# Microbial Adhesion and Removal from Bio-Surfaces

## - Adsorption Density of Microbes on Flat Surfaces -


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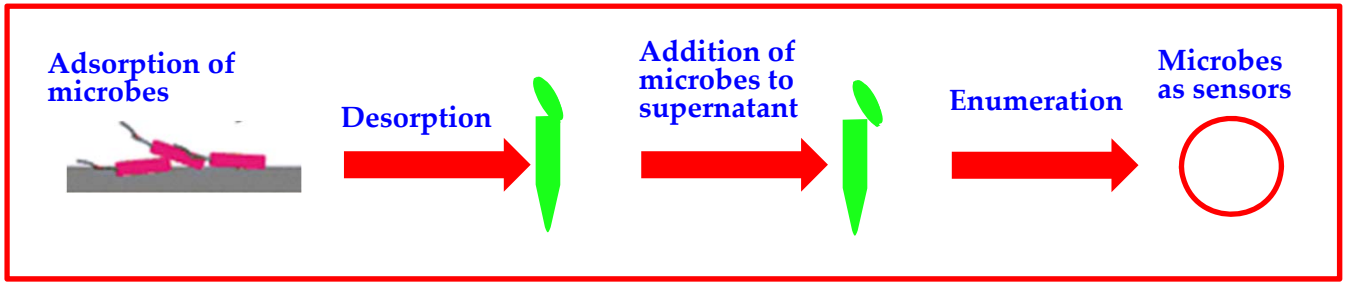
# Overview of the Presentation

**Research Team:** Vasanthakumar Balasubramanian (Vasanth), Brij Moudgil, J. Carnali, C. Walsh, and P. Chandar.

**Goal:** Establish techniques for adsorption density measurements on low surface area surfaces

**In this presentation**

**Microbes as sensors for determining polymer adsorption density on Vitroskin**




**Technical Information:**

Cell viability can be used as a detection parameter. For a given polymer, cell viability depends on free polymer concentration in solution (indirect Γ (adsorption density) measurement). Fluorescence-based protocol may also be used for enumeration of viable microbes on surfaces and in solution (direct Γ measurement).

**Industrial Relevance:** Unilever, Ecolab, Colgate-Palmolive, Johnson & Johnson, P&G etc…

# Current Scenario…



## Background:

- Microbial removal from surfaces using engineered particles demonstrated.
- Adsorption density measurements required to develop quantitative correlations.

### High Specific Surface Area Systems (e.g., fine particles)

1. Indirect (depletion) method – UV absorption, fluorescence, colorimetric methods, radioisotope-labeled fluorescently labeled molecules, TOC
2. Direct method – Radiolabeling, Fluorescence

### Low Specific Surface Area Systems (flat surfaces)

1. Indirect (depletion) method - requires new approaches.
2. Direct method – Radiolabeling, Fluorescence, SPR, OWLS, DPI, ATR-FTIR, SE, QCM-D, AFM

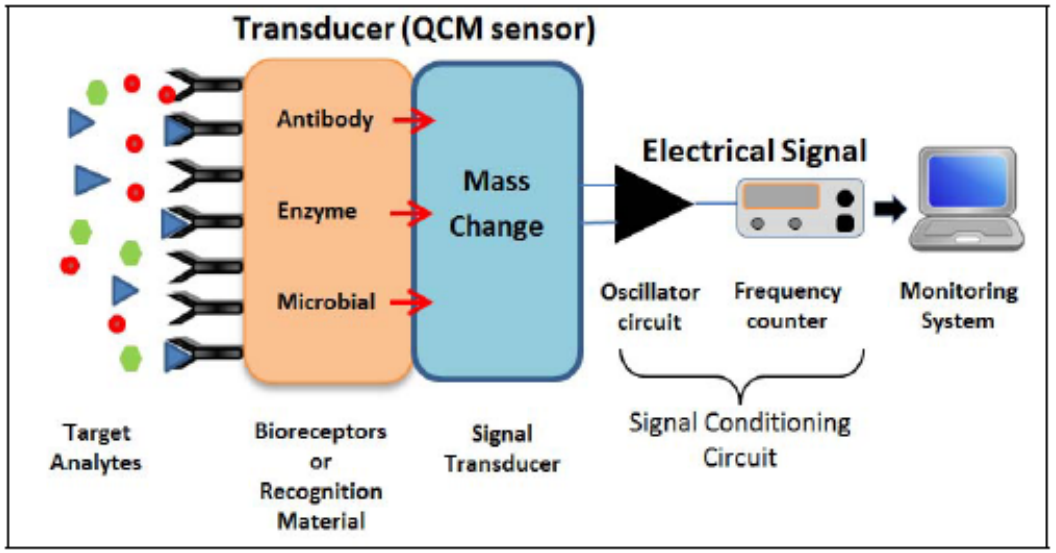
**Promising techniques - measurements on biosurfaces**

