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The Multistage™ Nanovector Delivery System: "From bench to…almost clinic"

Jason Sakamoto, Ph.D.
Co-Chair, Assistant Member
Department of Nanomedicine
The Methodist Hospital Research Institute

COO, The Alliance for NanoHealth
Houston Texas

Financial Interests

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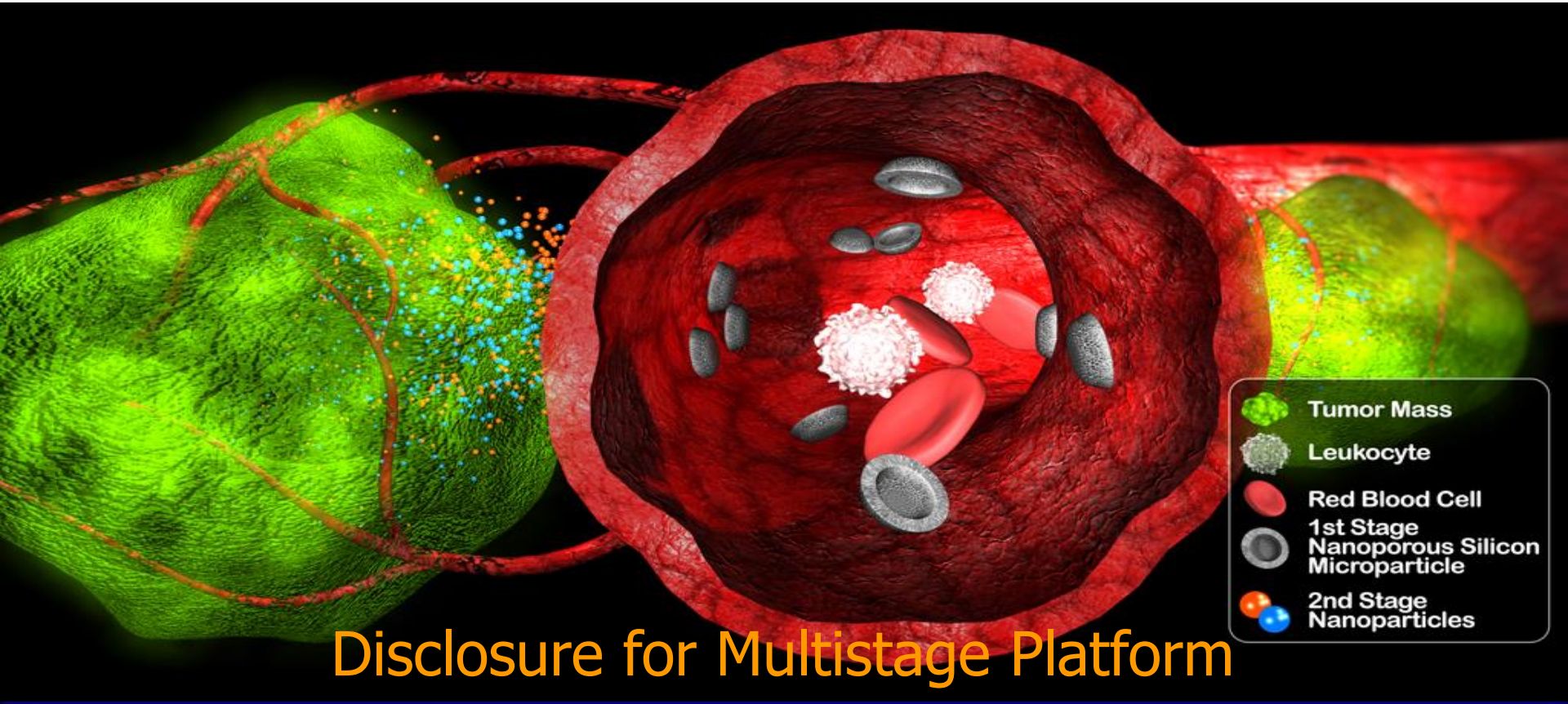
LEONARDO BIOSYSTEMS

Arrowhead Research CORPORATION



Disclosure for Multistage Platform

Benefits of Leveraging Academic Innovations

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LEONARDO BIOSYSTEMS

- Non-diluted Funding: Over $40M in Federal (NIH/DoD) funding
- Virtual R&D Department: 10 faculty members and their labs
- Keeping Pipelines Filled: Cancer, heart disease, transplant, regenerative medicine

Journal Covers 2005-2013

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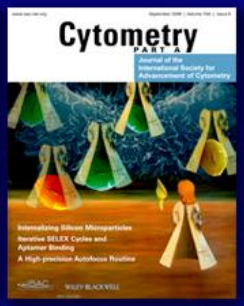

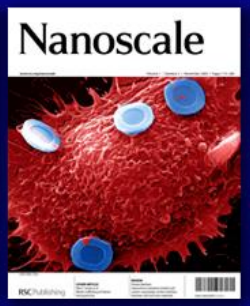

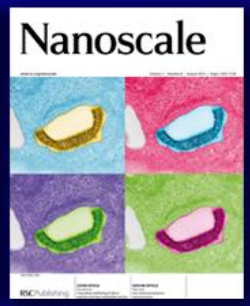

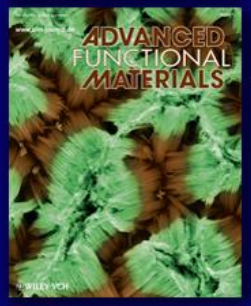

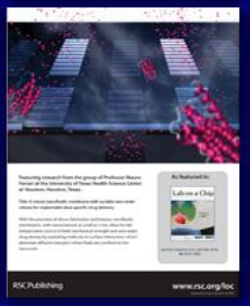

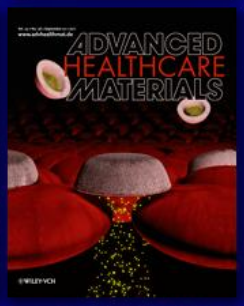

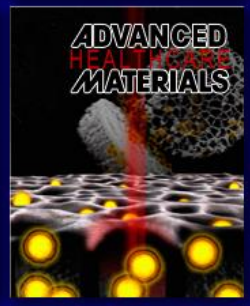

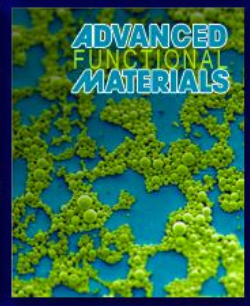

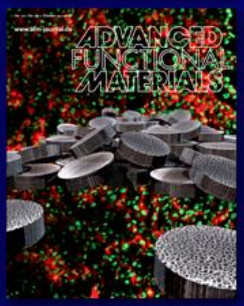

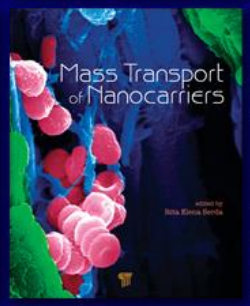

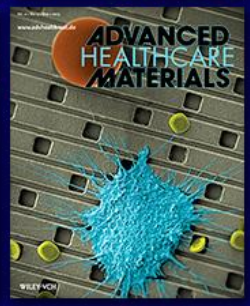

Challenges of Leveraging Academic Innovations

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LEONARDO BIOSYSTEMS

- Lack of Follow Through: Getting publications (and grants) more glamorous than translation work
- Tech Transfer Offices: Negotiating a reasonable licensing deal
- **Scale up and Manufacturing**: cGMP, mouse to man, QA/QC

Today's Panel Session: Technology Handoff

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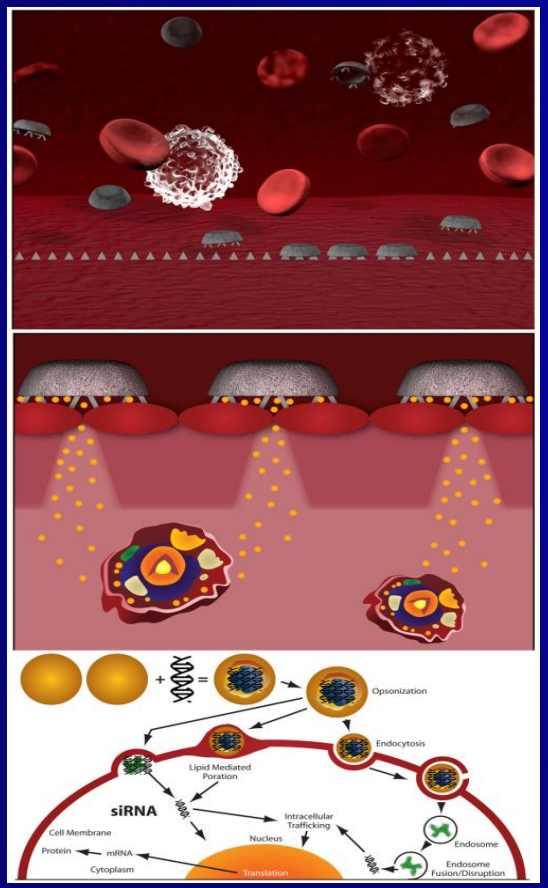
The Methodist Hospital Research Institute → LEONARDO BIOSYSTEMS

Goal → **IND Package** → Into the Clinic

Multi-Stage Drug Delivery System:

Mesoporous Silicon Microparticles



LETTERS

Mesoporous silicon particles as a multistage delivery system for imaging and therapeutic applications

ENNIO TASCIOTTI[1], XUEWU LIU[1], ROHAN BHAVANE[1], KEVIN PLANT[1], ASHLEY D. LEONARD[2], B. KATHERINE PRICE[2], MARK MING-CHENG CHENG[1], PAOLO DECUZZI[1,3], JAMES M. TOUR[2], FREDIKA ROBERTSON[4] AND MAURO FERRARI[1,4,5]*

[1]Nanomedicine, Brown Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, Texas 77030, USA
[2]Departments of Chemistry and Mechanical Engineering and Materials Science, and The Smalley Institute for Nanoscale Science and Technology, Rice University, Houston, Texas 77005, USA
[3]Center of Bio-/Nanotechnology and Engineering for Medicine, The University of Magna Graecia, Viale Europa – LOC. Germaneto, 88100, Catanzaro, Italy
[4]Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston Texas 77030, USA
[5]Department of Bioengineering, Rice University, Houston, Texas 77005, USA
*e-mail: mauro.ferrari@uth.tmc.edu

