Nanotechnology in Translational Research

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Richard J. Cote, M.D., FRCPPath, FCAP
Professor and Chair, Department of Pathology
Director, Biomedical Nanotechnology Institute
University of Miami Miller School of Medicine
Miami, Florida
**Nanotechnology** is the study of manipulating matter on an atomic and molecular scale. Generally, nanotechnology deals with *structures sized between 1 to 100 nanometre* in at least one dimension, and involves developing materials or devices possessing at least one dimension within that size.
Feynman, 1959: "There's Plenty of Room at the Bottom"

American physicist Richard Feynman at an American Physical Society meeting at Caltech on December 29, 1959 proposed "There's Plenty of Room at the Bottom."

He predicted the ability to:

- Manipulate individual atoms and molecules
- Use of one set of precise tools to build and operate another proportionally smaller set, scaling down successively.
Advantages of Going Nano

Features

- The scale of nanodevices, in particular sensors, allows for construction of devices that can detect thousands of different reactions in an extremely small area.
- Nanoparticles can be applied in targeted therapy and diagnostic imaging.
- Functionalized nanodevices for interaction at the biotic/abiotic interface will provide substantial benefits for implantable systems.
- Nanomaterials have unique physical properties that allow entirely novel approaches to diagnosis, therapy and functional restoration.

Benefits

- Nanotechnology permits exquisite feature control, enabling a large number of reactions in a small footprint.
- Can allow savings in reagents, analytes and the test materials such as serum/plasma samples.
- Can enable multiplexed reactions.
Some Landmark Nanotechnologies

**Microcantilever Transducers:**
Electromechanical Monolithic Resonator, US Pat.3417249 - Filed April 29, 1966

Microcantilevers enabled AFM development.

**Quantum Dots:** Discovered at the beginning of the 1980s by Alexei Ekimov (glass matrix) and by Louis E. Brus (colloidal solutions). The term "quantum dot" was coined by Mark Reed. QDots enabled biosensor development.

**Nanowire / Nanotube Field Effect Transistors:**
In 1952, Radushkevich and Lukyanovich observed 50 nm carbon nanotubes

FETs enabled multiplexed sensor development.
Diverse Applicability

**Nanotechnology** may be able to create many new materials and devices with a **vast range of applications**: 

- Medicine
- Electronics
- Biomaterials
- Energy production.
Early detection of extremely rare circulating tumor cells, or detection of trace amounts of serum cancer markers, or markers such as glucose in diabetes or cardiac enzymes in cardiovascular diseases is hard. Cost-effective assessment is urgently required.

<table>
<thead>
<tr>
<th>Examples of Current Barriers in Medicine</th>
<th>Nanobiotechnology Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early detection of extremely rare circulating tumor cells, or detection of trace amounts of serum cancer markers, or markers such as glucose in diabetes or cardiac enzymes in cardiovascular diseases is hard. Cost-effective assessment is urgently required.</td>
<td>Simpler, smaller and economical devices for disease testing and diagnosis, and monitoring therapeutic success</td>
</tr>
<tr>
<td>Orally or systemically administered drugs can adversely affect non-target cells and organs. The result is decreased treatment effectiveness due to unintended side effects and increased morbidity. Targeted drug-delivery needed.</td>
<td>Nanoparticles can deliver drugs specifically to the tumor site / disease lesion</td>
</tr>
<tr>
<td>Currently, there is no effective way to locally sense specific biomarkers inside the body, and to link such sensors to ‘talk’ to each other.</td>
<td>Implantable, networked, nanowire sensors may enable real-time biosensing, and allow actions, such as controlled drug release, inside the body.</td>
</tr>
</tbody>
</table>
Possible Applications of Nanotechnology in Cancer

• **Diagnosis**
  – Sensitive detection of serum tumor markers using multiplexed nanosensors
  – Multimarker evaluation of tissue using Quantum Dots

• **Imaging**
  – Targeted ferromagnetic nanoparticles for MRI

• **Therapy**
  – Targeted nanoparticles for drug delivery
# Tumor Markers Available for Nanosensing

