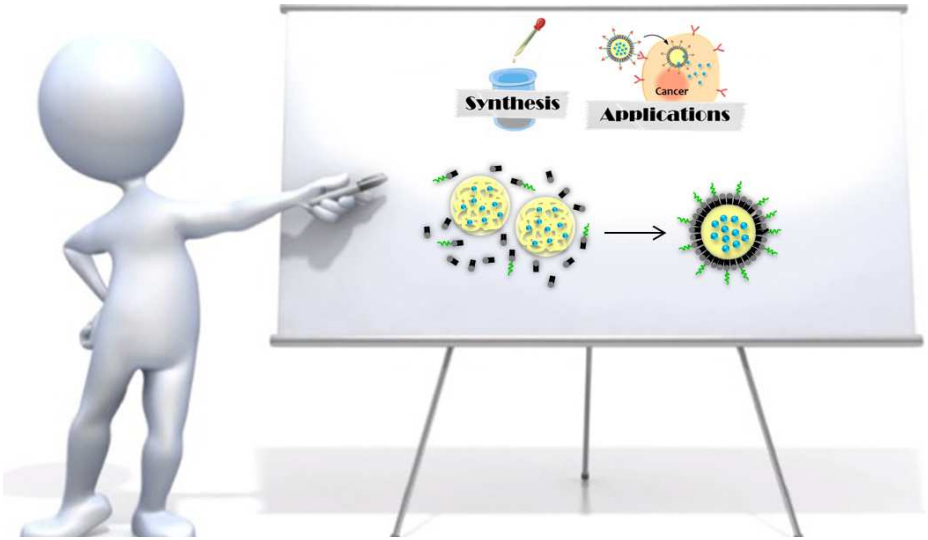
Nanotechnology for Drug and Vaccine Delivery

Presented by

Nuttawee Niamsiri, PhD

Department of Biotechnology, Faculty of Science, Mahidol University

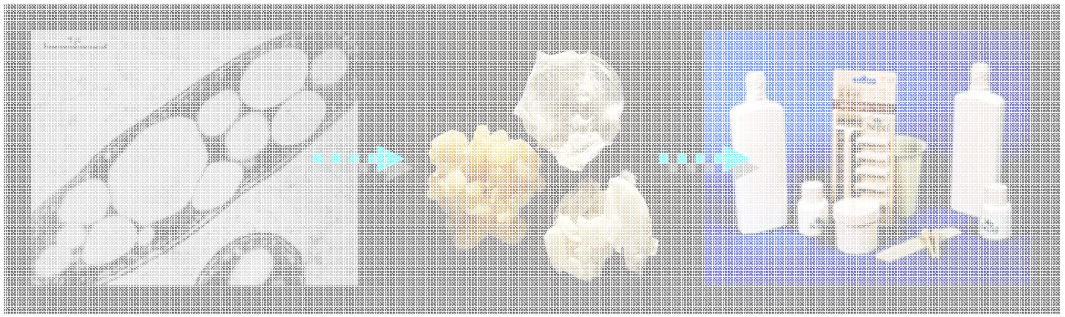


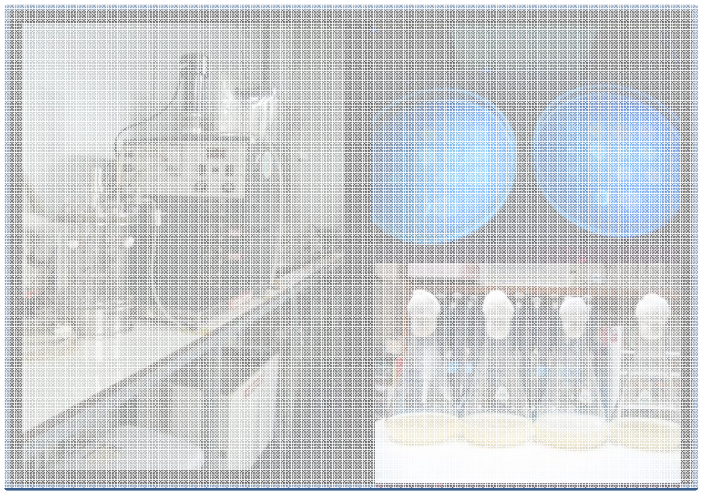
NAC2017
13th NSTDA Annual Conference
การประชุมวิชาการประจำปี สวทช. ครั้งที่ ๑๓

31st March 2017

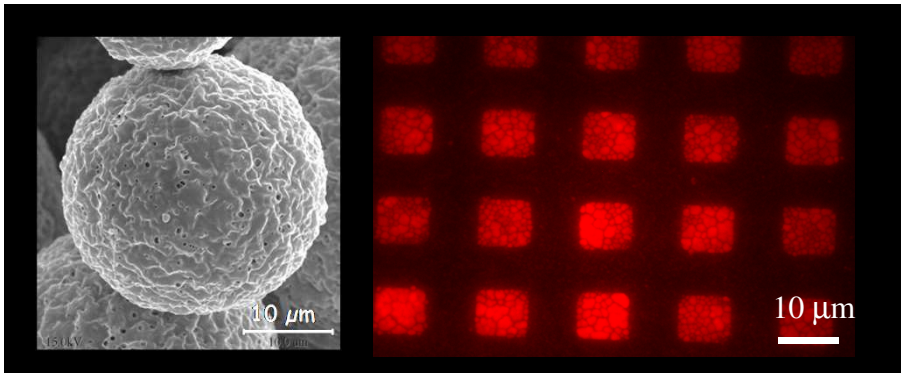
My Current Research Interests

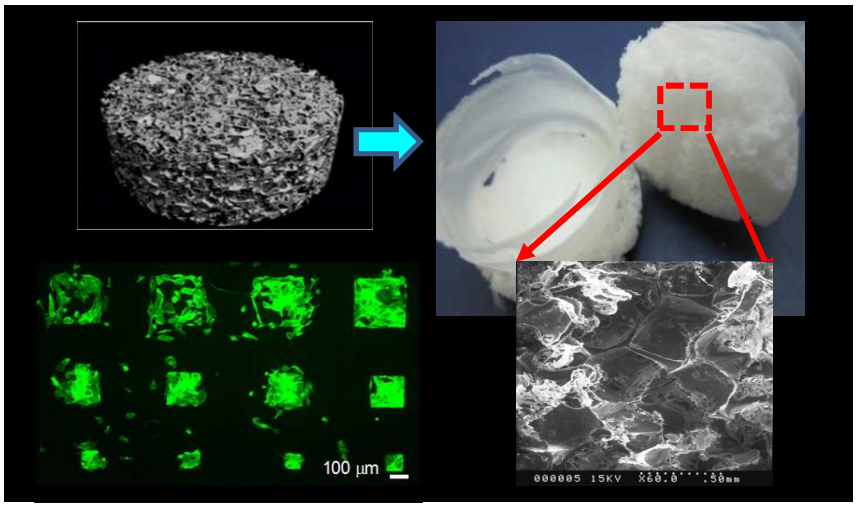
1) **Optimize the production of Polyhydroxyalknanoates (PHAs) in the bacterial system**
   - Screening novel PHA producers
   - Improve PHAs yield via fermentation strategies





2) **Develop novel applications or products based on PHAs**
   - Drug delivery & tissue engineering
   - Nanobiotechnology





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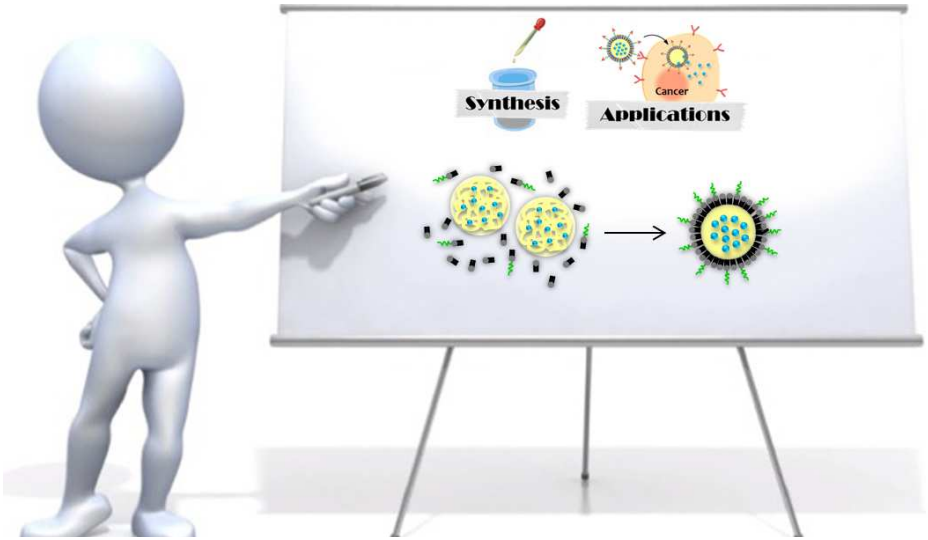

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...Outline of My Talk...

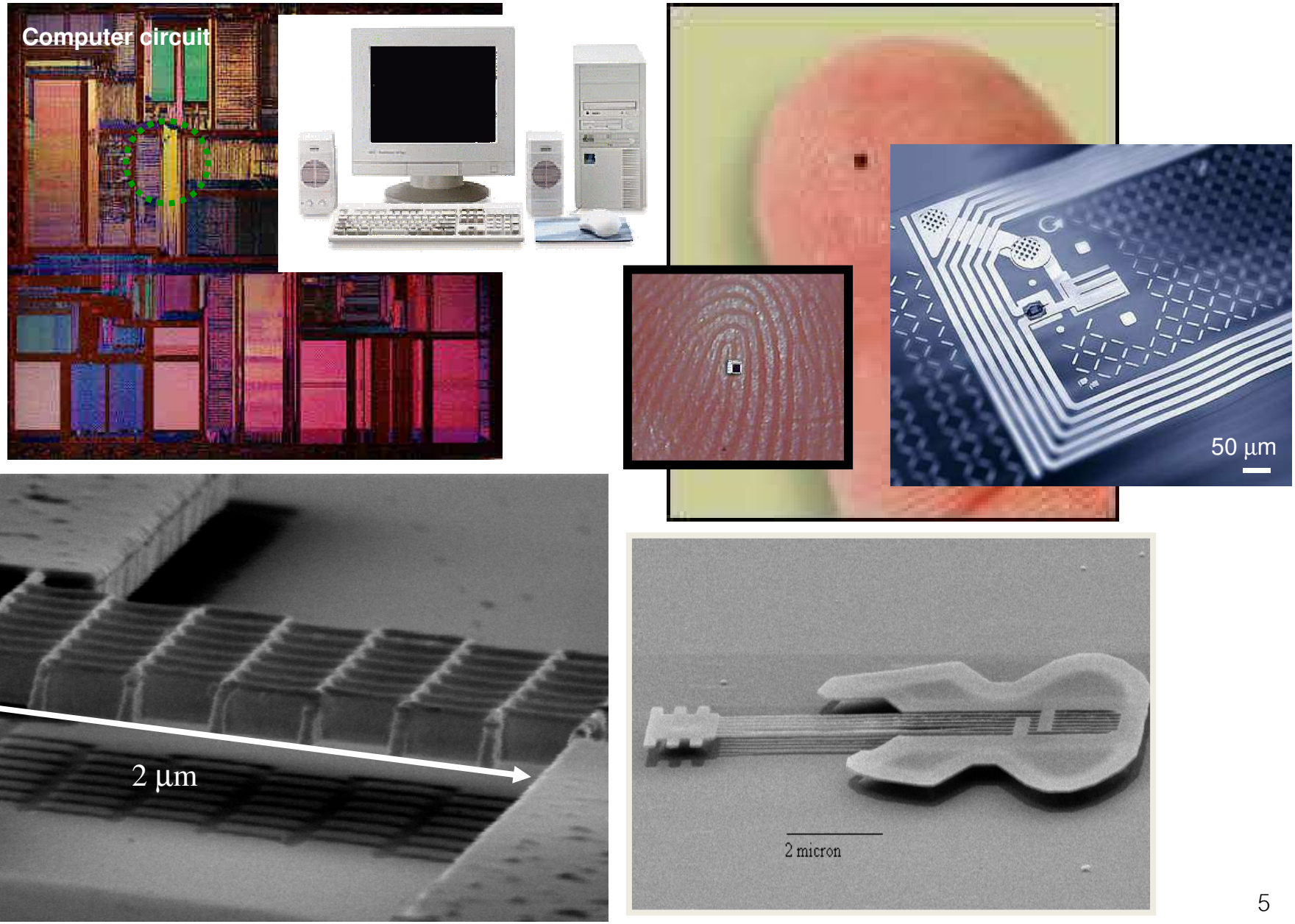
Nanotechnology

+

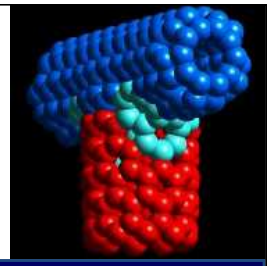
Drug delivery

Vaccine delivery

What is "Nanotechnology" ?



What is Nanotechnology ?

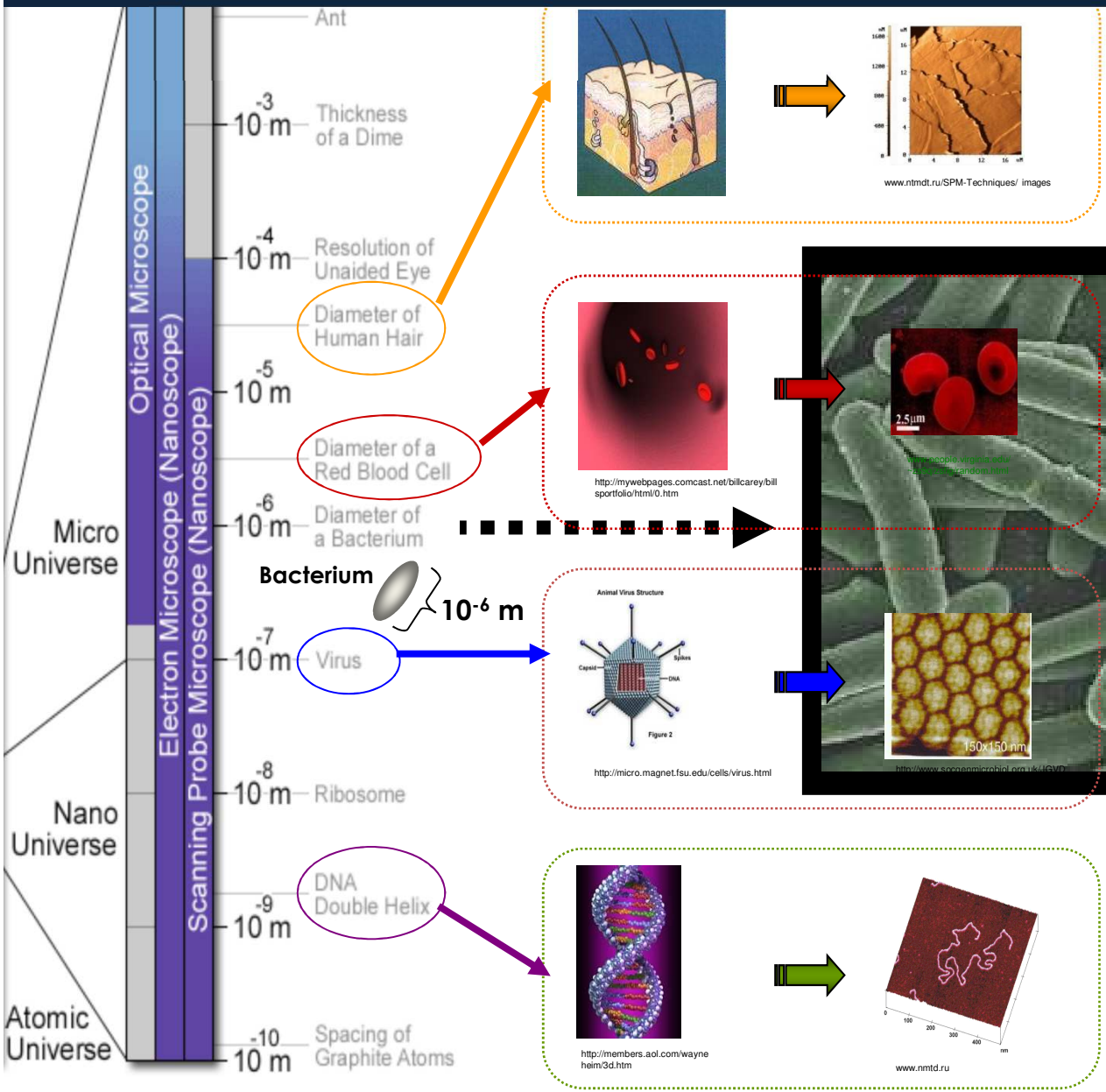


"นาโนเทคโนโลยี" หมายถึง เทคโนโลยีที่เกี่ยวข้องกับกระบวนการ สร้าง การสังเคราะห์ วัสดุ อุปกรณ์ เครื่องจักรหรือผลิตภัณฑ์ซึ่งมีขนาดเล็กมากในระดับนาโนเมตร เทียบเท่ากับระดับอนุภาคของโมเลกุลหรืออะตอม รวมถึงการออกแบบหรือการใช้เครื่องมือสร้างวัสดุที่อยู่ในระดับที่เล็กมาก หรือการเรียงอะตอมและโมเลกุลในตำแหน่งที่ต้องการ ได้อย่างแม่นยำ และถูกต้อง ทำให้โครงสร้างของวัสดุหรือสสารมีคุณสมบัติพิเศษ ไม่ว่าทางด้านฟิสิกส์ เคมี หรือชีวภาพ ส่งให้มีผลประโยชน์ต่อผู้ใช้สอย จาก http://www.thai-nano.com/whatnano.php

Nanotechnology

The manipulation, synthesis, fabrication, measurement, modeling or manufacture of **sub-1000 nanometer** scale matter.

