About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

Aptamer Based E-coli Detection in Waste Waters by SWCNTs Modified Biosensor System

Nimet Yildirim [1, 2], Jinyoung Lee [3], Hanchul Cho [3], HeaYeon Lee [3], Ahmed Busnaina [3], April Z. Gu[*2]

[1]Bioengineering PhD Program, Northeastern University, Boston, USA [2]Department of Civil and Environmental Engineering, Northeastern University, Boston, USA [3]The NSF Nanoscale Science and Engineering Center for High-rate Nanomanufacturing (CHN), Northeastern University, Boston, MA 02151, USA

Motivation

- E. coli is appropriate indicator for monitoring potential enteric pathogens in waters.
- A microbiological indicator for fecal contamination in water and foods.
- For drinking water, E.coli must be less than 1 CFU/100 mL (EPA).
- With traditional detection methods (like colony counting), detection takes more than 24 hours.
- PCR techniques needed extra DNA extraction steps.
- ELISA used antibodies, not stable for long time.
- Over the past decade, many biosensor systems have been developed for sensitive and reliable detection protocol.

1. Dufour, A.P. (1977) Escherichia coli : the fecal coliform. In Bacterial Indicators/health Hazards Associated with Water (eds A.W. Hoadley and B.J. Dutka), pp. 48–58, American Society for Testing and Materials, PA.
2. Bej, A.K., R. Steffan, J. DiCesare, L. Haff, and R.M. Atlas. 1990. Detection of coliform bacteria in water by polymerase chain reaction and gene probes. Appl. Environ. Microbiol. 56: 307-314.

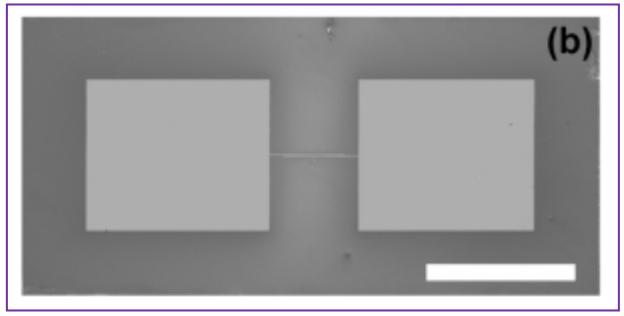
Introduction

SWCNTs Modified Biosensor System

- The flexible biosensor was fabricated by directed assembly using reusable template.
- Flexible template: polyethylene-naphthalate (PEN) film.
- A plain gold templates were fabricated as two electrode system.





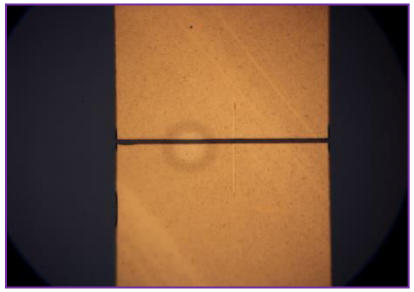


Yang Zhang *et al* 2013 *Nanotechnology* **24**

- The device was immersed into SWNTs suspension (0.001 wt%, 90 semiconducting SWNTs).
- DC power supplier was used to apply the potential (2~2.5V) between the two electrodes.
- Negatively charged SWNTs were attracted onto positive conductive patterns in template.

IV Measurement Procedure





- Conductance and resistance changes were measured between two gold electrodes.
- IV measurement was conducted before and after each experiment.

Introduction cont'd

Aptamers

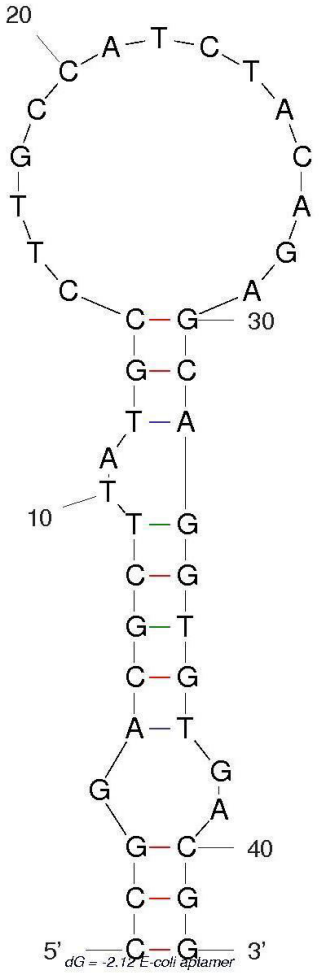
- Aptamers are single-stranded DNA, RNA, or modified nucleic acids.
- Specifically recognize target molecules.
- Obtained from a combinatorial library via an in vitro selection process known as the systematic evolution of ligands by exponential enrichment (SELEX) method.
- There are several advantages of aptamers over antibodies;

