A Three-Dimensional Sensor to Recognize Amyloid-β in Blood Plasma of Patients

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ABSTRACT: Detecting amyloid beta (Aβ) in unpurified blood to diagnose Alzheimer’s disease (AD) is challenging owing to low concentrations of Aβ and the presence of many other substances in the blood. Here, we propose a 3D sensor for AD diagnosis using blood plasma, with pairs of 3D silicon micropillar electrodes with a comprehensive circuit configuration. The sensor is developed with synthesized artificial peptide and impedance analysis based on a maximum signal-to-noise ratio. Its sensitivity and selectivity were verified using an in vitro test based on samples of human blood serum, which showed its feasibility for application in diagnosis of AD by testing blood plasma of the AD patient. The 3D sensor is designed to improve reliability by checking the impedance of each pair multiple times via constructing a reference pair and a working pair on the same sensor. Therefore, we demonstrate the ability of the 3D sensor to recognize cases of AD using blood plasma and introduce its potential as a self-health care sensor for AD patients.

1. INTRODUCTION

Alzheimer’s disease (AD) is a degenerative brain disease that is becoming an increasingly serious social issue due to the growing aging population. Many studies have investigated the exact cause of AD pathogenesis but have not yet identified it. Among the various causes that have been investigated, amyloid beta-peptide (Aβ) is widely known as a factor that accumulates in the brain and induces AD.1,2 Thus, Aβ positron emission tomography (PET) is used to diagnose Aβ pathology in AD patients along with cognitive intelligence tests, among others.3–5

Pathogenic Aβ-peptides result from a proteolytic process of amyloid precursor protein (APP) on the cell membrane. APP is a transmembrane protein that is frequently observed at the synapse sites of neuron cells; its function is not well defined but may involve regulation of synapse formation. β-secretase and γ-secretase are enzymes that cleave APP to Aβ-peptide by proteolysis. Once the APP N terminus is cleaved by β-secretase, a membrane-bound C99 fragment is formed and sequentially, the γ-secretase cleaves the C terminus of the C99 fragment. This proteolytic cascade generates pathogenic Aβ40 or Aβ42.6

The pathogenic characteristic of Aβ40 and Aβ42 essentially comes from their β-sheet-forming property. An intramolecular β-sheet formed from Aβ-peptides can be aggregated to a metastable oligomer through the interaction between the hydrophobic cores of adjacent monomers. Especially, Leu17, Phe19, Ala21, Leu34, Val36, and Val40 have a critical role in fibril propagation of Aβ-peptides because these compartments participate in hydrophobic clustering. There are several types of aggregate forms of Aβ (e.g., oligomers, protofibrils, and matured fibrils), but specific steps leading to AD have not yet been clearly defined.

Recent studies reported that Aβ was also found in biological fluids (blood and cerebrospinal fluid (CSF)), and Aβ-detecting methods using biological fluids have been developed.8–11 However, CSF can only be obtained via an invasive method such as a lumbar puncture, which is highly repulsive and inconveniencing to patients. To prevent repulsion from patients and provide a convenient detection procedure, there has been need for a new method for detecting Aβ in the blood.

Since Aβ concentrations of the blood are correlated with Aβ concentrations of the brain, detection using blood has been attempted as an easier diagnostic method of AD than PET.12 Most practical developed devices have used the human blood serum.13–15 However, the problem is that a high amount of Aβ is removed during the refinement process when collecting serum samples.16 To counteract this, a study using animal plasma to detect Aβ was attempted.17 Further, to apply the sensor to humans, the most critical issue of the Aβ sensor is accuracy and reliability as the blood plasma of real AD patients is more complex than blood serum or the managed animal plasma. Moreover, it is unclear which form of the Aβ aggregate...
prevails in the blood of a patient with AD; thus, we must design a target-binding peptide that can embrace every form of Aβ aggregate with high selectivity and sensitivity. Therefore, a sensor suitable for AD diagnosis from the blood of patients should be designed, and its accuracy and reliability should be verified in a real blood system of AD patients.

