Multilayered Nanoparticles for Drug/Gene Delivery in Nanomedicine

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Our Goal is to Design Autonomous Nanomedical Systems

**Definition:** Self-guiding, adaptive, multicomponent systems on the nanoscale for diagnostic and therapeutic prevention or treatment of disease

**Value:** These “smart” nanomedical systems can deal with changing conditions, are error-correcting, and can provide proper dose of therapeutic response on a cell-by-cell basis
Nanomedicine Concept of Regenerative Medicine “Fixing cells one cell at-a-time”

• Conventional cancer therapies try to cut out the bad cells (surgery), burn them out (radiation therapy), or poison the bad cells faster than the good cells (chemotherapy)! Conventional medicine removes diseased cells and does not attempt to fix them.

• Nanomedicine attempts to make smart decisions to either remove specific cells by induced apoptosis or repair them one cell-at-a-time (regenerative medicine). Single cell treatments will be based on molecular biosensor information that controls subsequent drug delivery to that single cell.
What is Nanomedicine?

Beyond the obvious application of nanotechnology to medicine, the approach is fundamentally different than conventional medicine:

- Nanomedicine uses “nano-tools” (e.g. smart nanoparticles) that are roughly 1000 times smaller than a cell (knives to microsurgery to nanosurgery …_)

- Nanomedicine is the treatment or repair (regenerative medicine, not just killing of diseased cells) of tissues and organs, WITHIN individually targeted cells, cell-by-cell (a nano “bottoms up”, rather than top-down approach)

- Nanomedicine typically combines use of molecular biosensors to provide for feedback control of treatment and repair. Most conventional medicine does not use feedback control. Drug use is targeted and adjusted appropriately for individual cell treatment at the proper dose for each cell (single cell medicine).
Concept: Smart Boolean Targeted, Programmed Sequence of Events, Multilayered Nanomedicine Systems with Biomolecular Sensors for Feedback Control of Gene/Drug Delivery within Single Cells

Cell targeting and entry
Intracellular targeting
Biomolecular sensing
Gene/drug delivery

Targeting molecules (e.g. an antibody, an DNA, RNA or peptide sequence, a ligand, a thioaptamer), in Boolean combinations for more precise nanoparticle delivery

Biomolecular sensors

Leary and Prow, US Patent pending 2004
Nanocrystals or Nanocapsules for Nanomedicine?

Semiconductor nanocrystals or “Quantum Dots™”
(Ref: Clark et al., 2004 submitted)

OR

Biodegradable “nanocapsules”
(Courtesy: Dr. Yuri Lvov)

Probable answer: Nanocrystals for ex-vivo optical diagnostics and some form of hybrid biodegradable nanocapsule with MRI contrast agent core for in-vivo simultaneous diagnostics and therapeutics (“theragnostics”).
Example: Targeted Nanocrystal Delivery

Nanocrystals coated with nothing, anti-CD95, HIV tat fragment, or a 6xArg peptide were incubated with live cells for 1 hour and imaged with confocal microscopy:

Streptavidin coated nanocrystals; EM courtesy of Dr. Popov

Blue = Hoechst 33342, DNA
Green = Nanocrystals
These are composite images including Nomarski scattering

Copyright: Tarl Prow, Ph.D. Thesis (Leary lab) 2004
Nanocapsule technology: LBL Assembly by Alternate Adsorption of Oppositely Charged Linear Polyions and Nanoparticles or Proteins

LBL (layer-by-layer) self-assembly

The LBL-assembly regimes for more than 40 different compounds have been established.

Work with collaborator: Dr. Yuri Lvov
A New and Better Approach to Nanocapsule Assembly?

Covalently Linked Biopolymers for Layer-by-Layer Assembly and Cleavable Layer-by-Layer Disassembly

Desired result: Stable, reproducible, long-term biocompatible, multilayered nanoparticles

A new collaboration at Purdue with Dr. Don Bergstrom…
A New Approach to Nanocapsule Targeting to Cells

Use 50-60 nt dsDNA aptamer molecules for cell targeting. Aptamers are (1) much smaller than antibodies, (2) have lower immunogenicity in-vivo, and (3) allow biodistribution studies of nanoparticles (NP) to single NP level using PCR amplification of NP-attached aptamers labeled with reporter molecules.
Example: Design and Construction of Multilayered Nanoparticle Systems

Ref: Prow, TW, Kotov, N.A., Lvov, Y.M., Rijnbrand, R., Leary, J.F.
“Nanoparticles, Molecular Biosensors, and Multispectral Confocal Microscopy”
Journal of Molecular Histology, Vol. 35, No.6, pp. 555-564, 2004
The Challenge: Optimal Drug Delivery to the Single Cell

A potential solution: Delivery a drug manufacturing (in-situ) factory, not a drug. Then manufacture exactly the optimal amount for that particular cell under feedback control of an upstream molecular biosensor.
Biomimicry – Can Nature Provide Some of the Answers?

