

# Nanotechnology & CBER Regulated Products

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# Center for Biologics Evaluation and Research

## CBER Mission

The mission of CBER is to protect and enhance the public health through the regulation of biological and related products including blood, vaccines, tissue, allergenics and certain biological therapeutics.

## Biological Product

means any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment or cure of diseases or injuries of man (Code of Federal Regulations, Chapter 21, Section 600.3 h).

# CBER Organization

**Office of the Center Director**

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graph TD; A[Office of the Center Director] --- B[Office of Blood Research and Review]; A --- C[Office of Vaccines Research and Review]; A --- D[Office of Cellular, Tissue and Gene Therapies]; A --- E[Office of Compliance and Biologics Quality]; A --- F[Office of Biostatistics and Epidemiology]; A --- G[Office of Information Technology]; A --- H[Office of Management]; A --- I[Office of Communication, Training & Manufacturers Assistance];
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**Office of Blood  
Research and Review**

**Office of Vaccines  
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**Office of Cellular,  
Tissue and Gene  
Therapies**

**Office of Compliance  
and Biologics Quality**

**Office of Biostatistics  
and Epidemiology**

**Office of Information  
Technology**

**Office of  
Management**

**Office of Communication,  
Training & Manufacturers  
Assistance**

# CDER & Nanotechnology

- Reps in FDA Nanotechnology Task Force
- Reps In Nanotech. Subcom. of the FDA/NCI Interagency Oncology Task Force
- Rep. in NHLBI DBDR WG Nanobiotechnology
- CDER Nanotechnology WG
- CDER conducts nanotechnology related research under FDA Critical Path Initiative

# CDER Regulatory Approach for Nanotechnology Related Products

- CDER regulated BLA products as well as CDER regulated devices (510k, PMA) are subject to premarket requirements
- CDER has the authority to obtain the detailed scientific information needed to review the safety and effectiveness of regulated products
- CDER did not yet approve/clear any product with a nanotechnology claim. Information on products under review are proprietary

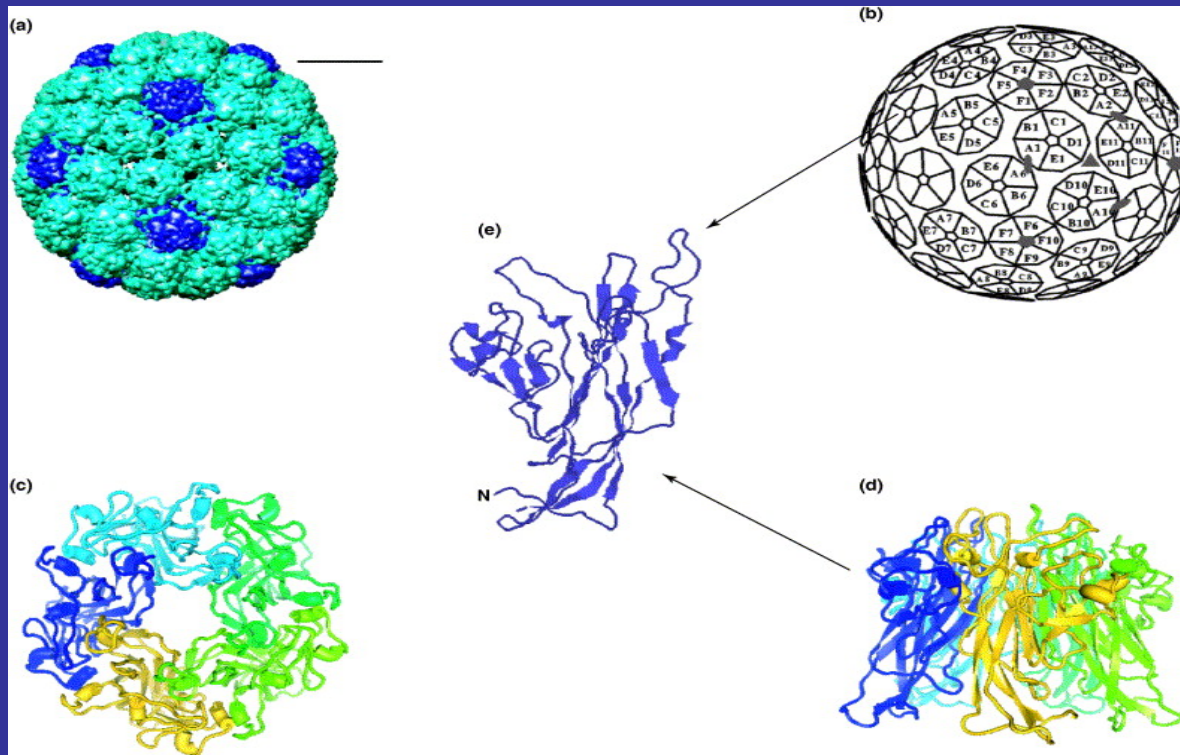
# Nanosized Biologics

Products containing nanosized particles derived from or intentionally produced in biologic systems

- Plasma protein products
- Blood substitutes
- Virus-like particle (VLP) vaccines
- Liposome vaccines and gene delivery systems,
- Adjuvants based on emulsion complexes, immunostimulatory complexes (ISCOMS)
- Tissue differentiation scaffolds