Nanotechnology Can Enable Interaction with the Multiscale Levels of Biology

Individuals

Organs

Cells

Protein and gene networks

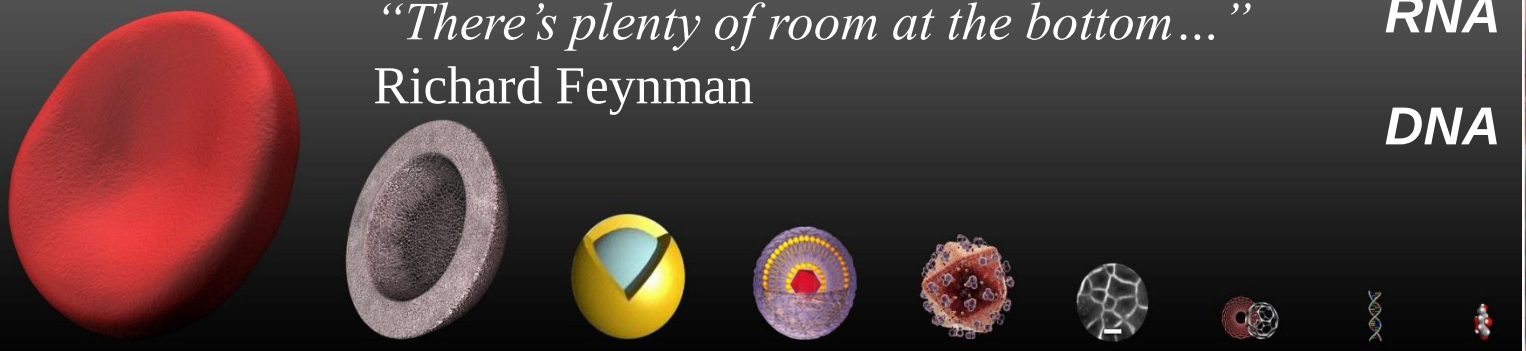
Protein interactions

Protein

RNA

DNA

"There's plenty of room at the bottom…"
Richard Feynman



Our Approach

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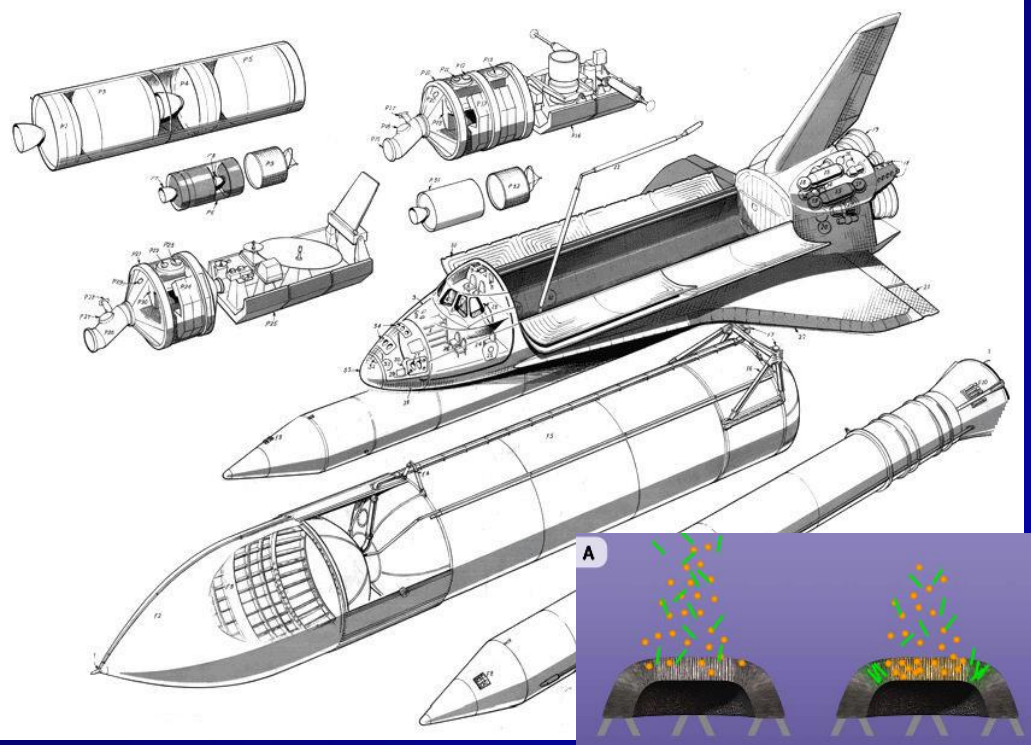
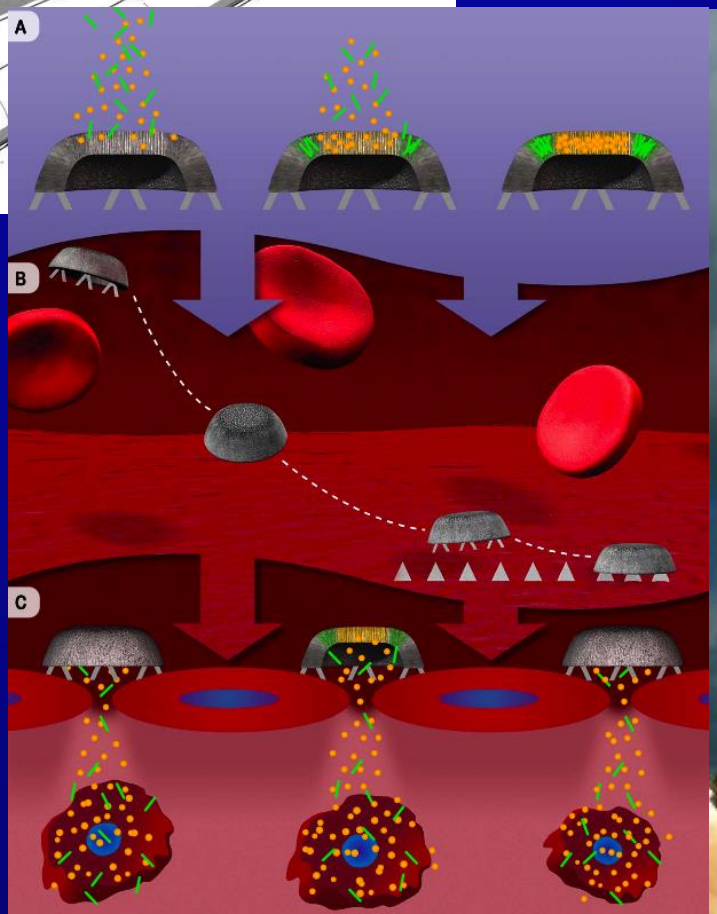
Decoupling Rx Challenges

LEONARDO BIOSYSTEMS

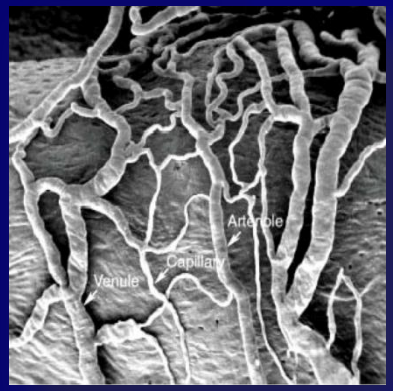
MultiStage Approach



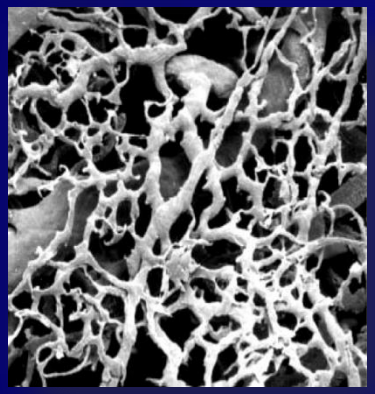


The Central Issue of Drug Delivery : Biological Barriers

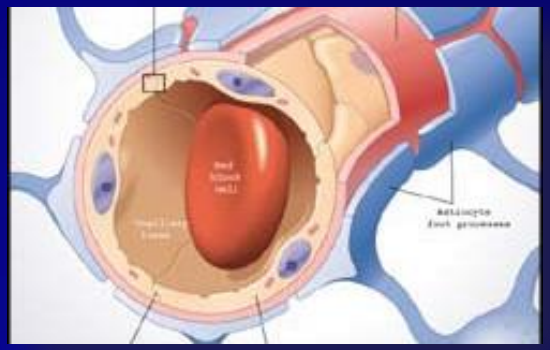
Normal vasculature

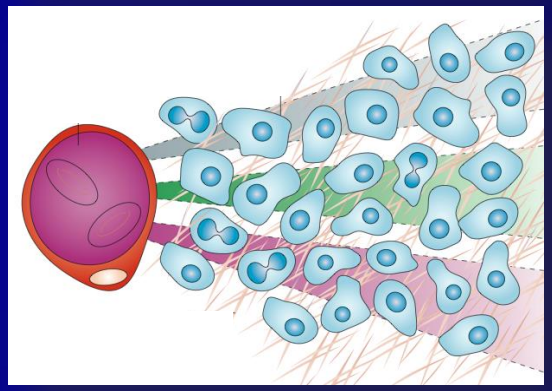


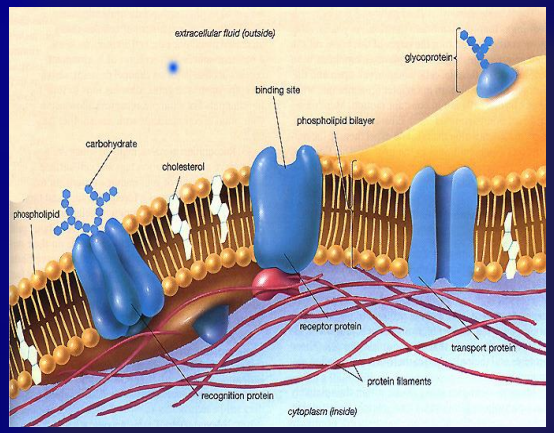
Tumor vasculature



- Hemo-Rheology
- Reticulo-Endothelial System
- Endothelial Barriers
- Tumor-Associated Interstitial Fluid Pressures
- Cell Membrane
- Ionic & Molecular Pumps
- Enzymatic Degradation
- Nuclear Membrane







New Delivery Strategy

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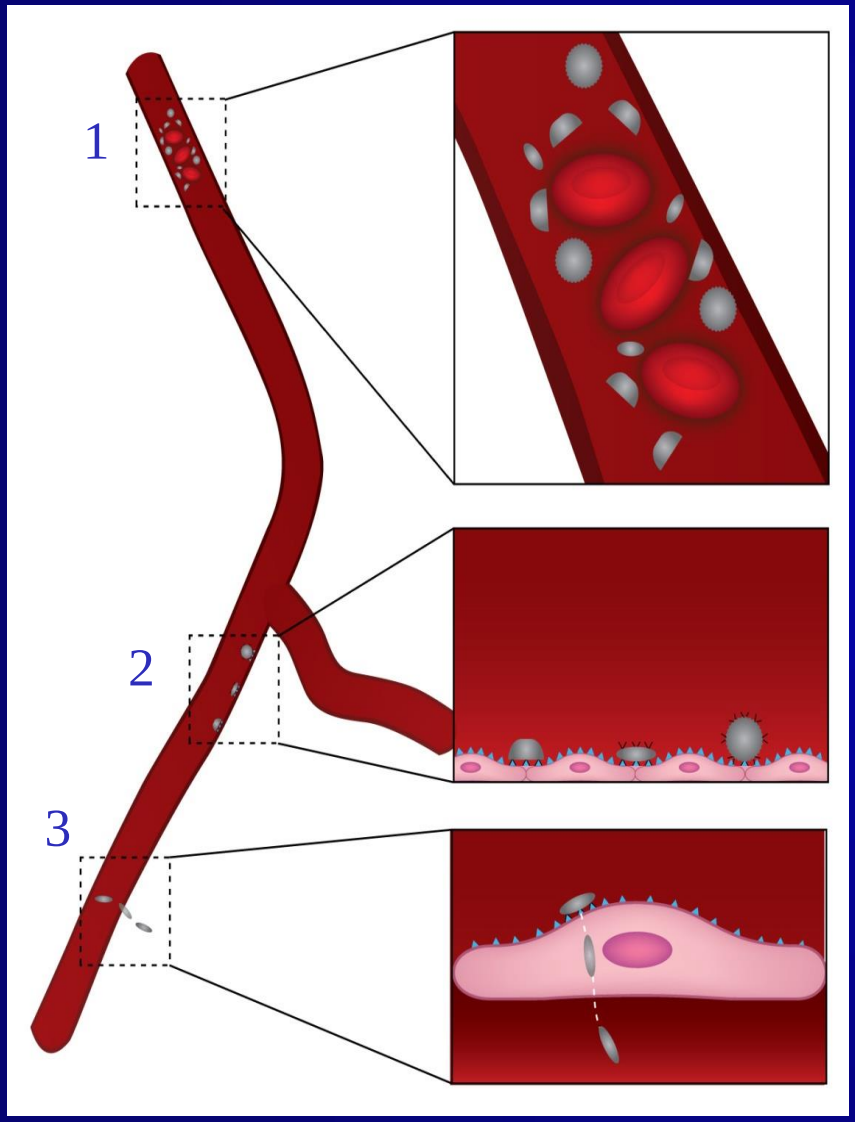
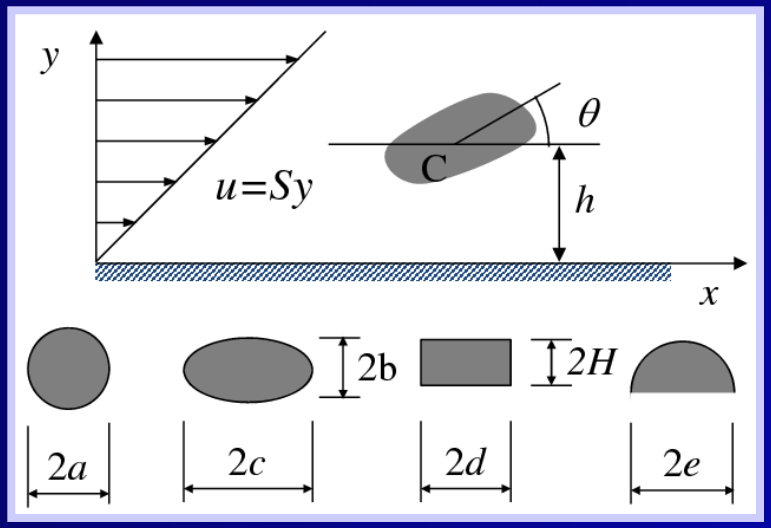