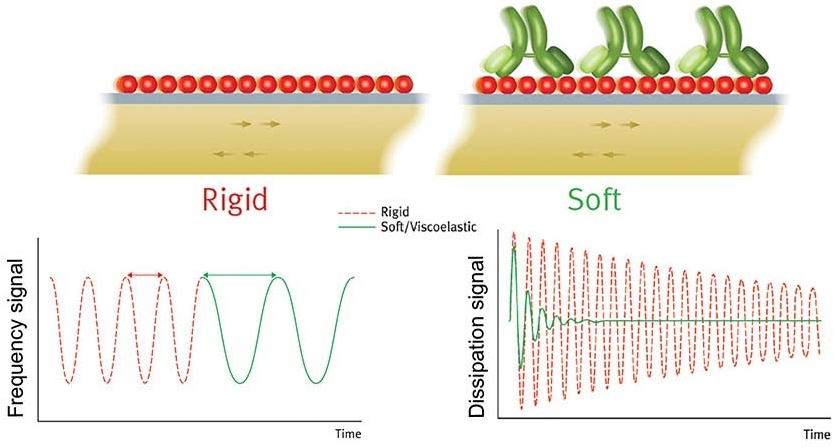
- QCM
- AFM
- Microbes as sensors

# Quartz Crystal Microbalance (QCM)

**The change in QCM frequency determines the mass of analyte adsorbed in ng/cm².**
**Sauerbrey equation**

$$\Delta m = -C'\Delta f$$




QCM sensing system

**Microbes immobilized using antibodies/ DNA/protein as bioreceptor (Quartz surface)**

***Bacillus thuringiensis*** **$10^7$ CFU – 60mg/cm²**
***Bacillus anthracis*** **$10^7$ CFU – 6mg/cm²**
**E.coli $10^8$ CFU - 10µg/cm²**

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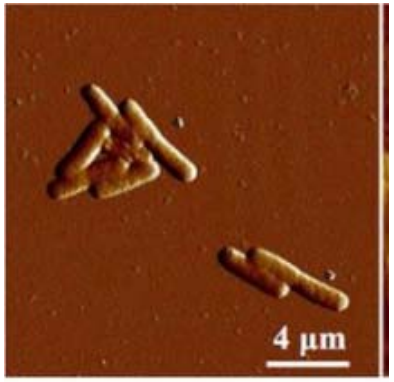
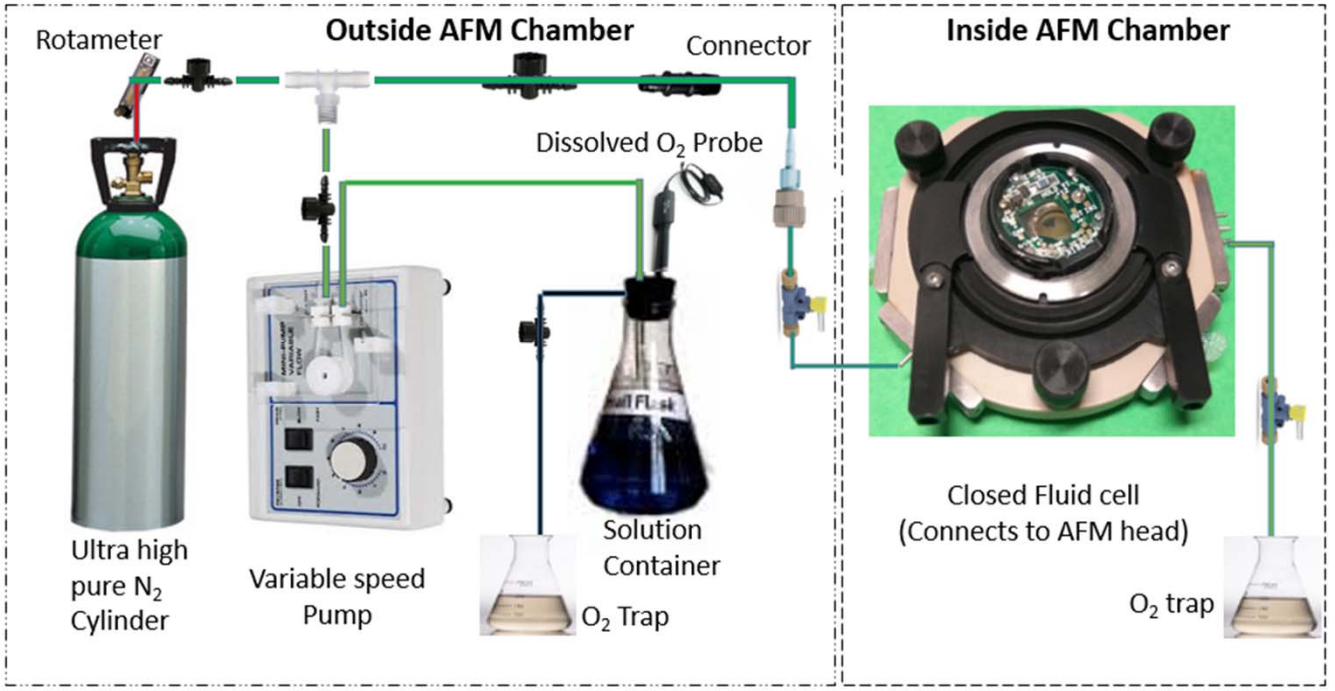
# Atomic Force Microscopy (AFM)