How Small is Micro- or Nano-?



A strand of hair seems pretty small to us but is *HUGE* in nanoscale terms, less than 1/10 of a millimeter:
~100 µm = 1000,000 nm

Even a red blood cell that is impossible to see by eye is around **~5 µm** – That is **5000 nm**

Small viruses can be considered to be on the nanoscale – Adenoviruses are **~80 nm** is size (can cause cold)

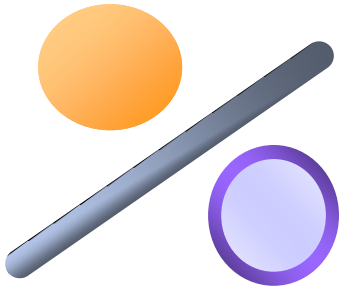
Double stranded DNA is **~2nm** in diameter which is 20Å – A gold atom is about 1.5Å in diameter

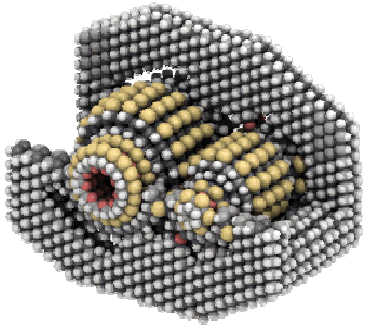
Two Main Concepts of Nanotechnology

I.

Nanotechnology can involve **behavior of small structures** (i.e. nanoscience). The general definition say this would be things from **1 to 100 nm in size**, but this is sometimes extended to larger systems (**up to 1000 nm**).

Nanosized structures can be used to create stronger materials, faster computers, and better medicine etc.



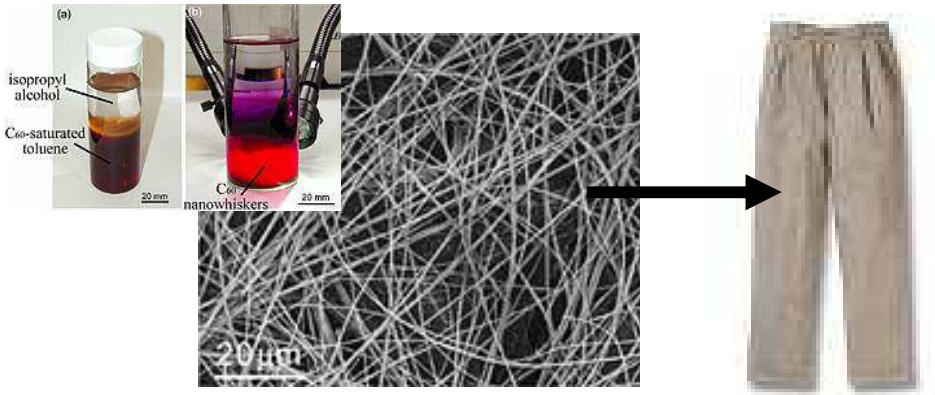


Nanotubes, nanoparticles etc.

II.

Nanotechnology can involve **Molecular manufacturing** that is an attempt to build mechanical/chemical manufacturing systems in a controlled manner to a functioning device or nano-structure.

Where researchers develop and apply materials at this scale to develop new products or methods; i.e., turning nanostructures into useable tools and applications.



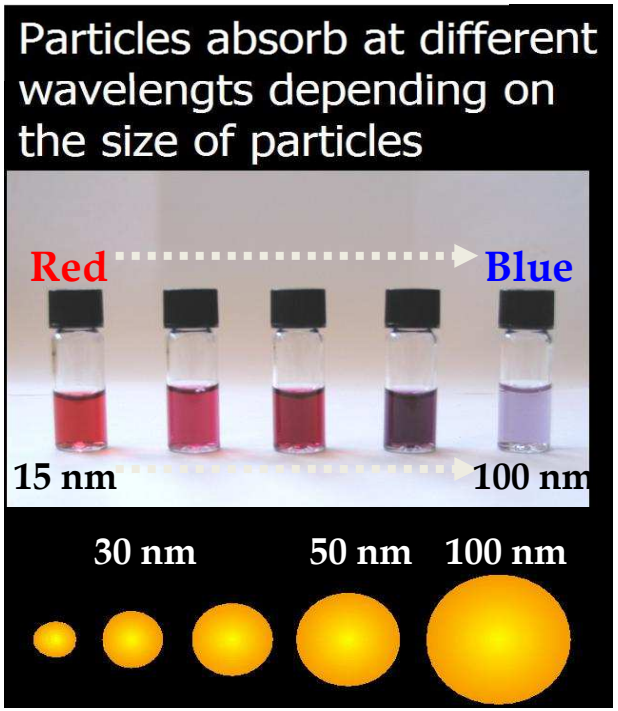
Nanotechnology can be described as the engineering of functional systems at the molecular scale

http://nanoengineer-1.com

Nanotechnology is <u>not</u> New!

- Gold nanoparticles are reflected as red.

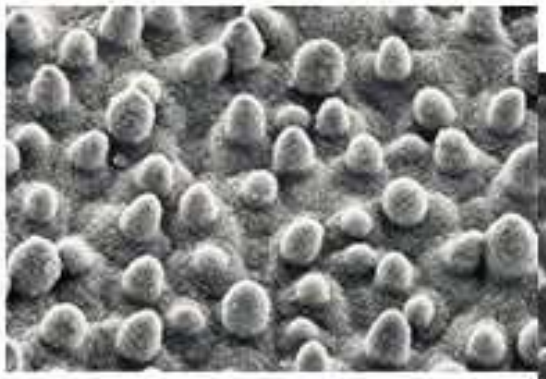
- <u>Ancient</u> stained-glass makers knew if they put finely ground gold or silver in the glass they would get beautiful colors.
- They used **nano-sized gold** and **silver particles**. *Optical properties change with the nanoscale*.

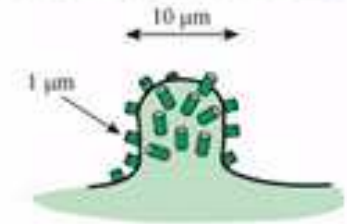


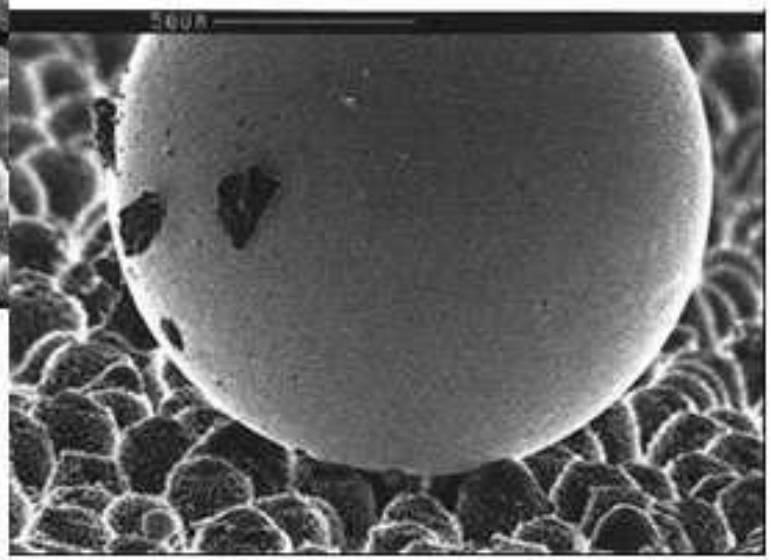
Example# 1: Self-Cleaning Surfaces (Lotus Effect)

- ผิวหน้าของใบบัว ประกอบด้วย (1) สาร Wax ที่กันน้ำ และ (2) โครงสร้างขนาดเล็ก คล้ายหนามจำนวนมหาศาลและมีการกระจายตัวอย่างเป็นระเบียบ โดยหนามแต่ละอันมีความเล็กขนาดนาโนเมตร ส่งผลให้ผิวด้านหน้าของใบบัวมีลักษณะขรุขระ

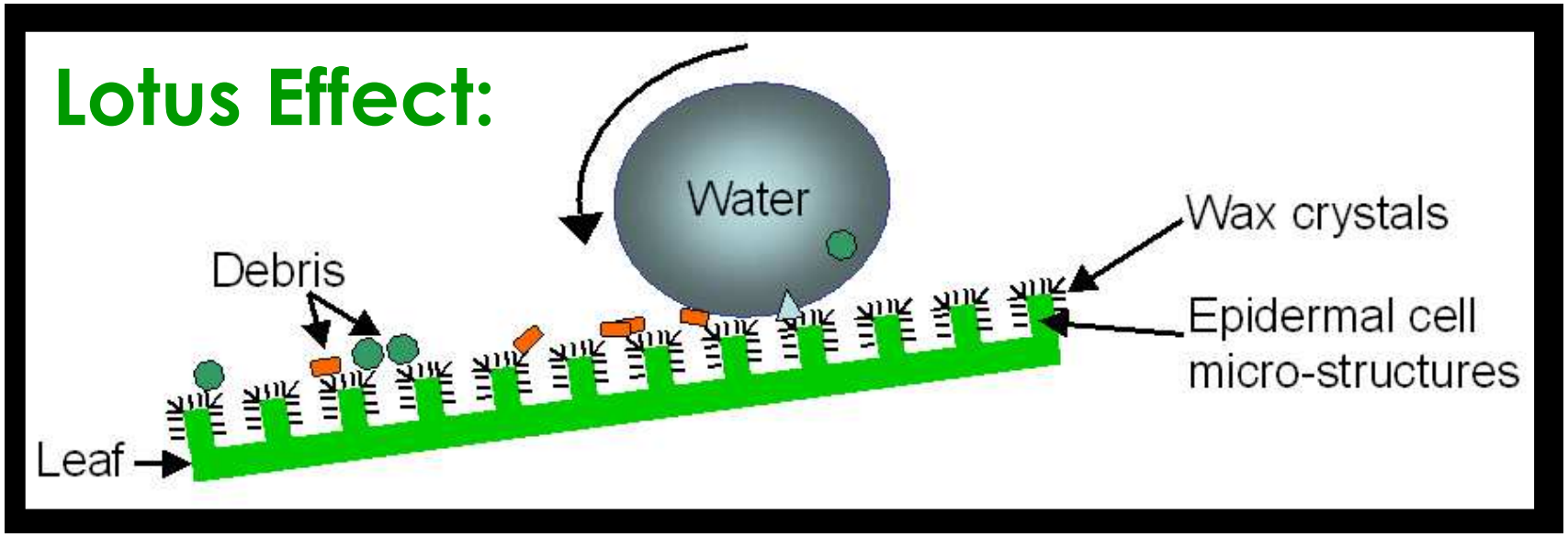
- Hydrophobicity และหนามเหล่านี้ส่งผล ทำให้เมื่อหยดน้ำตกลงมากระทบใบบัว ทำให้พื้นที่ผิวสัมผัสระหว่างน้ำกับใบบัวนั้นน้อยมาก



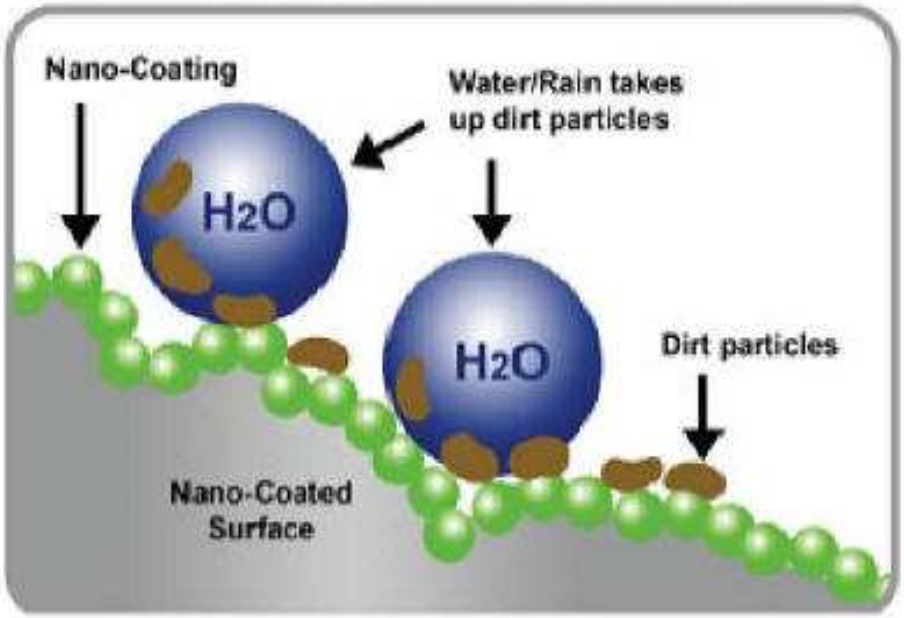


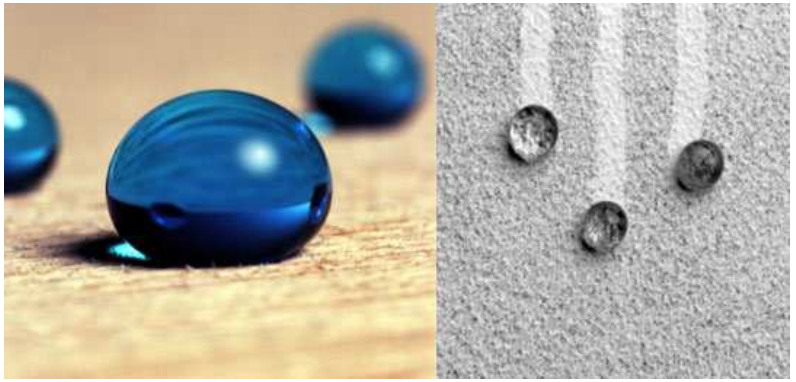


Scanning Electron Microscope (SEM)



… Biomimetics: Borrowing from Biology …

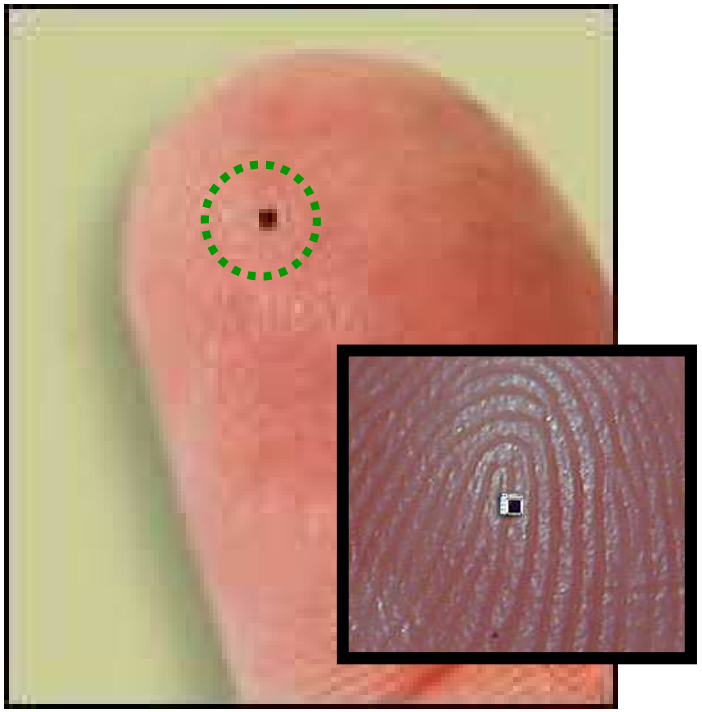




การสร้างเสื้อผ้าที่กันน้ำได้

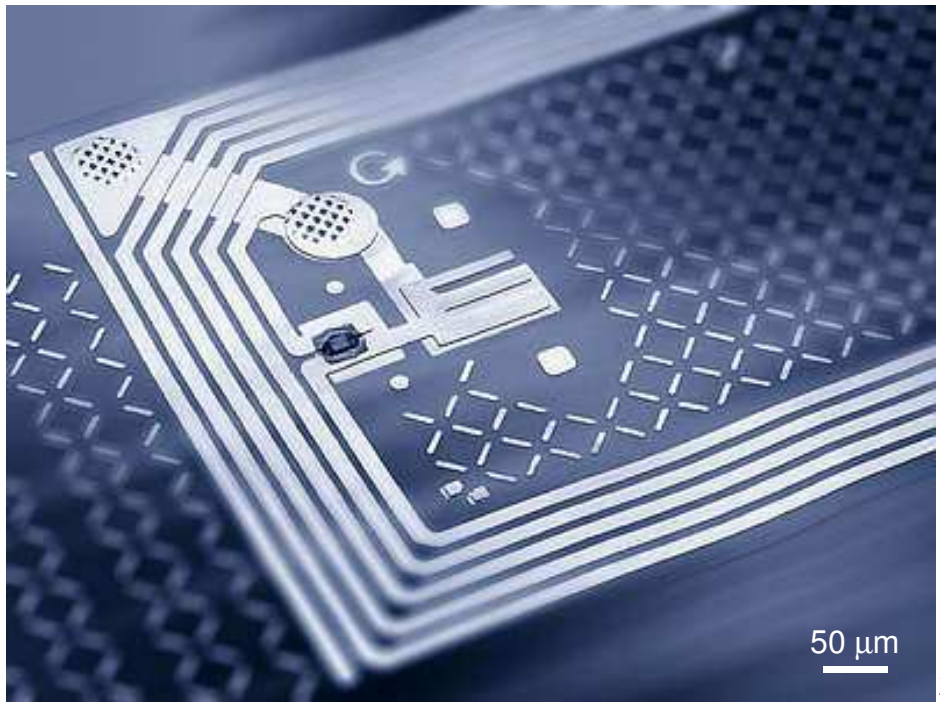
การผลิตสีที่มีคุณสมบัติในการทำความสะอาดตัวเองได้

