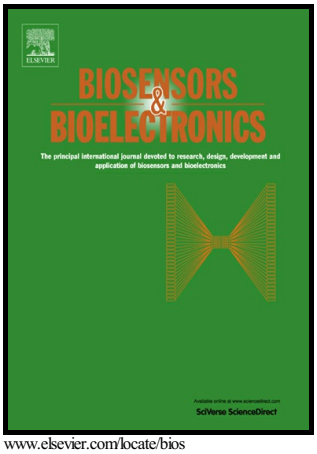

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Electrically-Receptive and Thermally-Responsive Paper-Based Sensor Chip for Rapid Detection of Bacterial Cells

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ABSTRACT

Although significant technological advancements have been made in the development of analytical biosensor chips for detecting bacterial strains (E. coli, S. Mutans and B. Subtilis), critical requirements i.e. limit of detection (LOD), fast time of response, ultra-sensitivity with high reproducibility and good shelf-life with robust sensing capability have yet to be met within a single sensor chip. In order to achieve these criteria, we present an electrically-receptive

thermally-responsive (ER-TR) sensor chip comprised of simple filter paper used as substrate coated with composite of poly(N-isopropylacrylamide) polymer (PNIPAm) – graphene nanoplatelet (GR) followed by evaporation of Au electrodes for capturing both Gram-positive (S. mutans and B. subtilis) and Gram-negative (E. coli) bacterial cells in real-time. Autoclave water, tap water, lake water and milk samples were tested with ER-TR chip with and without bacterial strains at varying concentration range 10^1 – 10^5 cells/mL. The sensor was integrated with in-house built printed circuit board (PCB) to transmit/receive electrical signals. The interaction of E. coli, S. mutans and B. subtilis cells with fibers of PNIPAm-GR resulted in a change of electrical resistance and the readout was monitored wirelessly in real-time using MATLAB algorithm. Finally, proposed ER-TR chip exhibited the reproducibility of 85-97% with shelf-life of up to four weeks after testing with lake water sample.

Keywords: Bacterial sensor, Filter paper, PNIPAm, Graphene, Lake water, Milk.

1. Introduction

According to world health organization reports, infectious diseases result in 26% of all deaths globally with high burden of nearly 45% of the all global diseases [1,2]. The United States department of agriculture (USDA) estimates loss of food produce and grains caused by major food pathogens and annual medical cost to be around $2.9-6.7 billion [3,4]. The detection of pathogens in water bodies, tap water, river water, raw food materials, food products, processing

and assembly lines, hospitals, ports of entry and drinking water supplies generally rely on conventional culturing techniques [5 – 7]. Numerous integrated techniques for the determination of E. coli have been reported in the last few years such as quartz crystal microbalance (QCM) [8,9], colorimetry [10-12], polymerase chain reaction (PCR) [13,14], fluorescence [15,16], electro-generated chemi-luminescence (ECL) [17] and electrochemical impedance spectroscopy (EIS) [18]. However, these techniques are highly dependent on trained users, limited to on-site availability and required extra time to process and normalize the data.

Electrochemical biosensors are widely recognized as powerful analytical tools due to a variety of contributions such as simple instrumentation, easy operation, low cost and short response time [our paper]. Analytical biosensor chip has been reported as a simple, rapid and inexpensive device that offers promising alternatives for detecting bacterial strains [19]. However, significant improvement is desired to operate the sensor with fast response time with low detection limit and good reproducibility with reasonable shelf-life. To achieve these parameters, extensive efforts have been made to introduce the different polymers [20-23], electrode materials [24-27] and antibody immobilization on electrodes [28-31]. As previously reported [32-37], polymers with electron mobility properties [38] have been used in many levels of microelectronics like electrostatic discharge (ESD) [39], and in other electronic devices like diodes, transistors, sensors, and actuators [24], [40]. Their high coloration efficiency, rapid switching ability, and diverse colors of conductive polymers make them efficient base material [41]. Here electrons moving along the molecule generate electrical current and conductivity can be tuned by chemical manipulation of the polymer backbone, by the nature of the dopant, by the degree of doping, and by blending with other polymers [42]. The sensing mechanisms of polymers might involve weight changes, ion adsorption and desorption, chain conformational

changes, redox reactions, volume and or charge transfer and screening. However, major drawbacks of such polymer based technology have been excessive or uncontrolled association with analyte, requirement of high amount of analyte as well as electrolyte and lesser possibility of reusability and reproducibility. Use of a non-conductive polymer with doped conductive carbon allotrope like graphene nanoplatelet is the strategy utilized to improve upon above shortcomings. Graphene-based composites have been used for biosensor development utilizing excellent electrical conductivity with high mechanical stiffness properties [43]. The use of a thermo-responsive-polymer (TRP) [44] is advantageous over other non-TRP due to its sensitivity towards a physiological temperature (approximately 37 °C), which is favorable to allow the growth of pathogens including E. coli. Poly(N-isopropylacrylamide) (PNIPAm), as a typical thermos-responsive polymer, has been studied extensively in the last decades for potential applications in sensing and drug delivering [42], [45-46].

In the present work, PNIPAm was utilized as a thermos-responsive material and found to shift the hydrophilic-hydrophobic balance as shown in Scheme 1. We hypothesized that an electrically-receptive membrane can be fabricated by combining graphene-nanoplatelet (GR) with thermo-responsive polymer (PNIPAm). Use of PNIPAm as matrix to make composite with various ratios of GR could be interesting in terms of its interaction with bacterial strains sensing and processing of electrical signals by implementing interdigitated Au electrodes (IDμE). The polymer chain in presence of bacterial samples shrinks dramatically as the temperature surpasses the lower critical solution temperature (LCST,32-33°C), which eventually changes the electrical properties when GR is further added to the polymer matrix. The prepared bacterial sensor has been developed for detecting E. coli cells present in different water bodies (tap water, lake water and milk samples). To show the broader applicability of developed sensor chip, we have

expanded our studies by adding two more bacterial strains namely B. subtilis and S. mutans. These bacterial strains cover both Gram-negative (E. Coli) and Gram-positive (B. subtilis and S. mutans) categories at various concentration range (10^1 – 10^5 cells/mL). E. coli, S. mutans and B. subtilis were detected repeatedly and recorded in real-time. A low-cost standalone miniaturized printed circuit board (PCB) was developed as a handheld device and integrated with ER-TR sensor chip. Uniformity of the PNIPAm-GR composite on the paper was achieved using optimized spin-coating technique, which ensured low roughness of the coated layer. Finally, recovery, shelf life and reusability of the bacterial sensor were evaluated over the period of time.