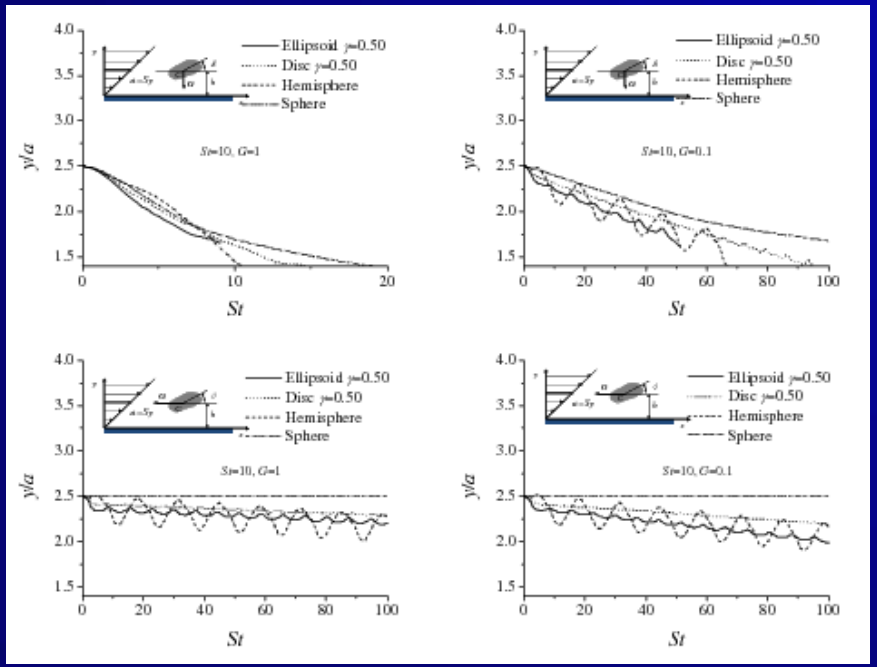
Breaking the problem down into:

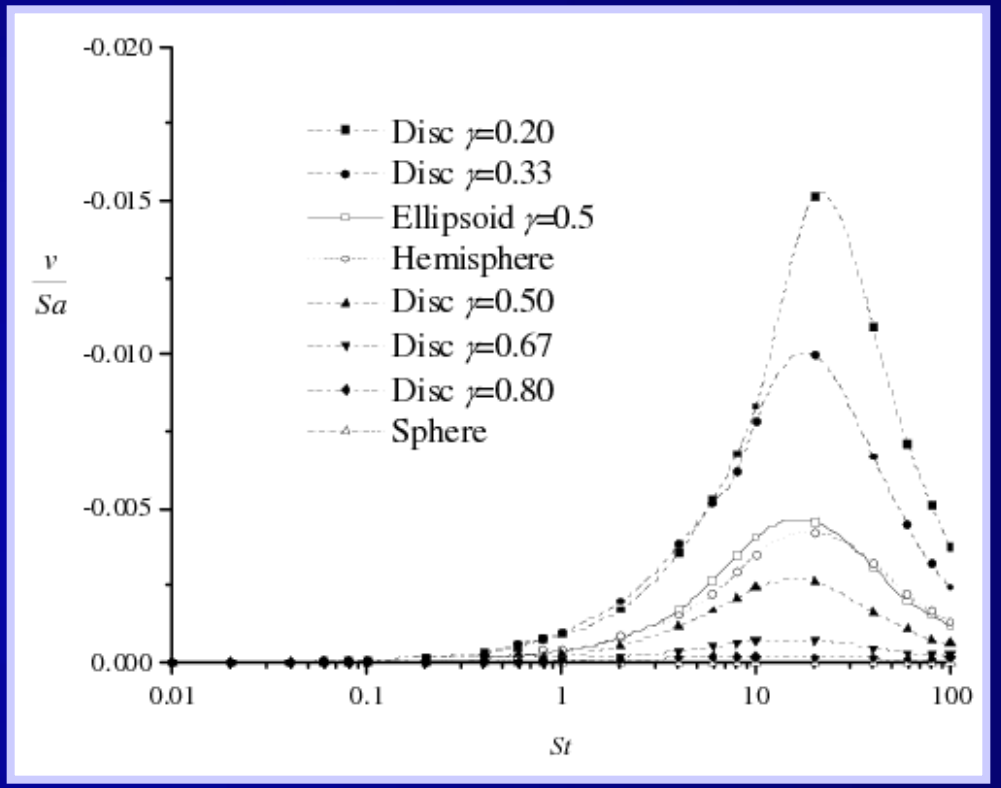
- ***Transport and Margination*** dynamics is the drifting of nanovectors towards the blood vessel walls
- ***Firm adhesion*** is the ability of a nanovector to recognize a vascular biological target and attach firmly at the blood vessels withstanding the hydrodynamic forces
- ***Internalization*** is referred to the ability of an adherent nanovector to control cellular uptake

Decuzzi et al., 2008, Pharm. Res.

Rational Design





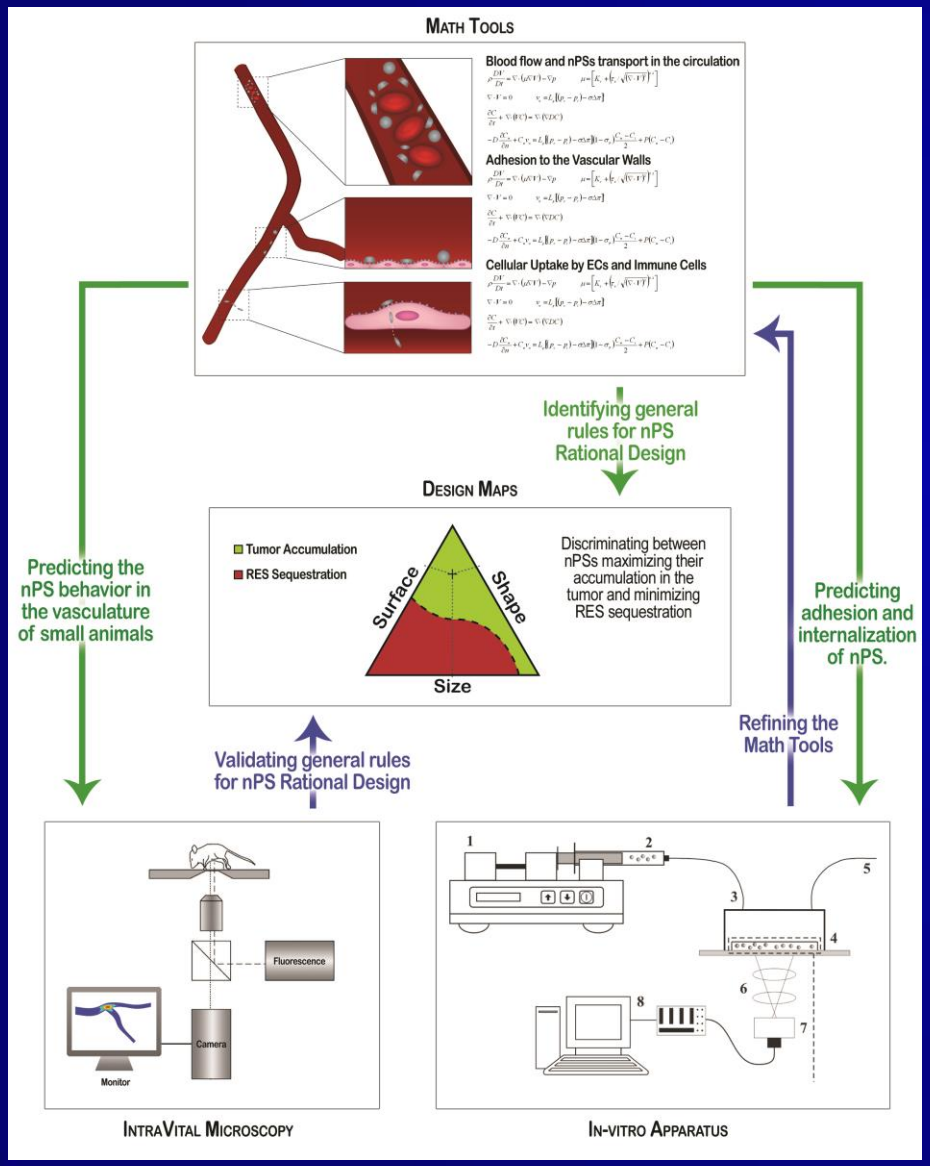


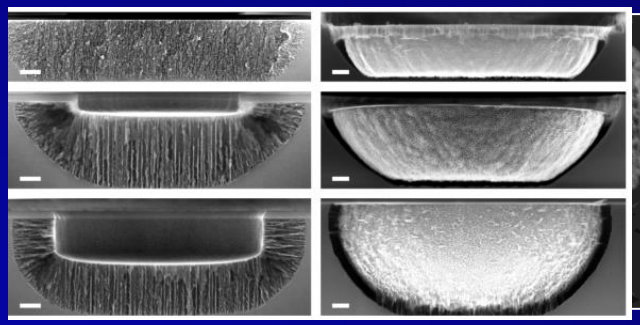
Discoidal particles with a smallest aspect ratio exhibit the largest margination propensity

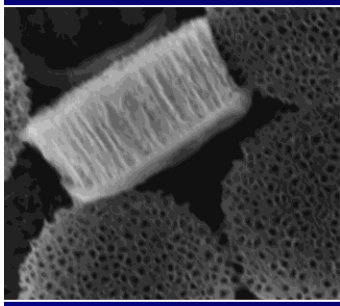
Lee et al., 2009, Nanotechnology

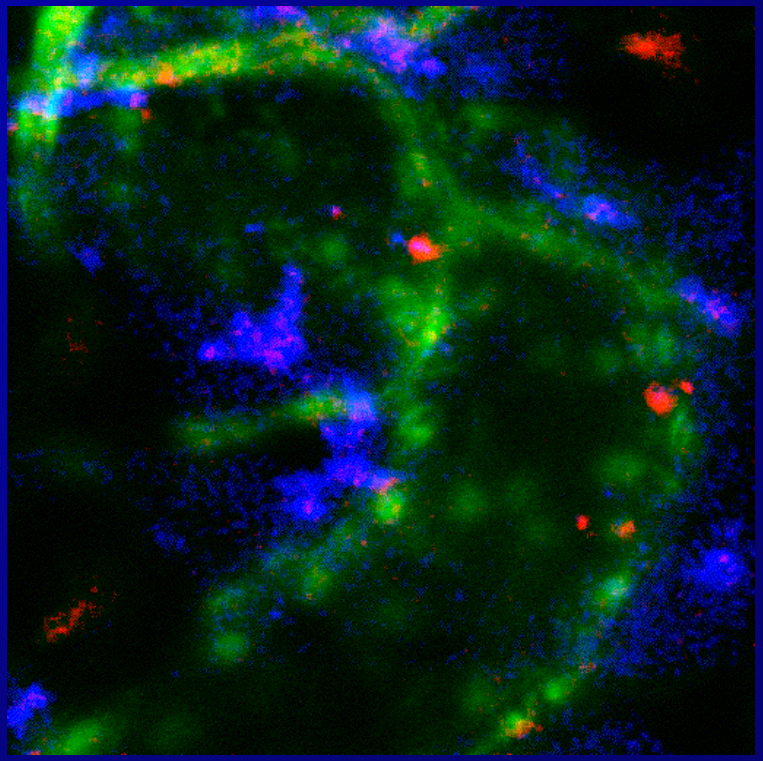
Integrated Framework for Rational Design

Paolo Decuzzi, Ph.D., Department of Nanomedicine, The Methodist Hospital Research Institute
pdecuzzi@tmhs.org