Example#2: Computing and Data Storage



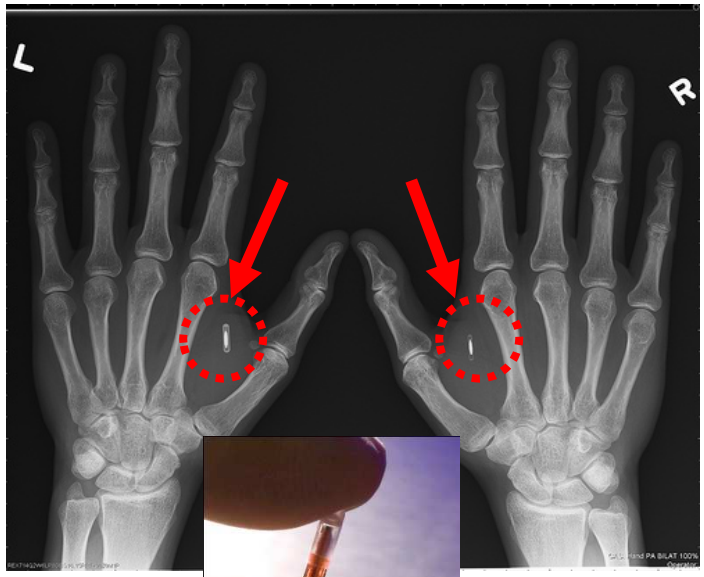
Smart Code Debuts Smallest Chip

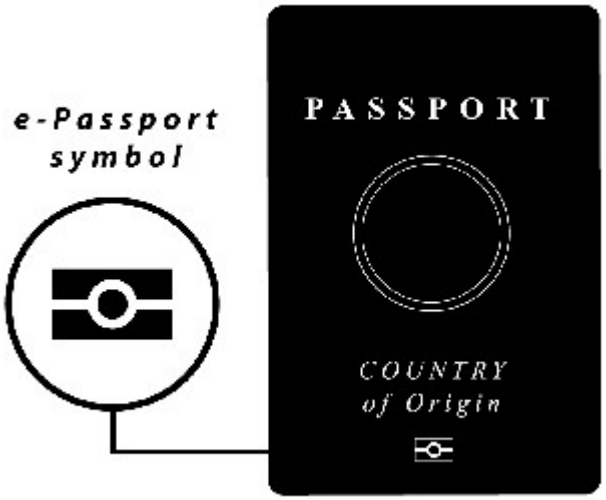
- The world's smallest and thinnest **Radio Frequency Identification chips** (RFID) tags were introduced this year by **Hitachi**.
- Tiny miracles of miniaturization, these RFID chips measure just 0.05 x 0.05 millimeters.
- RFID tags is used similar to bar code.



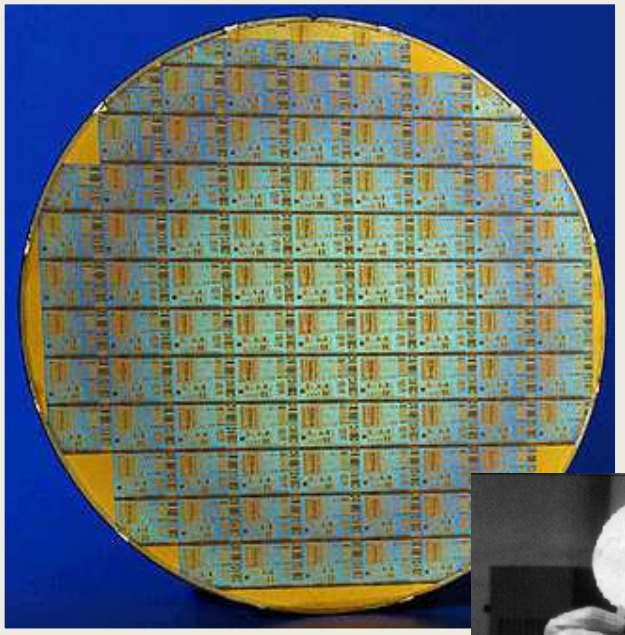
RFID ย่อมาจากคำว่า Radio Frequency Identification คือ ระบบเก็บข้อมูลทางอิเล็กทรอนิกส์ ที่เพิ่มความสามารถในการคำนวณและการรักษาความปลอดภัยของข้อมูล และส่งกำลังโดยคลื่นแม่เหล็กหรือคลื่นแม่เหล็กไฟฟ้าแทนการสัมผัสทางกายภาพ เป็นการเอาคลื่นวิทยุมาเป็นคลื่นพาหะ เพื่อใช้ในการสื่อสารข้อมูล

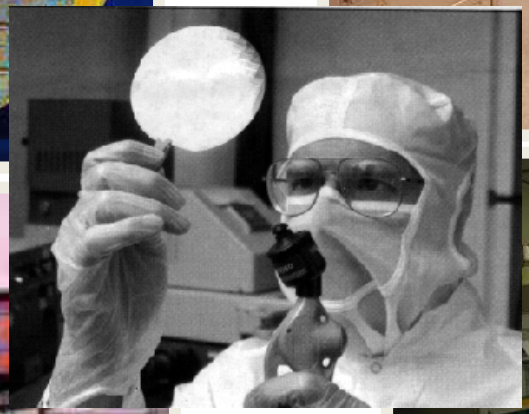
Applications of RFID Tags



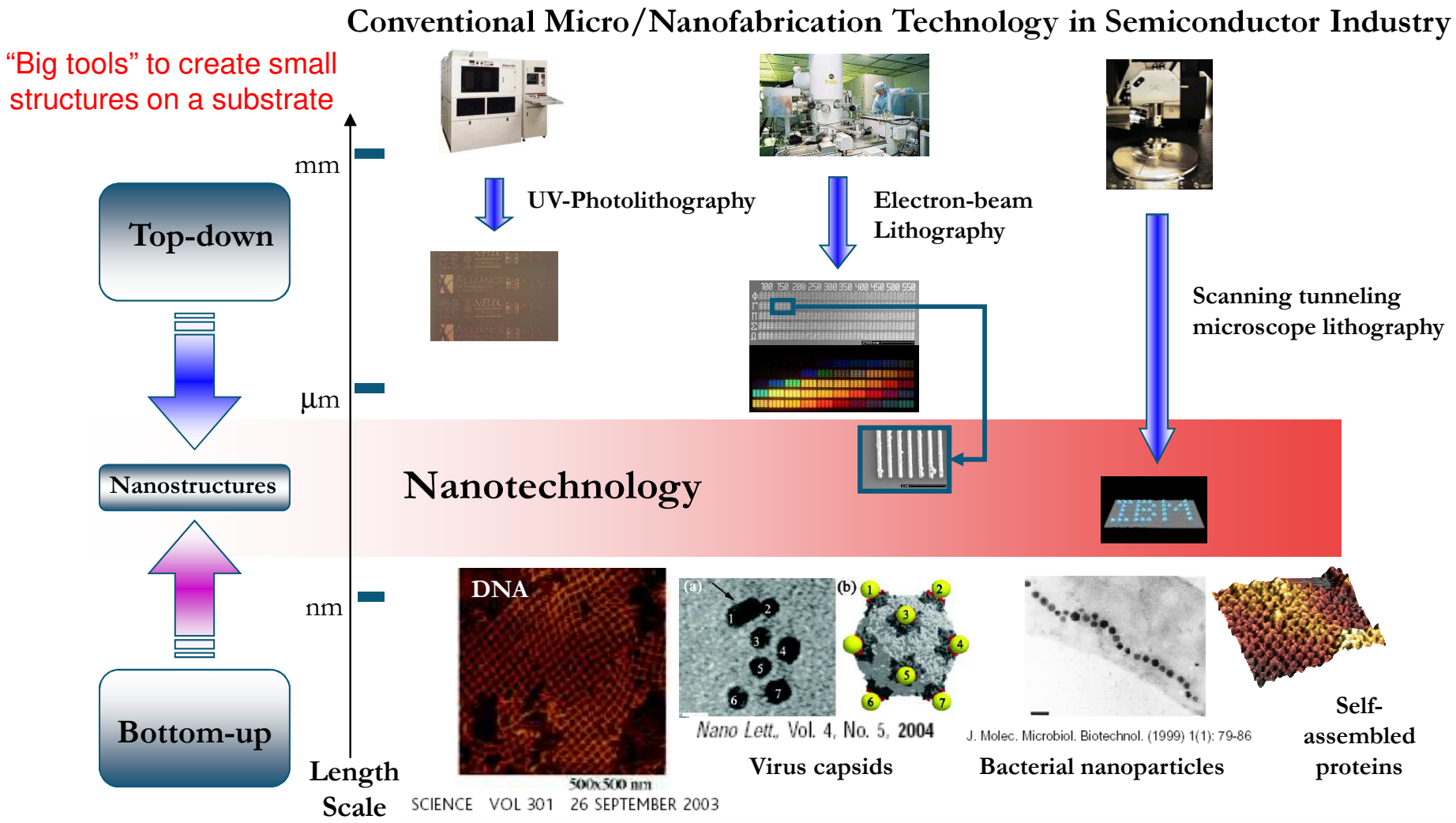


To create small-scale structures.. we ask "engineers" for a little help





Nanofabrication Approaches



Creating small structures using "biological systems"

Bionanofabrication – a process that takes advantage of structural specificity and/or catalytic efficiency of the biological systems to create various types of micro/nanostructures

Nanotechnology has been thought of as the new revolution in numerous fields including:

- Electronic and computing
- Semiconductor industry
- Materials science and manufacturing
- Biology and biotechnology
- Medicine and health
- Agriculture and veterinary
- Environmental and energy
- Food Science
- Textiles and cosmetics
- **Paint**, *et cetera*.

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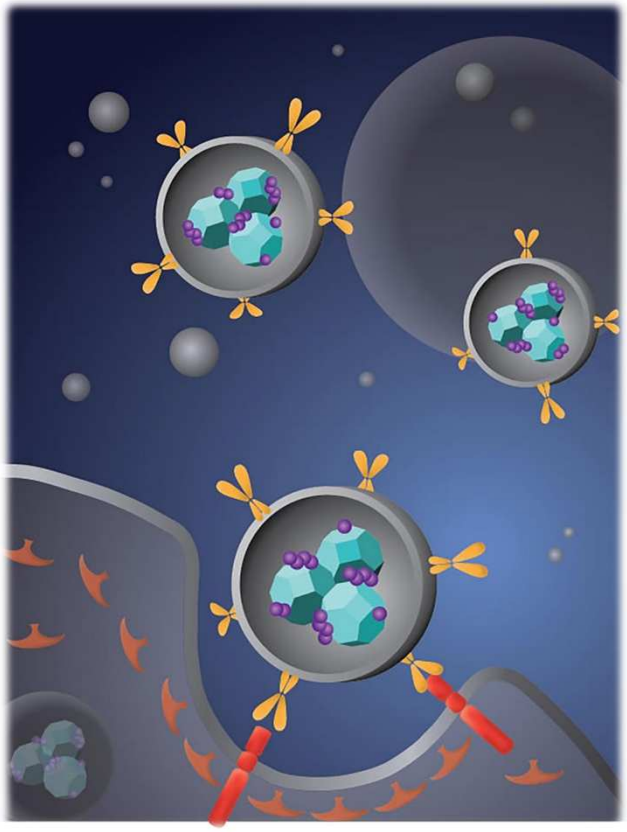
Why Nanotechnology in Drug delivery ?



Drug delivery through history

1656
Sir Christopher Wren (UK) used a quill attached to a small bladder to inject dogs with opium, then experimented on humans.

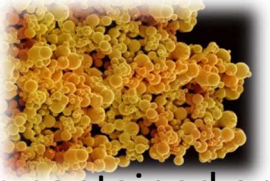
1830s
French physicians implanted pellets of morphine and other drugs beneath the skin with darning needles.

1843
William Brockedon (UK) got a patent for manufacturing pills by hammering dry medicinal ingredients into **a tablet**.

1855
Alexander Wood published the first paper on subcutaneous injection of drugs

Early 1950s
First time-release drug—Nitroglyn—for patients with angina. Multiple coatings on drug pellets in a capsule dissolved at different rates, sustained levels of drug longer in the body

1972
Tiny lipid bubbles called **liposomes** were first proposed as drug carriers; researchers surmised that they would slip more easily through tumors' leaky vessels and into cancerous tissue to deliver their payload.



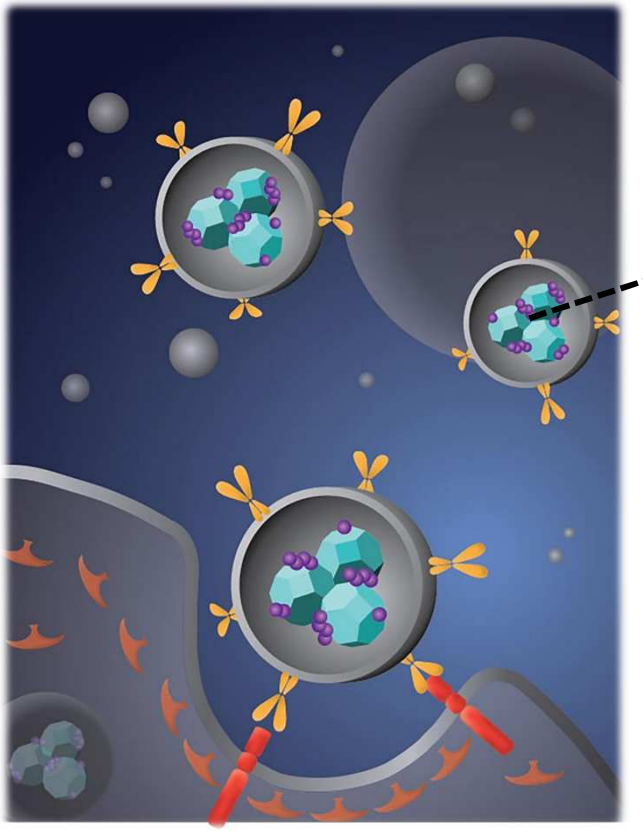
1981
The first **transdermal patch** contained an anti-motion-sickness drug that was absorbed by the skin directly into the circulation, bypassing the liver's filter.

1980s
Robert Langer (MIT) developed a **nano-carrier made of polymers** that slowly released drugs to kill cancer cells in the brain without toxicity to the rest of the body.

2003
The first **drug-eluting coronary stent** was approved by the FDA.

http://protomag.com/assets/delivering-drugs-through-history

Why Nanotechnology in Drug delivery ?



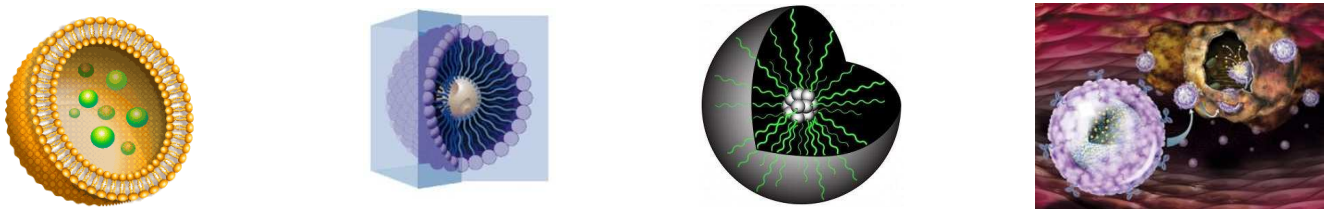
Chemical compounds
Proteins
DNA
RNA

Issues:

- Instability (i.e. light sensitive)
- Aggregation (i.e. hydrophobic drugs)
- Too fast action
- Non-specific delivery

Nanotechnology for Drug Delivery

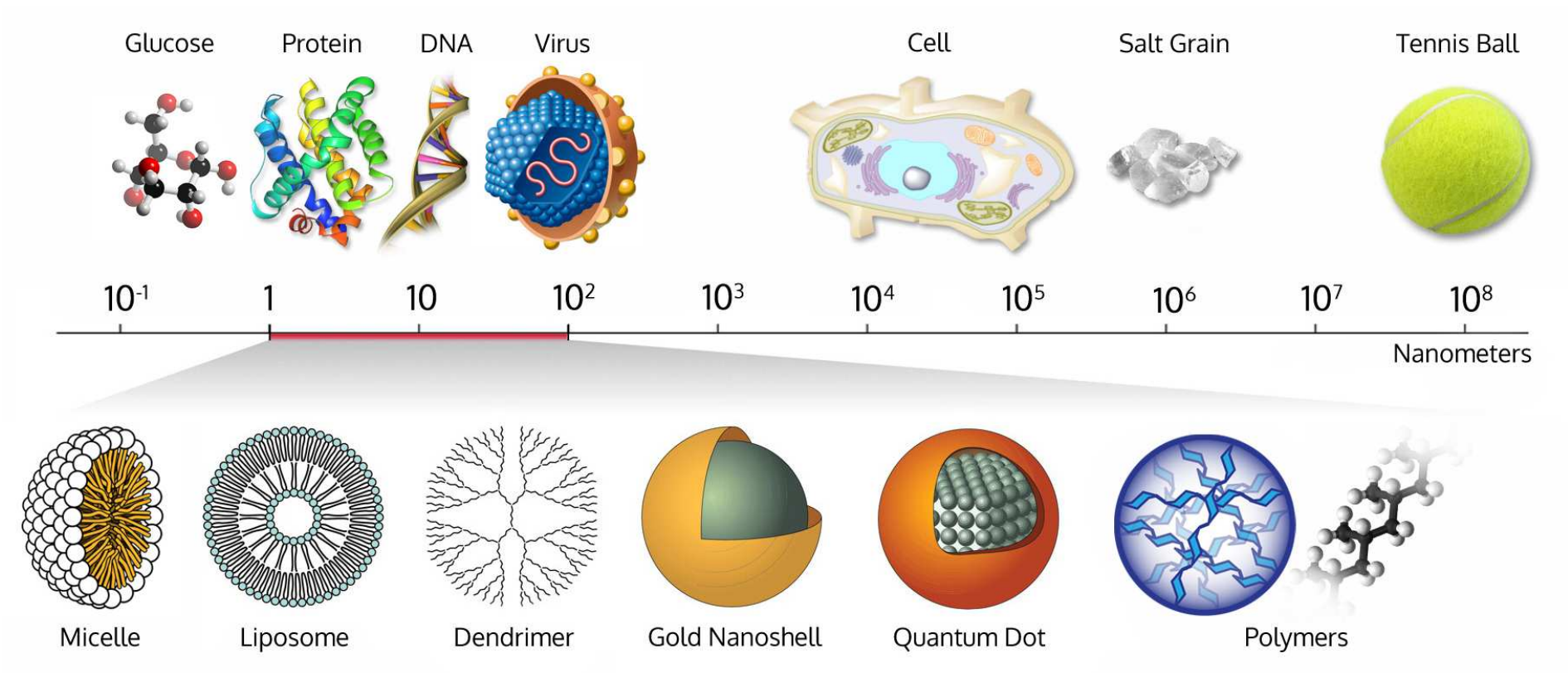
"Drug encapsulation"



