Functionalized Carbon Nanotube-based Microelectronic Sensor Array for Early Diagnosis of Alzheimer's Disease

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ABSTRACT

While there are no current therapies that can provide a definite cure to Alzheimer's Disease or stall its progress, early diagnosis may delay the symptoms thus offering optimistic opportunities from a preventative care standpoint. With that, we designed and developed a lab-on-a-chip by utilizing patterned microelectronic sensor devices for detecting specific biomarkers (Amyloid-β 1-42, Tau, and *p*-Tau) in relevant media. Our studies showed that the devices detected biomarker pair responses in phosphate-buffered saline, artificially-spiked cerebrospinal fluid, and clinical cerebrospinal fluid. Not only was a remarkable approximated limit of detection established (LOD ≈ 10fM–50fM), but also distinctive responses between non-pairing cross reactivity and pairing biomarkers were observed. Based on our results, these properly-fabricated devices reveal high specificity and a much higher sensitivity than most other Alzheimer's Disease testing platforms to date. Furthermore, this platform can aid in future R&D immunoassay data confirmation as well.

Keywords: carbon nanotubes, biosensors, alzheimer's disease, amyloid-β, tau

1. INTRODUCTION

Alzheimer's Disease (AD) is a severe and complex neurodegenerative disorder characterized by forms of dementia and cognitive decline. The cost of AD patient care in the United States was $259 billion for 2017, and unless addressed, this cost is estimated to reach $1.1 trillion by 2050 [1]. AD not only imposes a tremendous financial burden on the health care system, but also presents huge financial complications for the general economy. The standard method of AD diagnosis involves a combination of imaging and cognitive tests while definitive diagnosis is performed postmortem. Most of these tests are expensive and time-consuming, require highly-skilled personnel, and cannot be used as a tool for point-of-care applications. Because AD onset can occur decades before the manifestation of pathological hallmarks [2,3], a panel of biomarkers that can tap into this early window of opportunity will be beneficial in detecting, staging, treating, and monitoring the disease.

We designed and developed a lab-on-a-chip for the early diagnosis of the disease based on our patented innovative nanomaterials-enabled technology by utilizing patterned microelectronic sensor devices (MEDs) for detecting Food and Drug Administration-approved AD biomarkers (Amyloid-β 1-42, Tau, and *p*-Tau) [4a]. Technology of the MEDs involves the site-specific deposition of highly sensitive and robust single-walled carbon nanotubes (CNTs). It can serve as a simple, effective, and low-cost AD diagnostic tool in a point-of-care or clinical setting (Figure 1). Even with minimal power (0.25–2mW), CNT-based devices can attain high levels of sensitivity due to CNT's large surface area, high aspect ratio and the versatile surface chemistry that allows for a wide range of conjugation and functionalization options to obtain optimal sensing capabilities.

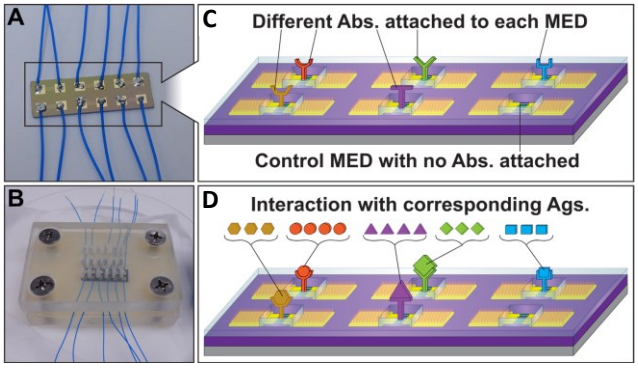

Figure 1: Photos of (**A**) MEDs configured in a multiplexed array and (**B**) a testing block. Illustrations of MEDs **(C)** conjugated with antibodies and **(D)** interacting with corresponding antigens.