Here, we studied the Aβ detection system based on human blood plasma collected from AD patients and healthy people. The developed 3D sensor system comprises a silicon micropillar (SiMP) as a 3D structure to form the spatial circuit (called as a comprehensive circuit) and an artificial peptide to recognize the Aβ selectively. The SiMP makes the sensor sensitive by constructing the comprehensive circuit, including the whole system, with proteins and peptides, around the SiMP. The SiMP 3D spatial circuit is more effective in detecting proteins in blood than the 2D electrode as proteins are diffused randomly. Compared to the 2D electrode, the SiMP electrode has higher reactivity with floating proteins due to the structural advantage and large exposure area. Furthermore, the impedance analysis, based on this 3D structured system, has outstanding sensitivity for detecting spatial impedance changes, similar to using tomographic imaging in the liquid solution. This heightened sensitivity enables the 3D sensor to be highly accurate even in low concentrations of Aβ in blood.

The SiMP is made of silicon 600 μm high and 200 μm wide, and it is designed to work as a pair as each electrode operates as a reference and working SiMP to increase the reliability of the 3D sensor. Regarding the high reliability of the sensor, the working SiMP is coated with a synthesized peptide for Aβ detection, while the reference SiMP is prepared without peptide. It is difficult to detect Aβ when in low concentrations in the blood, either with the 2D or single 3D electrode. Therefore, we designed a multiple detection system using several working SiMPs and reference SiMP pairs. Using this system, the 3D sensor’s reliability increases by increasing the statistical probability through the multiple detection and comparison of each pair. Although each working SiMP can change its impedance individually with the amount of reacted Aβ, reliability of the total 3D sensor increases through
considering that targeting peptides and Aβ-peptides work as an insulator, a bulky binding state of the ligand and substrates should be advantageous as it enhances the electrical signal of the SiMP sensor. Therefore, we have precisely designed an Aβ-targeting section, which does not interfere with the aggregation mechanism of Aβ fibril formation. A mimetic derivative from a hydrophobic core of an Aβ monomer would be beneficial because it can be disguised as a natural Aβ hydrophobic core or can promote aggregation by working as a nucleation core. One flaw that we encountered was its solubility because its origin is a hydrophobic core. Considering that fabrication and the working circumstance would be aqueous or a physiological condition, we should improve the solubility of the targeting peptide. This could be solved by adding a GS linker, which is a glycine–serine repeated sequence. Furthermore, it is advantageous for binding of the substrate by offering flexibility and space for efficient binding.

2. RESULTS AND DISCUSSION

2.1. Fabrication of the 3D Sensor. The 3D sensor was fabricated using an etch process to form the silicon micropillar (SiMP), whereas a complementary metal-oxide semiconductor (CMOS) process was used to construct the circuit (Supporting Information section S1). The etch process was divided into a dry etch process (deep reactive ion etching (DRIE)) and wet etch process. First, to make the SiMP for the comprehensive circuit, DRIE was applied to the Si substrate. The SiMP was designed with a positive-angle shape for durability and was located for operating the SiMPs as pairs.23 The SiMP was set with a distance of 800 μm between the SiMPs that worked as the pair, and the distance between the closest pairs was set at 2550 μm. For a positive SiMP angle, the SiMP was surrounded by protectors during the DRIE process.24 Next, the protectors around the SiMP were removed using the wet etch process. For the CMOS process, the circuit was constructed by exposing the electrode of the SiMP surface only. The electrode is formed by the step of Au deposition in the CMOS process, which is carried out by depositing Ti and Au sequentially through the e-beam evaporation (Figure 2). The circuit that was constructed between two of the SiMPs, each SiMP that was formed independently, made a comprehensive circuit through the solution. The circuit was designed with eight pairs of SiMPs, separating the reference and working SiMP pairs. The working SiMP was coated with the artificial peptide for recognition of Aβ by soaking the SiMP in a peptide solution. After fabricating the 3D sensor, it was bonded with a flexible-printed circuit board (FPCB) and printed circuit board (PCB) to minimize environmental noise. This system maximizes the signal-to-noise ratio through impedance analysis and adjustment of the frequency (1 kHz). The 3D sensor bonded with the FPCB was prepared in such a way that it would be easy to replace the sensor since the peptide loses its selective reaction after the binding process.