➢ much smaller than antibodies,

➢ more easily modified at terminal sites with several functional groups,

➢ more stable than antibodies.

E-coli Aptamer



- DNA aptamer for E-coli; can specifically distinguish the pathogen E. coli O157:H7 from other pathogens (Bruno et al. 2010).
- DNA library was first incubated with the E. coli K12 strain and the DNAs binding to the strain were discarded.
- The precluded DNAs were then used for the selection of O157:H7-specific aptamers.
- After 6 rounds of the subtractive cell-SELEX process, the selected aptamer was found to specifically bind to the O157:H7 serotype, but not to the K12 strain.

Bruno, J.G.; Carrillo, M.P.; Phillips, T.; Andrews, C.J. A novel screening method for competitive FRET-aptamers applied to E. coli assay development. J. Fluoresc. 2010, 20, 1211–1223.

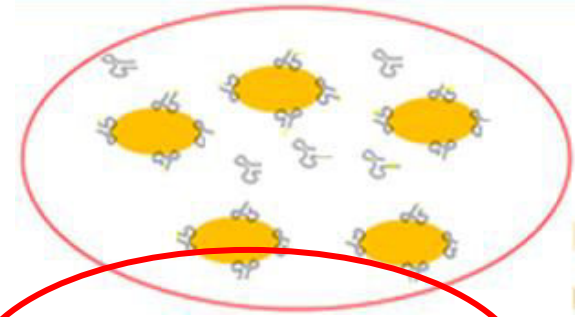
Biosensor Systems for E-coli Detection

Detection method	Recognition element/target	Detection range	Detection time	Enrichment or DNA-protein extraction	Regeneration	Ref.
DNA aptamer-based impedance biosensor	DNA-aptamer/ E. coli outer membrane proteins	1×10^{-7} to 2×10^{-6} M	90 minutes	E. coli OMPs extraction	15- 45% of regeneration	(Queirós et al. 2013)
DPV based electrochemical biosensor	DNA-aptamer/ E-coli surface lipopolysaccharide	2×10^2 to 2×10^7 $CFUml^1$	3.5 hours	NO	NO	(Luo et al. 2012)
Real-time PCR	DNA template of the RNA-aptamer/ E-coli surface protein	10^1 to 10^7 $CFUml^1$	60 minutes incubation + 40 minutes detection	DNA extraction	NO	(Lee et al. 2009)
DNA aptamer-based colorimetric detection	DNA aptamer/ whole E-coli cell	10^4 to 10^8 $CFUml^1$	2 hours	NO	NO	(Su et al. 2012)
DNA aptamer-based colorimetric detection	DNA aptamer/ whole E-coli cell	10^5 to 10^8 $CFUml^1$	20 minutes	NO	NO	(Wu et al. 2012)
Antibody modified microfluidic chip and real-time qPCR	Antibody/ whole E-coli cell	10^1 to 10^6 $CFUml^1$	Incubation + 74 minutes detection	DNA extraction	NO	(Dharmasiri et al. 2010)
Label free polyaniline based impedimetric biosensor	antibody	10^2 to 10^7 $CFUml^1$	10 minutes binding + few minutes detection	NO	NO	(Chowdhury et al. 2012)