<table>
<thead>
<tr>
<th>Tumor Markers</th>
<th>Cancers</th>
<th>Usual Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP (Alpha-feto protein)</td>
<td>Liver, germ cell cancer of ovaries or testes</td>
<td>Blood</td>
</tr>
<tr>
<td>B2M (Beta-2 microglobulin)</td>
<td>Multiple myeloma and lymphomas</td>
<td>Blood</td>
</tr>
<tr>
<td>CA 15-3 (Cancer antigen 15-3)</td>
<td>Breast and others including lung, ovarian</td>
<td>Blood</td>
</tr>
<tr>
<td>CA 19-9 (Cancer antigen 19-9)</td>
<td>Pancreatic, sometimes colorectal and bile ducts</td>
<td>Blood</td>
</tr>
<tr>
<td>CA 72-4 (Cancer antigen 72-4)</td>
<td>Ovarian</td>
<td>Blood</td>
</tr>
<tr>
<td>CA-125 (Cancer antigen 125)</td>
<td>Ovarian</td>
<td>Blood</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Thyroid medullary carcinoma</td>
<td>Blood</td>
</tr>
<tr>
<td>CEA (Carcino-embryonic antigen)</td>
<td>Colorectal, lung, breast, thyroid, pancreatic, liver, cervix, and bladder</td>
<td>Blood</td>
</tr>
<tr>
<td>EGFR (Her-1)</td>
<td>solid tumors, such as of the lung (non small cell), head and neck, colon, pancreas, or breast</td>
<td>Tissue</td>
</tr>
<tr>
<td>Estrogen receptors</td>
<td>Breast</td>
<td>Tissue</td>
</tr>
<tr>
<td>hCG (Human chorionic gonadotropin)</td>
<td>Testicular and trophoblastic</td>
<td>Blood, urine</td>
</tr>
<tr>
<td>Her-2/neu</td>
<td>Breast</td>
<td>Tissue</td>
</tr>
<tr>
<td>Monoclonal immunoglobulins</td>
<td>Multiple myeloma and Waldenstrom’s macroglobulinemia</td>
<td>Blood, urine</td>
</tr>
<tr>
<td>NSE (Neuron-specific enolase)</td>
<td>Neuroblastoma, small cell lung cancer</td>
<td>Blood</td>
</tr>
<tr>
<td>Progesterone receptors</td>
<td>Breast</td>
<td>Tissue</td>
</tr>
<tr>
<td>PSA (Prostate specific antigen), total and free</td>
<td>Prostate</td>
<td>Blood</td>
</tr>
<tr>
<td>Prostate-specific membrane antigen (PSMA)</td>
<td>Prostate</td>
<td>Blood</td>
</tr>
<tr>
<td>Prostatic acid phosphatase (PAP)</td>
<td>Metastatic prostate cancer, myeloma, lung cancer</td>
<td>Blood</td>
</tr>
<tr>
<td>S-100</td>
<td>Metastatic melanoma</td>
<td>Blood</td>
</tr>
<tr>
<td>TA-90</td>
<td>Metastatic melanoma</td>
<td>Blood</td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>Thyroid</td>
<td>Blood</td>
</tr>
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</table>
**Optomechanical Biosensing Using Cantilever Platform**

**Principle:** Cantilevers provide a nanomechanical response to biomolecular interactions via surface stress changes.

Interactions between Antigen-antibody / Nucleic acids / Any ligand-substrate

Specific interactions between probe and target molecules produce **change in surface stress** within a SAM on gold surface of cantilever, **sufficient to bend the cantilever**

Cantilever deflection can be **tracked by a laser** beam trained at its tip using a position sensitive detector

![Diagram of cantilever biosensing](image)
Specific and Sensitive Detection of PSA and DNA

Cantilever surface coated with antibody
Cantilever surface coated with template DNA
Challenged with PSA / non-target protein
Challenged with complementary / non-complementary DNA

Principle of Nanowire/Nanotube-based Sensing

Surface Modification

Bio-recognition
PSA Nanosensing

Complementary Detection of Prostate-Specific Antigen Using In$_2$O$_3$ Nanowires and Carbon Nanotubes
Chao Li,† Marco Curreli,† Henry Lin,§ Bo Lei,† F. N. Ishikawa,† Ram Datar,§ Richard J. Cote,§ Mark E. Thompson,† and Chongwu Zhou*†‡

(a) Clinically relevant levels of PSA can be detected in a PBS model system using both In$_2$O$_3$ nanowires (NW) and Carbon Nanotubes (CNT)

(b) Clinically relevant levels of PSA can be detected in diluted human female serum samples spiked with PSA using NW and CNT

(c) Ability to construct arrays of NW and NT sensors, and applying microfluidics platforms to the arrays.
(b) BSA used to block nonspecific binding. Upon increasing the concentration of BSA (from pure 0.01 X PBS) a stable baseline is seen up to 40µM BSA.

(c) Response to SARS N protein of a NW FET functionalized with antibody-mimic Fn: Addition of N protein changes the current.