2. Experimental

2.1.Preparation of PNIPAm – GR Suspension and Coating on Filter Paper

Graphene (GR) and Poly(N-isopropylacrylamide) (PNIPAm) were purchased from STERN Chemicals, USA and Sigma-Aldrich, Saint Louis, MO, USA, respectively. GR (2 mg/mL) was mixed with PNIPAm (16 mg/mL) to prepare suspension of 10 mL. Mixture of graphene-polymer (GR- PNIPAm) composite was probe sonicated at amplitude of 4, with pulse rate of 5 sec on and 1 sec off for 1 h with intermittent cycles of 30 min. Process was completed at controlled temperature of 37 °C. Spin coating technique (Table S1) was selected over conventional drop cast method to produce a thin and uniform layer of graphene-polymer composite with minimum surface defects. GP are carbon materials having “platelet” morphology and multifunctional properties. Each nanoplatelet of GP has a width of about 6-8 nm and length of ~25 µm. This morphology makes these platelets effective at providing connecting barrier properties. In addition, their pure graphitic composition provides significantly high electrical and thermal

conductivity. Samples were dried using nitrogen spray gun for 10 minutes and were further dried overnight under biosafety hood at RT to maintain sterility.

2.2.Fabrication of Au Based Electrodes

Interdigitated Micro-electrodes (IDμE) with thickness of 30 nm were fabricated directly on thin layer of PNIPAm-GR composite using a shadow mask with pre-defined pattern. This configuration ensures high conductivity and mechanical reliability for stable electrical signal transfer within sensing covered area of 1.2×1.2 mm^2. The design was initially created in AutoCAD 2014 and shadow mask was then printed at facility of Photo-Sciences Inc., Torrance, CA on nickel brass with thickness of 0.025 mm. Electron beam evaporator was finally used for depositing Au electrodes using shadow mask adjusted on thin layer of spin coated GR- PNIPAm.

2.3.Surface Characterization

Scanning electron microscopy (SEM) and Nuclear magnetic resonance (NMR) spectroscopy were employed for surface characterization of the developed ER-TR biosensor chip. Sensor chips were mounted on SEM sample holder using a piece of carbon tape. The images were taken using a Hitachi (Schaumburg, Illinois) S-4700 SEM with Oxford Instruments (Abingdon, Oxfordshire). Imaging was performed in a long working distance mode, with an accelerating voltage of 10kV, extracting current of 10 μA, and working distance of 25 mm for scanning. To understand, graphene was interacting with PNIPAm, we have done H1NMR study of 'graphene coated PNIPAm' and 'PNIPAm only' in CDCl3 solvent in VXR 500 MHZ machine.

2.4.Bacterial Contamination of Aqueous Medium

To mimic the bacterial contamination condition, a known number of bacterial cells (E. coli, S. mutans and B. subtilis) from Thermo-Fisher Scientific, USA were added to required volume of water. A stock of 1×10^6 cells/mL of E. coli was diluted with DI water to achieve 10^1 – 10^5

cells/mL. These suspensions were used for loading on ER-TR sensor chips in a required volume of generally 2 μL, or as described in specific experiment.

2.5.Real sample experiments and reproducibility

The experiments were demonstrated with autoclave water, tap water, lake water and milk samples for the detection of individual bacterial strain type (E. coli, S. mutans and B. subtilis). Autoclave water and tap water samples were used from our lab located at Medical Research Center, Carle Foundation Hospital Urbana IL. Lake water samples were collected from Crystal Lake at Urbana IL. Milk sample was collected from whole milk available in market. All samples were diluted 5 times with running buffer in order to reduce the media effect and then spiked with bacterial cell at varying concentration range (10^1 – 10^5 cells/mL). The mixture of each sample was vortexed and divided into two 10 mL vials. The reproducibility and the shelf life of the assay were also evaluated by monitoring the change in electrical resistance for lake water sample spiked with E. coli and S. mutans cells in real-time.

2.6.Miniaturized printed circuit board

The ER-TR biosensor chip was integrated with in-house built miniaturized printed circuit board (PCB). Microcontroller (ATmega328P) was reconfigured to control the input voltage of 5V, Bluetooth module (HC-05) and LCD were used to monitor the real-time response of the sensor and display the output electrical resistance across biosensor chip respectively. MATLAB was used to run the Bluetooth data (electrical signals transmit/receive to sensor and PCB). The Bluetooth chip used in this study was a commonly used BT module that has a standard baud rate of 9600 and operates at 2.4 GHz for its transmission with input voltage of 3.3V. To achieve the layer optimization and selecting appropriate electrical connection with respect to design rule checking (DRC), prototype was designed in standard Eagle software. Finally, layout design was

printed and integrated with battery and biosensor chip in our lab. Wire connection was performed using soldering and electrical conductive tape was used to connect Au electrodes pads with PCB terminals.

3. Results and Discussion

3.1.Biosensor development and characterization of PNIPAm-GR nanocomposite

Although, in literature, electrodes on polymer based biosensor have been produced using complex fabrication procedures [32-37] requiring various chemicals to be processed. Herein, we employed two step procedures to develop electrically-receptive thermally-responsive (ER-TR) sensor chip. Polymer-graphene suspension was spun on filter paper followed by direct Au electrode deposition. In suspension of PNIPAm-GR, polymer chain shrinks dramatically as the temperature exceeds the LCST, which eventually leads to a dramatic change of the electrical resistance properties of polymer-graphene matrix. PNIPAm-GR suspension was intelligently coated on filter paper for making it suitable to absorb water component during real-time measurement of samples. Strategy of using graphene nanoplatelets (GR) was chosen specifically for the purpose of not allowing the sensor chip to make a water-tight composite with PNIPAm polymer, rather keeping it edge-over-edge arrangement after spin coating. Polymer-graphene platelet with such arrangements would be channeling water component of the samples but restricting the percolation of other components including E. coli, S. mutans and B. subtilis cells. PNIPAm-GR composite was characterized by NMR to ensure graphene-PNIPAm interactions. As shown in Fig. S1, in case of ‘graphene coated PNIPAm’, showed two singlets at 1.250 and 1.137 ppm whereas ‘PNIPAm only’ showed one singlet at 1.135 ppm indicating graphene