Aspects of Improvement

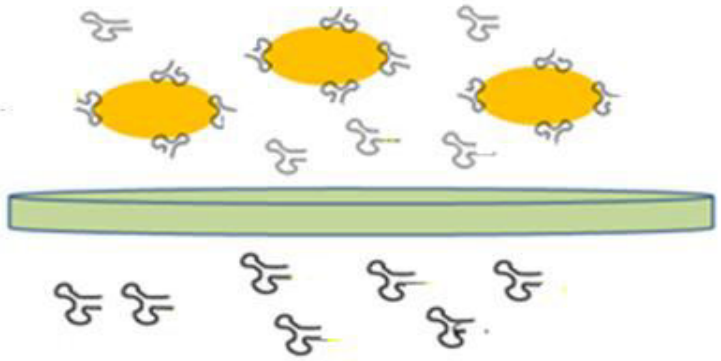
- Using E-coli specific DNA aptamer with high specificity and affinity without any reactivity loses in the harsh conditions
- DNA-aptamer directly binds to the surface lipopolysaccarides, so eliminate protein extraction step.
- SWCNTs modified biosensor system with using features of the electrochemical systems such as; low voltage need, small, cost effective and easy to use equipment.
- Improve sensitivity to detect the U.S. EPA allowable levels of E. coli and less than 1cells/ml infectious dose.

