Digital biosensor for human cerebrospinal fluid detection with single-use sensing strips

Cite as: J. Vac. Sci. Technol. B 40, 023202 (2022); https://doi.org/10.1116/6.0001576 Submitted: 21 October 2021 • Accepted: 22 December 2021 • Published Online: 01 February 2022

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ABSTRACT

Leakage of human cerebrospinal fluid (CSF) caused by trauma or other reasons presents exceptional challenges in clinical analysis and can have severe medical repercussions. Conventional test methods, including enzyme-linked immunosorbent assay and immunofixation electrophoresis testing, typically are performed at a few clinical reference laboratories, which may potentially delay proper diagnosis and treatment. At the same time, medical imaging can serve as a secondary diagnosis tool. This work presented here reports the use of a point-of-care electrochemical sensor for detection of beta-2-transferrin (B2T), a unique isomer of transferrin that is present exclusively in human CSF but is absent in other bodily fluids. Limits of detection were examined via serial dilution of human samples with known B2T concentrations down to 7×10^{-12} g B2T/ml while maintaining excellent sensitivity. Nine human samples with varying levels of B2T were compared using up to 100 times dilution to confirm the validity of sensor output across different patient samples.

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I. INTRODUCTION

Cerebrospinal fluid (CSF) is an extracellular fluid present uniquely in the brain and spinal cord, and it provides critical physiological functions such as shock absorption and waste transport.¹⁻¹¹ It also serves as the direct linkage for the extracranial space to the subarachnoid space.² A large variety of reasons could lead to the leakage of human CSF, for example, trauma, surgery, tumors, or congenital defects.¹² Spontaneous CSF leak also occurs with increased likelihood due to obesity, increased intracranial pressure (ICP), and obstructive sleep apnea (OSA).^{13–15} Cerebrospinal leaks can be manifested in clinical symptoms such as rhinorrhea (through the nose) and otorrhea (through the ear). If not diagnosed and treated properly, they can potentially result in life-threatening conditions such as meningitis, intracranial infection, and death. Enzyme-linked immunosorbent assay (ELISA) and immunofixation electrophoresis (IFE) testing are the golden standards for testing of CSF leaks. However, such biochemical assays need to be done offsite in clinical reference laboratories with trained personnel and have relatively long processing times of at least hours due to labor-intensive analysis. The turnaround time may run into days when including transportation and sample backlogs.¹¹ Depending on the method for sample collection and local concentration of CSF present, samples sent for testing may also require being concentrated up to tenfold to reach the minimum detection limit of ELISA and IFE. Therefore, it is vital to develop a rapid cerebrospinal fluid test with minimal turnaround time, capable of point-of-care use, and high sensitivity and specificity.

Beta-2-transferrin (B2T) is an isomer of human transferrin present uniquely in human cerebrospinal fluid but absent in other body fluids and tissues such as blood, tears, or mucus. Although transferrin of different variants can be found in other bodily fluids, such as tear and ear secretion, the pattern of isomer concentration differs between CSF and other bodily fluids and lays the foundation for the B2T assay for CSF leakage detection.⁶ Therefore, biochemical detection of traces of beta-2-transferrin by various assays with high sensitivity and specificity, such as ELISA and IFE, has become

the primary standard for clinical diagnosis, along with some secondary methods such as high-resolution computed tomography (CT) and magnetic resonance imaging (MRI) that facilitates localization of a leak¹³ but often fails to locate the specific site of the leakage. In the particular case that consistent clinical history and imaging findings have been observed as an indicator, the time-consuming B2T test might not be necessary.¹³ Kita et al. developed point-of-care CSF detection devices using lateral-flow immunoassay utilizing antibodies for beta-trace protein; however, the detection limit only ranges from 0.3 to 90 mg/l, which is higher than the range in commercially available products and could result in false negatives due to this high limit of detection (LOD).¹¹ It is possible to utilize various electrochemical methods to amplify the signal of biomolecular interaction.^{16,17} Detection utilizing the double drain/gate pulse method with Si metal-oxide-semiconductor field-effect transistor (MOSFET) has been employed^{4,7,18-20} but requires the use of complex fabrication techniques with metal lines on glass slides and complex readout setup requiring an external oscilloscope with a detection limit down to 10⁻¹⁰ g ČSF/ml with unspecified B2T concentration⁴ due to the lack of the ELISA assay. At the same time, biomarker detection using costly high electron mobility transistors (HEMTs) has been demonstrated in previous works;^{7,21}· however, the use of inexpensive Si-based MOSFETs has been shown to be sufficient for detection down to femtograms per milliliters for other biomarkers.¹⁸ This work presents the recent progress on the pulsed electrochemical detection of CSF with affordable plastic strips in a battery-powered handheld device with a low limit of detection.

