

Human Milk Shows Immunological Advantages over Organic Milk Samples for Infants in the Presence of Lipopolysaccharide (LPS) in 3D Energy Maps Using an Organic Nanobiomimetic Memristor/Memcapacitor

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Abstract: Human milk is well known for its immunological advantages of protection and support for healthy early childhood cognitive development and prevention of chronic diseases over cow milk for infants. However, little is known about how the immunological advantages are linked to reduce *Pathological High Frequency Oscillation* (pHFO) regarding neural synapse net energy outcomes when lipopolysaccharide (LPS) attacks at a clinical concentration range compared with that in cow milk in a 3D energy map. We developed a nanostructure biomimetic memristor/memcapacitor device with a dual function of chronoamperometric (CA) sensing/voltage sensing for the direct quantitative evaluation of immunological advantages between human milk and organic cow milk for infants in the presence of wide LPS concentration ranges; those ranges were between 5.0 pg/mL to 500 ng/mL and from 50 ng/mL to 1 µg/mL for both a CA and a voltage method, respectively. The Detection of Limit (DOL) results are as follows: 3.73×10^{-18} g LPS vs. 1.2×10^{-16} g LPS in 40 µL milk samples using the $3.11 \times 10^{-7} \text{cm}^3$ voltage sensor and the 0.031cm^2 CA sensor, respectively, under antibody-free and reagent-free conditions. The 3D energy map results show that cow milk is ten-times more prone to *E. Coli* attack, and the positive link was revealed that Pathological High Frequency Oscillation (pHFO) formations occurred over the studied LPS concentration range from 50 ng/mL up to 1000 ng/mL from *Rapid Eye Movement* (REM) sleep frequency, fast gamma frequency to *Sharp Wave-Ripple Complexes* (SPW- R) frequency. There had no pHFO with human milk samples at *Slow Wave Sleeping* (SWS), REM and SPW- R frequencies. The microbiota in the human milk samples successfully overcame the endotoxin attack from *E. coli* bacteria, however the pHFO only occurred at fast gamma frequency linked with the LPS level ≥ 500 ng/mL. Organic milk samples show an order of magnitude lower synapse energy density compared with human milk at SWS for with or without LPS, respectively. Copyright © 2016 IFSA Publishing, S. L.

Keywords: Nanobiomimetic neuronal memristor/memcapacitor, Spatial-temporal orientation, 3D energy map, Pathological High Frequency Oscillation (pHFO), Slow Wave Sleeping (SWS), Rapid Eye Movement (REM) sleeping, Dual sensing.

1. Introduction

There have been strong recommendations for mothers to breastfeed newborns and infants across the United States and around the world for the purpose of better immunological protection, the promotion and support of healthy early childhood cognitive development, and the prevention of obesity and other chronic diseases in order to fulfill critical public health goals published by the U.S. Center for Disease Control (CDC) and the World Health Organization (WHO) [1-2]. WHO published a detailed summary document that describes the advantages of human milk for infants' development. This document is a good guidance to mothers for obtaining knowledge in breastfeeding [3]. That human milk offers advantages over cow milk for babies' brain development is a well accepted truth. However, we are still far from the CDC's 2010 breastfeeding rate goal [2]. In publications on the topic of infant milk research, there are very few, if any, in-depth studies on the correlations between neural synapse energy output and milk quality comparing human breast milk and cow milk, in particular, analyzing the correlations between harmful *Lipopolysaccharide* (LPS) potentially exists in cow milk induced *pathological high frequency oscillation* (pHFO) and potentially more beneficial human milk induced good *high frequency oscillation* (HFO) in order to draw the attention of parents and support of health care professionals to impart and implement the CDC and WHO breastfeeding guidance.

Newborns sleep an average 16-18 hours per day, of which 50% of the time is spent in rapid eye movement sleep (REM), and 50% of time is in Slow-Wave Sleeping (SWS); sleep quality is dominated by SWS [4-5]. As the literature reported, REM usually takes place at greater than 10 Hz (α frequency) and SWS is between 0.1-3.5 Hz (δ frequency) [4-5].

A normal neural network circuitry constantly fires HFO (150-200 Hz) producing synchronization within the connection between the hippocampus and neocortex for the purpose of long term memory storage during SWS. Where pHFO (200-600 Hz) fires randomly, it leads to seizures and epilepsy [6-8]. The biggest problem in epilepsy research, as the Editor Noebels explained in the book, is that researchers are "*not clear how abnormal synchrony is generated during pHFO. Clearly there is a need for additional studies that will differentiate normal from pathologic HFO in vitro and in vivo.*" [6]. Our prior published works have advanced the first step by attempting to identify the difference between pHFO and HFO. We used a 3D mapping technology to detect a sub pM level β -amyloid induced abnormal neural network circuitry based on nanostructure biomimetic memcapacitors that have an amperometric/voltage sensing functions [9-12].

Lipopolysaccharide (LPS) is a common endotoxin from *E. coli* bacteria, and is a major cause of infectious diseases in over 20 million people

worldwide. LPS is a major contaminant found in commercially available proteins, and it is also the major contaminant in biological ingredients in drugs and injectables. Even small amounts 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 was unachievable [13-14]. *E. coli* bacteria covers 75 % its outer layer membrane with gram-negative endotoxin LPS, and it stimulates the host's immune response of cytokines [15-16]. Recently, researchers reported LPS penetrates the gut-immune-barrier (GIB) causing liver infection [17]; LPS leaking from the tight junction in the gut membrane into the blood stream causes many diseases, autism, obesity, diabetes, Alzheimer's, chronic pain, and inflammation [18-22]. Furthermore, LPS can break the blood-milk barrier and pass into cow milk and may cause harm, as reported from collected samples which were compromised by LPS and may have caused mastitis [23]. 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 necessary in the newborn infant's gut in the first week of life [24].