2. METHODS

2.1 Device Fabrication

MEDs were first individually extracted from whole wafers which were fabricated via photolithography and diced. As shown in Figure 2A, one 4-inch wafer yields more than 400 individual MEDs. The gold "fingers" of each electrode have a 2µm gap between them (Figure 2D) onto which the CNTs were deposited to create a chemiresistive device. Before CNT deposition, MEDs were first cleaned following a literature procedure using a series of solvents including micro90 solution, isopropanol, and acetone, each with 5-minute sonication and rinsing [4b]. The MEDs were then submerged in a piranha solution for 20 minutes for further washing and surface activation followed by a thorough rinse with deionized water (DI H_2O) and drying with clean a nitrogen jet. The clean MEDs were then processed using dielectrophoresis to deposit CNTs onto the finger gap area. CNTs were dispersed in

dimethylformamide (DMF) via sonication, then carefully pipetted into the well of a customized polytetrafluoroethylene cell block which contained the exposed fingers of a single MED. Once CNTs were deposited onto individual MEDs, they were then annealed at 200°C for 1 hour to improve contact of the CNTs with the fingers. Wires were soldered to the gold pads (Figure 2 B), and the MEDs were characterized by scanning electron microscopy (SEM) (Figure 2 C) and current-voltage (I-V) measurements. To anchor antibodies to the CNTs, MEDs were submerged in a 6mM 1-pyrenebutanoic acid, succinimidyl ester solution in DMF for 1 hour followed by a thorough rinse and air drying. Antibodies of interest were then diluted to working concentrations of 100nM and conjugated to individual MEDs followed by surface passivation using polysorbate-20 and 6-mercapto-1-hexanol to prevent non-specific binding and ensure reactivity with the target analyte. At this stage, MEDs were ready for testing.

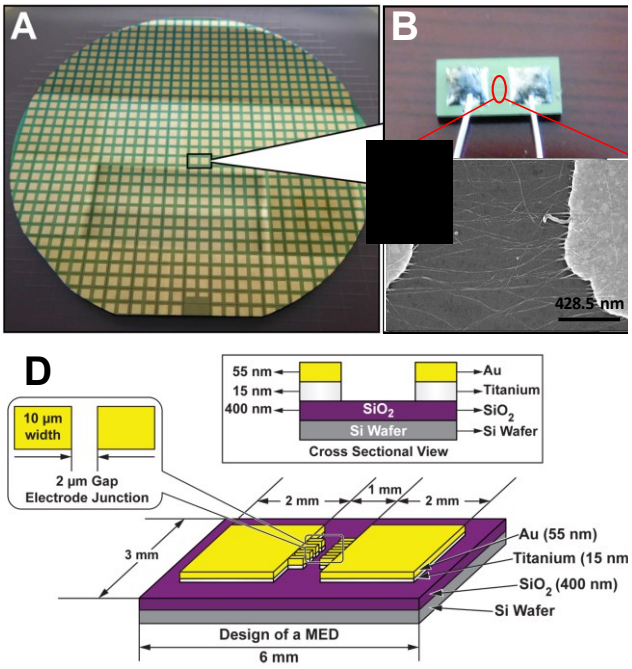

Figure 2: (**A**) 4-inch silicon wafer with over 400 patterned MEDs prepared by photolithography and dicing (**B**) Single MED after CNT deposition and wire-bonding; (**C**) SEM of an MED showing CNTs deposited between the finger electrodes. (**D**) Schematic showing the dimensionality of one MED.

2.2 Device Testing and Evaluation

We not only tested MEDs with specific antibody-antigen pairs, but also with non-pairing antibody-antigen biomarkers in various relevant media including: 1X phosphate-buffered saline (PBS), artificially-spiked cerebrospinal fluid (aCSF), and clinical cerebrospinal fluid (CSF). Antibody-conjugated MEDs were rinsed with 1X PBS followed by sterilized water. A base I-V curve was then taken. Next, 2µL of the antigen dilution was pipetted onto the reaction site and incubated at 40°C for ~20 minutes. The MEDs were again rinsed with 1X PBS and sterilized water and the I-V curve was measured. The same steps were then repeated until the I-V curves of antigen dilutions starting from low to high concentrations were recorded. Data was then processed and plotted, and the sensor response (S (%)) was calculated according to Equation 1 below:

$$S(\%) = \frac{I - I_0}{I_0} \times 100 = \left(\frac{\Delta I}{I_0}\right) \times 100 \qquad (1)$$