Fumiaki et al, *ACS Nano*, 200
Nanosensors for OvCa Serum Biomarker Detection

The limit of CA125 detection is 1000 times lower than the clinically relevant value for diagnosis.
Polysilicon Nanoribbon FET fabricated by top-down microfabrication, allowing more uniform device performance.
Combining Nanosensor Array with Microfluidics to Create a Functional Diagnostic Device

Nanosensors + Microfluidics = FET Array Device
Summary of Nanosensor Devices

Healthcare Applications

**Early Diagnostic:** Sensitive, low sample vol., rapid biomarker detection
- Cancer
- Cardiac Diseases
- **Infectious Diseases:** SARS, HIV, TB, Malarial Parasites, Influenza, etc.
  ✓ Allow non-PCR amplification based detection of DNA and RNA of viruses

**Therapeutic monitoring:** Low-cost, small footprint portable formats allow multiplexed, repeat analyte detection
- Also permit non-PCR-mediated detection of epigenetic changes

Other Applications
- Food stock Assessment
- Bio-warfare Agent Assessment
Near-term Nanobiotechnology Products that will Impact Human Healthcare

- Nanocapsulate **drugs**
- Nanoparticulate **Imaging Contrast Agents**
- Nanocantilever **Sensors and Actuators for Drug Delivery**
- Microfluidically controlled **diagnostic assay systems**
Nanoparticulate Therapeutic Delivery Vehicles

- **Nanoparticles**: Solid particle, can also be used in imaging
- **Nanoshells**: A core of silica and a metallic outer layer. Can be injected safely. Gold surface can be functionalized, typically used for cell-kill by external activation
- **Liposomes**: Lipid vesicles, can carry drugs encapsulated
- **Dendrimers**: An architectural class of nanoscale chemical polymers
- **Carbon nanotubes**: Can inject molecules into cells. Can be filled & capped, forming drug delivery devices

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**Abraxane**: A Novel Breast Cancer Therapeutic

Restoration of Health and Function: The Convergence of Multiple Technologies

- Hematopoietic stem cells
- Cardiac stem cells
- Endocrine function restoration in diabetes
- Bone, joint and tissue replacement
- Sight and neuromuscular restoration
Occult Metastases & Circulating Tumor Cells

• Primary event in cancer
• Recognized as holding extraordinary potential for disease management in cancer patients
  – Assess potential for disease progression
  – Determine need for systemic therapy & invasive Dx
  – Monitor therapeutic efficacy
  – Test molecular markers of cancer
  – Understand early molecular events in metastasis
• Discover new therapeutic targets
Impact of Occult Metastasis on Breast Cancer Outcome

Serial CTC Counts Predict Survival in Prostate Cancer

<table>
<thead>
<tr>
<th>Grp</th>
<th>Description</th>
<th>N (%)</th>
<th>Median OS in months (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;5 CTC at all draws</td>
<td>88 (38%)</td>
<td>&gt;26 (21.4 to ----)</td>
</tr>
<tr>
<td>2</td>
<td>&gt;5 CTC at BL &amp; &lt;5 CTC at last draw</td>
<td>45 (20%)</td>
<td>21.3 (18.4 to ----)</td>
</tr>
<tr>
<td>3</td>
<td>&lt;5 CTC at Early Draw &amp; &gt;5 CTC at Last Draw</td>
<td>26 (11%)</td>
<td>9.3 (8.2 to 11.3)</td>
</tr>
<tr>
<td>4</td>
<td>&gt;5 CTC at All Draws</td>
<td>71 (31%)</td>
<td>6.8 (5.8 to 10.3)</td>
</tr>
</tbody>
</table>

Kaplan–Meier plot for overall survival of CTC counts categories conversion at any time point following the early or late cycle of treatment. Survival times were calculated from the date of baseline (BL) CTC blood draw. Multivariate analysis was carried out using a Cox regression model with a time-dependant co-variable.
CTC are extremely rare events (~1/ml)
CTCs share many molecular traits with surrounding cells
Single-cell detection and analysis desirable

Challenge: Detection of CTC is Very Difficult

What Will an Ideal CTC Capture Platform Allow?

Efficient enrichment and capture of CTC from a solid tumors
Easy post-capture analysis
Simple process flow
Potential for point-of-care operation
Reliable and reproducible fabrication process
Current Methods for CTC Detection

Gradient Centrifugation

Specific Gravity

Blood

Centrifugation

Ficoll

Plasma

Enriched Lymphocyte

Erythrocytes Granulocytes

Immunomagnetic Separation

Surface Antigen

Ab-Magnetic beads

Mix

Cell mixture

Immunoaffinity ("CTC" Chip)*

Surface Antigen

Immunomagnetic Separation

Surface Antigen

Filtration†

Size

Cell mixture

50 μm


†G. Vona, et al, American Journal Of Pathology, 2000
Comparison of Cell Sizes

Mean (and range) of sizes of normal cellular components of blood, and certain cancer cell lines.