**Atomic force microscopy (AFM) was used to determine the adhesion forces between bacteria and substrate in water and to gain insight into the nano-scale surface morphology. Rastering - Tapping mode**

| Parameters | *E. coli* |
|---|---|
| Cell length/μm | 2.3 ± 0.5 |
| Cell width/μm | 1.3 ± 0.1 |
| Cell height/nm | 237 ± 28 |
| Cell envelop thickness/nm | 22 ± 4 |
| Pili thickness/nm | 4.8 ± 0.8 |




**Surface topographies of microbes on the substrate can be measured in tapping mode. Γ calculated from thickness and surface coverage.**

*Scientific Reports* volume5, 16857 (2015)

**Promising techniques - measurements on biosurfaces**

- QCM
- AFM
- Microbes as sensors

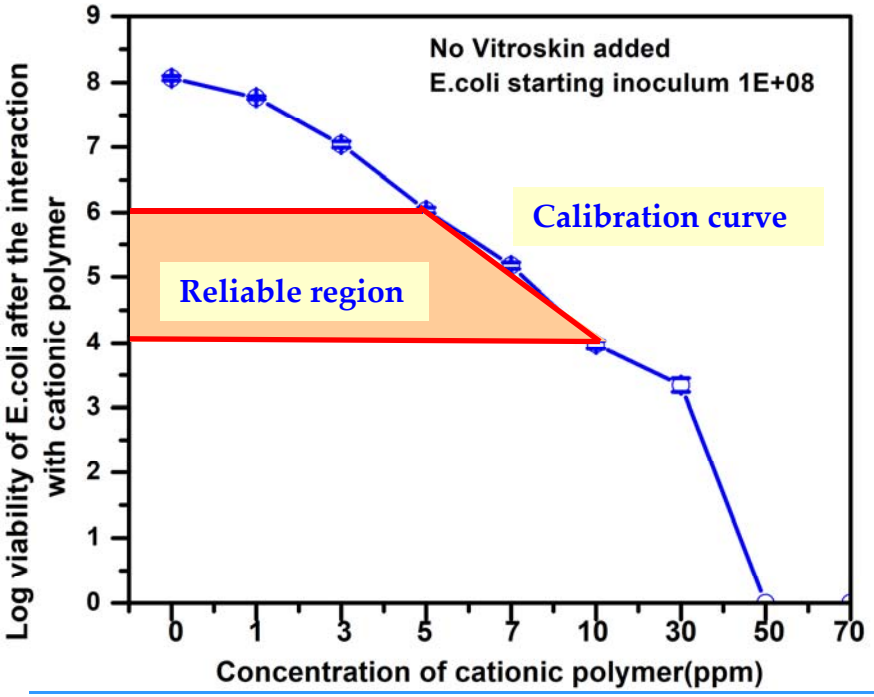
## Part - I Microbes as sensors for determining polymer adsorption density on Vitroskin

# Illustrative example: Cationic polymer adsorption density on Vitroskin using E.coli as a biosensor

## CPaSS Approach:

- Cell viability used as a detection parameter.
- Agar Plate method to determine cell viability.
- For a given polymer, cell viability depends on free polymer concentration in solution.

