BioMEMS Device for Real Time Drug Monitoring and Toxic Build-Up Prevention in Peritoneal Dialysis Patients

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Outline

I. Background & Clinical Need

II. Proposed Solution

   A. How it Works

   B. Fabrication & Device Properties

   C. Limitations

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What is peritonitis?

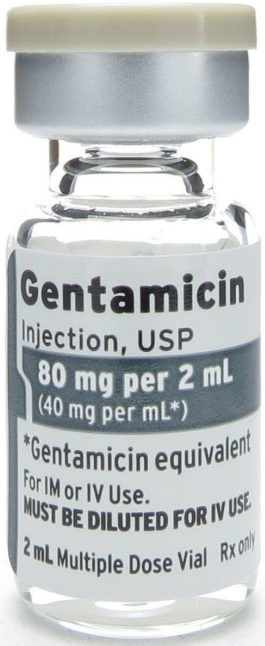
- Inflammation of the peritoneum, typically caused by a bacterial infection.
- Mortality for an episode of peritonitis is 5%.[1]
    - Rate in peritoneal dialysis patients is 1 episode per 2 years (2016).[2]

Why peritoneal dialysis patients?

- Peritoneal dialysis (PD) uses catheters to remove waste products from the blood when the kidneys cannot adequately do so. Infection may result from improper cleaning of the insertion site, unclean surroundings or contaminated equipment.[3]
- ~51,000 of end stage renal disease patients in the U.S. are treated with peritoneal dialysis.[2]

Treatment

- Gentamicin has a narrow therapeutic range.[4]
  - Can cause adverse reactions and may be irreversibly toxic if the concentration is too high[4]
- Gentamicin toxicity may result in:
  - Kidney and nerve damage, bilateral vestibulopathy and hearing loss[5]
- Current monitoring methods require skilled technicians, mL-scale sample volumes and long turnaround times.[2]
  - PETINIA: Particle-enhanced turbidimetric inhibition immunoassay
  - ELISA: Enzyme-linked immunosorbent assay



Gentamicin: an antibiotic commonly prescribed for peritonitis, used to treat severe bacterial infections.

https://www.mcguffmedical.com/gentamicin-sulfate-40mgml-mdv-2ml-25-vialstray

The Clinical Need

PD patients with peritonitis need **a safe way to monitor the concentration of gentamicin in real time** to ensure they are receiving effective dosages and to prevent adverse reactions and toxicity.

A BioMEMS Solution

We propose a CMOS-based BioMEMS device for label-free detection of gentamicin using a piezoresistive microcantilever (MCL) sensor.

- The biosensor will monitor drug level in the blood in real time, allowing for effective drug dosages to be adjusted appropriately.
- MCL sensor can complete detection in ~15 minutes[4]
- Requires only a microscale specimen (10 uL)[4]
- No sample modification via label-free detection
- Provides a low-cost portable solution

How it Works

Detection of Gentamicin - Immunosensing Overview

Target molecule: gentamicin

Biorecognition element: gold-coated MCL with antibody layer

- The microcantilever deflects due to adsorption stress.

Sensing element: embedded piezoresistive material

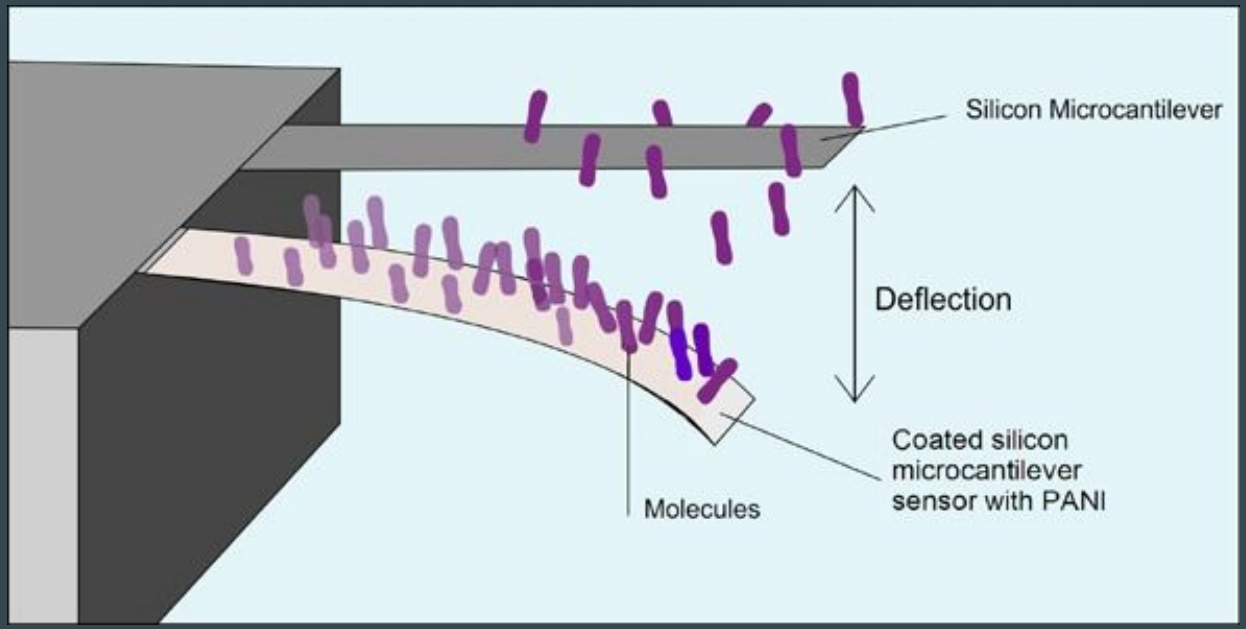
- The piezoresistive material gains the resistance-change signal of the deflection induced by the molecular recognizing generated surface stress of the MCL.[7]