Decuzzi et al., Pharm Res. 2009

MSV Checklist

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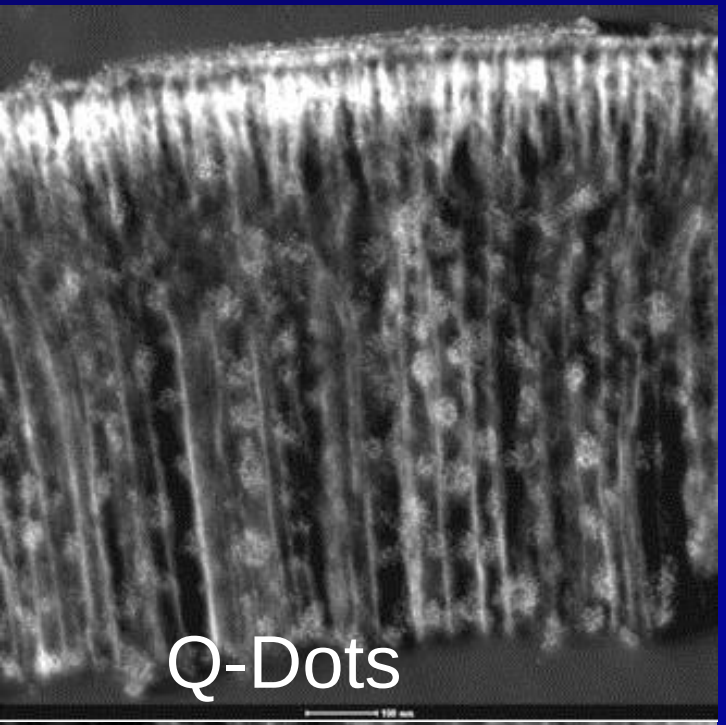
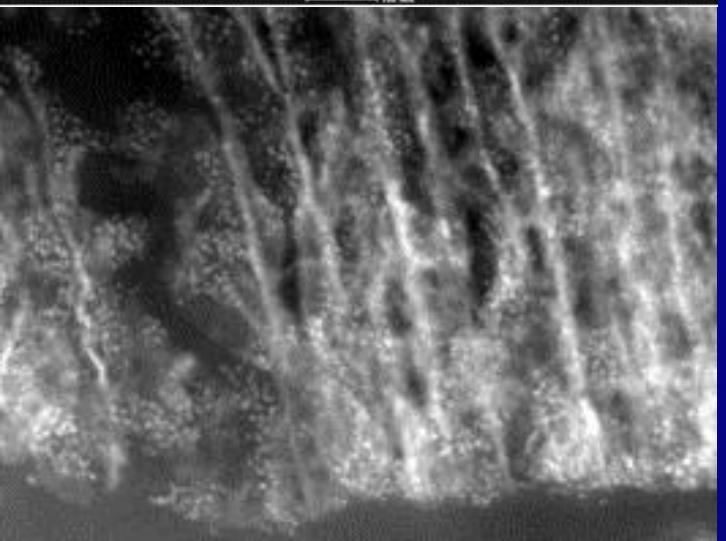
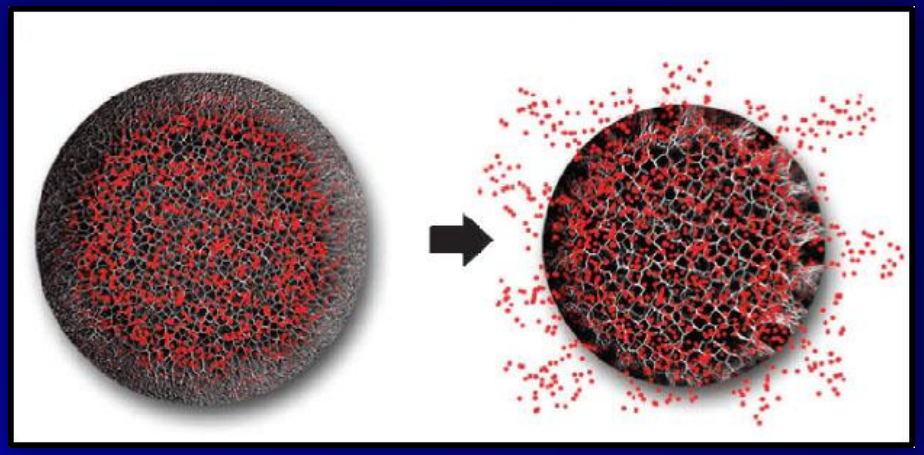
- Can you load them with drug?
  - What can you load them with?
- Can you get them to the tumor?
  - How does particle shape/size affect tumor accumulation?
- Can you kill the tumor?
  - Have you achieved tumor shrinkage in an animal model?
- How do you make these particles?
  - Can you scale up?

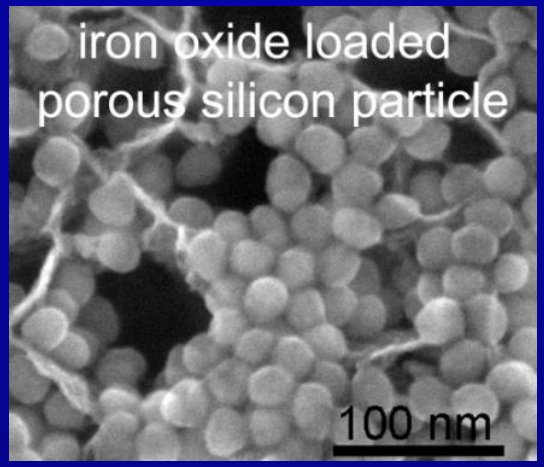
Loading 2nd Stage Vectors

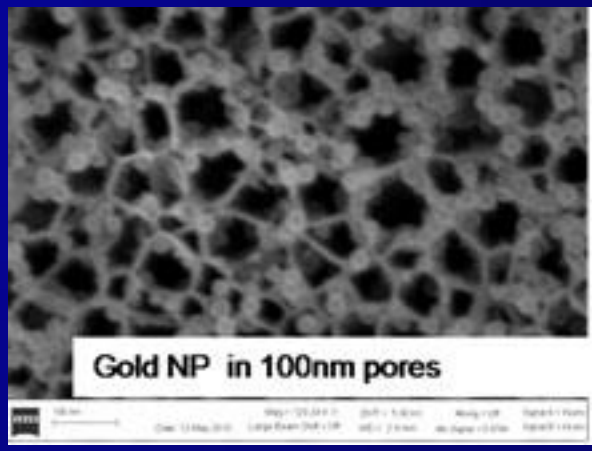
The Methodist Hospital Research Institute











- Liposomes
- Micelles

- CNT's
- Fullerenes

Ciro Chiappini and Xuewu Liu, et al

Biodistribution: Breast Cancer Model

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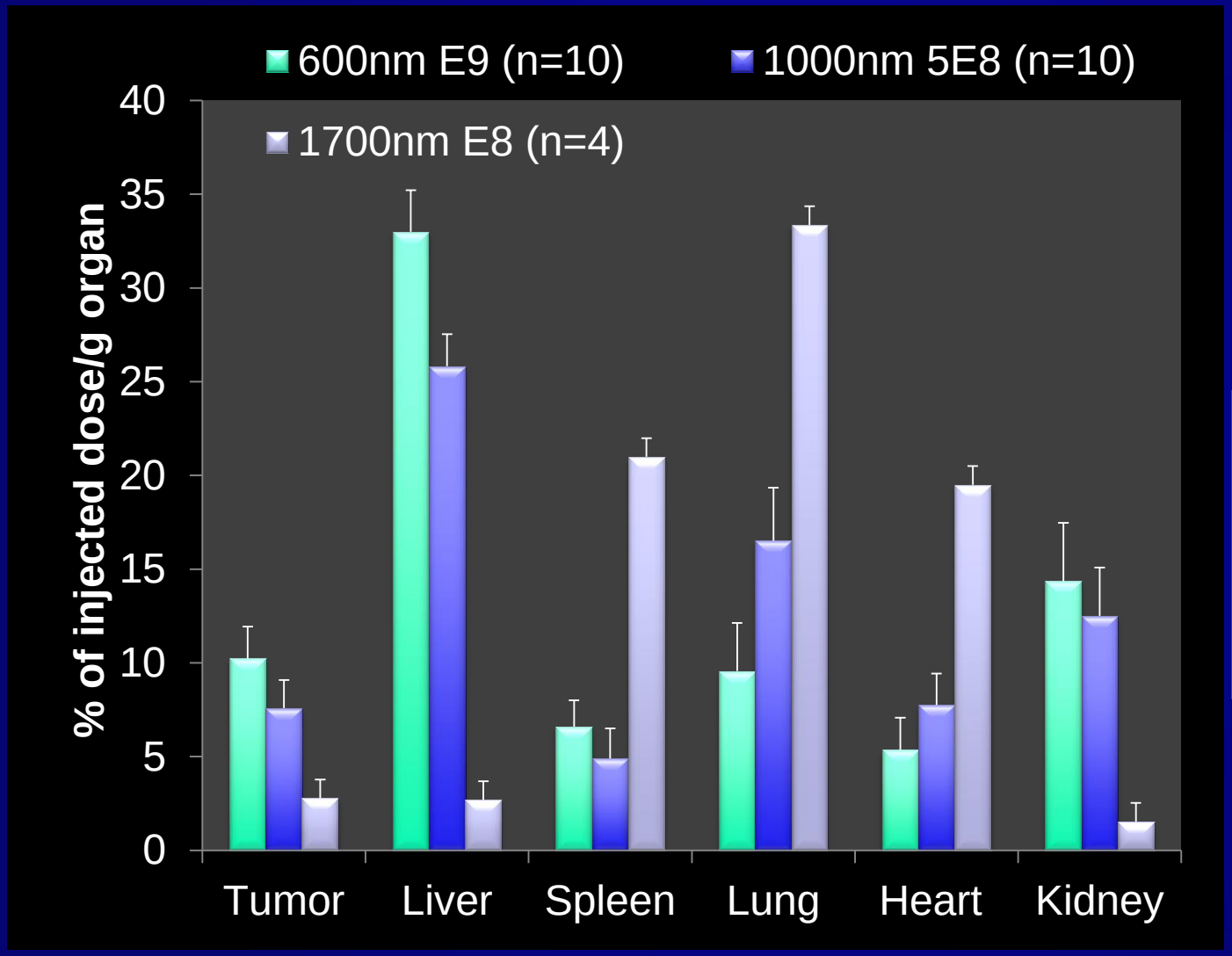

