Nanobiomimetic Memristor/memcapacitors' Function as a Voltage Sensor for Direct and Reagent-free Detection of sub pg *Lipopolysaccharide* (LPS) in Different Types of Milks for Infants

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ABSTRACT

Lipopolysaccharide (LPS) is a common endotoxin from *E. Coli* bacteria, and is the major source causing infectious diseases in over 20 million people worldwide. We developed a nanostructured biomimetic memristor/memcapacitor device for the direct measurement of single bacterium in sub pg LPS in human milk for infants under antibody-free, tracers-free, and reagent-free conditions by a double step chronopotentiometry (DSCPO) method. The linear concentration range is up to 0.5µg/mL in 40 µL specimen samples with a Detection of Limits (DOL) of 0.3 ng/mL with the energy density range between 123.2 and 0.11 µWHr/cm^3 using human milk specimens at 0.25 Hz and ±10 nA with an imprecision value 3.0% (n=12) against 9.8 to -0.042 µWHr/cm^3 for organic milk samples. The microbiota in the human milk initiated the warning signals while the LPS is present. Unfortunately, USDA certified organic milk for infants is lacking this protection for infants. Using our sensor as a model for a single neuron of a newborn's brain, we tested the impact of different types of milk upon the integrity of the reversible membrane action/resting potential and presented energy density maps ranging over 0.25- 300Hz. The results demonstrated human milk promotes brain development and memory over organic milk. Therefore, human milk is recommended for infants.

***Keywords*:** Nanobiomimetic memristor/memcapacitor; Nanostructured; LPS; Reversible membrane potential; Slow-Wave-sleeping; Voltage Sensor; Reagent-free; Tracer-free; Energy Density; Endotoxin;

INTRODUCTION

Lipopolysaccharide (LPS) is a common endotoxin from *E. Coli* bacteria, and is the major source causing infectious diseases over 20 million people worldwide. LPS is a major contaminant found in commercially available proteins, and it is also the major contaminate in biological ingredients in drugs and injectables, because even small amount of endotoxin can cause side effects such as endotoxic shock, injury, and even death; therefore a strengthened standard of drug purity is needed. However, removing LPS from pharmaceutical products, for intravenous application to 5 endotoxin units (EU) per kg of body weight per hr, is a challenge to researchers who thought this standard is unachievable [1-2]. E. Coli bacteria covers 75% its outer layer membrane with gram-negative exdotoxin LPS, and it stimulates the host's immune response of cytokines [3-4]. Recently, researchers reported LPS penetrates the gut-immune-barrier (GIB) causing liver infection [5]; LPS leaking from the tight junction in the gut membrane into the blood stream cause many diseases, autism, obesity, diabetes, Alzheimer's, chronic pain, and inflammation [6-10]. Furthermore, LPS can break the blood-milk barrier into the milk and may cause harm, as reported from collected cow milk, which was compromised by LPS, and may have caused mastitis [11]. A recently published paper reported human milk offers an advantage to correlate positively with gut microbiota and to maintain healthy oligosaccharide (HMO) isomers which are specific to human milk and that are necessary in the newborn infant's gut in the first week [12].

A paramount challenge was put on the researchers and industry as a whole for improving the LPS detection methods with more simplified procedure, more accuracy and precision, faster, and more affordable options. Because previously, a lack of sensitivity associated with the protein interference plus time consuming antibody and tracer assays hampered the ability to realize the unmet goals and fulfill these needs.

It is a well-accepted fact that breast-feeding offers more benefits for human babies' growth in nutritional and immune defense over cow milk [13-15], and it has been strongly recommended, as published by the World Health Organization [15]. We found very few tests or sensors, if any, to assess the energy outcomes at different neuronal synapse frequencies, such as slow-wave-sleeping and fast gamma frequency, between breast-feeding using human milk as compared with feeding organic cow milk in the presence of LPS challenge. We believe that this testing is important because not only it will increase our knowledge, but also it will provide first hand convincing evidence for preferring human milk for feeding infants in regards to the energy requirement for mental and physical development of infants. Our goal for this project is to develop a nanostructured memcapacitor/memristor sensor for antibody-free, reagent-free direct measurement of pg LPS, and to assess the energy outcome comparing human milk with cow milk. The

intention is that the memcapacitor/memristor device represents, in concept, a baby's single neuron to "feel" the energy gain or loss in the presence of LPS. This project is based on our prior experience in using the memristor/memcapacitor to mimic hippocampus-neocortex neuronal network circuitry [16-20].