From the Greek “bios” = life and “mimesis” = imitation…

“Biomimicry is a new science that studies nature’s models and then imitates or takes inspiration from these designs and processes to solve human problems.”

[from the preface of Biomimicry]
Concept of nanoparticle-based “nanofactories” (NF) manufacturing therapeutic genes inside living cells for single cell treatments.

The nanoparticle delivery system delivers the therapeutic gene template which uses the host cell machinery and local materials to manufacture therapeutic gene sequences that are expressed under biosensor-controlled delivery.
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Data: Single-Cell HCV Antiviral Ribozyme (Rz) Therapy

A: DNA staining (blue) of nuclei; B: HCV biosensor staining green
C: HCV NSE protein staining (red) D: Composite image A-C

(1 = Untreated cell) (2 = Rz treated cell)

Result: Rz treated cell # 2 shows decrease in HCV NSE protein (red)
Feasibility Study: Nanocapsule delivery of two reporter gene plasmids for possible binary transient gene therapy

Delivery of 2 different plasmids with lipid coated LBL. Huh-7 cells (Panels A. and B.) were transfected with a 1:1 mixture of pEGFP-C1 and pdsRed2-C1 or exposed to ~100nm layer-by-layer (LB) assembled nanocapsules containing a single layer of DNA (1:1 mixture of pEGFP-C1 and pdsRed2-C1) (Panels C. and D.). Although the transfection efficiency was low, there were cells expressing both EGFP (green) and dsRed (red) protein. All cells were counterstained with DAPI (blue).
High-throughput Cell Separation for Delivery of Highly Enriched Cell Subpopulations for Gene Expression Microarray Analysis of Nanoparticle-Treated Cells

LEAP™ (Laser-Enabled Analysis and Processing) has throughputs greater than 100,000 events/sec, high cell purity, yield and viability. It can process several cells or a billion cells with an expanded cell range including fragile cells. Another advantage is that it can analyze and purify biohazardous cells without generating aerosols.

Figure 1: A new cell separation technology (LEAF™, Oncosis, Inc.) first analyzes cells by high-speed, bright-field and/or fluorescence imaging. Then living cells not-of-interest are eliminated from the cell suspension or tissue. The laser can also be used to opto-inject cells at very high speeds for introduction of molecules into living cells without the need for conventional transfection techniques.

Shooting at cells inside 384-well plates to eliminate undesired cells and capture desired cells for subsequent gene expression microarray analysis.
Voyage of the Nano-Surgeons

NASA-funded scientists are crafting microscopic vessels that can venture into the human body and repair problems – one cell at a time.

http://science.nasa.gov/headlines/y2002/15jan_nano.htm

January 15, 2002: It's like a scene from the movie "Fantastic Voyage." A tiny vessel -- far smaller than a human cell -- tumbles through a patient's bloodstream, hunting down diseased cells and penetrating their membranes to deliver precise doses of medicines.

Only this isn't Hollywood. This is real science.

Right: Tiny capsules much smaller than these blood cells may someday be injected into people's bloodstreams to treat conditions ranging from cancer to radiation damage. Copyright 1999, Daniel Higgins, University of Illinois at Chicago.
Engineered multilayered nanoparticles targeted to radiation-damaged cells can initiate repair of damaged DNA using DNA repair genes manufactured inside individual living cells under the control of molecular biosensor switches.
Nanomedicine for Prevention of Radiation-Induced Cancer in Astronauts

Targeted nanoparticles

Seek out radiation-damaged cells

Cell entry and gene delivery

Expression of radiation-damage biosensor

Expression of biosensor

Does the cell show signs of radiation-induced stress?

No

Yes

Express therapeutic (DNA repair) genes
Nanoparticle Targeting Data

Targeting strategies already developed can detect one rare cell in a million other cells (similar to the expected frequency of cancer cells in astronauts exposed to space radiation)

Note: Nuclei of cells are counter-stained blue with a DNA dye
Concept: Ionizing Radiation Activates Biosensor Mediated DNA Repair Enzyme Expression

