# Industry Day Theme # Sustainable Medical Technologies (SMT)

IIT DELHI INDUSTRY DAY 2019

# Monitoring drug-bacteria interaction using dielectrophoresis and impedance spectroscopy: A combined approach

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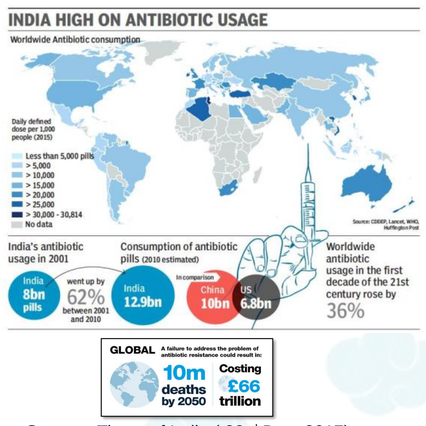
## Introduction and Motivation

✓ Bacterial infections have become a major challenge for global health as they kill over 15 million people annually worldwide.

✓ The problem is further complicated by high consumption of antibiotics and the absence of rapid diagnosis tools, leading to antimicrobial resistance.

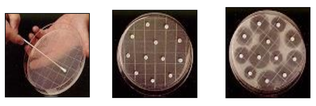
**Antimicrobial resistance has emerged as an alarming concern worldwide**



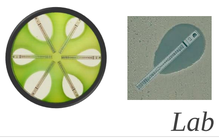

Source: Times of India ( 22nd Dec, 2017)

***Present techniques for antimicrobial susceptibility testing (AST):***

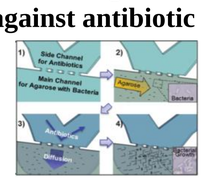
**Disk Diffusion:**



**E-Test:**



**Bacterial growth against antibiotic**




**Advantage:**
- Good accuracy and simple to perform

**Disadvantages:**
- Require sample preparation and pre-treatment
- Time consuming ( > 24 hr)
- Manually intensive

*Lab on a chip*, **2013**, *13*, 280

***MIC- minimal inhibitory concentration***

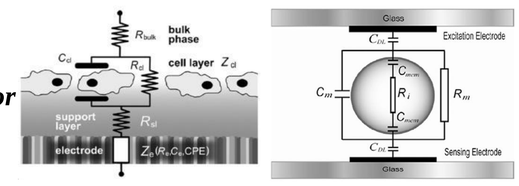
## DEP-IS Approach

**Dielectrophoresis (DEP)** allows separation of biological particles based on their distinct electrical properties.

**Impedance spectroscopy (IS)** is an electrical method to investigate cells and their electrophysiological response over a wide frequency range in real time. It is
- ✓ label-free
- ✓ high-throughput
- ✓ miniaturized and automated

Drawback:
✓ Require complex chip design and electrode surface modification to enhance impedance signal
✓ Lack of investigation of low conductive buffer to study bacterial cytoplasmic impedance response below 10 MHz frequency