Detection Procedure

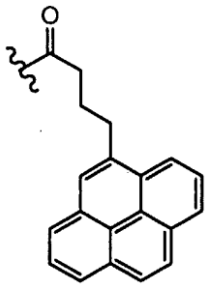


Mixing E-coli cells and aptamer

Filtration of the mixture with 0.22 µm pore size filter



Pumping to the sample cell

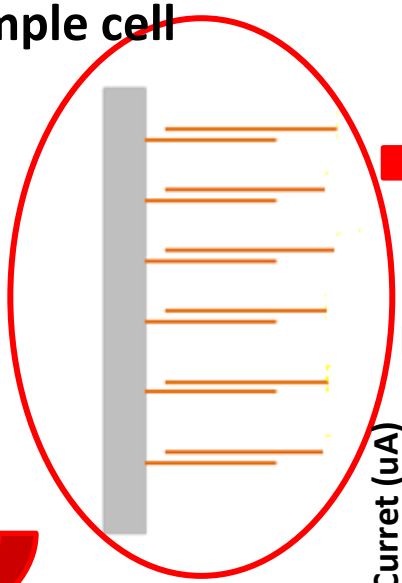


pyrene-butyric acid

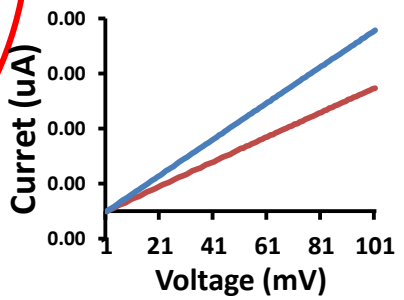


Probe-DNA immobilization onto the SWCTs surface

Hybridization of the aptamer and probe DNA

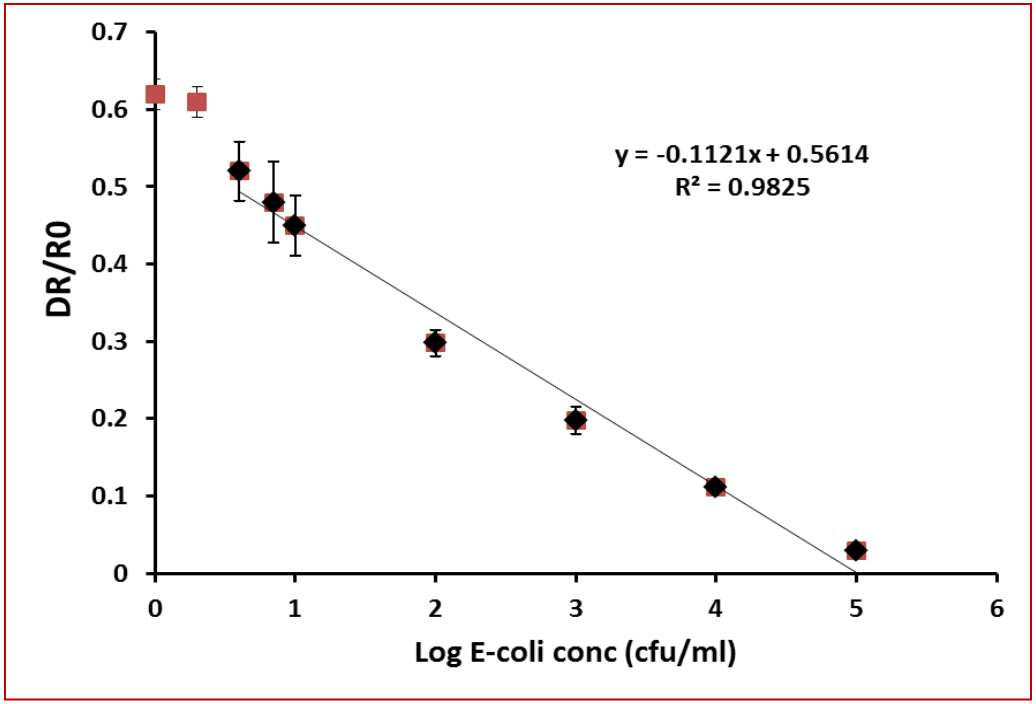


I-V Measurement



Regeneration with 0.5 % SDS

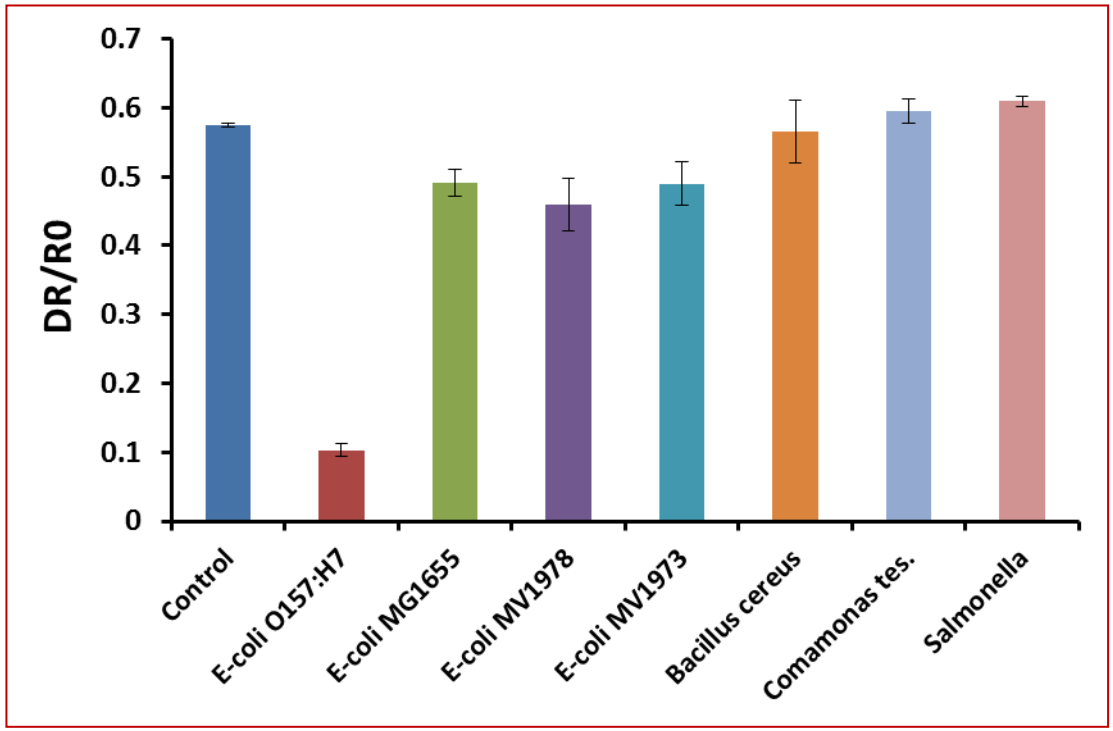
Dose-response Measurements of the Sensor



- Each data value is the average of three independent experimental results.
- The linear range between 4 cfu/ml and 10^5 cfu/ml since we got negligible signal with the E-coli concentration smaller than 4 cfu/ml.