Advantages of nanoparticles:

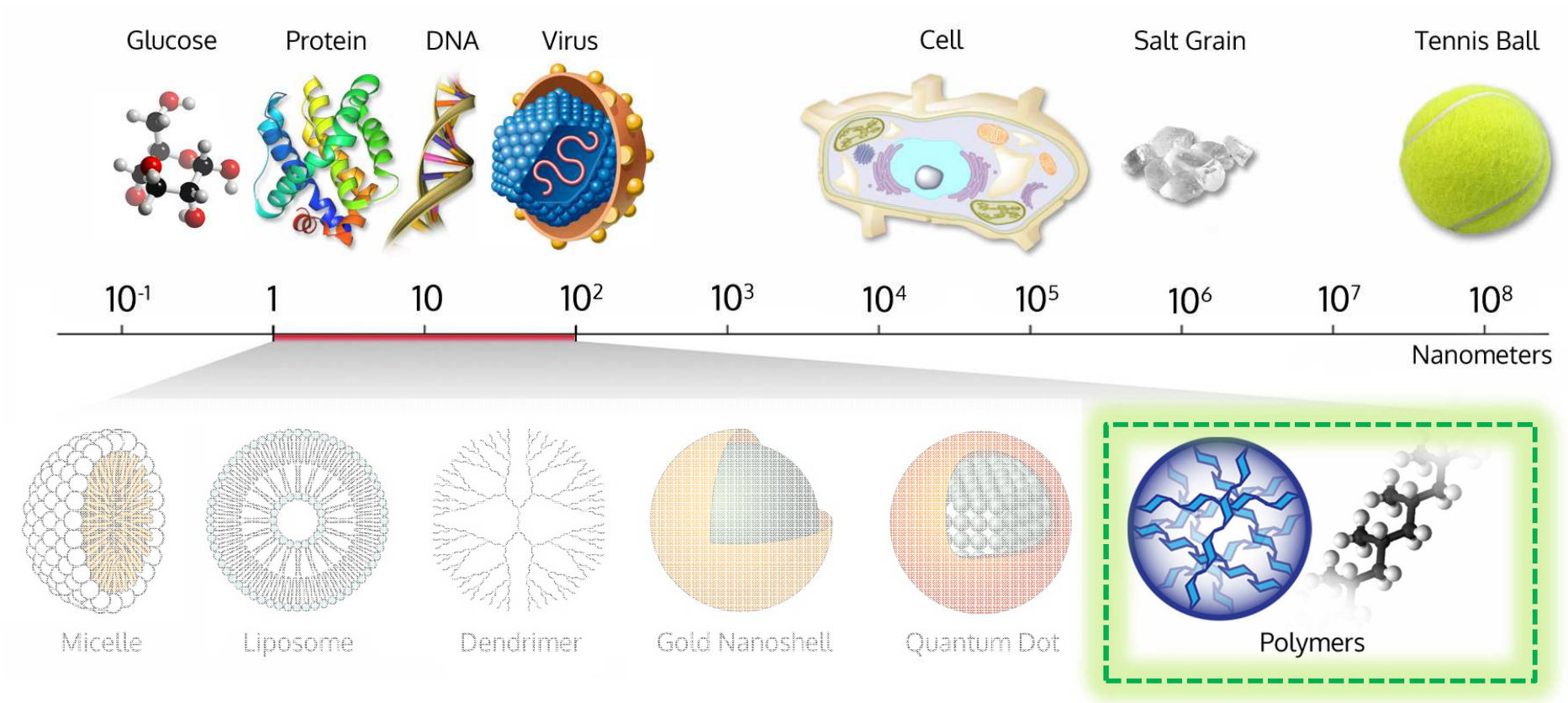
- Stability and long half-life
- Ability for slow released profile
- Feasible for surface modification for targeting purpose
- Passive transportation for sub-200 nm nanoparticles

Nano-carriers for Drug Delivery



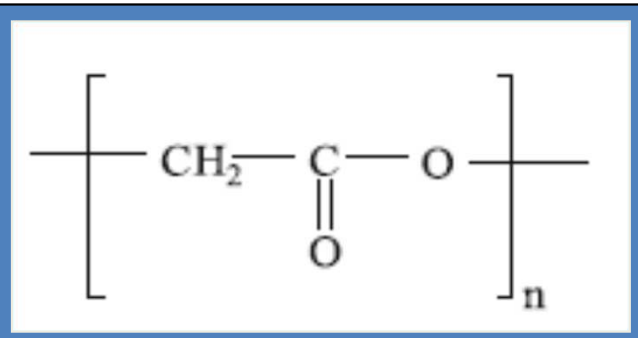
Special delivery vehicles such as liposomes, oil-based dispersions or micelle systems, dendrimer, inorganic and polymeric nanoparticles

Nano-carriers for Drug Delivery

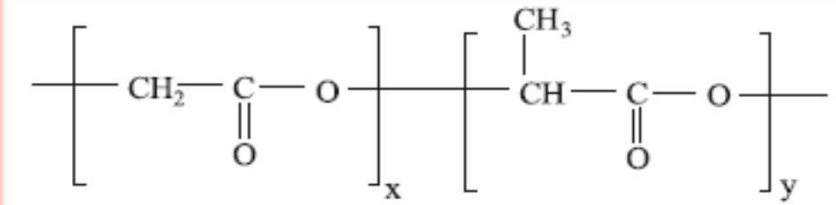


Special delivery vehicles such as liposomes, oil-based dispersions or micelle systems, dendrimer, inorganic and polymeric nanoparticles

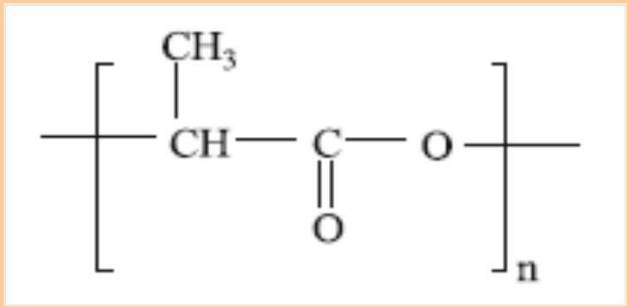
Biodegradable polymers in biomedical areas



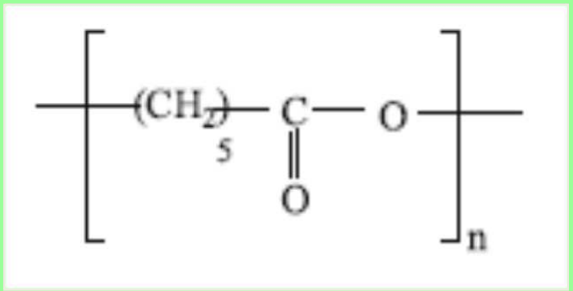
Poly(glycolic acid), PGA



Poly(lactide-*co*-glycolide), (PLGA)

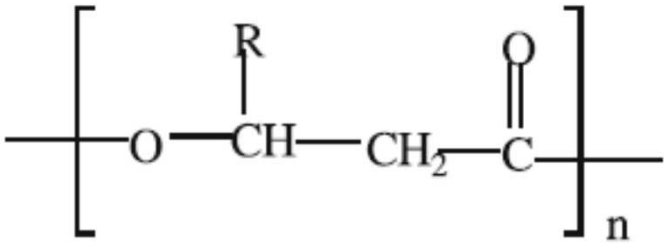


Poly(lactic acid), PLA



Poly(caprolactone), PCL

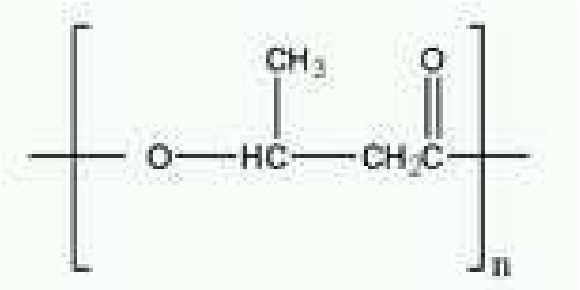
- Controlled drug delivery
- Scaffolds for tissue engineering



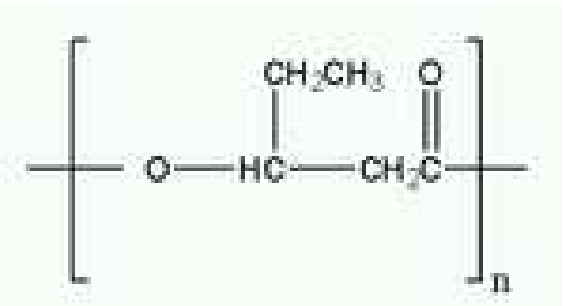
Polyhydroxyalkanoates, PHAs

PHAs Physical Properties for Controlled Drug Delivery

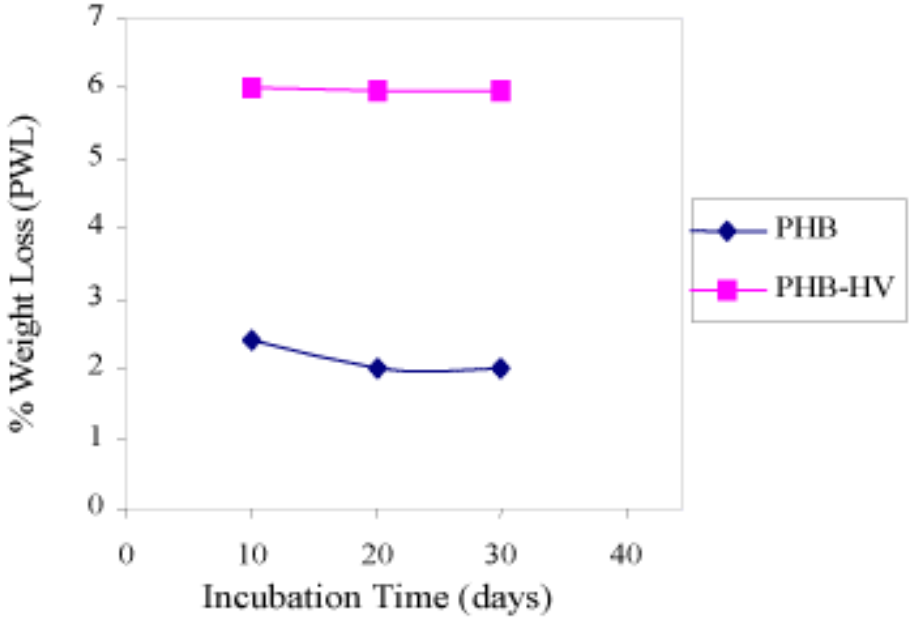
- **Biocompatibility and biodegradability**
- **Adjustable physical properties by types of PHA monomers**

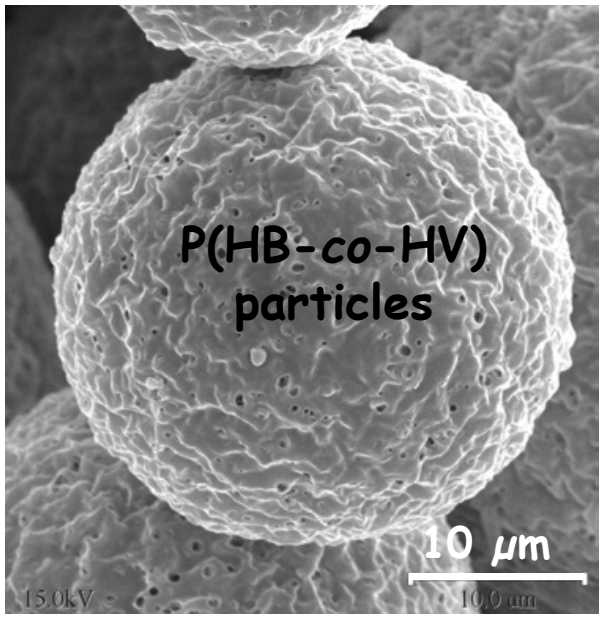


Polyhydroxybutyrate, PHB



Polyhydroxyvalerate, PHV

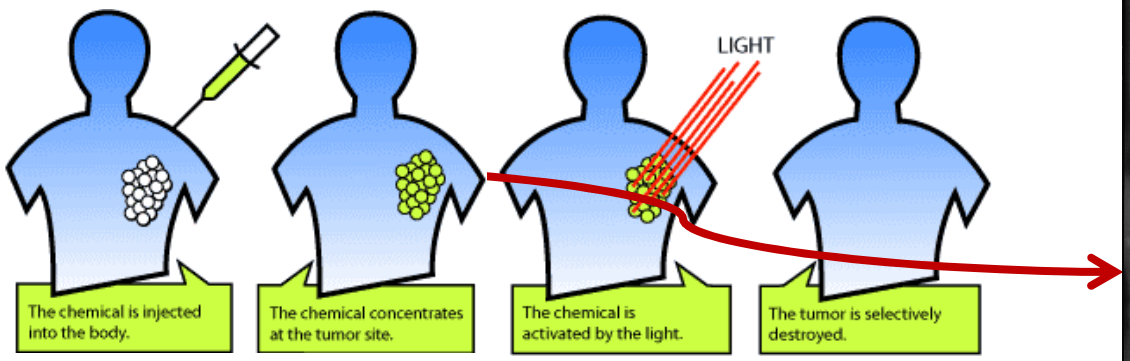


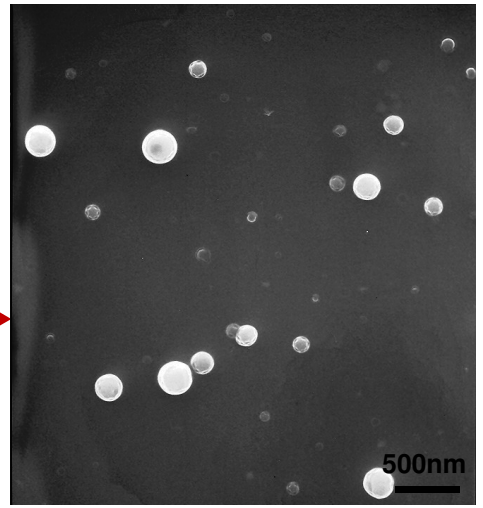


Maia, J. L. *et al.*, 2003

J. L. Maia, The effect of some processing conditions on the characteristics of biodegradable microspheres obtained by an emulsion solvent evaporation process, Braz. J. Chem. Eng. (2004)

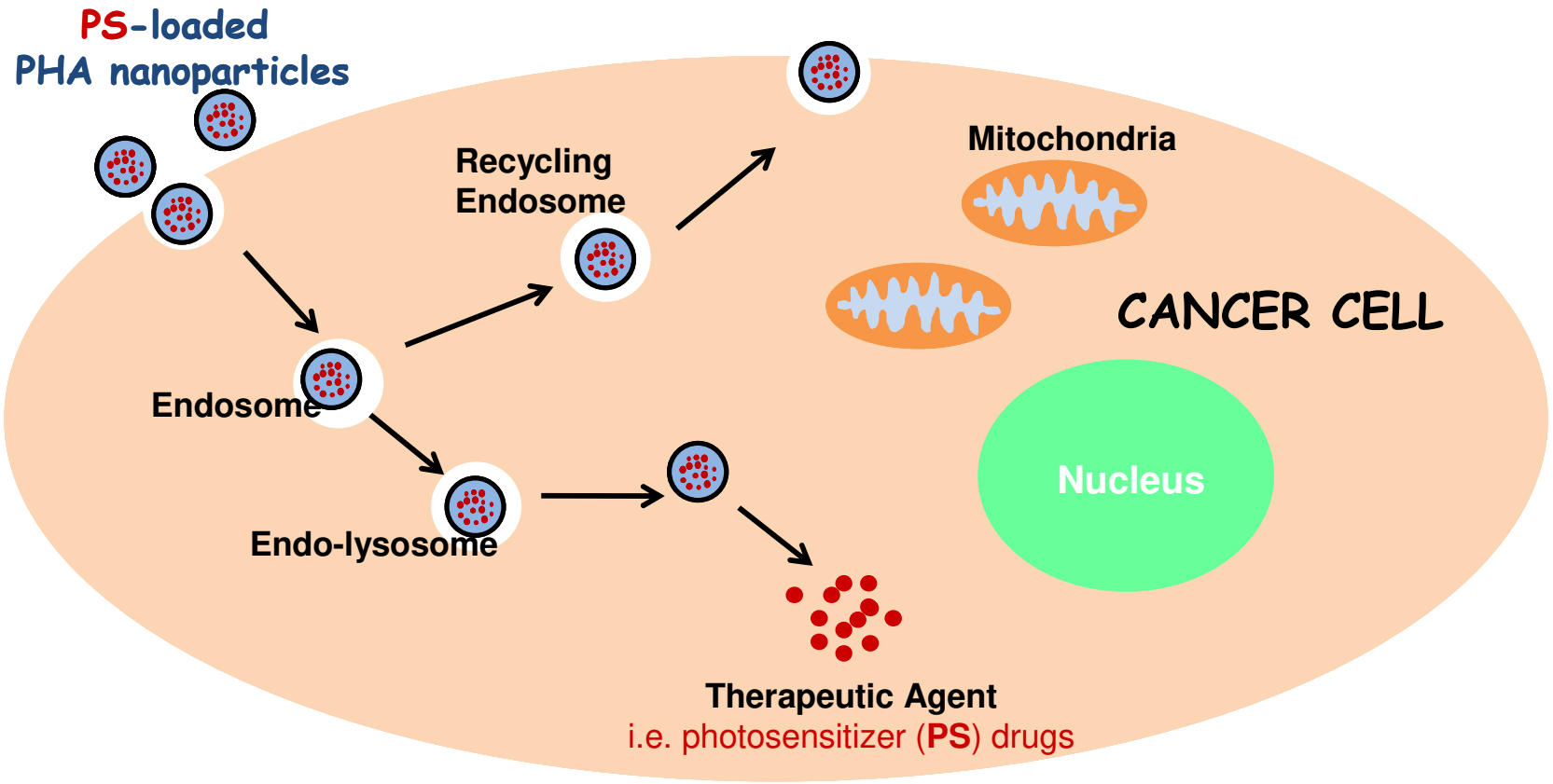
Development of polyhydroxyalkanoate sub-200 nm nanoparticles as anti-cancer drug delivers for photodynamic therapy