2.2. Design and Synthesis of the Aβ Recognition Peptide. As previously mentioned, the target-binding peptide should embrace all forms of Aβ aggregates and show high sensitivity and selectivity. The size of the folded Aβ42 monomer is approximately 3–4 nm, and the oligomer or protofibril will be above 5 nm.25 We presumed that adopting a short sequence of the hydrophobic core from the Aβ fibril should be beneficial because it can be trapped between any Aβ aggregation. Furthermore, this short Aβ derivative serves as a nucleation core for Aβ aggregation, which can lead to an enhanced sensor-detecting signal. In addition, the interposing principle of this segment is the electrostatic interaction and hydrophobic clustering of β-sheet formation so that high sensitivity can be imposed to target Aβ/β. From several hydrophobic core derivatives, a KLVFF sequence shows high binding affinity to Aβ42 (Figure 3a). A glycine–serine (GS) linker was added at the C terminus of the peptide derivative for flexibility and space for binding. An N-[1-(4,4-dimethyl-2,6-dioxacyclohexyldiene)ethyl] (Dde) group protecting lysine was added after the GS linker for carbocystetramethylrhodamine (TAMRA) coupling. Cysteine was placed at the C terminus of the whole peptide for the gold binding characteristic. A thiol functional group at the sidechain of cysteine was conjugated to the Au surface through a redox reaction.

We adopted an orthogonal peptide synthesis method for TAMRA conjugation. TAMRA is a derivative of rhodamine that is used as a fluorescence marker whose excitation...
wavelength is 555 nm and emission wavelength is 580 nm. We believed that coupling TAMRA to the designed peptide would be advantageous to measure binding characteristics. After backbone sequencing of the artificial peptide was synthesized, we deprotected the Dde protecting group at the ε-site of Lys14 prior to deprotection of the Fmoc protecting group at the peptide N terminus. Next, we conjugated 5(6)-TAMRA by amide bonding between the amine of the lysine sidechain and the carboxylic acid of TAMRA.

### 2.3. Chemical Sensor Test Using a Gold Nanoparticle (AuNP).

Before connecting the Aβ-targeting artificial peptide to an electronic sensor device, we confirmed applicability of the peptide to a gold-based sensor using a AuNP. First, to investigate the binding interaction between artificial peptides and the gold surface, we applied a TAMRA coupled with an artificial peptide to a citrate-removed AuNP colloid. A uniformly dispersed AuNP colloid shows a pink-red color, but when AuNP aggregates, the colloid color shifts to purple-blue. Due to the hydrophobic aggregation of the artificial peptide, the color of the AuNP colloid turned blue when more peptides were attached. At the optimal concentration, aggregation between AuNPs occurs drastically, thus the colloid color turns transparent because of precipitation. At this point, it can be roughly said that the peptide is completely attached to the AuNP. After the optimal concentration point, excess peptides assembled around attached peptides by forming a hydrophobic core. Consequently, a hydrophilic composition of peptides was exposed outside the AuNP, where suspension stability of the AuNP increased to the color shown in Figure 3b.

After identifying the binding characteristic and stoichiometry, we verified the binding behavior and numerical binding capacity of artificial peptides on the Au surface through a fluorescence polarization assay (Figure 4a). After processing the data, we found the $R^2$ square value of the sigmoidal curve fitting to be 0.995 (significant figures applied), almost equal to the Hill slope $R^2 = 1$. This result shows that our peptide exhibited cooperative binding behavior. This binding behavior of peptides gave the peptide–AuNP sensor a unique...
characteristic that has been previously mentioned: an enhancement of impedance signal intensity and a stabilized dispersion following optimal concentration. Next, we calculated the number of peptides that can bind to one AuNP by extrapolating the sigmoidal function. After analyzing the results, we proved that a rapid color change of the AuNP colloid can represent optimal binding quantity of the peptide to the AuNP. Therefore, we conducted further $\alpha\beta$-binding experiments based on the calculated result value.