# Virus-like Particles (VLPs)

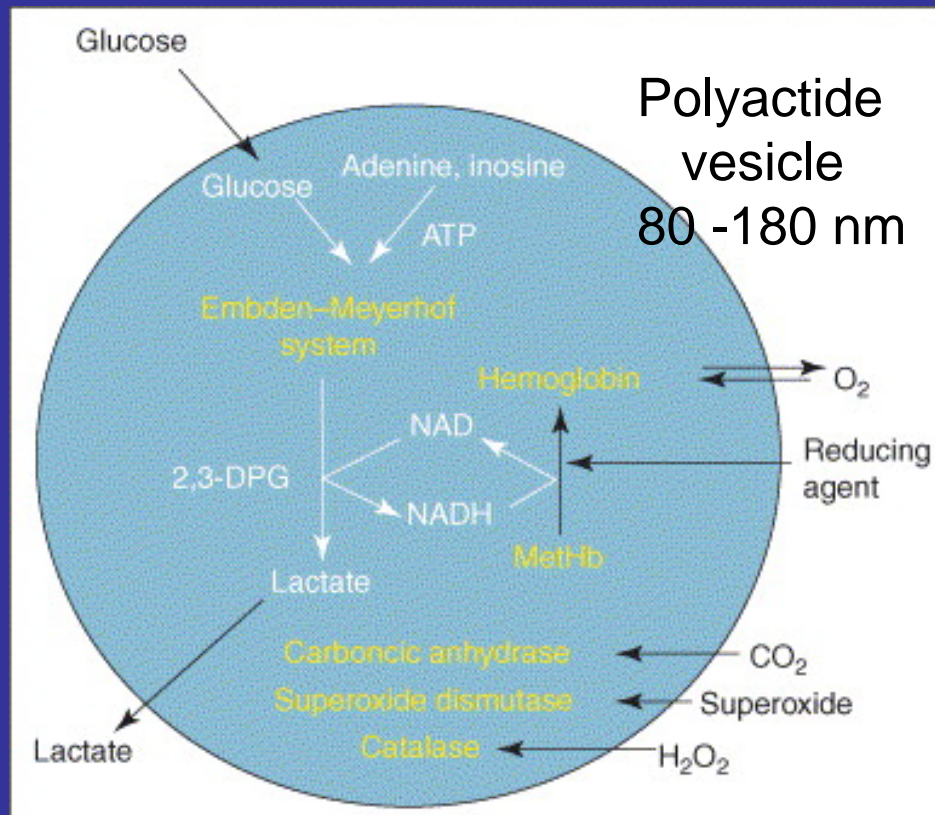
VLPs are defined as a replication-incompetent macromolecular protein assemblies that can be created from the minimal self-assembling structural proteins of phages and viruses. Here you can see a polyomavirus VLP consisted 72 capsomeres. Each capsomer is formed by five monomers. VLPs are of interest in vaccine development, also in gene therapy and drug delivery, however, their potential has yet to be fully realized.



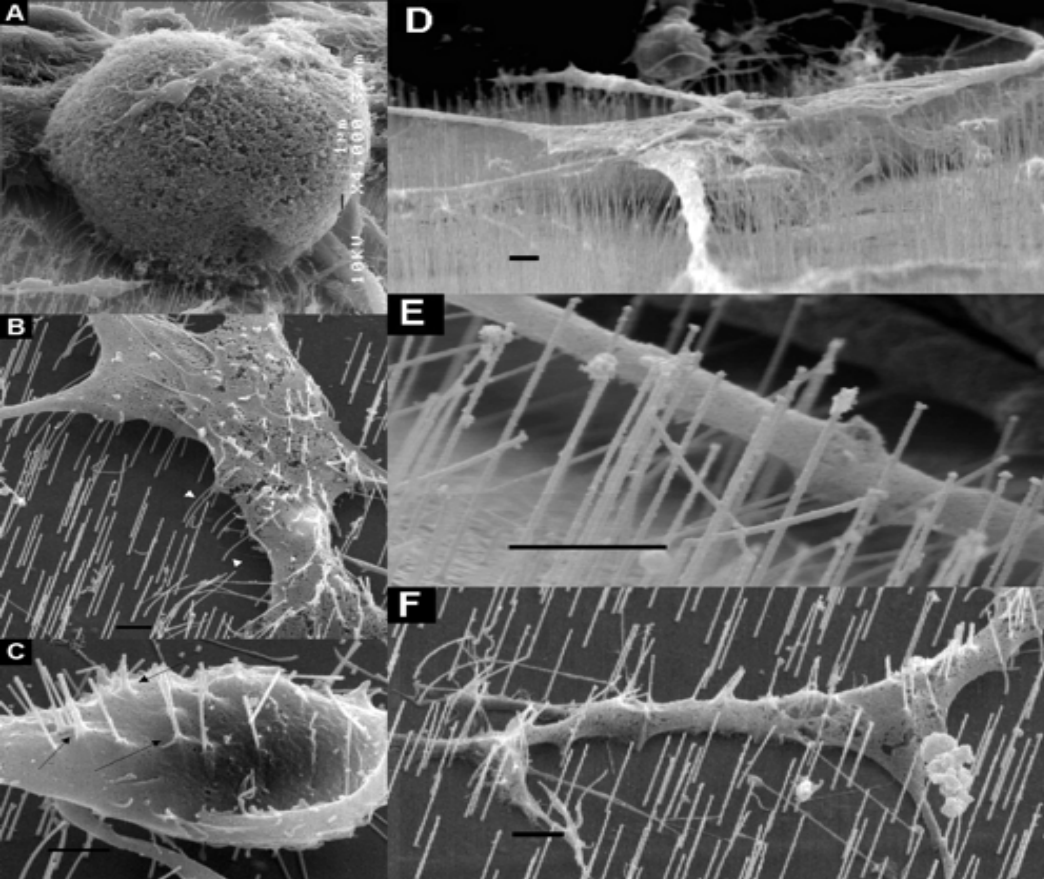
# Blood Substitutes Based on Nanobiotechnology

Chang T.M., Trends in Biotechnology 2006

Here is an example of nanotechnology based third-generation of RBC substitutes which contains Hb and the whole RBC enzyme system. This approach is based on biodegradable polymer – polylactide (PLA) vesicles 80 – 180 nm in size.



Nanodimension artificial RBCs with a PEG-PLA membrane



# Gallium Phosphide Nanowires as a Substrate for Cultured Neurons

Hallstrom W. *et al.*,  
Nanoletters 2007

Nanotechnology based tissue culture scaffolds is another important area how nanotech impacts development of biologics. Pictures here are showing how peripheral sensory neurons and other cells from dorsal root ganglia can adhere and survive on Gallium Phosphide nanowire surface. 50 nm wires can penetrate the cells without killing them. This is highly interesting system for various culturing studies, including guiding of regenerating axons, probing cells at extreme proximity, or intracellular drug delivery, generally wiring cells without harming them.