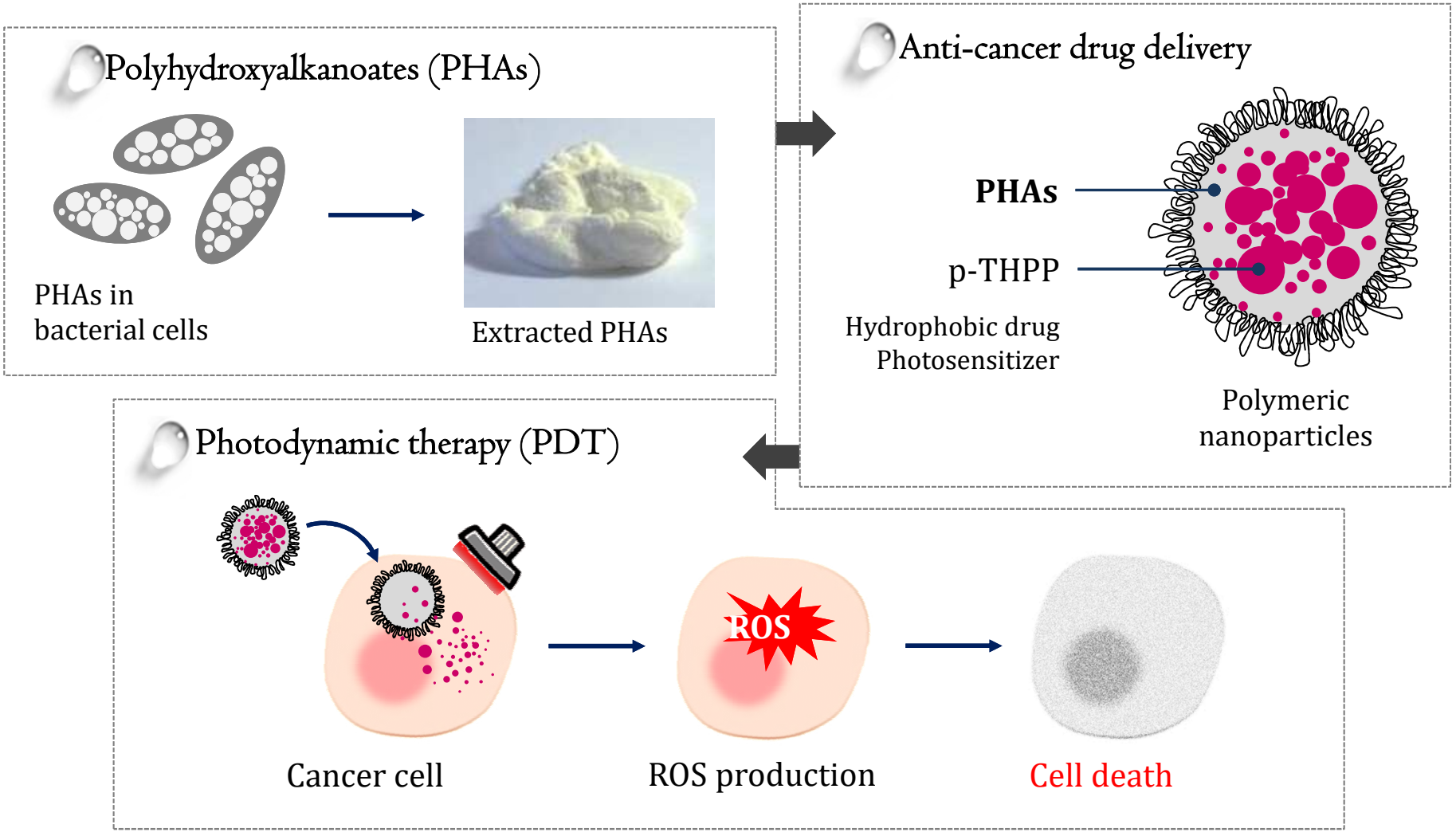


Development and characterization of bio-derived polyhydroxyalkanoate nanoparticles as a delivery system for hydrophobic photodynamic therapy agents.
Pramual, S., Assavanig, A., Sunintaboon, P., Berkgvist, M., Batt, C. A., Lirdprapamongkola, K., Svasti, J., N. Niamsiri,
J Mater Sci Mater Med 2016 Feb 28;27(2):40.

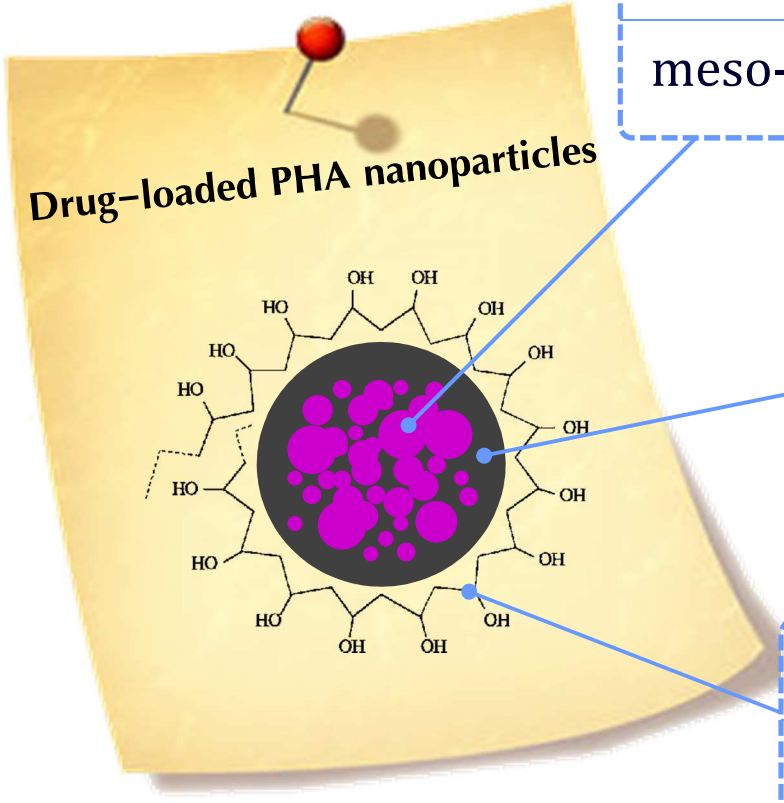
Why sub-200 nm encapsulation of drug can help killing more cancer cells?



The application of bacterial biopolymer as a nanocarrier for anti-cancer drug delivery in photodynamic therapy

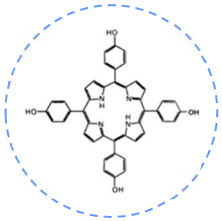


Our formulation strategy:



Anti-cancer drug :: p-THPP

meso-tetra(p-hydroxyphenyl)porphyrin



PHA types

- PHB
- P(HB-co-**12%**HV)
- P(HB-co-**50%**HV)

Stabilizer :: Poly(vinyl alcohol), **PVA**

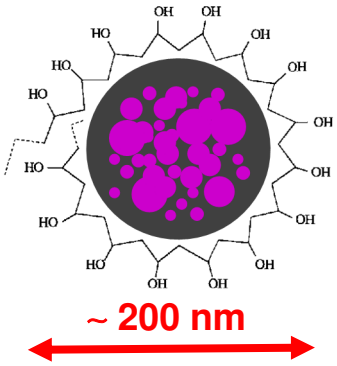
M_w = 30,000-70,000 [87-89% Hydrolyzed]

Nanoparticle characterizations

Initial drug loading: 10%

Polymer types	Size (nm)	Zeta potential (mV)	Drug loading (%)	Entrapment efficiency (%)
PHB	208.3 ± 3.1^{b}	-2.1	2.34 ± 0.50^{a}	23.44 ± 4.96^{a}
P(HB-co-**12%**HV)	198.2 ± 3.4^{a}	-1.0	4.10 ± 1.18^{b}	41.05 ± 11.78^{b}
P(HB-co-**50%**HV)	199.7 ± 5.9^{a}	-1.1	4.17 ± 0.45^{b}	41.71 ± 4.53^{b}

Mean with different superscript letters within the same bar are significantly different ($p \leq 0.05$) as determined by Duncan's test.



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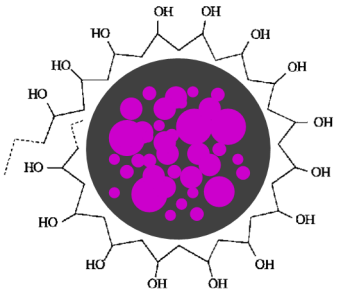
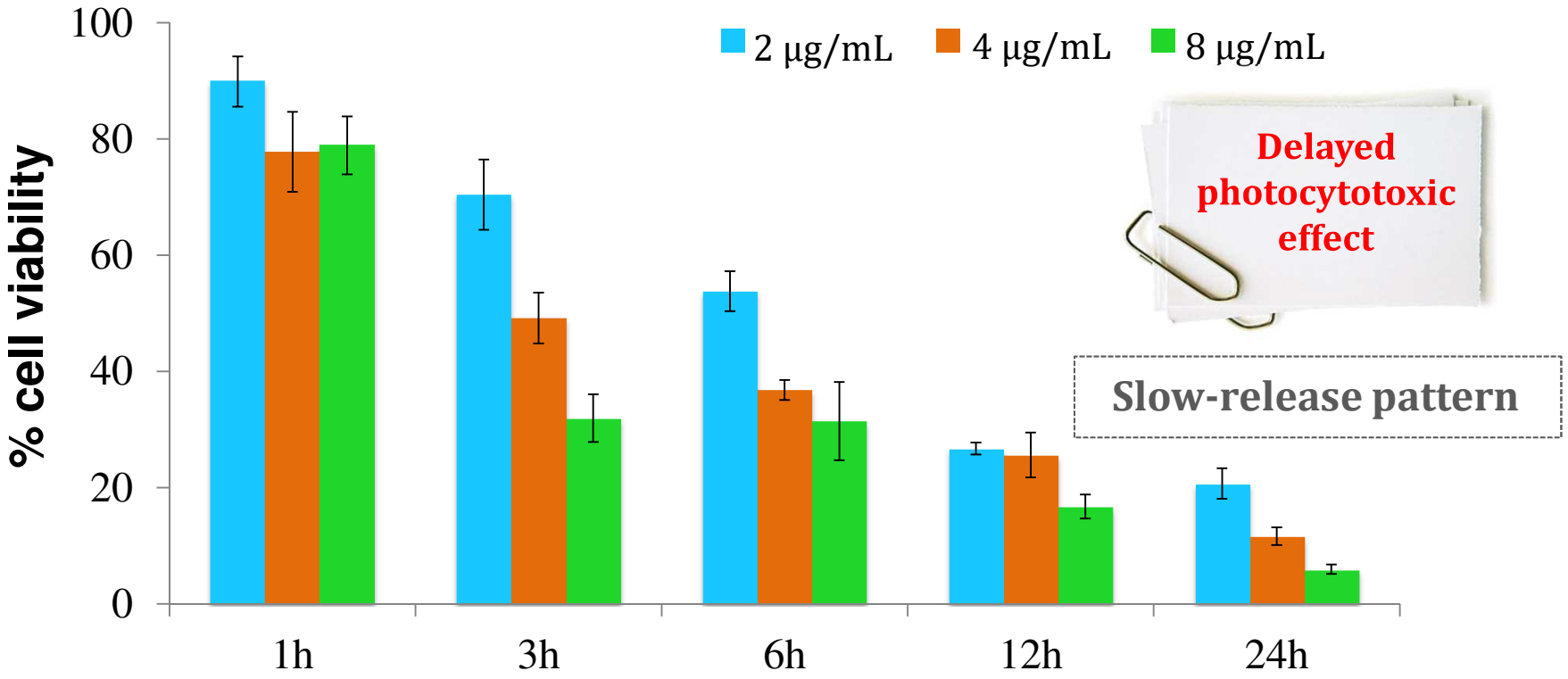


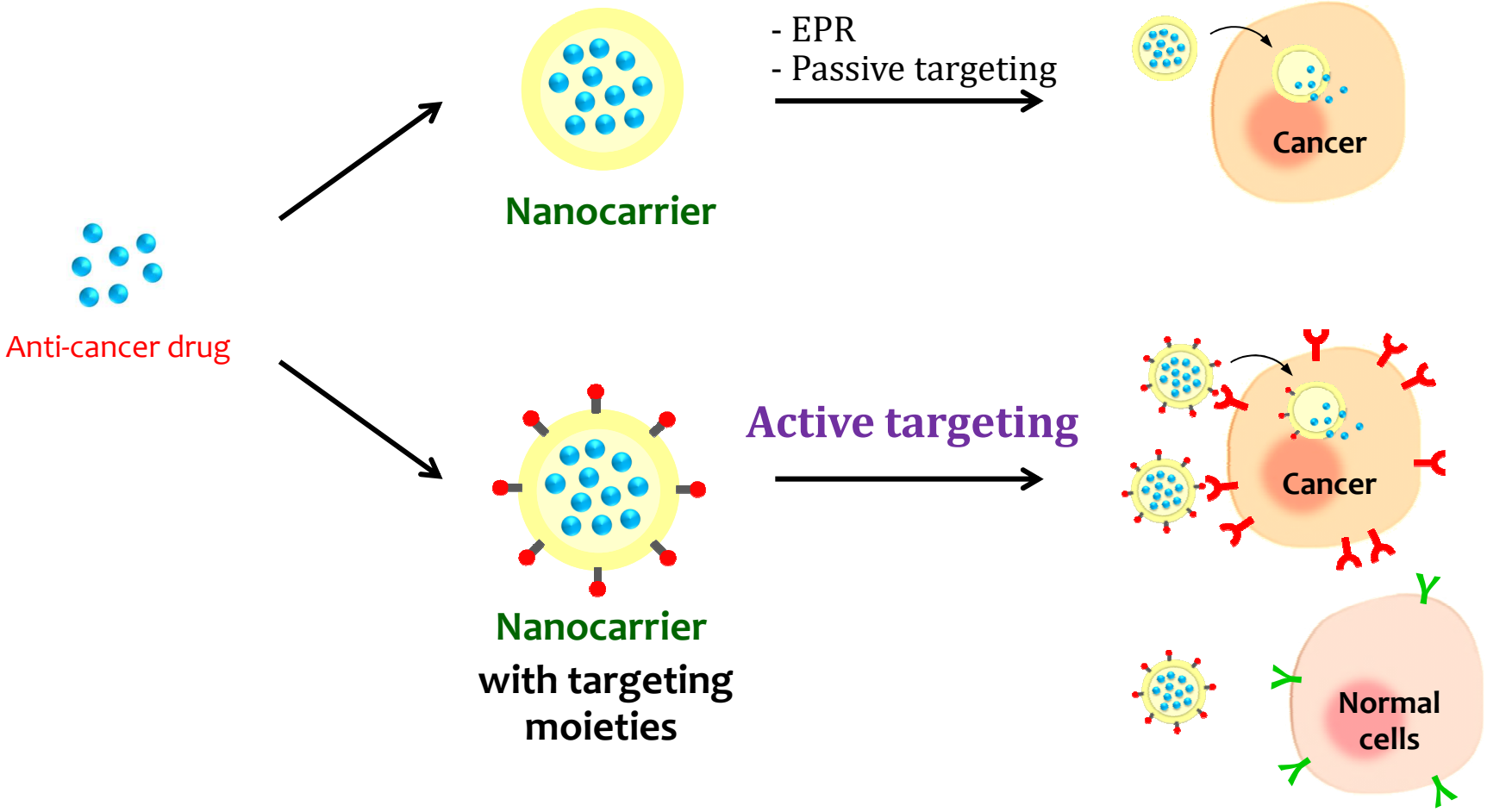
Photocytotoxicity testing by MTT assay

P(HB-co-50%HV) nanoparticles



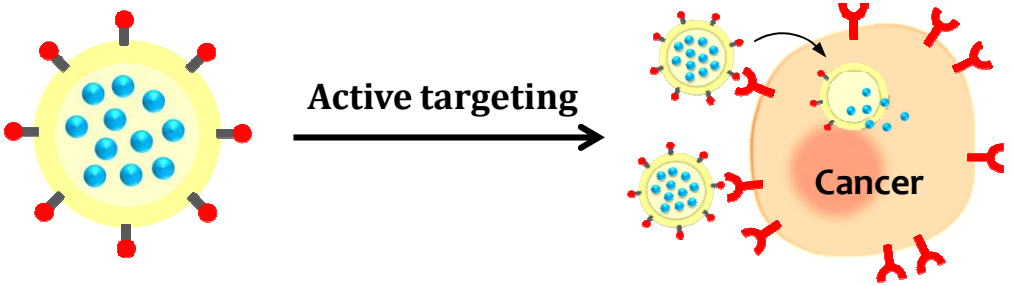
PHAs as a nanocarrier could promote a slow-release of hydrophobic anti-cancer drug for PDT.