Based on the above experiment, to observe definite color change, we removed the TAMRA fluorescence marker from the artificial peptide. Removal of TAMRA might affect binding capability of peptides, so we conducted a similar binding experiment with the TAMRA-removed artificial peptide (Figure 3b). Therefore, we adopted concentration candidates approximate to the average of two experiments. For the reference SiMP, increased impedance was noted when adding proteins, which caused increased resistance in the control and $\alpha\beta$ solutions. Shown the equation of the reference SiMP in Figure S3, the impedance of the reference SiMP is changed by the solution resistance. At the first IgG solution addition, however, the impedance decreased because of the lower impedance of the HBS solution than in PBS. The impedance of the reference SiMP is increased through continuous addition, thus the increase in the total solution resistance is represented to occur by adding a protein. In the reference SiMP pair, the total impedance changes is mainly affected by the solution resistance.

2.4. Impedance Analysis of the 3D Sensor. The 3D sensor circuit was assessed via impedance analysis. The SiMP in the 3D sensor constructs an open circuit in air condition. The open circuit is changed to the comprehensive circuit when the 3D sensor is soaked in the solution (Supporting Information section S3). In the 3D sensor, the reference SiMP pair and the working SiMP pair exist and construct different circuits depends on the peptide. Impedance analysis proceeded based on a $1 \times$ PBS solution, and this process proceeded every sensor before the experiment, so as to set as ground impedance of each working and reference SiMP pairs (Figure 5a). The 3D sensor was composed of one set of reference SiMPs without the peptide and seven sets of working SiMPs with the peptide. After peptide attachment on the working SiMPs, the impedance increased compared to before the peptide attachment (Figure 5b). The peptide can work as an insulator, thus the charge transfer resistance in the circuit was increased (Supporting Information section S3). Therefore, the impedance of the working SiMPs increased after coating with the peptide, while that of the reference SiMP was maintained.

2.5. Selectivity Test of the 3D Sensor Based on Human Blood Serum (HBS). The selectivity of the 3D sensor was confirmed using an in vitro test using an HBS solution. $\alpha\beta$ and other control solutions immunoglobulin G (IgG), concanavalin A (Con A), and vascular endothelial growth factor (VEGF) were prepared at 10 pM diluted in HBS. First, the 3D sensor was soaked in 400 $\mu$L of PBS and measured the impedance of the reference and working SiMP pairs to use as the standard impedance. On the 3D sensor soaked in PBS solution, 20 $\mu$L of control solutions was added in the following order: IgG, Con A, and VEGF. After, the $\alpha\beta$ solution diluted in HBS was added on the SiMP device. Each addition proceeded with the same PBS solution on the 3D sensor, and 20 $\mu$L of the PBS solution mixed with other solutions was removed per addition to maintain the total solution volume.
However, the impedance of working SiMP pairs decreased following the addition of the solutions. This impedance drop occurs by some peptide–protein reactions in the HBS solution. Since all proteins have a hydrophobic part for protein folding or interaction with a specific biomolecule,26−29 the control protein can react with the KLVFF segment of the peptide with strong hydrophobicity. However, for Aβ, the impedance rapidly decreased over 260% than other solutions. Even though some peptides already reacted with the control proteins due to the continuous test, this impedance reduction means that the Aβ−peptide reaction is stronger than other proteins (Figure 5c). The decrease in the impedance is occurred by the increase in the capacitance of the Ab described in Figure S3. In the working SiMP, the solution resistance also increases by the added protein, but the reaction of the peptide makes the increase in the capacitance greater.

Furthermore, this selectivity test proceeded in a continuous system with the same PBS solution, thus the measured concentration of the control and Aβ in the solutions was approximately 500 fM because the added solutions were diluted by the base PBS solution. Simultaneously, the other six sets of working SiMPs showed the same results (Supporting Information section S4). Therefore, the 3D sensor proves its high selectivity for the Aβ at a low concentration of approximately 500 fM, and the reliability of these multiple analyzing systems.