A paramount challenge was put on researchers and the pharmaceutical industry as a whole: to improve the LPS detection methods with a simplified procedure, more accurate and precision, and faster, more affordable options. 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 over feeding cow milk for human babies' growth in both nutritional content and immune defense [1-3, 25-27]. Our group's prior published work, based on a nanostructure biomimetic memristor/memcapacitor [28,] was able to directly measure sub pg LPS spiked in different types of milk under reagent-free conditions. In facing LPS challenge, we also demonstrated that human milk has energy outcomes at SWS an order of magnitude higher than cow milk [28]. Our goal for this project is to evaluate the immunological advantage of human milk vs. organic cow milk regarding the pHFO formation at LPS challenges. The intention is that the memcapacitor/memristor device is a sensor that represents, in concept, a baby's single neuron which is able to "feel" and react to the energy gain or loss in the presence of LPS. Our focus will be to determine how the pHFO occurs with dosage changes of LPS and the frequency change from SWS to 200 Hz. Of course, we will also demonstrate the memristor/memcapacitor's immunological capability in 3D contour mapping is based on a dual quantitative measurement of LPS in amperometric

/voltage method while showing the advantage of human milk over cow milk. This project is based on our prior experience in using the memristor/memcapacitor to mimic hippocampus-neocortex neuronal network circuitry [9-12, 28-32].

2. Experimental

2.1. Fabrication of the Nanostructure Self-Assembling Membrane (SAM) Gold Memristor/memcapacitor Chips

The nanostructure biomimetic SAM was freshly prepared according to the published procedures based on cross linked conductive polymers of triacetyl- β -cyclodextrin (TCD), polyethylene glycol diglycidyl ether (PEG), poly(4-vinylpyridine) (PVP) and β -CD copolymer with the appropriate amount of propositions on a gold chip [31-32]. 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 [31-32].

2.2. 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. Fig. 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.

2.3. Frequency Affects on Memristor/ Memcapacitor's Performance

Evaluations of frequency's affect on memristor performance were conducted by Cyclic Voltammetric method (CV) in pH 7.0 saline solution at room temperature from a scan rate of 1 Hz to 1 kHz without using any biological specimen. Data are to be used for comparison 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.

2.4. Assessing Energy Outcomes under Challenges of LPS

The Double Step Chronopotentiometry (DSCPO) method, as the voltage method, was used for assessing energy outcomes of slow-wave-sleeping (SWS) at 0.25 Hz and 200 Hz under the challenge of LPS at concentration ranges from 0, 50, 100, 500, to 1000 ng/mL of 4-5 levels with triplicates at ± 10 nA, respectively. Samples were 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. OriginPro 2016 (Origin Lab Corp., MA) was used for all statistical data analysis and figure plotting.

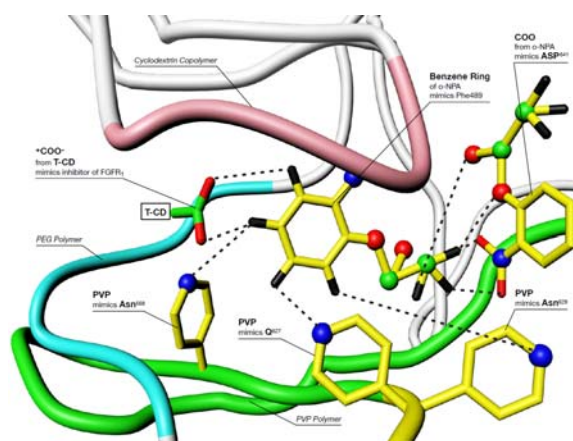


Fig. 1. 3D memcapacitor proposed conformational structure and electro-relay blocks in the presence of o-NPA.

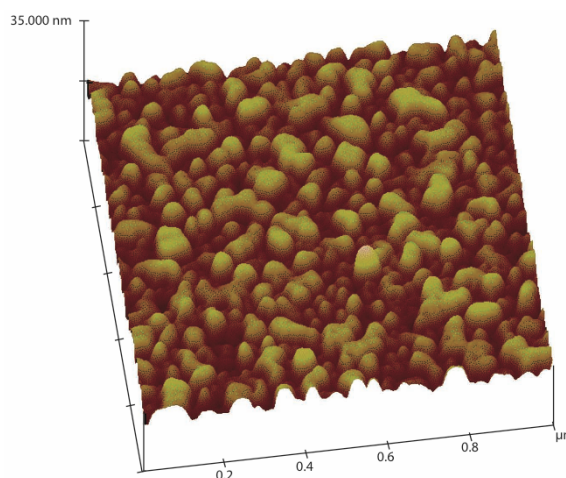


Fig. 2. 3D AFM image before adding o-NPA.

2.5. Quantitation of LPS

Quantitation of LPS was conducted with two methods. The first was a Chronoamperometric (CA) method under two steps of fixed potential: -50 mV and -400mV with each step duration of 100ms, and the data rate is 20 kHz at room temperature under the conditions of antibody-free, radioactive tracer-free and reagent-free in certified organic milk for infants with seven LPS challenge levels from 5.0 pg/mL to 500 ng/mL against controls, each sample run triplicates.

