-FET, Amyloid β-protein, Alzheimer's disease, Early diagnosis

1. INTRODUCTION

Since Alzheimer's disease (AD) was discovered 100 years ago by Alois Alzheimer [1], many researchers have strongly suggested that AD is characterized by distinct pathological abnormalities that include amyloid-β (Aβ) protein or tau-protein [2]. In the last decade, extensive research has been devoted to understanding neuronal death, protein dynamics in brain tissue and the biochemical mechanisms that link aggregation to toxicity [3]. To date, AD is usually diagnosed from patient medical history, collateral medical history from family and relatives, clinical observations including advanced medical imaging with via computed tomography (CT) or magnetic resonance imaging (MRI). In addition, single photon emission computed tomography (SPECT) or positron emission tomography (PET) can be used to help exclude other cerebral pathologies or subtypes of dementia [4-6]. However, these methods have limitations in terms of precision and early diagnosis, because they can only diagnose the disease through computed images, and they cannot diagnose AD in the early stages. In recent studies related to AD, AD-related that loss of olfactory function in AD has been investigated [7,8]. It was shown that early olfactory perceptual loss likely involves a nonfibrillar, versus fibrillar, Aβ related mechanism in the olfactory system, and nonfibrillar Aβ deposition is observed within the olfactory bulb.

Despite advances made in our understanding of AD through recent studies on neurotoxicity and olfactory sensory loss, research on Aβ peptide (mainly Aβ oligomer peptide) has proved challenging with engineering techniques such as traditional semiconductor devices, micro devices and so on.

To overcome this drawback, many researchers have attempted to use semiconductor devices and its materials such as ion-sensitive field effect transistors (ISFETs), nanowire FETs, carbon nanotube (CNT) devices and so on. Such biosensor devices have large sensitivity in regards to the variation of surface potential, and thus a slight change of surface potential by a bio-marker or via stimulation can be readily detected.

In addition, they offer other advantages such as high speed diagnosis, simple fabrication processes and the possibility of mass production. Nevertheless, thus far, we have never found a previous study on the early diagnosis for AD using p-FET. As previously mentioned, for simple diagnosis of AD, the detection of Aβ protein in neuron

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cells is a well-known method. In order to detect Aβ protein in cells, most approaches entail observations of the manifestation of cells bound with a fluorescent material in primary brain cells [9, 10]. In our present study, we fabricated photo-sensitive field effect transistor (p-FET) devices [11,12], and we suggest a new method of detecting a tiny amount of Aβ protein for early diagnosis of AD by using our devices.

2. BASIC PRINCIPLE

The basic principle of this approach is as follows: (1) Aβ protein uptake by beads that is placed on a photo sensitive area of p-FET. These beads are used to label the Aβ protein. (2) The cells, which conjugate the beads linked with Aβ, are also laid down on the photo sensitive channel region by a permanent magnet probe (home-made). It is then possible to observe the difference of the photo-current according to the presence of the magnetic beads with Aβ. (3) Early diagnosis of AD is possible in vivo if magnetic beads are possible to quantify in vivo with the conjugated cells. It is possible to theoretically interpret the amount of Aβ protein in our samples by calculating the variation of the photo-current. The responsivity is given as follows [13,14]

$$I_{ph} / P_{inc} = (I_{di} - I_{dd}) / EA \quad (1)$$

where I_{ph} is the generated drain current by light illumination, P_{inc} is the optical power incident on the channel of the device, Idi is the drain current under illumination, I_{dd} is the drain current in the dark, E is the irradiation of the incident light (power/area), A denotes the effective device area and R is I_{ph}/P_{inc}. In equation (1), the second term only expresses the photo current generated by illumination. When the transmittance of the beads is zero and magnetic beads are ideally distributed in the photo sensitive channel region, I_{di} can be expressed by