2.6. Sensitivity Test of the 3D Sensor Based on HBS.

To demonstrate the sensitivity of the 3D sensor, an in vitro test using the HBS solution with diluted Aβ was conducted. Aβ solutions were prepared from 1 nM to 1 fM, which are diluted 10-fold each step. The 3D sensor soaked in 400 μL of PBS measured the impedance of the reference working SiMP pairs. In the PBS solution on the 3D sensor, the Aβ-diluted HBS solution was continuously added at 20 μL per concentration. The total volume of PBS mixed with Aβ solution was maintained at 400 μL through the removal of PBS solution per every addition of Aβ solution. The impedance analysis was recorded for 5 min after each addition of Aβ solution to give enough time to diffuse.

The Aβ solution has a slight increase amount of 1% on the reference SiMP’s impedance, whereas a reduction of 8 times more than the reference SiMP is seen for the working SiMP impedance because of the Aβ−peptide reaction. This can be explained by the circuit changes because of the Aβ−peptide reaction. For the reference SiMP, the main effect on the impedance is caused by proteins in the solution, which increase the resistance of the solution. However, the main effect on the impedance of the working SiMP is attributable to the Aβ−peptide reaction, derived by increasing the capacitance of the sensor. The Aβ is bonded with the peptide on the SiMP surface, and this reaction makes the total impedance decrease through increasing the capacitance.

The 3D sensor detected an impedance change following the addition of 1 fM Aβ solution as seen in the different tendencies between the reference and working SiMP pair. Therefore, this sensitivity test shows that the Aβ detection limit of the 3D sensor is approximately 0.1 fM as a result of dilution from the added Aβ solution.

2.7. AD Patient Recognition Test of the 3D Sensor with Human Blood Plasma.

Figure 6. (a) Impedance analysis results of the reference and working SiMP pairs in the same 3D sensor using blood plasma from AD and healthy patients. Data was analyzed by a t test (* = p < 0.01 and *** = p < 0.0001, n = 5). (b) Comparison of impedance changes by the Aβ−peptide reaction of the 3D sensor for the blood plasma of the AD and healthy patient.
3D sensor for real AD patients, the human blood plasma from an AD and healthy patient was used. The test was conducted like the selectivity test based on the PBS solution. The SiMP device was set with two sets of reference SiMP pairs and six sets of working SiMP pairs to double check the impedance of the pairs. The impedance of the 3D sensor in the PBS solution was measured as a standard impedance to understand the impedance change difference between plasma of the healthy and AD patient. Because the human blood plasma is a complex liquid compared to HBS, the impedance measurement of each plasma was recorded 10 min after adding the plasma in the PBS solution.

Impedance from the reference SiMP increased after adding the blood plasma of the healthy and AD patient due to its higher impedance than PBS (Figure 6a). However, when the blood plasma of the healthy and AD patient was added to the 3D sensor, the impedance from the six working SiMP pairs decreased due to the peptide reaction. In addition, in the working SiMP pairs, the change of impedance, by the peptide reaction, is different between the blood plasma of the healthy and AD patient. For the AD patient’s blood plasma, the impedance decreased 143–271% more than the change in the healthy patient. This impedance change is consistent in all six working SiMP pairs after comparison, hence, the proven reliability of the 3D SiMP sensor.

The experiments in Figure 6a have demonstrated that the 3D sensor can recognize the plasma of AD patients through impedance changes. Here, additional experiments were designed to change the order of the human plasma addition to verify the Aβ–peptide reaction through the impedance change of each blood plasma. Subsequently, stronger reactions with the AD patient’s plasma than with the healthy patient were proven (Figure 6b). The results show that the 3D sensor rarely reacts with the blood plasma of the healthy patient after reacting with the blood plasma of the AD patient, whereas the blood plasma impedance changes for the AD patient after reacting with the blood plasma of the healthy patient. Therefore, the 3D sensor showed a stronger response to the blood plasma of the AD patient than in that of the healthy patient, indicating proof that the blood plasma of AD and healthy patients can be distinguished using the 3D sensor.

