Fundamentals of Nanotechnology: Relationship to Food Science and Technology

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Acknowledgements

Research supported by
USDA ARS Contract 1935-42000-035
National Defense University
(DAB J29-03-P-0022)

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Nanoscience and Nanotechnology

Nanoscience:
Fabrication, study and modeling of devices and structures where at least one dimension is 200 nm or smaller.

Nanotechnology:
Enables devices that are compact, portable, energy efficient, integrate sensing, and carry out complex functions of a full-scale laboratory.
Background: Microfluidics

Movement of fluids at microscopic level
Micron-sized channels and features

Applications

- Biosensors
- Micro-bioseparations
- Pathogen detection
Benefits: Microfluidic Systems

Miniaturization
- Consumes less reagents
- Enables higher sensitivity
- Shorter analysis time
Rapid Prototyping using PDMS

1. UV
2. High resolution transparency as mask
3. photoresist
4. SiO₂ or silicone substrate
5. Develop photoresist obtains master
6. Pour PDMS over master
7. Cure 70 °C for 1 hour
8. Release PDMS from master and seal against a flat substrate

Process takes a day or more

Lab-on-a-chip device cast in PDMS (channels are 30 microns wide)
The Need

Simple and rapid fabrication
Well defined surface chemistries
Flexibility and adaptability
Amendable for rapid prototyping techniques
Microfiber Assisted Fabrication in 15 min

SiO₂, glass, or PDMS substrate

Flat PDMS cover

Glass fiber (~12 µm diameter)

1 µL labeled avidin (green)

SiO₂ substrate

Glass substrate

1 nL/mm channel

t = 3 min
Mixing in Nanoliter Well

PDMS

Glass fiber (~12 µm dia)

SiO2 substrate

1 nL well at intersection

Labeled BSA (red, 200 µg/ml)

Labeled avidin (green, 200 µg/ml)

(b) t= ~3 minutes
Micro-scale Separation

Derivatize channels with ion exchange bioreceptor
Assemble micro-device
Use pre-derivatized particles
### Commercially Available Micro/Nano Particles

<table>
<thead>
<tr>
<th>Type</th>
<th>Surface</th>
<th>Chemistry</th>
<th>Size (µm)</th>
<th>Surface charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica</td>
<td>Hydroxyl</td>
<td>-OH</td>
<td>0.1-7.9</td>
<td>-</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Hydroxyl</td>
<td>-OH</td>
<td>0.7-7.9</td>
<td>-</td>
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<tr>
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<td>Carboxyl</td>
<td>-COOH</td>
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<tr>
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<td>Sulfonate</td>
<td>-SO_3</td>
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<tr>
<td>Polystyrene</td>
<td>Amino</td>
<td>-NH_3</td>
<td>0.7-7.9</td>
<td>+</td>
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<tr>
<td>Polystyrene</td>
<td>Dimethylamino</td>
<td>-NH(CH_3)_2</td>
<td>0.7-7.9</td>
<td>++</td>
</tr>
</tbody>
</table>

Data from Spherotec, Inc. Libertyville IL.
### Protein Coated Micro/Nano Particles

<table>
<thead>
<tr>
<th>Type</th>
<th>Surface</th>
<th>pl</th>
<th>Size (µm)</th>
<th>Surface Affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene</td>
<td>Antibody (IgG)</td>
<td>NA</td>
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<td>Binds protein A, G</td>
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<tr>
<td>Polystyrene</td>
<td>Avdin</td>
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<td>0.7-7.9</td>
<td>Binds to biotin</td>
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<tr>
<td>Polystyrene</td>
<td>Streptavidin</td>
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<td>0.7-7.9</td>
<td>Binds to biotin</td>
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<tr>
<td>Polystyrene</td>
<td>Biotin</td>
<td>NA</td>
<td>0.7-7.9</td>
<td>Binds to strept/avidin</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Protein A</td>
<td>4.9</td>
<td>0.7-7.9</td>
<td>Binds to IgG antibody</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Protein G</td>
<td>5.0</td>
<td>0.7-7.9</td>
<td>Binds to IgG antibody</td>
</tr>
</tbody>
</table>

Data from Spherotec, Inc. Libertyville IL.
Dip-coating Microfibers with Protein or Derivatized Particles

Glass fiber

Ultra-sonic cleaning in EtOH for 5 min

Dip-coat in protein or particle slurry

Glass fiber coated with particles
Glass Fiber Coated with Dimethylamino Beads

Dimethyl-amino microbeads, 800 nm

H

CH₃−N−CH₃

+ OH OH OH

Bare glass fiber, 12 µm dia
Glass Fiber Coated with Streptavidin Beads

Streptavidin microbeads, 800 nm

Streptavidin

Glass fiber pre-coated with biotin-BSA

Biotin
Micro-scale Separation

Derivatize channels with ion exchange or bioreceptor (in bead form)
Assemble micro-device
Carry out separation in flowing fluid
Sample size = 1 µL
Apply sample at 0.25 to 1 nL / sec
Microfluidic Separation Device

