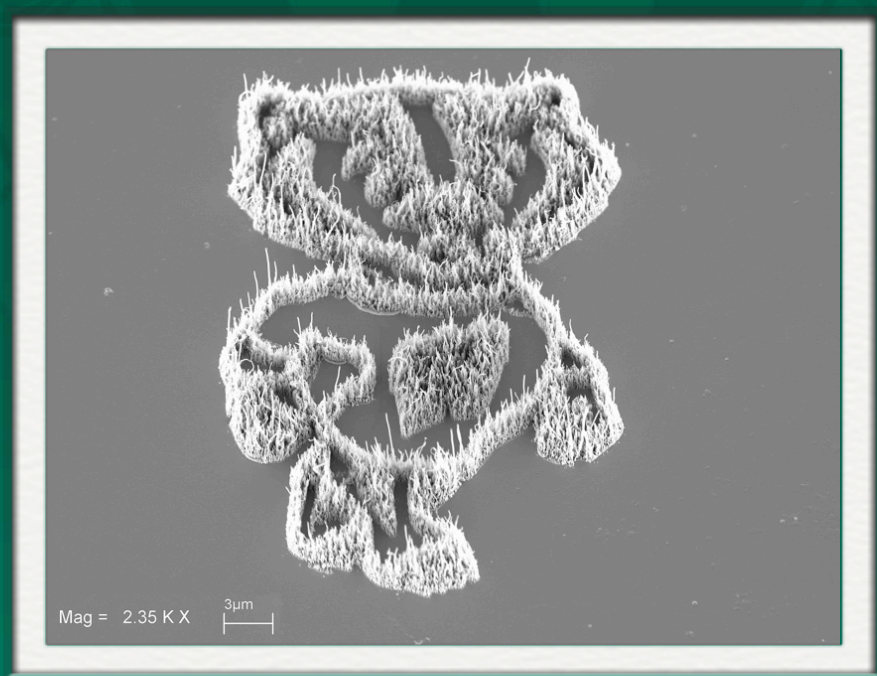


Nanotools for Toxicity Assessment of Nanomedicines

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July 22, 2008



Nano Bucky

Topics and Questions

1. Drug delivery systems using **nanotechnology**
2. Manufacturing **nanoparticle**-containing drugs
3. Identification of issues that need to be addressed by the FDA regarding **nanomaterial**-containing products:
 - Safety and Efficacy
 - Manufacturing
 - Regulatory procedures involved in the product approval
 - **Validation**

Nanotechnology Stats

- The demand for nanotechnology medical products will grow by more than 17% annually to reach \$53B in 2011.

The Freedonia Group, Nanotechnology in Health Care to 2011 Report; February 2007

- The Freedonia Group projects that nanotechnology will account for a drug delivery and biomedical product demand of \$3.7B in 2009, up from \$165M in 2004. The largest share of opportunities will emerge in pharmaceutical applications, which are expected to reach \$3.1B in 2009 and expand to \$18B in 2014.

The Freedonia Group



Nanotechnology Stats

- With at least 12 nanomedicines already approved and progressively more in active development, the next five years should see a steady succession of new nanotech-based drugs, imaging agents, and diagnostic products entering the marketplace. The most active areas of medical nanotechnology are in **drug delivery** and **in vivo imaging**.

AdvanceTech Monitor (2006)