# Nanotechnology Devices that Would be Regulated by CBER

Devices may contain nanomaterials as active or structural components or as plastic additives

Scientific challenges in material characterization and biocompatibility/toxicology review

New types of additional testing and characterization may be required

- Devices for collection, processing and storage of blood and plasma products (nanomaterials as plastic additives)
- Devices for pathogen reduction in blood products (fullerenes)
- In vitro diagnostics for screening of blood donors, blood typing, and for detection of viral and bacterial contamination of blood products (quantum dots, nanocantilevers, bio bar code based assays)

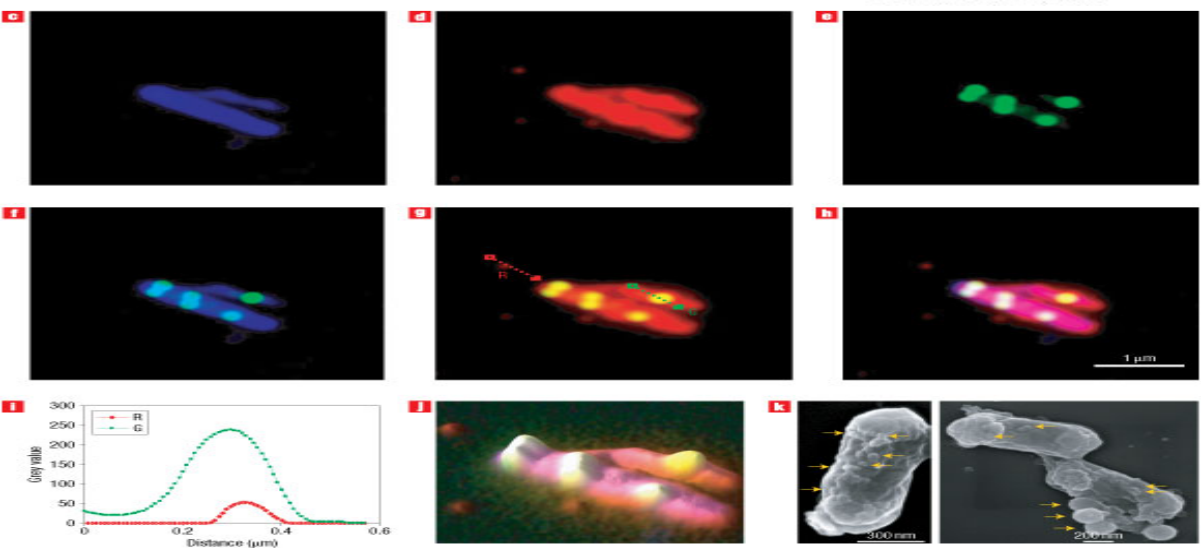
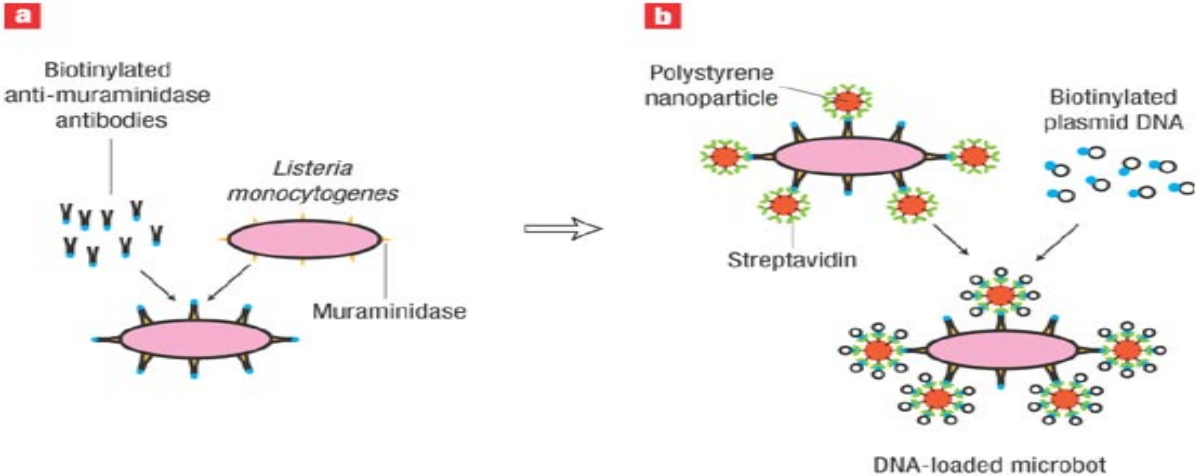
# Possible Combination Products

Assigned according primary mode of action

Intercenter collaborative review

- Nanosized biologics as drug delivery systems (albumin particles)
- Nanosized biologics as components of imaging devices (VLP as contrast agents)
- Polymer nanoparticle based vaccines





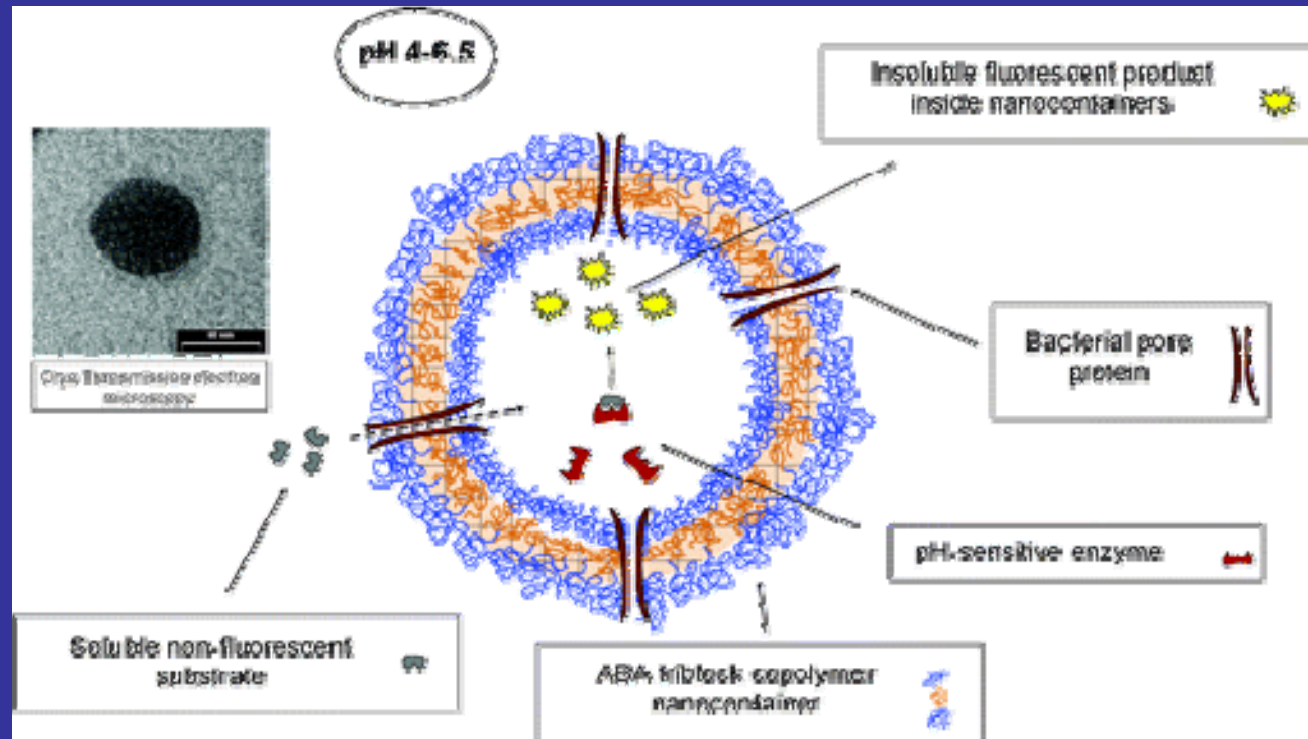
# Bacteria-mediated Delivery of Nanoparticles and Cargo into Cells