interacting with PNIPAm. PNIPAm-GR nanocomposite coated on filter paper was further characterized using SEM and AFM. The interaction of bacterial cells with PNIPAm-GR composite is responsible for resistance changes in sensing platform. In this regard, representative studies were performed with scanning electron microscopy (SEM) as shown in Fig. 1. PNIPAm-GR nanocomposite with magnified view of PNIPAm fiber with AFM is shown in Fig. 1a and E. Coli, B. subtilis and S. mutans cells tested on PNIPAm-GR/Au platform with magnified view in right panels of Fig. 1b, Fig. 1c and Fig. 1d respectively. High resolution image of an E. coli is shown in Fig. S3. Interdigitated Au microelectrodes was used to enhance the conductivity of the ER-TR sensor chip during real-time recording of bacterial strain and to maximize the resistance change, reduce the detection time, minimize interfering effects and work in a two-electrode system (vs. conventional three-electrode system), thus significantly benefiting the fabrication and performance of ER-TR sensor chip.

To evaluate the initial assessment of the developed ER-TR paper-based sensor and finding sensitivity, detection range and detection limit, two sensor chips were fabricated utilizing different PNIPAm concentration of 4 and 16 mg/mL. The amount of graphene (GR) in PNIPAm-GR suspension was kept constant at 2 mg/mL for both chips. The prepared ER-TR sensor chips were then tested with E. coli cells of concentration range from 10^1 to 10^5 cells/mL at similar ambient condition and it was found that ER-TR chip with PNIPAm-GR (16:2 (w/w) mg/mL) exhibited fast time of response, read data efficiently and revealed excellent stability and the strong adhesion between the thermoresponsive component PNIPAm and the conductive carbon allotrope of GR as compared to the sensor prepared with low concentration of PNIPAm-GR (4:2 (w/w) mg/mL).Thus, all subsequent experiments were demonstrated using PNIPAm-GR (16:2 (w/w) mg/mL).

3.2.Real-time sample *analysis (tap water, lake water and milk)*

Instead of employing conventional techniques (QCM, PCR, ECL, CV and EIS), the algorithm was programmed in MATLAB and electrical signals were readout and recorded efficiently using controller and BT modules of the PCB. Tap water, lake water and milk samples were used with and without bacterial strains (E. coli, S. mutans and B. subtilis). The proposed sensor chips were initially stored at 4 °C before testing with real samples. The detection mechanism of ER-TR chip was based on the change of LCST of PNIPAm (Scheme 1) once the samples of interest (tap water, lake water and milk) with and without bacterial strains (E. coli, B. subtilis and S. mutans) interacted with the chip surface. The base resistance of the bare biosensor chip was recorded for 3 min and then the samples were tested and spiked with known concentration range from 10^1 – 10^5 cells/mL. Control experiment was designed using autoclave water and change in resistance was monitored in real-time. Sample volume of 2 uL was pipette onto ER-TR sensor chip and a sudden jump in electrical resistance was measured and it gradually decreased after the time span of approximately 3 min and reached equilibrium state as shown Fig. S2. This ensured no existence of bacterial cells in the tested autoclave water sample. In follow up experiment, tap water sample was tested and bacterial strains (E. coli, S. mutans and B. subtilis) with known cells/mL were sequentially then spiked at concentration range of 10^1 – 10^5 cells/mL. The net change in resistance was monitored at each step as shown in Fig. 2a. Tap water sample without bacterial cells showed an increase in base resistance of ER-TR chip from 1.78 to 3.14 kΩ. After spiking with Gram-negative bacterial strain (E. coli), the base resistance increased to 4.17 kΩ for 10 cells/mL, 6.09 kΩ for 10^2 cells/mL, 6.33 kΩ for 10^3 cells/mL, 8.05 kΩ for 10^4 cells/mL and 12.93 kΩ for 10^5 cells/mL. We noted the percentage change in resistance for detecting B. subtilis

and S. mutans was almost 10.31 ± 2.43% and 37.88 ± 5.25% lesser than E. coli with similar concentration range (10^1 – 10^5 cells/mL). The primary reason for this reduction in resistance of B. subtilis and S. mutans is their morphology as shown in high resolution SEM images of Fig. 1c and Fig. 1d. Fig. 2b shows the calibration curves to explore range of detection for E. coli, B. subtilis and S. mutans with coefficient of linear regression 0.930, 0.925 and 0.934 respectively. Experiments were further repeated with tap water samples after autoclave procedure and spiked with E. coli, B. subtilis and S. mutans with concentration range (10^1 – 10^5 cells/mL) as shown in Fig. 2c. The net resistance of ER-TR sensor chip was increased to 3.77 kΩ for 10 cells/mL, 5.61 kΩ for 10^2 cells/mL, 6.02 kΩ for 10^3 cells/mL, 7.65 kΩ for 10^4 cells/mL and 11.34 kΩ for 10^5 cells/mL. These net values are less as compared with the tap water sample before autoclave. An additional resistance before autoclave could be due to other contaminants present in tap water. The coefficient of linear regression for E. coli, B. subtilis and S. mutans were improved after autoclave processing to 0.961, 0.970 and 0.958 respectively as shown in Fig. 2d.

Lake water samples were collected from Crystal Lake located at Urbana, IL USA and were tested directly with portable ER-TR sensor chip in real-time with and without bacterial strains (E. coli, B. subtilis and S. mutans) with concentration 10 and 100 cells/mL as shown in Fig. 3a. A significant change in resistance was observed when E. coli and B. subtilis cells with concentration 10 and 100 cells/mL were spiked individually. However, for lower concentration of S. mutans (10 cells/mL), there was a negligible change in resistance in comparison with 0 cells/mL. This confirms that ER-TR sensor chip is more sensitive towards E. coli and B. subtilis even for lower concentration of 10 cells/mL. To further investigate the affect the lower concentration of these bacterial strains (E. coli, B. subtilis and S. mutans), experiments were carried out using milk samples as shown in Fig. 3b. In comparison with tap water and lake water

samples, a tremendous increase in base resistance of ER-TR sensor chip from 1.803 kΩ to 4.9 kΩ was observed once the milk sample of 2 μL was pipette onto sensor chip. This could be because polymer-graphene composite which allows channeling the water component only but restricting the percolation of other components present in the milk. E. coli (Fig. 3b, left), B. subtilis (Fig. 3b, center) and S. mutans (Fig. 3c, right) were then spiked in milk samples with each concentration 10 and 100 cells/mL. The response time to detect signals after spiking bacterial strains varied from 3 – 5 min depending on the type of bacteria strain (positive/negative) and the concentration used in the study. However, a similar behavior was observed for lower concentration of S. mutans (10 cells/mL) and the change in resistance was also negligible even in case of milk sample. It is also noted that the response time to detect signal in milk sample for bacterial strains was increased 2 times in comparison with lake water sample.