Labeled **avidin (green; 10 µg/ml)** and **BSA (red; 10 µg/ml)** liquid mixture; \( t=0 \)

(a) Glass fiber coated with dimethylamino microbeads

\( t=\sim3 \text{ minutes} \)

(b) Glass fiber coated with biotinylated BSA

\( t=5 \text{ minutes} \)

Huang et al, 2003
Surface Effect in Microfluidic Channels (particle detection or capture)

Hydrophobic vs. Hydrophilic

- Surface chemistry of a microchannel is extremely important in the movement and control of fluids at microscopic level
Detection of GFP *E. coli* in moving fluid across microfiber device

1-2 µm GFP *E. coli* (~10^6 cells/ml) in PBS

linear velocity of 10 µm/s

1-2 µm GFP *E. coli* (~10^6 cells/ml) in PBS

Linear velocity of 100 µm /s
Counting Cells Using Micro-device

Diagram showing a PMT connected to an air slit and a microscope objective. The output connects to a HV power source and a microchip, which leads to an oscilloscope.
* 3-D IgG molecule is obtained from David Wild (http://www.techfak.uni-bielefeld.de/bcd/ForAll/Introd/antibody.html).
Specific Binding

Initial = 3.7*10^8 cells

Blank LG surface

Listeria on P66 Ab
E coli Does Not Bind

Initial= $3.7 \times 10^8$ cells

Blank LG surface  

E. Coli on P66 surfaces
Mixture of Two Bacteria

Initial = $3.7 \times 10^8$ cells

Listeria on P66 surface

E coli on P66 surface
Flow-through: Syringe + Holder

5 ml/min controlled by syringe pump

PBS containing *L. monocytogenes* or *E. coli*

P66 Membrane
Fluorescence Intensity

Integration time = 250 ms
SEM Images

Initial = $7.3 \times 10^7$ cells/ml x 50 ml = $3.7 \times 10^9$ cells

- Blank Membrane
- *E. coli* on P66 Membrane
- *L. monocytogenes* on P66 Membrane
Close-up of a Protein Biochip

Tip of a pin

Well = 5 nanoliters
30 to 50 wells/chip
Cross-section of a well on a Biochip

- Protein receptor
  - 20 nm size
- Target molecules bound to receptors
- Glass cover
- Receptors immobilized on electrode
- Pt electrode
- SiO$_2$ insulation
- Silicon substrate
Microchips can be produced in Large Numbers

Microchips for adsorption studies
PECVD fabricated oxide layer
SiO$_2$ with Pt patterns
To use chip

1. Sample fluid
2. Place fluid onto chip for interrogation
3. Electrically detect if receptor binds something present in the fluid

Complete steps 1 through 3 in three hours.

That “something” could be a pathogen that requires a rapid, preventative response.
Listeria monocytogenes

- Food-born pathogen
- Gram positive (1 µm x 3 µm)
- Growth temperature (1-45 °C)
- Acid and salt tolerant
- Cause listeriosis (fever, headache, meningitis, encephalitis, liver abscess, abortion & stillbirth)
- Annual cases >2,500; Mortality 20-28%

Bhunia, Jarat, 2001
Sample From Hotdog

- Oil
- Fat
- Aqueous Sample
- Solids
Rapid Cell Concentration and Recovery Needed

Separate cells from food or agricultural sample
Concentrate bacteria
Recover bacteria and introduce onto biochip
Design principles

Microfabricate chip.

Then

Prepare surface so that nothing adsorbs (biochemical equivalent of Teflon)

Fix protein receptor onto treated surface.

Use nanogram amounts of receptors, since these proteins cost $10,000 per gram

( but only pennies per 1000 nanograms)
Applications in Foods

Food Products

$13.1 billion in soda pop
7.5 billion in breakfast cereals
60.0 billion in fresh meats

Branded meats, precooked products, micro-waveable red meats

Livestock, poultry

Sales figures from Kilman, Wall Street Journal, Feb 20, 2002
Detect Gene Expression Products

Animal health monitoring
   Early warning of infectious disease
Biodegradable sensors
   Tracking foods during processing
Identity tracking and preservation

Nanoscale Science and Engineering for Agriculture and Food Systems, CSREES, USDA National Planning Workshop, 2002
Conclusions

Nanotechnology combined with biotechnology enables new sensors that are difficult to make otherwise.

Nanotechnology on microchip enables detection at a microscale: small, localized and rapid.

Bioseparations engineering for sample preparation is important.