EXPERIMENTAL

Fabrication of the Nanostructure Self-Assembling Membrane (SAM) Gold Memristor Chips

The nanostructured biomimetic SAM was freshly prepared according to the published procedures based on cross linked conductive polymers of triacetyl-ß-cyclodextrin (TCD), polyethylene glycol diglycidyl ether (PEG), poly(4-vinylpyridine) (PVP) and ß-CD copolymer with appropriate amount of propositions on gold chip [21-22]. The chemicals were purchased from Sigma and went through purification procedures before use. A mixture of o-nitrophenyl acetate (o-NPA) in a molar ratio 1000:1 to the TCD mixture was incubated for 2 hrs at 35°C; then the mixture was injected onto the gold surface and incubated for 48 hrs at 35°C. After that, we followed the clean procedures for completion of the SAM fabrication [21-22].

Characterization of the Membrane

The morphology of the AU/SAM was characterized using an Atomic Force Microscope (AFM) (model Multimode 8 ScanAsyst, Bruker, PA). Data Collected in PeakForce Tapping Mode. Probes used were ScanAsyst-air probes (Bruker, PA). The silicon tips on silicon nitride cantilevers have 2-5 nm radius. The nominal spring constant 0.4N/m was used. Figure 1 on the left illustrates 3D horizontal conformational structure of the memristor/memcapacitor in the presence of an embedded o-NPA. Fig. 2 shows the 3D AFM image before the o-NPA was embedded on gold.

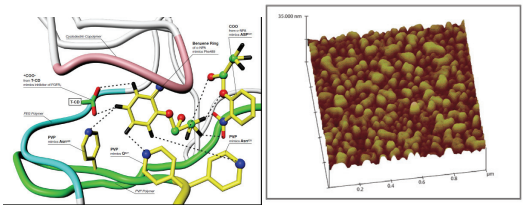


Fig. 1 (L) illustrates the 3D memcapacitor proposed conformational structure and electro-relay blocks in the presence of o-NPA. Fig. 2 (R) shows the 3D AFM image before adding o-NPA.

Frequency Affects on Memristor/Memcapacitor's Performance

Evaluations of frequency affect on memristors' performance were conducted by Cyclic Voltammetric method (CV) in pH 7.0 saline solution at room temperature from scan rate 1 Hz to 1KHz without using any biological specimen. Data are to be used for comparing between fresh human milk and USDA certified organic milk for infants with or without the presence of LPS covering the same range of real-time synapse action/resting potential pulses at different frequencies against controls.

Assessing Energy Outcomes under Challenges of LPS

The Double Step Chronopotentiometry (DSCPO) method was used for assessing energy outcomes of slow-wave-sleeping (SWS) at 0.25Hz **a**nd 200Hz under the challenge of LPS at concentration range from 0, 50, 100, 500, to 1000 ng/mL of 4-5 levels with triplicates at ±10 nA, respectively. Samples tested at each level without prior sample preparation, such as dilution or heating. The experiments were conducted at room temperature. The milk samples compared were human milk and USDA certified organic cow milk for infants, with and without LPS. Human milk was collected from a normal subject who breastfeeds a 1 month-old newborn (Lee Biosolutions Corp.,). An electrochemical workstation was used (Epsilon, BASi, IN) with a software package from BASi. Origin Pro 2016 (Origin Lab Corp., MA) was used for all statistical data analysis and figure plotting.

RESULTS AND DISCUSSIONS

Biomimetic *Fibroblast Growth Factor Receptor 1* (FGFR1) SAM membrane.

FGFR1 is one of family receptors of tyrosine kinases. It plays important roles in embryonic development, angiogenesis, wound healing, and malignant transformation, bone development, and metabolism [23-24]. Y. Zhang's group reported mice with deleted FGFR1 exhibited an increased mobilization of endothelial progenitor cells (EPCs) into peripheral blood undergoing endotoximia, and the endotoximia was induced by injection of LPS [24]. Our project's initial step is to build a model device such that the device's SAM membrane mimics the FGFR1 in the presence of LPS, which acts as a model metabolic product to access the FGFR1 function. By using this model to compare the effects of fresh human milk and organic cow milk at different frequencies of neuronal action/resting pulses at slow-wave sleeping (SWS) and fast gamma with or without LPS, energy efficiency or deficiency maps for infant brain cells will reveal the conclusions between the two distinct medias. Fig. 1 shows the electron-relay system and Fig. 2 is the AFM in a gold chip with the TCD/PEG/PVP/copolymer before adding o-NPA for embedding.