The second quantitation method was the voltage method, i.e., the DSCPO method, and the conditions were the same as described in the section of *Assessing Energy Outcomes under Challenges of LPS* by using human milk and organic cow milk samples under 4-5 LPS challenges from 50 ng/mL to 1000 ng/mL, respectively at ± 10 A against controls at 0.25 and 200 Hz, respectively. Freshly obtained samples were without pretreatment. Human milk cooled by dry ice was delivered to the laboratory, and it was brought to room temperature naturally without any heating before spiking the LPS. All water used was autoclaved and double distilled from Fisher Scientific. LPS was purchased from Sigma, and it was dissolved in autoclaved and filtered PBS pH 7.0 buffers.

2.6. A 3D Contour Mapping Method for Evaluation of Human Milk Immunological Advantage under the LPS Challenge

The data obtained from the quantitation using the voltage method was used for evaluation of human milk immunological advantage under LPS challenges compared with that of the organic cow milk samples in 3D mapping method. The energy density results were put into the "y" column, the spiked LPS concentration over 0.0 to 1000 ng/mL was put into the "x" column, and the frequency was at the "z" column having two levels of 0.25 to 200 Hz. After converting the three data columns into a random XYZ correlation matrix, one can plot the contour maps and analyze the spatiotemporal formation of the pHFO, if it exists among human milk or organic milk samples. The real-time data obtained from the DSCPO method was converted to volumetric energy density, $E = C_s \cdot (\Delta V)^2 / (2 \times 3600)$, where C_s is the specific volumetric capacitance, $C_s = [-i \cdot \Delta t / \Delta V] / L$, C_s is in F/cm^3 [33-34], Δt is the time in second, ΔV is the voltage in V, i is the current in Amps, and L is the volume in cm^3 .

3. Results and Discussions

3.1. 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 [35-36]. Y. Zhang's group reported mice with deleted FGFR1 exhibited an increased mobilization of endothelial progenitor cells (EPCs) into peripheral blood undergoing endotoxemia, and the endotoxemia was induced by injection of LPS [36]. Our project's initial step is to build a model device such that the device's SAM membrane mimics the FGFR1 receptor 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 SWS and fast gamma frequency with or without LPS conditions to find out whether or not milk samples are energy efficiency or deficiency on the biomimetic brain cells will provide useful information to reveal which type of milk samples is immunologically advantage to infants. 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.

3.2. Frequency Affects on Memristor/Memcapacitor's Performance

Fig. 3(a)'s i-V hysteresis curves were demonstrated with a switch point at the origin (0, 0) at almost all frequencies, except at kHz high frequency in the control medium PBS. When these perfect hysteresis behavior peaks occurred, especially at SWS frequency with a sensitive *Direct Electron-Transfer* (DET), and the switch point originates at origin, it indicates a healthy "newborn single neuron" exists before "feeding" it milk samples. Nonlinear frequency influence on current intensity is a characteristic of the memristor as reported in literature [9-12, 37-40]. Fig. 3(b) shows the controls in organic milk samples over 1 Hz to 1 kHz. The significant difference observed between the organic milk control, the PBS control, and the human milk control at SWS is that the organic milk did not have a butterfly type DET_{red} peak near -0.1 to -0.2 V, where as PBS and human milk had this peak and cross-points near the origin. Rather, the organic milk control had a strange DET_{red} peak at -0.595 V, and the control missed the cross-point near zero V. Further investigation is needed to determine what substances caused the unknown DET peak to occur. Fig. 3 (c) is the human milk controls with well-defined sensory DET butterfly peaks crossed near the origin at SWS. Fig. 3 (d) depicts that in human milk, 500 ng/mL LPS reduced the signal intensity at the SWS significantly. The LPS eliminated the original sensitive DET peaks, and that means the 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 (A β) that caused Alzheimer's sensory loss at SWS [9-12,

30]. 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. 3 (e).