Experiments were further extended with higher concentration (10^3 – 10^5 cells/mL) of E. coli, B. subtilis and S. mutans to investigate the behavior of ER-TR chip treating lake water and milk samples as shown in Fig. 4a,b and Fig. 4c,d respectively. The net change in resistance for concentration range 10^1 – 10^3 cells/mL was not significant in comparison with 10^4 – 10^5 cells/mL. This confirmed that PNIPAm-GRP was able to read signals both at lower and higher concentration of E. coli, B. subtilis and S. mutans spiked in lake water as well as in milk samples.

In comparison with previously reported sensor chips [10, 26, 27, 42, 47-57] to read bacterial strains, this work has shown excellent outcome (Table 1). Tokonami et al [47] reported a label-free E. coli identification using surface imprinting technology for the whole cell imprinting of bacteria on the surface of over oxidized polypyrole, which could detect the bacteria within range of 10^3 – 10^9 CFU/mL. In another study performed by Yilmas et al [48], the apple

juice was spiked with E. coli at diff erent concentrations and achieved the detection limit of 3.72 x 10^5 CFU/mL. Hesari et al [49] reported the biosensor chip for detection of E. coli cells in tap and lake water samples with response time ranging from 20 – 120 min depending on the concentration of of E. coli cells spiked in the respective sample. Recently, Idil et al [50] developed E. coli detection sensor chip using hybrid microcontact imprinting technology and capacitive biosensor technology. Nevertheless, the fabrication procedure itself involved number of steps and numerous reagents to immobilize E. coli cells onto the surface of the sensor and also required extra time to process the sample to detect the E. coli cells within the range of 10^2 – 10^5 CFU/mL. The work proposed in this study enables faster response time of about 3 – 5 min (tap water) and 5 – 10 min (lake water and milk) to detect E. coli, B. subtilis and S. mutans with wide range of concentration 10^1 – 10^5 cells/mL and lower limit of detection 5 cells/mL. Further, instead of employing complex fabrication and extra chemical processing, a simple filter paper was utilized as base material spin-coated with PNIPAm-GR suspension which substantially reduced the cost of the sensor chip.

3.3.Investigation of PNIPAm-GRP at lower temperature

Experiments were conducted in cold room (4°C) to study the characteristics of PNIPAm-GRP coated on filter paper after pipetting the E. coli (Fig. 5a) and S. Mutans (Fig. 5b). 100 cells/mL was tested in triplicate for both E. coli and S. mutans and the change of resistance for each testing was almost same with no significant change in comparison with base resistance of the sensor chip. Higher concentration 1000 cells/mL was also tested for both E. coli and S. mutans under similar conditions and the change of resistance was not observed as compared with 100 cells/mL. This confirmed that at higher temperature (≥ 32°C) polymer chain in presence of bacterial samples shrinks dramatically as the temperature surpasses the LCST (32-33°C) and the

developed sensor chip has limitations in studying the bacterial strains at lower temperatures (specifically $\leq 4°C$).

3.4.Recovery, Shelf Life and Reproducibility

Experiment was performed utilizing the real lake water samples collected from three site locations of Crystal Lake, Urbana IL. All experiments were carried out at RT and the results were then compared with autoclave water and DI water samples. Interestingly, sensor exhibited a significant change in electrical resistance for three lake water samples in comparison with autoclave water and DI water (Fig. 5c). This significant increase in resistance confirms the presence of contamination in lake water samples. Same samples were further tested after addition of E. coli (10^1, 10^2 and 10^3 cells/mL) and a recovery experiments were then demonstrated (Table 2). It clearly showed that response to bacterial population was significantly high. Thus, this paper-based sensor is significantly responsive to interpret the quality of a sample under investigation. Reproducibility of 88-97% at RT was achieved for three weeks (Fig S4). However, the response time to detect E. coli (1000 cells/mL) was increased from approximately 6 to 10 min. Since the base material was a simple filter paper, the mass-production of the biosensor chip can be doable at very low-cost.

4. Conclusion

This work has explored an electrically-receptive and thermally responsive (ER-TR) paper-based sensor chip integrated with hand held miniaturized instrument used for detecting both Gram-positive (B. subtilis and S. mutans) and Gram-negative (E. coli) bacterial strains. To the best of our knowledge, this is the first study utilizing nanocomposite of PNIPAm-GR as a base material

coated on filter paper to detect bacterial strains (E. coli, B. subtilis and S. mutans). Bacterial cells at concentration range of 10^1 – 10^5 cells/mL were spiked in autoclave water, tap water, lake water and milk samples. Fibers of PNIPAm-GR composite were used as a capturing technique to rapidly record the presence of bacterial cells in real samples with LoD 5 cells/mL. The present work has demonstrated the response time of 3 – 6 min in tap water and 6 – 10 min in lake water and milk samples. A recovery of 85-97% at RT was obtained when biosensor chip was tested with lake water. Overall, this portable bacterial sensor device has potential to develop bacterial diagnostics with significantly lower loading sample volume (2 μL). Results shown high possibility of developing the paper-based multilayer sensor chip at very low cost bacterial-chip integrated with battery operated PCB which could bring new adoptability to rural and remote areas where expensive and laborious equipment are not as easily applied.

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Notes

The authors declare no competing interest.

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Supporting information

Supporting file includes optimized spin coating details used to prepare nanocomposite of PNIPAm-GR on filter paper (Table S1). Real-time evaluation of the biosensor chip with PNIPAm-GR (2:2 (w/w) mg/mL) tested with autoclave water with and without E. coli (10^2 – 10^5 cells/mL) (Fig. S2). SEM image of sensor chip tested with autoclave water before and after addition of E. coli (Fig. S3) and high resolution TEM image of E. coli (Fig. S3). Shelf-life of ER-TR sensor chip (Fig. S4).