Akin D. *et al.*  
 Nature  
 Nanotechnology  
 2007

Here is a concept of gene delivery system based on nanopartiles attached to bacteria like *Listeria monocytogenes*. Cargo gene is loaded onto the nanoparticles, which are carried on the bacteria surface. When incubated with cells, the cargo carrying bacteria (microbots) were internalized and genes released from ananoparticles were expressed in the cells. The gene delivery also worked in vivo in mice.

# Toward Intelligent Nanosize Bioreactors: A pH-switchable, Channel-equipped, Functional Polymer Nanocontainer.

Broz P. *et al.*, *Nanoletters* 2006



Promising candidate structures for a targeted delivery of drugs or diagnostic agents are polymer-based vesicles consisting of diblock (hydrophilic- hydrophobic) or triblock (hydrophilic-hydrophobic-hydrophilic) copolymer building blocks. Revolutionary new concept is the combination of these polymer vesicles with multifunctional biological components such as transmembrane proteins, pore proteins or enzymes. Bacterial outer membrane protein F was integrated into the triblock polymer vesicles and remained functional

# CBER Nanotechnology Research Challenges under CP Initiative

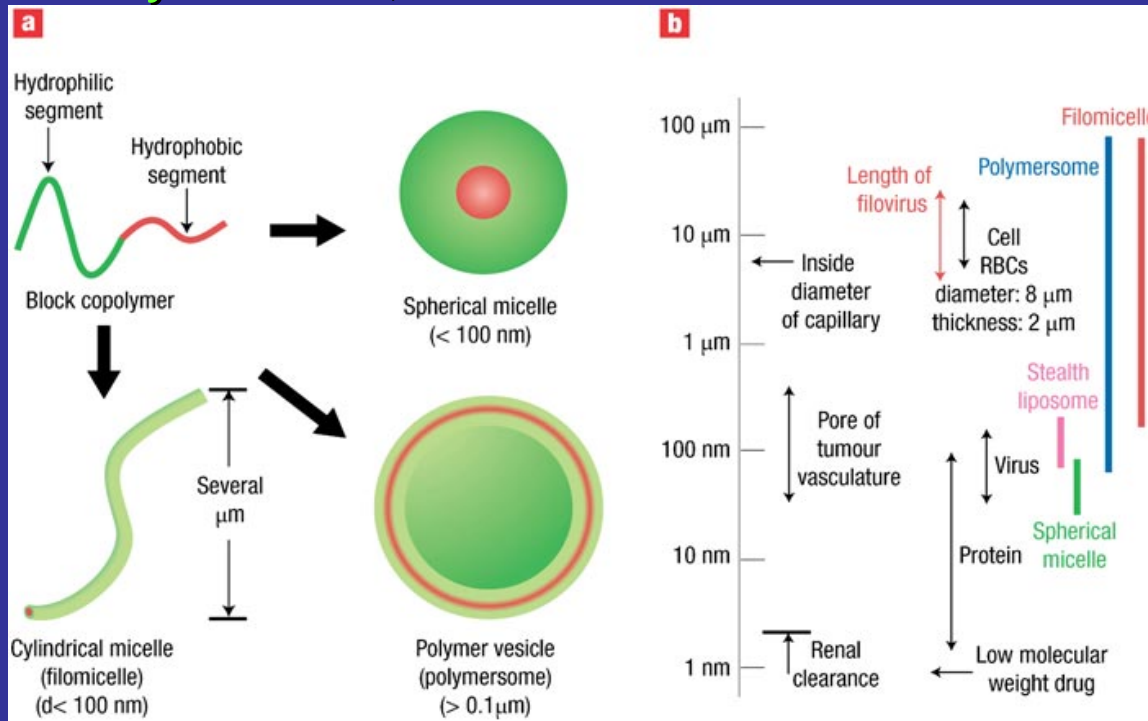
- Definition of naturally occurring vs. intentionally produced biologic nanoparticles
- Critical parameters for phys/chem characterization of biologic nanoparticles such as protein particles, liposomes, VLPs, membrane vesicles, ISCOMs
- Analytical tools for phys/chem characterization of biologic nanoparticles
- Tools for controlled manufacturing of biologic particles at nanoscale
- Tools for quality control of biologic nanoparticles

# CBER Nanotechnology Research Challenges under CP Initiative, cont.

- How the size distribution and other critical phys/chem characteristics of biologic nanoparticles impact or relate to safety and effectiveness of biologic products ?