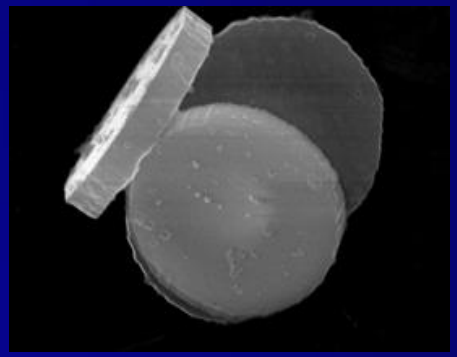

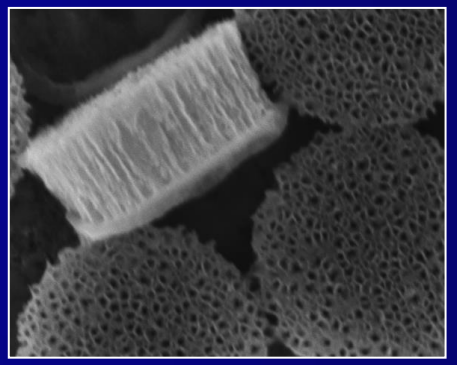

Orthotopic breast tumor in mice – ca 300 mm^3 – ICP analysis for Si

B. Godin,….Decuzzi, 2011

Biodistribution: Melanoma Model

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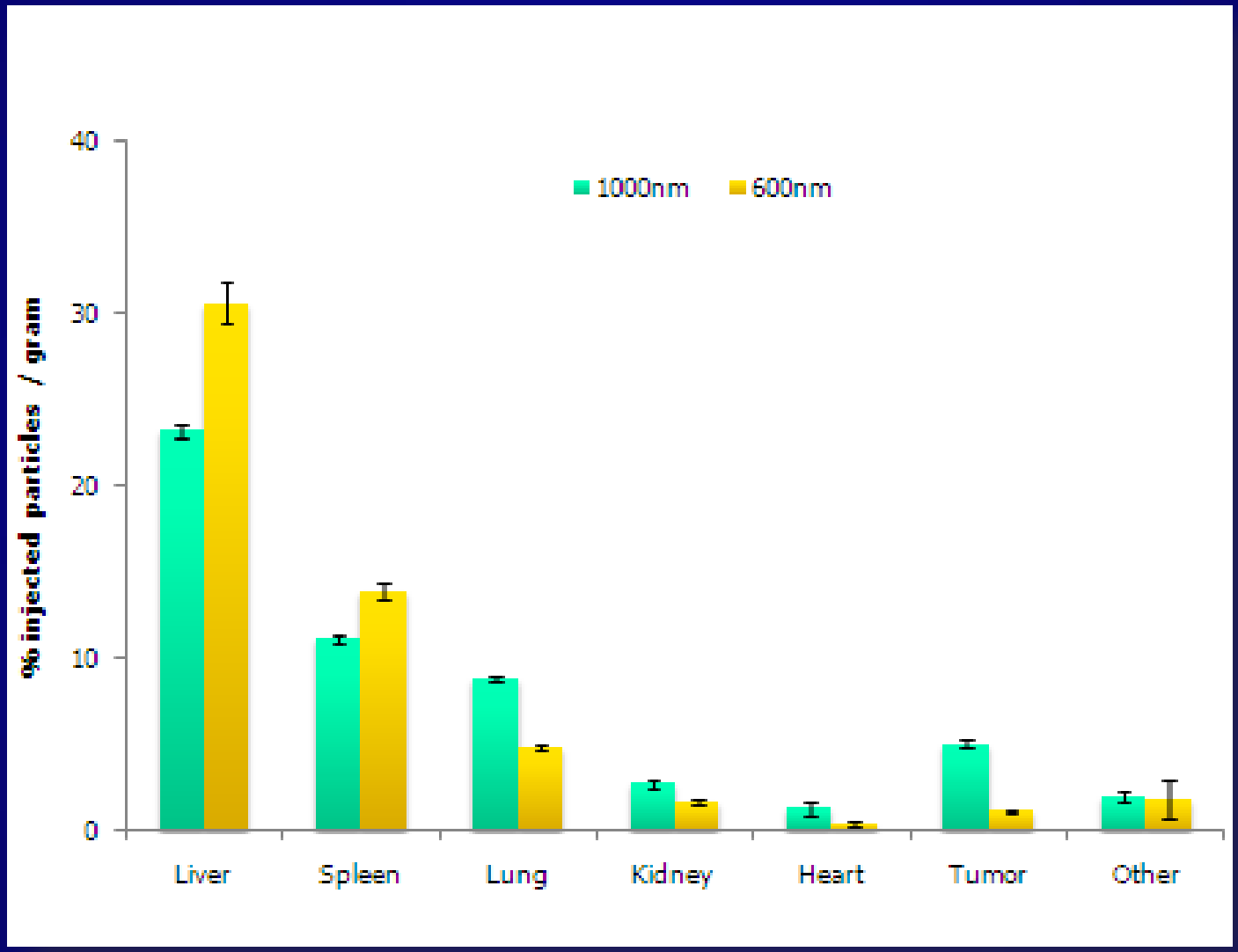
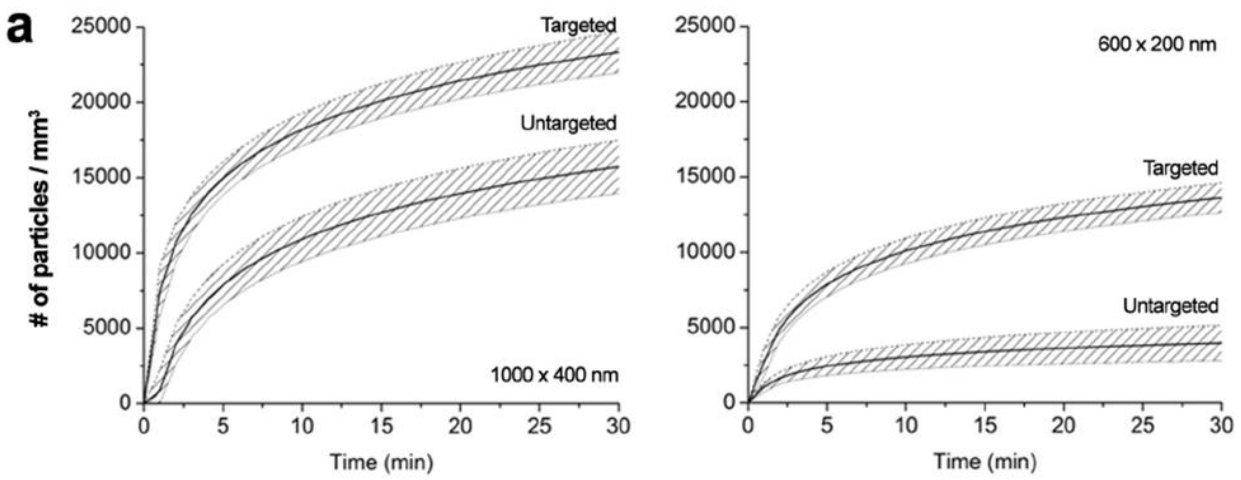


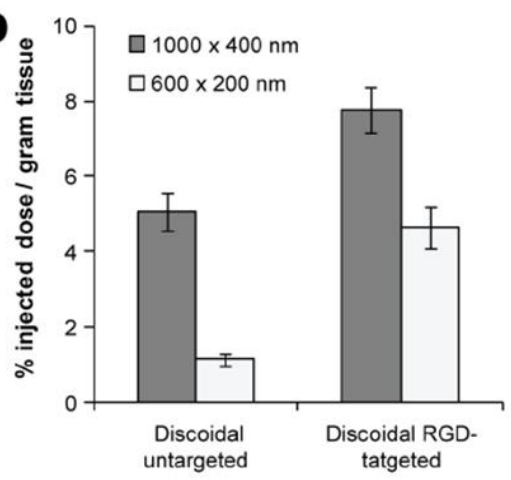
melanoma tumor in mice

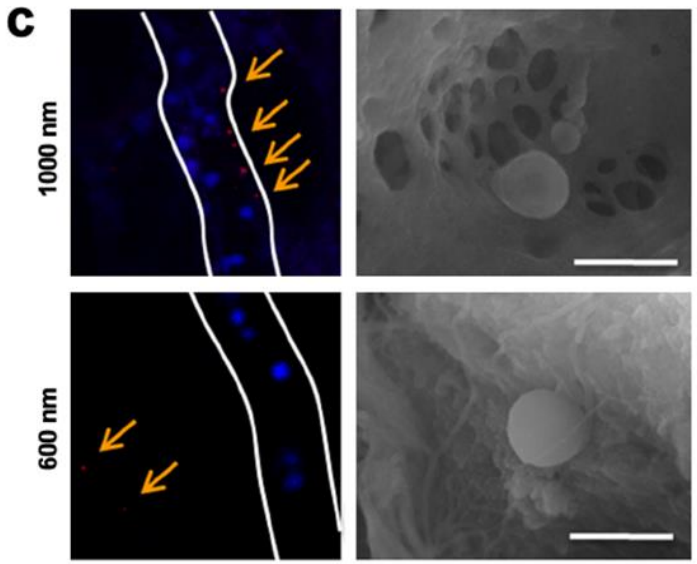
A. Van de Ven,….Decuzzi, 2011

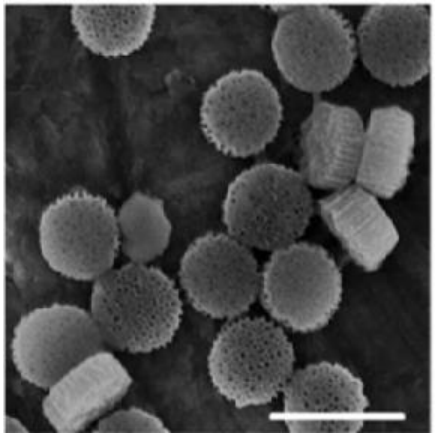
Biodistribution: Melanoma Model

Methodist The Methodist Hospital Research Institute



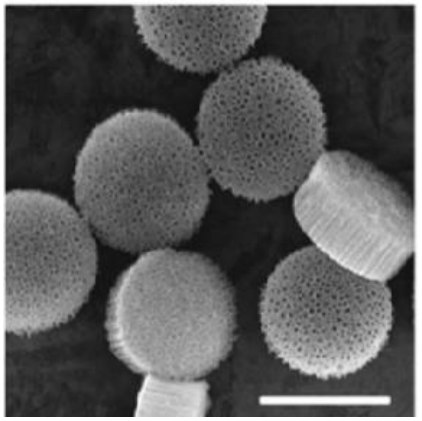






600 x 200 nm

1000 x 400 nm



A. Van de Ven,….Decuzzi, 2011

Multistage targeting to BM

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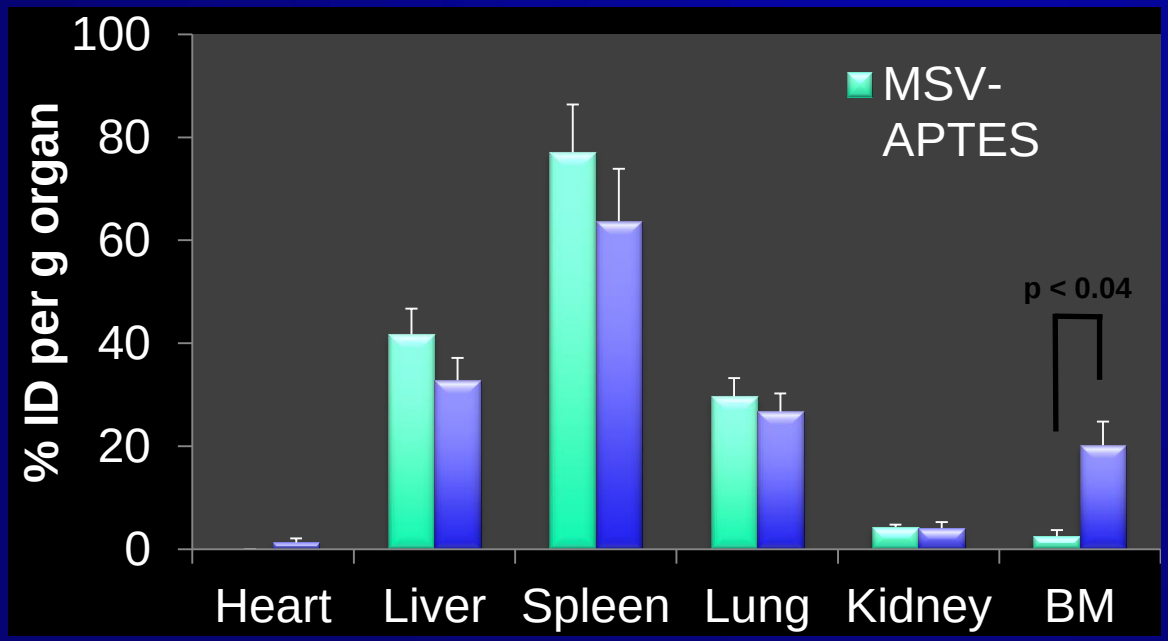
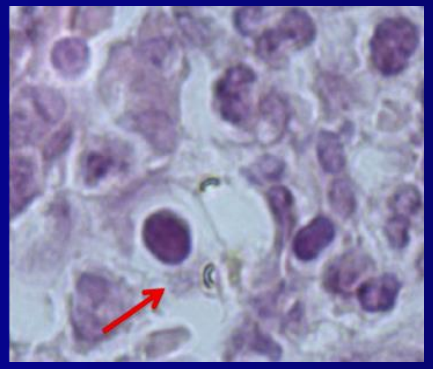
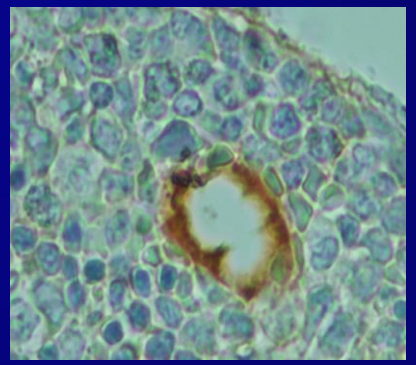
Bone marrow targeting of MSV achieved via E-selectin

- E-selectin overexpression confirmed on BM
- MSV conjugated to E-selectin aptamer (ESTA)
- 5 x 10^7 MSV (HEMI) i.v. injected in mice
- Major organs collected after 5 hours
- Silicon contents analyzed by ICP





Bone marrow histology



E-selectin expression in BM

Mann et al., Adv. Mater. 2011 (submitted)