3. RESULTS

Our studies showed that the MEDs successfully detected Amyloid-β 1-42, Tau, and *p*-Tau biomarker pair responses in each of the aforementioned media. We also demonstrated the ability to detect biomarker concentrations as low as 10fM–50fM and up to 100nM. Figure 3 A and D show the I-V curves for the detection of Amyloid-β 1-42 and Tau pairs in PBS, respectively. Figure 3 G shows the I-V curves for the detection of p-Tau in PBS, zoomed in at 1.5–2V region for clarity. In PBS, the MEDs revealed good sensor response S(%) ≈ 55% for Amyloid-β 1-42, 60% for Tau, and 50% for *p*-Tau, with distinct difference compared to the false positive and false negative controls (Figure 3 B, E, and H). General responses in aCSF ranged from 40–60%, while responses for Tau and *p*-Tau in CSF showed slightly lower values (26–31%) due to sample complexity. Although CSF provides the lowest response of the three media, which was expected due to its relatively low purity and inadequate stability, our preliminary tested pairs were still able to yield significant detection responses for the generation of an early calibration curve. Figure 4 summarizes the biomarker responses of Tau pairs in different media. Based on this data, PBS provides the most ideal conditions for the proof of concept and pair specificity. Furthermore, we were able to clearly distinguish responses between appropriate antibody-antigen pairs with that of controls as well as cross reactivity pairs (Figure 3 C, F, and I). MEDs conjugated with a particular antibody were tested against blank samples containing only PBS buffer and showed <5% or no response at all. Similarly, MEDs conjugated with a certain antibody tested against non-pairing antigens (i.e., Amyloid-β 1-42 cross reacted with Tau) revealed no greater than a 15% response.

Additional cross reactivity studies were performed by conjugating our devices with monoclonal (mOC) antibodies and testing against other Amyloid β42 aggregates under various conditions, which were then compared with dot and Western blot results from our collaborators at the University of California, Irvine. Figures 5 shows blot results from Dr. Glabe's research group relative to the tests performed on our devices [5]. The details are as following: Aβ42 was aggregated under 3 different conditions over a 10-day time period. 1µl aliquots were pipetted onto nitrocellulose membranes at time 0, and at the 3-day and 10-day time points. The membranes were then probed with the 23 mOC antibodies, along with