Measurement: MCL deflection and change in resistance

- Strain is applied to the piezoresistor which causes a change in resistance that can be electronically measured.[7]
- MCL deflection is proportional to analyte concentration.[8]

MCL Biosensing

1. Target molecules bind directly to the biorecognition element
2. Binding induces deflection of the MCL
3. MCL deflection is measured to detect changes in mass at the nanogram scale



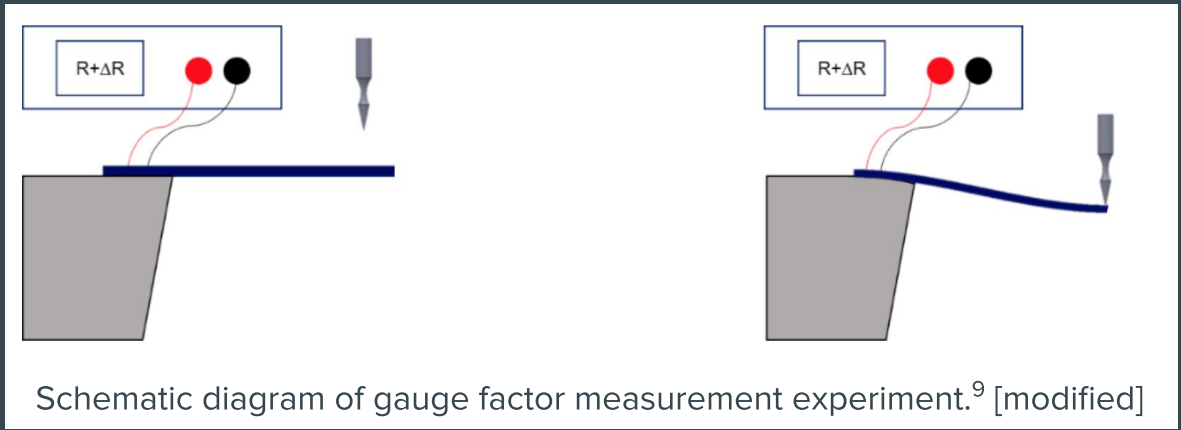
Schematic of MCL sensor principle.[8]

Mechanical Properties

Gauge Factor: $G = (\Delta R/R_0)/\varepsilon = 17.71$

where ΔR is change in resistance, R_0 is the reference resistance, and ε represents axial strain.[9]

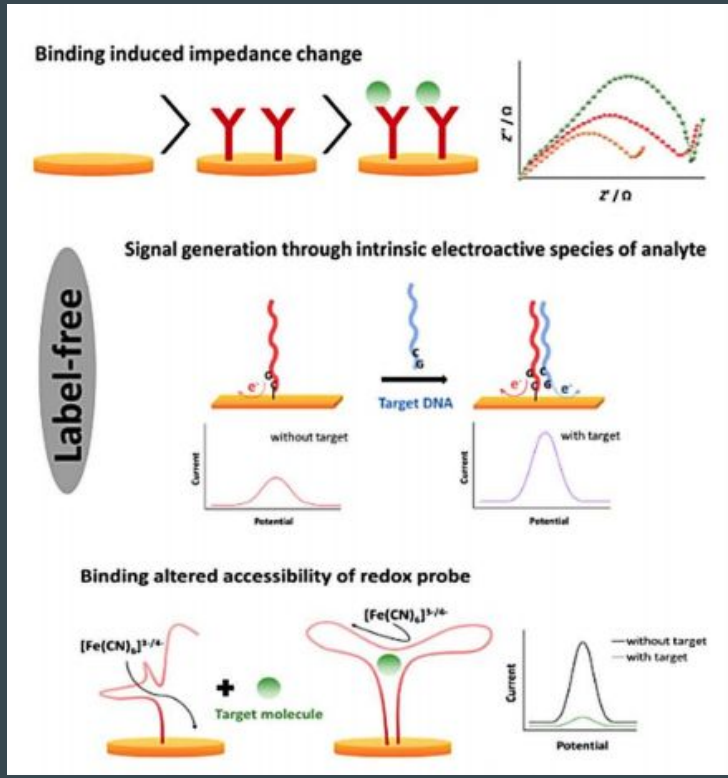
Resonance Frequency: Index that dictates whether the structure may have resonance in detection frequency.