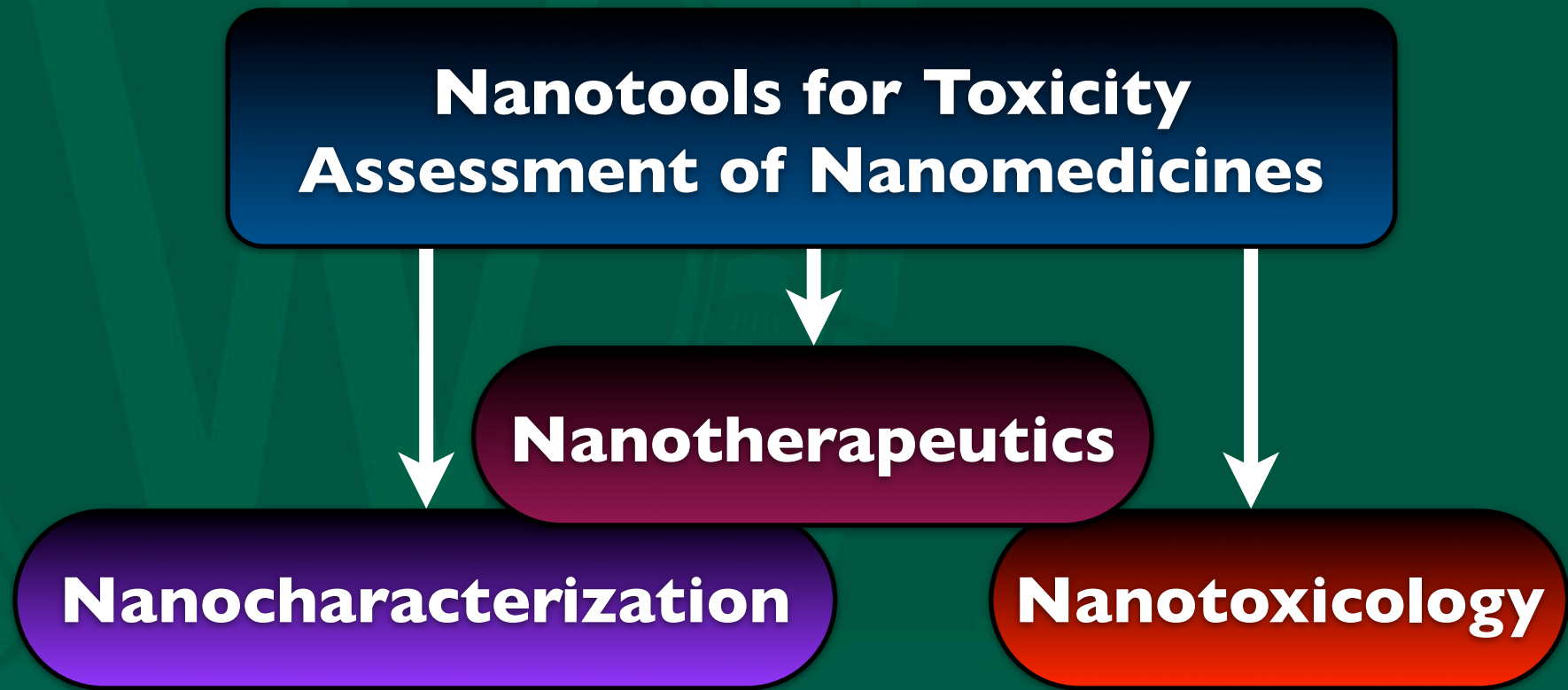


Nanotech-related EHS Research



Environmental, Health, and Safety Research Needs for Engineered Nanoscale Materials (September 2006)

Nanokit Development



What is **Nanotechnology** but a “...tool kit for manipulating matter at its finest scale.”

Science **304**:1732 (2004)

Medical Product Examples with Functional Nanotech Components

Medical products

Form of administration

- Oral
- Transdermal
- Inhalation
- Implant Device
- Injection

Analytical methods & instruments

- Micro-Array – Nanoarray
- Lab-on-a-Chip
- DNA/Cell/Protein-chip
- Diagnostic-Kit
- Biosensors
- Devices for magnetic cell separation
- Contrast agents

Medical Devices

- Special material & coatings for implants
- Active implants
- Surgery products
- Tissue engineering (Self-assembling scaffold)
- Wound healing products

Therapeutics

Field of application

Drug Delivery

Diagnostics

Analytical Tools & Instruments

Med. Material & Implants

Therapeutics

Functional nanotech component

- Liposomes
- polymer nanoparticles
- Nanocapsules
- Nanosuspensions
- Nanocrystals
- solid lipid nanoparticles
- Dendrimers
- Fullerenes
- Nanoshells
- Nanotubes
- polymer-protein conjugates
- polymer-drug conjugates
- polymeric micelles

- Perflurcarbon nanoparticles
- Fullerenes
- Dendrimers
- Quantum Dots
- Carbon nanotubes
- DNA-Hybride
- Gold/silver nanoparticles
- Superparamagnetic iron oxide particles

- (Bio)ceramics
- Nanospheres of hydroxyapatite
- Aluminium/Iron/Nickel/Titan alloys
- Biological composite material
- Nanoporous electrodes
- Gold/silver nanoparticles
- Carbon nanotubes/-fibers

- Magnetic nanoparticles for hyperthermia
- Dendrimer Drugs
- Fullerenes

Source: Ernst & Young

FDA and EU Regulatory Approval: Medicinal Product (Drug) vs. Medical Device

- Relies on ***principal intended action***: intended purpose, method of use

Medicinal Products (Drugs)