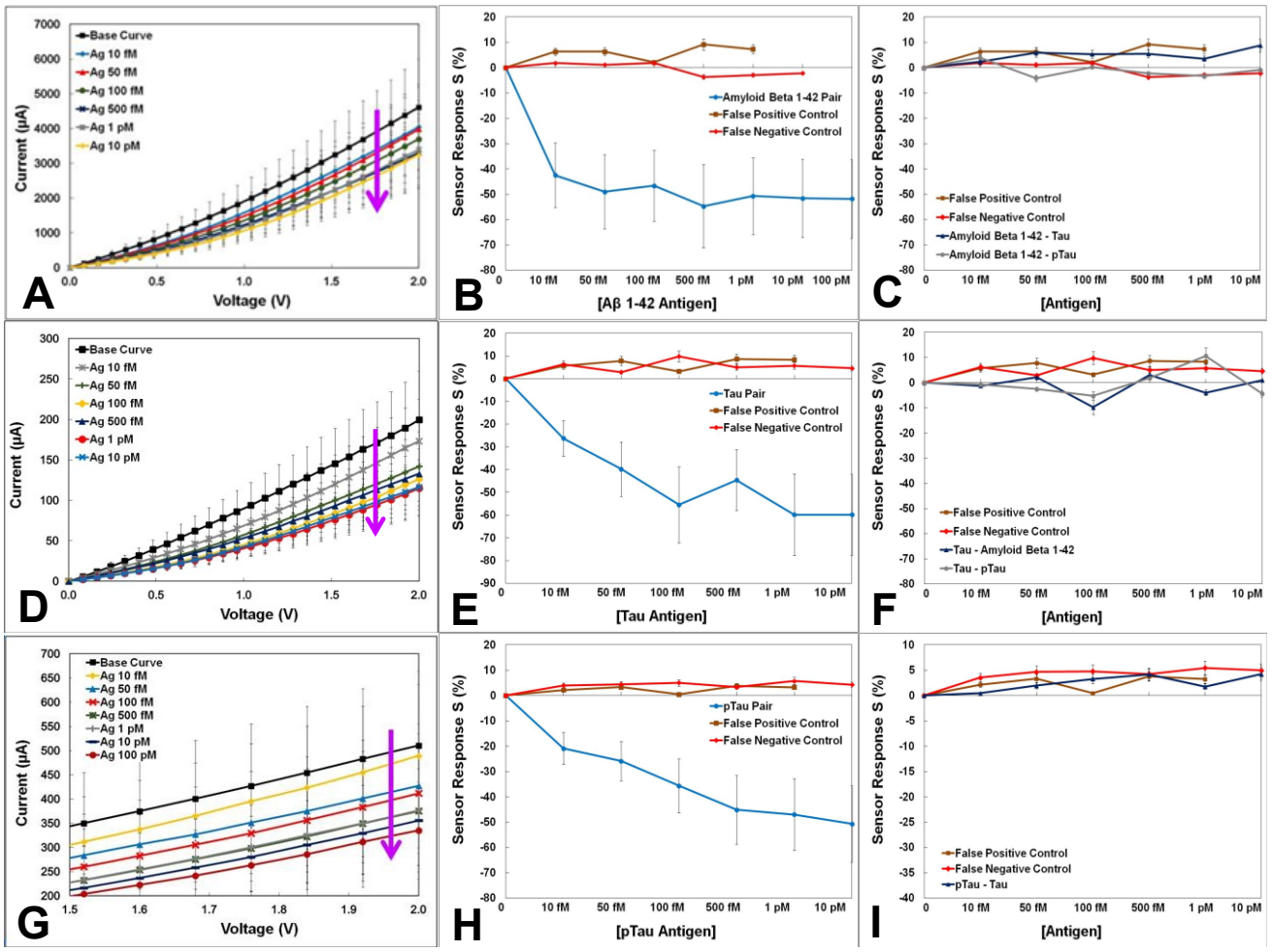

Figure 3: I-V curves for Amyloid-β 1-42 **(A)**, Tau **(D)**, and p-Tau pair **(G)** in PBS. S (%) of Amyloid-β 1-42 **(B)**, Tau **(E)**, and p-Tau pair **(H)** in comparison with false negative and false positive controls. S (%) of cross reactivity between non-pairing biomarkers in comparison with the same controls **(C, F, and I)**.

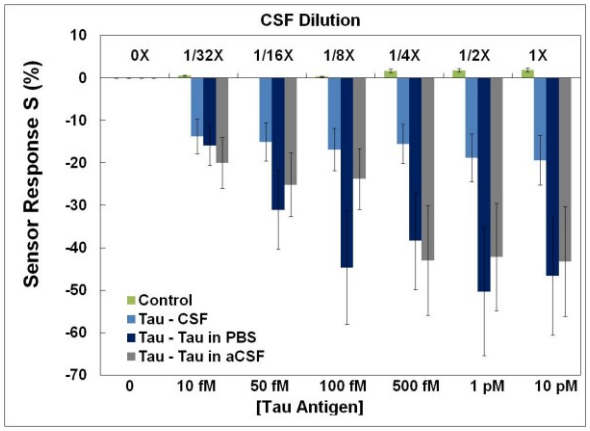

Figure 4: Tau pair tested in PBS, CSF and aCSF, showing a clearly concentration dependent response.

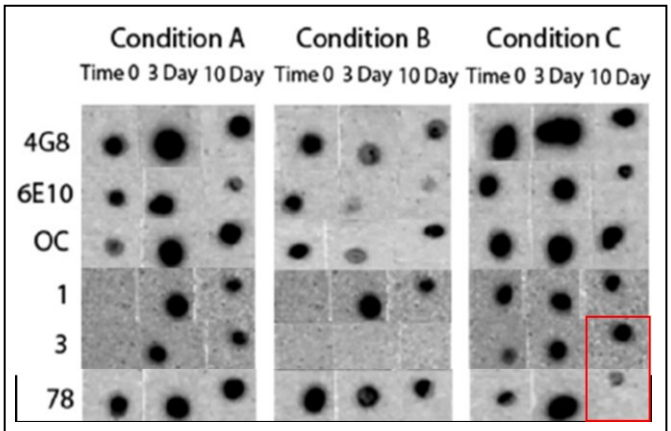

Figure 5: mOC3 and mOC78 dot blots showing the reactivity of Aβ42 aggregated under 3 different conditions over a 10-day time period.