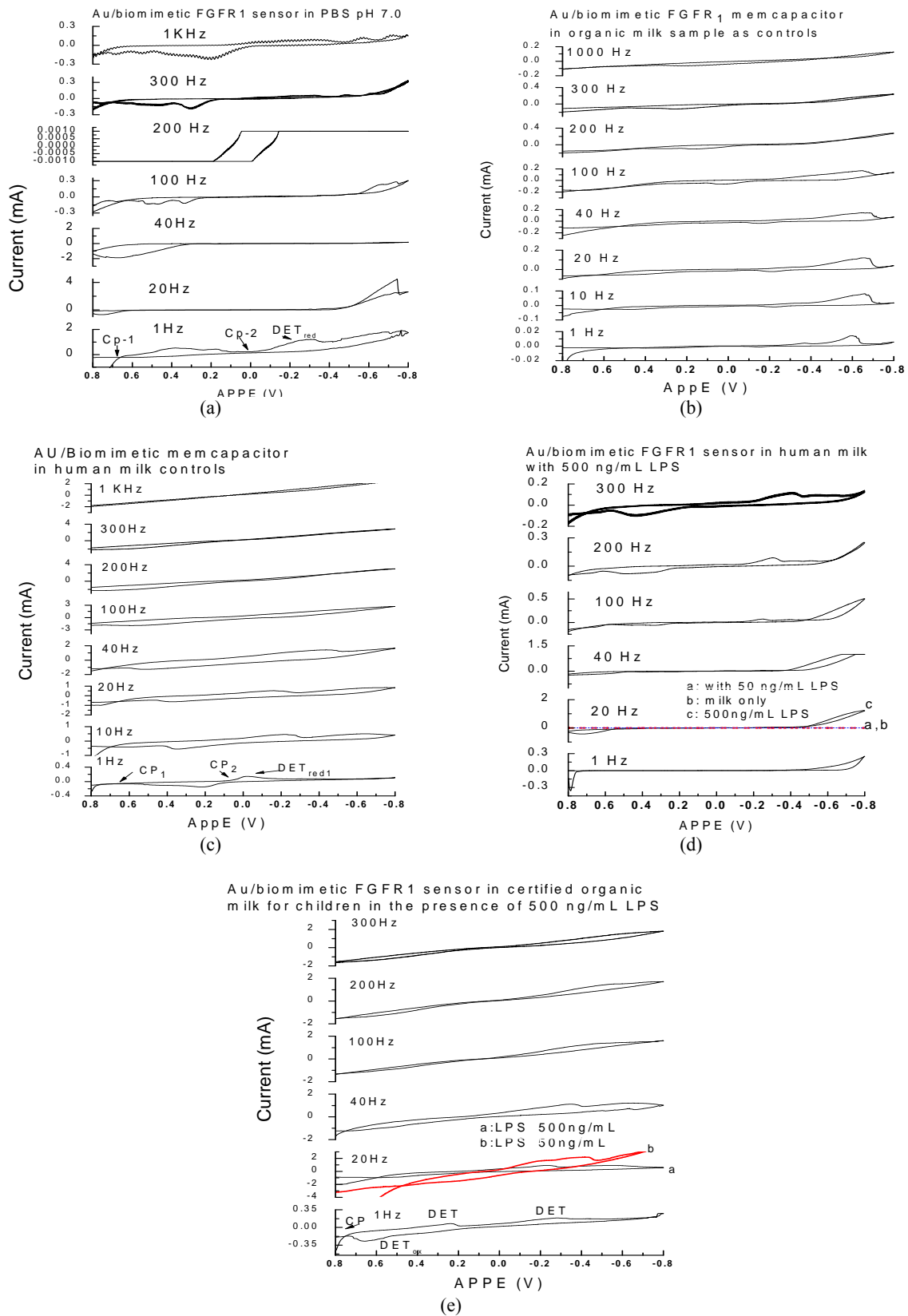


Fig. 3. (a) Frequency affects from 1 Hz to 1 kHz on hysteresis of the i-V curves of the memristor in PBS; (b) CV profiles of organic milk controls; (c) CV profiles of human milk controls, (d) CV profiles in 500 ng/mL LPS in human milk; (e) CV profiles in 500 ng/mL LPS in organic milk.

3.3. 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 voltage method. Fig. 4 (a-c) depict the synapse pulse control profiles using human milk, and compares samples using PBS media at 0.25, 40, 100, 200 and 250 Hz, respectively, without LPS. Curves overlap between the two media, and indicate human milk had no protein interference with the “single neuronal cell” as far as the neuron’s energy output is concerned. Fig. 4(d) compares the signal intensity when testing human milk in the presence of various LPS concentrations at 0.25 Hz,

and the results show the signal intensity is inversely proportional to a wide range of LPS concentrations from 50 ng/mL to 1000 ng/mL at 0.25 Hz. At 50 to 100 ng/mL, the biphasic pulse shape integrity is maintained; however the insert curves show that at 500 and 1000 ng/mL, the biphasic pulses are destroyed, and the cell net voltage intensity is close to zero. Fig. 4 (e) demonstrates a similar trend at 200 Hz, that the cell net voltage gets close to zero, at a higher concentration at 200 Hz, but at 50 ng/mL, the signal increases more than 30 % compared with that at zero LPS. This is a bad effect, a negative outcome of wasted energy. Fig. 5 (a) and 5 (b) depict LPS in organic milk’s impact on the synapse pulse intensity at 0.25 and 200 Hz, respectively.

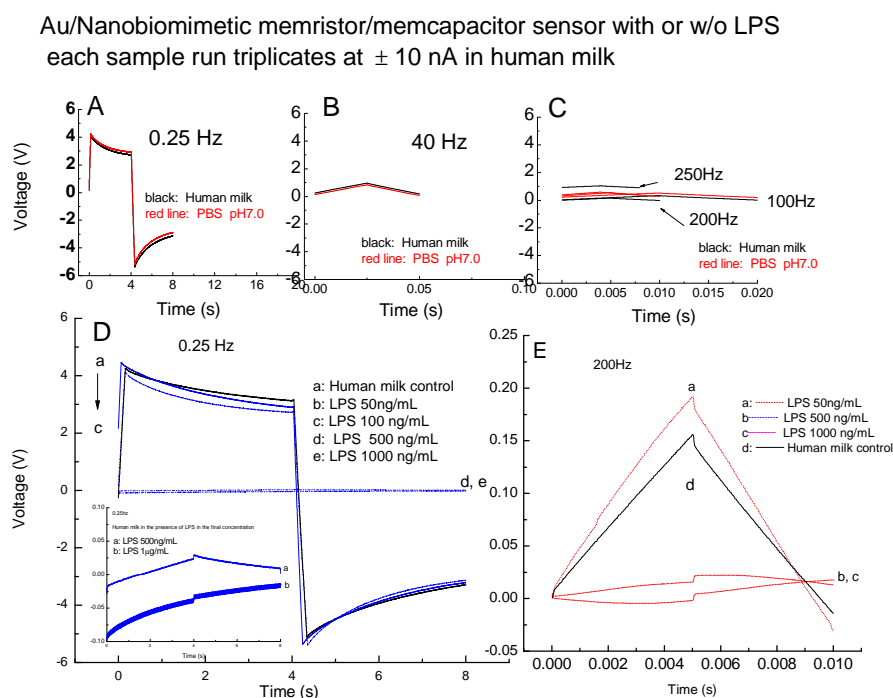


Fig. 4. (a, b, c) Single neuronal pulse at 0.25, 40 and 250 Hz compared in pH 7.0 PBS buffer (red curve) and the human milk (black curve), respectively without LPS; (d, e) LPS effects on human milk over 0, 50, and 100 to 1000 ng/mL at 0.25 and 200 Hz, respectively.