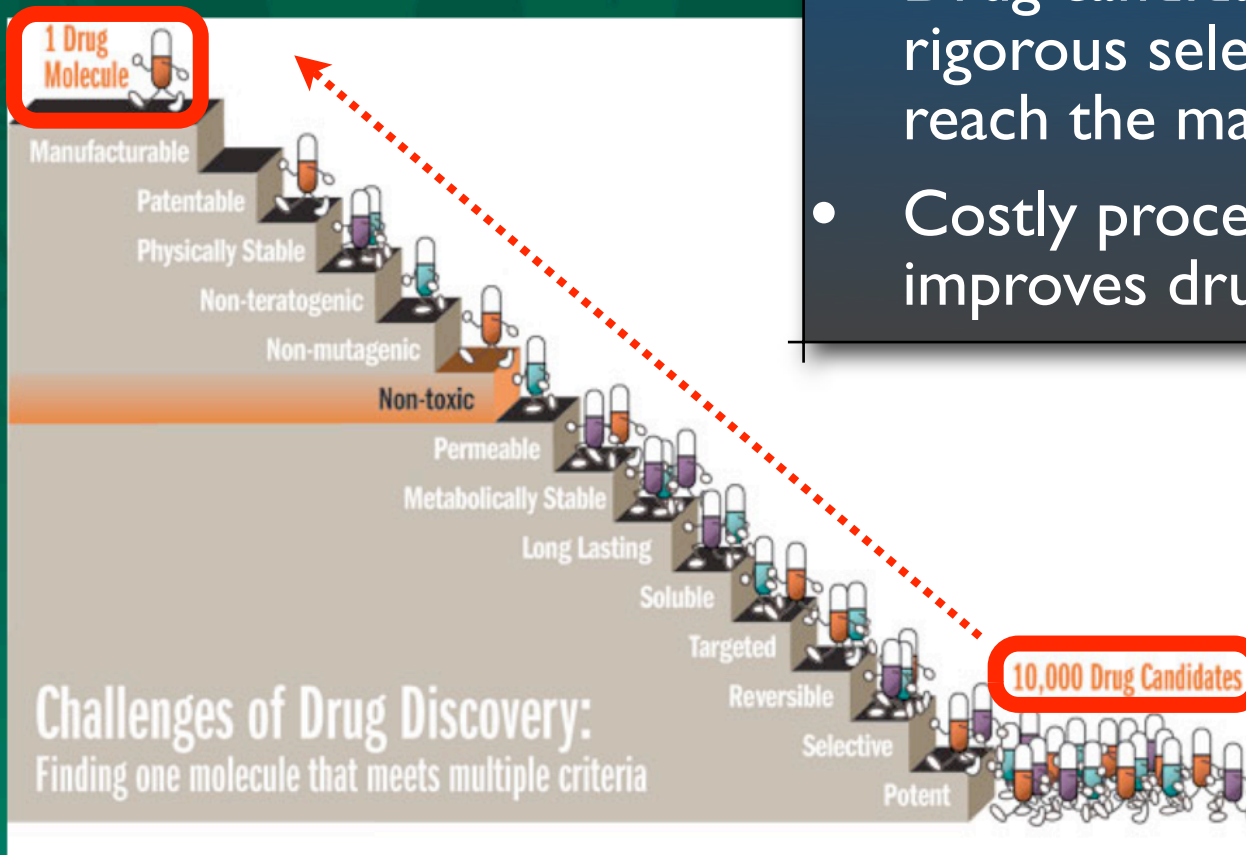
Action achieved by:
pharmacological
immunological, or
metabolic means

Medicinal Devices

Action achieved by:
physical means
mechanical action
structural action
replacement
support to organs, or
body functions

Successful Therapy Combines Drug Discovery and Delivery

- 70% of new drugs are insoluble; many are toxic
- **Pharmaceutics** improves drug therapeutic potential
- Drug candidates must meet numerous rigorous selection criteria to successfully reach the marketplace
- Costly process: **pharmaceutics** improves drug cost structures



Drug Discovery and Development Activities

Genetics - Target ID
Target Validation
Assay Development
HT Screening
Hit Identification
Hit to Lead
Lead Candidate
Preclinical
Phase I Clinical Trials
Phase II Clinical Trials
Phase III Clinical Trials
Phase IV-V
Market

Preclinical (3-4 yrs)

Clinical (4-6 yrs)

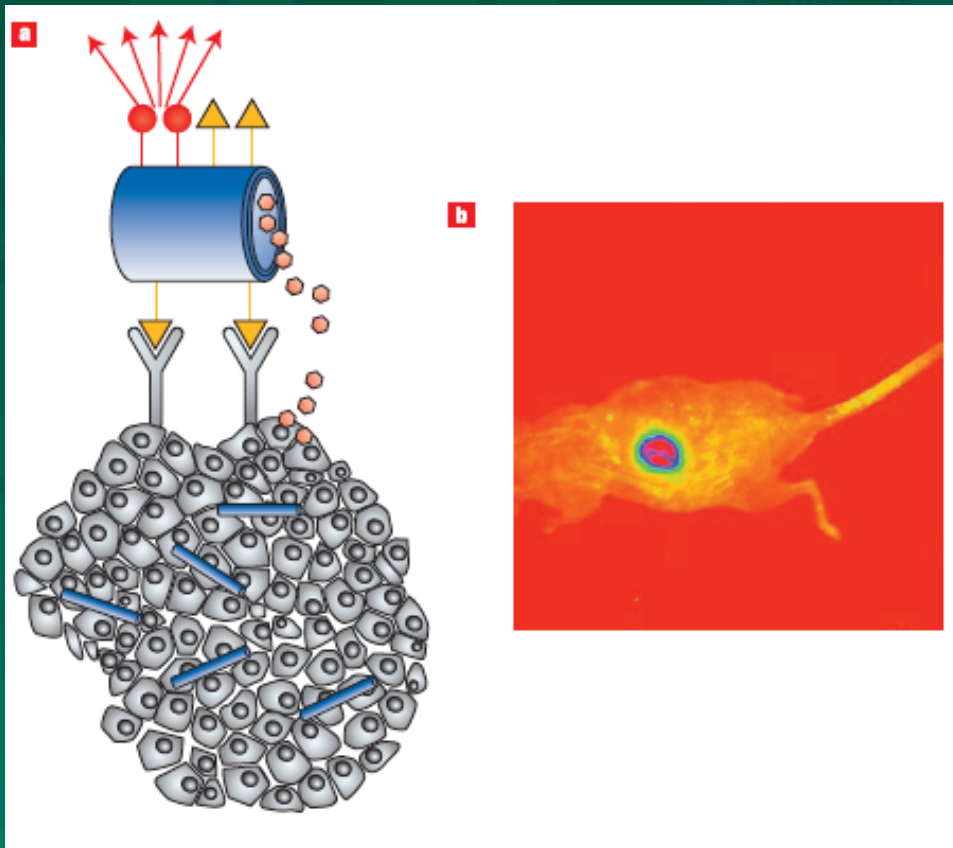
Approval (3 yrs)