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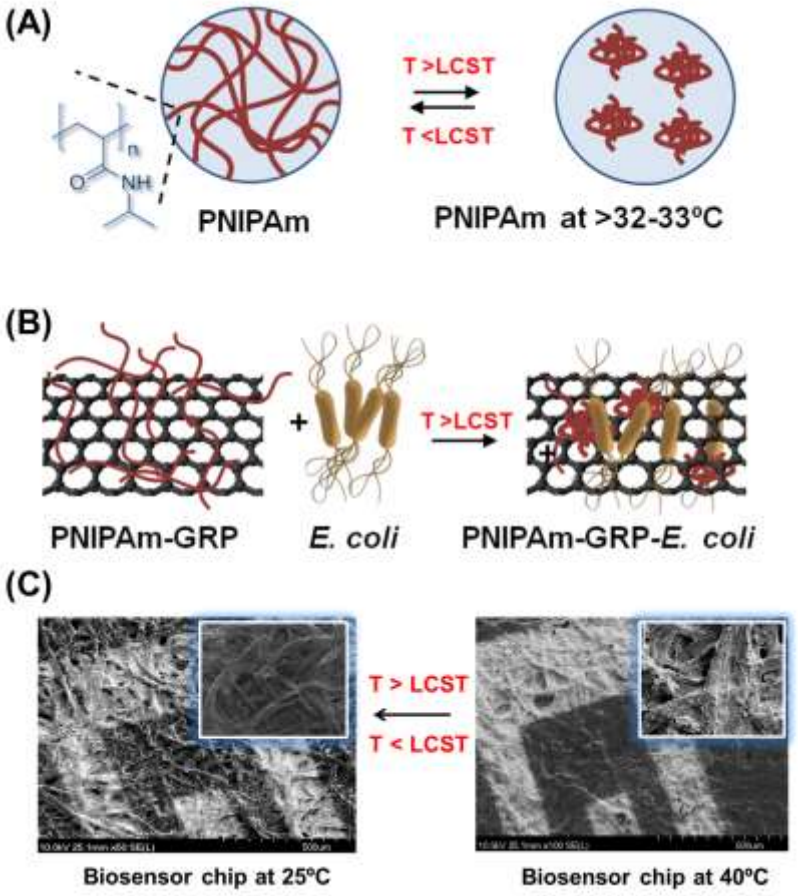
Table 1. Comparison of previously reported biosensors to evaluate E. Coli and other bacterial strains.

Materials	Bacterial strain	Methodology	Limit of Detection	Time	Labeling	Ref
MBs+magnetized graphite electrode	E. coli, Salmonella and Campylobacter	Amperometric / Voltammetry	$\mathbf{1.5 \times 10^3}$ in apple juice	80 min	Alkaline phosphatase	51
Bare IDME, MBs-Abs-cell attracted by a magnet	E. coli	Impedimetric	$\mathbf{7.4 \times 10^3}$ in buffer	35 min	Label-free	27
GCE/SWCNT/aptamers	E. coli surrogate CECT 675	Potentiometric	6 in milk, 26 in apple juice	Real-time	Label-free	52
SPCE/FeDC-AuNPs/Abs	E. coli	Amperometric	$\mathbf{5 \times 10^3}$ in milk	30 min	HRP	53
Overoxidized polypyrrole (OPPy) film	P. aeruginosa	Frequency	$\mathbf{10^3}$ in PBS, $\mathbf{10^6}$ in apple juice	3 min	Label-free	47
Au electrode/SAM of neutravidin/ biotin-Abs	E. coli	Impedimetric	10 in PBS	1 hr	Label-free	54
Quartz crystal Au electrodes/SAM of MPA/Abs	E. coli	Capacitive	$\mathbf{10^2}$ in buffer, $\mathbf{10^3}$in food samples	1 hr	Label-free	55
Thermally reduced monolayer graphene oxide (TRMGO) sheets	E. coli	Amperometric	10 in buffer	Real-time	Label-free	32
Magnetic nanoparticle-target-Gold nanoparticles (MNP-target-AuNP)	E. coli	Potentiometric	10 in buffer	1 hr	AuNP-label	26
Microcontact imprinted Au electrodes	E. coli	Voltammetry	70 in apple juice and river water	Real-time	Label-free	50
Fibers based on long-period gratings	E. coli	Colorimetric	$\mathbf{10^2}$ in buffer	NA	Label-free	56
4-mercaptophenylboronic acid (MPBA)-modified Au@Pt NPs	E. coli	Colorimetric	7 in buffer	40 min	Label-free	10
Paper/chitosan-glutaraldehyde/BSA/1st antibody/HRP-2nd	E. coli	Colorimetric	$\mathbf{10^4}$ in lixivium samples	3 hr	HRP	57

antibody/$\boldsymbol{TMB + H_2O_2}$						
Paper/graphene-PNIPAm/IDμE	E. coli, S. mutans and B. subtilis	Impedimetric	5 in DI water, tap water, lake water, milk	6 – 10 min	Label-free	This work

Table 2. Recovery tests for E. coli (10^1, 10^2 and 10^3 cells/mL) spiked in DI water before and after autoclave.

DI water sample #	**E. coli (cells/mL)**	**Resistance (Ω±SD)**		**Recovery (%)**
		Sample pre-incubated with E. coli	Autoclave samples spiked with E. coli	
1	10	66±17	72±11	91.7±0.6
2	100	390±33	378±24	96.9±1.2
3	1000	1056±79	1118±85	94.45±1.1



Scheme 1. Representation of PNIPAm aqueous solutions above or below LCST and its structural changes. The function of graphene nanoplatelets in delaying LCST and causing change in electronics of PNIPAm-GR substrate is also depicted. (A) PNIPAm with chemical structure in sol state (<LCST) and in gel state (>LCST), (B) PNIPAm in sol state with doped GR nanoplatelets and E. coli. Possibilities of bacterial samples exhibiting same effect due to cellular interactions (>LCST). (C) Paper-based biosensor chip with PNIPAm-GR prepared and stored at 4 °C (<LCST) for 1 week. SEM image was taken at 25°C. (D) As prepared SEM image of paper-based ER-TR biosensor chip at RT. Inset reveals the fibers of nanocomposite of PNIPAm-GR with Au deposited electrodes.