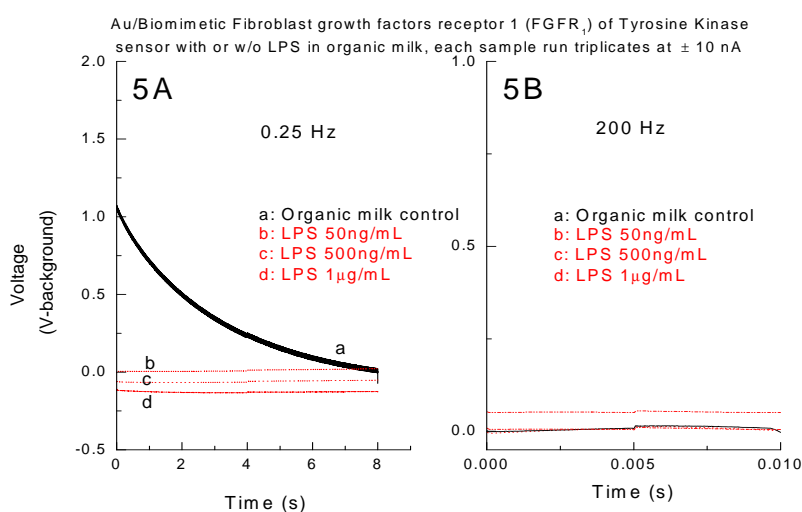


Fig. 5. (a, b) LPS effects on organic cow milk over 0, 50, and 500 to 1000 ng/mL at 0.25 and 200 Hz.

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 a 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 energy capability is critical to brain development in newborns. In the presence of LPS, regardless if the frequency is at SWS or at 200 Hz, 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.

3.4. Quantitation of LPS

The Voltage Method. Fig. 6 (a) 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 $Y = 125 - 0.25X$, $r = -0.9993$ ($n = 12$), $P < 0.0001$, $S_{y/x} = 2.0$. The Detection of Limits (DOL) is 0.3 ng/mL per cm^3 sensor, i.e., in a 40 μL sample, it detects 12 pg LPS using a 1 cm^3 sensor, and by the standard of 5 EU per ng of LPS, we are able to reach 0.06 EU in a 40 μL sample. Our sensor is $3.11 \times 10^{-7} \text{cm}^3$, and herein the DOL is $3.73 \times 10^{-18} \text{g}$ for LPS in our sensor. It means we are able to detect a single *E. coli* bacterium because an antigen is in 10^{-17}g range. As a matter of fact, the sensor only detects the biocommunications between the *E. coli* cell and the sensor due to the net energy changes. At 0.25 Hz, the energy density ranges between 123.2 and 0.11 $\mu\text{Whr}/\text{cm}^3$ using human milk specimens with an imprecision value 3.0 % compared the energy range of 9.8 to -0.042 $\mu\text{Whr}/\text{cm}^3$ for organic milk. Fig. 6 (b) shows the nonlinear curve for LPS at 200Hz using human milk. In contrast, Fig. 6 (c) and (d) shows there is 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.

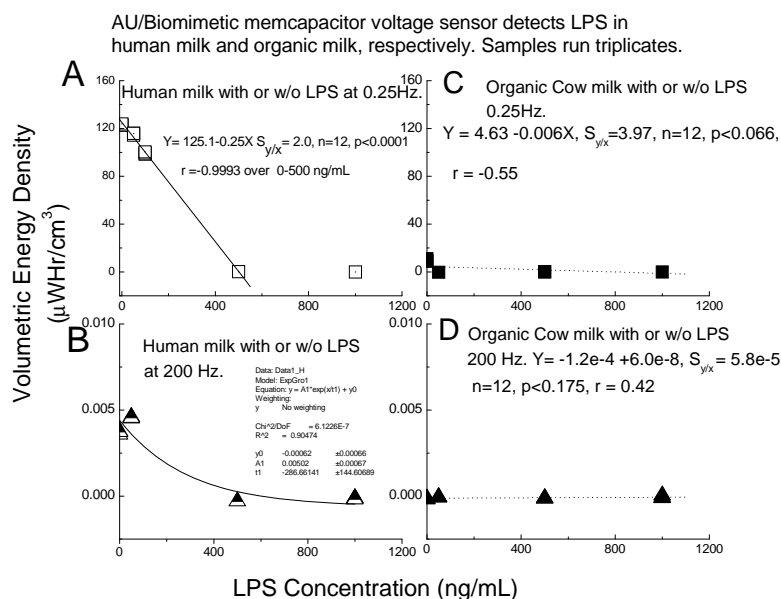


Fig. 6. (a, b) Quantitation calibration plots of LPS in human milk at 0.25 and 200 Hz respectively compared with LPS in organic milk at 0.25 and 200 Hz in (c, d).

The CA Method. The CA curve profiles were plotted using the biomimetic sensor in the presence of seven LPS concentration levels from 0, 5.0 pg/mL, 50.0 pg/mL, 5.0 ng/mL, 50 ng/mL, 125 ng/mL, and 250 ng/mL to 500 ng/mL against the control in organic milk samples as shown in Fig. 7. Fig. 8 depicts the lower level LPS's response curves, showing more clearly the significant increase in signals at 5 pg/mL LPS over the control. The CA

method for LPS produced a calibration curve with the regression equation $y = -0.09 + 0.02x$, $n = 21$, $S_{y/x} = 0.23$, $r = 0.998$ with $p < 0.0001$ covering the linear range from 5 pg/mL to 500 ng/mL in organic milk samples as shown in Fig. 9.