\$800M - \$1B

Drug Delivery Systems Using Nanotechnology

Promising applications

- Biosynthetic and Bioorganic Polymer Systems
- Theragnostics
- Multimodalities & Combination Therapy



- quantum dots
- ▲ tumor targeting ligand

Drug Delivery Systems Using Nanotechnology

Applications of these systems

- Synergistic and/or multi-modalities (hyperthermia/imaging/therapy)
- mRNA templates; increased biocompatibility; etc.
- Example: **CALAA-01** (Calando Pharmaceuticals); Phase I began May 2008
 - Targeted Anti-Neoplastic Polymer-drug Conjugate
 - Therapeutic: anti-R2 siRNA
 - Carrier: cyclodextrin-containing polymer
 - Targeting: Transferrin receptor
 - PEG-stabilized

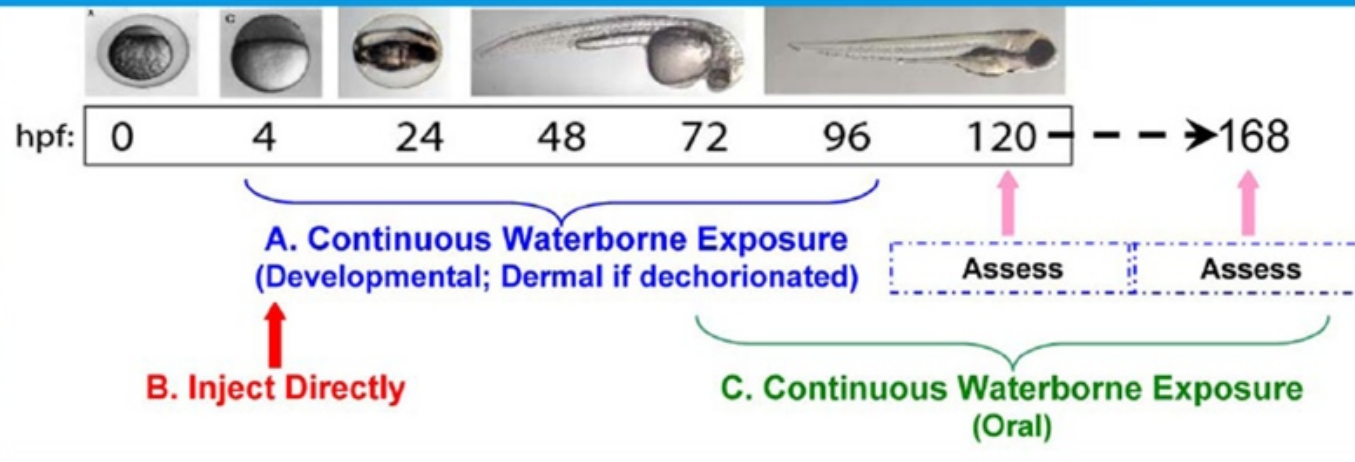
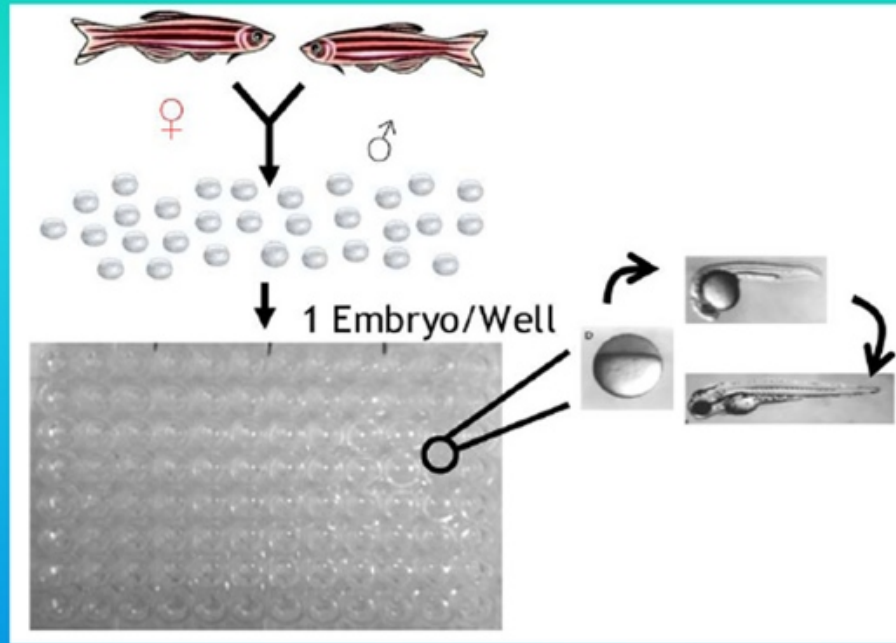
Drug Delivery Systems Using Nanotechnology