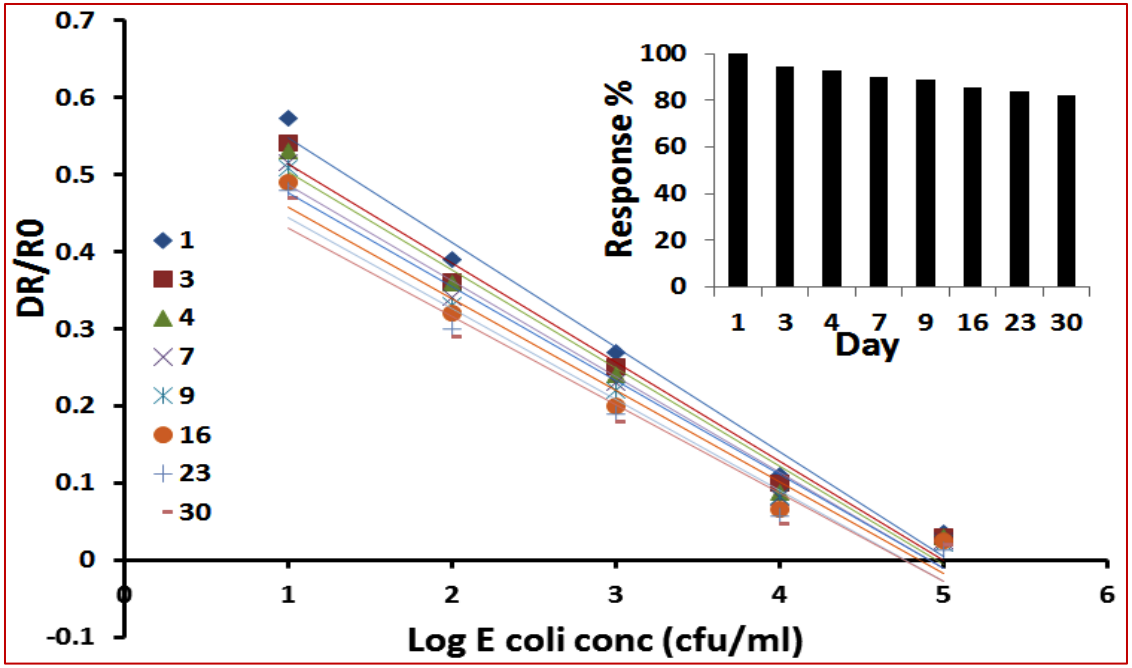
Assessment of Sensor Specificity

- All bacteria strains were applied at 2000 cfu/ml.



- Still have 10 % to 15 % of signal decrease with other pathogens.
- May come from just non-specific binding to the pathogens or loosing aptamers during filtration

Regeneration and Sensor Stability



- The sensor system is stable over 80 % with E-coli detection in30 days period.

Analysis of Spiked Wastewater Treatment Effluent Samples

	Spiked (cfu/ml)	Found (cfu/ml)	cv %	Recovery %
plant 1	10	8.7	3.2	87.7
	10^4	9255.1	18.1	92.5
plant 1	10	9.8	9.9	98.0
	10^4	10158.5	17.2	101.5
tap water	10	9.2	2.6	92.7
	10^4	8687.7	5.5	86.8

- Two duplicate experiments were performed for all samples.
- The recovery of all measured samples was between 93 and 118 %, and the parallel tests showed that the relativity coefficient was within 4.3-21.8 %.

Conclusion

- An aptamer-based biosensor for rapid and selective E-coli detection was developed with using a SWCNTs modified system.
- Low detection limit; 4 CFU/ml.
- When compare with the present studies, the new system is cost-effective, rapid (less than one hour), easy to use and reusable.
- The system is selective for E-coli O157:H7 .
- The biosensor evaluated in spiked wastewater samples.



APRIL Z. GU
Associate Professor and College of Engineering Faculty Scholar
Department of Civil and Environmental Engineering
471 Snell Engineering Center
360 Huntington Ave.
Boston, MA 02115

Northeastern University
Center for High-rate Nanomanufacturing



Questions ??

Thank you

Let Us Meet Again

We welcome you all to our future conferences of OMICS Group International

Please Visit:

www.omicsgroup.com

www.conferenceseries.com

www.pharmaceuticalconferences.com