Schematic diagram of gauge factor measurement experiment.[9] [modified]

Label-Free

- Label-free: the sample doesn't need to be modified
- Use of labels can produce unanticipated interactions
- Label-free benefits: highly sensitive, reduced required resources, allows for portability and real-time monitoring, less false positives/ negatives
- MCL biosensor: only label-free method that can measure the small molecules of a MW less than 2 kDa[10]

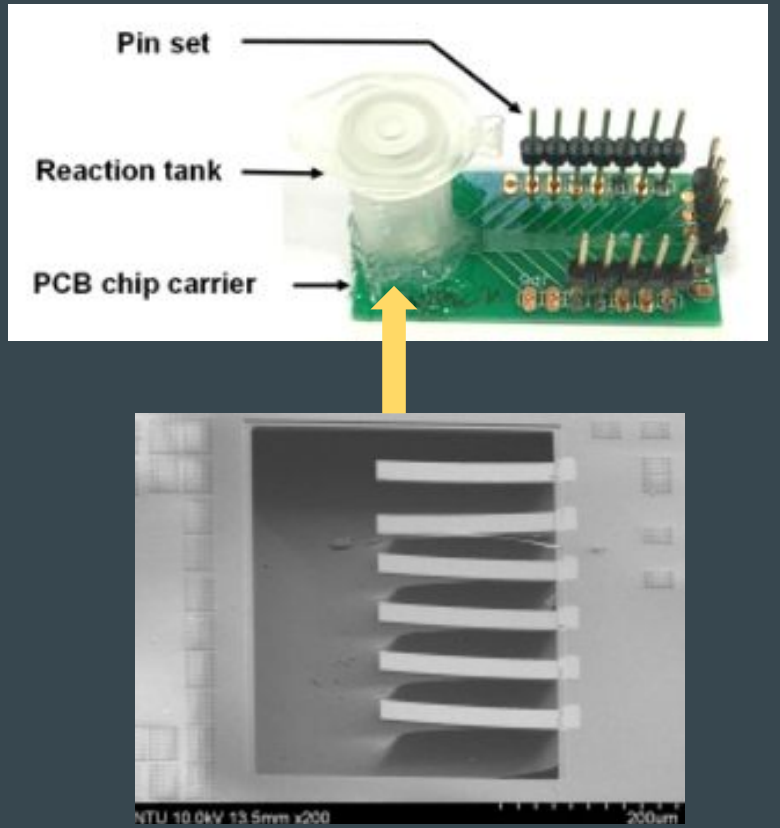


Wongkaew et al.