Active Nano-carriers for Drug Delivery



4

Active Targeting:



Moieties used as targeting agents:

- **Antibodies**
- Vitamins
- Carbohydrates
- Aptamers
- Peptides (Arg-Gly-Asp etc.)
- *trans*-activating transcriptional activator
- Proteins (e.g. lectins, and transferrin)

- *To be effective, the target agent/target affinity should be relatively high*
- *Target agent should also have low affinity to other binding sites.*
- *Multivalency is a plus.*

Endocytosis pathway	Particles size limit	Proteins involved	Stimuli
Phagocytosis	Up to 20 µm	Actin, dynamin	Antibody Fc region, complement, vitronectin, phosphatidylserine
Macropinocitosis	~1 µm	Actin	Growth factor, antigen binding
Caveolae-mediated	~80 nm	Caveolae, actin, dynamin	Cholera toxin, tetanus toxin, folic acid
Clathrin-mediated	~200 nm	Clathrin, actin, dynamin	Transferrin, low-density lipoprotein, EGF

Antibody-targeted Nanoparticles for Cancer Therapy Francois Fay, Christopher J Scott mmunotherapy. 2011;3(3):381-394. http://www.medscape.com/viewarticle/740169_3

Material systems in drug delivery & nanomedicine

Polymers

- Polyesters (PLGA/PCL/PHA etc.)
- Block-co-polymers (PEGs etc.)
- Hydrogels
- Natural polymers (chitosan/alginate etc.)
- PMMA/PET/PVA etc.
- Phosphorylcholine containing polymers

Inorganic

- Gold
- Silver
- Fe(ox)
- Silica
- Manganese(ox)
- Cobalt (ox)
- QDs

Carbon Nanotubes

- SWNT
- MWNT

Dendrimers/Dendrites

- PAMAM
- PPI
- MPA

Liposomes

- DPPC
- DOTMA
- DC-Chol
- DOPC
- DOPG

Biological

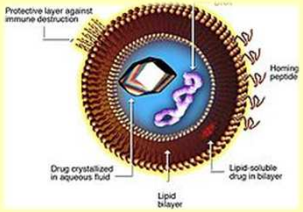
- Collagen/gelatin/fibrin
- Hyaluronan
- Recombinant proteins/conjugates
- Albumin
- Virus
- RNA/DNA (siRNA, assemblies)

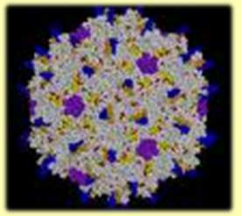
Several of these material systems can be (are) used on macro, micro, and/or nanoscale

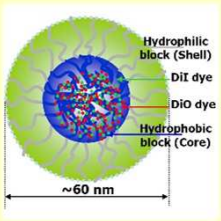
Nanotechnology

+

Drug delivery







Provide more effective "**Adjuvant**" system

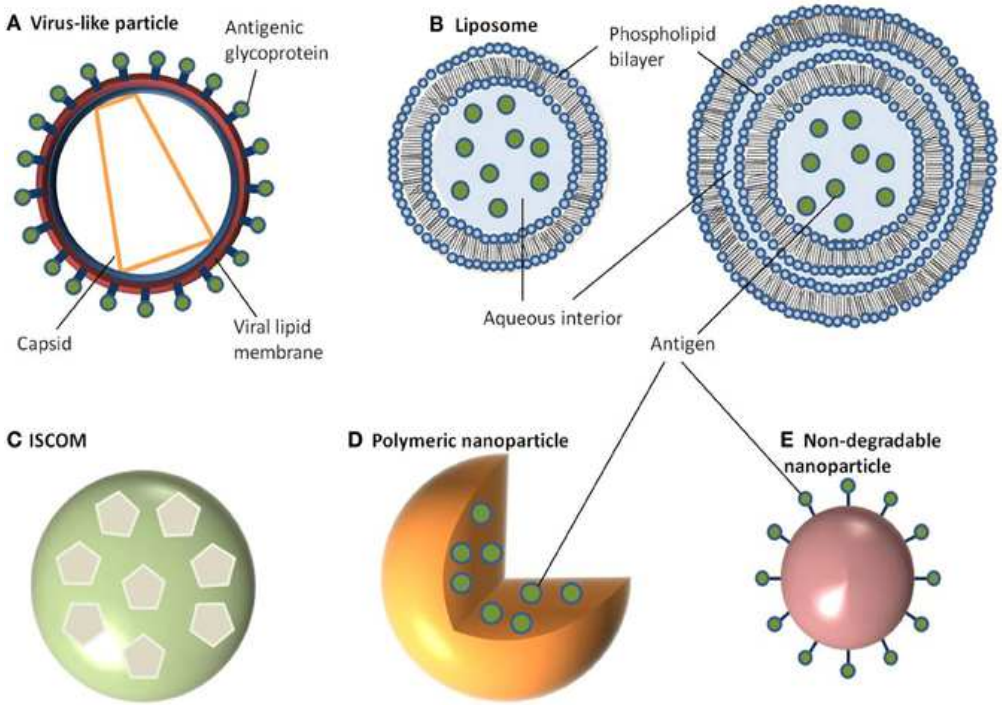
Vaccine delivery

Nanotechnology help improve:

- Instability of antigen
- Prevent aggregation
- Slow-released action
- Improve specific delivery
- **Increase immunomodulatory**

Nanotechnology for Vaccine Delivery

Biodegradable Nanoparticles as Vaccine Adjuvants and Delivery Systems: Regulation of Immune Responses by Nanoparticle-Based Vaccine



Adv Polym Sci (2012) 247: 31–64
DOI: 10.1007/12_2011_150

Published online: 20 October 2011

- The engineering of **nanostructure materials**, including nanoparticles, nanoemulsions or nanotubules, holds great promise for the development of new **immunomodulatory agents**, as such
  - Nanostructures can be used to more effectively manipulate or deliver immunologically active components to target sites.

- Successful **applications of nanotechnology** in the field of immunology will enable new generations of vaccines, adjuvants and immunomodulatory drugs that aim to improve clinical outcomes in response to a range of infectious and non-infectious diseases.

Table 1 – In vivo applications of nanocarriers for delivery of vaccines.

Delivery system	Composition	Antigen	Route	References
Liposome	DDA, TDB	Ag85B-ESAT-6	Intramuscular	[18]
	DDA, TDB	OVA	Intramuscular	[19]
	DDA, DODA, TDB	Ag85B-ESAT-6	Intramuscular	[20]
	Pegylated DDA, TDB	Ag85B-ESAT-6	Subcutaneous	[22]
	DDA, DSPC, cholesterol, TDB	Ag85B-ESAT-6	Subcutaneous	[23]
	MPL, DDA, TDB	OVA	Intraperitoneal	[24]
	DDA, TDB	Trivalent influenza vaccine	Subcutaneous	[25]
	DOPC, DOPG, MPB	OVA	Subcutaneous	[26]
	EPC, DOGS-NTA-Ni	His-tagged heat shock protein	Intradermal	[27]
	MDMPC, DMPG, cholesterol, MPL	Polyhistidinylated OVA	Subcutaneous	[28]
	Lecithin, cholesterol	Diphtheria toxoid	Subcutaneous	[29]
Emulsion	MF59	Hemagglutinin	Intramuscular	[34]
	MF59	Recombinant meningococcal B protein	Intramuscular	[36]
	MF59	Recombinant meningococcal B protein	Intramuscular	[35]
	W805EC	OVA	Intranasal	[39]
	W805EC	OVA	Intranasal	[40]
	GLA	Falciparum subunit	Subcutaneous	[41]
	GLA-SE	Plasmodium vivax subunit	Subcutaneous	[42]
	GLA-SE	Recombinant hemaagglutinin	Intramuscular	[44]
Synthetic polymer-based system	PLGA	OVA	Subcutaneous	[8]
	PLGA, polylactic acid	Hepatitis B surface antigen	Pulmonary	[45]
	Lipid-coated PLGA	OVA	Subcutaneous	[46]
	Lipid-coated PLGA	Malaria antigen	Subcutaneous	[47]
	Chitosan-coated polycaprolactone	H1N1 hemagglutinin	Intranasal	[48]
	Polyanhydrides	Yersinia pestis antigen	Intranasal	[49]
	Polylactic acid	Hepatitis B surface antigen	Subcutaneous	[51]
	Deacylated cationic polyethyleneimine	HIV CN54gp140 antigen	Pulmonary	[52]
	PEGylated poly [2-(N,N-dimethylamino) rthylmethacrylate]	HIV gag DNA	Intranasal	[53]
Natural biopolymer-based system	N-trimethyl chitosan	OVA	Intranasal	[54]
	Chitosan nanoparticles	HBsAg	Intraperitoneal	[56]
	Cholesteryl-conjugated pullulan	Clostridium botulinum type-A neurotoxin subunit antigen	Intranasal	[57]
Carbon-based system	SWCNT	Tuberculin purified protein derivative	Subcutaneous	[59]
	Carbon nanotube	Azoxystrobin	Subcutaneous Intraperitoneal	[60]
	Carbon magnetic nanoparticles	Hen egg lysozyme	Intravenous	[61]
	Carbon nanoparticles	Bovine serum albumin	Oral	[63]

Preparation of antigen-encapsulating polymeric nanoparticles by w/o/w emulsion method

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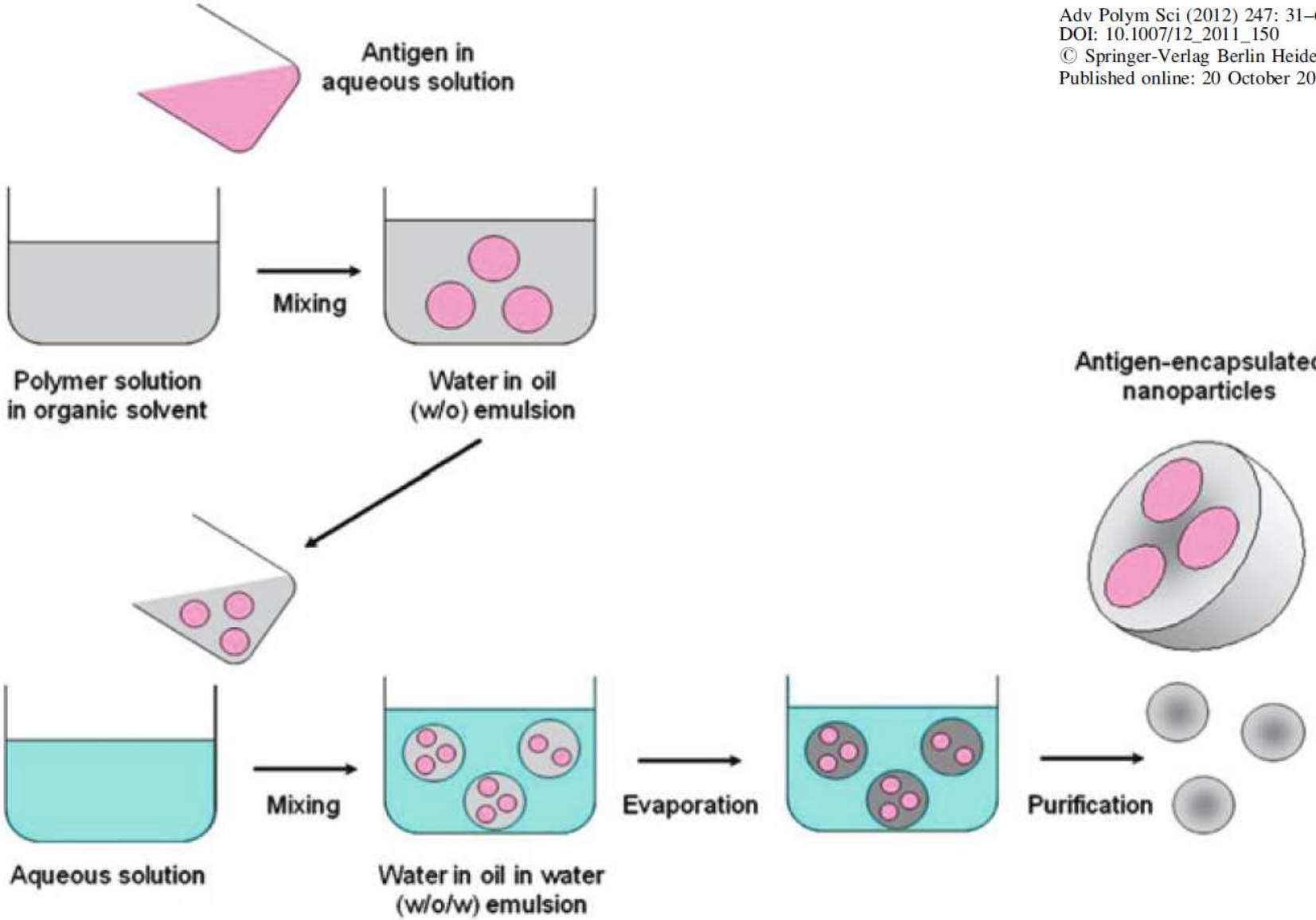