**Polymer adsorption density on Vitroskin can be determined based on cell viability measurements.**




| Initial polymer concentration (ppm) | Final polymer Concentration (ppm) | Adsorption density on Vitroskin μM/cm² (Depletion method) |
|---|---|---|
| 5 | 3 | 20 (not reliable) |
| 10 | ~6 | 40 |

**Sensitivity: Viable cell detection limit > $10^4$/ml**
**Reliability of viable cell measurement: ± 0.5 log**

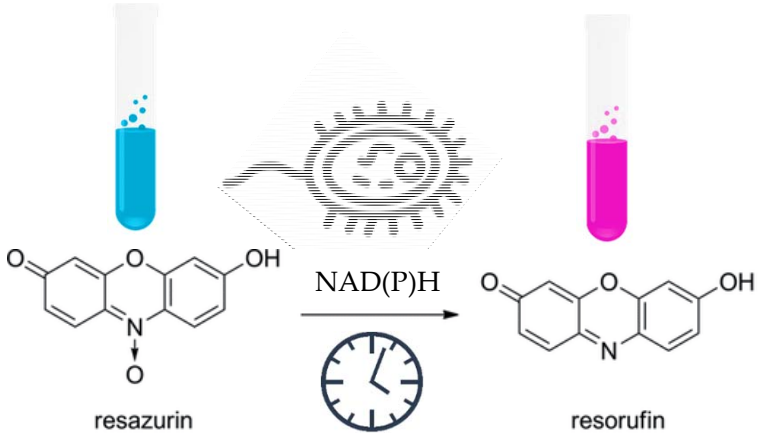
## Part – II CPaSS approach: Fluorescence-based protocol for enumeration of viable microbes on surfaces and in solution.