4. EXPERIMENTAL SECTION

4.1. Participants. We used blood samples from an AD patient and a healthy volunteer with healthy cognition recruited from Gachon University Gil Medical Center. Participants underwent a 3T-magnetic imaging scan, 18F-flutemetamol (FLUTE) PET scan, and detailed neuropsychological assessment.

The AD patient was diagnosed with AD dementia according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association criteria.30 The AD patient (female, 67 years old, 9 educational years) had a disease duration of 3 years. The mini-mental status examination (MMSE) score was 23, and the clinical dementia rating (CDR) was 0.5. Her amyloid PET showed an amyloid positive finding based on previous quantitative methods.31,32 A cognitive normal (CN) participant was enrolled from the community and met the following inclusion criteria: (1) a CDR score of 0 and (2) healthy level in neuropsychological assessment (defined as within 1.5 standard deviation of age and education n-corrected normative mean). The MMSE score of this CN participant (female, 69 years old, 12 educational years) was 30. Her amyloid PET showed amyloid negative.

All participants provided written informed consent, which is approved by Gachon University Gil Medical Center Institutional Review Board (GBIRB2018-350).

4.2. Collection of the Human Blood Plasma. Blood samples were collected in the morning, after an overnight fast, and collected in EDTA tubes by venipuncture. The blood samples were centrifuged for 15 min at 3000 rpm at 21 °C to obtain plasma. The plasma samples were aliquoted in 300 μL and immediately stored at −80 °C.
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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Science, ICT & Future Planning (grant nos.2018M3C7A1024654, 2020R1A2C2007578, and 2018M3D1A1058536); the Korean government (MSIP) (grant no. 2017R1A2B3011586); and the Korean Government (MSIT) (no. 2018M3C7A1056889) and supported by a grant from the Korea Healthcare Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare of the Republic of Korea (grant no. HI14C1135).

ABBREVIATIONS
Aβ, amyloid beta; AD, Alzheimer’s disease; APP, amyloid precursor protein; SiMP, silicon micropillar; PCB, printed circuit board; PET, positron emission tomography; DRIE, deep reactive ion etching; CSF, cerebrospinal fluid; TAMRA, carboxytetramethylrhodamine; SPPS, solid phase peptide synthesis; Fmoc, 9-fluorenylmethoxycarbonyl; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy; AuNP, gold nanoparticle; IgG, immunoglobulin G; Con A, concanavalin A; VEGF, vascular endothelial growth factor; HBS, human blood serum.