The DOL result is 0.1 pg/mL per one cm^2 sensor in organic milk, i.e., by the CA method we are able to detect $5.0 \times 10^{-4} \text{EU}$ *E. Coli* in 1 mL sample in 1 cm^2 sensor. Using this 0.031 cm^2 sensor, we are able to

detect *E. Coli* cells in the range of 0.2 - 5 cell assuming 5EU contains 2000-50000 *E. Coli* cells' activity [13]. The percentage of Pooled Relative

Standard Deviation (PRSD %) of the organic milk samples over the entire linear range is 2.0 %.

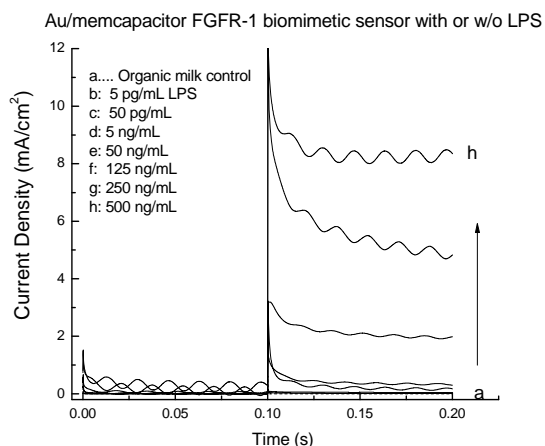


Fig. 7. CA curve profiles for with or without LPS in organic milk samples with each sample run triplicates.

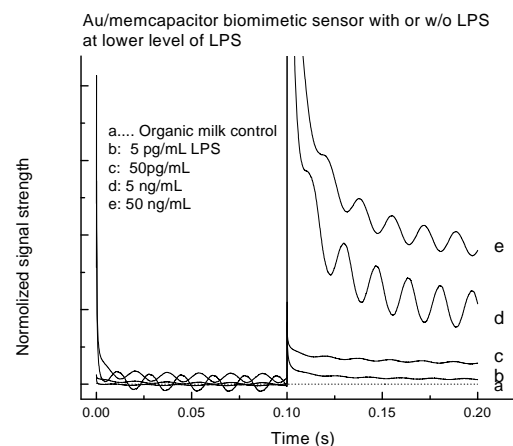


Fig. 8. Normalized CA curves at lower LPS concentration levels in organic milk.

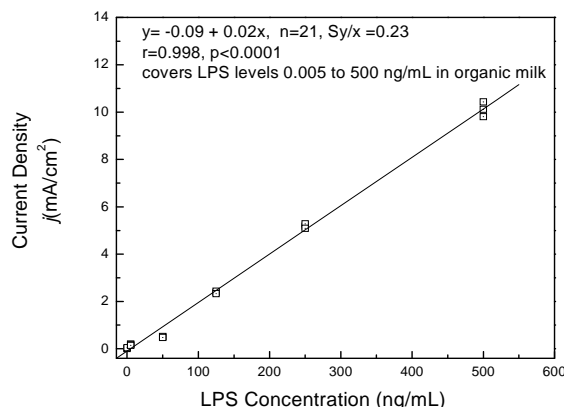


Fig. 9. Calibration curve using the CA method.

3.5. LPS Affects on the Energy Density in 3D Contour Maps

The energy density contour maps associated with the images are presented in Fig. 10 with energy density as Z, LPS concentration as X, and discharge pulse frequency as Y. Fig. 10 (a, b) depict the contour map of energy density vs. LPS concentration in the frequency change using the human milk samples and Fig. 10 (c, d) depict the contour map of energy density vs. LPS concentration in the frequency change using the organic milk samples. It is obvious that human milk produced tremendous higher energy (showing as the light in the image) with an intensity more than 10-fold higher, especially at SWS even in the presence of LPS.

3.6. Evaluation of Immunological Advantage under LPS Challenges

The comparisons of the immunological advantage under LPS challenges were evaluated

through the study of the formation of the pHFO using a 3D energy density map method. The energy density results were put into the "y" column, the spiked LPS concentration over 0.0 to 1000 ng/mL put into the "x" column and the frequency was into "z" column having two levels of 0.25 to 200 Hz. After converting the three data columns into a random XYZ correlation matrix, one can plot the contour maps and analyze the spatiotemporal formation of the pHFO if a pHFO exists among human milk or organic milk samples. Fig. 11 (a) depicts a 3D contour map of the relationship between energy density, LPS concentration and frequency using human milk. As we can see, at SWS, human milk held the highest neural synapse energy for with or without the presence of LPS challenge over the range from 0.0 ng/mL LPS to less than 200 ng/mL, until the LPS reaches 500 ng/mL, the energy gradually reduced to zero, in other words, human milk samples have an order of magnitude higher energy density at LPS=0 vs. organic milk at SWS and 200Hz, respectively as shown in Fig. 11 (a) compared with Fig. 11 (b). At 0.25 Hz, 50 ng/mL LPS caused 100 % energy reduce

in organic milk vs. human milk only 6.25% reduced. The rate of LPS reducing synapse energy is 10-times faster in organic milk samples than in human milk samples. It is estimated from the map at LPS