Frequency Affects on Memristor/Memcapacitor's Performance

Fig. 3's i-V hysteresis curve was demonstrated with a switch point at the origin (0, 0) at almost all frequencies, except at kHz high frequency in the control medium PBS only. When this perfect hysteresis behavior peaks, especially at SWS frequency with sensitive *Direct Electron-relay Transfer* (DET), and the switch point originates at origin, it indicates a healthy "newborn single neuron" exists before the feeding. Nonlinear frequency influence on current intensity is

the characteristics of the memristor reported in literature [17-20, 25-28]. Fig. 4R depicts 500 ng/mL LPS reduced the signal intensity at the SWS significantly in human milk. LPS eliminated the original sensitive DET peaks, and it means LPS first makes the neuron lose its sense of danger in the presence of toxins; this phenomenon matched our prior observations in the work of β-amyloid (Aβ) that caused Alzheimer's sensory loss at SWS [17-18, 20]. In the worst case, the cow milk with LPS impaired heavily the DET sensory ability of our model neuron as compared to that in human milk, as shown in Fig. 5.

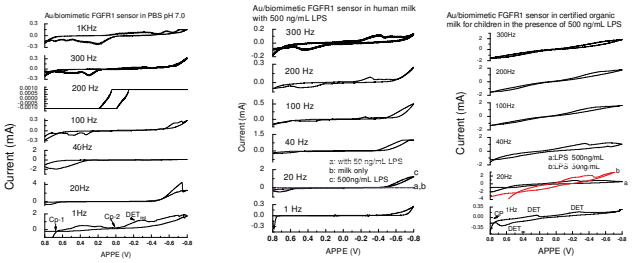


Fig 3 (L) Illustrates the frequency affects from 1 Hz to 1 KHz on hysteresis of the i-V curve of the memristor in PBS. Fig. 4 (m) depicts frequency affects with 500ng/mL LPS in human milk against control. Fig. 5(R) depicts the i-V curve in the presence of 500 ng/mL LPS in organic milk.

Assessing Energy Outcomes

Assessing energy outcomes was conducted by comparing human milk and certified organic milk, both with and without LPS, at 0.25 Hz and 200 Hz, respectively, using the DSCPO method. Fig. 6A, 6B and 6C depict the synapse pulse profiles using human milk and compared samples using PBS media at 0.25, 40, 100, 200 and 250Hz, respectively, without LPS. Curves overlap between the two media, and indicate human milk has no protein interference with the "single neuronal cell" as far as the energy concerns. Fig. 6D compares the signal intensity of human milk is inversely proportional to a wide range of LPS concentrations from 50 ng/mL to 1000 ng/mL at 0.25Hz. At 50 to 100 ng/mL, the biphasic pulse shape integrity is maintained; however the insert curves show that at 500 and 100 ng/mL, the biphasic pulses are destroyed, and the cell net voltage intensity is close to zero. Fig. 6E demonstrates a similar trend at a higher concentration at 200Hz that the cell net voltage closes to zero but at 50ng/mL the signal increases more than 30% compared with that at zero LPS, which is a bad effect of wasting energy. Fig. 7 depicts LPS in organic milk's impact on the synapse pulse intensity at 0.25 and 200Hz, respectively. First, in organic cow milk, without LPS, at SWS frequency, the net cell voltage is smaller than a 1/200 fraction of that when using human milk. Second, because of the cow milk's lack of critically needed microbiota and proteins, as compared to human milk, the biphasic integrity of the synapse pulses in the neuronal model, which is key to connect its sensory capacity to the energy capacitor at SWS, is no longer maintained. The single cell doesn't have enough energy to sustain a reversible membrane potential. This is critical to brain development in newborns. In the presence of LPS, regardless if the frequency is at SWS and 200Hz, all outcomes of neuronal pulse energy are close to zero. The chances of or opportunity for the neuronal cell to sense the presence of toxins, like LPS, when using cow milk are negligible, because the cell has no energy to sustain a reversible membrane potential.