# Nanocarriers Shape up for Long Life

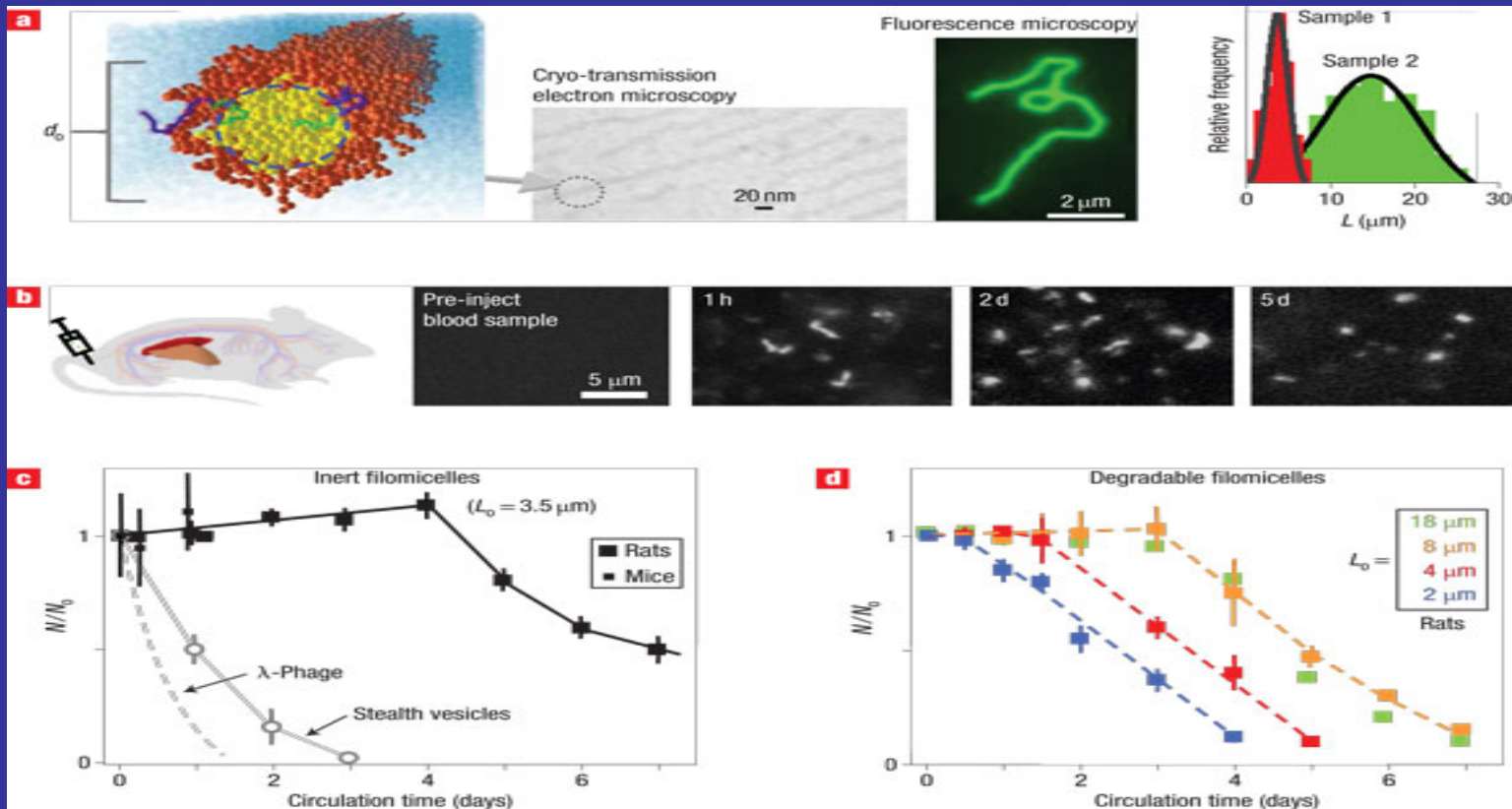
Nishiyama N., Nature Nanomedicine 2007



Delivery therapeutic or diagnostic agents is one of the most promising biomedical applications of nanotechnology. Liposomes and polymeric micelles or protein particles are most popular delivery system. In a classical concept nanocarriers are typically spherical and size and surface chemistry are always considered main features influencing their circulation half-time. Non-spherical particles have not received significant attention. As you know, many viruses and bacteria infecting mammals are filamentous – so why not to explore this strategy.

# Shape Effects of Filaments Versus Spherical Particles in Flow and Drug Delivery.

Geng Y. *et al.*, Nature Nanotechnology 2007



And here it is , Geng and colleagues showed recently that filamentous copolymer micelles remain in circulation ten times longer than their spherical counterparts. I believe that this observation will have great impact on future development of delivery systems.

# CBER Nanotechnology Research Goals in CP Initiative

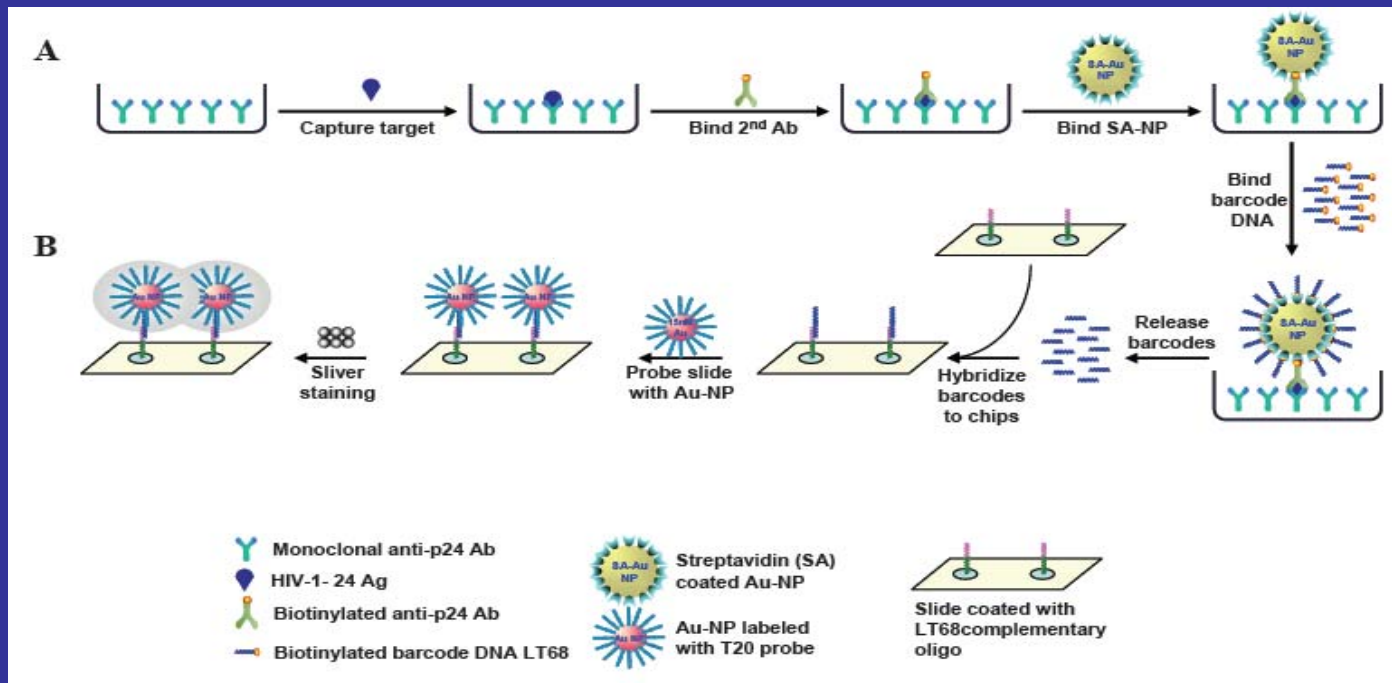
- Development of specific standardized, validated test methods and reference standards to evaluate quality and purity of the nanomaterials and biologic nanoparticles in vaccines, blood, tissues, cells and gene therapy products that are currently marketed, under clinical study or under preclinical development.
- Development of specific, standardized, validated test methods and reference standards to characterize nanomaterials and biologic nanoparticles “in vitro”, as well as to predict their clinical safety and efficacy “in vivo”.