<table>
<thead>
<tr>
<th>Normal blood components (% of cells)</th>
<th>Cancer cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>NCI-H522</td>
</tr>
<tr>
<td>RBC</td>
<td>J82</td>
</tr>
<tr>
<td>Basophils</td>
<td>RT4</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>HCC-70</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>MDA-MB-231</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>HT1080</td>
</tr>
<tr>
<td>Monocytes</td>
<td>T24</td>
</tr>
<tr>
<td></td>
<td>SK-BR-3</td>
</tr>
<tr>
<td></td>
<td>HCC-38</td>
</tr>
<tr>
<td></td>
<td>LnCaP</td>
</tr>
<tr>
<td></td>
<td>NCI-1395</td>
</tr>
</tbody>
</table>

Cell size (μm)

5.2 94.6 .001 .05 .1 .01 .01
Microfluidics Based Cell Separation

- Trapped Tumor Cell
- Parylene Membrane Filter
- Syringe 1
- PDMS Chamber
- MNCs
- RBC’s in the flow through

SEM of a Cell Captured on filter

Assembled Filter Device
Multiplexed Imaging *On-chip*

**FISH** analysis on microfilter

Her2/neu and chromosome 17 centromeric probes

**Spectral Imaging**

- **ALDH-1**
- **CD24**
- **CD44**
- **Pan CK**
Multi-Parameter Analysis of Isolated CTCs
Critical to New Cancer Diagnostics and Cancer Research Tools

- Laser microdissection enables single cell study.
- CGH demonstrates genomic study on single cell.
Microfilter Performance Vs CellSearch in Clinical Samples

Prostate Cancer
- Microfilter 27/28
- CellSearch 14/28

Colorectal Cancer
- Microfilter 10/12
- CellSearch 4/12

Breast Cancer
- Microfilter 11/11
- CellSearch 6/11

Bladder Cancer
- Microfilter 4/6
- CellSearch 3/6

Lin et al, Clin Cancer Res, 16(20), 5011-18, 2010
Viable CTC Capture and Culture

Viable cells (viability >90%) can be captured with high efficiency (>90%).

Cancer cells captured on microfilter and imaged under bright-field (left) and fluorescence (right) microscope for the same field.

Yellow arrows: Live captured cancer cells retaining green Calcein-AM
Red arrows: Dead cancer cells stained with PI
Black arrows: PBMCs.

SEM image showing captured tumor cell still in spherical shape.
“Patient Management” using CTC Detection
Using CTC in the Blood as a “Virtual” Tumor Biopsy

- Diagnosis → CTC
  - Quantitation
  - Target identification
  - Stem cell identification

- Monitoring
  - Quantitation
  - Target identification
  - Stem cell identification
  - Functional changes

- Real time therapeutic efficacy monitoring
- Assessment of changing targets
- Characterization of cells of interest
- Assessment of functional change

UHealth
UNIVERSITY OF MIAMI HEALTH SYSTEM
Pathology
Monitoring the Health Continuum

**Health Continuum**

1. **Predisposition**
2. **Prevention / Screening**
3. **Early Diagnosis**
4. **Prognostic Evaluation**
5. **Treatment**
6. **Therapeutic Monitoring**
7. **Functional Restoration**

**Approaches**

- Genetic susceptibility, Epigenomic changes, mutation analysis
- Imaging
- Proteomics & Genomics of Blood, body fluids
- Proteomics & Genomics of Blood, body fluids, CTC for early metastasis detection
- Proteomics & Genomics of Blood, body fluids
- Surgery, Chemotherapy, Radiation, New Drug Development, Targeted Therapy
- Imaging, Proteomics & Genomics of Blood & Body Fluids, CTC for Recurrence Detection, Remote @Home Monitoring
- Stem Cell Analysis

**Novel Technologies @ UM**

- Nanosensors
- Nanosensors
- CTC Filter
- Nanosensors
- Stem Cell Culture
- Nanosensors
- CTC Filter
- Single Cell Assays

**Informatics**

Actionable Data for Physicians
The Future of Clinical Diagnostics

- Combination of existing and novel tests based on cells, proteins and genes in one single platform
- High sensitivity, specificity and low cost
- Elimination of complicated laboratory procedures and equipment, simpler work-flow
- Bedside or in-office applications (point-of-care)
- Small size = increased portability
- Deliver advanced technologies world wide
  - Provide access to remote areas
  - Allow for new medical opportunities to underserved populations

Widely accessible patient specific disease management
Nanosensor Development Collaborators

Dr. Mark Thompson, USC     Dr. Chong wu Zhou, USC     Dr. Thomas Thundat, ORNL     Dr. Ram Datar, UM

Dr. Marco Curreli, USC     Dr. Fumi Ishikawa, USC     Dr. Henry Lin, ORNL     Dr. Arun Majumdar
CTC Microfilter Collaborators

Dr. Yu Chong Tai, Caltech
Dr. Ram Datar, UM
Dr. Marija Balic, Austria
Dr. Siyang Zheng, PennState
Dr. Henry Lin, ORNL
Anthony Williams, UM