5 EU/ng, human milk maintained 96.6 % original energy strength vs. organic milk only 49 % strength at 0.25 Hz.

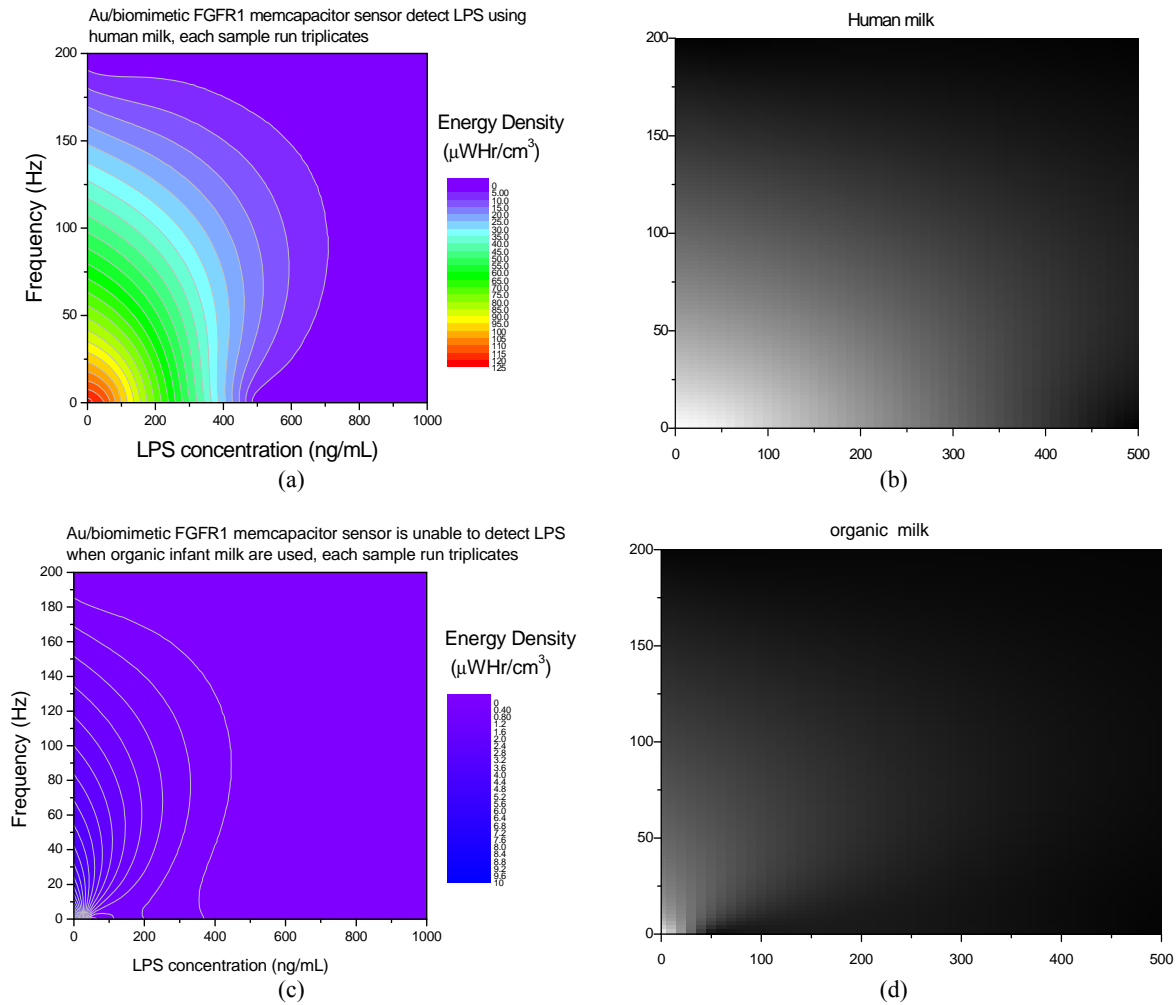


Fig. 10. (a, b) Energy density (as Z) contour map and the image are shown using human milk. The LPS concentration (as X) and synapse frequency (as Y). (c, d) Contour maps and images with LPS are shown using organic milk.

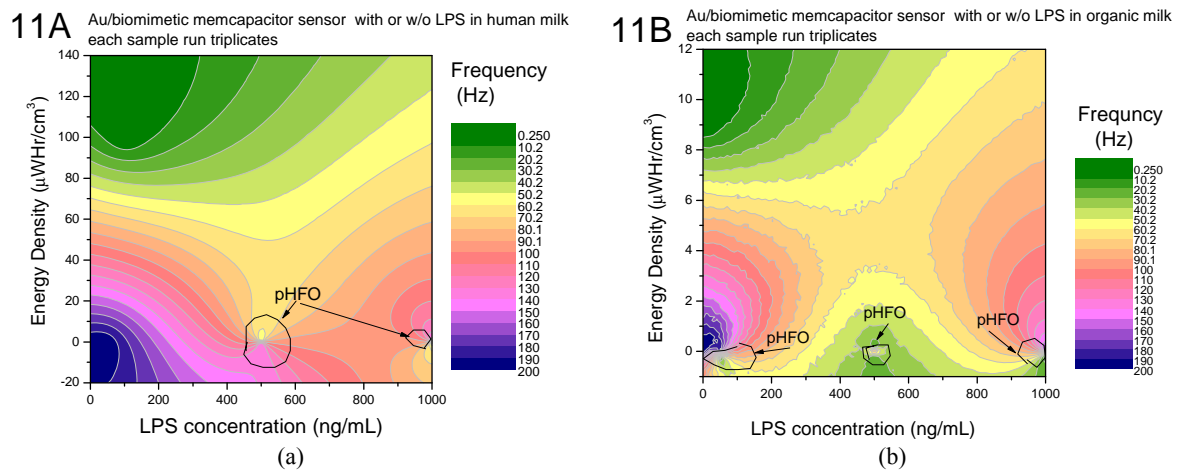


Fig. 11. (a) 3D energy distribution map vs. frequency and LPS concentrations in human milk, (b) 3D energy distribution map vs. frequency and LPS concentrations in organic milk.