REFERENCES
(1) Murphy, M. P.; LeVine, H., III Alzheimer’s Disease and the Amyloid-β Peptide. JAD 2010, 19, 311–323.
(2) Shoji, M.; Golde, T.; Ghiso, J.; Cheung, T.; Estus, S.; Shaffer, L.; Cai, X.; McKay, D.; Tintner, R.; Frangione, B.; et al. Production of the Alzheimer amyloid beta protein by healthy proteolytic processing. Science 1992, 258, 126–129.
(3) Nordberg, A.; Rinne, J. O.; Kadir, A.; Lingström, B. The use of PET in Alzheimer disease. Nat. Rev. Neurol. 2010, 6, 78–87.
(4) Vlassen, A. G.; Benzinger, T. L. S.; Morris, J. C. PET Amyloid-beta Imaging in Preclinical Alzheimer’s Disease. Biochim. Biophys. Acta. Mol. Basis Dis. 1822, 1822, 370–379.
(5) Marcus, C.; Mena, E.; Subramaniam, R. M. Brain PET in the Diagnosis of Alzheimer’s Disease. Clin. Nucl. Med. 2014, 39, e413–e426.
(6) Vassar, R.; Citron, M. Aβ-Generating Enzymes. Neuron 2000, 27, 419–422.
(7) Takahashi, T.; Mihara, H. Peptide and Protein Mimetics Inhibiting Amyloid β-Peptide Aggregation. Acc. Chem. Res. 2008, 41, 1309–1318.
(8) Mehta, P. D.; Pirttilä, T.; Mehta, S. P.; Seres, E. A.; Aisen, P. S.; Wisniewski, H. M. Plasma and Cerebrospinal Fluid Levels of Amyloid β Proteins 1-40 and 1-42 in Alzheimer Disease. Arch. Neurol. 2000, 57, 100.
(9) Jack, C. R., Jr.; Knopman, D. S.; Jagust, W. J.; Shaw, L. M.; Aisen, P. S.; Weiner, M. W.; Petersen, R. C.; Trojanowska, J. Q. Hypothetical Model of Dynamic Biomarkers of the Alzheimer’s Pathological Cascade. Lancet. Neurol. 2010, 9, 119–128.
(10) de la Escosura-Muñiz, A.; Plicha, Z.; Horák, D.; Merkoč, A. Alzheimer’s Disease Biomarkers Detection in Human Samples by Efficient Capturing through Porous Magnetic Microspheres and Labelling with Electrocatalytic Gold Nanoparticles. Biosens. Bioelectron. 2015, 67, 162–169.
(11) Prabhulkar, S.; Patytszek, R.; Cirrito, J. R.; Wu, Z.-Z.; Li, C.-Z. Microbiosensor for Alzheimer’s Disease Diagnostics: Detection of Amyloid Beta Biomarkers: Biosensor for Abeta Detection. J. Neurochem. 2012, 122, 374–381.
(12) Cho, S. M.; Kim, H. V.; Lee, S.; Kim, H. Y.; Kim, W.; Kim, T. S.; Kim, D. J.; Kim, Y. Correlations of Amyloid-β Concentrations between CSF and Plasma in Acute Alzheimer Mouse Model. Sci. Rep. 2014, 4, 1–4.
(13) Oh, J.; Yoo, G.; Chang, Y. W.; Kim, H. J.; Jose, J.; Kim, E.; Pyun, J.-C.; Yoo, K.-H. A Carbon Nanotube Metal Semiconductor Field Effect Transistor-based Biosensor for Detection of Amyloid Beta in Human Serum. Biosens. Bioelectron. 2013, 50, 345–350.
(14) Chu, Z.; Zhang, W.; You, Q.; Yao, X.; Liu, T.; Liu, G.; Zhang, G.; Gu, X.; Ma, Z.; Jin, W. A novel separation-sensing membrane performing precise real-time serum analysis during blood drawing. Angew. Chem. Int. Ed. 2020, 18701.
(15) Li, B.; Qi, J.; Fu, L.; Han, J.; Choo, J.; deMello, A. J.; Lin, B.; Chen, L. Integrated hand-powered centrifugation and paper-based diagnosis with blood-in/answer-out capabilities. Biosens. Bioelectron. 2020, 165, 112282.
(16) Lue, L.-F.; Guerra, A.; Walker, D. G. Amyloid Beta and Tau as Alzheimer’s Disease Blood Biomarkers: Promise from New Technologies. Neurol. Ther. 2017, 6, 25–36.
(17) Yoo, Y. K.; Kim, J.; Kim, G.; Kim, Y. S.; Kim, H. Y.; Lee, S.; Cho, W. W.; Kim, S.; Lee, S.-M.; Lee, B. C.; et al. A Highly Sensitive Plasma-based Amyloid-β Detection System through Medium-Changing and Noise Cancellation System for Early Diagnosis of the Alzheimer’s Disease. Sci. Rep. 2017, 7, 8882.
(18) Na, J.; Hong, M.-H.; Choi, J. S.; Kwak, H.; Song, S.; Kim, H.; Chae, Y.; Cheong, E.; Lee, J. H.; Lim, Y.-m.; et al. Real-Time Detection of Markers in Blood. Nano Lett. 2019, 19, 2291–2298.
(19) Qi, J.; Li, B.; Zhou, N.; Wang, X.; Deng, D.; Luo, L.; Chen, L. The strategy of antibody-free biomarker analysis by in-situ synthesized molecularly imprinted polymers on movable valve paper-based device. Biosens. Bioelectron. 2019, 142, 111533.
(20) Yang, Y.; Jia, J. A Multi-frequency Electrical Impedance Tomography System for Real-time 2D and 3D Imaging. Rev. Sci. Instrum. 2017, 88, No. 085110.
(21) González, G.; Huttunen, J. M. J.; Kolehmainen, V.; Seppänen, A.; Vauhkonen, M. Experimental Evaluation of 3D Electrical Impedance Tomography with Total Variation Prior. Inverse Probl. Sci. Eng. 2016, 24, 1411–1431.
(22) Liu, P.; Li, B.; Fu, L.; Huang, Y.; Man, M.; Qi, J.; Sun, X.; Kang, Q.; Shen, D.; Chen, L. Hybrid Three Dimensionally Printed Paper-Based Microfluidic Platform for Investigating a Cell’s Apoptosis and Intracellular Cross-Talk. ACS Sens. 2020, 5, 464–473.
(23) Miller, K.; Li, M.; Walsh, K.; Fu, X.-A. The Effects of DRIE Operational Parameters on Vertically Aligned Micropillar Arrays. J. Micromech. Microeng. 2013, 23, No. 035039.