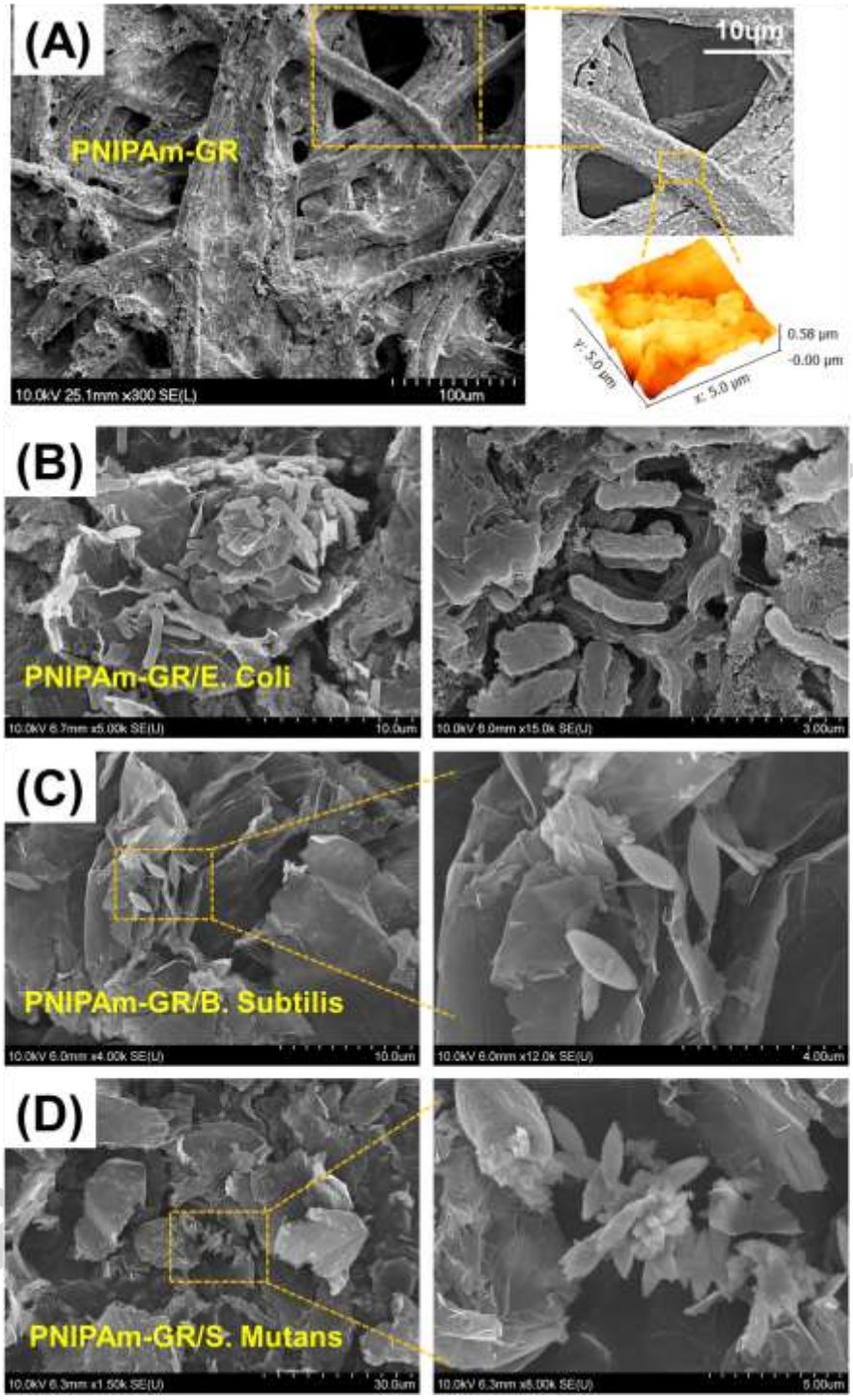


Fig. 1. Scanning electron microscopy performed on sensor platform (PNIPAm-GR) with and without bacterial strains. (A) PNIPAm-GR nanocomposite with magnified view of PNIPAm fiber with AFM. (B) E. Coli, (C) B. subtilis and (D) S. mutans cells on PNIPAm-GR with magnified view in right panels.