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6E10 and 4G8. The 3 aggregation conditions were: Condition A: peptide resuspended in 100mM sodium hydroxide (NaOH) and diluted in phosphate buffer; Condition B: peptide resuspended in hexafluoroisopropanol (HFIP) and diluted in water; Condition C: peptide resuspended in 100mM NaOH and diluted in *N′*-2-hydroxyethylpiperazine-*N′*-2 ethanesulphonic acid and sodium chloride buffer [5]. In the red box, we can see the noticeable dots for mOC3 and mOC78 signaling a reaction has occurred, which we comparatively saw in our devices. When we compare the signals and responses obtained from testing the mOC3 and mOC78 antibodies with Aβ42, condition C, day 10 antigens, our devices revealed the clear distinction of a reaction taking place just as shown on the dot blots. Therefore, our novel platform has the potential to not only become an alternative or supplemental benchmark for AD diagnostics, but also for future R&D immunoassay data confirmation.

4. CONCLUSIONS

In summary, the results provided confidence in the ability of our CNT functionalized MEDs to detect AD-specific biomarker antigens in PBS (Figure 6), aCSF, and CSF. We were able to generate signals and sensor responses well below the 100fM level thus providing a remarkable LOD. At 10fM–50fM, we were able to notice reasonable I-V curve trends and responses that remained distinct from the base curves and respective controls. With this, we have determined that our MEDs have excellent sensitivity down to femto-molar range. The fast and low-cost MEDs will offer the potential for a diagnostic tool to easily screen the early onset of AD and provide reasonable responses based on a patient's particular concentration of certain biomarkers. By improving fabrication methods, we found an optimized LOD (lowest possible concentration with S (%) > 10% from the base curve, controls, and cross reactivity) that will provide a highly necessary early-stage screening solution for AD. With this, being able to provide information about AD's early onset could help to determine a proper therapeutic path for patients. Additionally, by performing cross reactivity studies and obtaining remarkable expected results, we can highlight just how effective our devices are based on the specificity of paired reactions in comparison with proper controls and other interactions.

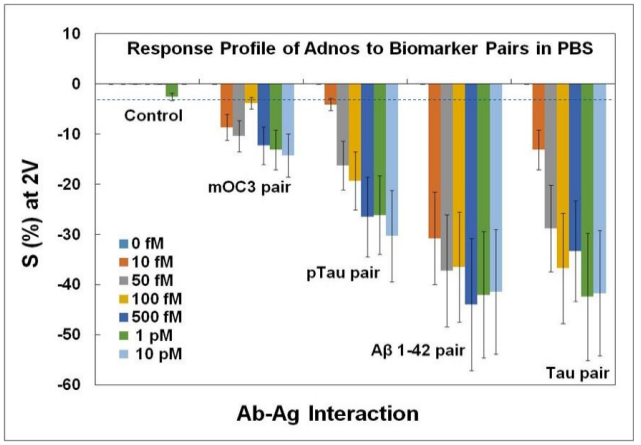

Figure 6: Summary of PBS pairing at each added concentration.

5. FUTURE WORKS

Currently, we are working on the optimization and further validation of MEDs. Additionally, further testing is taking place to study the cross-reactivity of the biosensor with similar, but non-AD biomarkers, such as those from another dementia-related illness. MEDs conjugated with AD antibodies like Tau are being tested in a similar fashion to the aforementioned cross-reactivity experiments.

Other potential works currently being studied involve the spin-off of the nanotechnological idea of using functionalized CNTs to detect other diseases with known biomarkers and detection of toxic industrial gases.

6. ACKNOWLEDGEMENTS

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