MSV Delivery of Doxil

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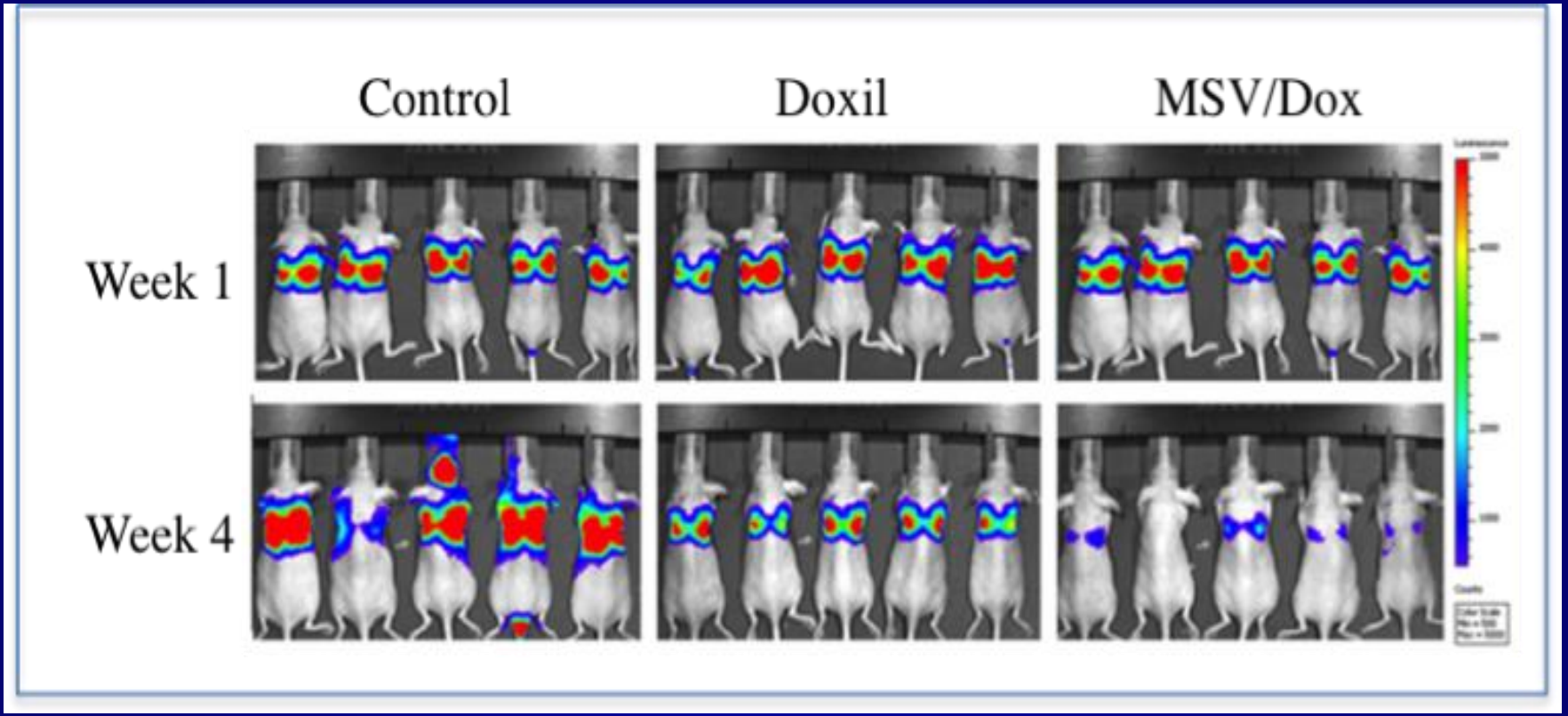

- Treatment of breast cancer lung metastasis with doxorubicin.
- The multistage vector-delivered polymeric doxorubicin (MSV/Dox) is more potent than the clinically available liposomal doxorubicin (DOXIL).

Haifa Shen, Ph.D., Department of Nanomedicine, The Methodist Hospital Research Institute
HShen@tmhs.org

MSV+ Neutral NanoLiposomal siRNA Rx

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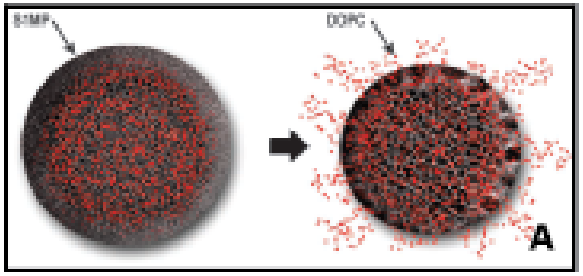
Cancer Research

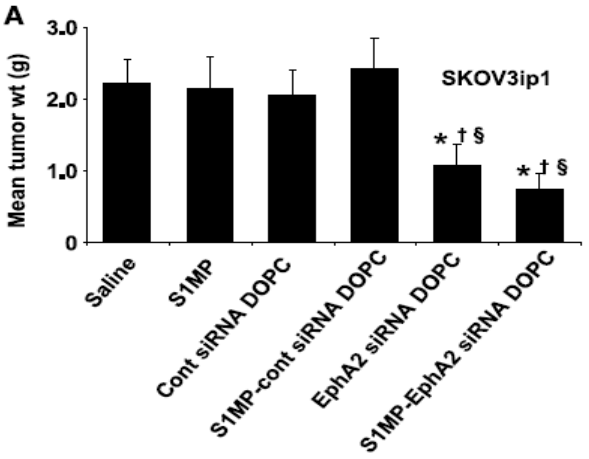
May 1, 2010 • Volume 70 • Number 9

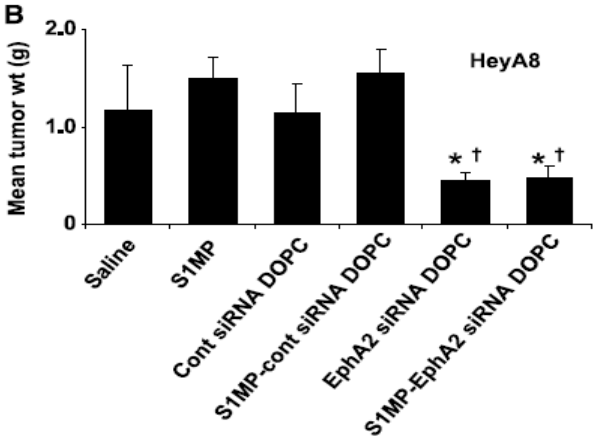
Sustained Small Interfering RNA Delivery by Mesoporous Silicon Particles

Takemi Tanaka[1,9], Lingegowda S. Mangala[2], Pablo E. Vivas-Mejia[11], René Nieves-Alicea[1], Aman P. Mann[1], Edna Mora[2,5,10,11], Hee-Dong Han[2], Mian M.K. Shahzad[2,8], Xuewu Liu[1,9], Rohan Bhavane[1], Jianhua Gu[1], Jean R. Fakhoury[1,9], Ciro Chiappini[9], Chunhua Lu[2], Koji Matsuo[2], Biana Godin[1], Rebecca L. Stone[2], Alpa M. Nick[2], Gabriel Lopez-Berestein[3,4,6,13], Anil K. Sood[2,3,6], and Mauro Ferrari[1,4,6,7,9]



Carrier	Administration	dosing	Gene silencing
L-siRNA	6	30ug	3-4 days
S1MP-L-siRNA	1	15 ug	20 days





* $P < 0.05$ compared to S1MP
§ $P < 0.05$ compared to saline
† $P < 0.05$ compared to S1MP-cont siRNA-DOPC

MSV-siRNA Rx + Small Molecule Drugs

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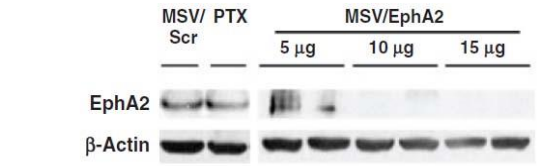
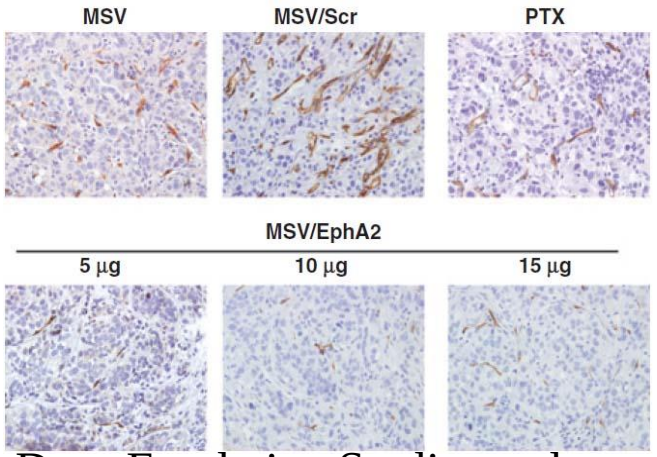
Cancer Therapy: Preclinical

Clinical Cancer Research

Enhancing Chemotherapy Response with Sustained EphA2 Silencing Using Multistage Vector Delivery

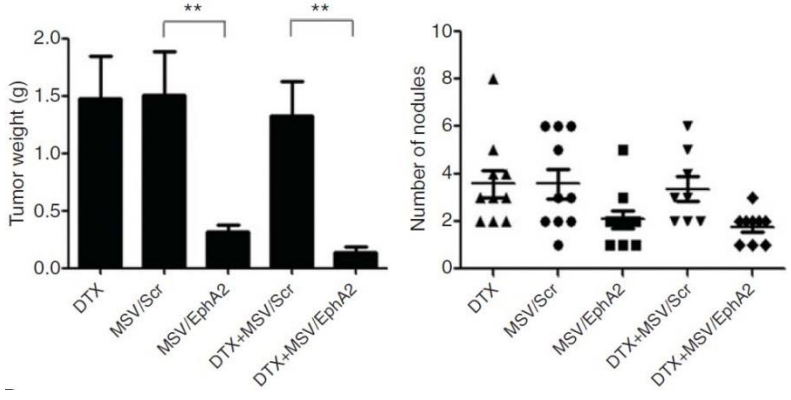
Haifa Shen[1,6], Cristian Rodriguez-Aguayo[2,8], Rong Xu[1], Vianey Gonzalez-Villasana[2], Junhua Mai[1], Yi Huang[1], Guodong Zhang[1], Xiaojing Guo[1], Litao Bai[1], Guoting Qin[1], Xiaoyong Deng[1], Qingpo Li[1], Donald R. Erm[1], Burcu Aslan[2], Xuewu Liu[1], Jason Sakamoto[1], Arturo Chavez-Reyes[8], Hee-Dong Han[3,4], Anil K. Sood[3,4,5], Mauro Ferrari[1,7], and Gabriel Lopez-Berestein[2,4,5,8]



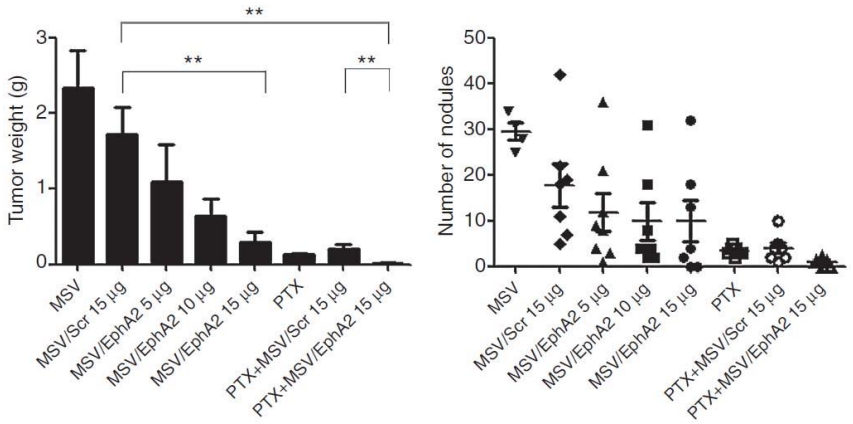


MSV-siRNA + Small Molecule Drugs

MSV-siRNA and Co-administration with Docetaxel



MSV-siRNA Dose Escalation Studies and Co-administration with Paclitaxel



Female athymic nude mice SKOV3ip2, HeyA8-MDR, cells were injected into the peritoneal cavity of nude, mice.2 weeks after tumor implantation.