$$I_{di} = I_{dd} + RE \cdot a_{bead} (n_s - n) \quad (2)$$

where a_{bead} is the area of a bead, ns is the number of magnetic beads under ideal saturation, and n denotes the number of beads in the area of the channel. The photo-current decreased in response to the presence of magnetic beads in the channel region. Therefore, the amount of magnetic beads can be calculated through the generated photo-current under illumination. In other words, it is possible to extract the quantity of Aβ proteins. In the same manner, using the cells containing magnetic beads, the photo-current can be expressed by

$$I_{di} = I_{dd} + RE \cdot a_{cell} (n_{s,cell} - n_{cell}) \quad (3)$$

where a_{cell} is the area of cells, $n_{s,cell}$ is the number of cells under ideal saturation, and n_{cell} provides the number of cells with magnetic beads.

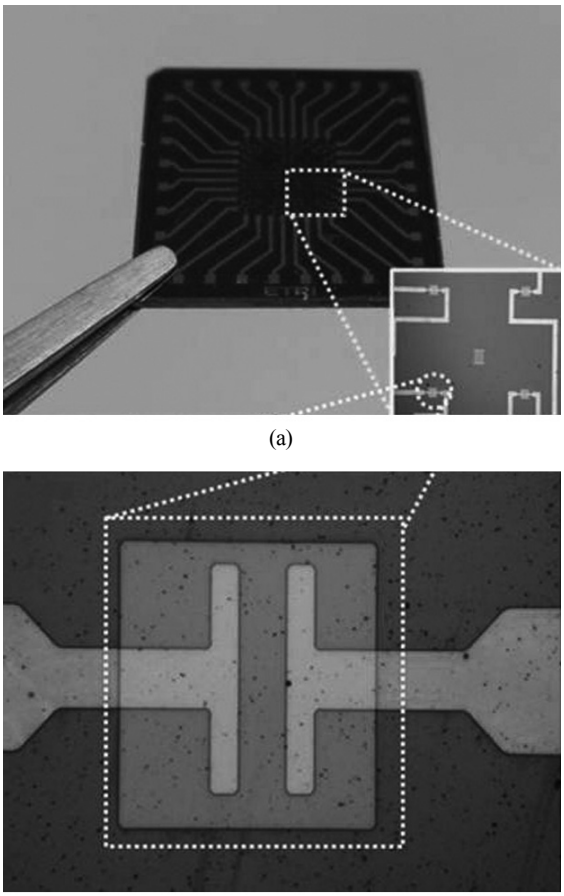
3. FABRICATION AND EXPERIMENT METHOD

For the detection regarding the amount of Aβ, we fabricated a 4 × 4 array of a p-FET device, which has a substrate (rear) gate structure. As shown in Figs. 1 (a) and (b), the starting material is a p-type Si substrate with a 100 nm thick thermal oxidation layer. This thermal oxide layer serves as a gate oxide of the rear gate. An amorphous Si layer with a 100 nm thickness was deposited by a low pressure chemical vapor deposition process (LPCVD) at 580℃. After defining the channel region with a rectangular shape of 100 x 100 μm^2, phosphorus *in-situ* doped poly-Si as source and drain electrodes was deposited by using LPCVD at 650℃, and continuously patterned with a photo-lithography process.

Prior to the bio related experiment, all surfaces were cleaned up and the poly (methyl methacrylate) (PMMA) passivation layer was coated using a spin coating system for electrical isolation. The electrical characteristics were measured using a semiconductor analysis system (HP4145).

Firstly, in order to determine the effect of the magnetic bead on photo-current behavior, we carried out multi-protein conjugating processes using magnetic beads sequentially bound to streptavidin, biotin, polyethylene glycol, and Aβ peptide. After these processes, magnetic beads are selectively located on the p-FET surface using a permanent magnet aligned under the sensing region.