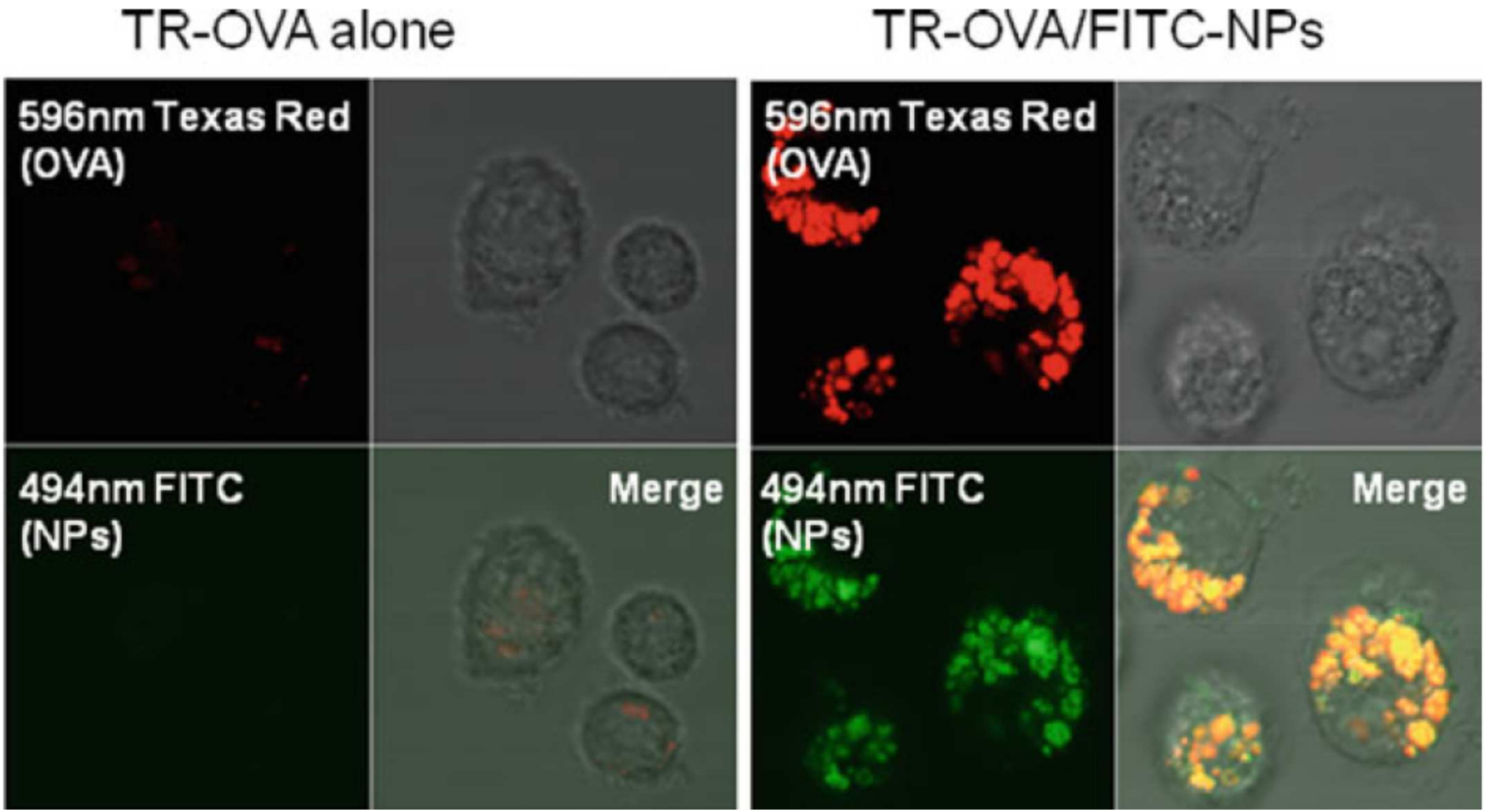
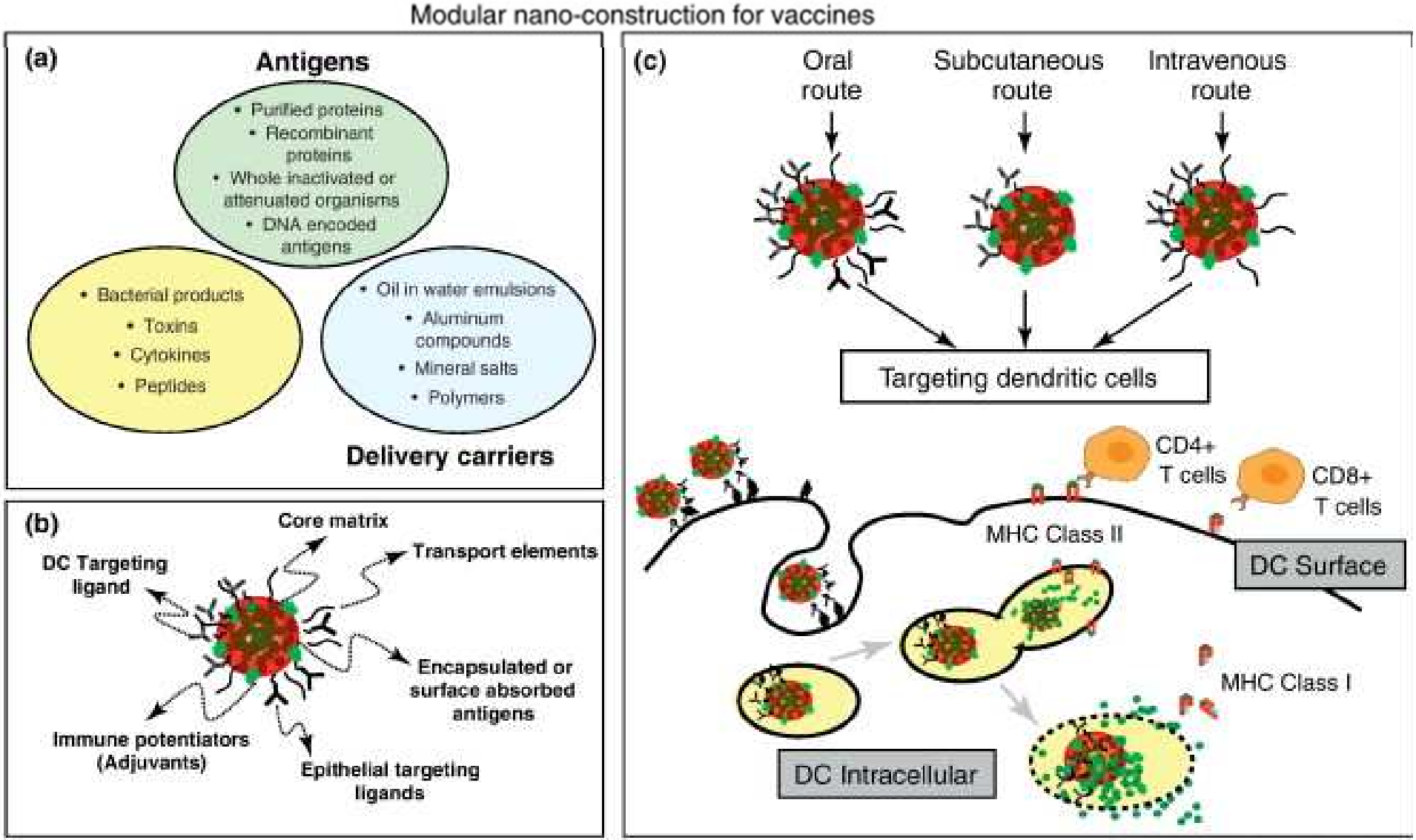
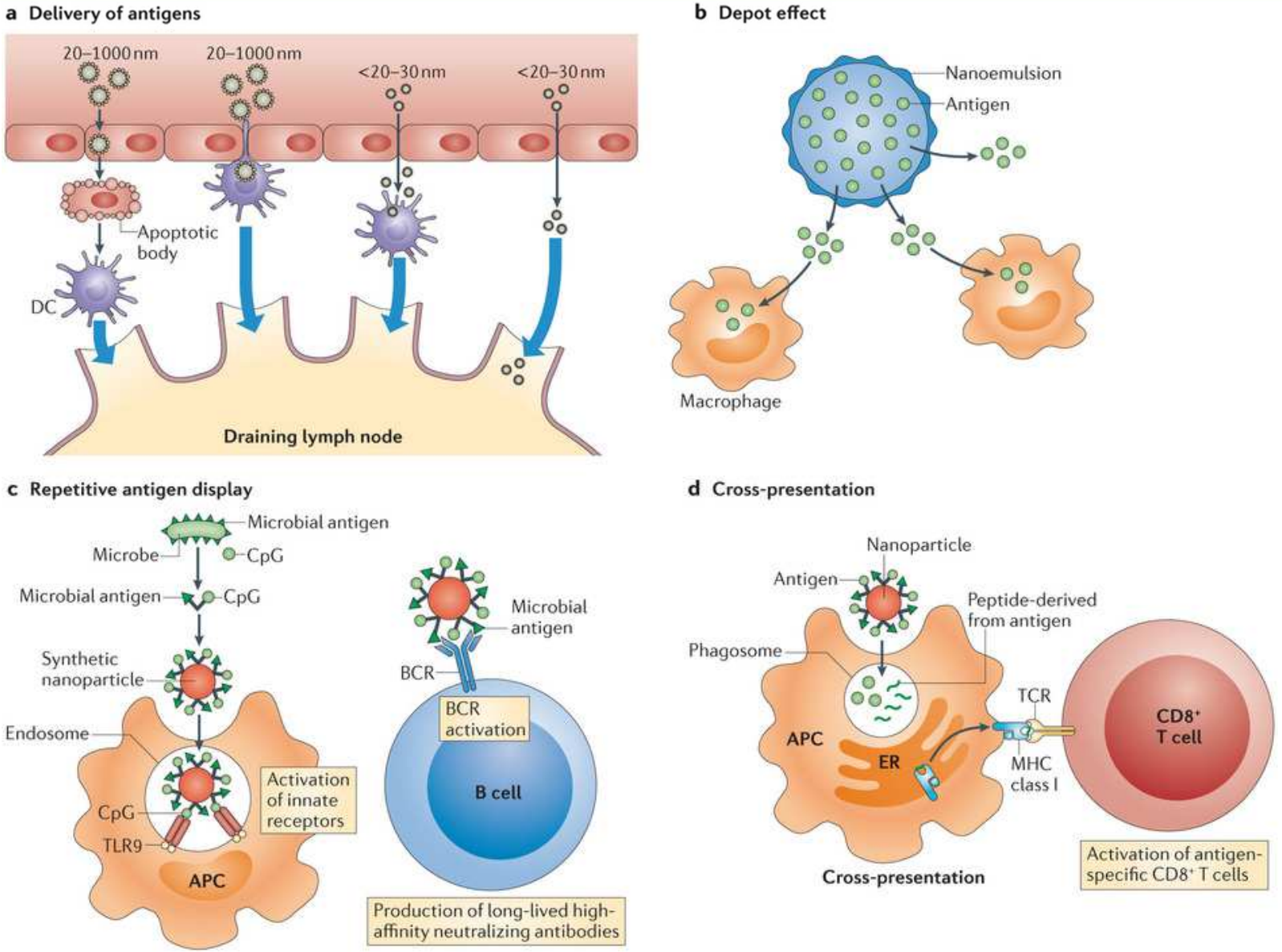


Fig. 13 Uptake of OVA-encapsulating γ-PGA-Phe nanoparticles by DCs. DCs were incubated with Texas Red-labeled OVA (*TR-OVA*) alone (**a**) or TR-OVA encapsulated within fluorescein-labeled nanoparticles (*TR-OVA/FITC-NPs*) (**b**). The intracellular localization of OVA (*red*) and NPs (*green*) was observed by confocal laser scanning microscopy

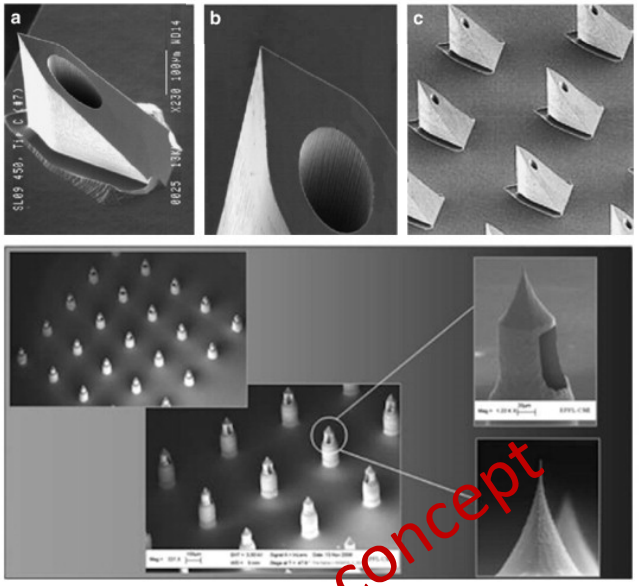
Improve Targeting for Vaccine Delivery

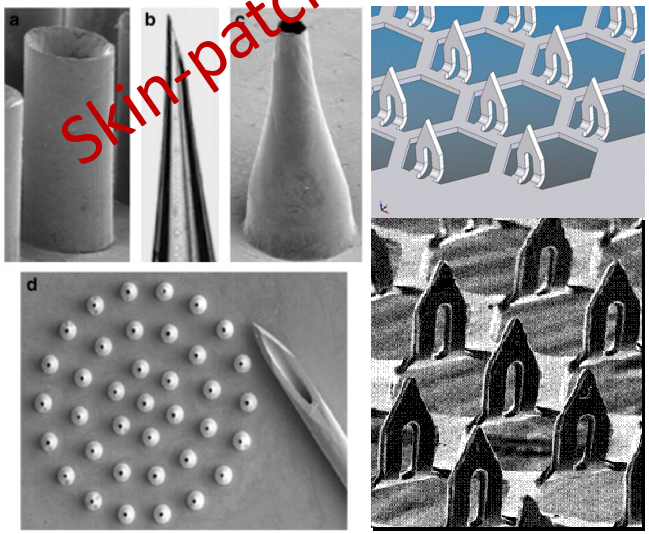


Nanoparticles can alter the induction of immune responses

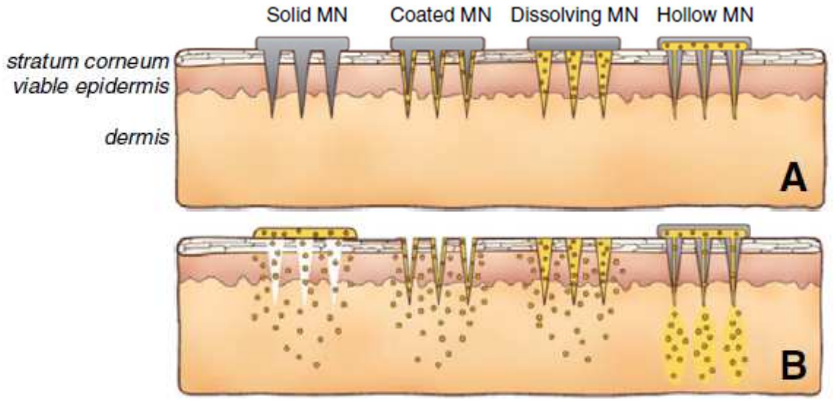


Microneedle in drug-delivery

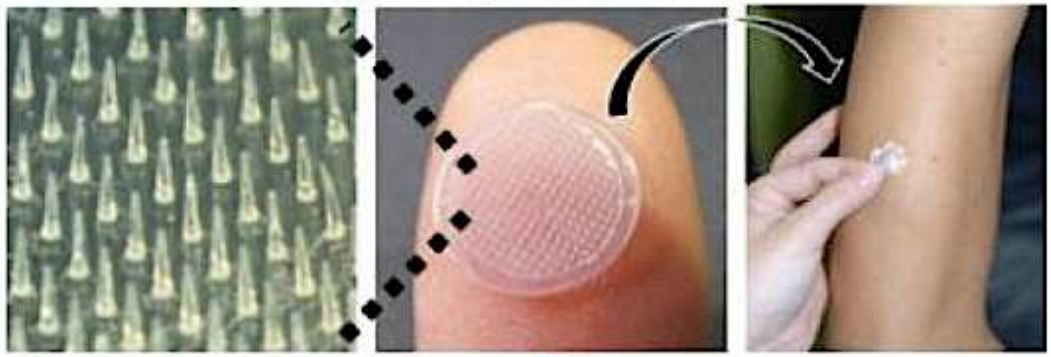


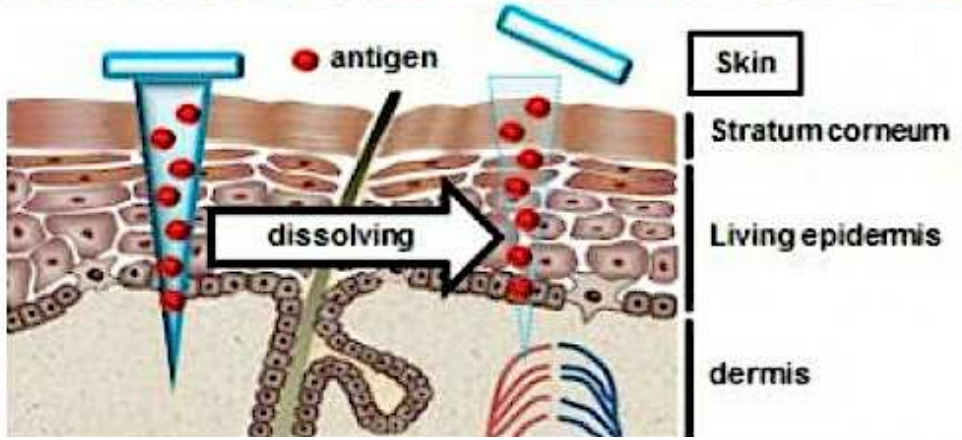


Skin-patch concept



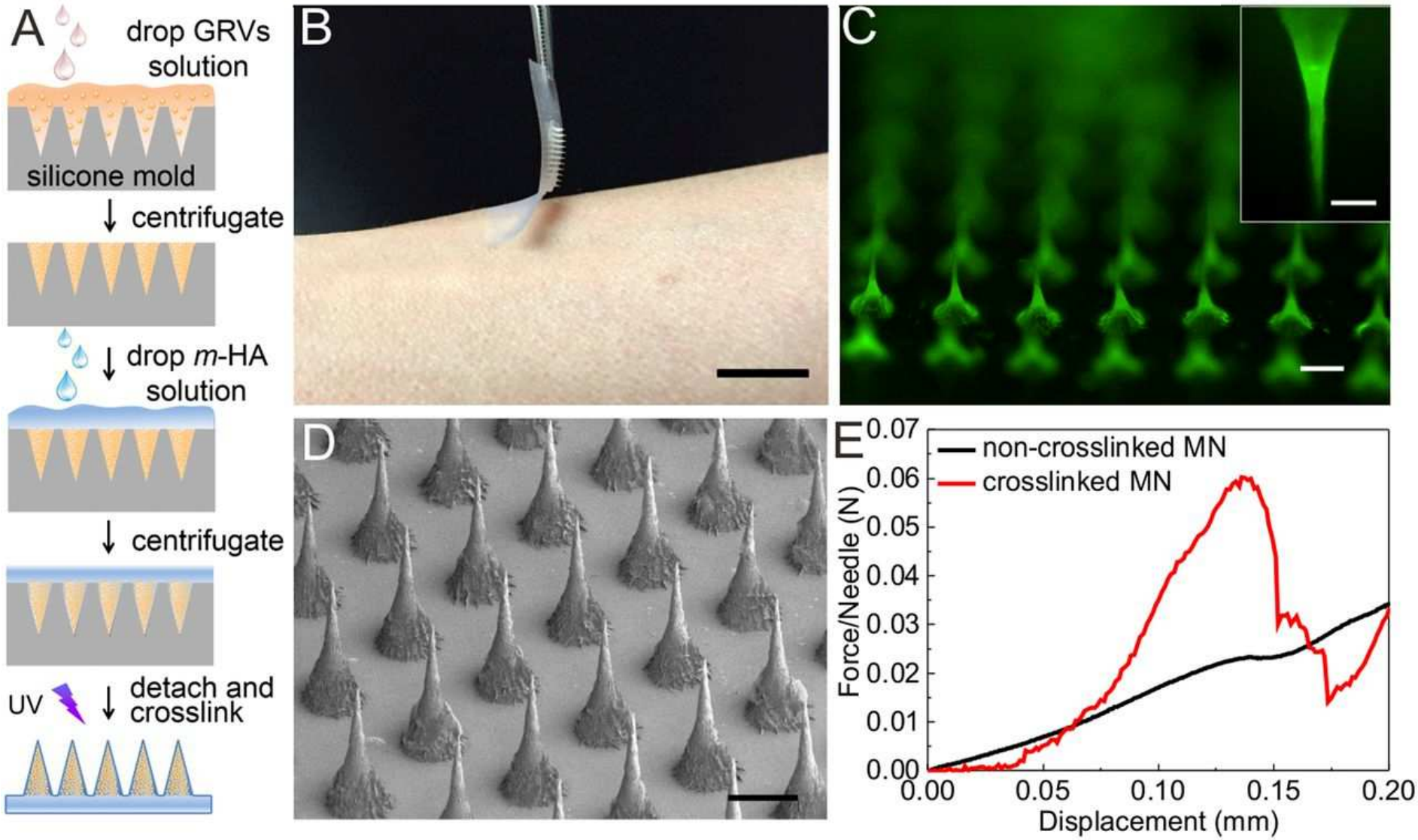
Dissolving microneedle patch





Reservoir-based drug delivery systems utilizing microtechnology. Advanced Drug Delivery Reviews Volume 64, Issue 14, November 2012, Pages 1590–1602