***Impedance for cell probing***



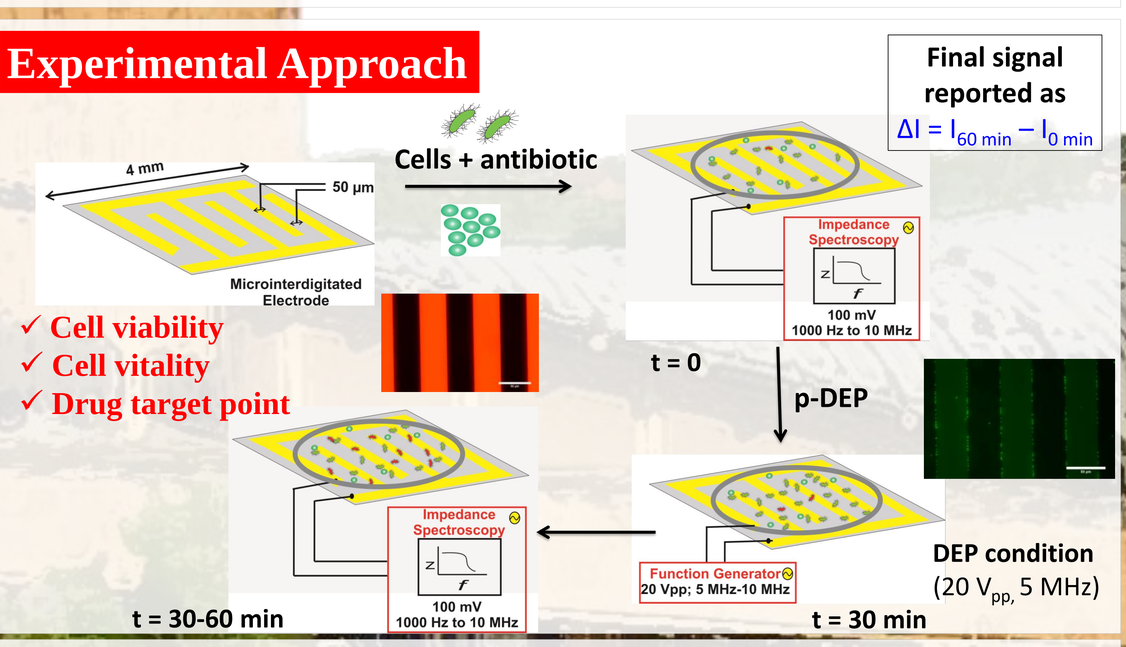

*Sci. Rep*, **2018**, *8*, 16109
*J Lab Autom*, **2014**, *19(1)*,42-9

*Biosen. Bioelec*, **2016**, *77*, 824

## Objective of our Research

To accelerate real-time cell viability analysis by measuring cell death by reducing typical AST time (>24 hr) to few hours.

## Experimental Approach

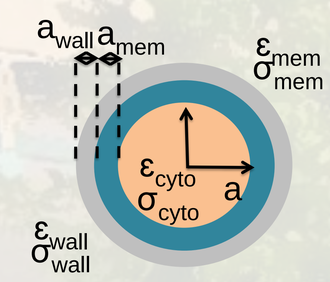


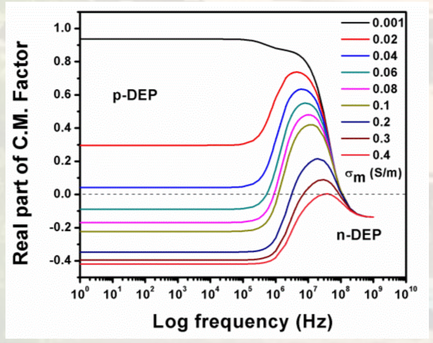

Final signal reported as $\Delta I = I_{60\ min} - I_{0\ min}$

- ✓ Cell viability
- ✓ Cell vitality
- ✓ Drug target point

## Theoretical double shell dielectric model

**pDEP condition**




**Positive DEP:** Particles are attracted to electric field intensity maxima ;
**Negative DEP:** Particles are repelled

***p-DEP: Conductivity < 0.4 S/m***

## Acknowledgement

**IIT-Delhi and CEERI-Pilani**

Chemical Engineering

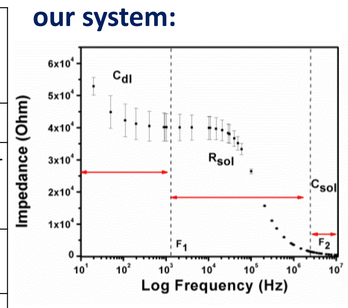
## Results and Discussion

### 1. Buffer optimization for DEP – IS Study

| Medium type | Conductivity (S/m) | \|S/N\| *S. typhi* in buffer | $F_1$ (kHz) | $F_2$ (MHz) | *Remarks* |
|---|---|---|---|---|---|
| DI water | 0.0001 | 41.83 | 0.002 | 0.022 | Unstable EDL |
| 0.1 mM PBS pH 7.4 | 0.02 | 144.31 | 1.2 | 4.5 | Unstable buffer; p-DEP; [S/N]↑↑ |
| **15 mM HEPES; pH 7.4** | **0.04** | **52.20** | **2.4** | **9** | **p-DEP, [S/N]↑** |
| 40 mM HEPES;pH 7.4 | 0.1 | 55.99 | 6 | 22 | p-DEP, [S/N]↑ |
| 40 mM HEPES; pH 8.5 | 0.2 | 909.46 | 12 | 45 | Not for biological particles (pH>7.4), [S/N]↑↑ |
| 10 mM PBS; pH 7.4 | 1.6 | 44.64 | 95 | 360 | Suppressed EDL; No p-DEP |

S/N= Signal/noise; *S.typhi* conc.= $10^7$cfu/mL; EDL- Electrical double layer

**Cut-off frequency of our system:**



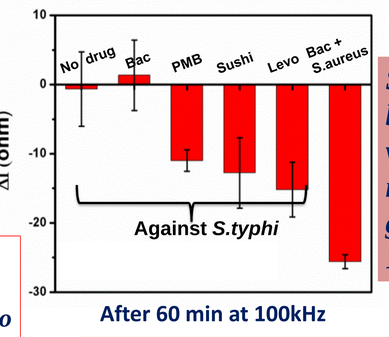

***15 mM HEPES buffer***
***✓ Allows high DEP bacterial capture***
***✓ characterize whole bacteria below 10 MHz***

### 2. Cell viability analysis against different class of antibiotics

| | Antibiotics | Action |
|---|---|---|
| ***S.typhi*** | Sushi peptide, Polymyxin B (PMB) | Rupture cell membrane |
| | Levofloxacin (levo) | Halts DNA replication |
| ***S.aureus*** | Bacitracin (Bac) (as control) | Attacks cell wall |

