Directed Assembly of Nanoparticles for Biosensing Applications

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Outline

- CHN Overview
  - NSEC CHN Vision and Mission
  - Applications Roadmap

- Biosensor Applications
  - Introduction
  - Current and Future trends
  - Vision
  - Capabilities, Users and Needs

- Research
  - Size-selective Directed Assembly of Nanoparticles
  - ELISA assay for nanoparticles to evaluate pH, stability and particle concentration
  - Detection results

- Summary
Past and Present (Nanoscience)

Manipulation of few atoms and SWNTs

STM 1981

AFM 1986

STM manipulation of atoms 1989

AFM manipulation of a SWNT 1999

Molecular logic gate 2002

Source: IBM
CHN Vision
The Path from Nanoscience to Nanomanufacturing

Future (Nanomanufacturing)

Manipulation of billions of Nanoelements

- Synthesis
- High-rate Directed Assembly
- Reliability

EHS, Quality & Process Control
Economics and Life Cycle

Our Mission: To bridge the gap between nanoscale scientific research and the creation of nanotechnology-based commercial products
Today, most diagnostic markers are single species, such as PSA. There is a need for diagnostic devices capable of quantitatively determining the level of multiple markers present in biological fluids or tissue to provide an accurate, quick and efficient diagnosis of a patient.

A variety of multiplex approaches are being developed at the present time, including:

- Antibody arrays with fluorescence or surface plasmon resonance detection (Lee, 2006 and Woodbury, 2002),
- Flow cytometry of coded nano-particles coated with capture agents (Morgan, 2004) and
- Use of liquid chromatography/mass spectrometry (Rifai, 2006).
A silicon nanowire device was used to detect biological and chemical species (Patolsky, 2005). The device offers the simultaneous detection of up to two oppositely charged viruses.

* Antibodies are not patterned (immobilized), so maximum sensitivity and density are not attained

Cantilevers are coated with antibodies to PSA. When PSA binds to the antibodies, the cantilever is deflected. The cantilever motion originates from the free-energy change induced by specific biomolecular binding.

Current and Future Needs

Why the Biosensor is Important?
- Current and planned implantable biosensors:
  - Cannot readily detect more than two biomarkers,
  - Are relatively large in size
  - Most are not biocompatible
  - Do not have potential for combining biosensing and drug release

Current and Future Needs
- Tremendous need for detection *multiple* biomarkers present biological fluids or tissue
- Need to increase sensitivity & selectivity of detection
- Need bio- compatibility and small size suitable for implantation
- Low cost
Goals

- Simultaneous measurement of multiple biomarkers with one device
- Very small size (can be as small as 100 µm x 100 µm)
- Can be made of all biocompatible material
- Low cost
- Future development will lead to a device where drugs are released based on the detected antigen.
- In-vivo measurement
- No issues with sample collection and storage
Capabilities, Users and Needs

Capability
- Nano-technology based device
- Detect several cancers at the same time
- Inserted intravenously into the blood stream
- High sensitivity
- High specificity
- Early detection

End User
- Patients
- Drug Development

Current Needs
- Early detection of cancers
- Low-cost test
- Quick and accurate results
- Effective monitoring for patients in remission

What Users Wanted:
- High sensitivity
- High specificity
- Early detection
- Focus on other chronic diseases
Nanotrench Template Directed Assembly Using Electrophoresis or Chemical Functionalization

Nanoparticle directed assembly

Selective directed assembly of nanoparticles

APL 2006
Size-selective Assembly Results

1 μm spaced array of 200nm and 100 nm fluorescent PSL particles assembled in 150nm deep circular trenches with the diameter of 225nm and 125nm.
Assembly of 320nm PSA IgG carboxyl polystyrene particles

The process is controlled by fine tuning key parameters such as:

- pH,
- Ionic strength,
- Particle concentration,
- Assembly voltage and time.
Nucleosome antigen specific mAb 2C5 was incubated at different pH values for 5, 15, 30, 60 min and their activity was measured using ELISA method.

The antibody maintained their specific activity at pH 10 and pH 11 for up to 60 min of incubation.

Activity after incubation at pH 4 was good till 30 min but decreased at 60 min. pH 3 Activity after incubation at pH 3 decreased within 5 min.
ELISA assay for PSL particles with mAb 2C5; Effect of Concentration

- Fixed amount of antibody mAb 2C5 (5 µg/mL) was incubated with varying concentrations (0 – 40 µg/mL) of nucleosome antigen to find amount of antigen that binds this fixed amount of antibody.
- Here it was observed that approx. 5 µg antibody can bind to approx. 4 µg antigen.
Sandwich Complex with Fluorescence Detection

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- Chip A: negative control; Chip B: incubation with PSA 1 mg/mL.

- Incubated with capture anti-PSA Ab followed by blocking with BSA. The control chip was not incubated with the detection antibody - just blocked.

- incubated the chip with human plasma spiked with 1 ug/mL (level typical for advanced prostate cancer).

- After washing incubated chips with fluorescently labeled detection antibody, we observed a strong signal for the chip with detection antibody only.
Using two monitors:

- one shows the control of the stages and the other showing the top and side view of the biosensor assembly region to merge vision information with motion control.
- This allows precise manipulation of the stages and the microscopes to facilitate the automated assembly process of the biosensor chip.
Questions?