II. MATERIAL AND METHOD

Disposable glucose test strips with carbon electrodes were used for functionalization with B2T antibody. The method for functionalization was described previously.^{4,18,20} In brief, the sensor strip was treated in ozone for 15 min to remove surface hydrocarbons and other contaminants. Then, the front electrode was subsequently electroplated with gold using a gold plating solution (Gold Plating Services, Kaysville, UT). The Au-plated electrodes were immersed for 4 h in 10 mM thioglycolic acid (TGA) in ethanol to form Au–S bonding. The binding efficacy for Au–S bonding with a

TABLE I. Human CSF sample B2T concentrations.

similar procedure was verified with electrical measurement and x-ray photoelectron spectroscopy, described previously.^{18,21,25} The test strips were then rinsed with acetonitrile, blow-dried, and immersed in equimolar N,N'-dicyclohexylcarbodi-imide (DCC, 0.1 mM) and N-hydroxysuccinimide (NHS, 0.1 mM) for 2 h and followed by isopropyl and DI water rinses. B2T antibody (mybiosource.com, San Diego, CA) was applied within the microfluidic channel and incubated for 18 h in a refrigerator at 4 °C. A self-assemble layer with carboxylic group terminal was formed on the Au electrode upon TGA incubation, and N-hydroxysuccinimide(NHS) esters were formed on top of this layer upon DCC/NHS incubation. Lysine and other terminal amine group reacts with the aforementioned NHS ester to form stable amide bonds.²⁶⁻²⁹ Human samples were diluted in 1% bovine serum albumin (BSA) in phosphate buffer solution (PBS) for all measurements.

Electrical measurements were made with an enhancement mode Silicon MOSFET (STMicroelectronics STP200N3LL) to amplify the electrical signal and provide the raw analog voltage for digital readout. The gate electrode of the MOSFET was externally connected to the sensor strip with a functionalized electrode, while a synchronous pair of electrical pulses were applied (~1.1 ms) between the drain electrode of the MOSFET and an auxiliary electrode on the sensor strips. An agilent infiniiVision DSO7054B oscilloscope was used to collect the analog drain waveform between the pull-up resistor and MOSFET, and the voltage at 750 μ s was extracted as an analog reading to calculate voltage gain between samples. A voltage-controlled oscillator (SN74S124N) was used to convert this analog waveform into discrete square waves of various frequencies with analog voltage as the input signal. A 12-bit asynchronous binary counter (SN74HC4040N) was used to record the number of pulses from the VCO output during the pulse duration. A built-in microcontroller was used to convert the 12-bit output into a digital reading on a liquid crystal display (LCD). Capacitance-voltage measurements of the strip were made using an Agilent 4284A precision LCR meter.

Human cerebrospinal fluid samples were collected from lumbar drains of nine patients in the Neurological Intensive Care Unit at the University of Florida Health Shands Hospital, and their B2T concentration is shown in Table I.

Human sample No.	Concentration (µg/ml) No. 1	Concentration (µg/ml) No. 2	Average concentration (µg/ml)	Standard deviation concentration (µg/ml)
1	19	17	18	1.4
2	24	20	22	2.8
3	9	8	8.5	0.7
4	5	6	5.5	0.7
5	9	9	9	0.0
6	1	1	1	0.0
7	4	1	2.5	2.1
8	70	75	72.5	3.5
9	0	1	0.5	0.7

III. DISCUSSION

Figure 1(a) shows the basic layout of the handheld device with a sensor strip installed. Unlike the previous model,¹⁸ the printed circuit board used in this work can be powered by a commercially available 9 V battery. The compact design allows handheld operation with a digital reading generated by a manual trigger. Standardized FFC/FPC connectors were used as strip connectors. Figure 1(b) shows the layout of the gold working electrode ($1035 \times 1500 \ \mu m^2$) and the auxiliary electrode ($450 \times 1500 \ \mu m^2$) adjacent to the working electrode. The scanning electron microscope (SEM) images in Fig. 2 show the gold surface formation after electrode-plated the graphite sensing electrode within the microfluidic channel.