Fabrication

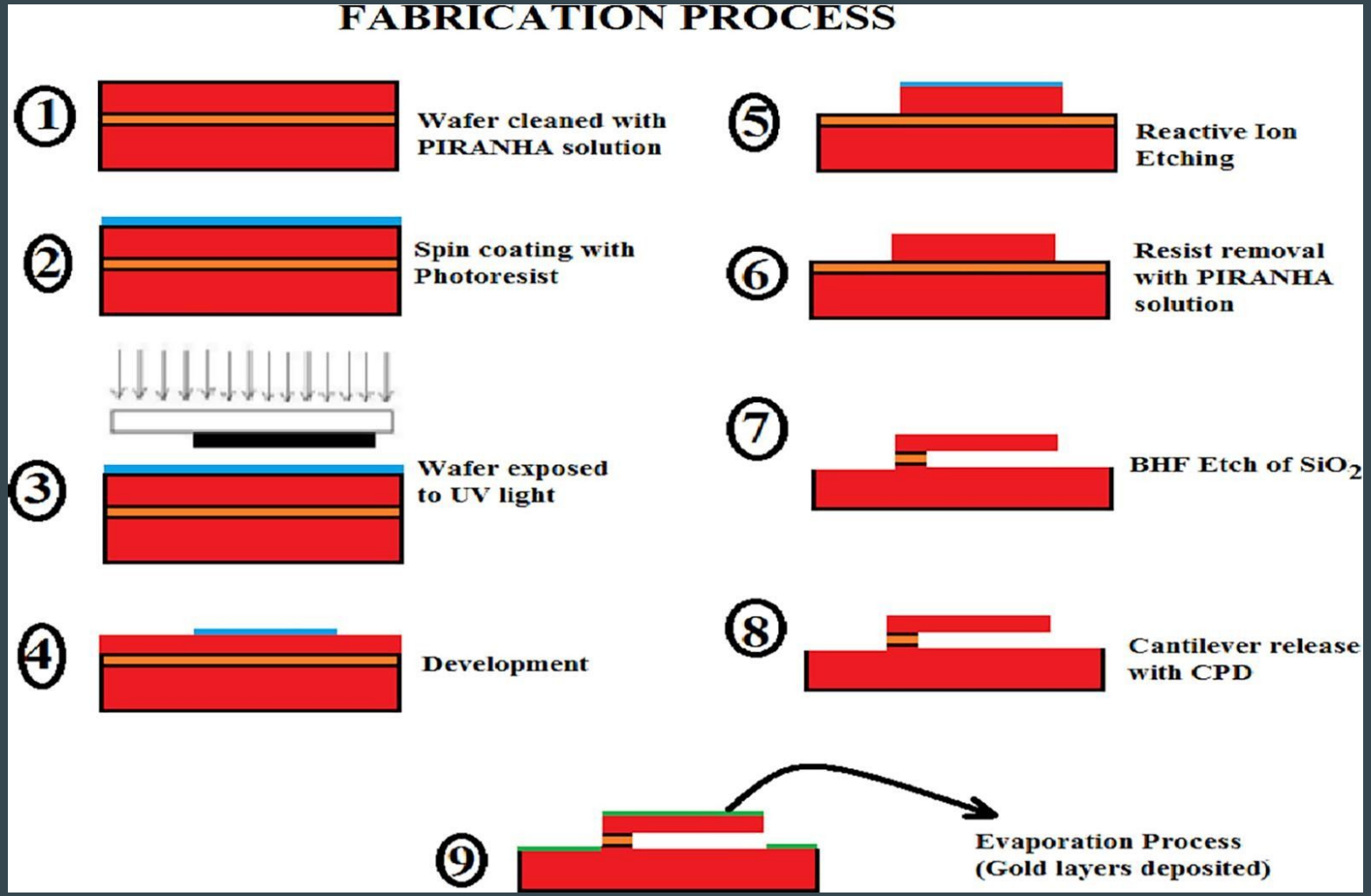
The MCL Sensing Chip

- The selected dimensions ensure that high sensitivity will be achieved.[4]
  - Each MCL is 4 µm thick, 40 µm wide, and 200 µm long.[4]
  - Chip size is 1.5 mm x 1.5 mm
  - Made out of 2 layers of polysilicon and 2 layers of metal
- 6 MCLs will be used for increased reliability.[4]
- The MCL Chip will be wire bonded to printed circuit board chip carrier for signal readout.
  - A parylene coating will be applied to protect the device from chemical reactions with various compounds including acids and alkaline solutions.
- A disposable reaction tank is integrated onto the carrier chip.[9]



The MCL Sensor - Fabrication Summary

1) Fabrication begins with a single side polished n-type silicon on insulator (SOI) wafer. This wafer will first be cleaned using a piranha solution, HF, and then rinsed with water.[11]
2) A positive photoresist will be coated on the wafer (AZ 5214) and then the wafer will be soft baked.[11]
3) To remove the layer of silicon below the photoresist, reactive ion etching will be used (gases include C_4F_8 and SF_6). Photoresist will be removed in piranha solution.[11]
4) A round of wet etching will be done to make the cantilever shape, this will be a wet etching processes done with a buffered hydrofluoric acid solution.[11]
5) Critical point drying will then be carried out at high pressure. This type of drying preserves the surface structure of specimen which could be damaged due to surface tension when changing from the liquid to the gaseous state. Isopropyl alcohol will be replaced with CO_2.[11]
6) Finally, an evaporation process will take place in order to deposit a layer of gold on the fabricated cantilever with titanium as an adhesion layer.[11]


FABRICATION PROCESS
1
Wafer cleaned with PIRANHA solution
2
Spin coating with Photoresist
3
Wafer exposed to UV light
4
Development
5
Reactive Ion Etching
6
Resist removal with PIRANHA solution
7
BHF Etch of SiO_2
8
Cantilever release with CPD
9
Evaporation Process (Gold layers deposited)