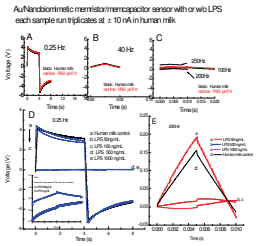


Fig. 6A, 6B and 6C depict the single neuronal pulse at 0.25, 40 and 250Hz compared in pH 7.0 PBS buffer (red curve) and the human milk (black curve), respectively without LPS. Fig. 6D and 6E compares the LPS effects on human milk over 0, 50 to 1000 ng/mL at 0.25 and 200Hz, respectively.

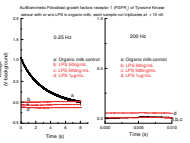


Fig. 7(L) and 7(R) compares the LPS effects on organic cow milk over 0, 50, 500 to 1000 ng/mL at 0.25 and 200Hz, respectively.

Quantitation of LPS

Fig. 8A illustrates the linear calibration curve at 0.25 Hz of volumetric energy density vs. LPS concentration range from 50 to 500 ng/mL using human milk, and it produced a linear regression equation Y= 125 – 0.25X, r=0.9993 (n=12), P<0.0001, Sy/x=2.0. The Detection of Limits (DOL) is 0.3 ng/mL, i.e., in a 40µL sample; it detects 12 pg LPS using a 1 cm^3 sensor, our sensor is $3.11x10^{-7}cm^3$. Herein the DOL in our sensor is $3.73x10^{-18}$g for LPS means we are able to detect a single E. Coli bacterium because an antigen is in 10^{-17}g range. At 0.25Hz, the energy density ranges between 123.2 and 0.11 µWHr/cm^3 using human milk specimens with an imprecision value 3.0% compared the energy range of 9.8 to -0.042 µWHr/cm^3 for organic milk. Fig. 8B shows the nonlinear curve for LPS at 200Hz using human milk. In contrast, Fig. 8C and 8D show no sensitivity towards LPS over the same concentration range using organic milk. Human milk offers more than 12.5-fold high energy than organic milk and 100-fold sensitivity for LPS than organic milk.

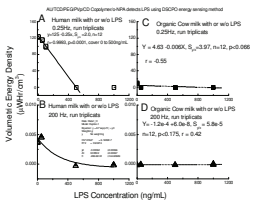
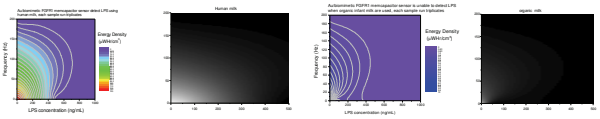


Fig. 8A and 8B illustrate the quantitation calibration plots of LPS in human milk at 0.25 and 200Hz, respectively compared with LPS in organic milk at 0.25 and 200Hz in Fig 8C and 8D, respectively.

Energy Density Map with Multiple Variables

The energy density contour maps associated with the images are presented in Fig. 9 with energy density as Z, LPS concentration as X, and discharge pulse frequency as Y. The Fig. 9A and B is for the human milk samples and Fig. 9C and D is for organic milk samples. It is obvious human milk produced tremendous energy showing as light, and the intensity is more than 10-fold higher, especially at SWS even in the presence of LPS.



A B C D

Fig. 9A, 9B depicts the energy density (as Z) contour map and image as a function of LPS concentration (as X) and synapse Frequency (as Y) in human milk compared with Fig. 9C and 9D using organic milk.

CONCLUSION

We have demonstrated the advantage of the memristor/memcapacitor device with the biomimetic FGFR1 membrane that plays favor with the microbiota in human milk and it offers warning signs in orders of magnitudes sensitive to the single bacterium's LPS presence in the human milk over organic milk. The same amount of LPS acted differently in organic milk, and the main difference is the absence of microbiota immune protection when it contacts with the neuronal sensor. Herein the LPS was able to suppress and distort the synapse signal at SWS that leads to a very weak and severally lower energy outcome hampering brain development. The technique offers enough sensitivity to energy changes in 78 fWHr with Detection of Limits (DOL) $3.73x10^{-18}$g of LPS under antibody-free, tracers-free, and reagent-free conditions for fast and real-time monitoring the quality of milk for improving of infants' health.

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