Microneedle in drug-delivery



http://www.pnas.org/content/112/27/8260/F8.large.jpg

Nano-delivery systems - Summary

In general “drug delivery systems" refers to:

- The construct of the drug/vaccine – its delivery vehicle
- Provides benefits (i.e. stable, controlled release, & active targeting) for delivery of drug/vaccine to target sites

Common vehicles for delivering drug/vaccine:

- Natural and synthetic polymers
- Liposomes

Methods of Administration:

- Enteral (Oral, feeding tube, rectal - stomach/GI tract adsorption)
- **Parenteral (Injection - Intravenous, intramuscular etc.)**

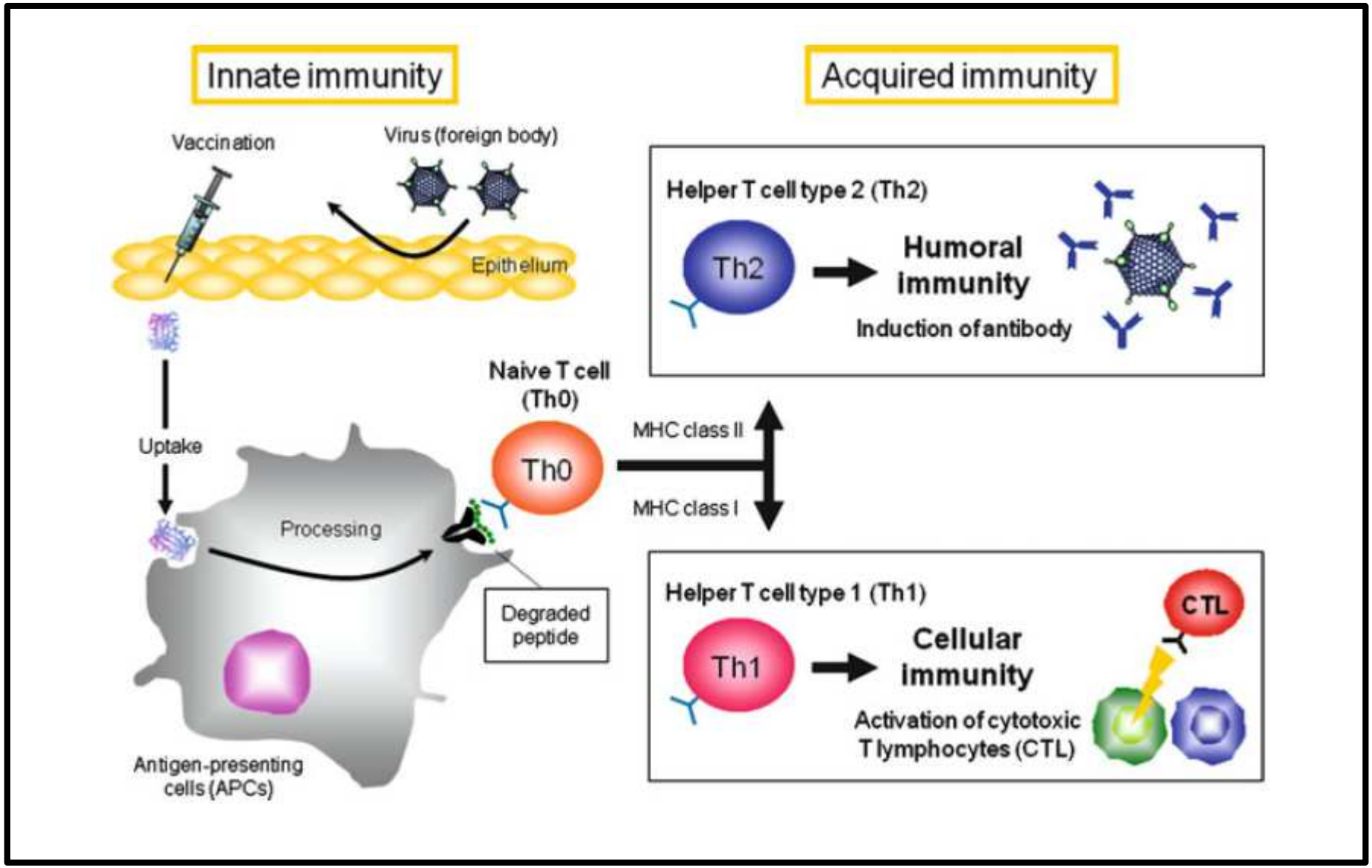
- Transmucosal (Under the tongue/nasal)
- Topical (Application on skin)

- **Transdermal (Medicated skin patches/adsorption through skin)**
- Inhalation and absorption of powdered drugs through the lungs.

- Implanted devices - drug release via external remote control (emerging tech.)

Thank you for your attention.

Induction immune responses by vaccination



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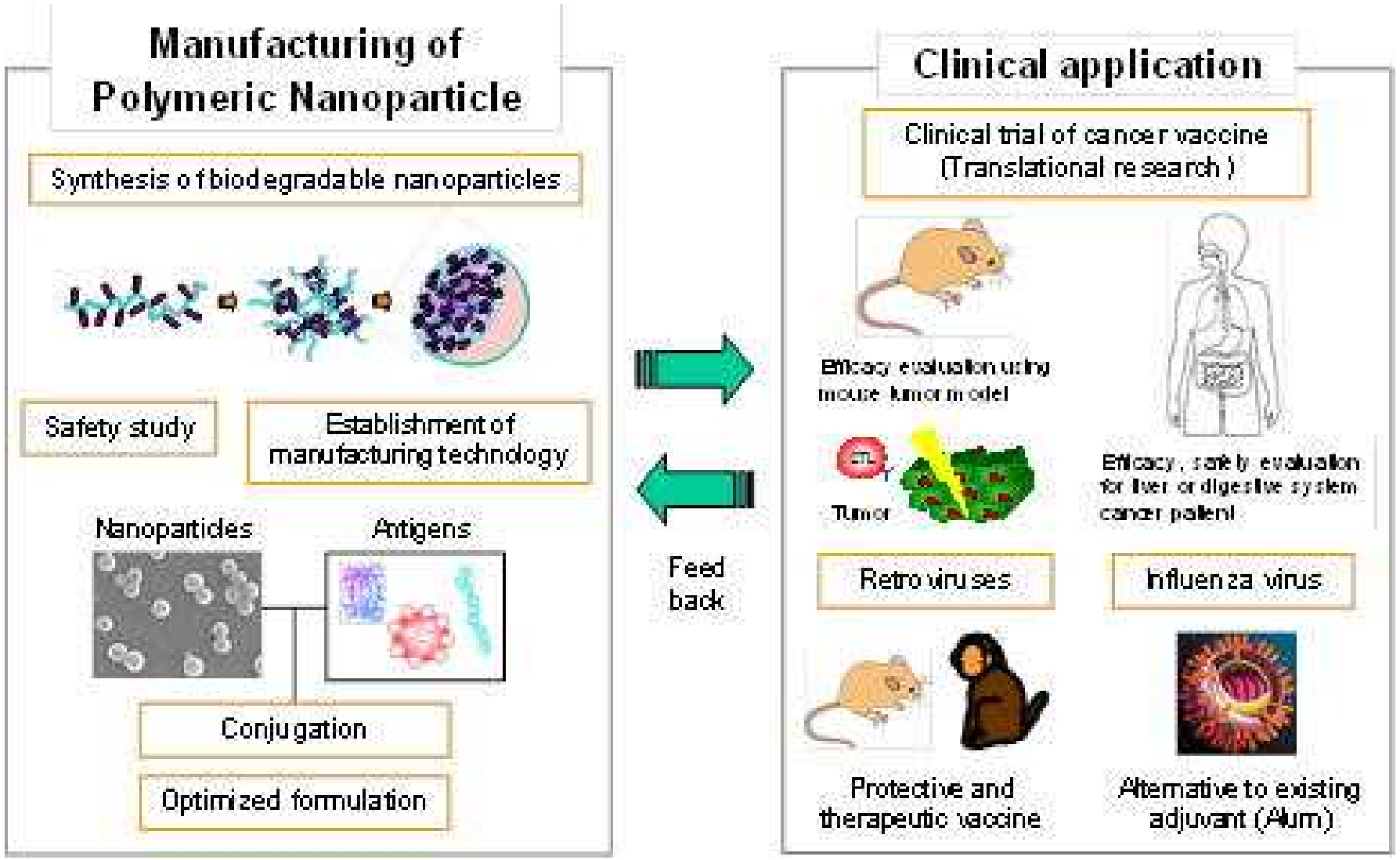
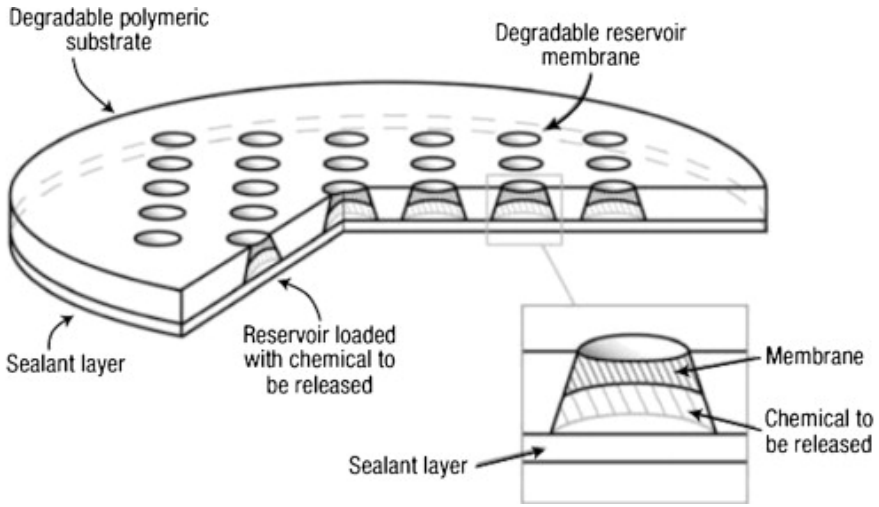
Nanotechnology for Vaccine Delivery



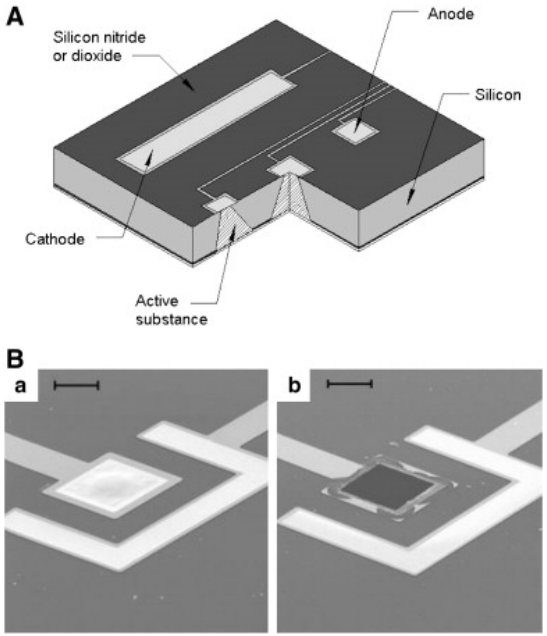
http://www.chem.eng.osaka-u.ac.jp/~akashi-lab/image22.jpg

MEMS technology in modern drug-delivery

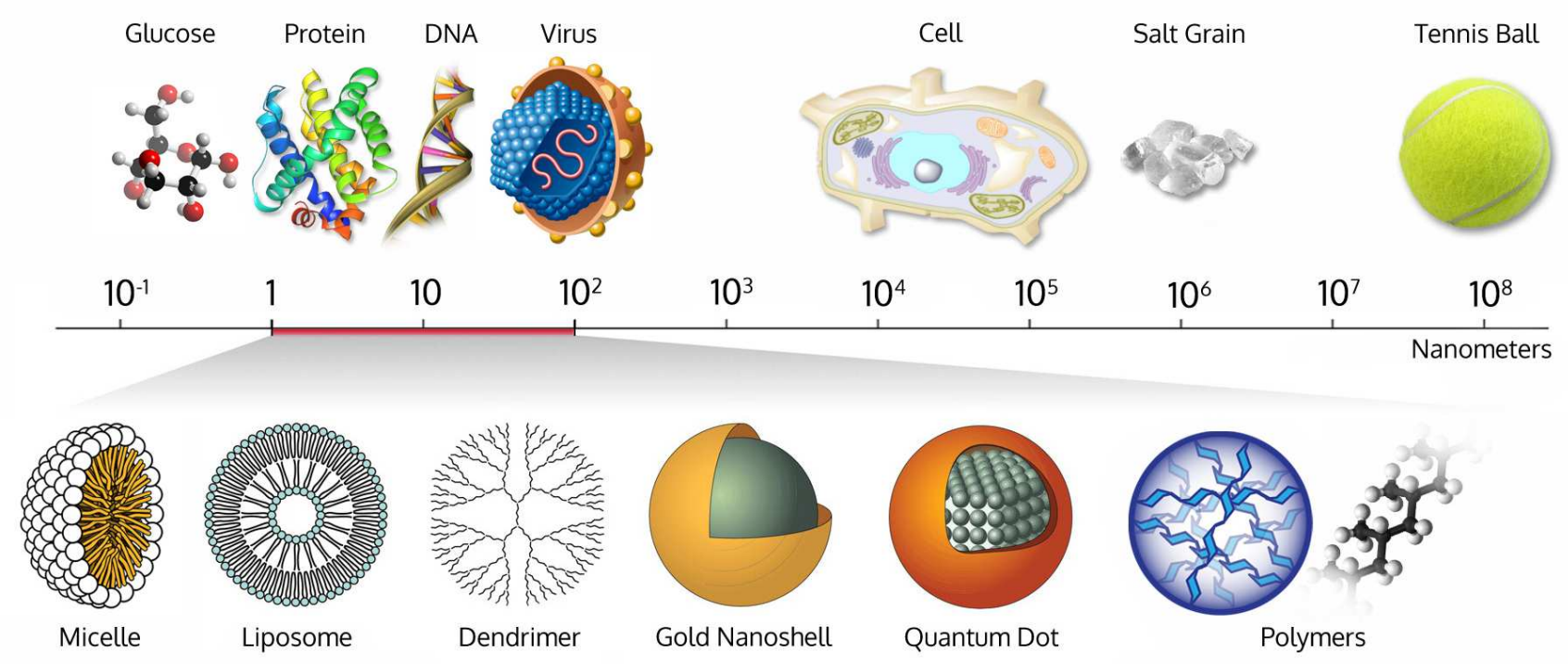
Passive, matrix controlled drug delivery system using bioerodible PLGA

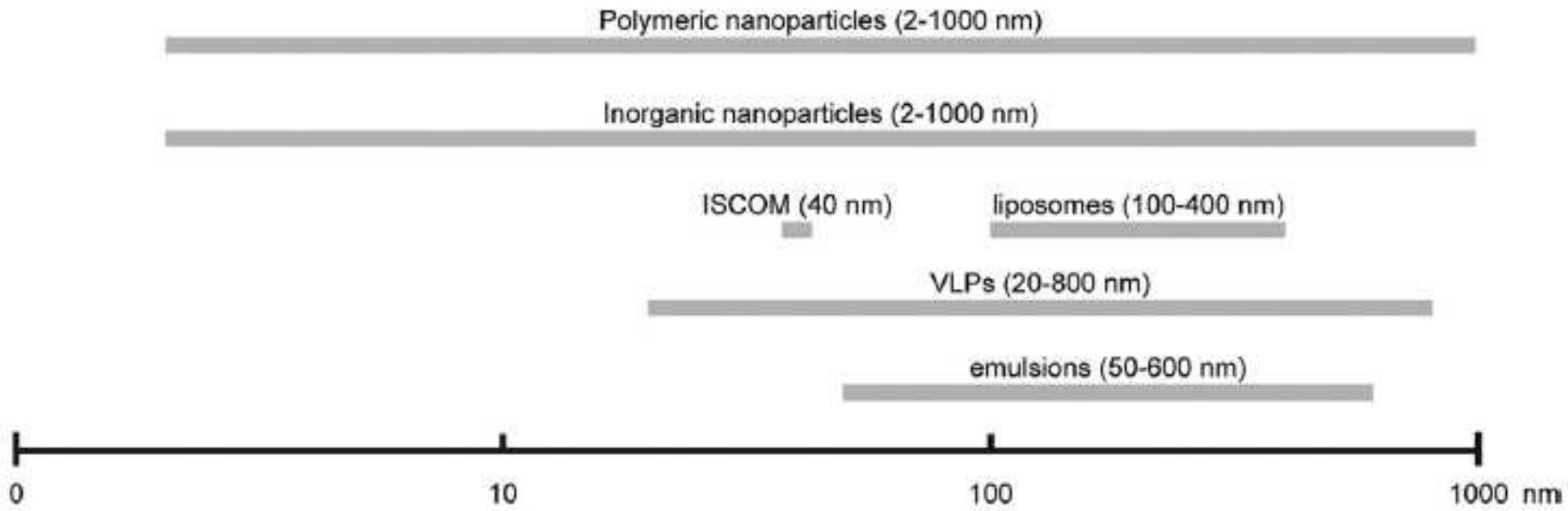


Reservoir-based drug delivery systems utilizing microtechnology. Advanced Drug Delivery Reviews Volume 64, Issue 14, November 2012, Pages 1590–1602



Electrochemically actuated drug delivery implant


Glucose
Protein
DNA
Virus
Cell
Salt Grain
Tennis Ball
10^{-1}
1
10
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
10^{7}
10^{8}
Nanometers
Micelle
Liposome
Dendrimer
Gold Nanoshell
Quantum Dot
Polymers


Polymeric nanoparticles (2-1000 nm)
Inorganic nanoparticles (2-1000 nm)
ISCOM (40 nm)
liposomes (100-400 nm)
VLPs (20-800 nm)
emulsions (50-600 nm)
0
10
100
1000 nm