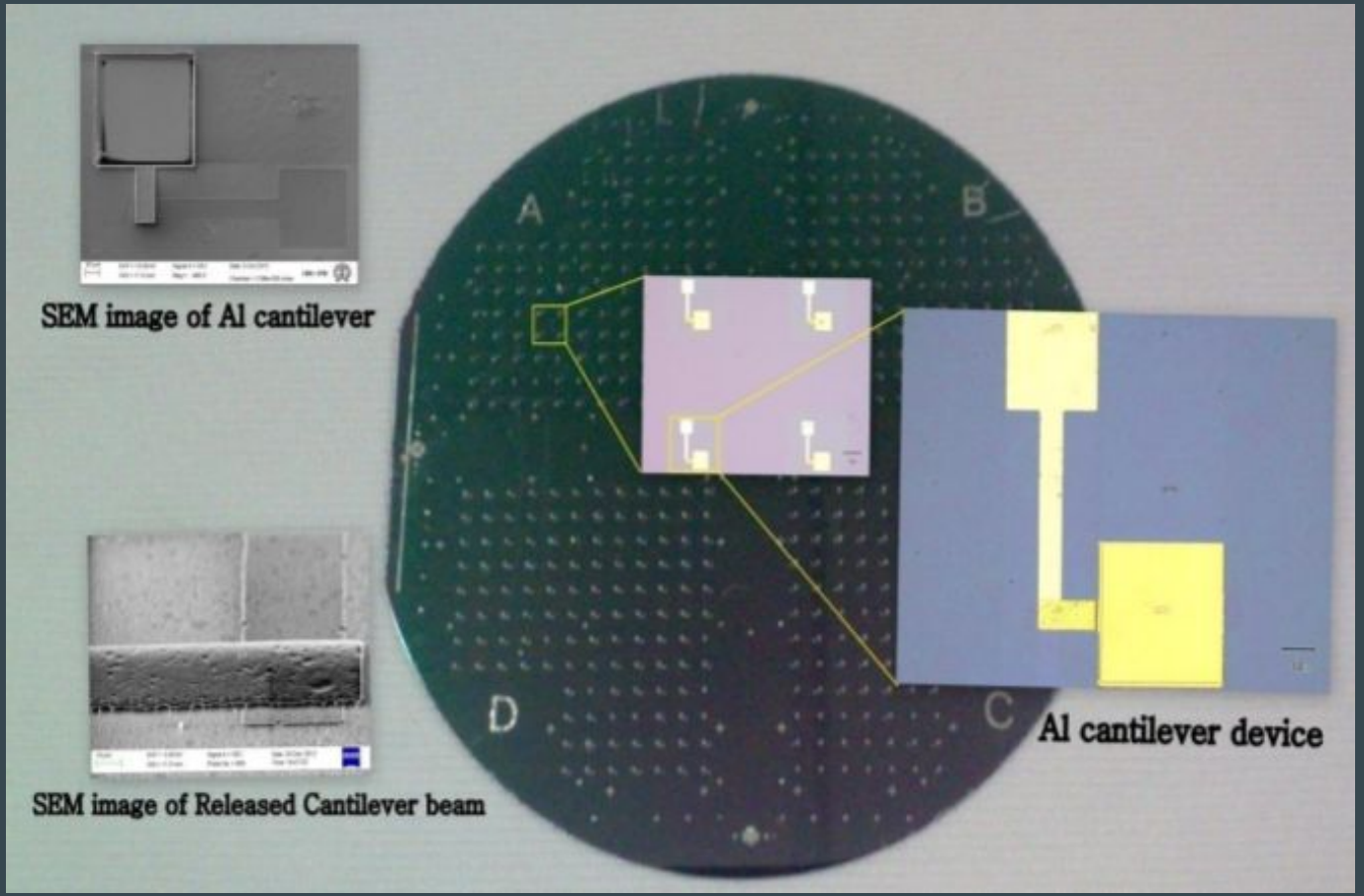


Image of fabricated cantilever.

Amiri et al.

Surface Functionalization

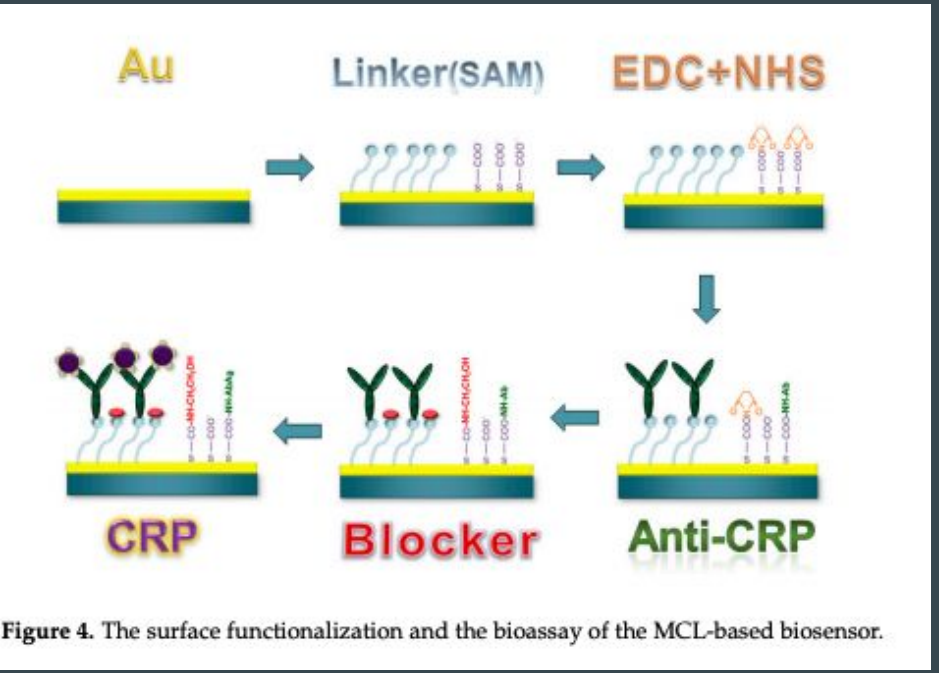


Figure 4. The surface functionalization and the bioassay of the MCL-based biosensor.

- Gold surface layer is applied and must be well cleaned
- Self-assembly monolayer (SAM) bonds covalently with the gold surface layer, using $SH(CH_2)_7COOH$ as the SAM[9]
- The gentamicin antibody (polyclonal) connects with the carbonyl group of the SAM
- An ethanolamine rinse washes away the SAM without antibody

Ku, YF, Huang, LS & Yen, YK.

Limitations of Current Design

- Sensitivity: limit of detection at 9.44 µg/mL[4]
  - Recall that gentamicin is given as a once a day dose at a concentration between 16 and 24 µg/mL
- Thermal Effect: very sensitive to temperature variations in its environment
- Noise: low signal-to-noise ratio
  - Can be difficult to reach a good baseline before each test

Method	Type	Sample	Linear range (µg/mL)	LOD (µg/mL)	Advantage	Disadvantage
[a]MCL-based sensor	Mechanical	PBS	10–50 (µg/mL)	9.44 (µg/mL)	Label free, portable, rapid response, real time detection	High noise level
[b]PETINIA	Optical	Serum	0–46 (µg/mL)	–	Sensitive and sufficient accuracy	Time-consuming Costly equipment
[c]ELISA	Optical	Serum	0.002–0.5 (µg/mL)	–	Protein-rich sample, high sensitivity, broad specificity	Expensive, not real-time, Label