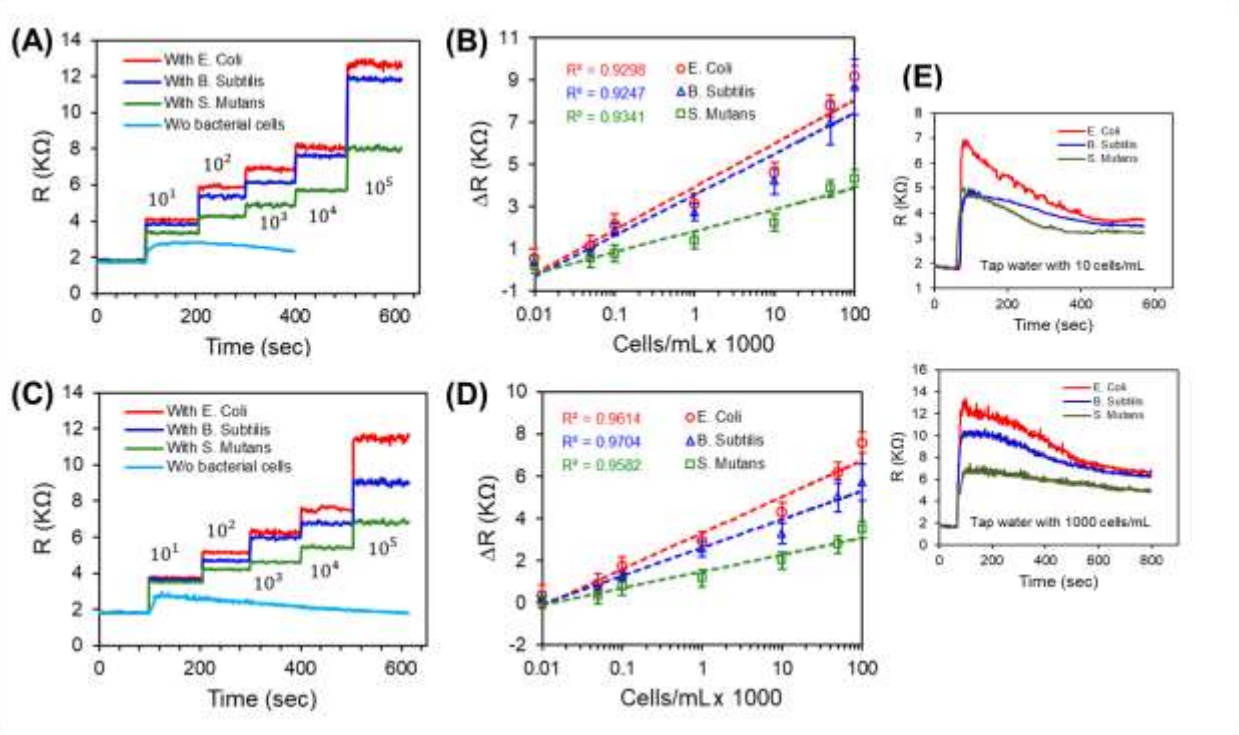


Fig. 2. Detection of bacterial strains (E. coli, B. subtilis and S. mutans) with concentration range from 10^1 – 10^5 cells/mL tested in Tap water before and after autoclave. (A) Tap water sample spiked with bacterial strains. The change in electrical resistance increased with increasing concentration range (10^1 – 10^5 cells/mL). (B) The calibration curves of the impedance magnitude changes versus the concentration of bacteria cells for the detection of E. coli, B. subtilis and S. mutans spiked in tap water sample. ΔR indicates the difference in resistance of the sensor chip before and after testing tap water samples. (C) Tap water sample after autoclave and spiked with similar bacterial strains. The change in resistance decreased in comparison with original tap water. (D) Improved linear regression of coefficient for E. coli (0.9314), B. subtilis (0.9704) and S. mutans (0.9582). (E) Real-time demonstration of E. Coli, B. Subtilis and S. Mutans with concentration 10 and 1000 cells/mL each spiked in tap water.

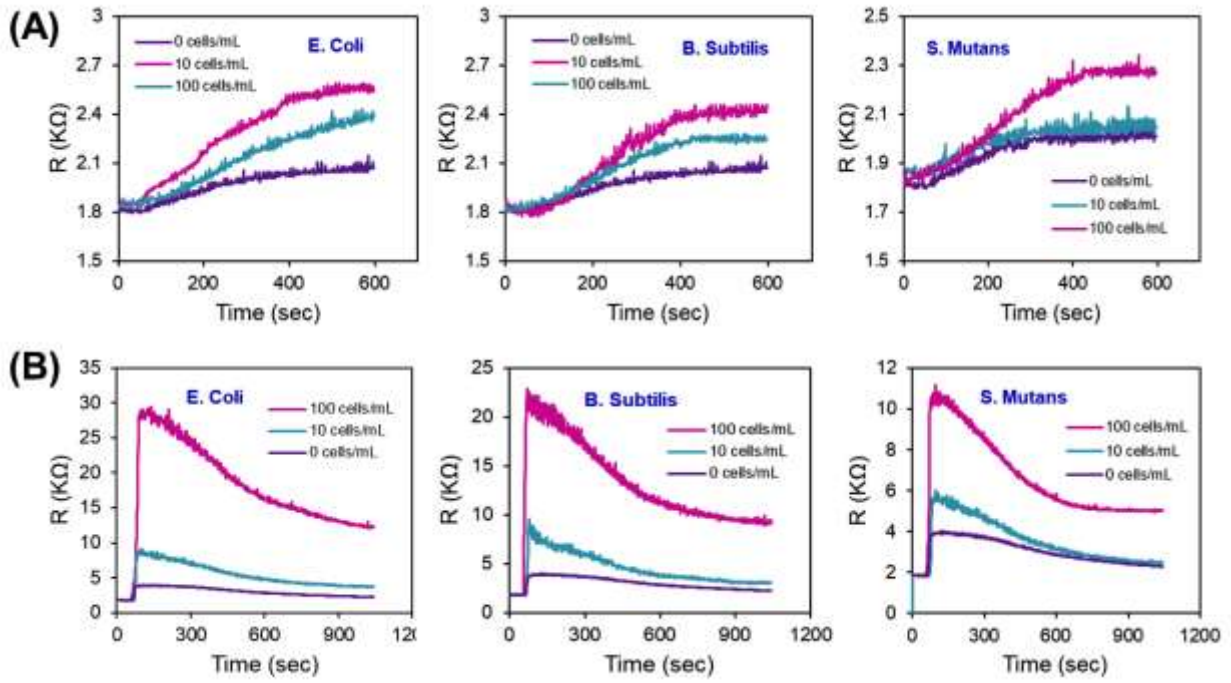


Fig. 3. Real-time evaluation of proposed sensor chip utilizing (A) whole milk and (B) lake water samples before and after addition of E. coli (left), B. subtilis (center) and S. mutans (right). Average time of response to achieve a stable electrical signal outcome detecting the presence of bacterial cells in milk and lake water sample varied from 6 to 10 min depending on the concentration range (10 – 100 cells/mL).