Small Molecule Drug Delivery: Micellar Paclitaxel

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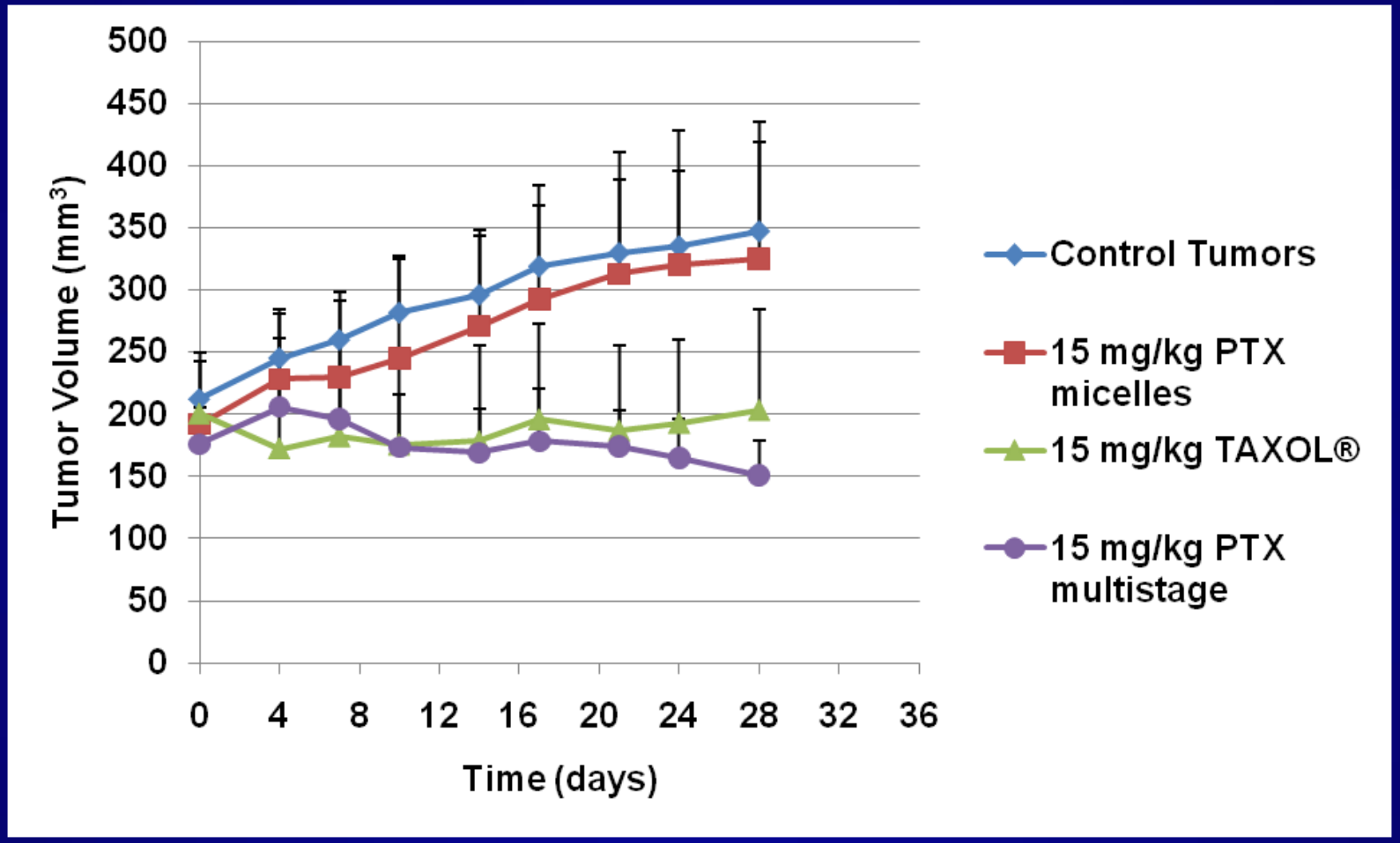

- One time intravenous injection of paclitaxel
- MDA-MB-468 breast tumors (triple negative) in mammary fat pad of nude mice

Blanco et al, (in preparation)

Fabrication of hemispherical porous silicon particles

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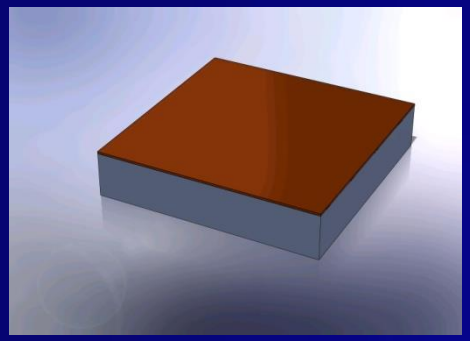
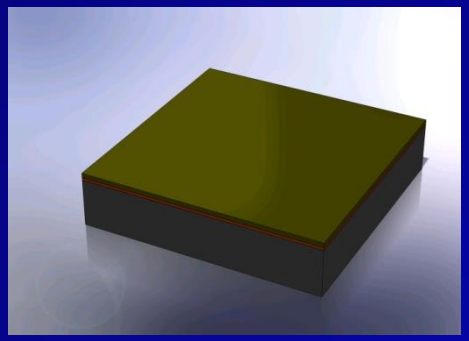
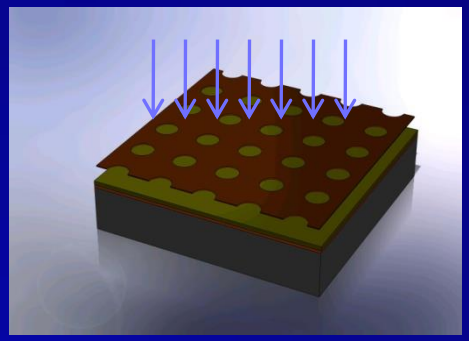


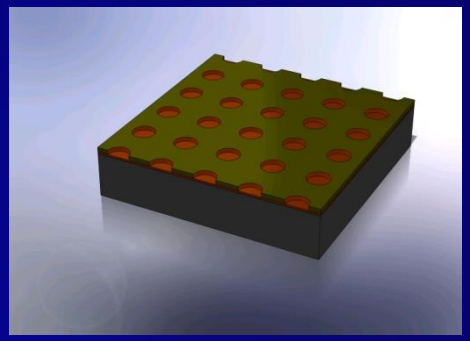
Si wafer+Si_3N_4 film



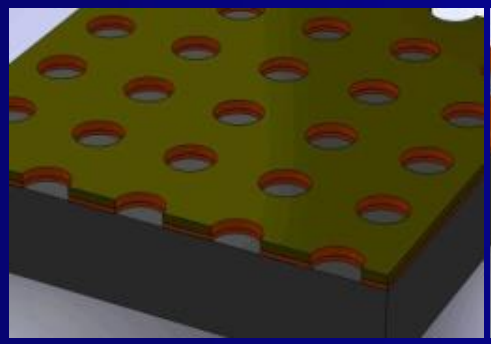
Photoresist-
photo sensitive polymer



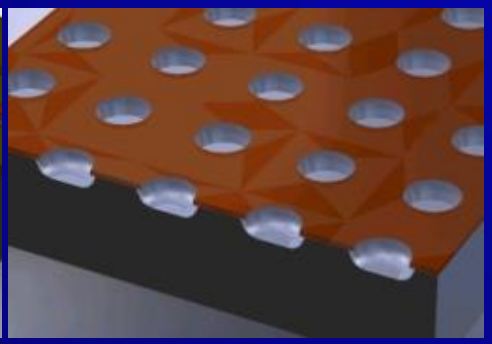
Patterning-
Mask+UV light



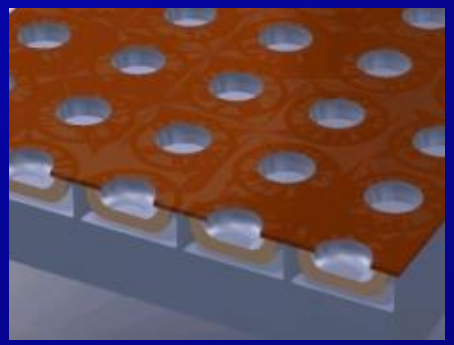
Film development



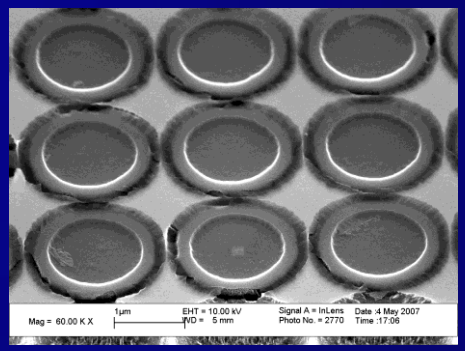
RIE transfer patterns to Si_3N_4 film



RIE of Si trench-
define shape



*Electrochemical etch to make porous silicon



Particles before release

Work performed by Dr. Xuewu Liu, Department of Nanomedicine, The Methodist Hospital Research Institute

Fabrication of porous silicon particles

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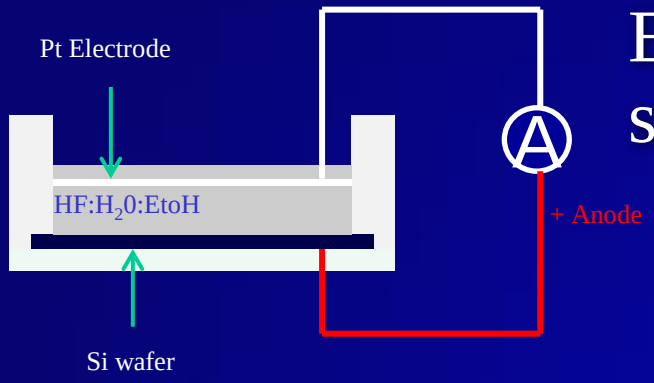
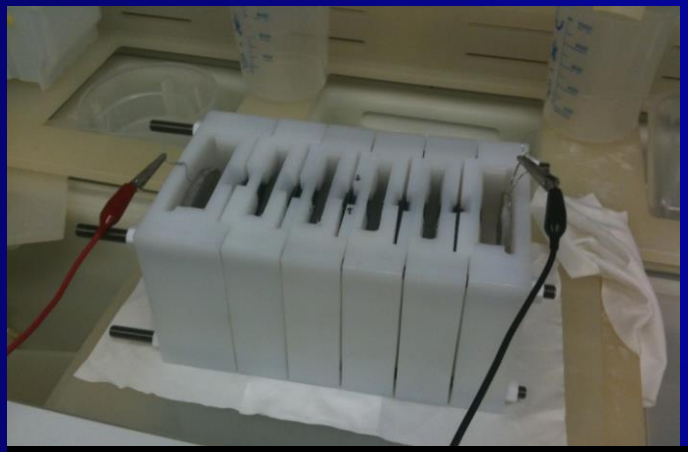
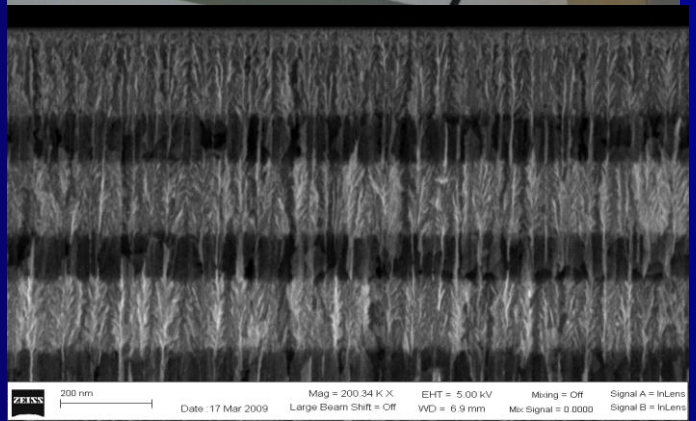


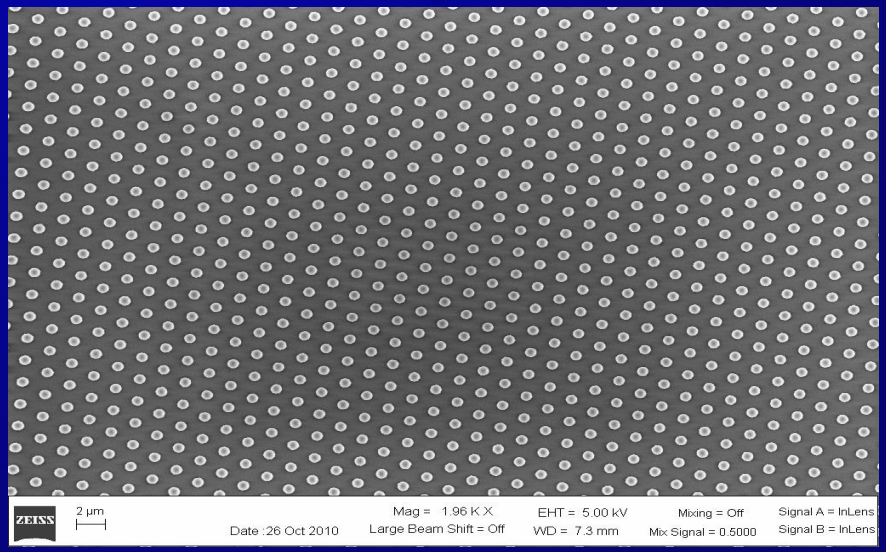
Electrochemical porosification of single crystalline silicon in HF solution

Particle fabrication: Combination of Microfabrication and Electrochemistry

- Porous structure by 2-step electrochemical etching.
- Photolithographic patterning for dimension and shape