Optimizing the MCL Biosensor

Thermal Effect Elimination

Limitation

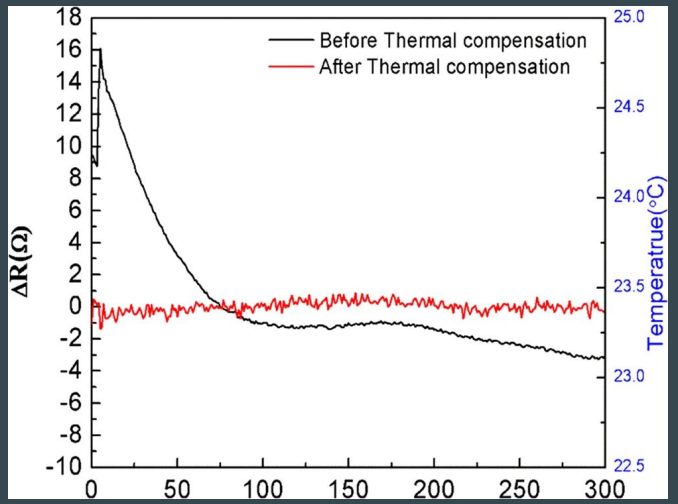
Temperature variations in the environment can change the resistance of the sensor which reduces detection accuracy.

Solution

1. Embed an aluminum temperature sensor into the sensing chip to monitor surrounding temperature
2. Perform temperature experiments to obtain the temperature-resistance relationship for the sensor
3. Correct the raw biomolecular signal to compensate for thermal effects and improve accuracy

Temperature function of the metal and MCL[12]

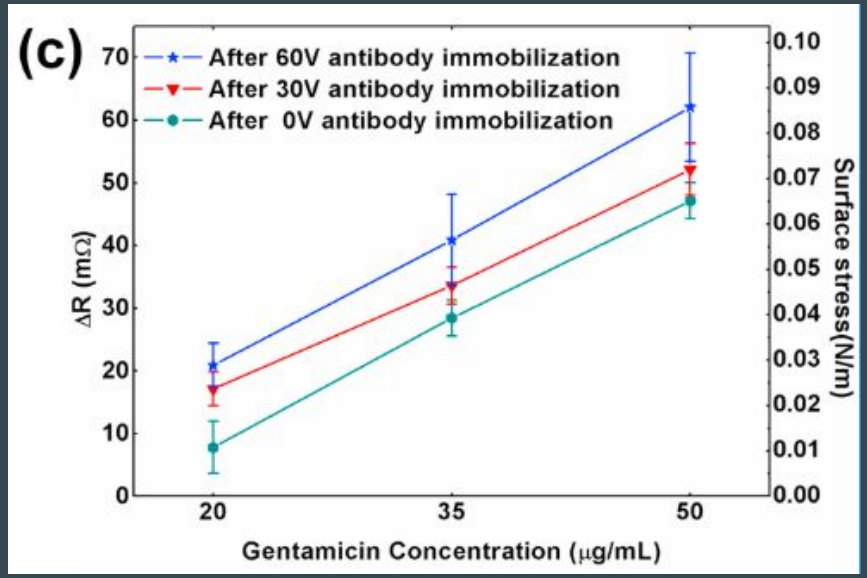
$$R = a(T-T_0)^2 + b(T-T_0) + c$$



Ku, YF, Huang, LS & Yen, YK.

Li & Yen.

Sensitivity Enhancement



Limitation

Detectable range of the sensor is too low. The ability to detect if the dosage is high enough to be in the target range is important.

Solution I

Apply an external electric field to improve antibody immobilization on the MCL surface.

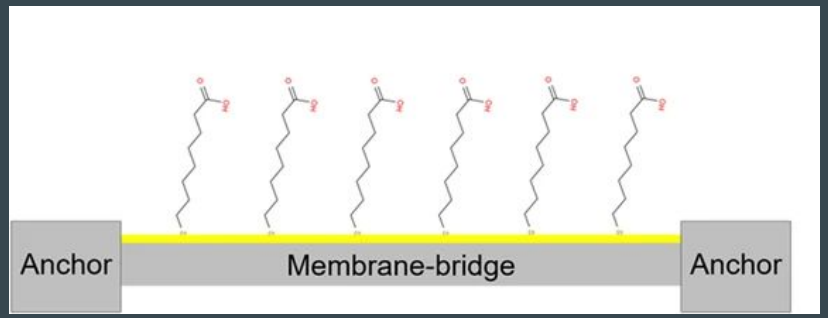
- Use gel electrophoresis to determine isoelectric point (pI)
  - pI: the point at which the molecule is electrically neutral
- The antibody (+) and gentamicin drug (-) hold opposite charges in a slightly alkaline solution which enhances binding.[4]
  - This technique can be applied for blood samples since blood is slightly basic.
- 1.8 fold enhancement in signal with 60 V electric field application.[4]