# CBER Nanotechnology Research Goals, cont.

- Evaluation and development of new toxicological methods and biocompatibility assays to assess the safety of nanomaterials and biologic nanoparticles in vaccine, blood, cell, tissue and gene products for human use.
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- Evaluation and development of standardized test methods to help ensure the accuracy of nanotechnology based assays for screening blood donors and for HIV diagnostic tests.

# Nanoparticle-Based biobarcode amplification assay (BCA) for sensitive and early detection of human immunodeficiency type capsid (p24) antigen

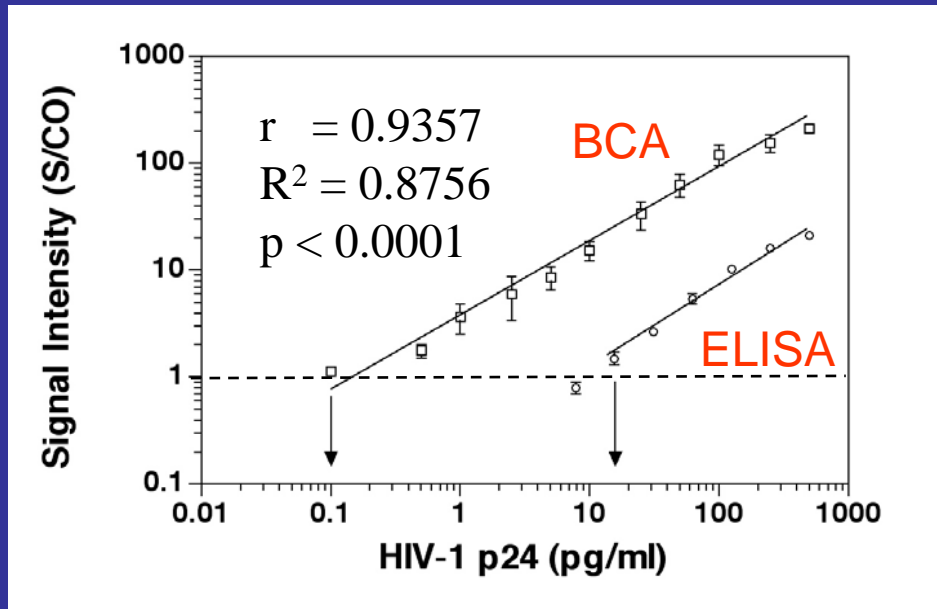
Tang S et al, *J. AIDS*, 2007; 46(2):231-7



In this collaborative study led by CBER investigators authors modified a nanoparticle-based biobarcode amplification (BCA) assay for early and sensitive detection of HIV-1 capsid (p24) antigen by using anti-p24 antibody-coated microplates to capture viral antigen (p24) and streptavidin-coated nanoparticle-based biobarcode DNAs for signal amplification, followed by detection using a chip-based scanometric method.

# Detection Limit: BCA vs ELISA

Tang S et al, *J. AIDS*, 2007; 46(2):231-7



Lower Limit of Detection (LOD)

BCA: 0.1 pg/ml or 0.1 ng/L

ELISA: 15 pg/ml

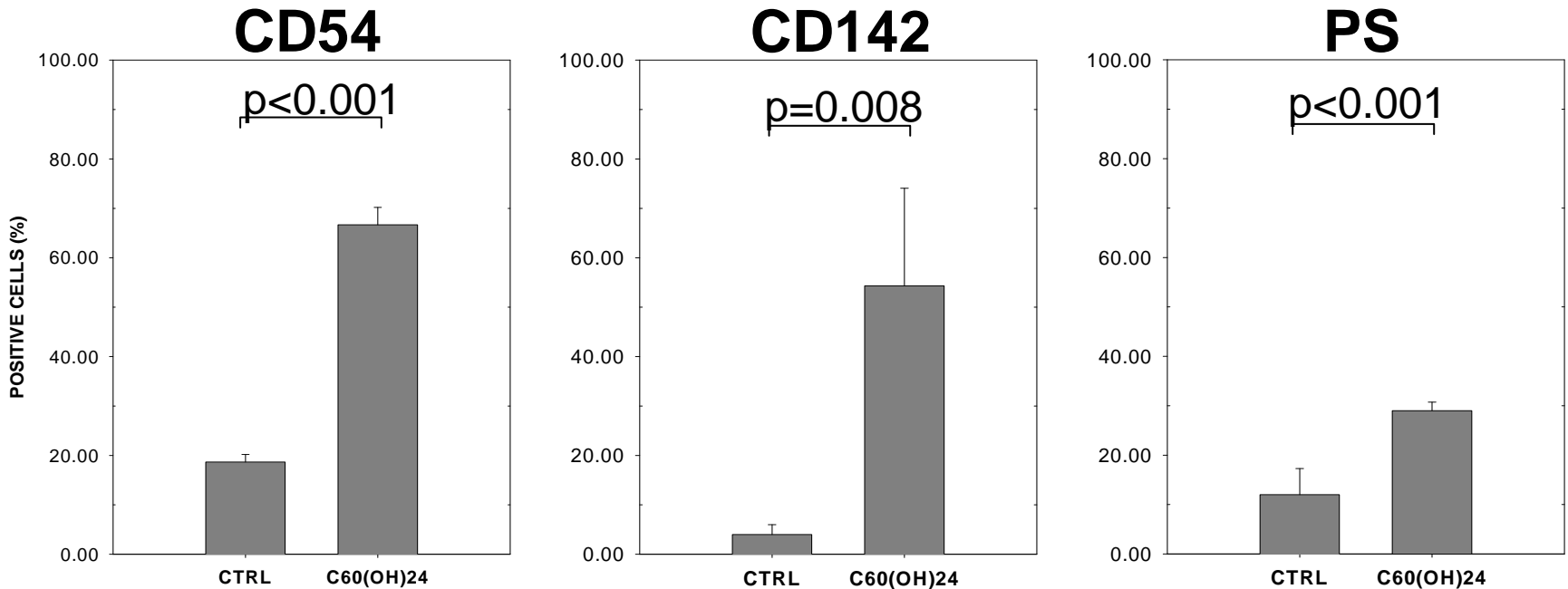
The modified BCA assay exhibited a linear dose-dependent pattern within the detection range of 0.1 to 500 pg/ml and was approximately 150-fold more sensitive than conventional enzyme-linked immunosorbent assay (ELISA). No false positive results were observed in 30 HIV-1-negative samples, while all 45 HIV-1 RNA positive samples were found HIV-1 p24 antigen positive by the BCA assay. In addition, the BCA assay detected HIV-1 infection 3 days earlier than ELISA in seroconversion samples.