Particle Fabrication

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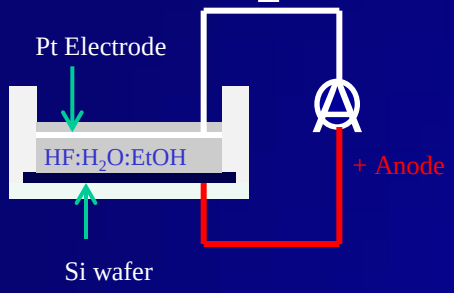
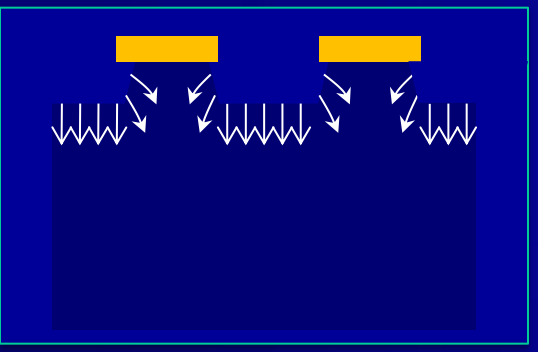
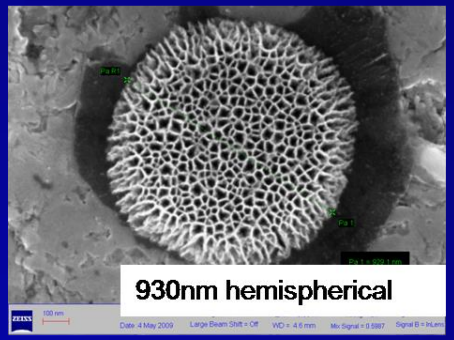
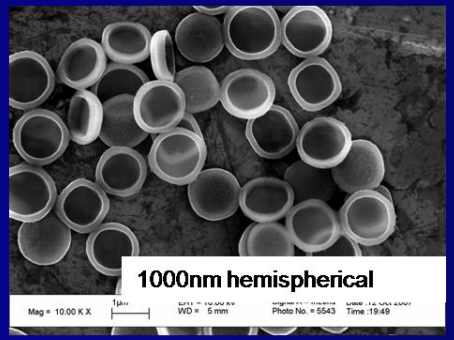
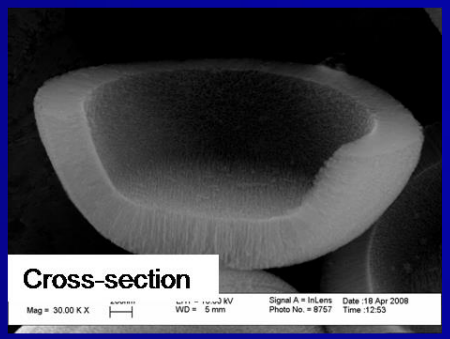
Hemispherical Particles:

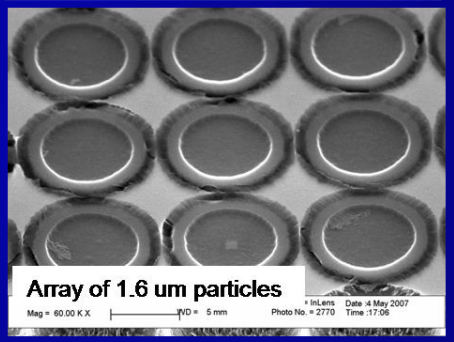


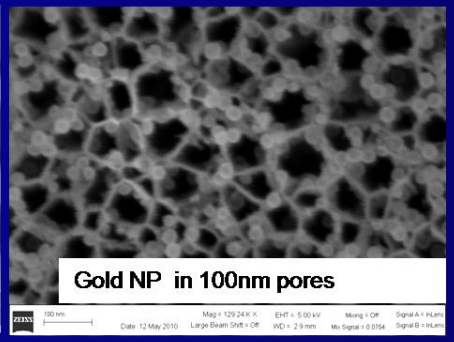












Combination of Microfabrication and Electrochemistry

- Photolithography: precise dimension and shape
- 2-step electrochemical etching: porous structure

Hemispherical particles:

- Shape controlled by pre-etched trenches
- Pore size determined by electrical current, HF concentration, surfactant
- Two domain pore size distribution
- Pore size 3nm-90nm
- Porosity 40%-65%
- **1.6E9 per 4" wafer**

Particle Fabrication

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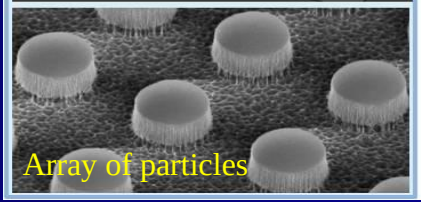
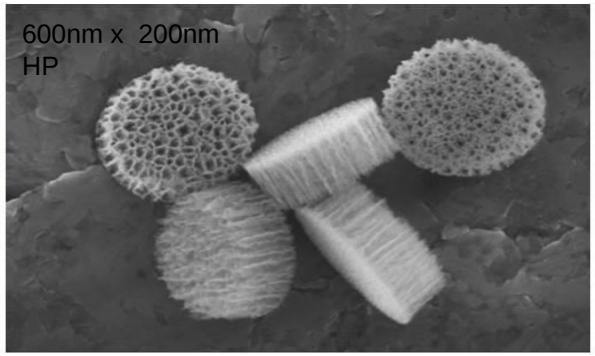
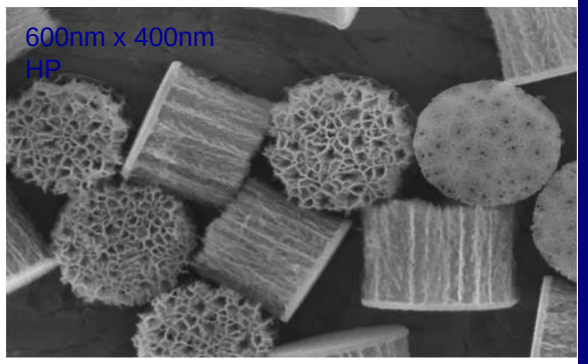
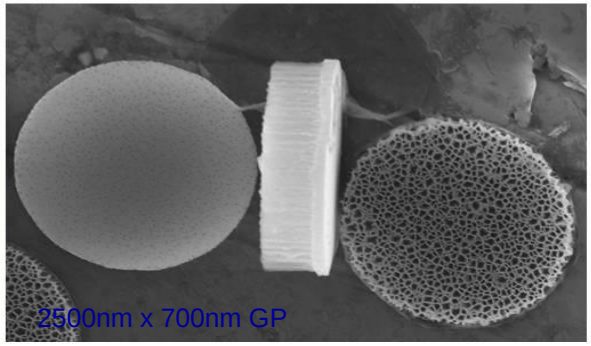
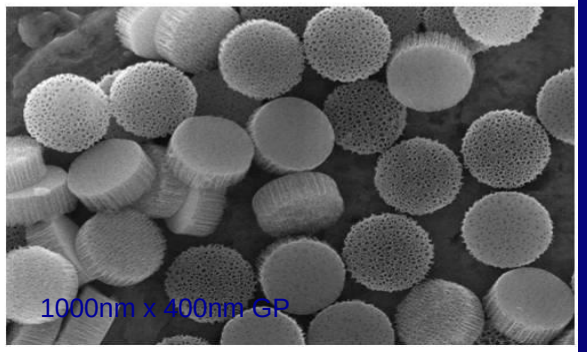
Discoidal Particles:











Combination of Microfabrication and Electrochemistry

- Photolithography: precise dimension and shape
- Deep reactive ion etching process and electrochemical etch

Discoidal particles:

- Particles as small as 400nm, as thin as 100nm
- One domain pore size distribution
- Pore size up to 150nm
- Easy to control the thickness and pore structure
- Allow to scale up the production: multilayer and nanosphere lithography
- **Projected: 1.3E11 particles (80x increase from 4" wafers)**

Scaling Up…(trying to)

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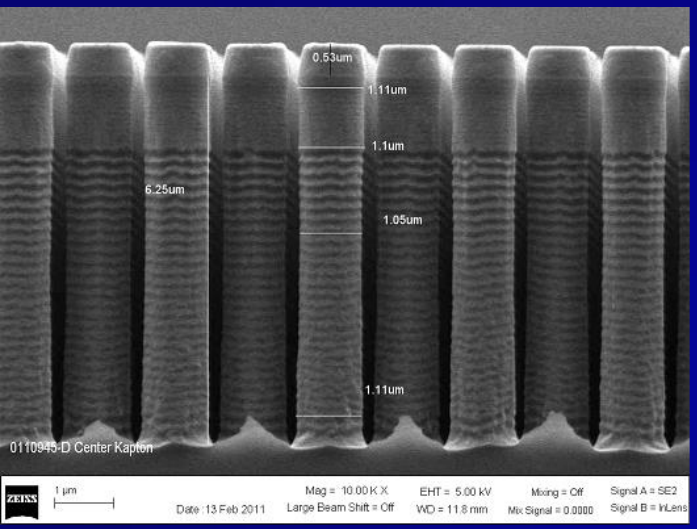
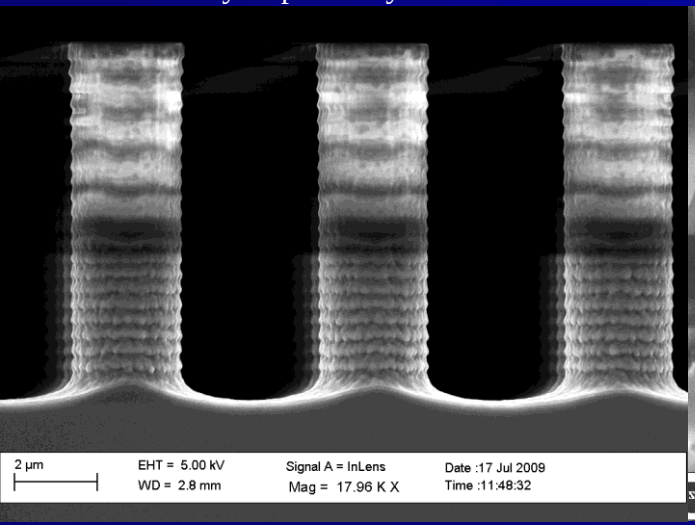
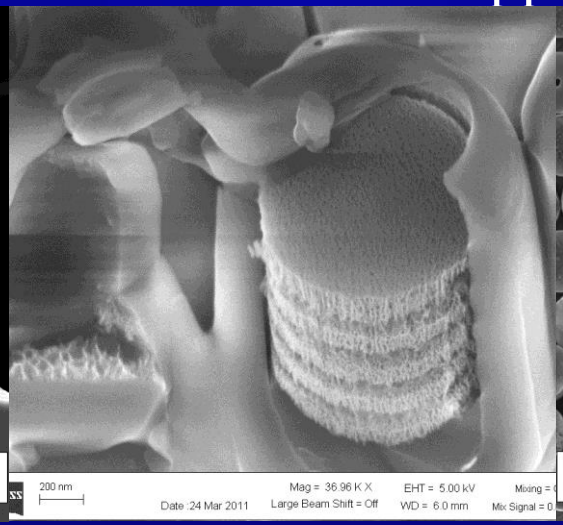


Form array of pillars by DSE

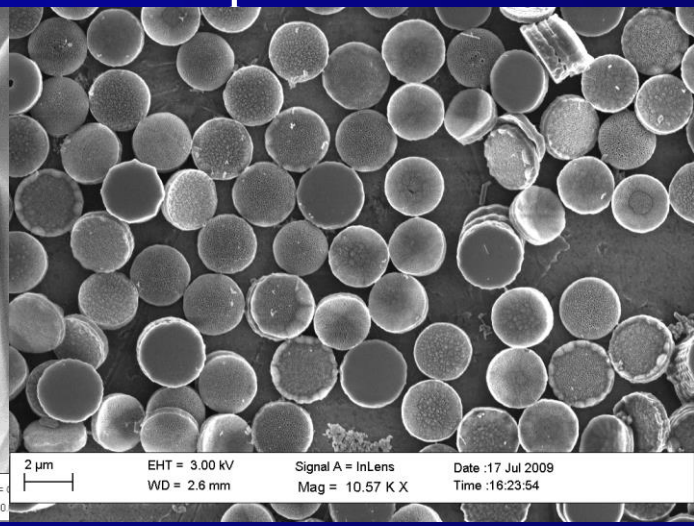
- One step photolithography process
- Deep RIE silicon etch to form pillars
- Multilayer Electrochemical etch
- Scale up production: ~20-80 times yield!
- Human dose approx 1E11 particles 4-5 wafers
- Mouse dose approx 4E9 particles



Si_3N_4 film + selective etch, then porosification by programmed four-step electrochemical etch



Closed view of multilayer particles



Released particles

Funding Acknowledgments

- DoD – Breast Cancer RP Innovator Award
- NCI – BRP R01, R33, PS-OC U54, CCNE U54
- FDA – Regulatory Science of Nanomedicine
- DoD/TATRC – NanoVector R&D Center
- State of Texas Emerging Technology Fund

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Thanks!

JHSakamoto@TMHS.ORG

713-441-7350