To optimize the transistor gate voltage to use in this test and maximize the sensitivity, the effect of the gate voltage applied to the sensor (and ultimately to the gate electrode of the transistor) was studied using undiluted, tenfold, and 100-fold diluted CSF samples from patient No. 8. Figures 3(a) and 3(b) show the



FIG. 1. Photograph of the printed circuit board during operation powered by a commercially available $9 \vee$ battery (a) and photograph of microfluidic channels with the functionalized Au surface (b).

sensor's analog and digital response. At gate voltage above 1.7 V, both readings are relatively insensitive to this CSF sample for this particular transistor type, and desired changes in a linear fashion have been observed across this concentration range using a gate voltage of around 1.67 V. Therefore, this voltage has been used throughout this work.

Figure 4 shows biofunctionalized sensor strips' small-signal capacitance-voltage measurement using an Agilent 4284A precision LCR meter. The working electrode was DC-biased from 0 to +2 V with an oscillator frequency of 1 kHz at ± 0.1 V $_{\rm rms}$. From 1% BSA solution (reference) to the highest tested concentration (~0.7 μ g B2T/ml), there is a consistent decrease from around 17 to 12 nF. This is direct proof of the self-assembled antibody layer reactivity without the use of MOSFET amplification and shows the capacitance variation due to the binding of antigen and antibody layer on the Au electrode surface.³⁰ The transistor's input (gate) capacitance was 5.2 nF with gate resistance of 100 M Ω . Given the resistance of the sensor strip varies between 6000 and 8500 Ω , the variation of resistance between different concentrations becomes insignificant to this analysis. Therefore, the sensor (17-12 nF) and MOS structure (5 nF) can be considered as two capacitors connected in series. With decreased small-signal capacitance due to an increase in the B2T concentration, the overall capacitance decreases and, therefore, reduces the gate capacitance charging time and improves the speed of transient response of the gate pulse.

To establish a correlation between analog and digital reading in our sensor system, a human sample (patient No. 8) serially diluted down to $7.25 \times 10^{-12} \,\mu$ g/ml was used. As shown in Fig. 5, a higher B2T concentration led to a faster transient response as a steeper drop in the drain voltage response. Note that the voltage at 750 μ s was extracted from the oscilloscope waveform to calculate the gain in the analog signal. The respective analog voltage value



FIG. 2. Scanning electron microscope (SEM) images of surface morphology for graphite sensing electrode before and after 30 s of gold plating.



FIG. 3. Analog sensor voltage extracted at 750 μs from waveform at different gate voltages (a) and digital readings (b) using undiluted and tenfold and 100-fold diluted of human CSF (from sample No. 8) with known B2T concentration.

and digital reading associated with each concentration were taken by pulsing the sensor ten times (1.1 ms pulse) and averaging the recorded readings for each pulse.

Over a range of 1% BSA (reference) down to 7.25×10^{-12} g/ml, Fig. 6(a) shows that the analog voltage (extracted at 750 μ s within waveform such as in Fig. 5) decreased linearly. Between reference (no B2T) to 7.25×10^{-12} g/ml, a net voltage drop of 929 mV was observed with a sensitivity of 111 mV/dec within the range 7.25×10^{-5} to 7.25×10^{-12} g/ml (a 767 mV drop in analog voltage), with standard deviation ranges from 9 to 47 mV among tested concentrations. Figure 6(b) shows the digital reading for the same



FIG. 4. Small-signal capacitance-voltage measurement for functionalized biosensor strip for the various concentration of diluted human CSF (from sample No. 8) with known B2T concentration.

concentration range with a net change of 711 mV with a sensitivity of 86.7/dec from maximum B2T concentrations down to the limit of detection, with a standard deviation between 3 and 33 in digital reading for all concentration within the tested range. These data show the capability of such a system not only being able to detect up to the maximum limit of ELISA but also nearly 5 orders of



FIG. 5. Typical MOSFET drain waveform used for digital reading conversion for B2T concentration range from 7.25×10^{-5} to 7.25×10^{-12} g B2T/ml.