Challenges in developing these systems

- “NanoDesign” complexity
- Long-term / chronic exposure studies are years away
- Lack of correlative *in vitro* and *in vivo* data
 - Are new testing models available? **YES!**
- **Zebrafish - bridging putative *in vitro* / *in vivo* assays**
 - Ability to assess developmental toxicity; phenotypic abnormalities that could be linked to genetic mutations; epigenetic potential; and limited long-term studies.

Zebrafish as a Nanotool for Toxicity

Methods



- Gold and silver nanoparticles (AuNP; AgNP)
- FDA approved polymers
Pluronics/Poloxamers
PEG

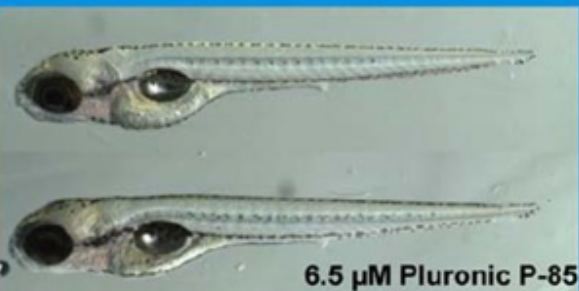
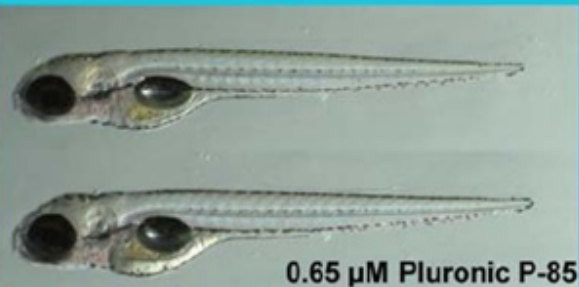
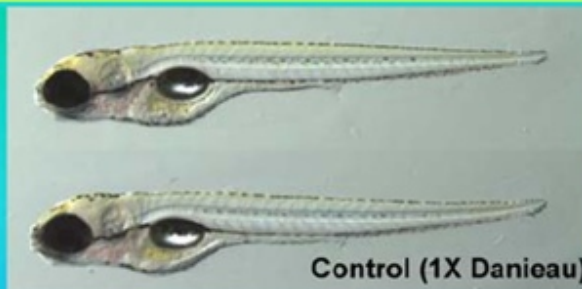
Zebrafish: Injections and Waterborne Exposures

Pluronic, also known as poloxamer

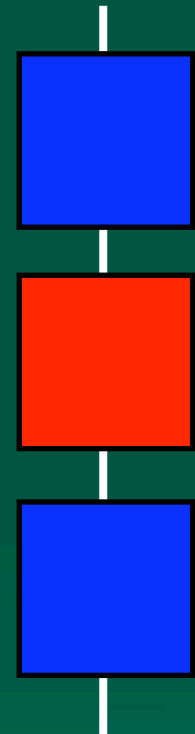
Exposure to Graded Concentrations of Pluronic P-85

☀ Injections:
At the CMC
mortality
percentages
and
incidences of
sublethal
effects are
highest
☀ However,
oral
exposures →
mirror early
waterborne