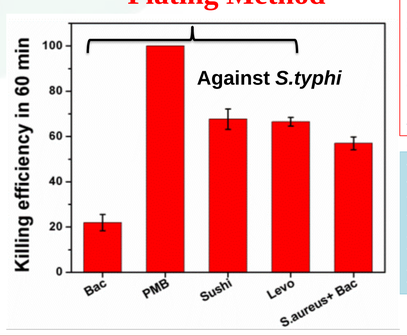
**S.typhi Conc.- $10^7$ cfu/mL; Antibiotic Conc.- 5 µM**

**DEP-IS data**




***Successful bacterial viability monitoring in under 1 hour!***

**Plating Method**

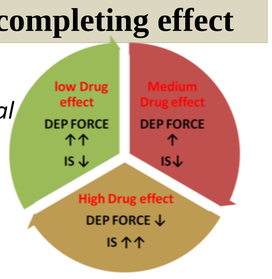



**Impedance drop order:** ***S.aures+bac >Levo ~ Sushi > PMB > Bac***

**Higher bacterial killing → High ionic release → impedance drop high**

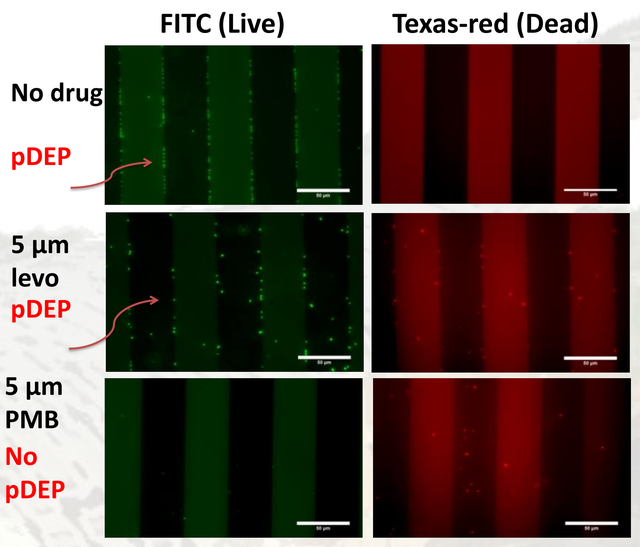
**IS and DEP completing effect**

***DEP force –*** *Enhance bacterial capture*
***IS –****Ionic release*

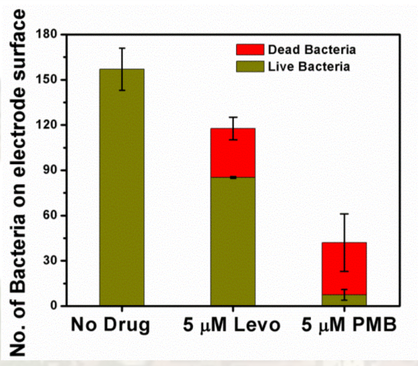



***DEP-IS data has different trend as compared to plating study***

### 3. Microscopic analysis at electrode surface

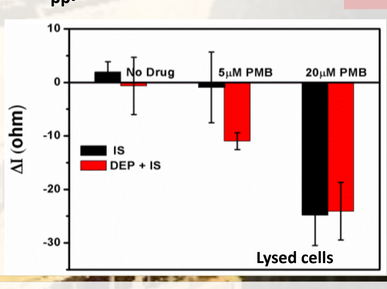



DEP capture (5 MHz, 20 $V_{pp}$) of bacteria for 30 min




- **High cell capture alone does not influence impedance signal**
- **Impedance change depends to ionic release**
- **Ionic release by dead cells contributes to impedance change and benefits from having cells closer to the electrode**

### 4. Role of DEP




***DEP enhances IS signal sensitivity as long as bacteria remain intact***

## Industrial Significance

Rapid bacterial analysis can aid in faster diagnosis of bacterial infections and accelerate the clinical decision-making process for antibiotic treatment, addressing the critical issue of antimicrobial resistance.

## Summary

- Buffer optimization for DEP + IS study
- Cell viability analysis against antibiotics by DEP + IS sensor
- Understanding IS and DEP competing effects by microscopic analysis
- Effect of DEP to enhance IS signal

## Future Plans

- ✓ Generate MIC curves of antibiotics
- ✓ Mathematical modeling of impedance data to elucidate bacterial-drug interaction