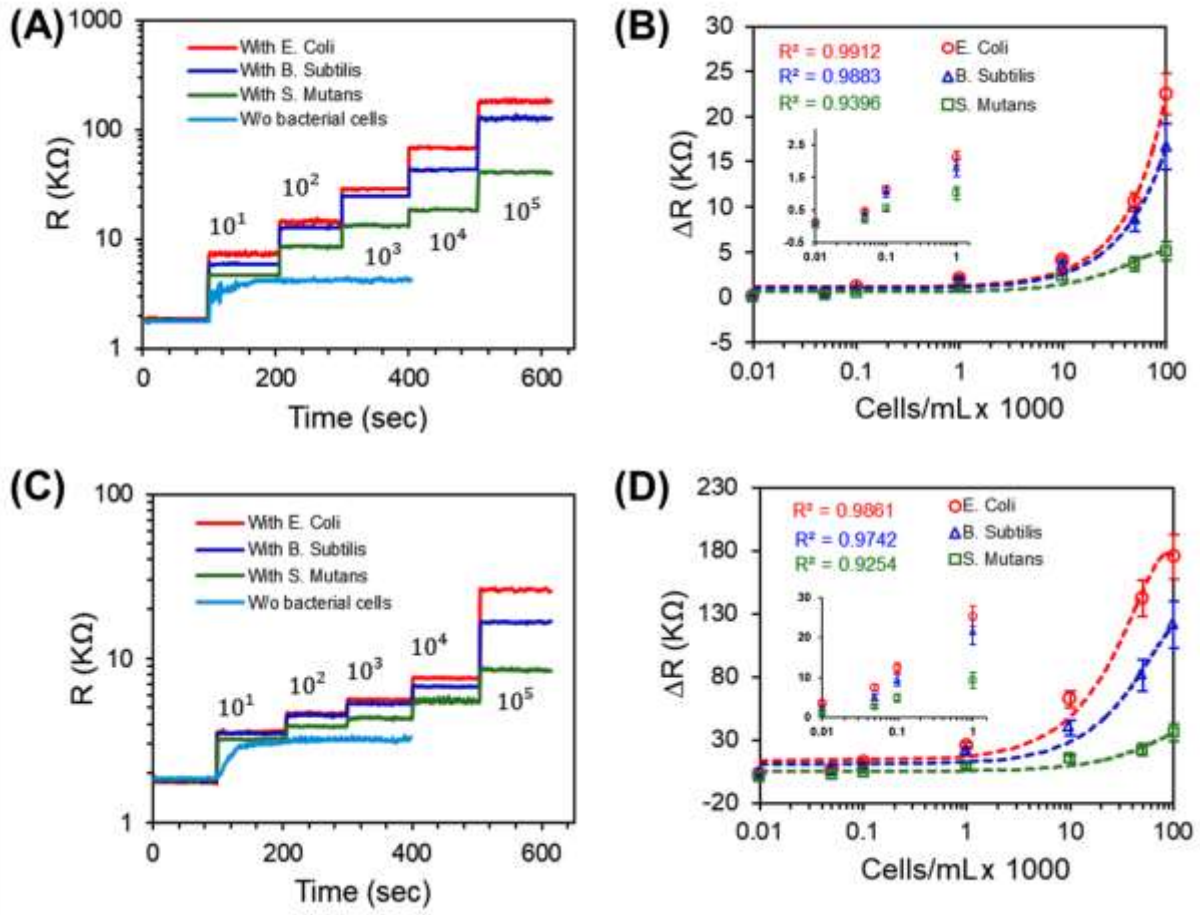


Fig. 4. Analytical assessment of ER-TR sensor chip utilizing whole milk and lake water samples. (A) Dynamic response of the sensor chip exposed to different concentrations (10^1 – 10^5 cells/mL) of E. coli, B. subtilis and S. mutans spiked in milk samples. (B) The calibration curve of the sensor chip tested with regression coefficient of 0.9912, 0.9883 and 0.9396 for E. coli, B. subtilis and S. mutans spiked in milk samples (4 µL) respectively. (C) (D) The calibration curves of the impedance magnitude changes versus the concentrations of bacterial cells represents the regression of 0.9861, 0.9742 and 0.9254 for E. coli, B. subtilis and S. mutans spiked in lake water samples (4 µL) respectively.

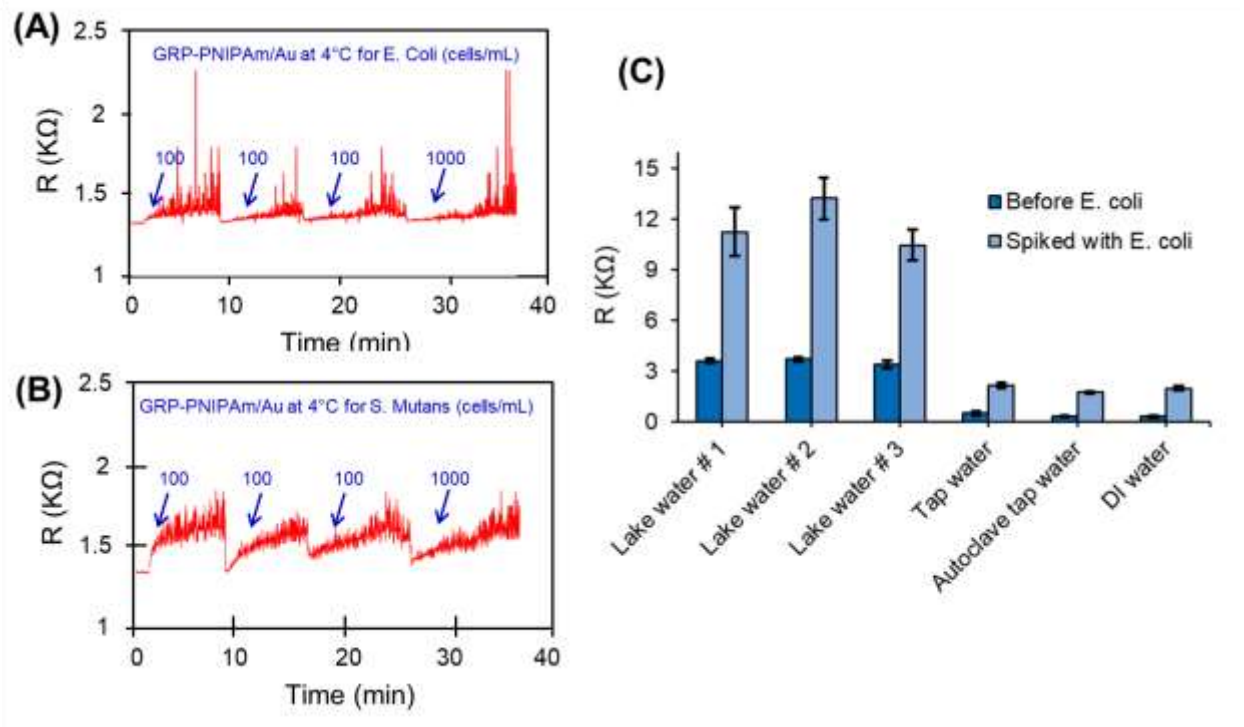


Fig. 5. Testing ER-TR sensor chip at lower temperature and recovery at RT. (A) Gram-positive (E. coli) and (B) Gram-negative (S. mutans) were tested in cold room (4°C) to study the operative behavior of PNIPAm-GRP coated on filter paper. (C) Testing lake water samples, tap water before and after autoclave and DI water with/without E. Coli (100 cells/mL).

Highlights

- Paper-Based Graphene-PNIPAm-Au sensor chip was developed as an integrated device.
- Bacterial cells were captured by fibers of GR-PNIPAm without using antibody conjugation.
- LCST of PNIPAm was controlled electrically using embedded IDμE on chip.
- Reproducibility of 85-97% and shelf life of up to 3 weeks were achieved.
- The platform tested for robustness with various bacterial strains and environmental or food samples