Waterborne



Pluronic

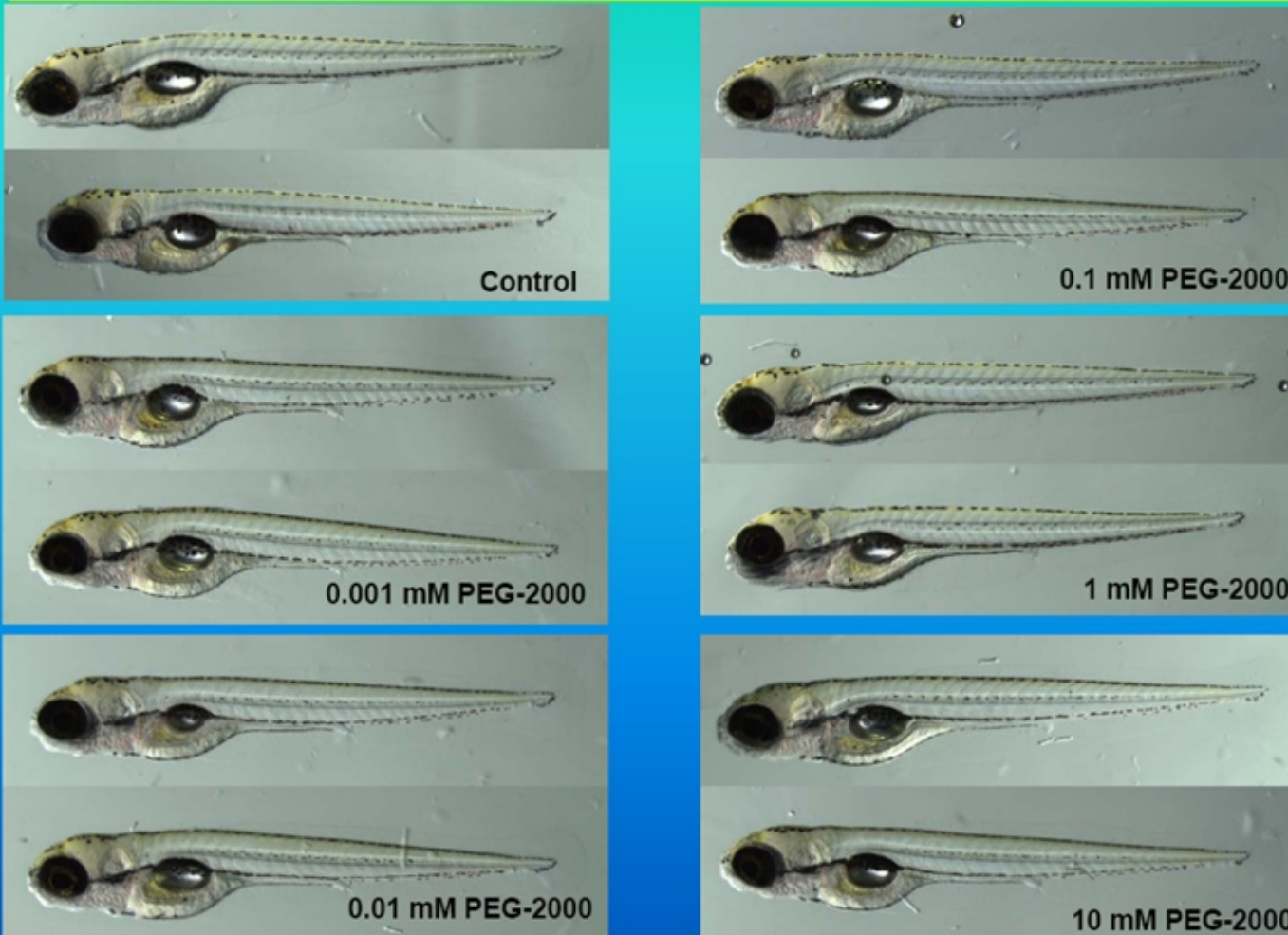


hydrophobic

hydrophilic

Zebrafish: Waterborne Exposure

Exposure to Graded Concentrations of PEG-2K



Drug Delivery Systems Using Nanotechnology

Are FDA requirements for preclinical assessment and QA adequate to safely evaluate nanomedicines?

- The short answer....no.
- Part of the problem is the exponential growth in nanotechnology coupled with the lack of concomitant screening assays.

What are the gaps?

- Lack of an *in vitro* model correlative to *in vivo* systems for predictive nanotoxicity.
- For example, Jahlen-Dechent's report of toxicity of gold nanoparticles: sulfated phosphene-derivatives used to quench AuNPs are highly toxic.

Gold Nanoparticles (AuNPs)

- Jähnen-Dechent *et al. Small* **3**:1941 (2007) “Size dependent cytotoxicity of gold nanoparticles”
 - 0.8 -15 nm AuNPs stabilized by triphenylphosphine derivatives
 - Tested with fibroblasts, epithelial cells, macrophages, melanoma cells
 - 1.4 nm AuNPs showed the highest toxicity with IC_{50} 30-56 μM
 - 15 nm AuNPs were **non-toxic** up to 60-100 higher concentrations
 - Conclusions: 1.4 nm AuNPs caused rapid cell death by necrosis within 12 h; 1.2 nm AuNPs caused rapid cell death by apoptosis (a difference of 0.2 nm!)