Sensitivity Enhancement

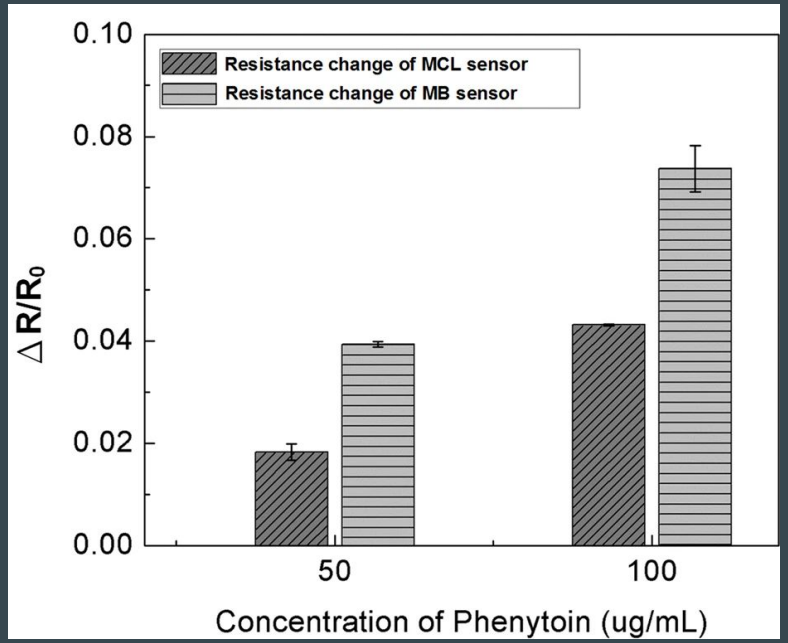
Solution II

Membrane-bridge (MB) configuration

- Can employ similar fabrication and surface functionalization techniques
- Re-characterize its mechanical properties

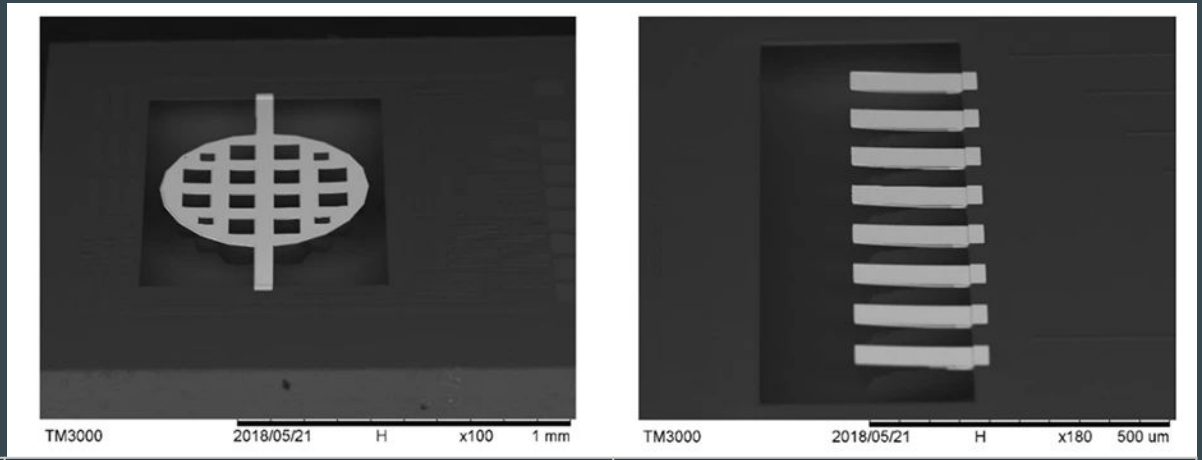


Yen, Y & Chiu, C



Results in greater resistance change than the MCL sensor at the same concentration of drug[9]

Biosensor Comparison



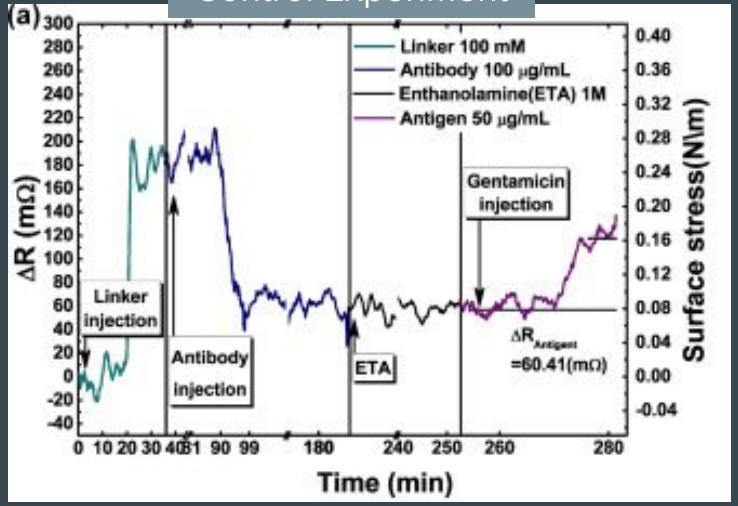
Type	Membrane-bridge	Microcantilever
Linear Detection Range	5-100 µg/mL	10-50 µg/mL
Limit of Detection	4.06 ± 0.15 µg/mL	9.44 µg/mL
Gauge Factor	21.0	17.7