Copyright: Tarl Prow, Ph.D. Thesis (Leary lab) 2004

Normal Cell

Irradiation

Irradiated Cell With DNA Damage

Cell With Repaired DNA

No transcription of DNA repair enzymes in normal cells

ARE proteins

ARE biosensor

X

Transcription of DNA Repair Enzymes Initiated by ARE Complex Binding

ARE proteins

Sequence to produce DNA repair enzyme

Expression

DNA repair
## Data: ROS Activated Biosensor in Living Human Cells

<table>
<thead>
<tr>
<th>Time (hrs):</th>
<th>0</th>
<th>24</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment:</td>
<td>Unstressed cells</td>
<td>Stressed cells</td>
<td>Fluorescence photographed</td>
</tr>
</tbody>
</table>

**Methods:** Cells were transfected with either ARE-GFP (stress biosensor) or TK-GFP (a control gene). 24 hours later the cells were stressed with a chemical to simulate space radiation stress. The cells were examined every 12 hours post treatment. Weak fluorescence was present at hour 48 and at hour 60 photographs were taken.

**Source:** Tarl Prow, Ph.D. Thesis (Leary lab) 2004

**Stressed cells**
- ARE-GFP+ (ARE biosensor fluoresces green in the presence of ARE stress proteins)

**Control cells**
- ARE-GFP+ background in unstressed cells

**Control cells** (No biosensors)
Feasibility example: Production and testing of novel UV-damage specific DNA repair enzymes with intracellular targeting sequences

1) Create genetically-modified DNA repair proteins that are specifically targeted to the cell’s nucleus or mitochondria to initiate repair at UV-induced DNA damage sites

   a) **Nuclear localization** signals PKKRKRRRL and PKKKRKRL at the C-terminus

   b) **Mitochondrial targeting** sequence MALHSMRKARERWSFIRA and MGVFCLGPWGLGRKLRRTFGKGPQQLSRLCGDHLQ at the N-terminus
Feasibility Results: Human cells transiently or stably transfected with missing DNA repair enzyme

T4 transfected DNA repair enzyme with no localization anchoring sequence, with transient expression

Wt-T4-PDG-GFP in CHO-XPG. Transient expression. 100x objective

T4 transfected DNA repair enzyme with mitochondrial localization anchoring sequence, with transient expression

MLS35-T4-PDG-GFP in CHO XPG. 100x objective

T4 transfected DNA repair enzyme with mitochondrial localization anchoring sequence, with stable expression

MLS18-T4-PDG-GFP in hXPA. 100x objective
Feasibility Example: A Strategy for accelerating DNA repair in human cells

• In humans, there is **ONLY ONE** mechanism to repair UV-induced damage to DNA
  – Immune system suppressed 8-24 hrs.
  – DNA damage removal takes 24-48 hrs.
• However, simpler organisms have **TWO** and sometimes **THREE** repair systems
• One of these repair systems is partially present in humans, **BUT** we are **MISSING** the **FIRST STEP**

Humans are missing this repair enzyme which can be transfected into human cells

• Using nanoparticle/biosensor technology we can supply this missing first step to enhance DNA repair in human cells
Feasibility Results: DNA comet assays show evidence of accelerated DNA repair in transfected human cells

<table>
<thead>
<tr>
<th>Hxpa DNA repair-deficient human cells</th>
<th>NLSI DNA repairable-human cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>No T4 treatment</td>
<td>No T4 treatment</td>
</tr>
<tr>
<td>T4 treated</td>
<td>T4 treated</td>
</tr>
</tbody>
</table>

**Un-irrad**

- **20 J 0h**
- **20 J 6h**

**Note:** Comet streaks show attempts to repair DNA damage which should be completed in about 6 hours as opposed to normal 72 hours. NLSI cells successfully repair, control Hxpa cells do not.
Our MCF Team and Current Collaborators

**Combinatorial chemistry/aptamers**
David Gorenstein (UTMB)
Xianbin Yang (UTMB)
Cagri Savran (Purdue)

**DNA Repair**
Stephen Lloyd (Oregon Health Sciences Center)

**Nanocrystal technology**
Nick Kotov (Univ. Michigan)
Jo Davisson (Purdue)

**Nanocapsule technology**
Yuri Lvov (Louisiana Tech U)
Don Bergstrom (Purdue)
Kinam Park (Purdue)

**Proteomics**
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Jo Davisson (Purdue)

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Tarl Prow** – nanotechnology; confocal microscopy; molecular biosensors for HCV
Peter Szaniszlo – HHV6/HIV; stem cells; microgenomics (UTMB)
Nan Wang – cell culture, molecular biology assays (UTMB)
Bill Rose–nanocapsule design (UTMB)

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Paul Robinson (Purdue)

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**Bioinformatics**
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**LEAP technology**
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**Microfluidics/engineering**
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* Texas A&M University
** Johns Hopkins University
*** recently deceased
A Few Relevant Recent References

- Leary, JF "Ultra High Speed Cell Sorting" Cytometry 67A:76–85, 2005