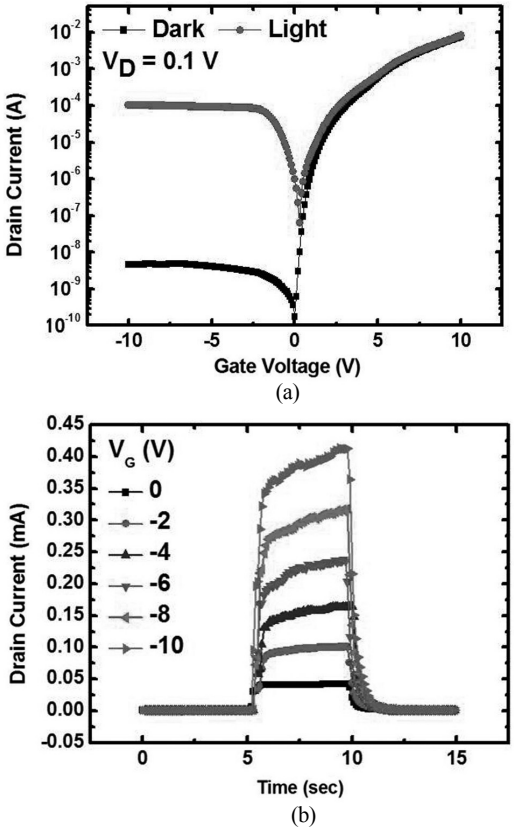
Additionally, we confirmed the possibility of cell sensing using p-FET and Raw264.7 treated with magnetic beads. Before processing the cell culture, PDMS well was attached on the surface of the p-FET device for isolation between source/drain electrode and cell culture media. Afterwards, Raw264.7 cells were incubated for 6 hours to uptake magnetic beads linked to the Aβ peptide.



(a)

(b)

Fig. 1. Optical images of photo-sensitive FET device. (a) The 4× 4 array of photo-sensitive FET device, (b) The photo-sensitive FET device with 100 μm × 100 μm (square). The green and orange part are electrodes (source and drain), the pink square is the photo-sensitive FET part (poly-Si).



(a)

(b)

Fig. 2. (a) Plot of sub-threshold and photo-current characteristics for the photo-sensitive FET devices, (b) Plot of photo-current change with various applied gate voltages.

4. RESULT AND SUMMARY

The sub-threshold and photo-current characteristics of the p-FET devices are shown in Fig. 2 (a). In a dark condition, the calculated threshold voltage and sub-threshold swing at V_D = 0.1 V are 1.6 V and 300 mV/dec, respectively. After the illumination of light with an intensity of 1mW/cm^2 on the channel, there is no noticeable change regarding the threshold voltage and sub-threshold swing. However, the leakage current is significantly increased from approximately1 μA more than 100 μA after illumination. This increase of the leakage current in the off-state is due to the generation of electron-hole pairs around the drain depletion region by illumination.

Fig. 2 (b) presents the photo-current change with various applied gate voltages. The sensing margin can be increased by controlling the applied rear gate voltage bias. Furthermore, the rear gate structure is of great advantage when diagnosing the live cells. In order to deal with live cells, the solutions, such as the cell culture media and PBS buffer, that has to be used for maintaining live cells. However, these solutions cause leakage current generation, thus making it difficult to precisely diagnose diseases. Accordingly, a sensing device with a rear gate electrode is a suitable device structure for handling live cells. In this study, in order to confirm the feasibility of diagnosis using the p-FET device, we used magnetic beads (Fluidmag-ARA, Chemicell, Germany) with sizes of 2.8 μm and 100 nm. Since the magnetic beads and Aβ are not directly connected, a chemical linker molecule is necessary for bonding between these two materials. The reaction between streptavidin coated on the magnetic beads and Aβ protein can be promoted by sequentially treating the linker chain and biotin molecules at 40℃ for 30 min.