Zebrafish: Gold Nanoparticle Exposure



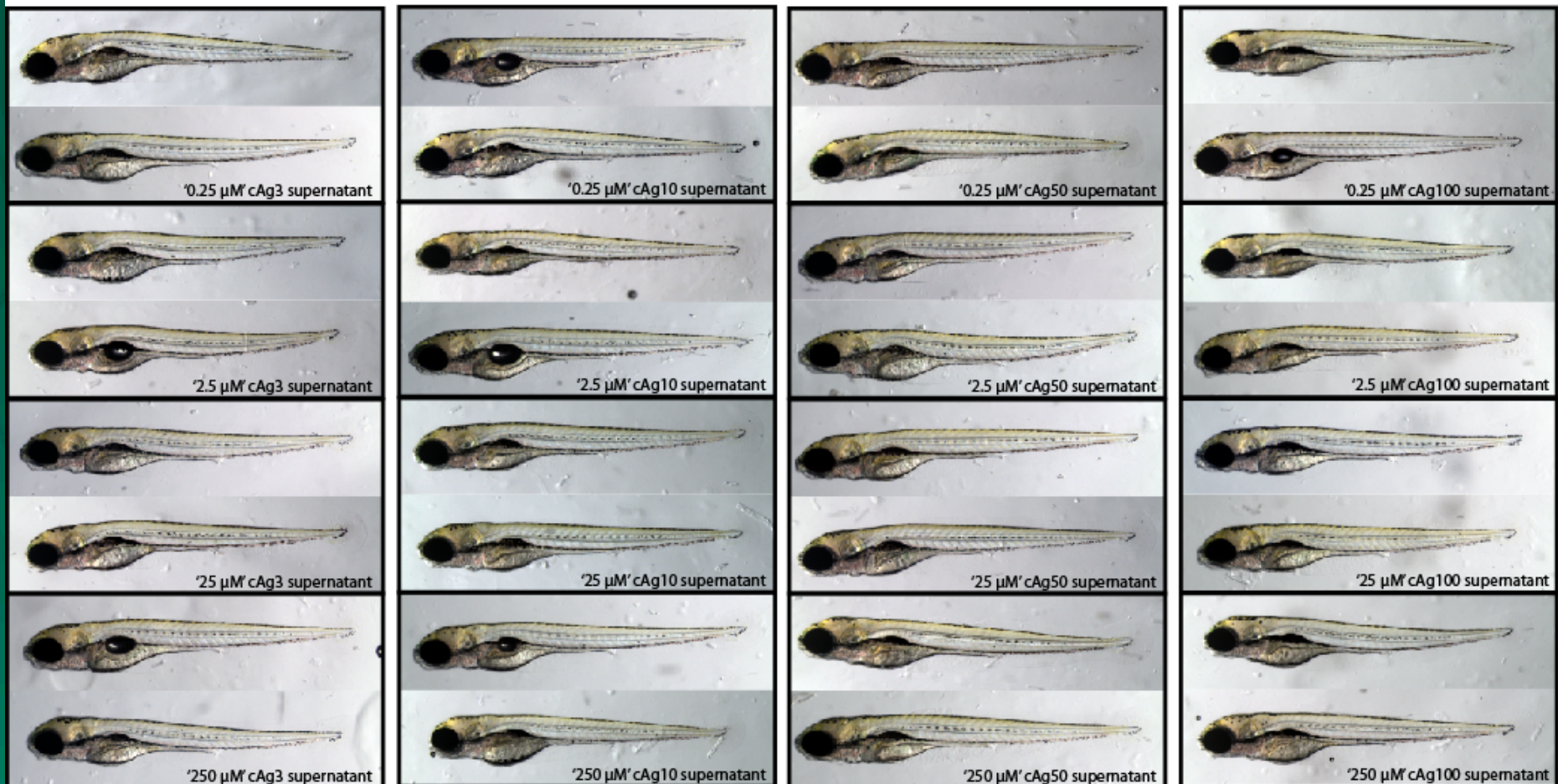
- Conclusion: No size dependent toxicity visible.

Zebrafish: Silver Nanoparticle Exposure



- Conclusion: Toxicity a function of size and concentration.

Zebrafish: Silver Nanoparticle Supernatant Exposure



Manufacturing Nanoparticle-Containing Drugs

The minimal characterization needs for nanomedicines?

NanoToolkit Development

- Toxicity - establishing an MTP or HTP assay for acute toxicity and predictive modeling
- Physicochemical characterization
 - **Molecular weight; particle size; surface charge** and associated distributions
 - **Stability** in aqueous media, plasma, protein adsorption
 - **Purity** (absence of lingering contaminants such as antioxidants or homopolymers)
 - **Reproducibility** of manufacture
 - **Drug release** and **biodegradability** profiles

Manufacturing Nanoparticle-Containing Drugs

What characteristics/parameters/features of nanomaterials need to be evaluated during development?