Yen, Y & Chiu, C

Testing
Phase I

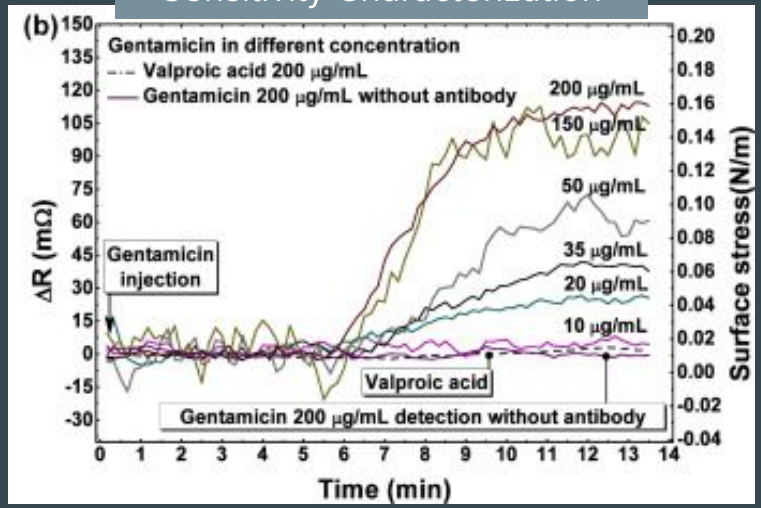
Li & Yen.

Control Experiment



- Visualize device dimensions with SEM microscope
- Confirm activation of antibody coating
- Measure lifetime and stability of the immobilized gentamicin antibody
  - X-ray photoelectron spectroscopy
- Characterize temperature effect and gauge factor

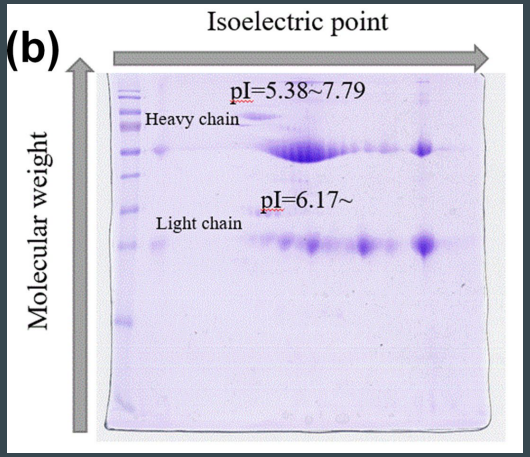
Sensitivity Characterization



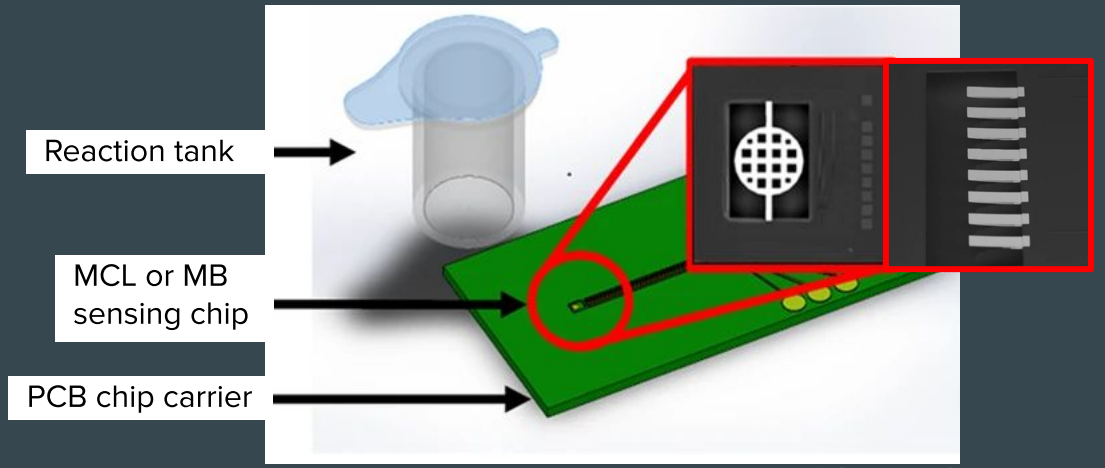
- Determine sensitivity and LOD over a range of gentamicin concentrations in a controlled solution
- Compare results to clinical techniques and previous MCL sensor results[4]

Testing

Phase II: Evaluating performance in human blood samples



Li & Yen.



Yen, Y & Chiu, C. [modified]

1. Optimize electric field for detecting gentamicin in blood
   - Control solution pH = 8.8
   - Blood pH ~ 7.4
2. Compare MB and MCL biosensors and evaluate against clinical techniques

Summary

Clinical Need PD patients with peritonitis need a safe way to monitor the concentration of gentamicin in real time to ensure they are receiving effective dosages and to prevent adverse reactions and toxicity.

Proposed Solution We proposed a CMOS-based BioMEMS device for label-free detection of gentamicin using a piezoresistive MCL or MB sensor.

Conclusion

- A BioMEMS device offers a robust and affordable solution for real-time drug monitoring for small molecules such as gentamicin.
- Improving sensitivity and optimizing accuracy in human blood samples are necessary for clinical application.
- Future work is needed to make this device a potential solution for point-of-care drug monitoring.

References

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(5) https://www.dizziness-and-balance.com/disorders/bilat/gentamicin%20toxicity.htm

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(7) Grogan, C, Raiteri, R, O'Connor, GM, et al. Characterisation of an antibody coated microcantilever as a potential immuno-based biosensor. *Biosens Bioelectron*. 2002;17(3):201-207.

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