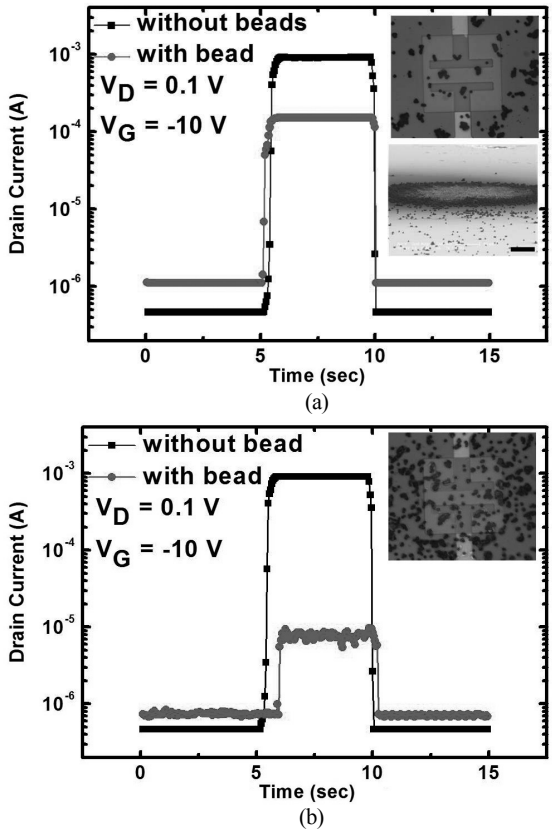


Fig. 3. Photo-current characteristics of p-FET according to the presence of mag-Aβ on the channel region. (a) A small quantity of mag-Aβ located in the channel region. (The inset shows optical image of selectively located magnetic beads), (b) A large number of mag-Aβ in the channel region.

In order to observe the photo-current changes with various amounts of magnetic beads, the magnetic beads were selectively reacted with Aβ protein or cells. In addition, the magnetic beads should be selectively located in the channel region. By aligning a permanent magnet probe with the channel region, the magnetic beads can be pulled down to the channel region. The left inset of Fig. 3 (a) illustrates a SEM image of selectively located magnetic beads in the shape of a circle with a diameter of roughly 200 μm.

We carried out the photo-current dependency on the quantity of Aβ protein uptake by magnet-beads (mag-Aβ) in the channel region. Fig. 3 (a) and (b) show the photo-current change of the p-FET device depending on the amount of modified mag-Aβ in the channel region. The p-FET device with a channel area of 100 $\times$ 100 μm^2 has a large photo-current of about 100 μA at V_D = 0.1 V and V_G = -10 V before the magnetic beads spread into the channel region. However, after situating the mag-Aβ in the channel region, a distinct decrease of the photo-current is observed, which is attributable to the opaque screen generated by the mag-Aβ. When a small amount of mag-Aβ is located in the channel region, the generated photo-current generation decreased slightly, as shown in Fig. 3(a). However, as shown in Fig. 3 (b), the presence of a large number of mag-Aβ in the channel region suppressed the electron-hole pair generation under the same illumination conditions, and resulted in a small photo-current.