**(invention disclosure submitted)**

# CPaSS Resazurin Fluorescence Dye Assay

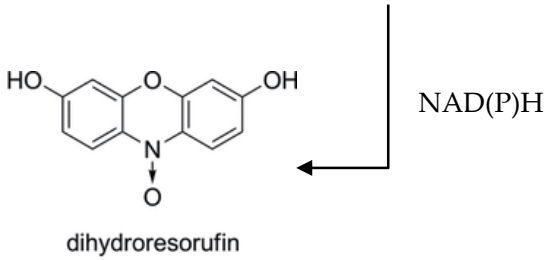


## PrestoBlue® Mechanism



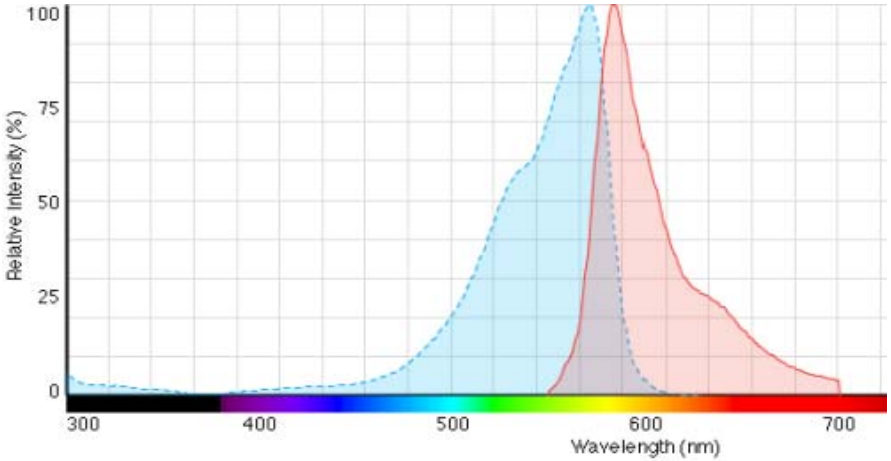

Non-Fluorescent

**Fluorescent**




Non-Fluorescent

## Resorufin Excitation and Emission



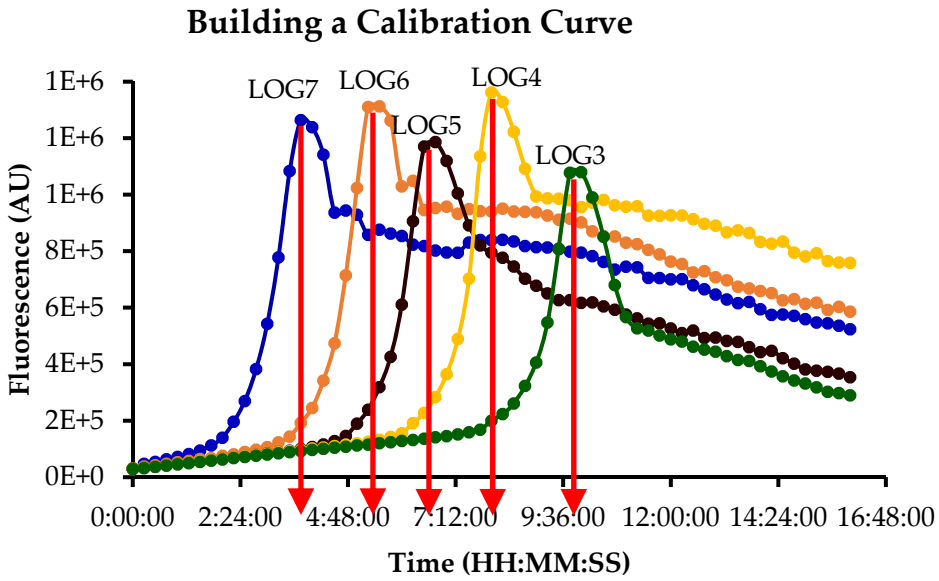

**PrestoBlue® uses the metabolically active resazurin, which is weakly fluorescent and is irreversibly reduced to highly fluorescent resorufin.**

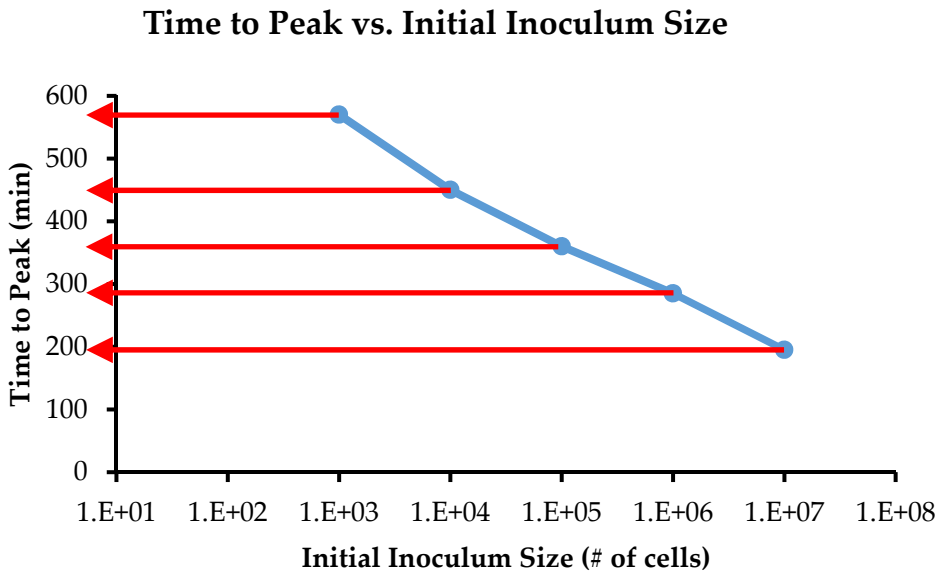
**Resazurin and resorufin freely diffuse through cell membranes, so readings are taken from the bulk solution.**