# Tang *et al.*: Summary

- For detection of HIV-1 p24, BCA assay could detect 0.1 pg/ml of HIV-1 p24 antigen compared with 15 pg/ml by conventional p24 antigen capture assays (ELISA), indicating that the current first generation BCA assay may be 150-fold more sensitive than the conventional ELISA.
- There is a linear relationship between the concentration of p24 antigen and the signal intensities at the range of 0.1 ~ 500 pg / ml.
- No false positive results were seen with 30 HIV-1 negative samples while all 45 HIV-1 positive samples were HIV-1 p24 positive by BCA assay.
- BCA assay can detect HIV-1 p24 around 3 days earlier than conventional ELISA.

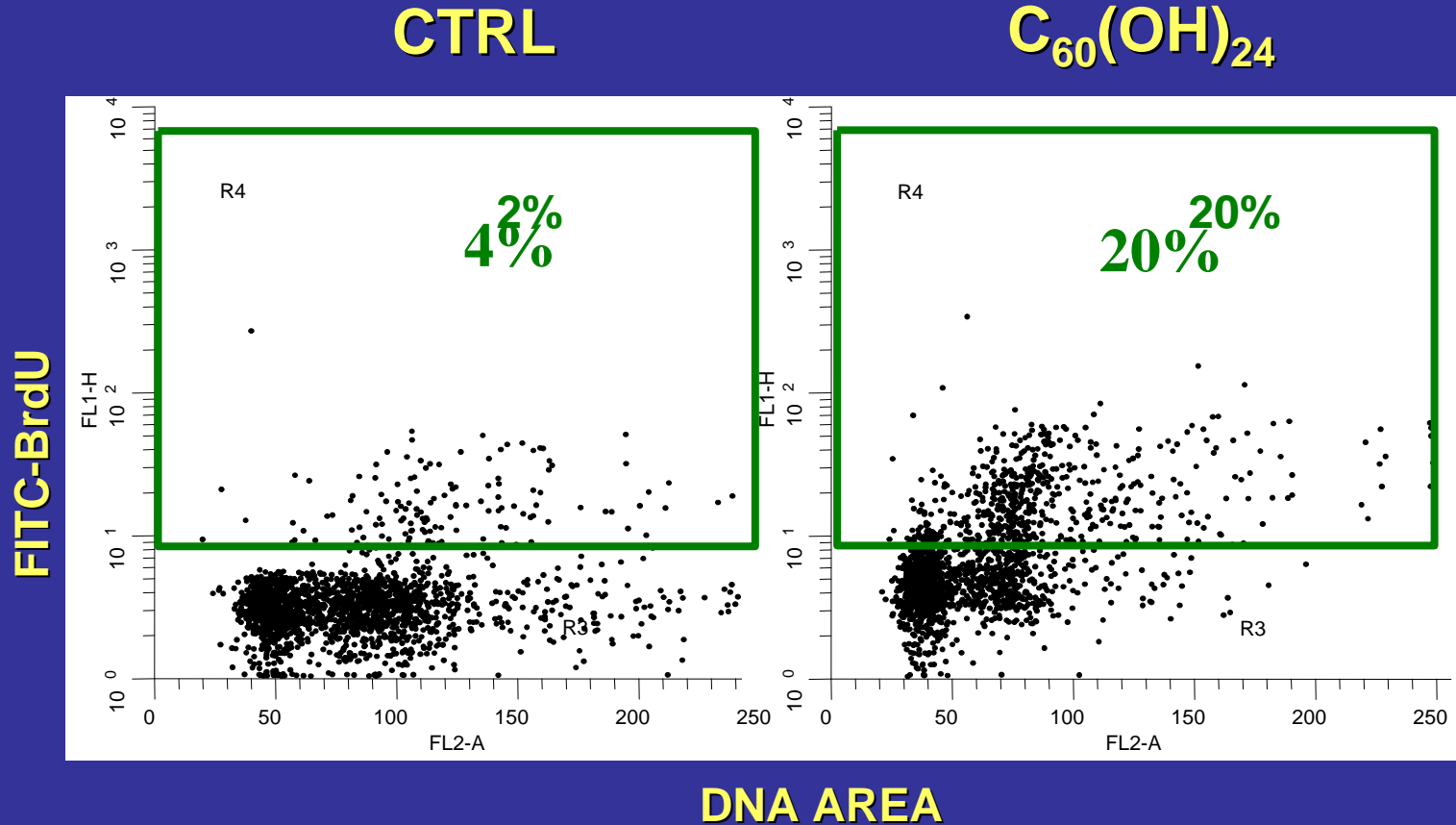
# Adverse Effects of Fullerenes on Endothelial Cells: Fullerenol $C_{60}(OH)_{24}$ Induced Tissue Factor and ICAM-1 Membrane Expression and Apoptosis In Vitro

Gelderman MP *et al. Int. J. Nanomedicine* 2008, 3(1):59-68.