FIG. 6. Average voltage drop (ΔV) extracted at 750 μ s from drain waveform for various concentrations of B2T in 1% BSA using human sample No. 8 (a) and average digital readings (b) using test strip functionalized with B2T antibody.

magnitude lower than the limit of detection compared to commercially available ELISA kits. This wide dynamic range capability makes it suitable for direct testing of clinical samples without the need of further concentration.

Figure 7 shows data for pure, tenfold, and 100-fold dilution of all nine clinical samples to confirm the validity of the aforementioned system. The 27 data points were collected from all nine patients' samples. The sensitivity for such detection was 93.8/dec, which is similar to the sensitivity for that of Fig. 6(b) for individual patient samples after serial dilution. This result demonstrates that (1) the positive correlation has been proven between all nine samples' B2T values and digital readouts from the circuit board and (2), as seen by similar sensitivity in digital reading between Fig. 6(b) (one patient, 86.7/dec) and Fig. 6 (nine patient, 93.8/dec),



FIG. 7. Average digital readings for all nine human samples with known B2T level as-is, after tenfold, and 100-fold dilution in 1% BSA using test strip functionalized with the B2T antibody.

the sensitivity of digital readout can be maintained even when measured across different patient samples and potentially exposed to various background ions, proteins, and contaminations such as blood and other bodily fluids. A summary table for comparison

 TABLE II.
 Comparison of various available detection methods for cerebrospinal fluid leak.

Method	Detection limit	Detection time	Reference
Enzyme-linked immunosorbent assay (ELISA).	1.25–80 µg/ml	1–5 h assay time 1–4 days turnaround time for commercial lab	31,32
Immunofixation electrophoresis (IFE)	2 µg/ml	2.5 h	33
Computed tomography (CT) and magnetic resonance imaging (MRI)	Non-quantitative	N/A	2–5,7,8,13,34
Electrical double pulse measurement (this work)	7 ng/ml to 73 μg/ml	Less than 1 s	This work

across different detection methods for CSF has been shown in Table II, showing the advantage for pulse measurement for biomarker detection, exemplified by B2T detection in this work.

IV. CONCLUSION

This work presents the detailed development of a handheld rapid biomarker detection system with transistor-based signal amplification. When applied in human biomarker detection, for example, human cerebrospinal fluid in this work, an actual limit of detection of 5 orders of magnitude lower than the currently available ELISA test has been achieved. The specificity has been studied by comparing cerebrospinal fluid across different patient samples and showed an excellent correlation between digital readout and their respective beta-2-transferrin values.

ACKNOWLEDGMENT

The work at UF is partially supported by Grant No. R01-DE025001 from National Institute of Dental and Craniofacial Research (NIDCR) at The National Institutes of Health (NIH).

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

¹C. Martín-Martín, G. Martínez-Capoccioni, R. Serramito-García, and F. Espinosa-Restrepo, BMC Res. Notes 5, 459 (2012).

²N. V. Vemuri, L. S. P. Karanam, V. Manchikanti, S. Dandamudi, S. K. Puvvada, and V. K. Vemuri, Indian J. Radiol. Imaging 27, 441 (2017).

³J. S. Zapalac, B. F. Marple, and N. D. Schwade, Otolaryngol. Head Neck Surg. 126, 669 (2016).

⁴P. H. Carey, J. Yang, F. Ren, C.-W. Chang, J. Lin, S. J. Pearton, B. Lobo, and M. E. Leon, J. Electrochem. Soc. 166, B708 (2019).

⁵D. E. Normansell, E. K. Stacy, C. F. Booker, and T. Z. Butler, Clin. Diagn. Lab. Immunol. 1, 68 (1994).

⁶T. Görögh, P. Rudolph, J. E. Meyer, J. A. Werner, B. M. Lippert, and S. Maune, Clin. Chem. 51, 1704 (2005).

⁷P. H. Carey et al., J. Electrochem. Soc. 167, 037507 (2019).

⁸O. Delaroche, P. Bordure, E. Lippert, and M. Sagniez, Clin. Chim. Acta 245, 93 (1996).

⁹L. Bernasconi, T. Pötzl, C. Steuer, A. Dellweg, F. Metternich, and A. R. Huber, Clin. Chem. Lab. Med. 55, 554 (2017).