- **Potential for utilization as a reporter for enumeration from surfaces.**

# Data Extraction





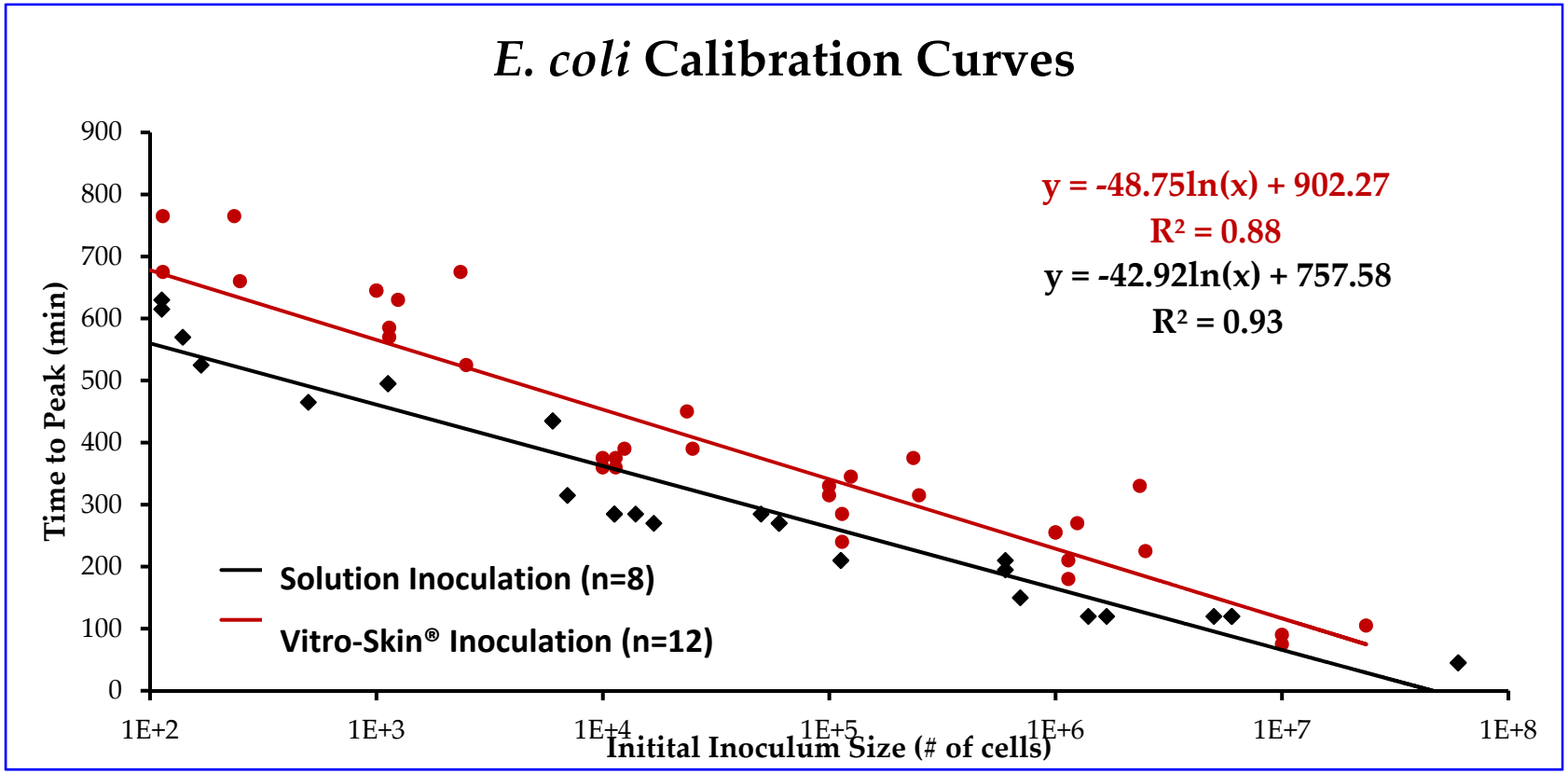

**Each curve represents a different initial inoculum size.**

**A unique lag phase exists for each inoculum size. By plotting the lag time, a LOG – linear curve is generated.**

**Since the independent variable is inoculum size, the dependent variable becomes lag time.**

# E.coli calibration curves in solution and at Vitroskin






| Initial inoculation CFU/cm² | Adsorption density on Vitroskin | |
|---|---|---|
| | CFU/cm² | pg/cm² |
| 1E+4 | 1E+3.2 | 1 |
| 1E+5 | 1E+3.8 | 1 |
| 1E+6 | 1E+4.7 | 10 |

**Advantages:** Lower cell count limit extended due to higher fluorescence sensitivity
- Reliable up to 1E+2 as compared to Agar plate method (1E+4)

# Summary and Future work



**Challenges in the adsorption of biomolecules on solid surfaces:**

- very small amounts of adsorbed material to be detected
- inherent difficulties to sense at an interface without interference from the solution.
- concomitance of several molecular phenomena (adsorption, desorption, conformational changes, and rearrangements)

- Microbes as sensors is a promising approach for adsorption measurements (depletion as well as direct methods).
- QCM and AFM may be used for direct adsorption density measurements. (QCM - limitation of substrates).
- Viability of AFM and QCM for microbial adsorption density need to be established.

# Thank you

**Acknowledgment**

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**Disclaimer**

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