Table 1 demonstrates the results of the comparison of LPS induced occurrence of the pHFO spatiotemporal locations associated with energy density and frequency using human milk and organic cow milk samples, respectively. Human milk offers significant immunological advantage by avoiding pHFO formation over 0.25 Hz to 100 Hz at LPS concentration of less than 500ng/mL; even when LPS ≥ 500 ng/mL, the pHFO frequency is within fast gamma frequency range around 110-120 Hz. Our observation was confirmed within the literature that breastfed babies have less risk for seizure and epilepsy compared with cow milk fed babies when they grow up to adulthood [41]. Report revealed cow milk fed babies have significantly higher blood phosphorus levels and lower blood calcium levels than are seen in breastfed babies [42]. The CDC's guidance for breastfeeding was well documented that breastfed babies are at an advantage in preventing chronic diseases [1-2]. Table 1 results show cow milk is ten-times more prone to *E. Coli* attack, and the positive link that was revealed is the formation of pHFO over the studied LPS concentration ranges which occurred in *Rapid Eye Movement* (REM) sleep (α frequency) to *Sharp Wave-Ripple Complexes* (SPW- R) (140-200 Hz) frequency [43].

Table 1. Comparison of the spatial-temporal pHFO locations induced by *E.Coli* in the 3D map^a in human milk and organic cow milk samples.

Milk	Frequency (Hz)	Energy Density $\mu\text{W}\cdot\text{hr}\cdot(\text{cm}^{-3})$ Mean (s.d.)	LPS (ng/mL)	pHFO
Human milk	120	0.10	500	yes
Human milk	120	-0.10	1000	yes
Human milk	0.25 (SWS)	115.5(0.99)	50	no
Human milk	0.25 (SWS)	123.2(0.58)	0	no
Organic milk	0.25 (SWS)	9.8(1.19)	0	no
Organic milk	0.25 (SWS)	-0.20(0.005)	50	no
Organic milk	20-40	-	50	yes
Organic milk	200	$-6.27e^{-5}(5.9e^{-6})$	50	yes
Organic milk	13-120	-	1000	yes
Organic milk	13-40	-	500	yes

^a this table does not take into account the influence of the cross-point and the DET peak influences.

Compared with human milk samples at SWS in the presence of 50 ng/mL LPS and without LPS, respectively, there were 577-fold and 12.6-fold lower neural synapse energy density results obtained in organic cow milk samples. The results provide strong evidence that the poor energy outcomes at SWS may have participated in the initiation of pHFO formation at higher frequency. The pHFO link to LPS

concentration at REM and SPW-R frequency provides an explanation to the literature which reports that cow milk fed babies are more frequently seen in sudden infant death syndrome (SIDS) than are seen in breast milk fed babies [44-45]. A more detailed literature review regarding the advantages of breastfeeding over cow milk feeding to infants to reduce SIDS was reported in the literature [46]. Because our evaluation of the pHFO formation does not include the influence of the cross-point and DET peak's sensory effects, we are therefore planning to study further the crucial factors impacting the pHFO dynamics so that when the results are obtained, we might be able to decipher and predict a fuller picture of the cause of SIDS.

4. Conclusion

We have demonstrated the advantage of the memristor/memcapacitor device with the biomimetic FGFR1 membrane: It behaves favorably with the microbiota in human milk, and it offers warning signs in orders of magnitude due to its sensitivity to a single bacterium's LPS presence in human milk over organic milk. The same amount of LPS acted differently in organic cow milk, and the main difference is the absence of beneficial microbiota immune protection when it contacts with the neuronal sensor. The 577-fold and 12.6-fold lower neural synapse energy density results in organic cow milk samples when compared to human milk samples at SWS in the presence of 50 ng/mL LPS and without LPS, respectively, explained that the initiation of pHFO was possibly due to poor energy outcomes hampering brain development. The dual function CA/voltage sensor memristor/memcapacitor device laid a foundation to quantitatively measure *E. Coli*'s biocommunication with the high sensitivity to energy changes in 78 fW hr with Detection of Limits (DOL) 3.73×10^{-18} g for the voltage method and a DOL of 1.2×10^{-16} g LPS for the CA method in 40 μL milk samples under antibody-free, tracers-free, and reagent-free conditions are demonstrated. Further study of the pHFO associated with sensory factors in a 3D dynamic mapping technology is needed to deepen our understanding of the immunological advantage of breastfeeding vs. cow milk feeding.

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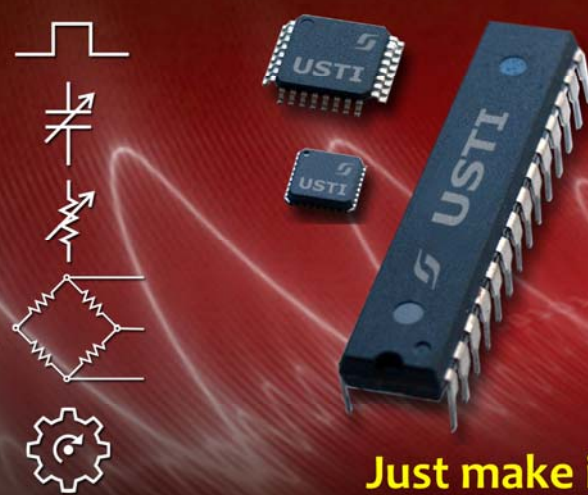
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