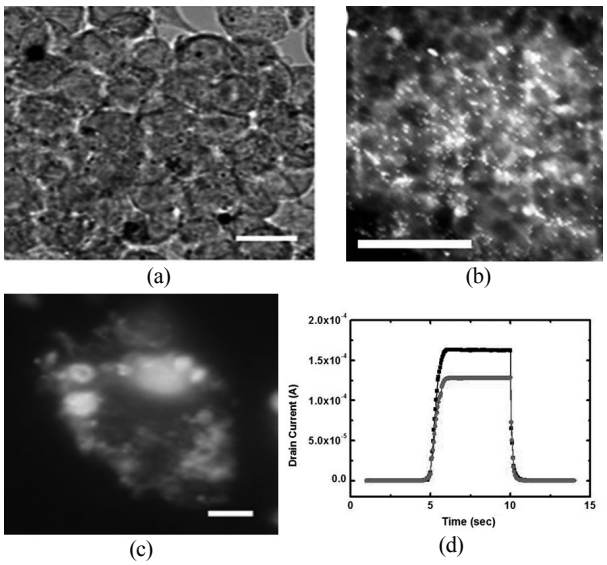


Fig. 4. (a) Image of RAW264.7 cells. Scale bar: 10 μm (cells shown fixed), (b) Image of RAW 264.7 cells on the photo-sensitive FET devices. The green strips are electrodes and the red strips are sensitivity parts. The cells have auto-fluorescence. Scale bar: 50 μm, (c) Image of ORN cells stained immediately after seeding. Scale bar: 1 μm (cells shown fixed). DAPI (blue), FITC (green), (d) Plot of the drain current vs the cells have been covered on the photo-sensitive FET device (red line) and without cells on the photo-sensitive FET device (black line, without opaque screen).

This indicates that Aβ proteins can be detected through the decreased level of photo-current, which is caused by the presence of mag-Aβ in the channel region. According to Eq. (3), the photo-current is proportional to the quantity (magnitude) of mag-Aβ. Therefore, we measured the parameter using an atomic force microscope. Although a scanning image cannot be provided here, it was found that Aβ aggregated on the beads in a quantity of roughly 10% relative to the diameter of the 100 nm magnetic beads. Thus, the quantity of Aβ will be approximately 0.1$\times$N, where N is the number of beads per unit area. In the same manner, it is also possible to bind or uptake magnetic beads

with Aβ protein to live cells. Therefore, we carried out a study involving, uptake of mag-Aβ to live RAW 264.7 cells (a microphage cell line, ATCC), human embryonic kidney (HEK 293, ATCC) cells, and olfactory receptor neurons (ORNs). Figs. 4 (a) and (b) present the RAW 264.7 cells (mag-Aβ was absorbed into RAW264.7 cells, and the cells were fixed) which are closely united together, and are entirely on the photo-sensitive FET devices in the PBS liquid, respectively. It is thus seen that RAW264.7 cells uptake mag-Aβ (which is shown by bright dots in Fig. 4 (b)), but HEK 293 cells only bind mag-Aβ physically (i.e. they show no uptake). We can clearly see that ORN cells uptake Aβ as shown in Fig. 4 (c), whereas ORN cells uptake mag-Aβ with a diameter of 100 nm, but do not uptake the beads with a size great than 100 nm. On average, the size of the mag-Aβ is 100 nm.

Fig. 4 (d) demonstrates a decrease of photo-current after situating RAW 264.7 cells in the channel region. Although an optical image is not provided, the RAW 264.7 cells also uptake mag-Aβ and occupy roughly 20% of the photo sensitive region in the p-FET. After all, this indicates that Aβ proteins with beads could be indirectly detected through the decreased level of photo-current. In case of Fig. 4 (b), we applied the following ratios to calculate the quantity of Aβ based on experimental results. i) ratioa(Ra); RAW cells occupied about 20% of the photo sensitive region, ii) ratiob(Rb); mag-Aβ occupied about 10% per RAW cell, iii) ratioc(Rc); Aβ aggregated on the beads in a quantity of roughly 10% relative to the diameter of the 100 nm magnetic beads. As a result, the magnitude of the Aβ was approximately (Ra) × (Rb) × (Rc) × 104 μm^2 × (molecular weight/unit area). Therefore, authors believed that the drain current reduction of about 50 μA was caused by the quantity (magnitude) of mag-Aβ in regards to the RAW 264.7 cells.

In summary, we developed a novel method to quantify Aβ protein by the use of p-FET. In the p-FET, we observed that the photo-current varies with the presence of the mag-Aβ, RAW 264.7 cells and ORN cells. The p-FET shows that the drain current reduction of about 50 μA is caused by the RAW 264.7 cells with mag-Aβ. Therefore, we suggest that our method can detect tiny amounts of Aβ for early diagnosis of AD through the use of p-FET devices.

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