(24) Hanein, Y.; Schabmueller, C. G. J.; Holman, G.; Lücke, P.; Denton, D. D.; Bühringer, K. F. High-aspect Ratio Submicrometer Needles for Intracellular Applications. J. Micromech. Microeng. 2003, 13, S91–S95.

(25) Xiao, Y.; Ma, B.; McElheny, D.; Parthasarathy, S.; Long, F.; Hoshi, M.; Nussinov, R.; Ishii, Y. Aβ(1-42) fibril structure illuminates self-recognition and replication of amyloid in Alzheimer’s disease. Nat. Struct. Mol. Biol. 2015, 22, 499–505.

(26) Dyson, H. J.; Wright, P. E.; Scheraga, H. A. The role of hydrophobic interactions in initiation and propagation of protein folding. Proc. Natl. Acad. Sci. U. S. A. 2006, 103, 13057–13061.

(27) Parker, C. W.; Osterland, C. K. Hydrophobic binding Sites on Immunoglobulins. Biochemistry 1970, 9, 1074–1082.

(28) Wang, J.; Edelman, G. Binding and functional properties of concanavalin A and its derivatives. I. Monovalent, divalent, and tetravalent derivatives stable at physiological pH. J. Biol. Chem. 1978, 253, 3000–3007. http://intl.jbc.org/cgi/content/abstract/253/9/3000

(29) Iyer, S.; Darley, P. I.; Acharya, K. R. Structural Insights into the Binding of Vascular Endothelial Growth Factor-B by VEGFR-1D2. J. Biol. Chem. 2010, 285, 23779–23789.

(30) McKhann, G.; Drachman, D.; Folstein, M.; Katzman, R.; Price, D.; Stadlan, E. M. Clinical Diagnosis of Alzheimer’s Disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. Neurology 1984, 34, 939–939.

(31) Kang, J. M.; Lee, S.-Y.; Seo, S.; Jeong, H. J.; Woo, S.-H.; Lee, H.; Lee, Y.-B.; Yeo, B. K.; Shin, D. H.; Park, K. H.; et al. Tau Positron Emission Tomography using [18F]THK5351 and Cerebral Glucose Hypometabolism in Alzheimer’s Disease. Neurobiol. Aging 2017, 59, 210–219.

(32) Thurfjell, L.; Lilja, J.; Lundqvist, R.; Buckley, C.; Smith, A.; Vandenberghe, R.; Sherwin, P. Automated Quantification of 18F-Flutemetamol PET Activity for Categorizing Scans as Negative or Positive for Brain Amyloid: Concordance with Visual Image Reads. J. Nucl. Med. 2014, 55, 1623–1628.