- Quantum size effects with nanoparticles
- Nanoparticle synthesis schemes
- Incommensurate reports between ***nanocharacterization*** of nanoparticles and ***nanotoxicity*** with cell-based assays: c.f.
 - Jähnen-Dechent *et al. Small* **3**:1941 (2007) “Size dependent cytotoxicity of gold nanoparticles”
 - Chan *et al. Nat Nanotech* **3**:145 (2008) “Nanoparticle-mediated cellular response is size-dependent”

Identifying Issues for the FDA Regarding Nanomaterial Containing Products

- It is well-known that the molecular weight fractions of polydisperse polymers induce alternative effects *in vivo*
 - Clean-up of nanodispersity will be key but expensive
- Cremophore-EL continues to be the drug delivery vehicle of choice for hydrophobic small molecules despite its well-known (fatal) toxicity
- Re-education of pharmaceutical development
- Emphasis of pharmaceutical chemistry to optimize formulations
- **NanoToolkit design** for predictive models of nanomedicine toxicity

Identifying Issues for the FDA Regarding Nanomaterial Containing Products

- Multivalent capacity
 - Biosynthetics - genetically engineered systems
 - Bioorganics - combination of recombinant and synthetic polymers
 - Proteins - albumin; antibodies; Fab
 - Polymers - dendrimers
- “Soluble” polymer systems vs. colloidal systems*
- Nanoparticles vs. micelles vs. polymer-drug conjugates*

*Extremely difficult to distinguish at the nano-scale

Emerging Technologies - Current Regulations

- Regulations on Advanced Therapies

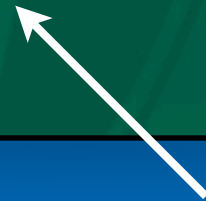
draft - proposed by European Commission, January 2007

Medical
Devices
93/42/EEC

?

Medicinal Products 2001/93/EC

Advanced Therapies



Medical
Devices

Tissue
Engineering

Cell
Therapy

Gene
Therapy

Biotech

Chemicals



SCHOOL OF
PHARMACY
University of Wisconsin-Madison

Source: PressBriefing/European Commission, Nov 2005
Courtesy: Dr. C. Camara, Medipol SA

Current and Emerging Technologies

Current and Emerging Technologies

- Current Theragnostics
 - Pulse monitors

Current and Emerging Technologies

- Current Theragnostics
 - Pulse monitors
 - Insulin pumps

Current and Emerging Technologies

- Current Theragnostics
 - Pulse monitors
 - Insulin pumps
 - ICDs

Current and Emerging Technologies

- Current Theragnostics
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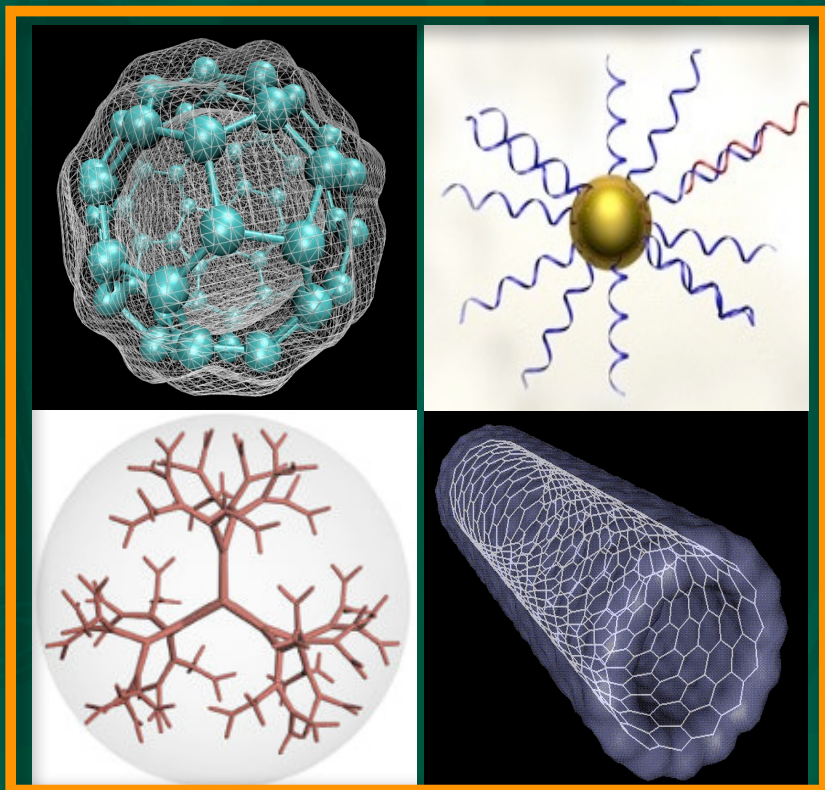
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Current and Emerging Technologies

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- ***Imaging agents***
- ***Theragnostics***
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 - nL insulin delivery control
- iNanomedicine (iMed) - technology pending
 - EKG / HR / RR
 - Acute med dispersal

Regulator/Institutional Oversight



**Safety and Efficacy of
Nanotechnology**



Future of Nanotechnology

- **VALIDATION**

- It's easy to enact new regulations, but it's tough to regulate them
- This is further complicated without standards and no established nanotools
- Establishing metric benchmarks for stability, size distributions, *in vitro* and *in vivo* will be important
- Increasing political and economic pressure to “deliver” nanotechnology to the market place
 - Currently visible with clothing, cosmetics, etc.
 - Nanomedicines are on the horizon with substantial investment and time to bring drugs to market
 - FDA hesitance will stifle commercialization

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