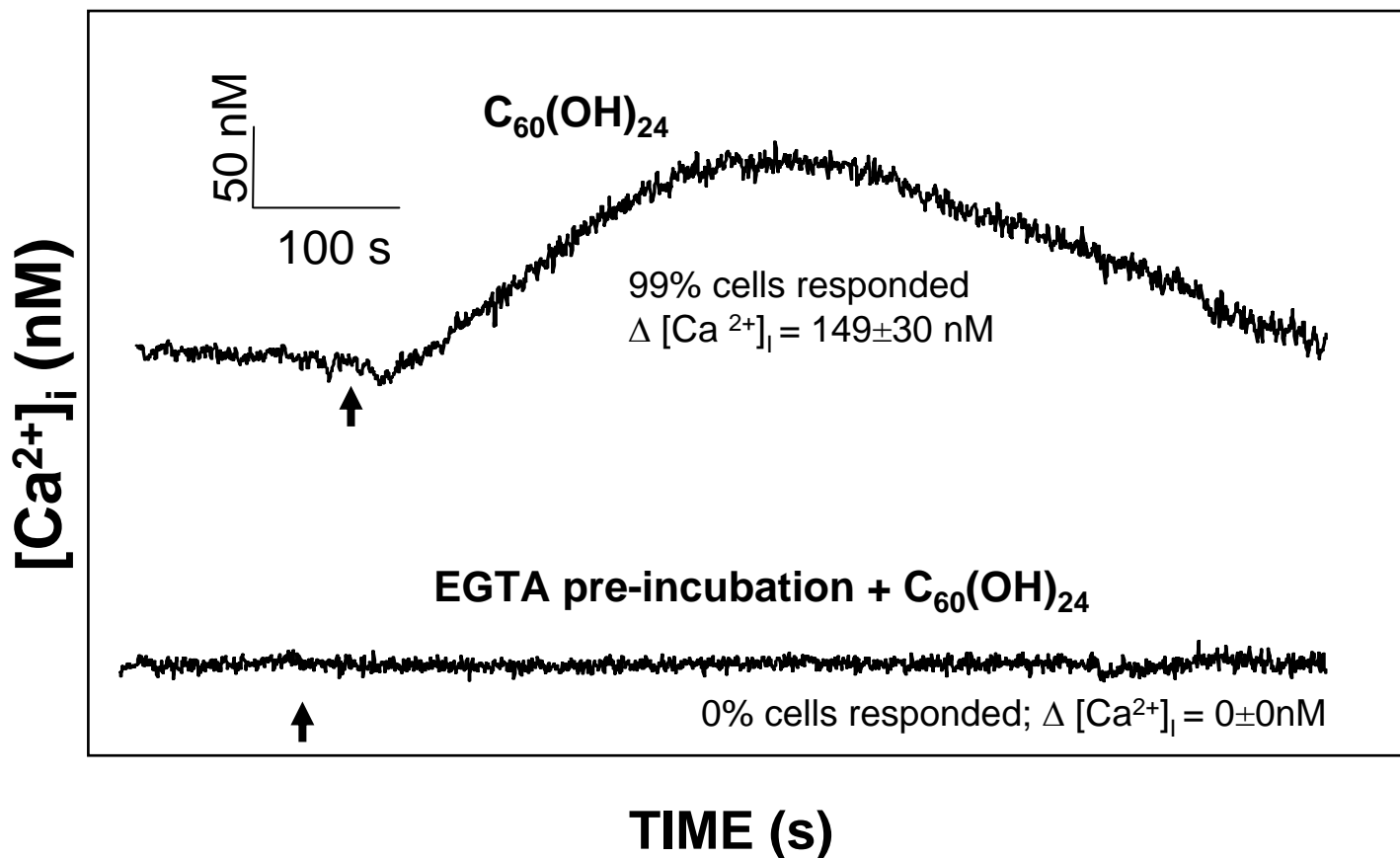
In this study from our laboratory, flow cytometric analysis of human umbilical vein endothelial cells (HUVECs) treated for 24 hrs with fullerenol  $C_{60}(OH)_{24}$  ( $100\mu\text{g}/\text{mL}$ ) showed significantly increased cell surface expression of ICAM-1 (CD54), tissue factor (CD142), and phosphatidylserine (PS).

# Flow cytometric TUNEL assay of human umbilical vein endothelial cells (HUVECs) treated 24 hrs with fullereneol $C_{60}(OH)_{24}$



Fullereneol  $C_{60}(OH)_{24}$  at  $100 \mu\text{g}/\text{mL}$  induced significant apoptosis (TUNEL) in cultured HUVECs.

# Calcium ion $\text{Ca}^{2+}$ influx induced by fullereneol $\text{C}_{60}(\text{OH})_{24}$ in cultured human umbilical vein endothelial cells (HUVECs)



Fullereneol  $\text{C}_{60}(\text{OH})_{24}$  induced in cultured HUVECs a concentration dependent increase of intracellular calcium  $[\text{Ca}^{2+}]_i$ . The activity could be inhibited by EGTA, suggesting that the source of  $[\text{Ca}^{2+}]_i$  in fullerene stimulated calcium flux is predominantly from the extracellular environment

# Gelderman MP et al: Summary

- HUVECs treated for 24 hrs with C60(OH)<sub>24</sub> at 100 μg/mL significantly increased cell surface expression of ICAM-1 (CD54), tissue factor (CD142), and phosphatidylserine (PS)
- C60(OH)<sub>24</sub> at 10 μg/mL significantly inhibited HUVEC proliferation and the cell cycle analysis showed G1 arrest of HUVECs by both C60 and C60(OH)<sub>24</sub>
- C60(OH)<sub>24</sub> at 100 μg/mL induced significant apoptosis (TUNEL) in cultured HUVECs.
- Both C60 and C<sub>60</sub>(OH)<sub>24</sub> induced in cultured HUVECs a concentration dependent increase of [Ca<sup>2+</sup>]<sub>i</sub>.
- The activity could be inhibited by EGTA, suggesting that the source of [Ca<sup>2+</sup>]<sub>i</sub> in fullerene stimulated calcium flux is predominantly from the extracellular environment.
- These findings warrant further studies on vascular biocompatibility of carbon nanostructures.

# Conclusions

- CBER has the authority to obtain the detailed scientific information needed to review the safety and effectiveness of CBER regulated nanotechnology products.
- Nanotechnology in development of biologics brings several scientific challenges ; many to be addressed under the FDA Critical Path Research Initiative.
- New types of nanotechnology related combination products are expected. Intercenter review and research collaboration is in progress.