¹⁰C. Meco and G. Oberascher, Laryngoscope 114, 991 (2004).

11 A. E. Kita, D. W. Bradbury, Z. D. Taylor, D. T. Kamei, and M. A. St. John, Otolaryngol. Head Neck Surg. 159, 824 (2018).

¹²E. L. Sanders, R. J. Clark, and J. A. Katzmann, Clin. Chem. 50, 2401 (2004).

¹³B. C. Lobo, M. M. Baumanis, and R. F. Nelson, Laryngoscope Invest. Otolaryngol. 2, 215 (2017).

14H. J. Son, A. Karkas, P. Buchanan, J. P. Giurintano, P. Theodosopoulos, M. L. Pensak, and R. N. Samy, Laryngoscope 124, 1204 (2014).

¹⁵R. F. Nelson, B. J. Gantz, and M. R. Hansen, Otol. Neurotol. 36, 476 (2015).

¹⁶B. Byrne, E. Stack, N. Gilmartin, and R. O'Kennedy, Sensors 9, 4407 (2009)

17 S. Y. Lu, S. S. Shan, J. Yang, C. W. Chang, F. Ren, J. Lin, S. Pearton, and Y. Te Liao, Proceedings of Annual International Conference of the IEEE Engineering in Medicine and Biology Society EMBS Berlin, Germany 23-27 July 2019 (IEEE, New York, 2019), pp. 5761-5764.

¹⁸M. Xian et al., J. Vac. Sci. Technol. B **39**, 033202 (2021).

¹⁹S.-S. Shan et al., IEEE Trans. Biomed. Circuits Syst. 14, 1362 (2020).

20 M. Xian, P. H. Carey, C. Fares, F. Ren, S. S. Shan, Y. Te Liao, J. F. Esquivel-Upshaw, and S. J. Pearton, 2020 IEEE Research and Applications of Photonics in Defense Conference, RAPID 2020-Proceedings (IEEE, New York, 2020).

²¹B. S. Kang, F. Ren, M. C. Kang, C. Lofton, W. Tan, S. J. Pearton, A. Dabiran, A. Osinsky, and P. P. Chow, Appl. Phys. Lett. 86, 1 (2005).
 ²²I. Sarangadharan *et al.*, Biosens. Bioelectron. 100, 282 (2018).

23 J. Yang, P. Carey, F. Ren, M. A. Mastro, K. Beers, S. J. Pearton, and I. I. Kravchenko, Appl. Phys. Lett. 113, 032101 (2018).

²⁴J. Yang, P. Carey, F. Ren, Y. L. Wang, M. L. Good, S. Jang, M. A. Mastro, and S. J. Pearton, Appl. Phys. Lett. 111, 202104 (2017).

25 B. S. Kang et al., Appl. Phys. Lett. 89, 122102 (2006).

²⁶M. Y. Bai and S. Z. Liu, Colloids Surf., B 117, 346 (2014).

27 T. Betancourt, J. D. Byrne, N. Sunaryo, S. W. Crowder, M. Kadapakkam, S. Patel, S. Casciato, and L. Brannon-Peppas, J. Biomed. Mater. Res., Part A 91, 263 (2009).

²⁸C. Ju, Y. Tang, H. Fan, and J. Chen, Anal. Chim. Acta 621, 200 (2008).

29 L. T. Tran, T. Q. Tran, H. P. Ho, X. T. Chu, and T. A. Mai, J. Nanomater. 2019, 9 (2019).

³⁰M. Y. Mulla, E. Tuccori, M. Magliulo, G. Lattanzi, G. Palazzo, K. Persaud, and L. Torsi, Nat. Commun. 6, 6010 (2015).

³¹Biomatik, 2021, https://www.biomatik.com/elisa-kits/ see: human-beta-2-transferrin-2tf-elisa-kit-cat-ekc32844/

32 ARUP Laboratories Test Directory, 2021, see: https://ltd.aruplab.com/Tests/ Pub/0050047/

33 C. Papadea and R. J. Schlosser, Clin. Chem. 51, 464 (2005).

34A. Warnecke, T. Averbeck, U. Wurster, M. Harmening, T. Lenarz, and T. Stöver, Arch. Otolaryngol. Neck Surg. 130, 1178 (2004).